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Synthesis of Trinucleoside Diphosphates with Polynucleotide Phosphorylase*

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ABSTRACT: Highly purified, primer-requiring preparations of polynucleotide phosphorylase have been used to synthesize triribonucleoside diphosphates of specific base composition and sequence. In this procedure, a particular nucleoside 5′-diphosphate is treated with a dinucleoside monophosphate under conditions favoring the synthesis of trinucleoside diphosphate rather than long-chain polymers.

The synthesis and characterization of fourteen of the sixty-four possible common trinucleoside diphosphates are reported. The method appears to be completely versatile.

In this paper we would like to report a general enzymic method for the synthesis of trinucleoside diphosphates of known sequence. The procedure is based on earlier work (Singer *et al.*, 1960a,b) which demonstrated that polynucleotide phosphorylase can add one or more nucleotide residues (from a nucleoside 5′-diphosphate) onto a suitable preformed oligonucleotide chain. Oligonucleotides such as pApApApU[1] and pApApApGpG were prepared and characterized.

The reaction we have used can be described as follows:

$$\mathrm{XpY} + \mathrm{NDP} \xrightarrow[\text{phosphorylase}]{\text{polynucleotide}} \mathrm{XpYpN} + \mathrm{P_i} \quad (1)$$

In equation (1), XpY is a dinucleoside monophosphate, NDP a nucleoside 5′-diphosphate, and XpYpN the trinucleoside diphosphate product. The procedure is general in that any given trinucleoside diphosphate can be synthesized; however, certain specific conditions are necessary in particular cases. The success of reaction (1) in synthesizing reasonable yields of specific trinucleoside diphosphates depends on several factors. (1) Highly purified polynucleotide phosphorylase from *Micrococcus lysodeikticus* (Singer and Guss, 1962; Singer and O'Brien, 1963) has an absolute requirement for an oligonucleotide primer for the synthesis of polyribonucleotides under the conditions used. (2) Dinucleoside monophosphates, as well as dinucleotides, can serve as primers for the phosphorylase (Singer *et al.*, 1960a; Thach *et al.*, 1964). (3) Dinucleoside monophosphates are not subject to phosphorolytic cleavage by the phosphorylase (Singer, 1958). Therefore rearrangement of the nucleotide sequence of the primer either by breakdown and resynthesis or by transnucleotidation (Singer *et al.*, 1959) is not a problem. (4) Conditions which limit long-chain polymer synthesis and thereby permit the accumulation of short-chain products are required. Such conditions have been studied further and are reported in this paper.

The need for a method such as described in this paper arose from recent work on the genetic code. A rapid, sensitive method for measuring the m-RNA–

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[1] Abbreviations: A, U, G, and C represent the nucleoside residues adenosine, uridine, guanosine, and cytidine, respectively. For polyribonucleotides of specific structure the letter p to the left of the nucleoside initial indicates a 5′-phosphate, the letter p to the right, a 3′-phosphate (see Heppel *et al.*, 1957). Thus, ApG is adenylyl-(3′,5′)-guanosine, and pApG is 5′-*O*-phosphoryladenylyl-(3′,5′)-guanosine. An A_{260} unit is the amount of material giving an absorbance of 1.0 in 1.0 ml of solution in a 1-cm light path. NDP represents a nucleoside 5′-diphosphate; ADP, UDP, GDP, CDP are the 5′-diphosphates of adenosine, uridine, guanosine, and cytidine, respectively; AMP, CMP, UMP, GMP are the 5′-phosphates of adenosine, cytidine, uridine, and guanosine, respectively.

dependent binding of specific [^{14}C]aminoacyl-s-RNA (soluble, or transfer RNA) to ribosomes has recently been reported (Nirenberg and Leder, 1964). It was shown that trinucleotides and trinucleoside diphosphates can serve as m-RNA in this system. Using trinucleoside diphosphates of known sequence, the base sequences of several RNA code words have been determined (Leder and Nirenberg, 1964a,b; Bernfield and Nirenberg, 1965). The extension of this work to the determination of the base sequence and genetic function of every possible triplet code word depends on obtaining, in pure form, the various specific trinucleotides. This requirement led to the experiments described in this paper.

Experimental Procedure

Materials. Nucleoside 5′-diphosphates were purchased from Schwarz BioResearch, Inc., Mount Vernon, N.Y. The dinucleoside monophosphate, ApA, was the generous gift of Dr. Fritz Rottman. It was synthesized by a dicyclohexylcarbodiimide condensation reaction using substrates prepared according to the methods of Lapidot and Khorana (1963) and Chla'dek and Smrt (1963). All other dinucleoside monophosphates were purchased from the Gallard-Schlessinger Chemical Manufacturing Corp., Garden City, N.Y. These compounds were further purified from contaminating materials by means of DEAE-cellulose column chromatography and were generously provided by Dr. Joel S. Trupin. The pUpU was prepared as previously described (Nirenberg and Leder, 1964). The T_2 ribonuclease of Takadiastase was the gift of Dr. George Rushizky (Rushizky and Sober, 1963). Snake venom phosphodiesterase was purchased from the Worthington Biochemical Corp., Freehold, N.J., and was further purified to free it of phosphomonoesterase activity (Keller, 1964). Polynucleotide phosphorylase with a specific activity of 51.5 units[2] per mg protein was purified from *M. lysodeikticus* according to published procedures (Singer and Guss, 1962; Singer and O'Brien, 1963). This preparation is equivalent to fraction VIII of Singer and O'Brien (1963). With such preparations, the polymerization of nucleoside 5′-diphosphates is dependent on the presence of oligonucleotides having a free, terminal C-3′ hydroxyl group (Singer and O'Brien, 1963).

Chromatographic and Electrophoretic Techniques. Chromatographic solvent I consisted of 1-propanol-water-ammonia, 55:35:10, v/v/v (Jones *et al.*, 1964). Solvent II consisted of 40 g ammonium sulfate dissolved in 100 ml of 0.1 M sodium phosphate, *p*H 7.0 (Rushizky and Knight, 1960). Electrophoresis was carried out at *p*H 2.7 in 0.05 M ammonium formate buffer on Whatman 3MM paper. The paper strips were subjected to 80 v/cm for from 20 to 45 minutes. Electrophoresis was also carried out at *p*H 3.5 in 0.05 M sodium formate buffer using Whatman no. 40 paper and 60 v/cm for 1 hour, and at *p*H 7.5 in 0.05 M triethylammonium acetate buffer using Whatman no. 40 paper and 6 v/cm for 16 hours. High-voltage electrophoresis was carried out in a Gilson Model D electrophorator. It should be noted that all the buffers used for preparative electrophoresis (*vide infra*) are volatile and can be removed by lyophilization. Nucleosides and nucleotides were visualized on paper strips by inspection in ultraviolet light.

Spectrophotometry. The concentrations of solutions of nucleosides and nucleotides were determined by measuring their absorbance at the appropriate λ_{max} and applying the corresponding extinction coefficients.[3] A Zeiss spectrophotometer was used. The concentrations of solutions of oligonucleotides were estimated by measuring their absorbance at 260 mμ at *p*H 7.0. An approximate extinction coefficient was calculated by averaging the ϵ_{260} of the constituent nucleotides. Complete spectra of the oligonucleotides were obtained with a Model 15 Cary recording spectrophotometer. For this purpose, solutions of oligonucleotides were prepared in 1 mM ammonium formate (*p*H 10). The *p*H was then adjusted to 7.0 with potassium phosphate and subsequently to 1.0 with concentrated HCl, without any appreciable change in volume. The accuracy and reproducibility of these procedures for *p*H adjustment were confirmed by direct determination of the *p*H of identical solutions devoid of nucleotides. Spectra were recorded at each *p*H, within 1 minute after adjustment of the *p*H.

Synthesis and Purification of Trinucleoside Diphosphates. All reaction mixtures (from 1 to 7 ml total volume) were 0.05 M in Tris, *p*H 9.0, 0.01 M in $MgCl_2$, 0.05 mM in EDTA, 10 mM in dinucleoside monophosphate, and contained 7 μg/ml of highly purified polynucleotide phosphorylase, and nucleoside 5′-diphosphate as indicated below. The various modifications used in specific syntheses are as follows: In procedure A, the concentration of nucleoside 5′-diphosphate was 10 mM. This procedure was used for UDP and GDP. In procedure B, 5 mM nucleoside 5′-diphosphate was used. This procedure was applied with ADP and CDP. Prior to the addition of enzyme, selected reaction mixtures of either type A or type B were heated to 70° for 5 minutes and then cooled to 37° (procedure C). Incubation with enzyme was at 37° for 120 minutes in each case. The total reaction mixture was then applied to sheets of Whatman 3MM paper (1 ml per sheet measuring 46 × 57 cm) and developed in solvent I for approximately 36–40 hours. The dinucleoside monophosphate and nucleoside 5′-diphosphate starting materials, as well as the trinucleoside diphosphate product and any other oligonucleotide-containing bands, were located under ultraviolet light and subsequently eluted with water. The eluted material was reapplied to What-

[2] The phosphorolysis assay of Singer and Guss (1962) was used. One unit is equivalent to the formation of 1 μmole of ADP from poly-A in 15 minutes.

[3] Properties of Nucleic Acid Derivatives, California Corp. for Biochemical Research, 1961.

man 3MM paper, electrophoresed at *p*H 2.7 as described, again eluted with water, and concentrated by lyophilization. In the preparation of ApGpA, the product was purified further by electrophoresis at *p*H 7.5. In solvent I, the dinucleoside monophosphates migrate faster than any other component of the reaction mixture. The R_F values of the oligonucleotides decrease with increasing chain length and they are readily separable. The nucleoside 5′-diphosphates migrate more slowly than the dinucleoside monophosphates and in some cases are separable from the trinucleoside diphosphate. In other cases, the trinucleoside diphosphate and nucleoside diphosphate are not separated in solvent I. However, in the cases studied, the higher mobility of the nucleoside 5′-diphosphates in the subsequent electrophoresis at *p*H 2.7 ensured the separation of these two components.

Characterization and Analysis of Trinucleoside Diphosphates. Each compound was subjected to two-dimensional paper chromatography in order to detect possible contaminants. For this purpose, 2 A_{260} units of each compound were applied to Whatman no. 40 paper. The papers were developed in solvent I for 18 hours and then, in a second direction, in solvent II for 3 hours. The migration of each compound was compared to the migration of 5′-AMP ($R_{5'\text{-AMP}}$).

Each compound was also degraded with T_2-ribonuclease and with snake venom phosphodiesterase in order to confirm its identity as the expected product. For digestion with T_2-ribonuclease the reaction mixtures (0.02 ml) contained 10 μmoles ammonium acetate, *p*H 4.5, 2 μg T_2-ribonuclease, and 3 A_{260} units of trinucleoside diphosphate. Incubation was for 6 hours at 37°. For digestion with venom phosphodiesterase the reaction mixtures (0.02 ml) contained 1 μmole ammonium carbonate, *p*H 9.0, 0.2 μmole $MgCl_2$, 1 μg snake venom phosphodiesterase, and 3 A_{260} units of trinucleoside diphosphate. Incubation was for 4 hours at 37°. In both instances the reaction mixtures were lyophilized, taken up in a small amount of water, applied to Whatman no. 40 paper, and subjected to electrophoresis at *p*H 3.5. Each ultraviolet light–absorbing spot was cut out and eluted in 0.01 M HCl, and its spectrum was read against appropriately eluted paper blanks. In order to confirm the identity of each spot, its spectrum was compared to that of a genuine reference sample of the particular nucleoside or nucleotide. Molar base ratios of digestion products were calculated on the basis of the spectrophotometrically determined concentrations of the eluate of each spot.

The products of digestion of a trinucleoside diphosphate by these two enzymes define uniquely the composition and sequence of the compound. Thus, T_2-ribonuclease yields a nucleoside from that end of the trinucleoside diphosphate bearing a free C-3′-hydroxyl group and 3′-monophosphates from all other residues (Rushizky and Sober, 1963). Venom phosphodiesterase yields a nucleoside from that end of the compound bearing a free C-5′-hydroxyl group and 5′-mononucleotides from all other residues (Razzell and Khorana, 1959; Hilmoe, 1959). The various possible products of each digestion are readily separated by electrophoresis at *p*H 3.5 (Smith, 1955).

Results

Table I lists fourteen trinucleoside diphosphates that have been prepared. The dinucleoside monophosphate and nucleoside 5′-diphosphate substrates are shown for each compound. In addition, the procedure used for synthesis is described, as well as the yield of trinucleoside diphosphate. As noted in Table I, there is considerable variation in the yields obtained. Several factors appear to influence the yields. Thus, when high concentrations of nucleoside 5′-diphosphate are used, polynucleotides of chain length ten or more accumulate and smaller oligonucleotides are not formed. This tendency to form long-chain polymers is especially noted in reactions utilizing ADP and slightly less so in those utilizing CDP. Therefore standard procedure B (see section on methods) was used in reactions involving ADP and CDP. However, even under the most favorable conditions devised so far, tetra-, penta-, hexa-, and heptanucleotides are generally formed in addition to the

TABLE I: Preparation of Trinucleoside Diphosphates.

Compound	Substrates for Synthesis: Dinucleoside Monophosphate	Substrates for Synthesis: Nucleoside Diphosphate	Procedure for Synthesis[a]	Yield[b] (%)
ApApC	ApA	CDP	B, C	6
ApApG	ApA	GDP	A	12
ApApU	ApA	UDP	A	32
ApUpC	ApU	CDP	B, C	6
ApUpG	ApU	GDP	A	4
ApUpU	ApU	UDP	A	15
ApCpG	ApC	GDP	A, C	3
ApCpU	ApC	UDP	A, C	12
ApGpC	ApG	CDP	B, C	21
ApGpU	ApG	UDP	A, C	27
ApGpA	ApG	ADP	B, C	13
GpApU	GpA	UDP	A	14
UpApU	UpA	UDP	A	5
GpCpU	GpC	UDP	A, C	6

[a] See section on methods for definitions of synthetic procedures. All compounds were isolated as described in the section on methods. [b] Equivalent to moles of pure trinucleoside diphosphate isolated × 100/moles of dinucleoside monophosphate starting material. The amounts of oligonucleotides were calculated from absorbancy measurements as described under Experimental Procedure.

TABLE II: Characterization of Trinucleoside Diphosphates.

Compound	$R_{5'AMP}$[a] Solvent[b] I	II	Molar Ratio of Degradation Products: T_2-Phosphodiesterase[c] Ap	Up	Gp	Cp	A	U	G	C	Snake Venom Phosphodiesterase[c] A	U	G	C	pA	pU	pG	pC
ApApC	1.07	0.25	2.00	*d*						0.92	1.00				0.96			1.02
ApApG	0.85	0.15	2.00						1.02		1.00				0.94		1.09	
ApApU	1.07	0.25	2.00					0.90			0.99				1.03	1.00		
ApUpC*	0.96	0.37	1.00	0.83						1.00	1.09					0.92		1.00
ApUpG	0.94	0.31	1.00	0.85					1.07		1.00					0.90	1.11	
ApUpU*	0.92	0.37	1.01	0.90				1.00			1.00					2.00		
ApCpG*	0.80	0.39	1.00			0.98			1.05		1.00						1.00	0.90
ApCpU	1.03	0.54	1.02			1.00		0.86			1.00					1.08		0.91
ApGpC	0.79	0.32	1.04		1.00					0.96	1.00						1.00	1.16
ApGpU*	0.85	0.34	1.27		1.00			0.96			0.99					1.00	1.00	
ApGpA	0.81	0.15	1.01		1.00		0.97				1.00				1.02		1.00	
GpApU	0.76	0.42	1.00		1.02			0.93					1.20		1.00	0.89		
UpApU	1.03	0.62	1.00	0.98				1.00				0.98			1.00	1.00		
GpCpU	0.84	0.72			1.03	1.00		0.90					0.84			1.01		1.00

[a] The $R_{5'AMP}$ is the mobility relative to that of 5′-AMP. These values were determined after isolation as described in Table I. Except for those compounds marked with an asterisk, only a single ultraviolet-absorbing spot was noted in each case. The contamination of the compounds marked with asterisks is described further in the text and in Table III. [b] The composition of the solvents is given in the section on methods. [c] The procedure for the degradation and isolation of products is described in the section on methods. [d] Blank spaces indicate the absence of the compound.

trinucleoside diphosphate. The one notable exception was in the synthesis of ApApG; only one longer oligonucleotide, presumably ApApGpG, was detected.

In addition, the nature of the nucleoside 5′-diphosphate used influences the yield. Thus, of the four diphosphates tested, UDP generally provided the highest yields of trinucleoside diphosphate (for example, ApApU, ApUpU, and GpApU). Furthermore, it was noted that in cases where the complementary bases cytosine and guanine were involved in the combination of dinucleoside monophosphate and nucleoside 5′-diphosphate, the yield of product could be significantly improved by heating the reaction mixture prior to addition of enzyme (procedure C, section on methods). This treatment presumably interferes with complementary base interaction. Recently the heat step has been used routinely in all syntheses.

Table II summarizes the data concerning the chromatographic behavior and characterization of the synthesized compounds. The trinucleoside diphosphates have approximately equal mobilities in solvent I, except that the presence of a guanosine residue generally slows migration. On the other hand, as shown previously (Rushizky *et al.*, 1961; Rushizky and Sober, 1962), solvent II frequently separates sequence isomers. All but four of the trinucleoside diphosphates migrated as single ultraviolet light-absorbing spots in both solvents. The four compounds marked with an asterisk in Table II were contaminated (less than 3%) with ultraviolet light-absorbing material. The $R_{5'\text{-AMP}}$ values of the contaminants, as well as estimates of the per cent contamination, are given in Table III.

As outlined in the section on methods, the analysis of the products of digestion with T_2-RNAase and with venom phosphodiesterase allows composition and

TABLE III: Contamination of Trinucleoside Diphosphates.

Compound	Trinucleoside Diphosphate $R_{5'\text{AMP}}$[a] Solvent[b] I	II	Contaminant $R_{5'\text{-AMP}}$[a] Solvent[b] I	II	Per Cent Contamination[c]
ApUpC	0.96	0.37	1.55	1.77	<3
ApUpU	0.92	0.37	0.65		<3
ApCpG	0.80	0.39	1.65	1.78	<3
ApGpU	0.85	0.34		0.73	<3

[a] See footnote *a*, Table II. [b] See section on methods. [c] The per cent contamination is an approximation obtained by visual comparison of the intensity of the spot on paper with the intensities of known amounts of nucleotides. More accurate estimation of the extent of contamination was difficult because the absorbance of eluates of these spots did not differ significantly from that of the corresponding paper blank.

TABLE IV: Trinucleoside Diphosphate Spectral Data.

Compound	λ_{max} at *p*H 10	7	1	λ_{min} at *p*H 10	7	1	A_{260}[a] at *p*H 10	7	1	280/260 at *p*H 10	7	1	270/260 at *p*H 10	7	1	250/260 at *p*H 10	7	1
ApApC	258	258	258	230	230	232	0.70	0.69	0.76	0.37	0.36	0.54	0.76	0.75	0.87	0.83	0.81	0.80
ApApG	256	256	256	228	229	230	0.70	0.70	0.76	0.33	0.33	0.34	0.67	0.69	0.67	0.89	0.91	0.87
ApApU	258	258	257	230	230	230	0.51	0.50	0.58	0.26	0.24	0.26	0.71	0.70	0.71	0.84	0.82	0.85
ApUpC	261	261	263	233	233	234	0.40	0.39	0.41	0.43	0.44	0.64	0.83	0.85	0.90	0.83	0.85	0.76
ApUpG	258	258	259	231	231	232	0.54	0.54	0.53	0.48	0.48	0.49	0.82	0.80	0.79	0.91	0.91	0.87
ApUpU	260	260	258	232	232	232	0.71	0.70	0.73	0.34	0.33	0.34	0.79	0.79	0.77	0.80	0.80	0.82
ApCpG	257	257	260	228	228	232	0.63	0.62	0.63	0.56	0.55	0.73	0.84	0.84	0.92	0.92	0.92	0.81
ApCpU	261	261	263	230	230	233	0.54	0.55	0.56	0.41	0.42	0.64	0.83	0.82	0.94	0.80	0.78	0.73
ApGpC	257	257	260	227	227	232	0.55	0.55	0.57	0.49	0.51	0.74	0.75	0.75	0.92	0.95	0.95	0.79
ApGpU	258	258	259	228	228	229	0.81	0.81	0.82	0.41	0.41	0.42	0.75	0.75	0.77	0.84	0.84	0.89
ApGpA	257	257	257	228	228	230	0.56	0.55	0.57	0.38	0.36	0.39	0.73	0.73	0.72	0.91	0.91	0.86
GpApU	258	258	259	227	227	228	0.72	0.70	0.72	0.43	0.44	0.36	0.83	0.83	0.79	0.85	0.86	0.85
UpApU	258	258	257	230	230	231	0.63	0.63	0.66	0.27	0.27	0.27	0.71	0.71	0.70	0.83	0.83	0.82
GpCpU	258	258	263	228	228	232	0.47	0.46	0.48	0.60	0.61	0.83	0.89	0.91	0.98	0.91	0.91	0.77

[a] A_{260} is the absorbance of the solutions at 260 mμ.

sequence assignments. Since at least 97% of each of the preparations migrates as one spot in two chromatographic systems which separate oligonucleotides on the basis of chain length, the ratio of nucleoside to nucleotide residues in the degradation products provides a measure of chain length. In this case both enzymatic digestions indicate that these compounds are, indeed, trinucleoside diphosphates. The deviations of the experimentally determined product ratios (Table II) from the theoretical values are compatible with the expected experimental error. The spectral data of Table IV are included for reference purposes.

Discussion

A number of methods for the preparation of oligoribonucleotides of known sequence have been described (Heppel *et al.*, 1955; Khorana, 1960; Michelson, 1961; Staehelin, 1963; Coutsogeorgopoulos and Khorana, 1964). Several of the methods depend upon enzymatic degradation of RNA and the difficult chromatographic separation of a large number of possible products, and are therefore of limited usefulness for preparative work. The usefulness of degradative enzymes is often further limited by their specificity; this disadvantage holds in methods involving either the degradative or synthetic capabilities of such enzymes (Heppel *et al.*, 1955; Bernfield and Nirenberg, 1964, 1965). Thach and coworkers (Thach *et al.*, 1964; Thach and Doty, 1965) have reported the use of polynucleotide phosphorylase for the synthesis of block copolymers of known sequence.

The method described in this paper appears to be quite general. There are sixty-four possible common trinucleoside diphosphates. We have described here the synthesis and characterization of fourteen. Recently, sixteen additional trinucleoside diphosphates have been synthesized and their characterization is in progress.[4] The particular compounds described here reflect only the availability of the dinucleoside monophosphates. Among the fourteen trinucleoside diphosphates listed in Table I are examples of the addition of CMP residues (from CDP) to A-, U-, and G-ended dinucleoside monophosphates; of UMP residues to A-, U-, and C-ended dinucleoside monophosphates; of an AMP residue to a G-ended dinucleoside monophosphate; of GMP to A-, U-, and C-ended dinucleoside monophosphates. The more recent syntheses[4] include examples of all the other possibilities. The lowest yield noted is in the case of ApCpG, where a GMP group (from GDP) was added to a terminal C residue in the dinucleoside monophosphate, ApC. In fact, this particular reaction was absolutely dependent on the heat step.

Thus, given all sixteen dinucleoside monophosphates, the synthesis of sixty-four trinucleoside diphosphates appears possible. The major difficulty is with yield. In certain cases low yields reflect the tendency of ADP to form long-chain polymers; in other cases, they reflect the resistance of GDP to polymerization (Grunberg-Manago *et al.*, 1956; Littauer and Kornberg, 1957; Verdanis and Hochster, 1961; Fresco and Su, 1962; Heppel, 1963; Brenneman and Singer, 1964). In general we have found considerably improved yields when large reaction mixtures are used, as a result of absolute rather than relative loss during purification. During preliminary trials we found, as might be expected, that the ratio of oligonucleotide to nucleoside diphosphate in the reaction mixture influences the yield of trinucleoside diphosphate. However, in addition to the XpY/NDP ratio, the absolute concentration of NDP is of critical importance. Thus, at higher NDP concentrations, long polymer molecules tend to form.

In experiments not reported here, pApA was successfully used to synthesize pApApU. The procedure was similar to the one described in this paper and represents an extension of earlier results (Singer *et al.*, 1960a). Thus this method may also be used for the synthesis of trinucleotides having a monoesterified phosphate at C-5′. Such compounds should prove especially useful in RNA code word recognition studies (Nirenberg and Leder, 1964).

The principal advantages of the enzymatic synthesis described are: (1) substrates and enzyme are readily available; (2) synthesis is rapid; (3) separation and purification of the products are relatively easy and further, pure, undegraded, and unincorporated dinucleoside is easily recovered; and (4) the reaction is theoretically, and thus far in our hands, completely versatile.

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