# SYNTHESIS OF OLIGO- AND POLYNUCLEOTIDES

## I. SYNTHESIS OF THREE PENTADEOXYRIBONUCLEOTIDES CORRESPONDING TO THE 5'-TERMINAL SECTON OF YEAST VALINE tRNA

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UDC 547.9

Oligo- and polynucleotides of a definite predetermined structure are of great interest for many investigations in the field of molecular biology and bioorganic chemistry. The synthesis of these substances opens up broad possibilities for the study of the specific interaction of nucleic acids with one another and with proteins – the phenomenon of "recognition," which is at the base of many fundamental biochemical processes. In particular, in the study of transport RNAs a fruitful approach to the determination of the functional topography of these important biopolymers is the method of "split molecules" developed by A. A. Baev et al., consisting in the selective cleavage of the polynucleotide chain of a tRNA with the aid of specific endonucleases and the self-assembly, from the fragments obtained, of molecular associates imitating the initial tRNA and possessing its activity to some degree or other [1]. It is obvious that in this process of self-assembly the replacement of individual fragments by synthetic oligonucleotides of predetermined structure will broaden the possibilities of the method and will permit additional information to be obtained concerning the functional value of different segments of the tRNA molecule; in this connection, the use not only of ribonucleotides but also of the more accessible deoxyribonucleotides appears promising, since hybrid DNA-RNA duplexes are similar in many parameters to two-stranded RNA.

In view of this, we have synthesized oligodeoxyribonucleotides reproducing the sequence of bases in various sections of yeast valine tRNA, for the study of which the method of "split molecules" has been used particularly widely [1]. The work was based on the principles and methods of constructing polynucleotide chains developed mainly by Khorana and his school [2]. As the first main aim we selected the two-stranded pentadecadeoxyribonucleotide (I), the A chain of which consists of the 15-membered 5'-terminal segment of the $tRNA^{Val}$ molecule.

5′ G G T T T C G T G G T C T A G 3′ (A)
3′ C C A A A G C A C C A G A T C 5′ (B)

I

The present paper describes the synthesis of three pentanucleotides present in chains A and B of this compound, namely: dpApApApCpC,* dpTpCpTpApG, dpCpTpApGpA.

The synthesis was effected by the scheme illustrated below, using deoxyribonucleoside 5'-phosphates as the starting materials. In order to protect the amino groups in them, these nucleotides were converted

* In this and the subsequent papers, a generally accepted system of abbreviations [3] is used which is based on the recommendations of the IUPAC-IUB Nomenclature Commission [4].

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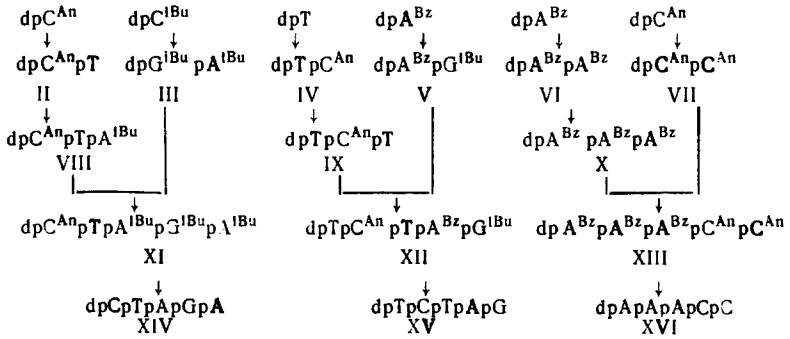
by the action of an excess of an acylating agent into the products of exhaustive N,O-acylation, which were then saponified with 1 N caustic soda.




In this way, we obtained $N_6$-benzoyldeoxyadenylic acid ($dpA^{Bz}$), $N_4$-anisoyldeoxycytidylic acid ($dpC^{An}$), and $N_2$-isobutyryldeoxyguanylic acid ($dpG^{iBu}$), which are commonly used in nucleotide synthesis. In addition, we obtained the previously unknown $N_6$-isobutyryl- and $N_6$, $O_3'$-diisobutyryldeoxyadenylic acids and showed that they are as suitable for the synthesis of oligonucleotides as the corresponding benzoyl derivatives; this decreases the variety of protective groups and simplifies the production of nucleotides with partially split-off N-acyl residues.

The oligonucleotide chain was constructed in the direction from the 5'- end to the 3'- end; the pentanucleotide was synthesized from the 5'-terminal trinucleotide, which, in its turn, was obtained by the method of successive growth of the chain and the 3'-terminal dinucleotide. The phosphoric acid residue in the nucleotide reacting with the 3'-hydroxyl (nucleoside component) was protected by a $\beta$-cyanoethyl residue, and in the nucleotide forming the phosphodiester bond by a phosphate group (nucleotide component) the 3'-hydroxyl was protected by an acetyl residue. An exception was the deoxyguanylic acid and also the deoxyadenylic acid in the synthesis of the pentanucleotide (XI), which in this case were used in the reaction in the form of the N,O-diisobutyryl derivatives, obtained by the exhaustive isobutyrylation of the nucleotide. Condensation with the formation of the phosphodiester bond was performed with the aid of mesitylenesulfonyl chloride or dicyclohexylcarbodiimide, which has been used repeatedly for similar purposes. After the hydrolytic elimination of the 5'- and 3'-protective groups, the mixture was chromatographed on DEAE-cellulose or DEAE-Sephadex, and the N-protective groups in the individual oligonucleotides were eliminated by ammonolysis. To determine its nucleotide composition, the oligomer obtained was subjected to exhaustive enzymatic hydrolysis to nucleosides or nucleotides and the monomers were separated by chromatography and their amounts were determined spectrophotometrically.

## EXPERIMENTAL

The starting materials used were the monodeoxyribonucleotides dpT, dpA, dpC, and dpG produced by the experimental chemical factory of the Novosibirsk Institue of Organic Chemistry of the Siberian Branch of the Academy of Sciences of the USSR (Novosibirsk); to convert them into the pyridinium form, they were passed through a column of Dowex 50 ($PyH^+$) in a solution of 2 M aqueous pyridine and were then freeze-dried. To obtain absolute pyridine, a commercial preparation of "ch" ["pure"] grade was boiled for several hours over granulated KOH and was then distilled over KOH, TsCl, BaO, and $P_2O_5$. Chromatography was performed on Whatman No. 1 paper (strips 47 cm long) in a descending flow in the 96% EtOH–1 M $AcONH_4$ (7 : 3) system with pH 7.5 (wherever there is no statement to the contrary). Paper electrophoresis was performed in 0.05 M triethylammonium bicarbonate buffer (TEAB, pH 7.5) at a voltage of 22 V/cm for 4 h.

The products of the oligonucleotide synthesis were separated by chromatography on columns of DE-23 and DE-32 diethylaminoethylcellulose or of A-25 diethylaminoethyl-Sephadex in the bicarbonate form. The reaction mixture was deposited on the column in dilute aqueous pyridine solution with a concentration of nucleotide material not greater than 5-7 mM and containing not more than 20% of pyridine at a rate of passage of the solution not greater than 0.07 ml/min · $cm^2$. The column was eluted with 0.05 M TEAB in 10% ethanol until the pyridine had been completely eliminated (as monitored by the optical density of the eluate at 260 nm), after which it was eluted with a linear gradient of TEAB. All the chromatographic operations were performed at 2-4°C. The eluates were diluted (twofold) with pyridine and were evaporated in vacuum at 20°C, the triethylamine being eliminated completely by a repeat evaporation with pyridine. After

evaporation, the residue was freeze-dried or was precipitated with ether from pyridine. The yield was determined in optical units (1 $OU_{280}$ of the substance corresponds to unit optical density of a 1-cm layer of solution at 280 nm, and was calculated (as a percentage of theoretical) from the number of moles of reaction product and of the starting material not taken in excess; as the value of the molecular extinction of an oligonucleotide at a given wavelength we took the sum of the extinctions of the mononucleosides $\varepsilon_{267}$ 9600 for dpT, $\varepsilon_{302}$ 22,430 for $dpC^{An}$, $\varepsilon_{259}$ 16,700 for $dpG^{iBu}$, $\varepsilon_{280}$ 18,300 for $dpA^{Bz}$ [5], $\varepsilon_{274}$ 16,700 for $dpA^{iBu}$ (see below) without taking hypochromism into account.

1. $N_2,O_{3'}$-Diisobutyryldeoxyguanosine 5'-Phosphate ($dpG^{iBu}$-OiBu). A suspension of 6.0 g of the pyridinium salt of dpG in 150 ml of pyridine and 90 ml of isobutyric anhydride was stirred at 0°C for 5 days; then 75 ml of methanol and, after 30 min, 150 ml of 20% aqueous pyridine were added. The solution was left at room temperature for 16 h and was evaporated several times with the addition first of 20% aqueous pyridine and then of water. After freeze-drying from 5% aqueous pyridine, the yield of $dpG^{iBu}$-OiBu was 7.35 g (96%); $R_{dpT}$ 2.14, $\lambda_{max}$ 258 nm, $\lambda_{min}$ 227 nm.

2. $N_2$-Isobutyryldeoxyguanosine 5'-Phosphate ($dpG^{iBu}$). With cooling, 90 ml of 2 N NaOH was added to a solution of 3 g of $dpG^{iBu}$-OiBu in 90 ml of 50% aqueous pyridine, and the mixture was kept at 0°C for 7 min and was neutralized to pH 8 by adding, with stirring, Dowex 50×8 ($PyH^+$). The solution was passed through a column with a fresh portion of Dowex, the resin was washed with 2.5 liters of 2 M aqueous pyridine, the eluate was concentrated in vacuum to a syrupy state, and the residue was freeze-dried. The yield of $dpG^{iBu}$ was 2.67 g (98%); $R_{dpT}$ 1.27; $\lambda_{max}$ 258 nm, $\lambda_{min}$ 227 nm.

3. $N_6$-Benzoyldeoxyadenosine 5'-Phosphate ($dpA^{Bz}$). A solution of 2 g of the pyridinium salt of dpA in 25 ml of pyridine was shaken with 25 ml of BzCl for 3-4 h, and after decomposition with water at 0°C the mixture was extracted with chloroform. The extracted $dpA^{Bz}$-OBz was treated with 1 N NaOH in 50% aqueous pyridine (0°C for 20 min), the solution was neutralized to pH 8.0 by the addition of Dowex 50×8 ($PyH^+$), the solid matter was filtered off, the filtrate was concentrated to 1/10 of its initial volume, the excess of benzoic acid was eliminated by repeated extraction with ether, the aqueous layer was evaporated, and the residue was precipitated with ether from pyridine. The yield of $dpA^{Bz}$ was 2.45 g (91%); $R_{dpT}$ 1.03; $\lambda_{max}$ 280 nm, $\lambda_{min}$ 245 nm (see [6]).

4. $N_6,O_3$-Diisobutyryldeoxyadenosine 5'-Phosphate ($dpA^{iBu}$-OiBu) and $N_6$-Isobutyryldeoxyadenosine 5'-Phosphate ($dpA^{iBu}$). A mixture of 1.72 g of the pyridinium salt of dpA, 15 ml of isobutyric anhydride, and 40 ml of pyridine was stirred for 72 h, cooled to 0°C, treated with 15 ml of methanol, kept at room temperature for 1 h, and evaporated. Evaporation with methanol was repeated three times, and the residue was precipitated with ether from pyridine. The yield of $dpA^{iBu}$-OiBu was 1.6 g (87%); $R_{dpT}$ 1.90.

The hydrolysis of the $dpA^{iBu}$-OiBu with 1 N NaOH (15 min at 0°C) gave a quantitative yield of $dpA^{iBu}$; $R_{dpT}$ 1.40, $\lambda_{max}$ 274 nm (ε 16,700), $\lambda_{min}$ 230 nm. Ammonolysis of the diisobutyrate (conc. $NH_4OH$, 72 h at 20°C) gave the initial dpA.

5. $N_4$-Anisoyldeoxycytidine 5'-Phosphate ($dpC^{An}$) was obtained similarly from $dpA^{Bz}$ (expt. 3). Yield 95%, $R_{dpT}$ 1.02, $\lambda_{max}$ 302 nm, $\lambda_{min}$ 235 nm (see [7]).

6. Thymidine 5'-(β-Cyanoethyl Phosphate) (dCEpT). To a solution of 7 g of the pyridinium salt of dpT in 100 ml of pyridine was added 70 ml of ethylene cyanohydrin, 50 g of N,N'-dicyclohexylcarbodiimide (DCC), and 4 g of Dowex 50×8 ($PyH^+$) which had been dried by repeated evaporation with pyridine. To dry the reaction mixture further, 10 ml of the pyridine was distilled off in vacuum, and the suspension was stirred at 20°C for 48 h. Then 200 ml of water was added and the excess of DCC was extracted with cyclohexane (5×200 ml), after which the aqueous pyridine solution was allowed to stand for 16 h and was filtered, the filtrate was concentrated in vacuum, and the residue was evaporated several times with pyridine and was finally precipited from pyridine with ether at −10°C. The yield of dCEpT was 6.2 g (82%), $R_{dpT}$ 2.02, $u_{dpT}$ 0.63 (see [8]).

The cyanoethyl derivatives of the other nucleotides were obtained similarly: $dCEpA^{Bz}$ (yield 81%, $R_{dpT}$ 2.02, $u_{dpT}$ 0.65), $dCEpC^{An}$ (yield 88%, $R_{dpT}$ 2.00, $u_{dpT}$ 0.55), $dCEpG^{iBu}$ (yield 92%, $R_{dpT}$ 1.97, $u_{dpT}$ 0.55), $dCEpC^{An}pT$ (yield 75%, $R_{dpT}$ 1.83, $u_{dpT}$ 0.62), $dCEpC^{An}pTpA^{iBu}$ (yield 85%, $R_{dpT}$ 1.72), $dCEpTpC^{An}$ (yield 77%, $u_{dpT}$ 0.60), $dCEpTpC^{An}$ pT (yield 76%, $u_{dpT}$ 0.77), $dCEpA^{Bz}pA^{Bz}$ (yield 85%, $R_{dpT}$ 1.70), $dCEpA^{Bz}pA^{Bz}$ (yield 80%, $R_{dpT}$ 1.65).

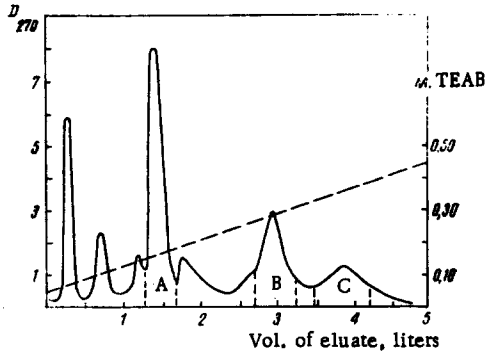



Fig. 1

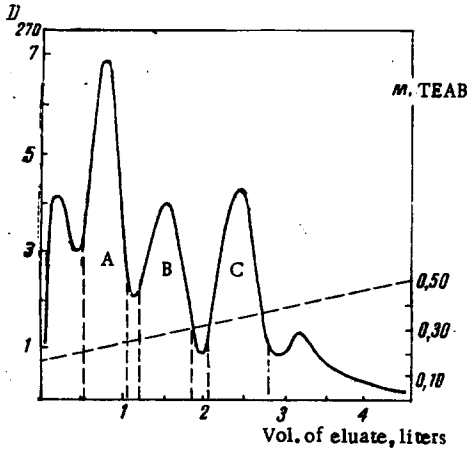



Fig. 2

Fig. 1. Isolation of dpC$^{An}$pTpA$^{iBu}$pG$^{iBu}$pA$^{iBu}$ (XI): peak A contains 1600 OU (28%) of dpG$^{iBu}$ pA$^{iBu}$ (III); peak B 750 OU (17%) of dpC$^{An}$ pTpA$^{iBu}$ (VIII); and peak C 750 OU (18%) of the pentanucleotide (XI).

Fig. 2. Isolation of dpTpC$^{An}$pTpA$^{Bz}$pG$^{iBu}$ (XII): peak A contains 1820 OU (23%) of dpA$^{Bz}$pG$^{iBu}$ (V); peak B 970 OU (35%) dpTpC$^{An}$pT (IX) contaminated with 90 OU of the pyrophosphate (V); and peak C 1230 OU (25%) of the pentanucleotide (XII).

7. 3'-O-Acetylthymidine 5'-Phosphate (dpT-OAc). A suspension of 2.0 g of the pyridinium salt of dpT in 50 ml of pyridine and 20 ml of $Ac_2O$ was stirred at 20°C for 4 h and, with cooling, it was treated with methanol and then with water and the mixture was left for 12 h. After evaporation and precipitation with ether from pyridine, the yield of dpT-OAc was 2.07 g (93%), $R_{dpT}$ 1.32, $\lambda_{max}$ 267 nm, $\lambda_{min}$ 235 nm.

The other 3'-O-acetyl derivatives of nucleotides were obtained similarly: dpC$^{An}$-OAc (yield 90%, $R_{dpT}$ 1.22), dpA$^{Bz}$-OAc (yield 92%, $R_{dpT}$ 1.32), dpA$^{Bz}$pG$^{iBu}$-OAc (yield 93%, $R_{dpT}$ 1.27), dpC$^{An}$pC$^{An}$-OAc (yield 94%, $R_{dpT}$ 1.15).

8. dpC$^{An}$pT (II). A mixture of 1.53 g (2.7 mmoles) of dCEpC$^{An}$ and 3.04 g (6.9 mmoles) of dpT-OAc (in the form of the pyridinium salts) was dried by evaporation with pyridine (5×4 ml) and dissolved in 15 ml of pyridine, and then 2.2 g (10.5 mmoles) of mesitylenesulfonyl chloride was added, and the mixture was left at 20°C for 3 h. It was cooled to 0°C, and 21 ml of a 1 M pyridine solution of triethylamine and 21 ml of water were added and this mixture was left for 12 h. With cooling, 57 ml of 2 N NaOH was added and after being kept at 0°C for 20 min the reaction solution was rapidly neutralized to pH 8.0 by the addition of Dowex 50×8 ($PyH^+$). The resin was filtered off and washed with 2 M aqueous pyridine. The combined filtrate (0.5 liter) was deposited on a column of DEAE-cellulose ($HCO_3^-$) (3.3×100 cm) and was chromatographed in a linear gradient of TEAB in 10% ethanol (5 liters 0.05 M–7 liters 0.03 M), 20-ml fractions being collected and their absorption being measured at 270 nm. Fractions 150-233 yielded dpT (34,000 $OU_{270}$, 51%), fractions 265-365 yielded dpC$^{An}$ (5200 $OU_{302}$, 8.5%), and fractions 440-530 yielded dpC$^{An}$pT (II) (26,650 $OE_{275}$, 41%), $R_{dpT}$ 0.99, $\lambda_{max}$ 280, 302 nm, $\lambda_{min}$ 235, 293 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.83, $\varepsilon_{270}/\varepsilon_{260}$ 1.17, $\varepsilon_{280}/\varepsilon_{260}$ 1.23, $\varepsilon_{290}/\varepsilon_{260}$ 1.17 (see [9]).

9. dpC$^{An}$pTpA$^{iBu}$ (VIII) was obtained by condensing 476 mg (0.5 mmole) of dCEpC$^{An}$pT and 560 mg (1 mmole) of dpA$^{iBu}$-OiBu under the conditions of expt. 8. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$) (3.5×50 cm) in a linear gradient of TEAB in 15% ethanol (3.5 liters 0.05 M–3.5 liters 0.30 M), 11-ml fractions being collected and their absorption at 270 nm being measured. Fractions 105-152 yielded dpA$^{iBu}$ (3500 $OU_{270}$, 22%), fractions 200-250 yielded dpC$^{An}$pT (1200 $OU_{270}$, 7%), and fractions 300-400 yielded the trinucleotide (VIII) (5360 $OU_{270}$, 30%), $R_{dpT}$ 1.13, $\lambda_{max}$ 276, 302 nm, $\lambda_{min}$ 236, 297 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.76, $\varepsilon_{270}/\varepsilon_{260}$ 1.24, $\varepsilon_{280}/\varepsilon_{260}$ 1.22, $\varepsilon_{290}/\varepsilon_{260}$ 0.93.

10. dpG$^{iBu}$pA$^{iBu}$ (III). A mixture of 0.8 g (1.45 mmole) of dCEpG$^{iBu}$, 1.6 g (2.9 mmoles) of dpA$^{iBu}$-OiBu, and 0.7 g of previously dried Dowex 50×8 ($PyH^+$) was evaporated with pyridine (4×3 ml), and then 10 ml of pyridine and 2 g of DCC were added and the mixture was stirred for 72 h. Then it was treated with 50 ml of water and was extracted with cyclohexane (3×100 ml). The aqueous pyridine layer was filtered and, with cooling, the filtrate was treated with an equal volume of 2 N NaOH and then the mixture was kept

TABLE 1. Properties of the Unprotected Oligonucleotides

| Oligonucleotide | * $R_{dpT}$ | UV spectrum | | | | | | Found on enzymatic hydrolysis (relative proportion) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\lambda_{max}$ | $\lambda_{min}$ | $\frac{\varepsilon_{250}}{\varepsilon_{260}}$ | $\frac{\varepsilon_{270}}{\varepsilon_{260}}$ | $\frac{\varepsilon_{280}}{\varepsilon_{260}}$ | $\frac{\varepsilon_{290}}{\varepsilon_{260}}$ | dpC | dpT | dpA | dpG | dC] | dT | dA | dG |
| dpCpT | 1,05 | 268 | 234 | 0,78 | 1,05 | 0,76 | | 1,00 | 1,10 | | | | | | |
| dpTpC | 0,95 | 267 | 237 | 0,75 | 1,09 | 0,79 | | | | | | 1,07 | 1,00 | | |
| dpGpA | 0,93 | 257 | 225 | 0,92 | 0,76 | 0,42 | | | | 1,10 | 1,00 | | | | |
| dpApG | 0,85 | 255 | 225 | 1,03 | 0,72 | 0,40 | | | | | | | | 1,00 | 1,05 |
| dpCpC | 0,74 | 270 | 248 | 0,91 | 1,20 | 1,03 | | 1,00 | | | | 1,05 | | | |
| dpApA | 0,78 | 259 | 228 | 0,79 | 0,80 | 0,40 | | | | 1,00 | | | | 1,04 | |
| dpApApA | 0,59 | 259 | 228 | 0,78 | 0,78 | 0,37 | | | | 2,02 | | | | 1,00 | |
| dpCpTpA | 0,91 | 262 | 234 | 0,75 | 0,95 | 0,53 | | 1,00 | 1,05 | 1,09 | | | | | |
| dpTpCpT | 0,88 | 267 | 236 | 0,71 | 1,10 | 0,79 | | | | | | 1,00 | 2,16 | | |
| dpCpTpApGpA (XIV) | 0,30 | 259 | 230 | 0,90 | 0,82 | 0,56 | | 1,03 | 1,01 | 2,21 | 1,00 | | | | |
| dpTpCpTpApG (XV) | 0,38 | 262 | 232 | 0,85 | 0,91 | 0,60 | | | | | | 1,07 | 2,08 | 1,08 | 1,00 |
| dpApApApCpC (XVI) | 0,23 | 259 | 237 | 0,78 | 0,87 | 0,57 | | 1,00 | | 1,46 | | | | | |

* In the n-PrOH−25% $NH_4OH-H_2O$ (55:10:35) system.

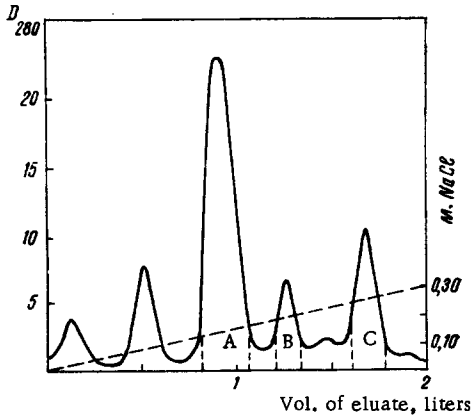



Fig. 3. Isolation of $dpA^{Bz}pA^{Bz}pA^{Bz}$-$pC^{An}pC^{An}$ (XIII): peak A contains 2500 OU (51%) of $dpC^{An}pC^{An}$ (VII); peak B 450 OU of $dpA^{Bz}pA^{Bz}pA^{Bz}$ (X), and peak C 905 OU (28%) of the pentanucleotide (XIII).

at room temperature for 20 min and was neutralized with Dowex 50×8 ($PyH^+$) to pH 8.0. The resin was filtered off, and the filtrate was deposited on a column of DEAE-cellulose ($HCO_3^-$) (3.5×100 cm) and chromatographed in a linear gradient of TEAB in 15% ethanol (6 liters 0.05 M−6 liters 0.25M), 35-ml fractions being collected and their absorption at 270 nm being determined. Fractions 15-100 yielded $dpA^{iBu}$ (22,300 $OU_{274}$, 53%), and fractions 145-225 yielded $dpG^{iBu}pA^{iBu}$ (III) (12,000 $OU_{264}$, 28%), $R_{dpT}$ 1.40, $\lambda_{max}$ 264, 274 nm, $\lambda_{min}$ 230, 270 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.86, $\varepsilon_{270}/\varepsilon_{260}$ 0.99, $\varepsilon_{280}/\varepsilon_{260}$ 0.89, $\varepsilon_{290}/\varepsilon_{260}$ 0.53.

11. $dpC^{An}pTpA^{iBu}pG^{iBu}pA^{iBu}$ (XI) was obtained by condensing 83 mg (4300 $OU_{270}$, 0.065 mmole) of $dCEpC^{An}pT$-$pA^{iBu}$ and 192 mg (5700 $OU_{264}$, 0.19 mmole) of $dpG^{iBu}pA^{iBu}$-OiBu under the conditions of expt. 8. Chromatography was performed on a column of DE-23 cellulose ($HCO_3^-$) (2.5× 42 cm) in a linear gradient of TEAB in 20% ethanol (2.5 liters 0.05 M−2.5 liters 0.45 M); the rate of elution was 30 ml/h and the volume of the fractions was 12 ml (Fig. 1). The yield of the pentanucleotide (XI) was 28 mg (750 $OU_{270}$, 18%), $R_{dpT}$ 0.18, $\lambda_{max}$ 272 nm, $\lambda_{min}$ 238 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.88, $\varepsilon_{270}/\varepsilon_{260}$ 1.14, $\varepsilon_{280}/\varepsilon_{260}$ 1.03, $\varepsilon_{290}/\varepsilon_{260}$ 0.76.

12. $dpTpC^{An}$ (IV) was obtained by condensing 2.3 g (5 mmoles) of dCEpT and 3.94 g (7 mmoles) of $dpC^{An}$-OAc under the conditions of expt. 8. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$) (4.5×100 cm) in a linear gradient of TEAB in 10% ethanol (10 liters 0.05 M−10 liters 0.30 M), 14-ml fractions being collected and their absorption at 270 nm being measured. Fractions 100-300 yielded dpT (4200 $OU_{270}$, 85%), fractions 400-650 $dpC^{An}$ (56,200 $OU_{302}$, 36%), and fractions 700-1050 $dpTpC^{An}$ (IV) (42,000 $OU_{302}$, 37%), $R_{dpT}$ 1.02, $\lambda_{max}$ 272, 302 nm, $\lambda_{min}$ 237, 295 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.77, $\varepsilon_{270}/\varepsilon_{260}$ 1.16, $\varepsilon_{280}/\varepsilon_{260}$ 1.13, $\varepsilon_{290}/\varepsilon_{260}$ 1.07 (see [8]).

13. $dpTpC^{An}pT$ (IX) was obtained by condensing 1.07 g (1.1 mmole) of $dCEpTpC^{An}$ and 2.07 g (4.7 mmoles) of dpT-OAc under the conditions of expt. 8. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$) (4.5×90 cm) in a linear gradient of TEAB in 10% ethanol (8 liters 0.05 M−8 liters 0.30 M), 10-ml fractions being collected and the absorption at 270 nm being determined. Fractions 200-450 yielded dpT (11,000 $OU_{270}$, 25%), fractions 900-1000 $dpTpC^{An}$ (IV) (790 $OU_{302}$, 4%), and fractions 1150-1350 the trinucleotide (IX) (723 mg, 12,700 $OU_{302}$, 51%), $R_{dpT}$ 1.20 [in the iso-$PrCO_2H$−25% $NH_4OH-H_2O$ (66:1:33) system], $\lambda_{max}$ 270, 302, $\lambda_{min}$ 238, 300 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.77, $\varepsilon_{270}/\varepsilon_{260}$ 1.14, $\varepsilon_{280}/\varepsilon_{260}$ 1.09, $\varepsilon_{290}/\varepsilon_{260}$ 0.89 (see [5]).

14. $dpA^{Bz}pG^{iBu}$ (V) was obtained by condensing 2.27 g (4 mmoles) of $dCEpA^{Bz}$ and 2.86 (5 mmoles) of $dpG^{iBu}$-OiBu under the conditions of expt. 8. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$) (4.5×80 cm) in a linear gradient of TEAB in 10% ethanol (8 liters 0.05 M−8 liters 0.20 M),

14-ml fractions being collected and the absorption at 270 nm being determined. Fractions 100-300 yielded $dpG^{iBu}$ (31,000 $OU_{258}$, 37%), fractions 300-500 $dpA^{Bz}$ (21,000 $OU_{280}$, 29%), fractions 80-1100 $dpA^{Bz}pG^{iBu}$ (V) (40,000 $OU_{270}$, 29%), $R_{dpT}$ 1.63 (in the same system as in expt. 13), $\lambda_{max}$ 266, 282 nm, $\lambda_{min}$ 231, 270 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.86, $\varepsilon_{270}/\varepsilon_{260}$ 1.03, $\varepsilon_{280}/\varepsilon_{260}$ 1.12, $\varepsilon_{290}/\varepsilon_{260}$ 0.85 (see [9]).

15. $dpTpC^{An}pTpA^{Bz}pG^{iBu}$ (XII) was obtained by condensing 90 mg (2700 $OU_{302}$, 0.065 mmole) of $dCEpTpC^{An}pT$ and 250 mg (8200 $OU_{270}$, 0.24 mmole) of $dpA^{Bz}pG^{iBu}$-OAc under the conditions of expt. 8. Chromatography was performed on a column of DE-32 cellulose ($HCO_3^-$) (2.5×90 cm) in a linear gradient of TEAB in 30% ethanol (2 liters 0.15 M–2 liters 0.5 M); rate of elution 40 ml/h; fraction volume 10 ml (Fig. 2). The yield of the pentanucleotide (XII) was 37 mg (1230 $OU_{270}$, 25%), $R_{dpT}$ 1.26 (in the same system as in expt. 13), $\lambda_{max}$ 264 nm, $\lambda_{min}$ 236 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.87, $\varepsilon_{270}/\varepsilon_{260}$ 1.00, $\varepsilon_{280}/\varepsilon_{260}$ 0.93, $\varepsilon_{290}/\varepsilon_{260}$ 0.69.

16. $dpA^{Bz}pA^{Bz}$ (VI) was obtained by condensing 5 g (8.6 mmole) of $dCEpA^{Bz}$ and 5.3 g (9.55 mmoles) of $dpA^{Bz}$-OAc under the conditions of expt. 8. The reaction mixture was separated by chromatography on a column of A-25 DEAE-Molselekt ($HCO_3^-$), (3.6×55 cm) in a linear gradient of TEAB in 10% ethanol (5 liters 0.05 M–5 liters 0.4 M), 22-ml fractions being collected and their absorption at 280 nm being measured. Fractions 130-235 yielded $dpA^{Bz}$ (111,900 $OU_{280}$, 34%) and fractions 340-439 yielded $dpA^{Bz}pA^{Bz}$ (12,200 $OU_{280}$, 39%), $R_{dpT}$ 1.10, $\lambda_{max}$ 280 nm, $\lambda_{min}$ 244 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.93, $\varepsilon_{270}/\varepsilon_{260}$ 1.09, $\varepsilon_{280}/\varepsilon_{260}$ 1.45, $\varepsilon_{290}/\varepsilon_{260}$ 1.31 (see [10]).

17. $dpA^{Bz}pA^{Bz}pA^{Bz}$ (X) was obtained by condensing 2.65 g (2.5 mmoles) of $dCEpA^{Bz}pA^{Bz}$ and 5.55 g (10 mmoles) of $dpA^{Bz}$-OAc under the conditions of expt. 8. The reaction mixture was separated by chromatography on a column of A-25 DEAE-Molselekt ($HCO_3^-$) (5.7×21 cm) in a linear gradient of TEAB in 10% ethanol (7 liters 0.05 M–7 liters 0.45 M), 16-ml fractions being collected and their absorption at 280 nm being measured. Fractions 230-400 yielded $dpA^{Bz}$ (48,000 $OU_{280}$, 26%), fractions 435-465 $dpA^{Bz}pA^{Bz}$ (20,300 $OU_{280}$, 22%), and fractions 690-811 the trinucleotide (X) (38,500 $OU_{280}$, 28%), $R_{dpT}$ 1.01, $\lambda_{max}$ 280 nm, $\lambda_{min}$ 245 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.95, $\varepsilon_{270}/\varepsilon_{260}$ 1.11, $\varepsilon_{280}/\varepsilon_{260}$ 1.42, $\varepsilon_{290}/\varepsilon_{260}$ 1.27.

18. $dpC^{An}pC^{An}$ (VII) was obtained by condensing 2.6 g (3.85 mmoles) of $dCEpC^{An}$ and 2.8 g (4.95 mmoles) of $dpC^{An}$-OAc under the conditions of expt. 8. The reaction mixture was separated by chromatography on a column of DEAE-cellulose ($HCO_3^-$) (4.2×110 cm) in a linear gradient of TEAB in 10% ethanol (9 liters 0.05 M–9 liters 0.30 M), 22-ml fractions being collected and the absorption at 302 nm being measured. Fractions 210-341 yielded $dpC^{An}$ (54,000 $OU_{302}$, 27%) and fractions 440-645 $dpC^{An}pC^{An}$ (VII) (72,500 $OU_{302}$, 41%), $R_{dpT}$ 1.03, $\lambda_{max}$ 302 nm, $\lambda_{min}$ 236 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.91, $\varepsilon_{270}/\varepsilon_{260}$ 1.18, $\varepsilon_{280}/\varepsilon_{260}$ 1.47, $\varepsilon_{290}/\varepsilon_{260}$ 1.65 (see [10]).

19. $dpA^{Bz}pA^{Bz}pA^{Bz}pC^{An}pC^{An}$ (XIII) was obtained by condensing 48 mg (1760 $OU_{280}$, 0.032 mmole) of $dCEpA^{Bz}pA^{Bz}pA^{Bz}$ and 134 mg (5800 $OU_{302}$, 0.128 mmole) of $dpC^{An}pC^{An}$-OAc under the conditions of experiment 8. Chromatography was performed on a column of A-25 DEAE-Sephadex ($Cl^-$) (1×34 cm) in a linear gradient of NaCl in 0.01 M tris-HCl (pH 7.5) in 7 M urea (1 liter of buffer and 1 liter of 0.3 M NaCl in the buffer); the rate of elution was 42 ml/h and the volume of the fractions 7 ml (Fig. 3). The yield of the pentanucleotide (XIII) was 23 mg (905 $OU_{280}$, 28%), $R_{dpT}$ 0.24, $\lambda_{max}$ 280 nm, $\lambda_{min}$ 240 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.88, $\varepsilon_{270}/\varepsilon_{260}$ 1.16, $\varepsilon_{280}/\varepsilon_{260}$ 1.41, $\varepsilon_{290}/\varepsilon_{260}$ 1.35.

Analysis of the Oligonucleotides. The protective groups were removed from the bases by the action of $NH_4OH$ (2 ml of conc. aqueous $NH_3$ per 20 OU of oligonucleotide, 72 h at 20°C or 3 h at 50°C) with subsequent evaporation and freeze-drying. The substance obtained was hydrolyzed completely by the phosphodiesterase from kufi (Vipera lebetina) venom (to the nucleoside 5'-phosphates) or crude snake venom (to the nucleosides). The mixture of monomers formed was separated by paper chromatography and elution with water. The amount of each of the hydrolysis products was determined spectrophotometrically in 0.1 N HCl at a wavelength corresponding to the absorption maximum; as standard values of the molecular extinctions we used literature figures [11]. The results of the analyses are given in Table 1.

## SUMMARY

The directed chemical synthesis of three pentadeoxyribonucleotides, dpCpTpApGpA, dpTpCTpApG, and dpApApApCpC, corresponding to the 5'-terminal segment of yeast valine tRNA, has been effected.

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