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FORMATION OF PHOSPHODIESTER LINKAGES BY OXIDATION OF A PHOSPHORAMIDATE

Eiko Ohtsuka, Shigeo Morioka, and Morio Ikehara
Faculty of Pharmaceutical Sciences, Osaka University
Toyonaka, Osaka, Japan.

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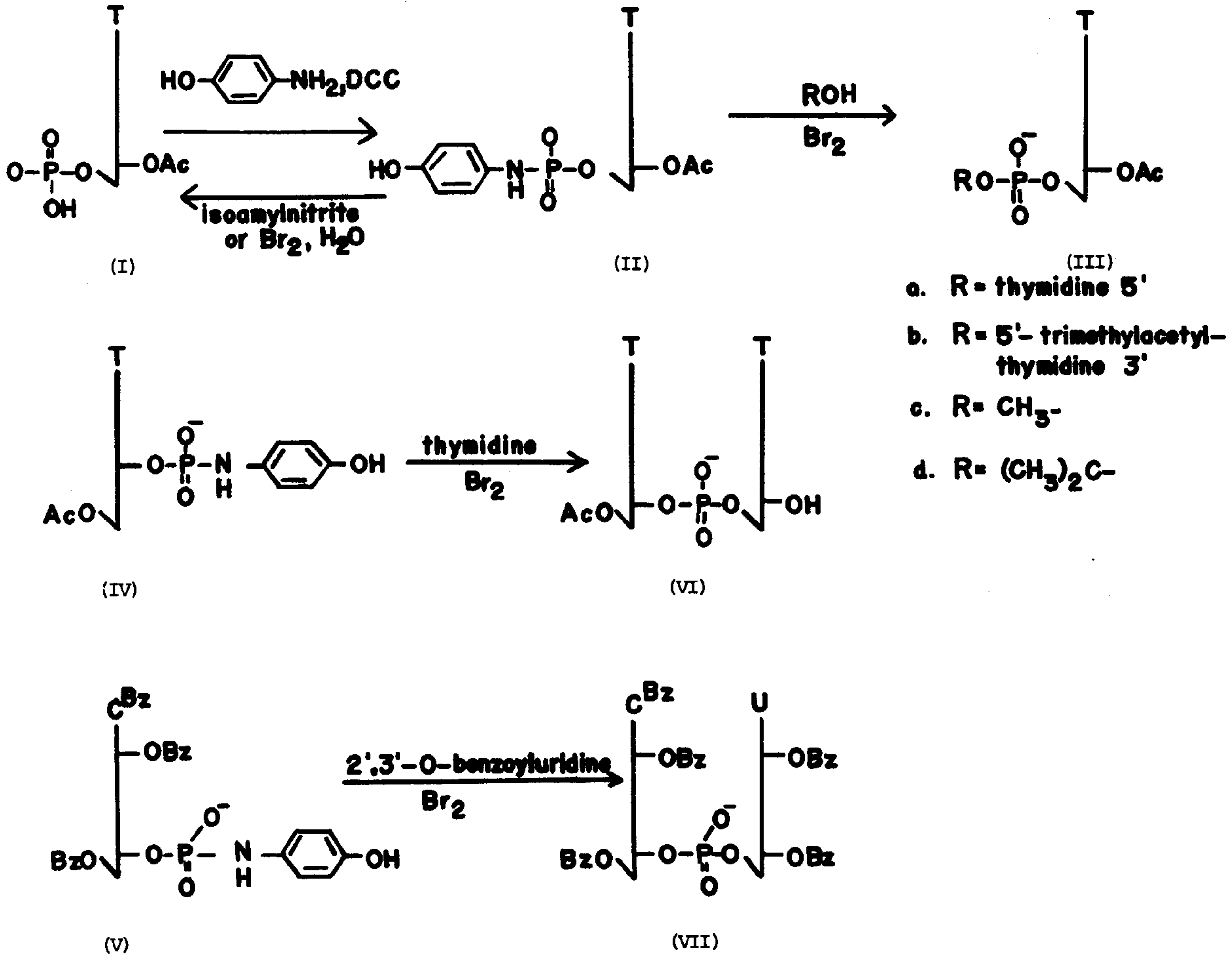
The activation of phosphomonoesters to form phosphodiesters is one of the most important problems in the synthetic study of oligonucleotides. Comparative studies of the activating reagents of nucleotides have shown that dicyclohexylcarbodiimide (DCC) and arylsulfonylchlorides give relatively good results$^{1)}$. Active intermediates have not been isolated in these reactions. It is desirable to use preactivated phosphomonoesters in the synthesis of phosphodiester linkages. Nucleoside S-alkyl phosphorothioates$^{2)}$ and nucleoside quinolphosphates$^{3)}$ are known to be activated by oxidation. Aromatic phosphoramidates have been extensively used as protecting groups of phosphomonoesters in the synthesis of ribo$^{4)}$- and deoxyribo$^{5)}$-oligonucleotides. In this communication we report that p-hydroxyanilidates of mononucleotides are oxidized to form phosphodiesters.

3'-O-Acetylthymidine 5'-phosphoro-p-hydroxyanilidate (II) was synthesized using a similar method as described for thymidine 5'-phosphoranilidate$^{5)}$ in a yield of 67%. 3'-O-Acetylthymidine 5'-phosphate (I) and p-hydroxyaniline were allowed to react with DCC at 28° for 26 hr. The product was isolated as pyridinium salt by passing through a column of pyridinium Dowex 50x2 and extracting with 1:1 n-butanol-ethylacetate. Impurities were removed by extraction with methylene chloride before the ion exchange treatment. 5'-O-Acetylthymidine 3'-phosphoro-p-hydroxyanilidate (IV) and N,2',5'-tribenzoylcytidine 3'-phosphoro-p-hydroxyanilidate (V) were prepared similarly. The amidate (II) could be converted quantitatively to 3'-O-acetylthymidine 5'-phosphate either by treatment with isoamylnitrite in pyridine-acetic acid or by oxidation with bromine or iodine in aqueous acetate buffer at pH 7.5.

For the formation of phosphodiesters (III), compound (II) was activated by oxidation with bromine in anhydrous conditions in the presence of thymidine, 5'-O-trimethylacetylthymidine$^{6)}$, isopropanol, or methanol. Other amidates IV and V were also allowed to react with thymidine and

Chart



a. R = thymidine 5'

b. R = 5'- trimethylacetyl-thymidine 3'

c. R = CH_3-

d. R = $(CH_3)_2C$-

Table I

Alcohol	Amt. (mmol)	Amidate	Amt. (mmol)	Solvent (ml)	Br_2 (mmol)	Time (hr)	Product	Yield (%)
Thymidine	0.20	II	0.10	DMF 0.2	0.39	24	IIIa	58.2
Thymidine	0.22	IV	0.11	DMF 0.2	0.39	24	VI	45.7
5-Trimethyl- acetyl thymidine	0.20	II	0.10	DMF 0.2	0.39	24	IIIb	36.5
Methanol	0.10	II	0.05	DMF 0.1	0.20	4	IIIc	86.6
Methanol	0.11[a]	II	0.05	0	0.20	4	IIIc	91.3
Isopropanol	0.13	II	0.05	DMF 0.1	0.20	24	IIId	51.6
Isopropanol	0.12[a]	II	0.05	0	0.20	24	IIId	81.0
2',3'-O-Dibenzoyl-uridine	0.1	V	0.05	DMF 0.2	0.20	1	VII	24.3

a) amount in ml.

2',3'-O-dibenzoyluridine to yield 5'-O-acetylthymidylyl-(3'-5')-thymidine (VI) and N,2',5'-O-tribenzoylcytidylyl-(3'-5')-2',3'-O-dibenzoyluridine (VII), respectively. The results are summarized in Table I. The yield of the products were estimated by extracting the spots in electrophoresis at pH 7.5 and measuring absorbance of the spots: thymidylic acid, Rm 1.0; the pyrophosphate, 0.73; an unidentified compound, 0.62; dinucleoside monophosphate, 0.40; alcoholesters of thymidine 5'-phosphate, 0.60. The results show that methanol gives higher yield compared with isopropanol and larger excess of the primary alcohol gives almost quantitative reaction. Unprotected thymidine was found to be linked at the 5'-hydroxyl group exclusively. Reaction between the primary hydroxyl group of the nucleoside and the activated 3'-phosphate seemed to be more favored than the one between the 3'-hydroxyl group of thymidine and the activated 5'-phosphate. All products were characterized by digestion with venom phosphodiesterase and spleen phosphodiesterase. Polymerization of mono- and di-nucleotides by this method is under investigation.

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