# SYNTHESIS OF OLIGO- AND POLYNUCLEOTIDES

## II. SYNTHESIS OF PENTADEOXYRIBONUCLEOTIDE BLOCKS CORRESPONDING TO SEGMENT 1-10 OF YEAST VALINE tRNA

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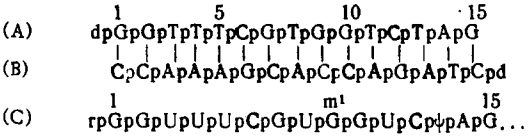
UDC 547.9

In the preceding paper, together with two other groups, we described the synthesis of three pentanucleotide segments (A11-15, B1-5, and B11-15) of the two-stranded pentadecadeoxyribonucleotide (A)·(B) corresponding to the 5'-terminal section 1-15 of yeast valine tRNA [1].




Continuing this work, we have synthesized three other segments of this two-stranded pentadecanucleotide, namely: A1-5 (V) and two mutually complementary segments – A6-10 (XII) and B6-10 (XVIII). The synthesis was performed by the block method in accordance with the scheme shown below, several different routes being used to obtain the pentanucleotides dpCpGpTpGpG (XII) and dpCpCpApCpG (XVIII) in order to study the properties of the intermediate oligonucleotides and to select the optimum variant of the method of synthesis. In the creation of an internucleotide bond the blocking of the 5'-phosphate residue and the 3'-hydroxyl of the deoxyribose moiety and of the amino groups in the pyrimidine and purine nuclei was effected by means of the same protective groups as previously [1]; as condensing agents we used triisopropylbenzenesulfonyl chloride, mesitylenesulfonyl chloride, and dicyclohexylcarbodiimide. (See scheme on following page.)

The pentanucleotides obtained at the present time and previously, (V), (XII), dpTpCpTpApG, dpCpTpApGpA, (XVIII), and dpApApApCpC, taken together, reproduce the complete nucleotide sequence of both chains (A and B) of the 15-membered segment of the double-helical DNA, including the 5'-terminal section (C) of yeast valine tRNA.

## EXPERIMENTAL

For general experimental information, see [1]. The work was performed with monodeoxyribonucleotides produced by the experimental chemical factory of the Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Academy of Sciences of the USSR (Novosibirsk). Ion-exchange chromatography was performed on DE-23 or DE-32 DEAE-cellulose (Whatman, England) and A-25 DEAE-Sephadex (Farmacia, Sweden). The protected oligonucleotides were chromatographed on FN3 or Whatman No. 1 paper in the EtOH–1 M $AcONH_4$ (7:3) system (pH 7.5), and the products of the elimination of the N-protective groups were chromatographed in the n-PrOH–conc. $NH_4OH$–$H_2O$ (55:10:35) system. The UV spectra were taken in neutral aqueous solutions. The monomeric composition of the oligonucleotides after the elimination of the N-protective groups was determined by hydrolysis with the phosphodiesterase of snake venom or

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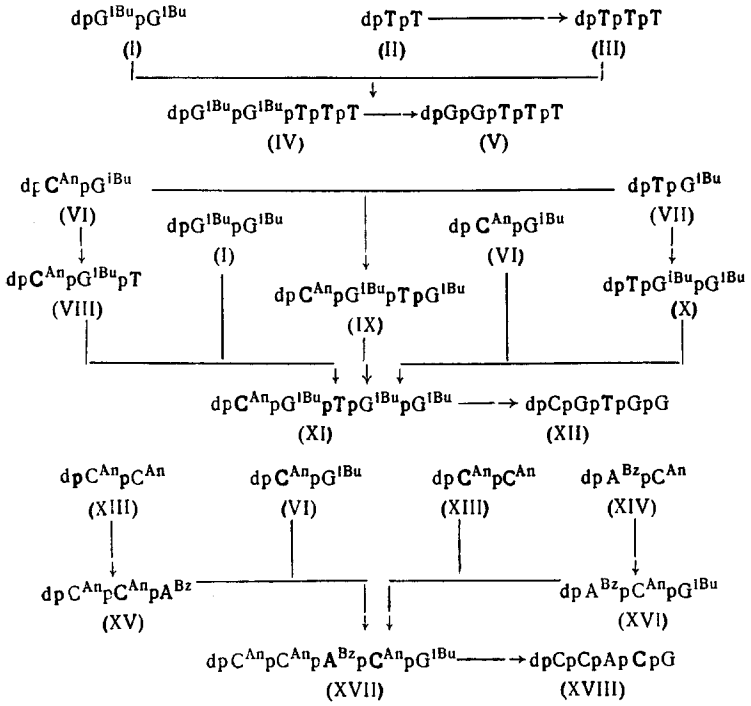
Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 402-410, May-June, 1973. Original article submitted January 26, 1973.




(for homooligomers) with alkaline phosphatase, and then phosphodiesterase (see [1]). The cyanoethyl and acetyl derivatives of the mono- and oligonucleotides were obtained as described in the preceding paper [1].

1. $dpG^{iBu}pG^{iBu}$. A mixture of the pyridinium salts of $dCEpG^{iBu}$ (0.9 g, 1.4 mmole) and $dpG^{iBu}$-iBu (1.2 g, 2.1 mmoles) was dried by evaporation with pyridine (3×50 ml) and dissolved in 8 ml of pyridine, and then 1.4 g (4.6 mmoles) of triisopropylbenzenesulfonyl chloride was added and the mixture was left at 20°C for 5 h. It was cooled to −50°C, 12 ml of a 1 M pyridine solution of triethylamine and 20 ml of water were added, and then the mixture was left at 20°C for 16 h. After this, it was mixed with 2 N NaOH (equal volumes) at 0°C, kept at 0°C for 10 min, neutralized with Dowex 50×8 ($PyH^+$) to pH 8, and then the resin was filtered off and was washed with 2 M aqueous pyridine. The combined filtrate (900 ml) was transferred to a column of DEAE-cellulose ($HCO_3^-$, 3.6×30 cm) previously equilibrated with 0.05 M triethylammonium bicarbonate (TEAB) and chromatography was performed in a concentration gradient of TEAB in 10% ethanol (2 liters 0.05 M−2 liters 0.2 M; 0.5 liter 0.2 M−0.5 liter 0.3 M), with the collection of 12-ml fractions in 10 min and measurement of the absorption at 260 nm. Fractions 260-350 yielded 19,000 $OU_{260}$ (40%) of the dinucleotide (I) $R_{dpT}$ 1.40, $\lambda$ max 258 nm, $\lambda$ min 228 nm; $\varepsilon_{250}/\varepsilon_{260}$ 0.85, $\varepsilon_{270}/\varepsilon_{260}$ 0.78, $\varepsilon_{280}/\varepsilon_{260}$ 0.70, $\varepsilon_{290}/\varepsilon_{260}$ 0.55. Fractions 130-210 gave 17% of the initial $dpG^{iBu}$.

After the elimination of the N-protective groups by ammonolysis (25% $NH_4OH$, 72 hours at 20°C), dpGpG was obtained with $R_{dpT}$ 0.55, monomer composition dpG: dG 1.00: 1.09.

2. dpTpT (II) was obtained by the reaction of 3.35 g (7.35 mmoles) of dCEpT and 3.2 g (7.25 mmoles) of dpT-Ac under the conditions of expt. 1. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 4×80 cm; 5 liters 0.05 M−5 liters 0.30 M TEAB in 10% ethanol, 18-ml fractions/10 min). Fractions 310-460 yielded 70,000 $OU_{267}$ 50% of dpTpT, $R_{dpT}$ 0.85, $\lambda_{max}$ 267 nm, $\lambda_{min}$ 233 nm, monomer composition dT: dpT 1.09: 1.00. The amount of dpT recovered was 16%.

3. dpTpTpT (III) was obtained by the reaction of 0.76 g (0.9 mmole) of dCEpTpT and 0.80 g (1.8 mmole) of dpT-Ac under the conditions of experiment 1. Chromatography was performed on a column of DEAE-Sephadex ($HCO_3^-$, 2×30 cm; 2 liters of 0.05 M−2 liters of 0.4 M TEAB in 10% ethanol, 12-ml fractions/10 min). Fractions 192-240 yielded 8800 $OU_{267}$ (36%) of the trinucleotide (III), $R_{dpT}$ 0.60, $\lambda_{max}$ 267 nm, $\lambda_{min}$ 233 nm; monomer composition dT: dpT 1.0: 1.95. The amount of dpT recovered was 70% and of dpTpT 45%.

4. $dpG^{iBu}pG^{iBu}pTpTpT$ (IV) was obtained by the reaction of 260 mg (0.24 mmoles) of $dCEpG^{iBu}pG^{iBu}$ and 400 mg (0.33 mmole) of dpTpTpT-Ac by the method of expt. 1.

Chromatography was performed on a column of DE-32 cellulose ($HCO_3^-$, 1.8×40 cm) in a concentration gradient of TEAB in 10% ethanol (0.8 liter of 0.05 M−0.8 liter of 0.25 M; 0.8 liter of 0.25 M−0.8 liter

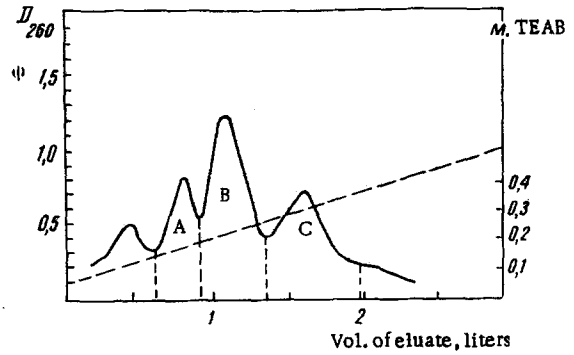



Fig. 1

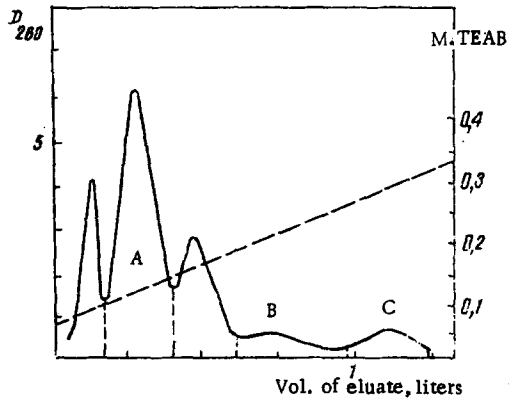



Fig. 2

Fig. 1. Isolation of dpG$^{iBu}$pG$^{iBu}$pTpTpT (IV) (expt. 4): peak A contains 2700 $OU_{260}$ of the dinucleotide (I), peak B 5200 $OU_{260}$ of the trinucleotide (III), and peak C 4300 $OU_{260}$ of the pentanucleotide (IV).

Fig. 2. Isolation of dpC$^{An}$pG$^{iBu}$pTpG$^{iBu}$pG$^{iBu}$ (XI) (experiment 10A): peak A contains 1250 $OU_{260}$ of dpG$^{iBu}$; peak B 250 $OU_{260}$ of the tetranucleotide (IX); and peak C 200 $OU_{260}$ of the pentanucleotide (XI).

of 0.5 M) at a rate of elution of 67 ml/h with a fraction volume of 10 ml (Fig. 1). The yield of pentanucleotide (IV) was 4300 $OU_{260}$ (0.075 mmole, 32%), $R_{dpT}$ 0.50, $\lambda$ max 262 nm, $\lambda$ min 232 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.80, $\varepsilon_{270}/\varepsilon_{260}$ 0.93, $\varepsilon_{280}/\varepsilon_{260}$ 0.70, $\varepsilon_{290}/\varepsilon_{260}$ 0.42. The amount of dinucleotide (I) recovered was 34% and of the trinucleotide (III) 55%.

After elimination of the N-protective groups by ammonolysis, the unprotected pentanucleotide (V) was obtained, $R_{dpT}$ 0.35, $\lambda$max 262 nm, $\lambda$min 233 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.89, $\varepsilon_{270}/\varepsilon_{260}$ 0.93, $\varepsilon_{280}/\varepsilon_{260}$ 0.69, $\varepsilon_{290}/\varepsilon_{260}$ 0.30; nucleotide composition dpG : dpT 2.05 : 3.00.

5. dpC$^{An}$pG$^{iBu}$ (VI). A. Compound (VI) was obtained by condensing 2.65 g (4.7 mmoles) of dCEpC$^{An}$ and 3.9 g (6.9 mmoles) of dpG$^{iBu}$-iBu under the conditions of experiment 1. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 4×85 cm) in a linear concentration gradient of TEAB in 10% ethanol (10 liters of 0.05 M–10 liters of 0.26 M), 18-ml fractions being collected every 7 min and their absorption at 280 nm being measured. Fractions 601-810 yielded 74,800 $OU_{280}$ (57%) of the dinucleotide (VI), $R_{dpT}$ 1.12, $\lambda$ max 262, 286 nm, $\lambda$ min 233, 270 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.85, $\varepsilon_{270}/\varepsilon_{260}$ 1.00, $\varepsilon_{280}/\varepsilon_{260}$ 1.07, $\varepsilon_{290}/\varepsilon_{260}$ 1.07, $\varepsilon_{300}/\varepsilon_{260}$ 0.94, and fractions 381-460 yielded 6300 $OU_{302}$ (4%) of the pyrophosphate dC$^{An}$-5'pp5'-dC$^{An}$,* $R_{dpT}$ 1.37. The amount of dpG$^{iBu}$ recovered was 40% (fractions 81-200) and of dpC$^{An}$ 5% (fractions 316-380).

Ammonolysis of the dinucleotide (VI) gave dpCpG, $R_{dpT}$ 0.64, $\lambda$ max 253, 270 (shoulder) nm, $\lambda$ min 218 nm, $\varepsilon_{250}/\varepsilon_{260}$ 1.01, $\varepsilon_{270}/\varepsilon_{260}$ 0.94, $\varepsilon_{280}/\varepsilon_{260}$ 0.78, $\varepsilon_{290}/\varepsilon_{260}$ 0.33. Nucleotide composition, dpC : dpG 1.00 : 1.01.

B. A mixture of the pyridinium salts of dCEpC$^{An}$ (0.85 g, 1.5 mmole) and dpG$^{iBu}$-iBu (1.2 g, 2.1 mmoles) was dried by being evaporated with pyridine five times and was then dissolved in 10 ml of pyridine after which 3.2 g (15.5 mmoles) of dicyclohexylcarbodiimide and 2 g of Dowex 50×8 ($PyH^+$) were added and the mixture was stirred at 20°C for 72 h. After the addition of 10 ml of water, the unchanged carbodiimide was extracted with hexane (3×20 ml), the aqueous pyridine solution was allowed to stand for 12 h, and the urea that had deposited was filtered off. The filtrate was subjected to treatment with alkali, as in expt. 1, and after neutralization it was chromatographed on a column of DEAE-cellulose ($HCO_3^-$, 4×75 cm, 5 liters of 0.05 M–5 liters of 0.30 M TEAB in 10% ethanol). The yield of the dinucleotide (VI) was 23,450 $OU_{280}$ (54%); the recovery of the dpG$^{iBu}$ was 27% and of dpC$^{An}$ 22%.

6. dpC$^{An}$pG$^{iBu}$pT (VIII).† A. Compound (VIII) was obtained by the condensation of 1.17 g (1 mmole) of dCEpC$^{An}$pG$^{iBu}$ and 1.40 g (3.5 mmoles) of dpT-Ac in the presence of 1.45 g (6.5 mmoles) of mesitylene-

* This pyrophosphate was converted quantitatively into dCEpC$^{An}$ under standard cyanoethylation conditions (see [1]).

† E. P. Badosov took part in this section of the work (expts. 6 and 9B).

sulfonyl chloride (3 h at 20°C). After working up as in expt. 1, the mixture was chromatographed on a column of DEAE-cellulose ($HCO_3^-$, 2.5×77 cm) in a linear concentration gradient of TEAB in 10% ethanol (3.5 liters of 0.05 M–3.5 liters of 0.35 M), 16-ml fractions being collected every 10 min and their absorption at 270 nm being determined. Fractions 230-380 yielded 16,000 $OU_{270}$ (46%) of the trinucleotide (VIII) $R_{dpT}$ 1.00, $\lambda_{max}$ 263, 275 nm, $\lambda_{min}$ 235, 270 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.85, $\varepsilon_{270}/\varepsilon_{260}$ 0.98, $\varepsilon_{280}/\varepsilon_{260}$ 0.96, $\varepsilon_{290}/\varepsilon_{260}$ 0.84. The recovery of dpT was 41% (fractions 30-120).

The ammonolysis of (VIII) gave dpCpGpT, $R_{dpT}$ 0.60, $\lambda_{max}$ 255, 265 (shoulder) nm, $\lambda_{min}$ 230 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.91, $\varepsilon_{270}/\varepsilon_{260}$ 0.97, $\varepsilon_{280}/\varepsilon_{260}$ 0.74, $\varepsilon_{290}/\varepsilon_{260}$ 0.20; nucleotide composition dpC : dpG : dpT 1.00 : 1.02 : 1.07.

B. Compound (VIII) was obtained by condensing 180 mg (0.17 mmole) of $dCEpC^{An}pG^{iBu}$ and 300 mg (0.68 mmole) of dpT-Ac under the conditions of experiment 5B. Yield 2090 $OU_{270}$ (36%).

7. $dpTpG^{iBu}$ (VII) was obtained by condensing 450 mg (1 mmole) of dCEpT and 630 mg (1.1 mmole) of $dpG^{iBu}$-iBu under the conditions of experiment 6A (4 h at 20°C). The mixture was chromatographed on a column of DEAE-cellulose ($HCO_3^-$, 3×50 cm, 2.4 liters of 0.05 M–2.4 liters of 0.25 M TEAB in 10% ethanol, 9-ml fractions/5 min, absorption at 260 nm). Fractions 260-360 yielded 9000 $OU_{260}$ (36%) of the dinucleotide (VII), $R_{dpT}$ 1.20, $\lambda_{max}$ 261 nm, $\lambda_{min}$ 233 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.75, $\varepsilon_{270}/\varepsilon_{260}$ 0.90, $\varepsilon_{280}/\varepsilon_{260}$ 0.72, $\varepsilon_{290}/\varepsilon_{260}$ 0.43. The recovery of dpT was 35% (fractions 100-156) and of $dpG^{iBu}$ 40%.

The ammonolysis of (VII) gave dpTpG, $R_{dpT}$ 0.65, $\lambda_{max}$ 259 nm, $\lambda_{min}$ 232 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.94, $\varepsilon_{270}/\varepsilon_{260}$ 0.92, $\varepsilon_{280}/\varepsilon_{260}$ 0.71, $\varepsilon_{290}/\varepsilon_{260}$ 0.28; nucleotide composition dpT : dpG 1.00 : 1.10.

8. $dpTpG^{iBu}pG^{iBu}$ (X) was obtained by the reaction of 0.65 g (0.67 mmole) of $dCEpTpG^{iBu}$ and 1.07 g (1.9 mmole) of $dpG^{iBu}$-iBu under the conditions of expt. 6A (2 h at 20°C). The mixture was chromatographed on a column of DEAE-cellulose ($HCO_3^-$, 2×40 cm, 4.5 liters of 0.05 M–4.5 liters of 0.4 M TEAB in 30% ethanol, 18-ml fractions/10 min, absorption at 270 nm). Fractions 404-480 yielded 6000 $OU_{270}$ (26%) of the trinucleotide (X), $\lambda_{max}$ 258 nm, $\lambda_{min}$ 235 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.87, $\varepsilon_{270}/\varepsilon_{260}$ 0.86, $\varepsilon_{280}/\varepsilon_{260}$ 0.70, $\varepsilon_{290}/\varepsilon_{260}$ 0.45. The recovery of $dpG^{iBu}$ was 6% (fractions 290-318) and of $dpTpG^{iBu}$ 10% (fractions 336-386).

Ammonolysis yielded dpTpGpG, $R_{dpT}$ 0.49, $\lambda_{max}$ 257 nm, $\lambda_{min}$ 230 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.97, $\varepsilon_{270}/\varepsilon_{260}$ 0.85, $\varepsilon_{280}/\varepsilon_{260}$ 0.65, $\varepsilon_{290}/\varepsilon_{260}$ 0.34; nucleotide composition dpT : dpG 1.00 : 1.95.

9. $dpC^{An}pG^{iBu}pTpG^{iBu}$ (IX) was obtained by the reaction of 180 mg (0.18 mmole) of $dCEpC^{An}pG^{iBu}$, 230 mg (0.24 mmole) of $dpTpG^{iBu}$-Ac, and 250 mg (1.4 mmole) of mesitylenesulfonyl chloride (3 h at 20°C). The reaction mixture was chromatographed on a column of DEAE-cellulose ($HCO_3^-$, 1.5×40 cm, 2 liters of 0.05 M–2 liters of 0.4 M TEAB in 10% ethanol), with the collection of 7-ml fractions/10 min and the measurement of the absorption at 260 nm. Fractions 280-350 yielded 930 $OU_{260}$ (11%) of the tetranucleotide (IX), $R_{dpT}$ 0.80, $\lambda_{max}$ 260, 275 (shoulder) nm, $\lambda_{min}$ 235 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.84, $\varepsilon_{270}/\varepsilon_{260}$ 0.92, $\varepsilon_{280}/\varepsilon_{260}$ 0.88, $\varepsilon_{290}/\varepsilon_{260}$ 0.77. The recovery of $dpTpG^{iBu}$ was 55% (fractions 118-180) and of $dpC^{An}pG^{iBu}$ 22% (fractions 181-240).

The ammonolysis of (IX) yielded dpCpGpTpG, $R_{dpT}$ 0.60, $\lambda_{max}$ 256 nm, $\lambda_{min}$ 235 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.96, $\varepsilon_{270}/\varepsilon_{260}$ 0.94, $\varepsilon_{280}/\varepsilon_{260}$ 0.72, $\varepsilon_{290}/\varepsilon_{260}$ 0.30; nucleotide composition dpT : dpC : dpG 1.00 : 1.00 : 1.94.

10. $dpC^{An}pG^{iBu}pTpG^{iBu}pG^{iBu}$ (XI). A. Compound (XI) was obtained by the reaction of 900 $OU_{260}$ (0.017 mmole) of $dCEpC^{An}pG^{iBu}pTpG^{iBu}$ and 2800 $OU_{260}$ (0.17 mmole) of $pdG^{iBu}$-iBu in the presence of 100 mg (0.5 mmole) of mesitylenesulfonyl chloride in 1 ml of pyridine (4 h at 20°C). Chromatography was performed on a column of DE-23 cellulose ($HCO_3^-$, 1×35 cm) in a linear concentration gradient of TEAB in 10% ethanol (0.7 liter of 0.05 M–0.7 liter of 0.35 M), at a rate of elution of 30 ml/h and with a fraction volume of 5 ml (Fig. 2). The yield of pentanucleotide (XI) was 200 $OU_{260}$ (0.003 mmole, 17%), $R_{dpT}$ 0.60, $\lambda_{max}$ 259, 275 (shoulder) nm, $\lambda_{min}$ 228 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.85, $\varepsilon_{270}/\varepsilon_{260}$ 0.88, $\varepsilon_{280}/\varepsilon_{260}$ 0.83, $\varepsilon_{290}/\varepsilon_{260}$ 0.69. The recovery of $dpG^{iBu}$ was 45% and of the tetranucleotide (IX) 27%.

The ammonolysis of (XI) gave the unprotected pentanucleotide (XII), $R_{dpT}$ 0.25, $\lambda_{max}$ 256, 272 (shoulder) nm, $\lambda_{min}$ 235 nm, $\varepsilon_{250}/\varepsilon_{260}$ 1.03, $\varepsilon_{270}/\varepsilon_{260}$ 0.88, $\varepsilon_{280}/\varepsilon_{260}$ 0.68, $\varepsilon_{290}/\varepsilon_{260}$ 0.32; nucleotide composition dC : dpT : dpG 1.10 : 1.00 : 3.20.

B. Compound (XI) was obtained by the reaction of 2000 $OU_{270}$ (0.055 mmole) of $dCEpC^{An}pG^{iBu}pT$ ($PyH^+$) and 4000 $OU_{260}$ (0.12 mmole) of $dpG^{iBu}pG^{iBu}$-Ac($Et_3NH^+$) under the conditions of expt. 9. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 1.5×51 cm, 1.5 liter of 0.05 M–1.5 liter of 0.35 M TEAB in 10% ethanol, 13-ml fractions/15 min). Fractions 100-190 yielded a mixture of the pentanucleotide (XI) and the initial trinucleotide (VIII) [recovery of the dinucleotide (I) 52%]. This mixture was

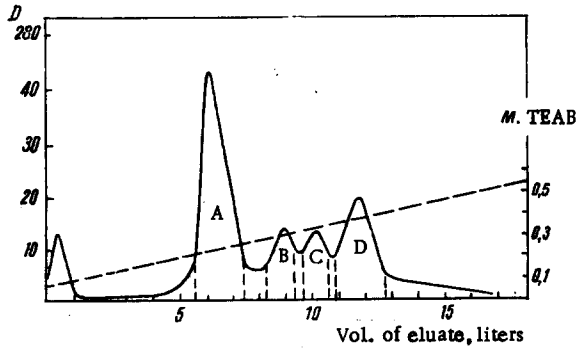



Fig. 3. Isolation of $dpC^{An}pC^{An}pA^{Bz}pC^{An}pG^{iBu}$ (XVII) (expt. 15B): peak A contains 48,700 $OU_{280}$ of the dinucleotide (VI); peak B 14,300 $OU_{280}$ of the trinucleotide (XV); peak C 15,200 $OU_{280}$ of the unidentified substance; peak D 21,000 $OU_{280}$ of the pentanucleotide (XVII).

rechromatographed (column 1.5×25 cm, 0.75 liter of 0.15 M–0.75 liter of 0.35 M TEAB in 10% ethanol, 4.5-ml fractions). Fractions 110-270 yielded 900 $OU_{260}$ (20%) of the pentanucleotide (IX).

C. Compound (XI) was obtained by the reaction of 3440 $OU_{270}$ (0.13 mmole) of $dCEpC^{An}pG^{iBu}$ and 4470 $OU_{270}$ (0.13 mmole) of $dpTpG^{iBu}pG^{iBu}$-Ac under the conditions of expt. 9; yield 37%.

11. $dpC^{An}pC^{An}$ (XIII). A. This was obtained by the reaction of 1.2 g (2.2 mmoles) of $dCEpC^{An}$ and 2.0 g (3.6 mmoles) of $dpC^{An}$-Ac under the conditions of expt. 1. Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 4.5×90 cm, 5 liters of 0.05 M–5 liters of 0.35 M TEAB in 10% ethanol, 20-ml fractions/12 min). Fractions 350-450 yielded 74,000 $OU_{302}$ (76%) of the dinucleotide (XIII), $\lambda_{max}$ 302 nm, $\lambda_{min}$ 238 nm (see [1]). The recovery of $dpC^{An}$ was 9% (fractions 150-225).

B. The condensation of 1.6 g (2.8 mmoles) of $dCEpC^{An}$ with 1.3 g (2.3 mmoles) of $dpC^{An}$-Ac was carried out similarly with the aid of 2.45 g (11.5 mmoles) of mesitylenesulfonyl chloride in 15 ml of pyridine (4 h at 20°C). Chromatography was performed on a column of DEAE-Sephadex ($HCO_3^-$, 3.5×50 cm, 5 liters of 0.07 M–5 liters of 0.55 M TEAB in 10% ethanol, 19-ml fractions/10 min). Fractions 300-490 yielded 60,000 $OU_{302}$ (58%) of the dinucleotide (XIII). The recovery of $dpC^{An}$ was 13% (fractions 180-250).

12. $dpC^{An}pC^{An}pA^{Bz}$ (XV). Compound (XV) was obtained from 2.25 g (2.0 mmoles) of $dCEpC^{An}pG^{An}$ and 2.0 g (3.6 mmoles) of $dpA^{Bz}$-Ac in the presence of 4.9 g (16.5 mmoles) of triisopropylbenzenesulfonyl chloride in 20 ml of pyridine (4.5 h at 25°C) (see expt. 1). Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 4×60 cm, 5 liters of 0.05 M–5 liters of 0.35 M TEAB in 15% ethanol, 18-ml fractions/11 min, absorption at 280 nm). Fractions 430-540 yielded 47,000 $OU_{280}$ (45%) of the trinucleotide (XV), $R_{dpT}$ 0.81, $\lambda_{max}$ 287 nm, $\lambda_{min}$ 237 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.86, $\varepsilon_{270}/\varepsilon_{260}$ 1.21, $\varepsilon_{280}/\varepsilon_{260}$ 1.49, $\varepsilon_{290}/\varepsilon_{260}$ 1.61, $\varepsilon_{300}/\varepsilon_{260}$ 1.40. Fractions 556-640 yielded 13,300 $OU_{302}$ of an unidentified substance $R_{dpT}$ 1.32, $\lambda_{max}$ 302 nm, $\lambda_{min}$ 238 nm. The recovery of $dpA^{Bz}$ was 62% (fractions 71-170) and of the dinucleotide (XIII) 20% (fractions 300-425).

The ammonolysis of (XV) yielded dpCpCpA, $R_{dpT}$ 0.59, $\lambda_{max}$ 264 nm, $\lambda_{min}$ 227 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.83, $\varepsilon_{270}/\varepsilon_{260}$ 0.93, $\varepsilon_{280}/\varepsilon_{260}$ 0.57, $\varepsilon_{290}/\varepsilon_{260}$ 0.16, nucleotide composition dpC : dpA 1.93 : 1.00.

13. $dpA^{Bz}pC^{An}$ (XIV). A. This was obtained from 2.45 g (4.3 mmoles) of $dCEpA^{Bz}$ and 2.5 g (4.4 mmoles) of $dpC^{An}$-Ac under the conditions of expt. 9 (5 h at 20°C). Chromatography was performed on a column of DEAE-Sephadex ($HCO_3^-$, 3×35 cm, 5 liters of 0.05 M–5 liters of 0.3 M TEAB in 10% ethanol, 25-ml fractions/16 min, absorption at 280 nm). Fractions 272-344 yielded 46,300 $OU_{280}$ (30%) of the dinucleotide (XIV), $R_{dpT}$ 1.05, $\lambda_{max}$ 285 nm, $\lambda_{min}$ 240 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.91, $\varepsilon_{270}/\varepsilon_{260}$ 1.18, $\varepsilon_{290}/\varepsilon_{260}$ 1.54, $\varepsilon_{290}/\varepsilon_{260}$ 1.58, $\varepsilon_{300}/\varepsilon_{260}$ 1.33. The total recovery of $dpC^{An}$ and $dpA^{Bz}$ was 24%.

Ammonolysis gave dpApC, $R_{dpT}$ 0.70, $\lambda_{max}$ 262 nm, $\lambda_{min}$ 236 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.74, $\varepsilon_{270}/\varepsilon_{260}$ 0.80, $\varepsilon_{280}/\varepsilon_{260}$ 0.36, nucleotide composition dpA : dpC 1.00 : 1.08.

B. Compound (XIV) was obtained by the condensation of 1.05 g (1.85 mmole) of $dCEpA^{Bz}$ and 1.55 g (2.8 mmoles) of $dpC^{An}$-Ac by the procedure of expt. 5B. Chromatography was performed as in experiment

13A (column 2.5×45 cm, 35-ml fractions/24 min). Fractions 155-195 yielded 23,500 $OU_{280}$ (36%) of the dinucleotide (XIV). The recovery of $dpA^{Bz}$ was 44% (fractions 60-90) and of $dpC^{An}$ 47% (fractions 97-125).

14. $dpA^{Bz}pC^{An}pG^{iBu}$ (XVI) was obtained by the condensation of 1.05 g (1.0 mmole) of $dCEpA^{Bz}pC^{An}$ and 2.45 g (4.2 mmole) of $dpG^{iBu}$-iBu by the method of expt. 9 ( 5 h at 20°C). Chromatography was performed on a column of DEAE-Sephadex ($HCO_3^-$, 3×50 cm, 3 liters of 0.05 M−3 liters of 0.3 M and 3 liters of 0.3 M−3 liters of 0.4 M TEAB in 10% ethanol, 30-ml fractions/22 min, absorption at 280 nm). Fractions 268-340 yielded 14,000 $OU_{280}$ (28%) of the trinucleotide (XVI), $R_{dpT}$ 0.80, $\lambda_{max}$ 282 nm, $\lambda_{min}$ 238 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.86, $\varepsilon_{270}/\varepsilon_{260}$ 1.03, $\varepsilon_{280}/\varepsilon_{260}$ 1.18, $\varepsilon_{290}/\varepsilon_{260}$ 1.11, $\varepsilon_{300}/\varepsilon_{260}$ 0.85. The recovery of $dpG^{iBu}$ was 50% (fractions 88-120) and of the dinucleotide (IV) 16% (fractions 180-216).

The ammonolysis of (XVI) gave dpApCpG, $R_{dpT}$ 0.40, $\lambda_{max}$ 257 nm, $\lambda_{min}$ 228 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.93, $\varepsilon_{270}/\varepsilon_{260}$ 0.89, $\varepsilon_{280}/\varepsilon_{260}$ 0.58, $\varepsilon_{290}/\varepsilon_{260}$ 0.22, nucleotide composition dpA : dpC : dpG 100 : 1.06 : 0.97.

15. $dpC^{An}pC^{An}pA^{Bz}pC^{An}pG^{iBu}$ (XVII). A. Compound (XVII) was obtained by the condensation of 535 mg (13,000 $OU_{302}$, 0.5 mmole) of $dCEpC^{An}pC^{An}$ and 740 mg (26,000 $OU_{280}$, 0.5 mmole) of $dpA^{Bz}pC^{An}pG^{iBu}$-Ac in the presence of 0.39 g (1.8 mmole) of mesitylenesulfonyl chloride in 5 ml of pyridine (3 h at 20°C). Chromatography was performed on a column of DEAE-cellulose ($HCO_3^-$, 3.5×55 cm, 3.5 liters of 0.05 M−3.5 liters of 0.4 M TEAB in 30% ethanol, 12-ml fractions/10 min, absorption at 280 nm). Fractions 510-590 yielded 7800 $OU_{280}$ (21%) of the pentanucleotide (XVII). The recovery of $dpC^{An}pC^{An}$ was 44% (fractions 405-450) and of $dpA^{Bz}pC^{An}pG^{iBu}$ 34% (fractions 451-500).

The pentanucleotide (XVII) (2500 $OU_{280}$) was rechromatographed on a column of DEAE-Sephadex ($Cl^-$, 1.6×35 cm) in a linear concentration gradient of NaCl in 0.02 M tris-HCl (pH 7.5) in 7 M urea (1 liter of 0.02 M buffer and 1 liter of 0.25 M NaCl in the buffer), 6-ml fractions being collected every 12 min. The combined fractions 266-280 were diluted with water to a volume of 1200 ml and were deposited on a column of DEAE-cellulose ($HCO_3^-$, 1.2×45 cm) and then the column was washed with 0.05 M TEAB until the reaction for $Cl^-$ was negative, after which the substance was eluted with 120 ml of 1 M TEAB (pH 7.5). This gave 2280 $OU_{280}$ of the pentanucleotide (XVII), $R_{dpT}$ 0.43, $\lambda_{max}$ 287 nm, $\lambda_{min}$ 239 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.42, $\varepsilon_{270}/\varepsilon_{260}$ 1.10, $\varepsilon_{280}/\varepsilon_{260}$ 1.19, $\varepsilon_{290}/\varepsilon_{260}$ 1.20, $\varepsilon_{300}/\varepsilon_{260}$ 1.19.

Ammonolysis yielded the unprotected pentanucleotide (XVIII), $R_{dpT}$ 0.23, $\lambda_{max}$ 261 nm, $\lambda_{min}$ 228 nm, $\varepsilon_{250}/\varepsilon_{260}$ 0.91, $\varepsilon_{270}/\varepsilon_{260}$ 0.95, $\varepsilon_{280}/\varepsilon_{260}$ 0.66, $\varepsilon_{290}/\varepsilon_{260}$ 0.26, nucleotide composition dpC : dpA : dpG 2.94 : 1.00 : 1.01.

B. Compound (XVII) was obtained by the condensation of 2.0 g (1.3 mmole) of $dCEpC^{An}pC^{An}pA^{Bz}$ and 2.2 g (2.2 mmole) of $dpC^{An}pG^{IBu}$-Ac by the method of expt. 1.

Chromatography was performed on a column of DE-23 cellulose ($HCO_3^-$, 3.8×66 cm) in a linear concentration gradient of TEAB in 20% ethanol (9 liters of 0.05 M−9 liters of 0.6M) with a rate of elution of 80 ml/h and a fraction volume of 16 ml (Fig. 3). Yield: 21,000 $OU_{280}$ (19%); the recovery of the dinucleotide (XIII) was 65% and of the trinucleotide (XVI) 21%.

## SUMMARY

The chemical synthesis of three pentadeoxyribonucleotides – dpGpGpTpTpT, dpCpGpTpGpG and dpCpCpApCpG – corresponding to the 5'-terminal segment of yeast valine tRNA has been effected.

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