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PROTECTION OF THE PHOSPHORYL GROUP IN OLIGONUCLEOTIDES IN THE FORM OF A DIANILIDATE. DIANILIDOPHOSPHOCHLORIDATE AS PHOSPHORYLATING AGENT

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In the synthesis of oligonucleotidic blocks, it is necessary to start from substances bearing a phosphoryl group which must be properly protected during the synthesis of internucleotidic bonds and which, moreover, is easily deblocked while the other protecting groups on the oligonucleotidic block must remain intact. In the diester synthesis, the phosphoryl group may be preferably protected in the form of an aromatic phosphamidate[1]; this protecting group can be removed under very mild conditions (amyl nitrite in pyridine-acetic acid). The presence of one ionisable function in the phosphomonoester amidate is, however, not suitable for the synthesis of the oligonucleotides investigated in the present time in this Laboratory (the so-called combined synthesis during which the neutral triesters are isolated[2]). We have thus searched for a completely neutral blocking of the phosphoryl group. Protection of the phosphoryl group in the form of a bis(2,2,2-trichloroethyl) ester[3] was not satisfactory. The attempted application of nucleoside phosphodithioate S,S-diethyl ester failed since this neutral triester (in contrast to the phosphothioate O,S-diester[4]) is stable towards the action of either iodine or sodium periodate.

The nucleoside phosphodianilidate may also be considered as a neutral form of the phosphate. Substances of this type are accessible by reaction of hydroxy compounds with dianilidophosphochloridate (I) or -bromidate[5]. From these two agents we have used compound I because it is well defined and more stable. The reaction of compound I in pyridine with polyhydroxy compounds is claimed by Zetzsche and Büttiker[6] to afford phosphoester dianilidate while other authors[7] deny the possibility of such a reaction.

In the present paper I wish to report that the treatment of 5′-O-dimethoxytritylthymidine with agent I in pyridine at 20°C affords quantitatively 5′-O--dimethoxytritylthymidine 3′-phosphodianilidate (II). Compound II is stable towards 2,3,5-triisopropylbenzenesulfonyl chloride (20 h, 20°C). By the action of cold 80% aqueous acetic acid, compound II is quantitatively converted into thymidine 3′-phosphodianilidate, a key intermediate with a neutral protecting group. On treatment with amyl nitrite in 1:1 pyridine-acetic acid, compound II is transformed to 5′-O-dimethoxytritylthymidine 3′-phosphate.

The stability of agent I (it may be crystallised from aqueous ethanol), the stability of its solutions in anhydrous pyridine, the quantitative reaction with the secondary hydroxylic function, the possibility to isolate the neutral intermediate on silica gel (no contamination of the final product by inorganic phosphate), and finally, mild deblocking of the phosphoryl group while the acid labile as well as the alkali labile protecting groups remain intact[1,8], all these properties appear to make from compound I the most advantageous phosphorylating agent of all. The wide applicability of agent I was demonstrated by some preliminary experiments. Thus, uridine 2′,3′-bis-phosphate (R_{Up} in 7:1:2 2-propanol - concd. aq. ammonia - water, 0.62) was obtained in a quantitative yield from 5′-O-trityluridine and uridine afforded the 2′,3′,5′-tris-phosphate (R_{Up}, 0.35).

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