SYNTHESIS OF OLIGONUCLEOTIDES CONTAINING 3'-TERMINAL NUCLEOTIDES WITH INVERTED CONFIGURATIONS OF THE SUGAR RESIDUES

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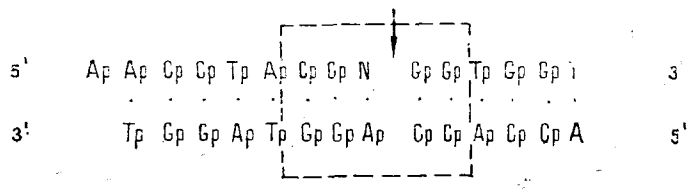
To study the substrate specificity of T4 DNA ligase a series of short DNA duplexes each of which contains in the ligation site a nucleoside with a modified sugar residue is proposed. The synthesis has been performed on previously undescribed mixed dinucleoside phosphates containing deoxycytidine and one of the following modified nucleosides: deoxyxylothymidine and xylo-, lyxo-, and arabinouridines. By the phosphotriester method in solution, using the above-mentioned dinucleoside phosphates, nonanucleotides containing at the 3'-end nucleosides with inverted configurations at the carbon atoms in positions 2' and 3' have been obtained.

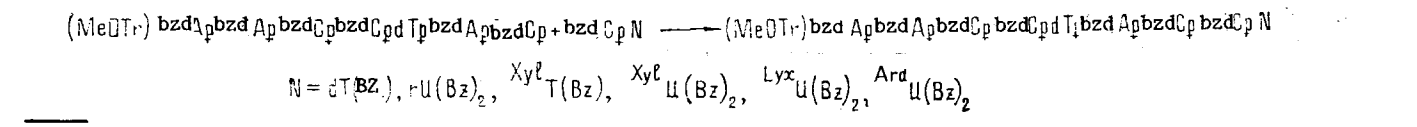
Oligonucleotides with solitary replacements of natural nucleosides by nucleosides with inverted configurations at the carbon atoms in positions 2' and 3' may serve as convenient models for studying the functioning of the enzymes of nucleic acid metabolism. Thus, using synthetic oligonucleotides containing recognition sites for the restriction endonuclease EcoRI, Ohtsuka et al. [1] first showed that the replacement of one deoxyadenosine residue by an arabinoadenosine residue considerably retarded the cleavage of the substrate. In a development of these investigations it appeared desirable to expand the range of modifications of the sugar-phosphate backbone and, by using the advances of modern oligonucleotide chemistry, to obtain a set of substrates for enzymological studies.

In the present communication we describe the synthesis of nine-membered oligonucleotides bearing at the 3'-end various pyrimidine nucleosides with inverted configurations at the carbon atoms in positions 2' and 3'. Each of these oligonucleotides is one of the three components in a series of duplexes constructed for studying the substrate specificity of T4 DNA ligase. Furthermore, the primary structure of each of these duplexes includes a recognition site of the restriction endonuclease EcoRII (enclosed by the dashed line) with modifications in the central A•T pair



where N=dT, rU, ^{Xyl}T*, ^{Xyl}U, ^{Ara}U, ^{Lyx}U (the arrow shows the point of junction of the oligonucleotide blocks; the symbol d has been omitted).

The oligonucleotides were synthesized by the block phosphototriester method in solution. Since the desired oligomers differed only by the structure of the 3'-terminal unit, they were obtained by condensing one and the same completely protected heptanucleotide with different dinucleoside phosphates by the scheme on the following page.†



* ^{Xyl}T represents 1-2'-deoxy-β-D-xylofuranosyl thymine; ^{Xyl}U — 1-β-D-xylofuranosyluracil; ^{Ara}U — 1-β-D-arabinofuranosyluracil; and ^{Lyx}U — 1-β-D-lyxofuranosyluracil.
†The symbol p denotes an internucleotide phosphate with a p-chlorophenyl protective group.

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TABLE 1. Yields* of Dinucleoside Phosphates and Some of Their Characteristics

Compound	Yield, %	R_f**	Degree of hydrolysis by snake venom PDE, %
I. d(CpT)	80	0,5	100
d $Cp^{Xyl}T$	86	0,5	45
d CprU	78	0,6	100
II. d $Cp^{Xyl}U$	76	0,6	23
III. d $Cp^{Lyx}U$	80	0,6	10
IV. d $Cp^{Ara}U$	85	0,6	59

*The yields are given for the condensation products — completely blocked nucleoside phosphates.
**The chromatographic mobilities are given for the completely protected dinucleoside phosphates in the chloroform—methanol (9:1) system.

Nucelosides with inverted configurations of the carbon atoms in positions 2' and 3' were obtained from the corresponding natural nucleoside via the anhydroderivatives.

The structures of the modified nucleosides were confirmed by the results of PMR and CD spectroscopy. Thus, for example, for ^{Ara}U the values of the chemical shifts (ppm) of the spin—spin coupling constants (Hz, in parentheses) were 8.22 d* ($J_{5,6}$ = 7.8) for H-6; 6.8 d ($J_{1',2'}$ = 4) for H-1'; 5.68 d ($J_{5,6}$ = 8) for H-5; 4.96-4.8 m for H-2' + H-3'; 4.44 m for H-4'; 4.24 m for H-5'. For ^{Lyx}U — 8.4 d ($J_{5,6}$ = 8) for H-6; 6.75 d ($J_{1',2'}$ = 6) H-1'; 5.78 d ($J_{5,6}$ = 8) for H-5; 4.88-4.62 m for H-2' + H-3' + H-4'; 4.41 m for H-5', respectively. As can be seen from the CD spectra (Fig. 1), the amplitude of the positive band rises sharply on passing from ribo- to arabinouridine, which agrees with information in the literature [2].

For their use as nucleotide components in the synthesis of oligonucleotides, the anomalous nucleosides were blocked by the following scheme:

$$N \xrightarrow{MeOTrCl} MeOTrN \xrightarrow[DMAP]{BzCOCl} MeOTrN(Bz)_n \xrightarrow[CHCl_3]{20\%\ CCl_3COOH} N(Bz)_n$$

n=1, N=dT, ^{Xyl}T

n=2, N=rU, ^{Xyl}U, ^{Lyx}U, ^{Ara}U .

The course of the reaction was monitored with the aid of TLC, and it was found that the chromatographic characteristics of the derivatives of the anomalous nucleosides were practically indistinguishable from the characteristics of the analogous derivatives of the unmodified nucleosides. The nucleoside components obtained in this way were condensed with (MeOTr)BzdCp(ClPh). It must be mentioned that merely by increasing the excess of anomalous nucleoside derivatives twofold in comparison with the nucleotide it was possible to achieve 80-85% yields of the dinucleoside phosphates. When the 1.5-fold excess of nucleotide component that is standard for the phosphotriester method was used, however, the yields did not exceed 45-50%. To prove the structures of the nucleoside phosphates obtained, the protective groups were removed from part of them and the deblocked dinucleoside phosphates were isolated by the successive use of preparative paper chromatography and electrophoresis. In a study of the hydrolysis of the dinucleoside phosphates by snake venom phosphodiesterase (PDE) a considerable retardation of the cleavage of these compounds was observed as compared with control samples — d(CpT) and dCpRU (Table 1). These results are in harmony with those obtained previously in the hydrolysis by PDE of other dinucleoside phosphates also containing nucleosides with an inverted configuration at the carbon atom in position 2' or 3' [3].

On the synthesis of the nonanucleotides from heptanucleotides and dinucleoside phosphates containing anomalous sugars, the latter were added to the condensation in twofold

*Symbols: d — doublet; m — multiplet.

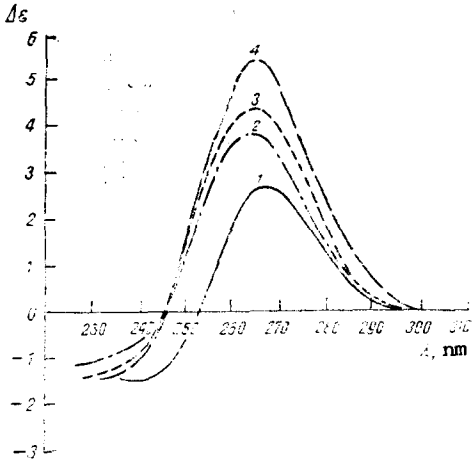

Fig. 1. CD spectra of xylouridine (2), lyxouridine (3), and arabinouridine (4) in 0.02 M phosphate buffer, pH 7.0; 20°C. The CD spectrum of uridine (1) is given for comparison [2].

excess as compared with the heptamer, which enabled an 80-90% yield of the desired nucleosides to be achieved and considerably facilitated their isolation. After complete deblockage, the nonanucleotides were isolated by the successive use of ion-exchange and reversed-phase chromatography.

The primary structures of (AACCTACC)rU and of d(AACCTACCT) were confirmed by the Maxam-Gilbert method [4]. In addition, the presence of terminal cis-glycol groupings in d(AACCTACC)rI and d(AACCTACC)^{Lyx}U was shown (see the Experimental part).

EXPERIMENTAL

The work was carried out with nucleosides from the chemical factory, Omutnisk, and snake venom phosphodiesterase (EC 3.1.4), 1-methylimidazole, 1,2,4-triazole, 3,4,6-triisopropylbenzenesulfonyl chloride (TPS), and dimethylaminopyridine (DMAP) from the FRG.

UV spectra were recorded on a Specord UV-VIS spectrophotometer (GRD), and CD spectra on a Jouan-III dichrograph (France). The coefficients of molar extinction for the nucleosides with the inverted configuration of the carbohydrate fragment were taken as equal to the extinction coefficients for their natural analogs. ^{1}H NMR spectra were recorded on a Varian XL 100 spectrometer (USA) in deuterated pyridine with tetramethylsilane as internal standard.

TLC was performed on Silufol UV_{254} plates (Serva) or Kieselgel 60 F_{254} (Merck) in the chloroform–methanol (9:1) system; chromatography on ethanol–1 M ammonium acetate, pH 7.5, (7:3) system.

Column chromatography was carried out on silica gel L 40/100 (Chemapol, Czechoslovakia). The course of elution was followed with the aid of an ISCO UA-5 flow-throgh microspectrophotometer (USA) and by TLC.

Vertical electrophoresis was performed in a Labor instrument (Hungary) in 0.05 M triethylammonium bicarbonate buffer (pH 7.5) at a voltage of 1000 V for 2 h.

^{Xyl}T was obtained by the modification of deoxythimidine [2], and ^{Lyx}U and ^{Ara}U via anhydro derivatives of uridine [2, 3, 5]. ^{Xyl}T was kindly provided by I. A. Mikhailopulo, Institute of Bioorganic Chemistry of the Belorussian SSR Academy of Sciences. N-Benzoylnucleosides were synthesized by the use of intermediate protection of the hydroxy groups with chlorotrimethylsilane [6]. The monomethoxytritylation of O-benzoylation of the nucleosides were effected by standard methods [7].

The Dinucleoside Phosphates (I-IV) (see Table 1) were obtained by condensing (MeOTr)-bzdCp(ClpH) with a twofold excess of O-benzoylated anomalous nucleosides in absolute pyridine using equivalents of TPS and 6 equivalents of 1-methylimidazole [8]. After the end of the reaction (TLC monitoring), the reaction mixture was chromatographed on a column of silica gel. The blocked dinucleoside phosphates were eluted by the use of a linear gradient of ethanol in chloroform (from 0 to 5%). The fractions were evaporated. For analysis, the protective groups were removed from part of the substance by successive treatment with a solution of ammonia in pyridine (50°C, 20 h) and then with 80% acetic acid (20°C, 30 min).

(MeOTr)bzdCpdT(Bz), (MeOTr)bzdCprU(Bz), (MeOTr)bzdApbzdApbzdApbzdApdTpbzdCpbzdCp

were obtained by standard methods [8].

The Nonanucleotides were obtained in a similar manner to the dinucleoside phosphates (I-IV). After deblocking, they were isolated by the successive use of ion-exchange chromatography on the resin Polysil-CA [9] in a Tracor chromatograph (Holland), and then HPLC on Zorbax C-8 resin in an Altex chromatograph (USA). The purity of the oligonucleosides obtained was 85-98%.

The qualitative reaction for a cis-glycol grouping was performed by treating 0.5 OU of oligonucleotide with 0.1 M $NaIO_4$ followed by β-elimination [10].

Hydrolysis with Snake Venom PDE was carried out in 0.2 M Tris buffer (pH 8.5) containing 0.04 M $MgCl_2$ under standard conditions (37°C, 1 h). After hydrolysis, the reaction mixtures were analyzed by TLC in n-propanol—ammonia—water (55:10:35).

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SUMMARY

1. The synthesis has been performed of previously undescribed mixed dinucleoside phosphates containing deoxycytidine and one of the following modified nucleosides: deoxyxylothimidine and xylo-, lyxo-, and arabinouridines.

2. Using the phosphotriester method in solution with the above-mentioned dinucleoside phosphates, nonanucleotides containing at the 3'-end nucleosides with inverted configurations of the carbon atoms in positions 2' and 3' have been obtained.

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