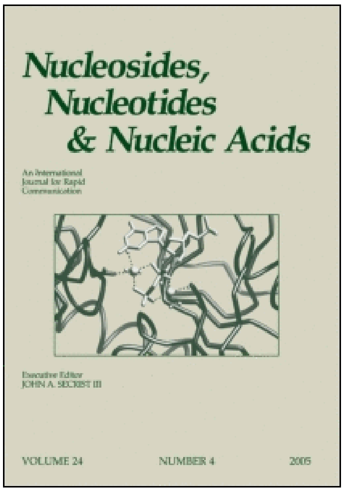

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

N, N-Diisopropyl-O-Methyl-O-4-Nitrophenyl Phosphoramidite. New Flexible Phosphitylating Reagent in the Synthesis of Thio- and Seleno-Oligonucleotides

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To cite this Article Heliński, Jan , Dabkowski, Wojciech and Michalski, Jan(1993) 'N, N-Diisopropyl-O-Methyl-O-4-Nitrophenyl Phosphoramidite. New Flexible Phosphitylating Reagent in the Synthesis of Thio- and Seleno-Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 12: 6, 597 — 604

To link to this Article: DOI: 10.1080/07328319308019014

URL: http://dx.doi.org/10.1080/07328319308019014

NUCLEOSIDES & NUCLEOTIDES, 12(6), 597-604 (1993)

N,N-DIISOPROPYL-O-METHYL-O-4-NITROPHENYL PHOSPHORAMIDITE. NEW FLEXIBLE PHOSPHITYLATING REAGENT IN THE SYNTHESIS OF THIO- AND SELENO- OLIGONUCLEOTIDES

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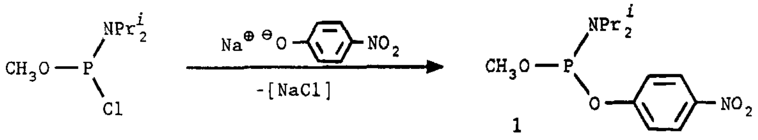
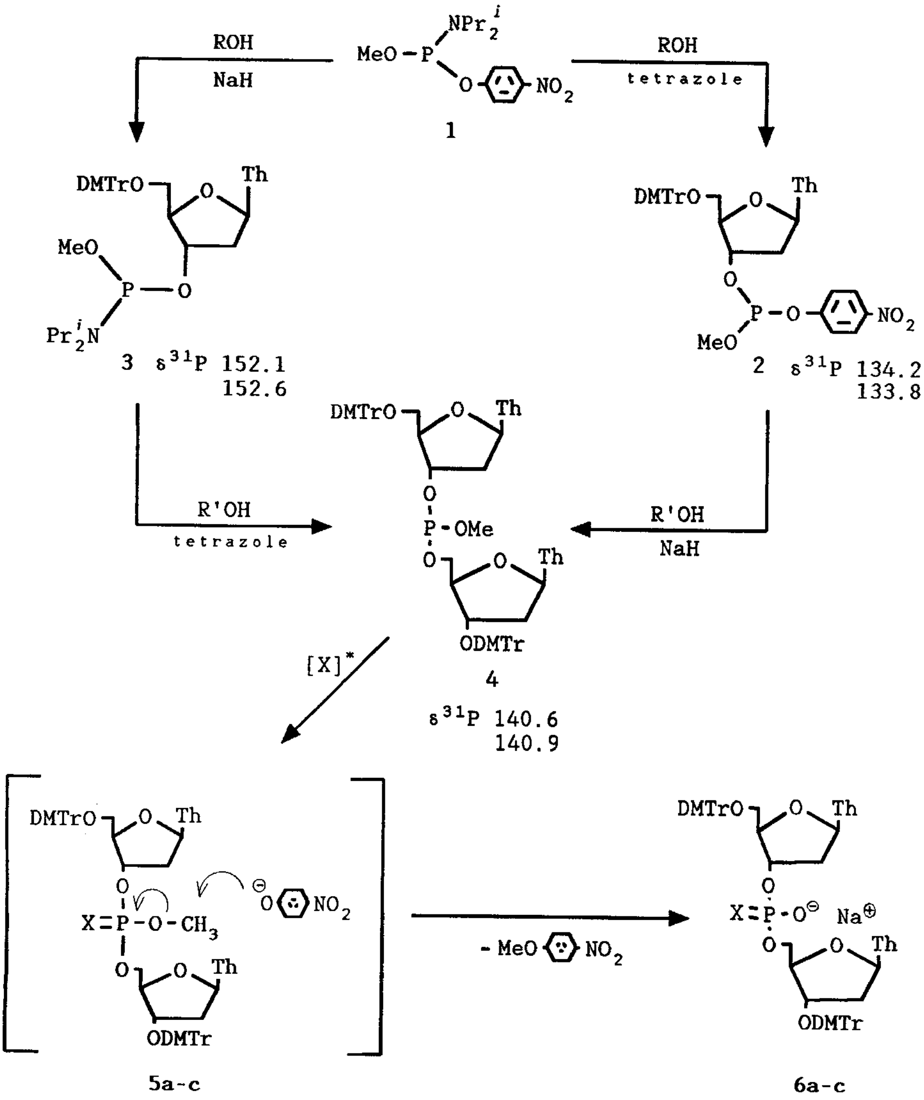
Abstract: Internucleoside coupling by the title reagent, illustrated by synthesis of dinucleoside and trinucleoside thiophosphates and selenophosphates is accomplished by a new strategy involving the displacement of 4-nitrophenoxy group. Sodium 4-nitrophenolate formed in situ facilitates the oxidation of the intermediate P^{III} structures and acts as a demethylating reagent.

Phosphitylating reagents in oligonucleotide synthesis have been widely used since the classic work of Letsinger.[1] Many important improvements have been made since then, for instance, the use of phosphoramidites containing the diisopropylamino group.[2] The 4-nitrophenoxy group attached to the tetracoordinate phosphorus centre exhibits high propensity to act as a leaving group in nucleophilic displacement reactions. This ability has been widely used in phosphorus chemistry and biochemistry.[3] In contrast, the utilization of the 4-nitrophenoxy group in phosphitylation procedures has not been sufficiently explored.[4] Studies on phosphitylating reagents containing aryloxy leaving groups have been undertaken in this Laboratory. Recently, we have shown that the 4-nitrophenoxy group attached to the P^{III} centre indeed acts as an excellent leaving group in the formation of 3',5'-dinucleosidyl-P-methylphosphonites which are readily transformed into the corresponding P-methylphosphonates and P-methylthiophosphonates.[5]

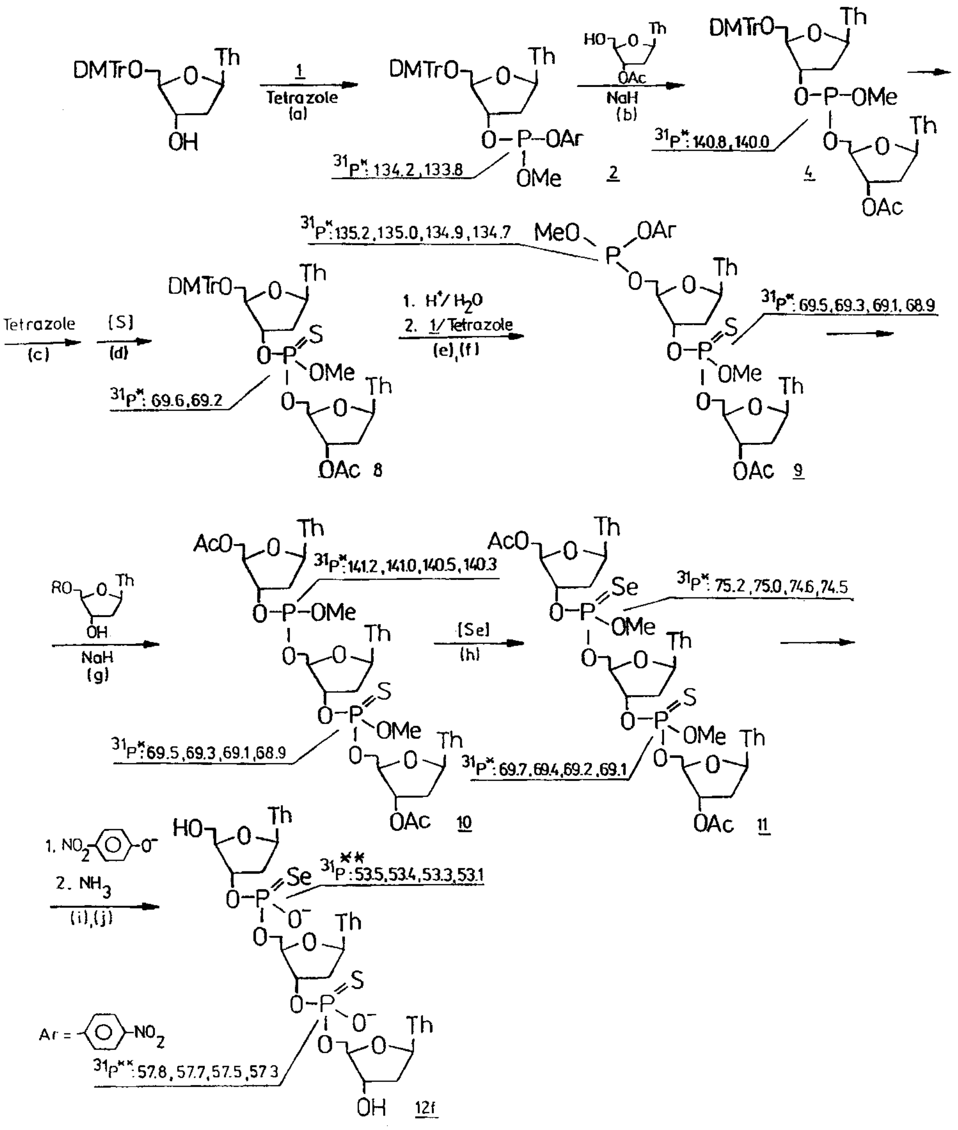
In this paper we demonstrate the use of N,N-diisopropyl-O-methyl-O-4-nitrophenylphosphoramidite 1 in the synthesis of oligonucleotide phosphates, thiophosphates and selenophosphates. The phosphoramidite 1 is readily available from the N,N-diisopropylmethylphosphonoamidic chloride and can be stored at ambient temperature without decomposition.





* catalyzed by 4-nitrophenolate
ROH = 5'-O-dimethoxytritylthymidine
R'OH = 3'-O-dimethoxytritylthymidine
X = O, S, Se

Scheme 1



Scheme 2

Chemical shifts relative to external 85% H_3PO_4

*in $CDCl_3$; **in C_5D_5N

Internucleoside linkage formed with the aid of 1 can be constructed either with the assistance of tetrazole activation of the diisopropylo-amino group of 1 or sodium hydride activation of the nucleoside substrate. Both routes are highly selective. The use of reagent 1 is illustrated by reactions represented in Schemes 1 and 2 which lead to dinucleotides **6a-c** and the trinucleotides of type **12**. These compounds comprise a variety of combinations in which nucleosidyl residues are linked by phosphate, thiophosphate and selenophosphate bridges. Remarkable fast oxidations of the phosphite **4** and immediate demethylation of the **5a-c** leading to the phosphate **6a**, the thiophosphate **6b** and selenophosphate **6c** caused by the 4-nitrophenolate anion formed in situ take place. The demethylation step is so rapid that we failed to observe intermediate esters **5a-c** by ^{31}P n.m.r. spectroscopy.

All reactions leading to phosphate **6a** and its sulfur **6b** and selenium **6c** analogues can be readily performed as a one flask procedure with very good yield. The advantage of this procedure lies in the stability of the new phosphitylating reagent containing a 4-nitrophenoxy group combined with the ability of the sodium 4-nitrophenoxide to act as an oxidation activator and efficient demethylation reagent. Standard addition of elemental sulfur or selenium to oligonucleotides containing P^{III} linkages proceeds slowly and requires special conditions.[6] The usual practice in demethylation of the structures **5** requires the use of thiophenol, unfavorably known for its unpleasant smell. The activation properties of sodium 4-nitrophenolate and its pronounced ability to dealkylate methyl phosphates, thiophosphates and selenophosphates were additionally confirmed in model experiments, on a number of O-methyl phosphates and phosphonates. Detailed mechanistic studies are in progress.[7]

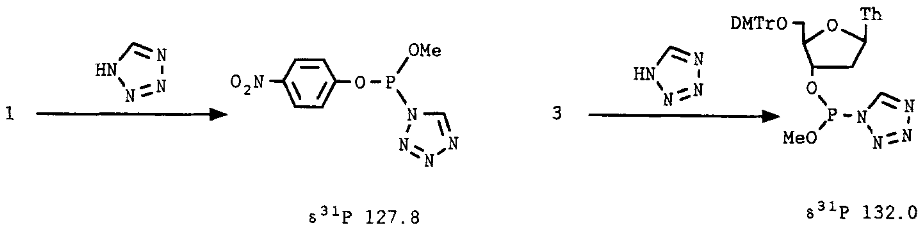
The structure and purity of compounds **1**, **2**, **3** was clearly delineated from their spectral ^{31}P n.m.r. data. Compounds **4**[8] and **6a-c**[9] are known and their properties agree with those described by other authors. The structure of **6a** (X=O) was also confirmed by the transformation of **6a** into the trimethylsilyl phosphate **7a**. Phosphorothioate **6b** (X=S) and selenoate **6c** (X=Se) were converted by the reaction with methyl iodide into methyl esters **7b** and **7c**.

$$\text{6a (X=O)} \xrightarrow{Me_3SiCl} RO(R'O)P(O)OSiMe_3,\ \text{7a}\ \delta^{31}P\ -7.3,\ -12.5$$

$$\text{6b (X=S)} \xrightarrow{MeI} RO(R'O)P(O)SMe,\ \text{7b}\ \delta^{31}P\ +31.0,\ +29.4$$

$$\text{6c (X=Se)} \xrightarrow{MeI} RO(R'O)P(O)SeMe,\ \text{7c}\ \delta^{31}\ +24.1,\ +25.6$$

This methodology is even more clearly illustrated by the synthesis of six structural combinations of trinucleotide thiophosphates and selenophosphates **12a-f**. In the typical protocol given in Scheme 2 which describes the synthesis of trinucleotide **12f** steps (a) and (f) involve the activation of the phosphitylating reagent by tetrazole and steps (b) and (g) the activation of the nucleoside substrate by sodium hydride. In steps (d) and (h) the addition of elemental sulfur or selenium is effectively promoted by the presence of sodium 4-nitrophenolate. In step (i) fast demethylation is achieved by the reaction with sodium 4-nitrophenolate leading to 4-nitroanisole.Step (c) illustrates the acidification by tetrazole which is required in order to avoid dealkylation which otherwise proceeds immediately after the addition of elemental sulfur. Phosphitylation reactions with the use of amidate **1** are not affected by the presence of anionic



Scheme 3

phosphate groups. Therefore a modified strategy in which the dealkylation by 4-nitrophenolate precedes phosphitylation is also practicable.

In phosphitylations described by Scheme 2, which are induced by the tetrazole, intermediate formation of tetrazolides was clearly observed by ^{31}P n.m.r. spectroscopy (Scheme 3). These observations confirm the intermediacy of phosphortetrazolidites in phosphitylating procedures involving phosphoramidites.[11]

EXPERIMENTAL

N,N-Diisopropyl-O-methyl-O-4-nitrophenylphosphoramidite **1**. The solution of N,N-diisopropylamine (0.01 M) and triethylamine (0.01 M) in dry THF (50 mL) was added dropwise at -10°C under a nitrogen atmosphere to the solution of methoxydichlorophosphine (0.01 M) in dry THF (50 mL) over 1 h. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 1 h. The triethylamine hydrochloride was removed by filtration and the solution of N,N'-diisopropylaminomethoxychlorophosphine (0.01 M) in dry THF (50 mL) was added dropwise at r.t. for 10 min. to the sodium 4-nitrophenolate (0.01 M) in dry THF (50 mL). After 0.5 h sodium chloride was filtered off. The filtrate was evaporated to dryness and crude **1** purified by column chromatography [Kieselgel 60, Et_2O: n-hexane:triethylamine 50:50:0.5 v/v R_f: 0.22] to give N,N-diisopropyl-O-methyl-O-4-nitrophenylphosphoramidite **1** [$\delta^{31}P$ ($CDCl_3$): 144.5 ppm]. Yield of **1** isolated: 95% (oil).

One-flask synthesis of dinucleotides 6a-c: The amidite **1** (1.1 mM) was treated with 5'-O-dimethoxytritylthymidine (1.0 mM) in the presence of sublimed tetrazole (1.1 mM) in dry THF (30 mL) at 20°C. The reaction was completed within 0.5 h to yield **2** in 100% yield (^{31}P n.m.r.). This reaction mixture was allowed to react with 3'-O-dimethoxytritylthymidine (1.1 mM) and NaH (2.2 mM) dissolved in dry THF (10 mL) at 20°C. After 10 min. the phosphite **4** was formed in 100% yield (^{31}P n.m.r.). The phosphite **4** can also be obtained by an analogous procedure via the intermediate formation of **3**. The reaction mixture containing phosphite **4** was treated either by passing through molecular O_2 or adding elemental sulfur (selenium). After 2 h the crude solutions of **6a-c** were purified by

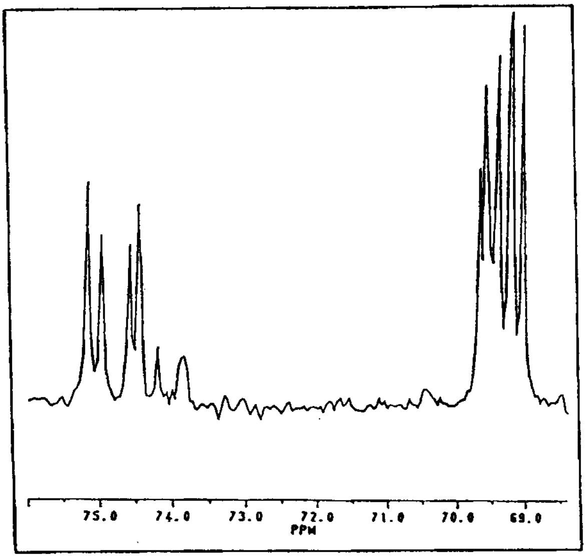


Figure 1.
The 81-MHz ^{31}P NMR spectrum of the compound **11**

silica-gel chromatography yielding dinucleotides **6a-c** in over 90% yield of products isolated **6a**[9a] (X=O): $\delta^{31}P$ (C_5D_5N)=+3.9, **6b**[9b,c,d] (X=S): $\delta^{31}P$-(C_5D_5N)= 58.7, 58.4 (1:1), **6c**[9c] (X=Se): $\delta^{31}P$ (C_5D_5N)= 51.1, 52.0 (1:1).

Synthesis of O-5'-O-(Di-p-methoxytrityl)thymidin-3'-yl, O-3'-O-(Di-p-methoxytrityl)thymidin-5'-yl-O-methyl phosphite 4 (Procedure b): To a suspension of NaH (1.0 mM) in dry THF (10 mL) 5'-O-dimethoxytritylthymidine (1.0 mM) was added. After 10 min. the amidite 1 (1.1 mM) in dry THF (10 mL) was added at 20°C. The reaction mixture was stirred for 10 min. at 20°. The reaction mixture was stirred for 10 min. at 20°C. The resulting 5'-O-DMT-thymidine-3'-O-methyl-N,N-diisopropylphosphoramidite **3** was allowed to react with 3'-O-dimethoxytritylthymidine (1.0 mM) and sublimed tetrazole (2.2 mM) in dry THF (10 mL) at 20°C. After 10 min. the dinucleoside phosphite **4** was formed in 100% yield (^{31}P n.m.r.).

One-flask synthesis of trinucleotide 8f: d[T_P(Se)T_P(S)T].

Step (a): the amidite **1** (1.1 mM) was treated with 5'-O-dimethoxytritylthymidine (1.0 mM) in the presence of sublimed tetrazole (1.1 mM) in THF (30 mL) to yield **2** (0.5 h).

Step (b): addition of 3'-O-acetylthymidine (1.1 mM) and NaH (2.2 mM) dissolved in THF (10 mL) gave **4** (10 min).

Steps (c) and (d): reaction mixture containing **4** was acidified by tetrazole (1.1 mM) and allowed to react with elemental sulfur (1.1 mM) to give **8** (0.5 h).

Step (e): removal of DMT group from **8** by acid hydrolysis (benzenesulfonic acid 2% solution in $CHCl_3$, 0°C) gave **8a** d[T_P(S,OMe)T(Ac)] (5 min).

Step (f): **8a** was phosphitylated by **1** (1.1 mM) in the presence of sublimed tetrazole (1.1 mM) to give **9** (0.5 h).

Step (g): **9** was condensed with 5'-O-acetylthymidine (1.0 mM) in THF in the presence of NaH (2.0 mM) (10 mL) to give **10** (69.3, 69.5).

Step (h): addition of elemental selenium (1.0 mM) gave **11** (0.5 h).

Step (i): demethylation by sodium 4-nitrophenolate (1.0 mM) gave **12a**, d[AcT_P(S)TAc] (10 min).

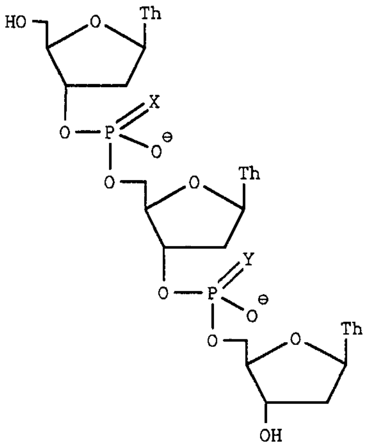
Step (j): deacetylation by NH_3/H_2O (35%, 50°C, 3 h) gave **12f**.

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 (0-10%) in 1,2-dichloroethane. The product **12f** was isolated in 86% yield. By analogues protocol were synthesized trinucleotides **12a-e**. Structures of **12a-f** were confirmed by FAB mass spectroscopy.[10] All operations except step (e) and (j) were performed at 20° in rigorously dry THF under argon.

^{31}P spectroscopic data of compounds 12a-e *

	P(X)	^{31}P	P(Y)	^{31}P
12a	O	+3.9	O	+3.9
12b	S	57.9, 57.8	S	57.6, 57.8
12c	Se	53.6, 53.5	Se	53.4, 53.2
12d	O	+3.9	S	57.8, 57.6
12e	O	+3.9	Se	53.4, 53.3

* chemical shifts relative to external 85% H_3PO_4



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7. For example: the addition of elemental sulfur to diisopropylmethylphosphite is faster by a factor of three at 0°C in THF solution. The diisopropylmethylphosphorothionate formed in this reaction is demethylated at once to give sodium diisopropylphosphorothioate and 4-nitroanizole.
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10. Mass spectrometric $(M+Na)^+$ data of compounds **12a-f**: **12a**: 871.6, **12b**: 903.7, **12c**: 997.5, **12d**: 887.7, **12e**: 934.6, **12f**: 950.6.
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Received 1/26/93
Accepted 4/22/93