STEPWISE SYNTHESIS OF DEOXYRIBO-OLIGONUCLEOTIDES

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We wish to report the synthesis of a deoxyribo-trinucleotide of the type d-pXpYpY, needed as a substrate in the study of nuclease activity, which involved stepwise chemical synthesis of a trinucleoside diphosphate by the methods of Khorana and his school[1] and 5'-phosphorylation of XpYpY by enzymatic phosphate transfer.

N^6-anisoyl-deoxycytidine 5'-phosphate[2] (Ia)(Fig. 1) was converted to its acetate (Ib) and condensed with 5'-tritylthymidine[3] (II) by the usual procedure (dicyclohexylcarbodiimide in pyridine). The resulting protected dinucleoside phosphate (IIIa) was carefully deacetylated and the IIIb thus obtained (yield: 40%) was separated on a DEAE-cellulose column by a triethylammonium bicarbonate in methanol linear gradient. Structure was confirmed by transformation to TpC[4] and to pTpC[5] via Tener's phosphorylation procedure[6], and by enzymatic degradation; thus, purified venom diesterase acting on the completely unmasked TpC (IIIc) gave rise to T and pC, spleen diesterase gave Tp and C, whereas crude venom diesterase gave T and C.

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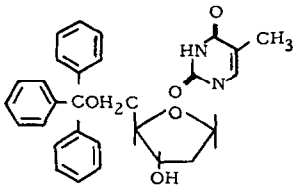
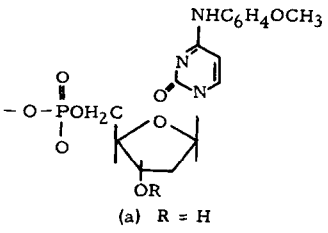


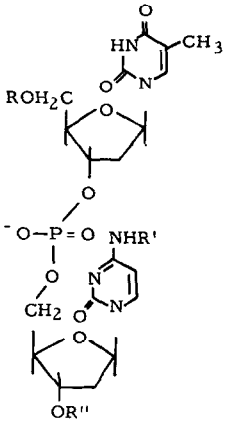
II



I

(a) R = H

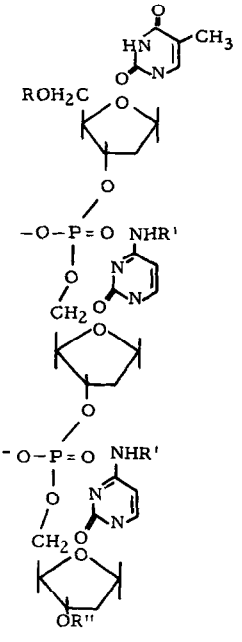
(b) R = acetate



III (a) R = trityl
R' = anisoyl
R'' = acetate

(b) R = trityl
R' = anisoyl
R'' = H

(c) R = R' = R''



IV (a) R = trityl
R' = anisoyl
R'' = acetate

(b) R = trityl
R' = R'' = H

(c) R = R' = R'' = H

Fig. 1

Further condensation of IIIb with a second pC-unit in its masked form (Ib) gave the masked trinucleoside diphosphate 5'-trityl-thymidylyl-(3' ⟶ 5') - [N^6-anisoyl-2-deoxycytidyl] - (3' ⟶ 5') - [N^6-anisoyl-3'-acetyl] -2-deoxycytidine (IVa). The latter, upon alkaline treatment, gave the deacylated product IVb which could be isolated by preparative paper chromatography (yield from IIIb: 27.5%). Subsequent removal of

the trityl blocking group gave the trinucleoside diphosphate proper, TpCpC (IVc)[1], which was homogeneous on a DEAE-cellulose column, had the proper ultraviolet spectrum, and upon enzymatic degradation as above, gave the expected products in the predicted quantities.

Chemical phosphorylation of a properly blocked derivative of IV did not prove successful: this was accomplished, albeit in low yield (5%), by transphosphorylation of IVc from phenyl phosphate with an enzyme preparation from malt diastase[7]. The resulting deoxy-trinucleotide (pTpCpC, V) was separated on a DEAE-cellulose column and its structure ascertained by enzymatic degradation and physical properties.

The fact that IVc served as a phosphate acceptor for transphorylation suggests that other substrates of the type $X(pY)_n$ may also be phosphorylated in this manner, and that the method may be generally useful in synthesis.

The question whether the E. coli phosphodiesterase described by Lehman[8] degrades oligonucleotides starting from the 3'-OH end only, or whether degradation is also possible by fission from the 5'-monophosphate end could now be examined. As seen in Fig. 2, path (a) corresponds to the former supposition and would result in the generation of pC and pTpC (the enzyme does not attack substrates below the trinucleotide level). On the other hand, additional optional hydrolysis via path (b) would also give pT and pCpC. (The supposition that (b) only is operative could be eliminated by other data.)

When pTpCpC (V) was submitted to the action of the E. coli phosphodiesterase†, only pTpC and pC could be detected by paper chromatography. Within the errors of the method, this would indicate

† This experiment was kindly carried out by Professor I. R. Lehman.

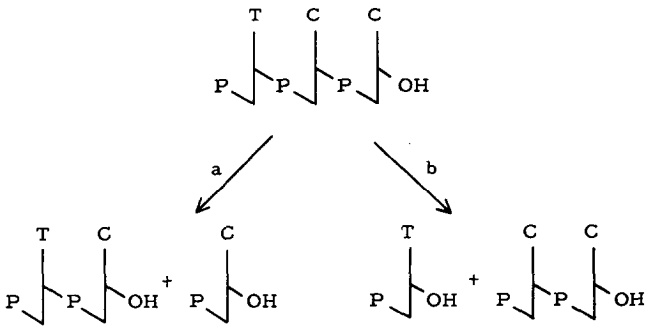


Fig. 2

that path (a) is the only one operative, a result in conformance with other data[9].

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