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SYNTHESIS OF THYMIDINEPHOSPHOROTHIOYL - ($O^{3'} \rightarrow O^{5'}$)-THYMIDINE[+] via PHOSPHOROTHIOIC ACID O,O,S-TRIESTER

A. Malkievicz[++] and J. Smrt

Institute of Organic Chemistry and Biochemistry,

Czechoslovak Academy of Sciences, Prague, Czechoslovakia

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There are possible two types of dinucleoside phosphate analogues derived from phosphorothioic acid. The O,S-diester was prepared (Cook[1]) by reaction of nucleoside 3′-phosphorothioate with 5′-deoxy-5′-iodonucleoside. The synthesis of the biochemically more interesting O,O-diesters was reported by Eckstein[2,3]. In this synthesis, the free hydroxylic functions of the starting nucleoside 5′-phosphorothioates are blocked and the resulting protected compounds are condensed by the action of triisopropylbenzenesulfonyl chloride with a nucleoside bearing a free C-3′ hydroxylic function. In this procedure, however, the sulfur atom is to a considerable extent split off and the final product contains mostly the phosphoric acid derivatives.

In the present communication we wish to report a novel synthesis of a dinucleoside O,O-phosphorothioate with the use of the so called triester synthesis. In this synthesis, the starting nucleoside 5′-S-(2-cyanoethyl)phosphorothioate[1] (I) is converted by reaction with a nucleoside derivative bearing a

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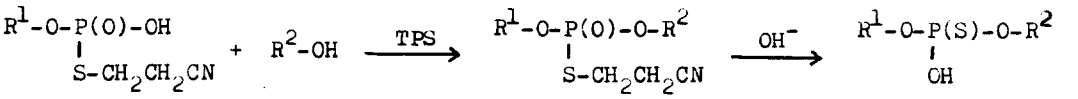
[+] A nomenclature is proposed analogous to that of oligonucleotides, i.e., the internucleotidic bond is designated ($O^{3'} \rightarrow O^{5'}$). This designation shows that in the present case a phosphorothioic acid O,O-diester is involved.

[++] Present address : Institute of Technology, Department of Chemistry, Lodz, Poland.

free hydroxylic function (II) in the presence of triisopropylbenzenesulfonyl chloride to phosphorothioic acid O,O,S-triester III; β-elimination of the 2-cyanoethyl group affords the O,O-diester IV.



I II III IV

R^1, $C^{5'}$-nucleosidyl-; R^2, $C^{3'}$-nucleosidyl-; TPS, triisopropylbenzenesulfonyl chloride.

Thus, thymidine 5′-S-(2-cyanoethyl)phosphorothioate[1] is converted by the action of acetic anhydride in pyridine to the $C^{3'}$-acetyl derivative which is condensed in the form of the triethylammonium salt with two equivalents of 5′-O-dimethoxytritylthymidine by the action of three equivalents of triisopropylbenzenesulfonyl chloride. After 20 hours at room temperature, 5′-O-dimethoxytritylthymidinephosphorothioyl-($O^{3'}\rightarrow O^{5'}$)-3′-O-acetylthymidine is isolated by preparative thin-layer chromatography on silica gel in 80% yield. (Anal. Calcd. for $C_{46}H_{50}N_5O_{14}PS$: N, 7.29; P, 3.23; S, 3.33. Found: N, 6.97; P, 2.83; S, 3.17). On treatment with 90% acetic acid for 2 hours, the latter compound is converted quantitatively to thymidinephosphorothioyl-($O^{3'}\rightarrow O^{5'}$)-3′-O-acetylthymidine. (Anal. Calcd. for $C_{25}H_{32}N_5O_{12}PS$: N, 10.66; P, 4.64; S, 4.87. Found: N, 10.45; P, 4.39; S, 4.72). On treatment with a mixture of concentrated aqueous ammonia and methanol, this O,O,S-triester is converted quantitatively to thymidinephosphorothioyl-($O^{3'}\rightarrow O^{5'}$)-thymidine[2].

The use of this approach to the synthesis of phosphorothioic analogues of oligonucleotides of the deoxyribo and ribo series is in progress.

REFERENCES

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