SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES CONTAINING 1,2-DIDEOXY-D-RIBOFURANOSE

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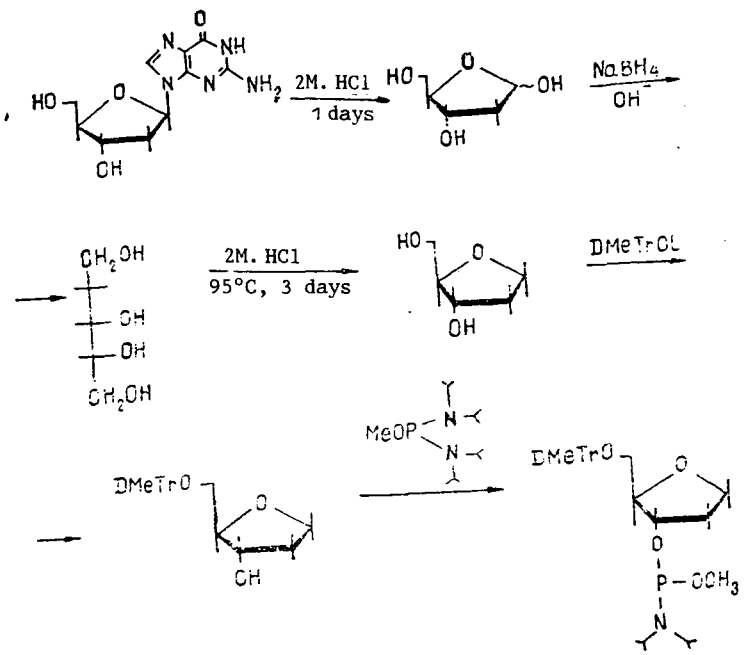
The directed search for method of synthesizing oligodeoxyribonucleotides with nonnucleotide inserts is continuing. These compounds are of interest from the point of view of the propects of their use as antisense oligonucleotides resistant to the action of nucleases. The authors of the present paper have constructed DNA duplexes in which one of the nucleosides (dT or dG) in the section recognizing the restriction endonucleases EcoRII and MvaI ($CC^{A}/_{T}GG$) has been replaced by 1,2-dideoxy-D-ribofuranose.

The study of the interaction of substrates modified in this way with the enzyme EcoRII and MvaI will permit the roles of the individual heterocyclic bases at the stages of the binding and cleavage of DNA by these endonucleases to be established.

DNA duplexes containing 1,2-dideoxy-d-ribofuranose in one of the chains were obtained by the co-annealing of oligodeoxyribonucleotide (I) or (II) and ACCACCAGGTAGGT and also of (III) and ACCTACCTGGTGGT (Table 1). Compounds (I)-(III) were synthesized by the solid-phase phosphoramidite method on the polymeric supports Silokhrom C-80 and CPG [1]. The oligonucleotide chain was grown in a Viktoriya-4M automatic synthesizer, and the modified unit was introduced into the column with the aid of syringe. The oligodeoxyribonucle otides synthesized were isolated by reversed-phase HLPC. The nucleotide sequences of the compounds were confirmed by the Maxam–Gilbert method [2] (Fig. 1). The results on the synthesis and isolation of the modified oligodeoxyribonucleotides are presented in Table 1.

1,2-Dideoxy-D-ribofuranose – a nucleoside analog not containing a heterocyclic base – was obtained by the scheme given below. It combines two methods of synthesizing this compound that



have been developed previously [3, 4]. The starting material for the synthesis was the readily available domestic preparation deoxyguanosine. The reactions were performed under mild

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TABLE 1. Synthesis of Oligonucleotides Containing 1,2-Dideoxy-D-ribofuranose

Oligonucleotide	Weight of polymer, mg	Type of polymer	Yield with respect to Tr, %**		Amt. of sub. after isolation, OU_{260}	Yield, %
			overall	per stage		
I. d(ACCTACCXGGTGGT)*	36	C-80	39	93	1.9	1,6
II. d(ACCTACCTXGTGGT)	20	C-80	—	—	1,5	0,9
III. d(ACCACCAGXTAGGT)	44	CPG	—	—	6,0	5.3
	26	C-80	—	—	6,8	3,0
IV. d(ACCAXCGXGCT)	58	C-80	16	85	6,0	1,7
V. d(XCCACCGCGCT)	29	C-80	45	92	21	10,8
VI. d(AGAGTAACCpXp)	65	C-80	57	93	35	20,9

*X – 1,2-Dideoxy-D-ribofuranose.

**Determined spectrophotometrically from the amount of dimethoxy trityl carbinol (E_{499} 71,700 $M^{-1} \cdot cm^{-1}$ in perchloric acid–ethanol (3:2)[7].

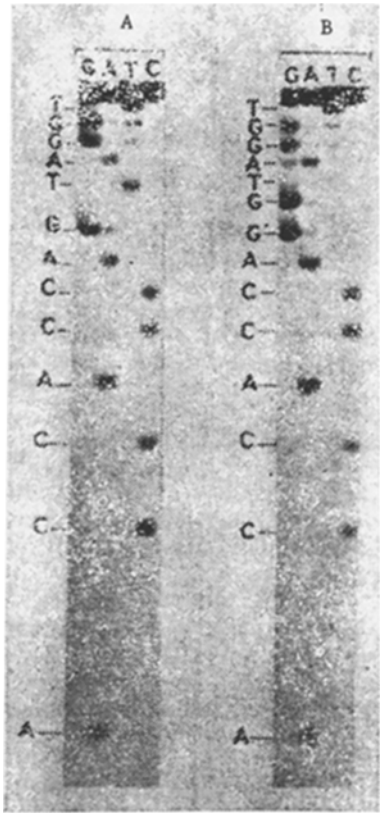

Fig. 1. Analysis by the Maxam-Gilbert method of the nucleotide sequences ACCACCAGXTAGGT (A) and ACCACCAGGTAGGT (B).

mild conditions without isolation at the intermediate stages. The chromatographic properties and the PMR and UV spectra of the 5-dimethoxytrityl-1,2-dideoxy-d-ribofuranose that we had synthesized agreed with those given in the literature [3]. 5-O-(4,4′-Dimethoxytrityl)-1,2-dideoxy-d-ribofuranose-3-yl methyl N,N-diisopropylphosphoramidite was obtained by a standard procedure [5] and was used in the synthesis without additional purification.

Besides compounds (I-III), we synthesized oligodeoxyribonucleotides (IV-VI), in which two nucleosides in the center of the chain or the terminal nucleotide had been replaced by 1,2-dideoxy-D-ribofuranose (Table 1). In the last case, the oligodeoxyribonucleotide contained a 3′-terminal phosphate group, introduced by the procedure of [6].

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