

## Template-directed Synthesis of Oligonucleotide. Part 3.<sup>1</sup> Condensation of Nucleotides in the Presence of Nucleic Acid Base-binding Cross-linked Poly(4-vinylpyridine)

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Cationic polynucleotide analogues such as derivatives of nucleic acid base-binding poly(4-vinylpyridine) are capable of interacting strongly with nucleotides. Thus, the cross-linked poly(4-vinylpyridine) containing adenine, [PVP]<sub>2</sub>-C<sub>2</sub>'-Ade<sub>61</sub>, showed considerable selectivity in interacting with the complementary nucleotide. The selective adsorption of nucleotides onto [PVP]<sub>2</sub>-C<sub>2</sub>'-Ade<sub>61</sub> have been studied together with the selective adsorption of the complementary nucleotide in water and in pyridine. Selectivities were 26 to 40% in water and 33 to 44% in pyridine. Using [PVP]<sub>2</sub>-C<sub>2</sub>'-Ade<sub>61</sub> as a template, template-directed non-enzymatic condensations of nucleotides were carried out in both water and pyridine. The total conversion was greater in pyridine than in water. Considerable amounts of complementary oligonucleotides with the template were obtained. The selectivity as a whole in the condensation depended on the selectivity in the adsorption.

THERE are two approaches to the non-enzymatic synthesis of oligonucleotides of definite sequence, stepwise and template-directed condensations of nucleotides. For the latter, two kinds of templates have been employed, polynucleotides<sup>2-13</sup> and synthetic neutral polynucleotide analogues.<sup>14</sup> Results of template-directed condensations of mononucleotides on polynucleotides show both a low percentage conversion and a low degree of polymerization. This is thought to result from coulombic repulsion in the interaction between the polynucleotide and the condensing nucleotide. A synthetic neutral polynucleotide analogue, poly(1-vinyluracil), poly(vU), has been used as the template and its efficiency as the template has been compared with that of polyuridylic acid, poly(U).<sup>13</sup> Generally, both were found to be equally effective although the former was more effective for a hydrolysing condensation of adenosine cyclic 2',3'-phosphate. Cross-linked polystyrene-supported neutral oligonucleotides having a definite sequence have also been used as the template, a considerable amount of partly complementary oligonucleotides being obtained.<sup>14</sup>

None of these reported templates have coulombic attractive interaction with the condensing nucleotide. Here, in contrast, we report template-directed condensations of nucleotides in the presence of polynucleotide analogues having a positive charge [*e.g.* nucleic acid base-binding cross-linked poly(4-vinylpyridine)].

### RESULTS AND DISCUSSION

Nucleic acid base-binding poly(4-vinylpyridine)s and their derivatives used in the work described are shown below. Suffix *n*, *m*, *p*, and *l* show the content of nucleic acid base moiety, the content of unsubstituted moiety, the degree of cross-linking, and the length of methylene chain, respectively.

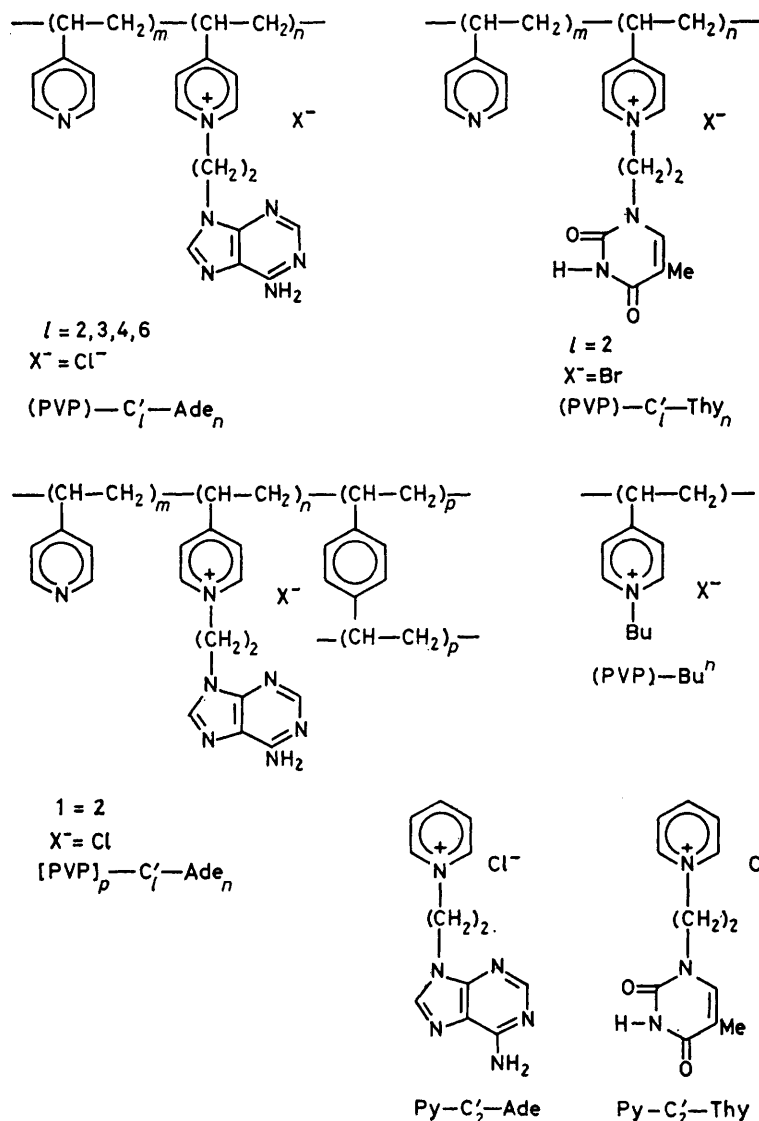
Interactions of linear poly(4-vinylpyridine)s having a nucleic acid base such as (PVP)-C<sub>l</sub>'-Ade<sub>n</sub> and (PVP)-

C<sub>l</sub>'-Thy<sub>n</sub> with nucleotides were remarkable even at relatively low concentration.<sup>1</sup> Table 1 shows maximum hypochromicities in curves of mixtures consisting of cationic polynucleotide analogues, (PVP)-C<sub>l</sub>'-Ade<sub>n</sub> or (PVP)-C<sub>l</sub>'-Thy<sub>n</sub>, and nucleotides. The polynucleotide analogue and the nucleotide ratio where the maximum hypochromicity was given, varied. In addition, purine-purine interaction of bases was stronger than that of purine-pyrimidine bases and pyrimidine-pyrimidine bases. On the other hand, hypochromicities in curves of the mixture consisting of (PVP)-Bu<sup>n</sup> and nucleotides were very low. This suggests that an interaction between the nucleotide and (PVP)-Bu<sup>n</sup> and an interaction between the nucleotides which are drawn toward (PVP)-Bu<sup>n</sup> are both disregarded. Further, no hypochromicity was observed in solutions of mixed cationic mononucleotide analogues such as Py-C<sub>2</sub>'-Ade or Py-C<sub>2</sub>'-Thy and nucleotides. Thus, it may be concluded that the interactions tabulated in Table 1 arise predominantly as a result of vertical stacking between nucleic acid base moieties under the influence of coulombic attraction between the polynucleotide analogues and the nucleotides. Similar observations have been reported for the interactions between polynucleotide analogues and polynucleotides such as poly(A), poly(U), or nucleic acid bases.<sup>15</sup> For mixtures consisting of poly(iminoethylene) or commercial poly(ethylenimine) having nucleic acid base and nucleotides<sup>16</sup> showed similar behaviour.

In contrast, the base-base interaction in the heterogeneous system resulted in a considerable complementary base-base interaction. Table 2 lists the affinity chromatographic results for nucleotides with the cross-linked adenine-containing poly(4-vinylpyridine), [PVP]<sub>2</sub>-C<sub>2</sub>'-Ade<sub>61</sub>. In both water and pyridine solvent systems, the total amount of adsorbed nucleotide on [PVP]<sub>2</sub>-C<sub>2</sub>'-Ade<sub>61</sub> was both nearly constant and independent of the nucleotide used. This implies that the amount of the adsorbed nucleotide depends on the total number of the quater-

nized pyridine moiety in  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ . The selectivity \* of Urd-5'-P, the complementary nucleotide, in

worth considering. Such phenomena have been observed in several heterogeneous systems.<sup>17-21</sup> In general,



the adsorption on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  is expressed by equations (1) and (2):

for a binary system

$$\frac{(\text{Urd-5'-P}) - (\text{Ado-5'-P})}{(\text{Urd-5'-P}) + (\text{Ado-5'-P})} \times 100 \quad (1)$$

for a ternary system

$$\frac{(\text{Urd-5'-P}) - \frac{1}{2}[(\text{Ado-5'-P}) + (\text{Cyd-5'-P})]}{(\text{Urd-5'-P}) + (\text{Ado-5'-P}) + (\text{Cyd-5'-P})} \times 100 \quad (2)$$

where the parentheses show the amount of each adsorbed nucleotide in the adsorption. The order of the addition of the nucleotide to  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  did not affect the selectivity. This means that the adsorption is reversible. In this affinity chromatography, even if simultaneous vertical stacking of bases is not excluded, the appearance of considerable complementary base-base interaction is

water interferes with the hydrogen-bonding. The tendency of heterogeneous  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  to inter-

\* For a binary system, a selectivity for  $n_1$  is defined as  $n_1 - n_2$ . To normalize the selectivity, a factor of  $1/(n_1 + n_2)$  should be multiplied. Then the selectivity,  $S$ , is expressed as

$$S = \frac{n_1 - n_2}{n_1 + n_2} \times 100$$

For a ternary system, a selectivity for  $n_1$  is defined as  $n_1 - \frac{1}{2}(n_2 + n_3)$ . To normalize the selectivity, a factor of  $1/(n_1 + n_2 + n_3)$  should be multiplied. Then the selectivity,  $S$ , is expressed as

$$S = \frac{n_1 - \frac{1}{2}(n_2 + n_3)}{n_1 + n_2 + n_3} \times 100$$

In general, when each  $n_m$  contributes in the same weight, the selectivity,  $S$ , for  $i$ th component in an  $m$  component system is expressed as

$$S = \frac{n_i - \frac{1}{m-1} \sum_{m \neq i} n_m}{\sum_m n_m} \times 100$$

where  $n_m$  shows the amount of  $m$ 'th component.

act with the complementary base is due to the hydrophobicity of a matrix formed by the cross-linked polymer.

The template-directed condensation of a nucleotide was carried out as follows using  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ .

of 2',5' and 3',5' linked oligomers. However, after 80% digestion the 3',5' linked oligomer had been predominantly formed, the remainder being the 2',5' linked compound. Further, chromatographic evidence

TABLE 1

Maximum hypochromicities in curves of mixtures consisting of polynucleotide analogues and nucleotides <sup>a</sup>

Analogue	Nucleotide	Hypochromicity(%)	Ratio <sup>b</sup>	Wavelength <sup>c</sup> (nm)	Concentration <sup>d</sup> (M)
(PVP)-C <sub>2</sub> '-Ade <sub>48</sub>	Ado-5'-P	11.8 ± 0.2	1 : 0.6	260	4.8 × 10 <sup>-4</sup>
	Guo-5'-P	7.5 ± 0.2	1 : 0.5	255	4.8 × 10 <sup>-4</sup>
	Cyd-5'-P	6.7 ± 0.2	1 : 2.2	265	4.8 × 10 <sup>-4</sup>
	Urd-5'-P	5.4 ± 0.2	1 : 2.5	260	4.8 × 10 <sup>-4</sup>
	ATP	ppt.	—	—	4.8 × 10 <sup>-4</sup>
(PVP)-C <sub>3</sub> '-Ade <sub>66</sub>	Ado-5'-P	9.8 ± 0.3	1 : 1.1	260	5.7 × 10 <sup>-4</sup>
	Cyd-5'-P	6.9 ± 0.3	1 : 0.8	265	5.7 × 10 <sup>-4</sup>
	Urd-5'-P	6.0 ± 0.3	1 : 0.6	260	5.7 × 10 <sup>-4</sup>
	Ado-5'-P	9.7 ± 0.3	1 : 1.3	260	5.7 × 10 <sup>-4</sup>
(PVP)-C <sub>4</sub> '-Ade <sub>39</sub>	Cyd-5'-P	7.0 ± 0.3	1 : 0.6	265	5.7 × 10 <sup>-4</sup>
	Urd-5'-P	6.1 ± 0.3	1 : 0.4	260	5.7 × 10 <sup>-4</sup>
	Ado-5'-P	10.0 ± 0.3	1 : 1.1	260	5.7 × 10 <sup>-4</sup>
(PVP)-C <sub>6</sub> '-Ade <sub>47</sub>	Cyd-5'-P	7.0 ± 0.3	1 : 0.8	265	5.7 × 10 <sup>-4</sup>
	Urd-5'-P	5.9 ± 0.3	1 : 0.4	260	5.7 × 10 <sup>-4</sup>
	Ado-5'-P	10.9 ± 0.4	1 : 0.8	263	6.7 × 10 <sup>-5</sup>
	Guo-5'-P	9.0 ± 0.4	1 : 4.0	259	6.7 × 10 <sup>-5</sup>
(PVP)-C <sub>2</sub> '-Thy <sub>100</sub>	Cyd-5'-P	4.3 ± 0.4	1 : 1.0	269	6.7 × 10 <sup>-5</sup>
	Urd-5'-P	5.3 ± 0.4	1 : 0.4	264	6.7 × 10 <sup>-5</sup>
	Ado-5'-P	1.5 ± 0.2	1 : 1.0	260	6.7 × 10 <sup>-4</sup>
	Urd-5'-P	0.6 ± 0.2	1 : 1.0	260	6.7 × 10 <sup>-4</sup>
(PVP)-Bu <sup>n</sup>	Ado-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>
	Urd-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>
Py-C <sub>2</sub> '-Ade	Ado-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>
	Urd-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>
Py-C <sub>2</sub> '-Thy	Ado-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>
	Urd-5'-P	0	—	—	6.7 × 10 <sup>-4</sup>

<sup>a</sup> Disodium salts of nucleotides were used. <sup>b</sup> This shows the ratio of the polynucleotide analogue and nucleotide where the maximal hypochromicity was given. <sup>c</sup> This shows the wavelength where the hypochromicity was calculated. <sup>d</sup> Concentration shows total concentration of nucleic acid bases in the mixture.

$[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  was allowed to adsorb nucleotides (pyridinium form) in pyridine and was then dried, suspended in solvents, and treated with carbodi-imides. The results are tabulated in Table 3. Total conversions

showed little evidence for the presence of an oligomer linked through the heterocyclic base. The selectivity of Urd-5'-P in the condensation on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  is expressed by equation (3) where (Urd-5'-P) represents

TABLE 2

Selective adsorption of nucleotide on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ <sup>a</sup>

Nucleotide (mmol)	H <sub>2</sub> O (ml)	Adsorbed nucleotide (mmol)	Selectivity <sup>b</sup> (%)
Ado-5'-P (0.50)	1.0	Ado-5'-P (0.19)	
Urd-5'-P (0.50)	1.0	Urd-5'-P (0.17)	
Ado-5'-P (0.50) Urd-5'-P (0.50)	1.0	Ado-5'-P (0.07) Urd-5'-P (0.12)	26.3
[Ado-5'-P (0.50) Urd-5'-P (0.50)]	2.0	[Ado-5'-P (0.03) Urd-5'-P (0.12)]	40.0
Cyd-5'-P (0.50)		Cyd-5'-P (0.05)	
Pyridine (ml)			
Ado-5'-P (0.50)	1.0	Ado-5'-P (0.20)	
Urd-5'-P (0.50)	1.0	Urd-5'-P (0.20)	
Ado-5'-P (0.50) Urd-5'-P (0.50)	1.0	Ado-5'-P (0.08) Urd-5'-P (0.16)	33.3
[Ado-5'-P (0.50) Urd-5'-P (0.50)]	1.0	[Ado-5'-P (0.40) Urd-5'-P (0.15)]	43.8
Cyd-5'-P (0.50)		Cyd-5'-P (0.05)	

<sup>a</sup>  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ ,  $2.5 \times 10^{-4}$  mol eq.; Column, 2 mm i.d., Eluant, 20 ml. <sup>b</sup> See the text.

were relatively low, however, the complementary nucleotide moiety, Urd-5'-P, being found in the products in larger amounts than other nucleotide moieties. In addition, the complementary homogeneous dinucleotide, pUpU, was obtained in considerably larger amounts than other dinucleotides such as pApA and pCpC. In pyridine, a considerable amount of the complementary homogeneous trinucleotide, pUpUpU, was obtained. When the reaction products were treated with snake venom phosphodiesterase, *ca.* 80% of these products were digested to some components. From literature precedents<sup>9,12</sup> the major product expected using unprotected ribonucleoside 5'-phosphate, would be a mixture

the amount of Urd-5'-P moiety found in the products. As shown in Table 3, the selectivity in the adsorption

$$\frac{(\text{Urd-5'-P})}{(\text{Total Product})} \times 100 \quad (3)$$

has a considerable effect on the selectivity in the condensation. This shows the directive influence of  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  on these condensation reactions. Further, more of the complementary nucleotide moiety, Urd-5'-P, was formed in the pyridine system than in water system.

Taking account of the accuracy in the analysis, pyridine seems to stabilize the complementary interaction between  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  and the nucleotide.

Thus the relatively higher selectivity in the condensation compared with that in the adsorption, even in the water system, suggests that the hydrophobic matrix of the  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  has a tendency to reject water molecules and stabilize the adsorption selectivity. On the other hand, since in the course of the condensation reaction, nucleotides adsorbed on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  will be desorbed in part, the desorption rate of the non-

up to 6 h at 5 °C, until no further optical density change occurred. U.v. measurements were carried out on a Hitachi EPS-3T spectrophotometer at 15 °C. The hypochromicity

$$\text{Hypochromicity (\%)} = \left(1 - \frac{I_{a+b}}{mI_a + nI_b}\right) \times 100 \quad (4)$$

was calculated according to equation (4) where  $m$  and  $n$  are volume fractions of the solutions a and b, and  $I_a$ ,  $I_b$ , and

TABLE 3  
Condensation of nucleotide adsorbed on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$

No.	Nucleotide adsorbed on $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ (mmol)	Condensation reagent	Solvent	Main product <sup>a</sup> (μmol)	Conversion (%)	Selectivity <sup>b</sup> (%)
1	Ado-5'-P (0.08) Urd-5'-P (0.16)	EDC	Water	EDC-pU (2.35) EDC-pA (1.07) pUpU (2.35) pUpA (0.55) pApU (0.47)	4.2	47.3
2	Ado-5'-P (0.08) Urd-5'-P (0.16)	DCC	Pyridine	DCC-pU (1.88) DCC-pA (1.07) pUpU (4.71) pUpUpU (0.78) pApA (1.33) pUpA (0.42) pApU (0.42)	7.9	56.4
3	Ado-5'-P (0.04) Urd-5'-P (0.15) Cyd-5'-P (0.05)	EDC	Water	EDC-pU (1.88) EDC-pA (0.53) EDC-pC (0.80) pUpU (2.82) pApA (0.80) pCpC (0.68) pUpA (0.30) pApU (0.21) pUpC (0.38) pCpU (0.27) pApC (0.16) pCpA (0.16)	6.2	38.2
4	Ado-5'-P (0.04) Urd-5'-P (0.15) Cyd-5'-P (0.05)	DCC	Pyridine	DCC-pU (1.88) DCC-pA (0.53) DCC-pC (0.80) pUpU (9.18) pUpUpU (1.25) pApA (2.00) pCpC (0.39) pUpA (0.42) pApU (0.42) pUpC (0.65) pCpU (0.65) pApC (0.40) pCpA (0.40)	15.0	58.9

<sup>a</sup> EDC-pU, EDC-pA, EDC-pC, DCC-pU, DCC-pA, and DCC-pC are adducts of nucleotides with the condensing reagents, respectively. Any product which is not cited in the Table, is negligible compared with the main products. <sup>b</sup> See the text.

complementary nucleotide might be higher than that of the complementary one.

These results suggest the occurrence of condensation of nucleotides on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ . In addition, the simultaneous reaction between the nucleotides on  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$  and the nucleotides desorbed was not excluded. It is concluded that a template-directed condensation of nucleotide took place. And this implies a promising prospect for the use of this material as a template molecule in the template synthesis of oligonucleotide.

#### EXPERIMENTAL

*U.v. Spectroscopic Measurement.*—Standardized solutions of the polynucleotide analogues, nucleotides, and nucleosides were mixed in varying proportions. The solutions were not buffered. The mixtures were stored for periods of

$I_{a+b}$  are absorbances of solutions of a, b, and the mixture solution, respectively.

*Materials.*—Poly(4-vinylpyridine) *N*-alkylated with  $\omega$ -halogenoalkylated nucleic acid bases,  $(\text{PVP})\text{-C}_1'\text{-Ade}_n$ ,  $(\text{PVP})\text{-C}_1'\text{-Thy}_n$ , cross-linked poly(4-vinylpyridine-co-divinylbenzene) *N*-alkylated with 9-(2-chloroethyl)adenine,  $[\text{PVP}]_2\text{-C}_2'\text{-Ade}_{61}$ , poly(1-butyl-4-vinylpyridinium bromide),  $(\text{PVP})\text{-Bu}_n$ , 1-[2-(adenin-9-yl)ethyl]pyridinium chloride,  $\text{Py-C}_2'\text{-Ade}$ , and 1-[2-(thymine-1-yl)ethyl]pyridinium chloride,  $\text{Py-C}_2'\text{-Thy}$ , described elsewhere, were used.

*Enzymes.*—Snake venom phosphodiesterase (SV) and beef spleen phosphodiesterase (BS) were purchased from Nutritional Biochemicals Corp. and wheat germ acid phosphatase (WG) was purchased from Worthington Biochemical Corp.

*Condensation of Nucleotide.*—Nucleotides were converted into the pyridinium form by Dowex 50 (pyridinium form) resin before use.



**Condensation of Nucleotide adsorbed on  $[PVP]_2\text{-C}_2'\text{-Ade}_{61}$ .**—Nucleotides (pyridinium form) were adsorbed on  $[PVP]_2\text{-C}_2'\text{-Ade}_{61}$  in pyridine and washed with pyridine, as stated above. The nucleotide-containing  $[PVP]_2\text{-C}_2'\text{-Ade}_{61}$  was completely dried after which 150 mg were suspended in 5 ml of water and 5 ml of pyridine, respectively. In total 0.5 g of condensing reagent, 1-ethyl-3-(3-dimethylaminopropyl)carbodi-imide (EDC) (for water system) and  $NN'$ -dicyclohexylcarbodi-imide (DCC) (for pyridine system) were added to the suspensions. The mixtures were allowed to react with stirring at a temperature of 5 °C. Every 20 h, 0.125 g of the condensing reagent was added. After 50 h, the solvent was removed under reduced pressure, and 20 ml of 1M-NaCl was added, heated to 70 °C for 5 min, and then filtered. (Almost all of the nucleotide material was removed.) The filtrate was collected and dried and treated with a small amount of acetic acid; the resulting precipitate was filtered off and the filtrate collected and analysed.

**Analyses of Condensation Products.**—The filtrate which contained the condensing products was subjected to paper chromatography, electrophoresis, and liquid chromatography. Product spots from both the paper chromatography and the electrophoresis were cut out and eluted by soaking for 24 h in 20% ethanol-water. The solutions so obtained were concentrated and analysed by the following procedure. One aliquot was subjected to liquid chromatography and another to u.v. spectrophotometry in order to identify the species of nucleic acid base moieties present. A further aliquot was degraded with a small amount of an aqueous solution of SV preparation (0.01 mg/ml) for 12 h at 38 °C in 0.3M-Tris buffer (pH 8.8) contained 0.1M-MgCl<sub>2</sub>. The resulting solution was subjected to paper chromatography in order to identify both the species and the quantity present. The direction of the linkage in the heterogeneous dinucleotides, (pA,pC), (pA,pU), and (pC,pU), were analysed by the following procedure. The heterogeneous dinucleotide was incubated in 0.2 ml of 0.15M-sodium acetate buffer (pH 5.4) with 0.2 ml of WG preparation (0.1 mg/ml), at 38 °C for 12 h. The resulting solution was applied on the top of Sephadex G-25 (coarse) column (70 × 1.2 cm i.d.) and was eluted with 0.02M-ammonium hydrogencarbonate solution. The elute was lyophilized. The lyophilized material was degraded with BS preparation (0.01 mg/ml) for 12 h in 0.1M-citrate buffer (pH 6.5) at 38 °C and the resulting solution was concentrated and subjected to paper chromatography.

**General Methods of Analysis of Products.**—Paper chromatography was performed by the descending technique using Whatman 3 MM paper. The solvent system used was ethanol-1M-ammonium acetate (pH 6.7) (5 : 2) :  $R_F$  values were pUpU (0.40), pApA (0.25), pCpC (0.31), (pA,pU) (0.30), (pC,pU) (0.36), (pA,pC) (0.28), pUpUpU (0.41), EDC-pU, -pA, -pC (ca. 0.80), and DCC-pU, -pA, -pC (ca. 0.90), respectively. Errors were of the order of a few percent.

U.v. spectra were determined with a Hitachi EPS-3T recording spectrophotometer.

Paper electrophoreses (e.p.) were carried out using a Savant flat-plate type electrophoresis apparatus at a potential of 30 V/cm with 1/15M-phosphate buffer (pH 7.0) on Whatman 3 MM paper for ca. 100 min. Electrophoretic mobilities to anode were relative to that of Urd-5'-P: pUpU (1.05), pApA (0.95), pCpC (1.03), (pA,pU) (0.90), (pC,pU) (1.09), (pA,pC) (1.01), pUpUpU (1.04), EDC-pU, -pA, and -pC (0.28), and DCC-pU, -pA, and -pC (0.44), respectively.

Liquid chromatography (l.c.) was carried out with a Shimadzu-Dupont 830 column on Permaphase AAX, by an exponential gradient of 0.002M-KH<sub>2</sub>PO<sub>4</sub> (pH 3.2) to 0.5M-KH<sub>2</sub>PO<sub>4</sub> (pH 4.5) at the gradient rate of 3%/min. Retention volume (ml) were: pUpU (10.1), pApA (8.5), pCpC (7.9), pUpUpU (18.5), EDC-pU (1.4), EDC-pA (1.1), EDC-pC (0.9), DCC-pU (2.2), DCC-pA (2.0), and DCC-pC (1.7), respectively.

At every measurement of the optical density ( $A_{260}$ ), appropriate blanks were cut from other areas of the chromatograms and soaked in the same solvent. The errors inherent in the paper chromatography and in the electrophoresis are of the order of 20%. Conversions of products are based on the assumption that the u.v. absorption of the products are the same as u.v. absorptions of constituent nucleotides.

Structures of adducts of the condensing reagents to nucleotides, EDC-pU, EDC-pA, EDC-pC, DCC-pU, DCC-pA, and DCC-pC were not determined. However, there is some evidence that those materials are the adducts of nucleotides, with the condensing reagents as follows:  $\epsilon_{\text{max}}/\epsilon_{\text{min}}$  were slightly larger than those of the corresponding nucleotides; mobilities in electrophoresis were smaller than those of the corresponding nucleotides: some materials reduced by NaBH<sub>4</sub> showed similar  $\epsilon_{\text{max}}/\epsilon_{\text{min}}$  values to those of the corresponding nucleotides. Materials treated with 2M-NaOH or 0.1M-HCl were identified by the corresponding nucleotides. Since EDC-pU, -pA, and -pC were difficult to separate by paper chromatography and electrophoresis, liquid chromatography was adopted. The assignment of peaks in the chromatography was done with a product in the condensation of a single corresponding nucleotide component system.

**Experiment 1 in Table 3.**—pUpU and pApA which showed the same retention times as authentic compounds when subjected to liquid chromatography, were digested with SV in ca. 80%, to give, respectively, pU and pA as the sole products. The heterogeneous dinucleotide consisting of pU and pA, (pU,pA), was digested with SV to give pU and pA in a roughly equal quantities. WG pre-treated (pU,pA) was digested with BS to give U, A, Up, and Ap in a ratio of 1 : 1.3 : 1.2 : 1.

**Experiment 2 in Table 3.**—Analyses of pUpU, pUpUpU, and pApA, achieved by the digestion with SV, gave pU or pA as the sole products. The ratio of pUpU and pUpUpU was determined by liquid chromatography since the two spots obtained by paper chromatography were incompletely separated. (pU,pA) was digested with SV to give pU and pA in a roughly equal quantities. WG pre-treated (pU,pA) was digested with BS to give similar quantities of U, A, Up, and Ap.

**Experiment 3 in Table 3.**—The analytical procedure and results for pUpU and pApA were similar to those described above. The mixture of pCpC and (pU,pA) was digested with SV to give pC, pU, and pA roughly in the ratio 8 : 3 : 3. Heterogeneous dinucleotides consisting of the corresponding nucleotide, (pU,pC), and (pA,pC), were digested with SV to give similar amounts of the corresponding nucleotides. A WG pre-treated mixture of pCpC and (pU,pA) ( $R_F = 0.3$ ) was digested with BS to give U, A, C, Up, Ap, and Cp in a ratio of 1 : 1.3 : 3.3 : 1.3 : 1.1 : 2.9. WG pre-treated (pU,pC) was digested with BS to give U, C, Up, and Cp in a ratio of 1 : 1.4 : 1.2 : 0.9. WG pre-treated (pA,pC) was digested with BS to give similar quantities of A, C, Ap, and Cp.

**Experiment 4 in Table 3.**—The analytical procedure and

results for pUpU, pApA, (pU,pC), (pA,pC) and a mixture of pCpC and (pU,pA) were similar to those described above. The ratio of pUpU and pUpUpU was determined by liquid chromatography. A WG pre-treated mixture of pCpC and (pU,pA) ( $R_F = 0.3$ ) was digested with BS to give U, A, C, Up, Ap, and Cp in a ratio of 1 : 1 : 1 : 0.9 : 0.9 : 0.8. WG pre-treated (pU,pC) was digested with BS to give U, C, Up, and Cp in a ratio of 1 : 1.1 : 0.9 : 0.9. WG pre-treated (pA,pC) was digested with BS to give A, C, Ap, and Cp in a ratio of 1 : 1 : 0.9 : 0.9.

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