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SYNTHESIS OF P^1,P^2-DINUCLEOTIDE PYROPHOSPHATES

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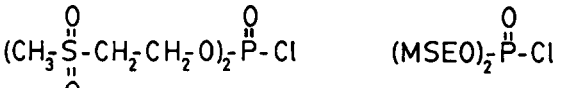
Abstract: The three possible pyrophosphate-linked dimers of pdAp were synthesized in high yield in a convenient way using two fully protected 3´,5´-deoxyadenosine diphosphate intermediates which were prepared via a phosphotriester approach.

Pyrophosphate-linked, oligonucleotide analogues are of interest because of their interactions with polynucleotides as well as their possible significance in chemical evolution research. [1] An efficient method was required for the synthesis of defined oligomers of 2´-deoxynucleotide-3´,5´-diphosphates both to aid in establishing the identities of products of oligomerization reactions and to serve as starting materials and possibly templates for such reactions.

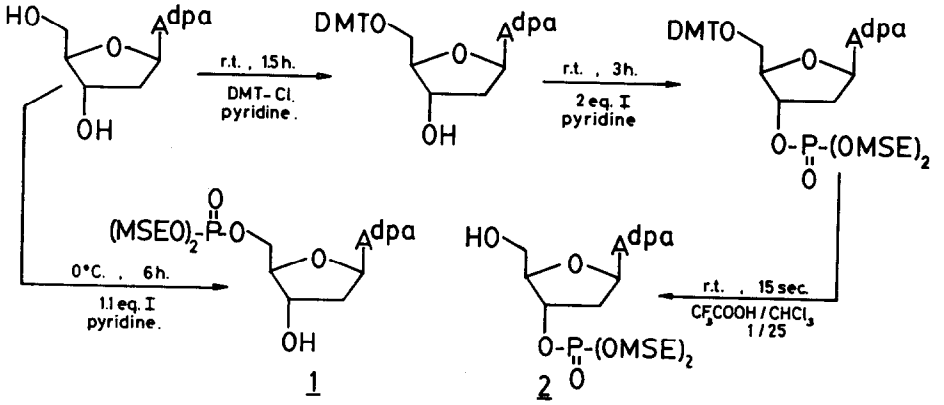
The introduction of a terminal pyrophosphate function on a nucleoside is based on the classical reaction between a phosphodiester and phosphoric acid [2,3]. In modern nucleic acid chemistry, the intermediate diesters are prepared by a triester method [4,5]. To form an internucleosidic pyrophosphate, reaction must be between a diester and monoester. Such examples are few [6,7]. Here we describe the synthesis of dimers containing both internucleosidic pyrophosphate as well as terminal phosphate groups, in which both intermediates are prepared by a single, straight-forward phosphotriester method. We first introduce the terminal phosphate group into the 3´- or 5´- position of a properly protected deoxynucleoside. For this purpose we chose bis[2(methylsulphonyl)ethyl]- phosphochloridate (I) as phosphorylating agent [8]:



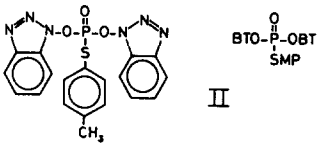
I

The MSE protecting groups are stable under the further conditions of the reaction and can easily be removed by β-elimination under mild alkaline hydrolysis.

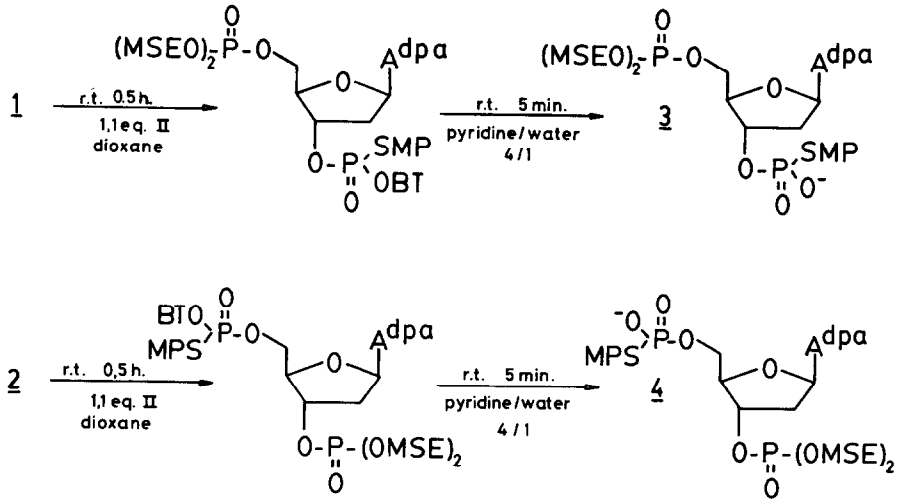
As an additional advantage of this reagent, we found that it can be introduced selectively into the 5 -position, thus shortening the synthesis route to 1 considerably:



To introduce an activatable phosphate group into the remaining 3´- and 5´-positions of 1 and 2 , we used van Boom´s versatile bifunctional reagent S-4-methylphenyl-O,O-bis[1-benzotriazolyl]phosphorothioate (II) [5]:

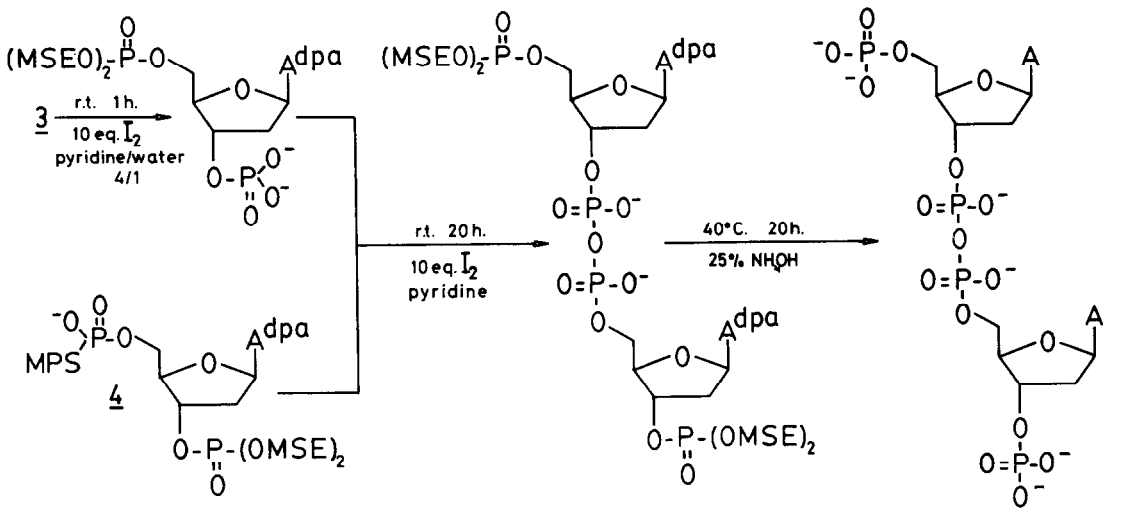


Thus 1 and 2 react with II to form phosphotriesters, from which the OBT-group can be hydrolyzed selectively to form the phosphodiesters 3 and 4 , which constitute the key intermediates in this synthesis:



Compounds 3 and 4 are obtained as triethylammonium salts, can be purified by silica-gel chromatography and can be stored in vacuo over P_2O_5 for long periods of time.

To form the pyrophosphate, the MPS-group of 3 or 4 is removed by oxidation with 10 equivalents of iodine in pyridine/water(4/1). After coevaporation with dry pyridine to remove all traces of water, 1 equivalent of 3 or 4 is added and the mixture is stirred overnight. Thus, dimerization of 3 leads to the 3´-3´ product; 4 gives the 5´-5´ isomer; while a combination of 3 and 4 results in formation of the 3´-5´ pyrophosphate dimer:



Treatment with 25% NH_4OH at 50°C for 24h is sufficient to remove all protecting groups from the products. Purification is by ion-exchange chromatography on DEAE-Sephadex (A25), using a linear gradient of 0.05 to 0.8 M TEAB. Yields are over 85%. The structures of the products have been confirmed by HPLC analysis, enzymatic degradation and ^{31}P-NMR. [9]

Presently we are extending this method to the other common deoxynucleosides, as well as to the synthesis of longer pyrophosphate-linked oligomers on a solid-phase support.

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Abbreviations:
DMT, dimethoxytrityl; MSE, methylsulphonylethyl; SMP = MPS, 4-methylphenylsulphenyl; OBT = BTO, 1-benzotriazolyl; dpa, diphenylacetyl; A, adenine.

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[9] ^{31}P-NMR shifts relative to phosphoric acid:
3'-3': 2.7 and -10.1 ppm.
5'-5': 3.0 and -8.5 ppm.
3'-5': 3.5, 3.2, -8.6 and -9.5 ppm.

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