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N,N-BIS[2-OXO-3-OXAZOLIDINYL]PHOSPHORODIAMIDIC CHLORIDE: A NOVEL COUPLING REAGENT IN THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES.

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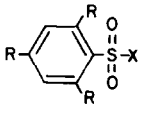
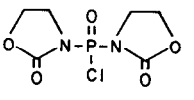
Abstract: Use of a novel coupling reagent, N,N-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (BOPDC) in the synthesis of oligodeoxyribonucleotides is described. This reagent allows the synthesis of phosphotriesters in high yields (70-80%) without detectable side reactions. Synthesis of a hexanucleotide, d(A-A-C-C-C-G) is presented as an example.

In the past decade, oligodeoxyribonucleotides of defined sequences have played an important role in studies of DNA structure[1] and DNA-protein interactions[2] and gene isolation[3], synthesis[4] and manipulation[5]. These and many other applications of the oligonucleotides in the chemistry and biology of nucleic acids have prompted the development of newer and highly efficient synthetic methods. Most of the methods commonly employed involve formation of a triester linkage, which is subsequently converted into the desired phosphodiester bond. A key step in phosphotriester synthesis is the use of a coupling reagent in activation of a phosphodiester[6]. The activated phosphodiester or the symmetrical phosphoric anhydride intermediate(s) rapidly react with the appropriate hydroxy group of the nucleoside to form a phosphotriester. The coupling reagents most commonly used are sulfonyl chlorides (1a)[7], and their corresponding



1a R = H, CH_3, $CH(CH_3)_2$; X = Cl.
1b X = 3-nitro-1,2,4-triazole.
1c X = tetrazole.



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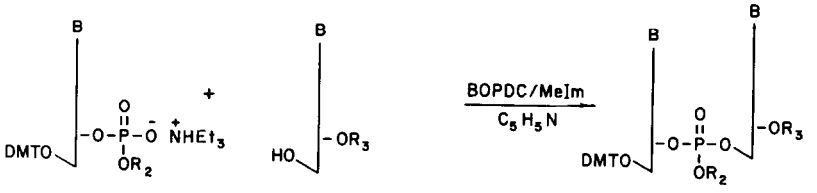
N, N-bis[2-oxo-3-oxazolidinyl] phosphorodiamidic chloride. (BOPDC)



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R_2 = o-Cl. Ph, R_3 = Bz or Cl $C_6H_4O(O)POCH_2CH_2CN$.
B = 6-N-benzoyladenin-9-yl, 4-N-benzoylcytosin-1-yl, 2-N-isobutyrylguanin-9-yl.

azole derivatives (1b and 1c)[8,9]. However, these coupling reagents suffer from the following deficiencies: (1) arylsulfonyl chlorides and the corresponding azole derivatives are unstable on prolonged storage[8], (2) reaction is slow and invariably leads to sulfonation of the hydroxy group of the nucleoside component[9], (3) nucleoside bases, guanine and thymine, are modified[10].

TO circumvent these problems, a coupling reagent, N,N-bis[2-oxo-3-oxazolidinyl]phosphorodiamidic chloride (BOPDC), was investigated in the synthesis of oligodeoxyribonucleotides. The choice of BOPDC was based on the observations of Palomo-Coll et. al.[11,12], that carboxylic acids formed symmetrical carboxylic acid anhydrides on reaction with this reagent. Since symmetrical phosphoric anhydrides have been suggested as intermediates in the synthesis of phosphotriesters by Kan et. al.[13], it was of interest to determine whether BOPDC would activate phosphodiester leading to phosphotriester. Synthesis of di, tri, and oligonucleotides was undertaken by employing BOPDC as the coupling reagent. The results summarized in Table 1 show that oligonucleotides can be readily synthesized in high yields (reported yields are of chromatographically purified products).

Table 1

Oligodeoxynucleotides	Yields %	Oligodeoxynucleotides	Yields %
DMT-ApAp*	80	DMT-ApG-OBz	73
DMT-ApCp*	70	DMT-CpG-OBz	77
DMT-CpCp*	83	DMT-ApGpGp*	67
DMT-CpAp*	76	DMT-CpCpCpG-OBz	74
		DMT-ApApCpCpCpG-OBz	83

Abbreviations: DMT = 4,4'-dimethoxytrityl; C = 4-N-benzoyl-2'-deoxycytidine; G = 2-N-isobutyryl--2'-deoxyguanosine; A = 6-N-benzoyl-2'-deoxyadenosine; p = 2-chlorophenylphosphate; p* = $ClC_6H_4O(O)POCH_2CH_2CN$ Bz = benzoyl. These abbreviations are based on a simplified scheme suggested by Reese[16].

In a typical experiment, a suitably protected phosphodiester (3, 0.11 mmol), an appropriately protected hydroxyl component (4, 0.10 mmol), and N-methylimidazole (MeIm, 0.3 mmol) were dissolved in dry pyridine (5 ml) and the solution was evaporated to dryness. The residue was dissolved in dry pyridine (2 ml), BOPDC (0.3 mmol) was added and the solution was stirred at room temperature for 30 min. After the usual work-up[14], the desired product was isolated by flash chromatography (the column was developed with increasing proportions of methanol in methylene chloride). The isolated oligonucleotides were homogeneous as judged by silica gel and reverse-phase silica gel TLC.

Removal of the protecting groups by the standard procedure[15], resulted in fully unprotected oligonucleotides. One of the deblocked oligonucleotide, hexamer, d(A-A-C-C-C-G), was examined by HPLC. The HPLC profile in Fig. 2 (the column was eluted with a sharp (A) and a shallow (B) gradient of acetonitrile) clearly shows that major component in the crude mixture is the hexanucleotide (62%). Similar results were obtained with other oligonucleotides listed in Table 1.

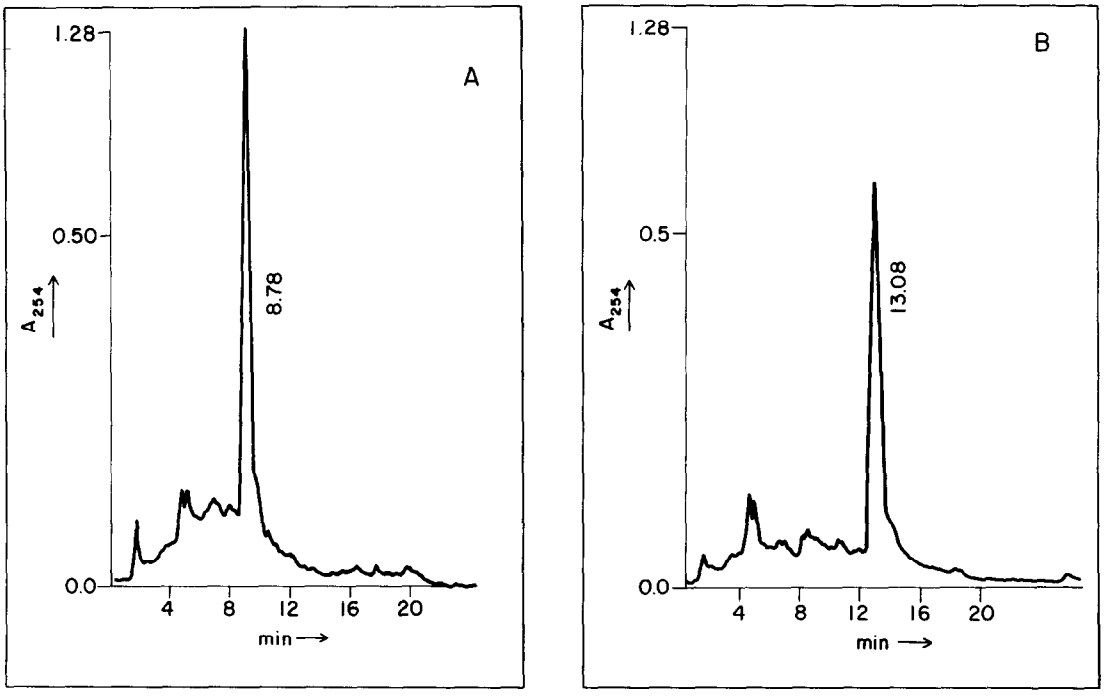

Fig. 2: HPLC was performed on C-18 (microsorb, Rainin) column using CH_3CN/0.1M aqueous TEAA linear gradient. (A) 6-15% 10 min. (B) 6-15% 20 min. Flow = 1.5 ml/min.

The reactivity of BOPDC with the 5'-hydroxy group and the bases of the nucleosides was investigated. This was accomplished by treating each of the appropriately protected nucleoside with BOPDC under the reaction conditions used for coupling. Analysis of the reaction products showed no difference in Rf values on TLC., and in UV and NMR spectral data before or after reacting with BOPDC. These studies show that no detectable side products are formed during BOPDC mediated coupling reactions.

In summary, BOPDC is an effective reagent in the synthesis of oligonucleotides. The synthesis of phosphotriesters proceeds rapidly without detectable side reactions.

Acknowledgements

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