"Nonisomerizable" 2′- and 3′-*O*-Aminoacyl Dinucleoside Phosphates. Chemical Synthesis and Acceptor Activity in the Ribosomal Peptidyltransferase Reaction†

Stanislav Chládek,* David Ringer, and Kevin Quiggle

ABSTRACT: The chemical synthesis of C-A(2′Me)Phe (XIII), C-A(2′Phe)Me (XIIa), C-A(2′Phe)H (XIIb), and C-A-Phe (XV) as well as the precursory nucleoside derivatives, A-(2′Me)Phe (Vb), A(2′Phe)Me (Va), and A(2′Phe)H (IX), is described. "Nonisomerizable analogs" of the 3′ terminus of AA-tRNA compounds, XIII, XIIa, XIIb, Va, Vb, and IX, were tested as acceptor substrates of ribosomal peptidyltransferase. The 3′-*O*-aminoacyl derivatives C-A(2′Me)Phe (XIII), C-A(2′H)Phe, and A(2′Me)Phe (Vb) were found to be active acceptors of the Ac-Phe residue in the peptidyltransferase reaction, whereas the 2′-*O*-aminoacyl derivatives C-A(2′Phe)Me (XIIa), C-A(2′Phe)H (XIIb), A(2′Phe)Me (Va), and A(2′Phe)H (IX) were completely inactive. The results indicate that peptidyltransferase exclusively utilizes 3′-*O*-AA-tRNA as an acceptor substrate.

The exact location of the aminoacyl residue on the terminal adenosine unit of AA-tRNA[1] has been a matter of considerable interest (Zachau and Feldman, 1965). But, because of the extremely rapid $2' \rightleftarrows 3'$ acyl migration within the cis vicinal diol of the terminal adenosine residue (with a half-time estimated at *ca.* 2×10^{-4} sec, Griffin *et al.*, 1966), a definitive assignment of the position of the aminoacyl group in native AA-tRNA cannot be made using conventional chemical and physical methods. Furthermore, in an enzyme-mediated system such as protein biosynthesis, it is probable that a specific isomer of AA-tRNA is used in each step, although the same isomer may not be used throughout all of the stages; indeed, this now appears to be the case. On the basis of the structure and activity of the antibiotic puromycin, which can be considered a simple 3′-*O*-aminoacyl-tRNA analog, and the inactivity of its 2′ analog in the inhibition of polypeptide synthesis (Nathans and Neidle, 1963), it has been assumed that 3′-*O*-aminoacyl-tRNA is an active species in protein biosynthesis. Moreover, we have recently observed (Chládek *et al.*, 1973) that the 3′-phenylalanyl ester of "open-chain adenosine," a "nonisomerizable" analog of 3′-AA-tRNA, is a fair acceptor in the peptidyltransferase reaction, whereas its 2′ isomer is inactive.[2] Recent studies by Ofengand and Chen (1972) and Sprinzl and Cramer (1973) have shown that it is 2′-*O*-aminoacyl-tRNA that is formed by enzymic aminoacylation of tRNA. On this basis, we felt that further studies were necessary in order to elucidate the role of the individual 2′ and 3′ isomers of AA-tRNA in the different steps of protein biosynthesis.

Since it is well known that 2′(3′)-*O*-aminoacyl oligonucleotides can be used conveniently as tools for investigating the structural requirements of certain steps of protein biosynthesis, we felt that some of the desired studies could be pursued effectively using these relatively simple structural analogs of AA-tRNA. Accordingly, we have synthesized several aminoacyl oligonucleotides in which the aminoacyl residue esterifies specifically the 2′- or 3′-hydroxyl group of the 3′-terminal adenosine residue. We have achieved this by the replacement of ribose by 2′- and 3′-deoxyribose and by 2′- and 3′-*O*-methylribose. In this communication the chemical syntheses of several "nonisomerizable" dinucleoside phosphates (XIIa, XIIb, and XIII) are reported along with the parent nucleosides (Va, Vb, and IX). As the initial part of our biochemical studies with these substrates, we report the acceptor activity of compounds Va, Vb, IX, XIIa, XIIb, and XIII in the ribosomal peptidyltransferase reaction.

Results and Discussion

Synthesis. The synthesis of the aminoacyl dinucleoside phosphates XII and XIII follows the general procedure outlined in a previous paper (Chládek and Žemlička, 1974). The method consists of the phosphodiester condensation of a suitably protected nucleoside 3′-phosphate with a 2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-aminoacyl)nucleoside. Because all protecting groups must be removed under mildly acidic conditions to ensure the integrity of the aminoacyl group, we have chosen N^4-dimethylaminomethylene-2′,5′-di-*O*-tetrahydropyranylcytidine 3′-phosphate (Ia) as the nucleotide com-

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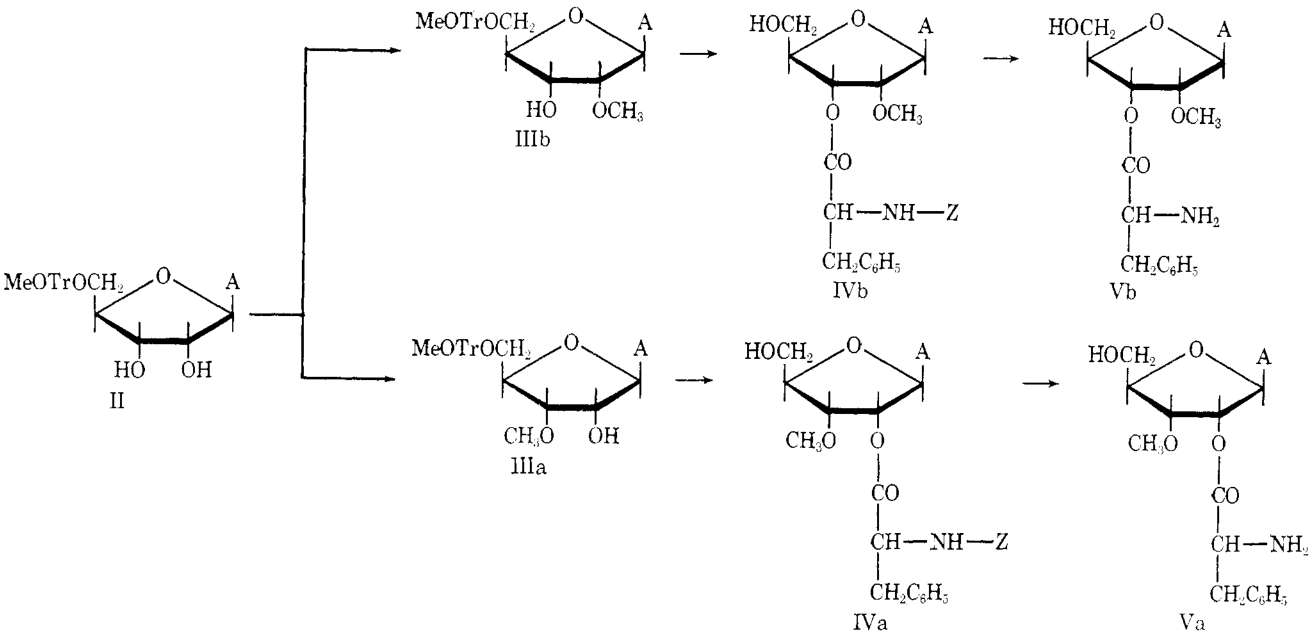
† From the Michigan Cancer Foundation, Detroit, Michigan 48201. *Received February 12, 1974.* This investigation was supported in part by U. S. Public Health Service Research Grant No. GM-19111-02 from the National Institute of General Medical Sciences and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. This paper is No. XIX in the series: Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides. For a preceding report of this series (Paper XVIII), see Chládek and Žemlička (1974). For a preliminary report of a part of this work, see Ringer and Chládek (1974a).

[1] Abbreviations used are: AA-tRNA, aminoacyl transfer ribonucleic acid; Ac-Phe-tRNA, *N*-acetyl-L-phenylalanyl transfer ribonucleic acid; Ac-Phe, *N*-acetyl-L-phenylalanine; A-Phe, 2′(3′)-*O*-L-phenylalanyladenosine; A(2′Me)Phe, 2′-*O*-methyl-3′-*O*-L-phenylalanyladenosine (Vb); A(2′H)Phe, 2′-deoxy-3′-*O*-L-phenylalanyladenosine; (A(2′Phe)-Me, 3′-*O*-methyl-2′-*O*-L-phenylalanyladenosine (Va); A(2′Phe)H, 3′-deoxy-2′-*O*-L-phenylalanyladenosine (IX); C-A-Phe, cytidylyl(3′–5′)-2′(3′)-*O*-L-phenylalanyladenosine (XV); C-A(2′Me)Phe, cytidylyl-(3′–5′)-2′-*O*-methyl-3′-*O*-L-phenylalanyladenosine (XIII); C-A(2′H)-Phe, cytidylyl(3′–5′)-2′-deoxy-3′-*O*-L-phenylalanyladenosine; C-A(2′-Phe)Me, cytidylyl(3′–5′)-2′-*O*-L-phenylalanyl-3′-*O*-methyladenosine (XIIa); C-A(2′Phe)H, cytidylyl(3′–5′)-3′-deoxy-2′-*O*-L-phenylalanyladenosine (XIIb); Z, *N*-benzyloxycarbonyl; Z-Phe, *N*-benzyloxycarbonyl-L-phenylalanine; MeOTr, *p*-methoxytrityl; Thp, tetrahydropyranyl.

[2] For the appropriate nomenclature of the "open-chain analogs," see Chládek *et al.* (1973).

SCHEME I



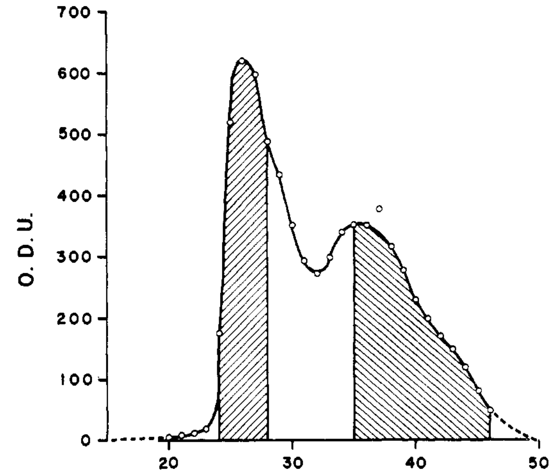


FIGURE 1: Separation of IIIa and IIIb by column chromatography. The mixture of isomers was dissolved in methanol-dioxane-water (5:2:3) (*ca.* 100 ml) and applied to a Dowex 1 (OH$^-$) column (200–400 mesh, Sigma, St. Louis, Mo.) (3 × 70 cm) and the column was eluted by the same solvent. The flow rate was 3.3 ml/min and 10-ml fractions were taken. Fractions 24–28 contained IIIb, 29–34 a mixture of IIIa and IIIb, and 35–46 contained IIIa. The purity of IIIa and IIIb was checked by nmr spectroscopy (Martin *et al.*, 1968).

ponent. Its precursor, 2′,5′-di-*O*-tetrahydropyranylcytidine 3′-phosphate (Ib), was prepared by the acid-catalyzed addition of *N*-dimethylaminomethylenecytidine 3′-phosphate to dihydropyran by the method described earlier for 2′,5′-di-*O*-(1-ethoxyethyl)cytidine 3′-phosphate (Holý *et al.*, 1969). The dimethylaminomethylene group was split during the workup and the nucleotide Ib was isolated easily in high yield by precipitation as its barium salt.[3] This preparation is more convenient than the preparation of the 1-ethoxyethyl derivative (Holý *et al.*, 1969), since it does not require extensive purification by column chromatography. Moreover, the quantitative removal of the tetrahydropyranyl groups from the nucleotide Ib can be achieved under very mild conditions by 0.05 N HCl at room temperature (Griffin *et al.*, 1968).

The two isomeric derivatives, 2′-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)-3′-*O*-methyladenosine (IVa) and 3′-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)-2′-*O*-methyladenosine (IVb), were prepared from pure 3′-*O*-methyl-5′-methoxytrityladenosine (IIIa) and its 2′ isomer (IIIb), respectively (Scheme I). The latter were obtained by the reaction of 5′-methoxytrityladenosine (Chládek and Žemlička, 1974) with diazomethane and stannous chloride (Robins and Naik, 1971). The reaction proceeded quantitatively, but the mixture of the 3′ and 2′ derivatives IIIa and IIIb could not be separated by silica gel chromatography, unlike the analogous 3′- and 2′-*O*-benzyl derivatives (S. Chládek, unpublished results). However, the isomers were separated (Figure 1) on a Dekker column (Gin and Dekker, 1968). The nmr spectrum of each of the methyl derivatives IIIa and IIIb showed no detectable contamination by the second isomer. The dimethylaminomethylene derivatives of IIIa and IIIb were then aminoacylated by the standard procedure (Chládek *et al.*, 1970) with *N*-benzyloxycarbonyl-L-phenylalanine and dicyclohexylcarbodiimide. The aminoacyl derivatives protected at the aminoacyl moiety (IVa and IVb) were isolated by thin-layer chromatography (tlc) in good yield after removal of the dimethylaminomethylene and 5′-methoxytrityl groups. Similarly, we have prepared 2′-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)-3′-deoxyadenosine (VIII) (Scheme II) *via* the 5′-*O*-*p*-methoxytrityl derivative (VII) by aminoacylation of the *N*-dimethylaminomethylene derivative of VII and subsequent removal of protecting groups. The unprotected L-phenylalanyl derivatives (Va, Vb, and IX) were obtained by hydrogenolysis of the *N*-benzyloxycarbonyl groups from the intermediates IVa, IVb, and VIII, and were characterized by the usual criteria.[4]

The condensation of the protected nucleotide Ia with the *N*-benzyloxycarbonyl-L-phenylalanylnucleosides IVa, IVb, and VIII was effected (Scheme III) by dicyclohexylcarbodiimide. The fully protected derivatives were deblocked by treat-

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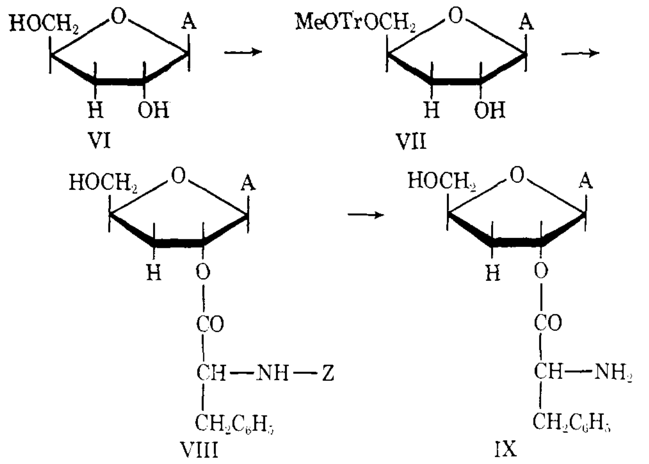
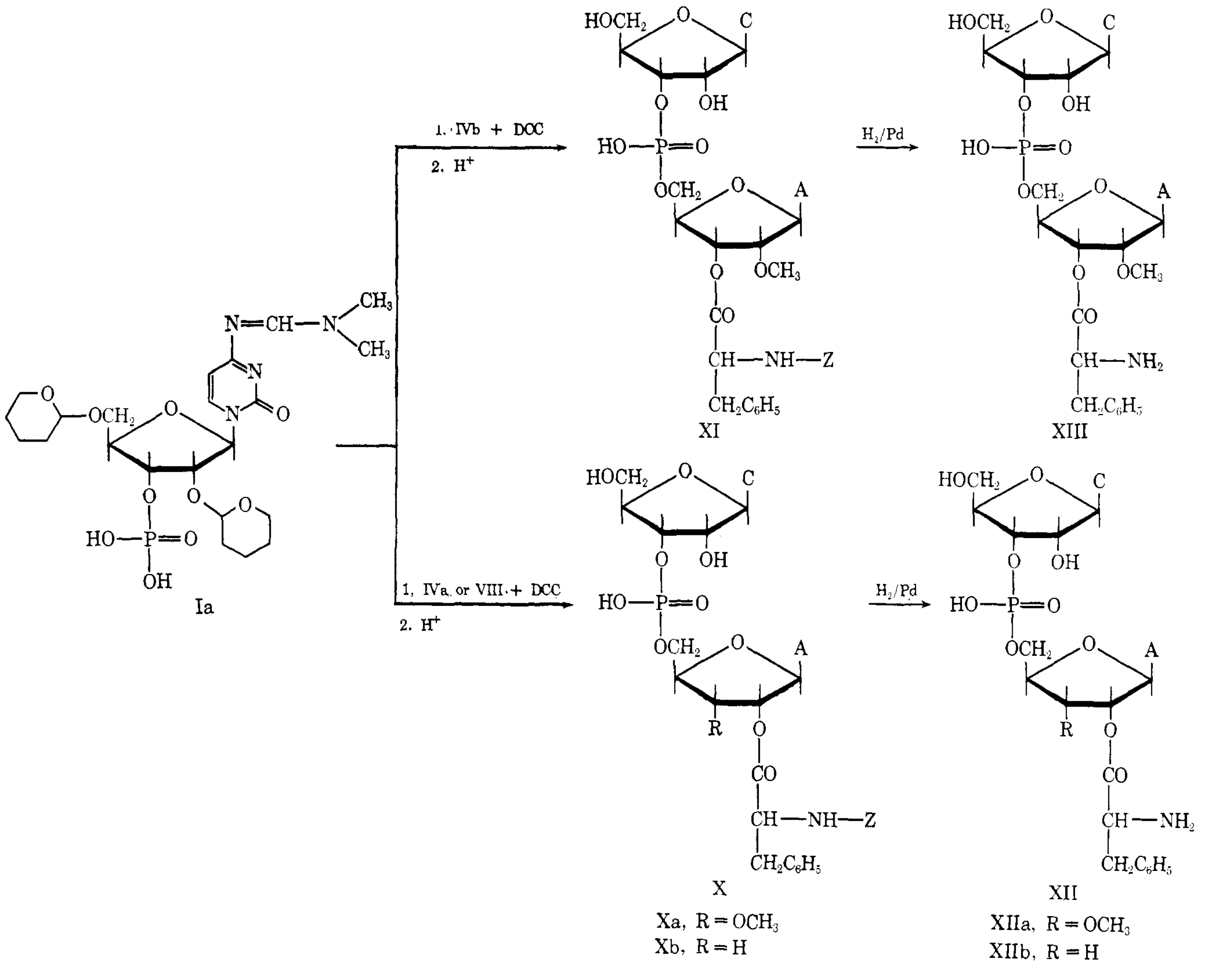
[3] N^4-Acetyl-2′,5′-di-*O*-tetrahydropyranylcytidine 3′-phosphate was used by Harris *et al.* (1972), but the details of the preparation are not given.

[4] A(2′Me)Phe (Vb) was prepared earlier by Pozdnyakov *et al.* (1972) by a different procedure, but no details of preparation have been given.

Scheme II



Scheme III



ment with chromatographic system S_2 to split the *N*-dimethylaminomethylene group and 0.05 N HCl to remove the tetrahydropyranyl groups. Under the conditions used, the removal of the protecting groups was quantitative. The resulting *N*-benzyloxycarbonyl-L-phenylalanyl derivatives Xa, Xb, and XI were isolated by microcrystalline cellulose tlc. Cytidylyl(3′-5′)-2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)adenosine (XIV) was prepared by the same method. The final step of the synthesis was hydrogenolysis of the benzyloxycarbonyl group from the amino acid moiety. This step was accomplished in high yields and the final products (XIIa, XIIb, XIII, and XV) were characterized by the usual criteria—paper chromatography, paper electrophoresis, tlc, uv spectra, and alkaline hydrolysis to the parent dinucleoside phosphates, and L-phenylalanine. The nearly quantitative digestion of the aminoacyl dinucleoside phosphates XIIa, XIIb, XIII, and XV by pancreatic ribonuclease proves that only negligible isomerization of the 3′-5′-phosphodiester linkage takes place during the synthesis despite the fact that only acid-labile groups were used.

Biochemical Results. Figure 2 shows the acceptor activity of the 2′- and 3′-*O*-aminoacyl dinucleoside phosphates in the peptidyltransferase reaction with the Ac-Phe-tRNA·poly(U)·70S ribosome (*E. coli*) complex. It is apparent that only the 3′-esters act as acceptors, whereas no activity was observed for the 2′-esters over a wide range of concentrations.[5] The products, formed by the peptidyltransferase reaction, were analyzed as described earlier (Rychlík *et al.*, 1969). In the case

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[5] When the methyl derivatives XIIa and XIII are tested at concentrations above 5 × 10^{-5} M, precipitation of compounds and ribosomes occurs and therefore data are not reported. Although there is no precipitation at high concentrations of the deoxy derivatives C-A(2′H)Phe and C-A(2′Phe)H (XIIb), the latter is essentially inactive at 10^{-4} to 10^{-3} M.

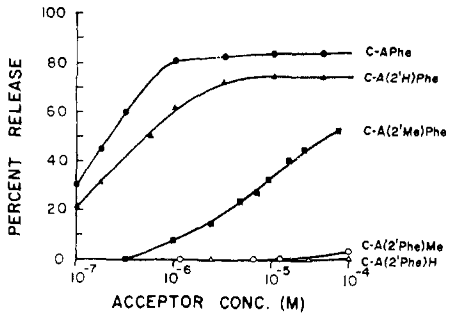

FIGURE 2: 2′- and 3′-*O*-L-phenylalanyl dinucleoside phosphate dependent release of Ac-[^{3}H]Phe residue from Ac-[^{3}H]Phe-tRNA in the peptidyltransferase reaction. Percent release represents the acceptor-dependent decrease in CCl_3COOH precipitated counts trapped by Millipore membranes. For other details, see Materials and Methods. (●) C-A-Phe (Ringer and Chládek, 1974b); (▲) C-A(2′H)Phe (Ringer and Chládek, 1974b); (■) C-A(2′Me)Phe; (○) C-A(2′Phe)Me; (△) C-A(2′Phe)H.

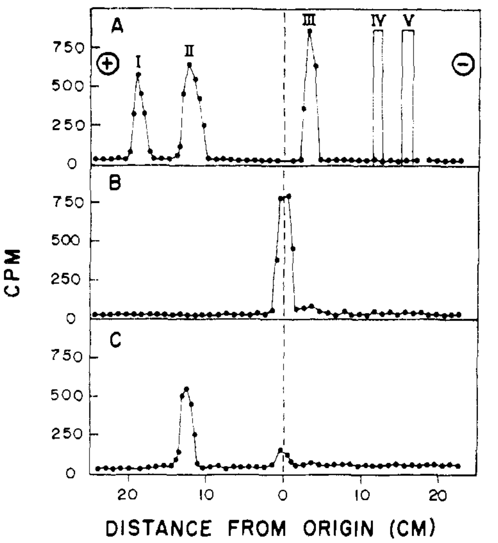

FIGURE 3: Paper electrophoretic analysis of products formed by transfer of the Ac-Phe residue from Ac-Phe-tRNA to the acceptor substrate C-A-Phe. A is a composite of the distance of migration of the following individually run control markers: I, Ac-Phe; II, Ac-Phe-Phe; III, Phe; IV, C-A; and V, C-A-Phe. Below A is the electrophoretic analysis of the peptidyltransferase reaction between Ac-Phe-tRNA and C-A-Phe before (B) and after (C) alkaline hydrolysis. Components of the reaction mixture, conditions and procedure are described in Materials and Methods

of Ac-Phe transfer to C-A-Phe (XV), C-A(Ac-Phe-Phe) is formed which gives Ac-Phe-Phe after saponification (Figure 3). As can be seen from Figure 4, which shows the acceptor activity of the nucleoside derivatives Va, Vb, IX, and A(2′)H)-Phe, only A(2′Me)Phe (Vb) is active.[6] The very low activity of A(2′H)Phe has been observed earlier (Rychlík *et al.*, 1969) and we have found that the 2′-*O*-aminoacyl derivatives Va and IX are also inactive.

In general, the acceptor activity of the substrate increases sharply with the addition of a cytidine 3′-phosphate residue to the 2′(3′)-*O*-aminoacyl derivatives of adenosine (Rychlík *et al.*, 1967; Symons *et al.*, 1969; Hengesh and Morris, 1973; Ringer and Chládek, 1974b). We have attributed this fact to the existence of a locus at the peptidyltransferase A site that recognizes the penultimate cytidine 3′-phosphate residue (Ringer and Chládek, 1974b). The slight decrease of the acceptor activity of C-A(2′H)Phe compared to that of C-A-Phe indicates that the 2′-OH plays a relatively minor role in the acceptor activity of C-A-Phe (Ringer and Chládek, 1974b). It would seem unlikely that replacement of ribose in C-A-Phe by 2′-deoxyribose in C-A(2′H)Phe would result in a profound conformational change of the molecule in a manner that prevents C-A(2′H)Phe from acting as an acceptor at the peptidyltransferase center. Thus, we interpret the failure of C-A(2′-Phe)H (XIIb) to act as acceptor in the peptidyltransferase reaction as a consequence of the "wrong position" of the aminoacyl residue (*i.e.*, on the 2′-hydroxyl group) rather than as a result of conformational changes. This interpretation is strongly supported by comparison (*vide infra*) of the peptidyltransferase activities of C-A(2′Me)Phe (XIII) and C-A(2′Phe)-Me (XIIa); again, the 2′-*O*-ester XIIa is inactive. This conclusion is in agreement with the recent report of Sprinzl and Cramer (1973) who observed that Phe-tRNA with 3′-deoxyadenosine (Phe at the 2′ position) incorporated in the 3′ terminus (prepared by enzymic aminoacylation) is not a substrate for poly(U)-directed poly(Phe) synthesis. The isomeric compound, Phe-tRNA with 2′-deoxyadenosine incorporated at the 3′ terminus (Phe residue at the 3′ position), could not be prepared by these authors (Sprinzl and Cramer, 1973) because of specificity of the enzymic charging for the 2′-hydroxyl group. The 3′ analog of Phe-tRNA which has a 3′-amino-3′-deoxyadenosine residue incorporated at the 3′ terminus ("puromycin-tRNA") has been shown to be an acceptor in the peptidyltransferase reaction (Fraser and Rich, 1973). There is not a profound difference in the acceptor activities of A(2′Me)Phe (Vb) and C-A(2′Me)Phe (XIII) (Figures 2 and 4). However, the former is a much weaker acceptor than A-Phe. Also, there is a very sharp decrease of activity from C-A-Phe (XV) to C-A(2′Me)Phe (XIII) as observed from the extent of reaction (Figure 2) or reaction rate (Figure 5). We assume that the low activity of A(2′Me)Phe (Vb) and C-A(2′Me)Phe (XIII) can be attributed to steric hindrance by the 2′-*O*-methyl group in the peptidyltransferase reaction.[7]

The results presented here are fully consistent with the report of Hussain and Ofengand (1973) who showed that the 2′-phenylalanyl ester of the "open-chain" analog of adenosine[8] was inactive in the peptidyltransferase reaction, whereas a mixture of the 2′ and 3′ isomers prepared by chemical synthesis was active. We recently reported that the open-chain analog of 3′-A-Phe is moderately active and that its 2′ isomer is inactive (Chládek *et al.*, 1973). Both isomers were prepared by chemical synthesis.[9] Therefore, we can conclude that the 3′ isomer of AA-tRNA is the only one which can react at the peptidyltransferase center as an acceptor of the peptide chain. However, this fact does not necessarily mean that 3′-*O*-AA-tRNA is required for all steps of protein biosynthesis at the ribosomal level. We have recently shown (Ringer and Chládek, 1974a), using the same models described in this communica-

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[6] The acceptor activity of A(2′Me)Phe (Vb) has been observed (Pozdnyakov *et al.*, 1972) but because of considerable differences in assay systems it is impossible to correlate our results with theirs in a quantitative manner.

[7] Interestingly, Hecht *et al.* (1973) observed that when the terminal adenosine unit of the 3′ end of tRNA was replaced with either 2′-*O*-methyladenosine or 3′-*O*-methyladenosine there was no charging by aminoacylsynthetase.

[8] This compound was prepared from tRNA, which was oxidized by periodate, reduced by borohydride, charged enzymatically (presumably at the 2′ position), and then digested by pancreatic RNase.

[9] In the case of the open-chain analog, however, there is a substantial decrease in acceptor activity compared to A-Phe that is attributed to the absence of the relatively rigid ribofuranose ring (Chládek *et al.*, 1973).

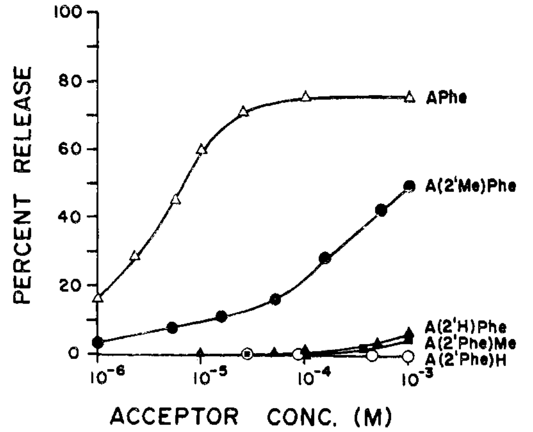

FIGURE 4: 2′- and 3′-*O*-L-phenylalanyl nucleoside dependent release of the Ac-[^{3}H]Phe residue from Ac-[^{3}H]Phe-tRNA in the peptidyltransferase reaction. Per cent release represents the acceptor dependent decrease in CCl_3COOH precipitated counts trapped by Millipore membranes. For other details, see Materials and Methods. (△) A-Phe; (●) A(2′Me)Phe; (▲) A(2′H)Phe; (■) A(2 Phe)Me; (○) A(2′Phe)H

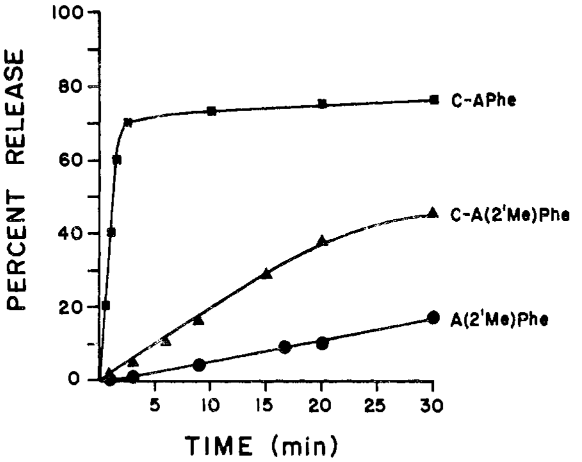

FIGURE 5: Time course of Ac-[^{3}H]Phe residue transfer from Ac-[^{3}H]Phe-tRNA to A(2′Me)Phe and C-A(2′Me)Phe. The per cent Ac-[^{3}H]Phe residue released during the peptidyltransferase reaction from the donor molecule was determined, as described in Figures 2 and 4. Acceptor substrate concentration was the same for all reactions, 5.0×10^{-5} M. (■) C-A-Phe; (▲) C-A(2′Me)Phe; (●) A(2′Me)Phe.

tion, that the 3′ terminus of 2′-*O*-AA-tRNA may interact with the peptidyltransferase A-site or with a site that is closely related. Indeed, if it is 2′-*O*-AA-tRNA which is bound to the ribosome in the EF-T_u dependent process, one must postulate the existence of a ribosome-catalyzed 2′ → 3′ transacylation. This "isomerase" would then supply the "correct" 3′ isomer of AA-tRNA that is required for the subsequent step of peptide-bond formation.

Materials and Methods

General Methods

Chromatography. Paper chromatography was performed by the descending technique on Whatman No. 1 and 3MM paper using the following solvent systems: S_1, 2-propanol–concentrated ammonium hydroxide–water (7:1:2) and S_2, 1-butanol–acetic acid–water (5:2:3). R_F values are given in Table I. Microcrystalline cellulose was used for preparative (2-mm thick, 20 × 20 cm) plates (Chládek and Žemlička, 1974) with system S_3, 1-butanol saturated with 10% acetic acid. Thin-layer chromatography was performed on silica gel coated aluminum foils (Merck, Darmstadt, Germany) in solvents S_4, $CHCl_3$–CH_3OH (95:5); S_5, $CHCl_3$–CH_3OH (9:1); S_6, $CHCl_3$–CH_3OH (4:1). Preparative tlc was also performed on 5-mm thick nonadhering (loose) layers of silica gel (15 × 36 cm), as described (Chládek and Žemlička, 1974).

TABLE I: R_F Values in Paper Chromatography of Starting Compounds, Products, and Authentic Specimens.

Compound	S_1	S_2	Compound	S_1	S_2
A	0.60	0.50	Thp_2Cp Ib	0.54	
3′-dA	0.66		C-A(Z-Phe) XIV	*a*	0.68
Cp	0 09	0.13	C-A(2′Z-Phe)Me Xa	*b*	0.70
C-A	0.20		C-A(2′Z-Phe)H Xb	*b*	0.69
C-A(2′Me)	0.30		C-A(2′Me)(Z-Phe) XI	*b*	0.70
C-A-Me	0.30		C-A-Phe XV	*a*	0.41[c]
C-3′-dA	0.24		C-A(2′Phe)Me XIIa	*a*	0.49[c]
A(2′Me)	0.70		C-A(2′Phe)H XIIb	*a*	0.43[c]
A-Me	0.70		C-A(2′Me)Phe XIII	*a*	0.49[c]
Phe	0.69[c]	0.70[c]			

[a] Decomposition. [b] Partial decomposition. [c] Color reaction with ninhydrin.

Paper electrophoresis was conducted on a Savant flat plate, using 1 M acetic acid as a buffer on Whatman No. 1 paper at 40 V/cm for 2 hr. Electrophoretic mobilities are given in Table II.

Uv-absorbing compounds were detected using a Mineralight lamp, ninhydrin-positive substances were detected by spraying with 0.1% ninhydrin in ethanol, and compounds containing the *p*-methoxytrityl group were detected by spraying with perchloric acid. Uv spectra were obtained using a Beckman Model DB-GT grating spectrophotometer. Yields of oligonucleotides were determined spectrophotometrically at pH 2.0 (0.01 N HCl) using the following extinction coefficients: C-A, ϵ_{260} 21,200; C-A(Me), ϵ_{260} 21,200; C-3′-dA, ϵ_{260} 21,700 (calculated using ϵ_{260} 14,900 for 3′-dA).

Nmr spectra were recorded on a Varian A-60A spectrophotometer; $(CH_3)_4Si$ was used as an internal standard with $CDCl_3$ and CD_3COCD_3, and sodium 2,2-dimethyl-2-silapentane-5-sulfonate was used with CD_3SOCD_3. The solvents, reagents and chemicals used were as described previously (Chládek and Žemlička, 1974).

Pancreatic ribonuclease degradation was performed with 0.5–1 μmol of compound which was dissolved in 0.2 ml of pancreatic ribonuclease (Sigma, St. Louis, Mo.) solution (1 mg of enzyme/1 ml of 0.1 N Tris-HCl, pH 7.8). The solution was

TABLE II: Relative Mobility of Products in the Paper Electrophoresis (1 M Acetic Acid, pH 3.4, Mobility of Cp = 1.00).

Compound	Mobility	Compound	Mobility
A	−8.7	C-A(2′Z-Phe)H Xb	−4.1
3′-dA	−9.7	C-A(2′Phe)H XIIb	−7.1
A-Me	−7.5	C-A(Z-Phe) XIV	−4.1
Phe	−4.0	C-A-Phe XV	−7.5
A(2′Phe)H IX	−15.3	C-A(2′Z-Phe)Me Xa	−3.2
A(2′Phe)Me Va	−16.7	C-A(2′Phe)Me XIIa	−7.6
A(2′Me)Phe Vb	−17.8	C-A(2′Me)(Z-Phe) XI	−3.2
		C-A(2′Me)Phe XIII	−7.5

incubated for 3.5 hr at 37°, after which the reaction mixture was chromatographed (Whatman No. 3MM) in system S_1. The spots of the products of enzymic degradation were eluted with 0.01 N HCl (24 hr) and the optical density of the eluates was measured.

Starting Materials. Cytidine 3′-phosphate was prepared as described by Lohrmann and Khorana (1964); its purity was checked as described earlier, and it was shown by nmr spectroscopy to be free of its 2′ isomer. 2′(3′)-*O*-(*N*-Benzyloxycarbonyl-L-phenylalanyl)adenosine (XVI) was prepared as described previously (Chládek *et al.*, 1970), as was 5′-*p*-methoxytrityladenosine (Chládek and Žemlička, 1974). C-A(2′H)Phe was prepared as described earlier (Chládek and Žemlička, 1974). Cordycepin (3′-deoxyadenosine) was prepared by the method of Russell *et al.* (1973) and was characterized by its mp, tlc, uv, and nmr spectra. *p*-Methoxytrityl chloride (*p*-anisylchlorodiphenylmethane) (Aldrich, Milwaukee, Wis.) was purified by crystallization from petroleum ether containing a trace of acetyl chloride (mp 122–124°). Dihydropyran was purchased from Aldrich (Milwaukee, Wis.).

5′-O-p-Methoxytritylcordycepin (3′-Deoxy-5′-O-p-methoxytrityladenosine) (VII). Cordycepin (0.25 g, 1 mmol) (VI) was stirred with dimethylformamide (2 ml) and dimethylformamide dimethyl acetal (0.2 ml) for 17 hr at room temperature. The solution was evaporated to dryness *in vacuo*, and the uv spectrum (95% ethanol) of the residue showed quantitative conversion of its *N*-dimethylaminomethylene derivative (λ_{max} 312 nm, λ_{min} 254 nm). The residue was coevaporated with anhydrous pyridine (three times) and dissolved in anhydrous pyridine (5 ml); *p*-methoxytrityl chloride (0.34 g, 1.1 mmol) was added, and the reaction mixture was stirred at room temperature for 17 hr. After that time, tlc in systems S_5 and S_6 showed quantitative conversion of the *p*-methoxytrityl derivative together with some formation of the di-*p*-methoxytrityl derivative. The reaction mixture was cooled in ice, quenched by the addition of methanol (5 ml), and the solution was evaporated *in vacuo* to a gum that was distributed between chloroform and water. The organic layer was washed with water, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was dissolved in methanol saturated with ammonia at 0° (25 ml), kept for 15 hr at room temperature, and then evaporated *in vacuo*. The residue was dissolved in chloroform (*ca.* 3 ml), and applied to two tlc plates, and the plates were developed in system S_5. The main band of product was eluted with system S_5, the eluate evaporated *in vacuo*, and the residue triturated with chloroform–petroleum ether (bp 30–60°) to give a solid product (VII) that was dried *in vacuo* and shown to be chromatographically uniform by tlc (S_5, R_F 0.38). The yield was 0.34 g (65%): uv (95% ethanol) λ_{max} (nm) 262 (ϵ 14,500), 236; λ_{min} (nm) 247 (ϵ 12,150); nmr (CD_3COCD_3 + D_2O), 8.15 (H_8, s, 1), 8.12 (H_2, s, 1), 7.30, 6.87, 6.74 (phenyls + methoxyphenyl, m, 14), 6.04 ($H_{1'}$, d, $J_{1'2'}$ = 2 Hz, 1), 3.76 (methoxyl, s, 3).

2′-O-(N-Benzyloxycarbonyl-L-phenylalanyl)cordycepin [2′-O-(N-Benzyloxycarbonyl-L-phenylalanyl)-3′-deoxyadenosine] (VIII). Compound VII (0.28 g, 0.53 mmol) was dissolved in dimethylformamide (8 ml) and dimethylformamide dimethyl acetal (0.8 ml) and kept at ambient temperature for 13 hr, after which the solution was evaporated *in vacuo* to dryness. The uv spectra (95% ethanol) showed quantitative conversion to the *N*-dimethylaminomethylene derivative (λ_{max} 313, 234 nm, λ_{min} 259, 226 nm). The residue was coevaporated with pyridine (three times), dissolved in pyridine (2 ml), Z-Phe (0.32 g, 1.06 mmol) was added, the solution was cooled in ice and mixed with a cold solution of dicyclohexylcarbodiimide (0.33 g, 1.60 mmol) in pyridine (1.5 ml). The reaction mixture was kept in ice for 1 hr and at room temperature for 4 days. The reaction was quenched by ice, petroleum ether (bp 30–60°) (10 ml) was added, the mixture was diluted with pyridine (*ca.* 5 ml), and the precipitated dicyclohexylurea was removed by filtration. The filtrate was evaporated *in vacuo*, the residue coevaporated with toluene (three times) and extracted with petroleum ether (bp 30–60°) (3 × 10 ml). After evaporation, the residue was dissolved in 80% acetic acid (20 ml) and the solution was freeze-dried after being kept for 17 hr at room temperature. The residue was dissolved in chromatographic system S_2, and after 24 hr the solution was evaporated to dryness *in vacuo;* the residue was repeatedly co-freeze-dried with dioxane to remove the last traces of solvents. The residue was dissolved in methanol–chloroform and applied to two silica gel plates and developed (twice) with system S_5. The major band was eluted with system S_5, the eluate evaporated *in vacuo*, the residue triturated with chloroform–petroleum ether (bp 30–60°), and the solid product (VIII) was collected (0.14 g, 50%). The product was chromatographically uniform (system S_5, R_F 0.25); uv (95% ethanol) λ_{max} (nm) 260 (ϵ 14,400); λ_{min} (nm) 227 (ϵ 3060), 250/260 = 0.77, 280/260 = 0.30; nmr (CH_3COCD_3), 8.15 (H_8, s, 1), 8.12 (H_2, s, 1), 7.24, 7.18 (phenyls, 2s, 10), 6.75 (NH_2, s, 2), 6.04 ($H_{1'}$, d, $J_{1',2'}$ = 2.5 Hz, 1), 5.02 (CH_2 from benzyloxycarbonyl, s, 2).

5′-O-p-Methoxytrityl-3′-O-methyladenosine (IIIa) and 5′-O-p-Methoxytrityl-2′-O-methyladenosine (IIIb). A solution of diazomethane in 1,2-dimethoxyethane was prepared (Khwaja and Robins, 1966) from 21 g of nitrosomethylurea and stored overnight over sodium hydroxide pellets at −20°. This solution was filtered and added dropwise with stirring to a suspension of 5′-*O*-*p*-methoxytrityladenosine (II) (2.69 g, 5 mmol) (Chládek and Žemlička, 1974) in 300 ml of 1 mM $SnCl_2 \cdot H_2O$ in methanol. A clear solution was obtained upon completion of the addition of the diazomethane. Tlc (S_4) showed that methylation was complete. The methanol solution was evaporated to dryness *in vacuo* and the resulting material co-freeze-dried with dioxane. The product was applied to a column of Dowex 1 (OH^-) (Gin and Dekker, 1968). Details of the chromatographic procedure are given in Figure 1. The isomers were eluted in two overlapping bands; the 2′-*O*-methyl isomer (IIIb) was eluted first; yields after freeze-drying: 2′-*O*-methyl derivative IIIb (0.93 g), mixed isomers IIIa and IIIb (0.81 g), 3′-*O*-methyl derivative IIIa (1.11 g); total yield = 100%.

Compound IIIa was chromatographically uniform (tlc, S_4, R_F 0.4): uv (95% ethanol) λ_{max} (nm) 260; λ_{min} (nm) 247; nmr (CD_3COCD_3) 8.10 (H_8, H_2, s, 2), 7.30, 6.87, 6.72 (phenyls + methoxyphenyl, m, 14), 6.50 (NH_2, s, 2), 5.99 ($H_{1'}$, d, $J_{1',2'}$ = 4 Hz, 1), 3.77 (aromatic methoxyl, s, 3), 3.47 (3′-*O*-methyl, s, 3); (CD_3COCD_3 + D_2O) 8.20 (H_8, s, 1), 8.14 (H_2, s, 1), 6.04 ($H_{1'}$, d, $J_{1',2'}$ = 4.5 Hz, 1).

Compound IIIb was chromatographically uniform (tlc, S_4, R_F 0.4): uv (95% ethanol) λ_{max} (nm) 260; λ_{min} (nm) 247; nmr (CD_3COCD_3) 8.09 (H_8, H_2, s, 2), 7.35, 6.84, 6.68 (phenyls + methoxyphenyl, m, 14), 6.10 ($H_{1'}$, d, $J_{1',2'}$ = 4 Hz, 1), 3.72 (aromatic methoxyl, s, 3), 3.44 (2′-*O*-methyl, s, 3); (CD_3COCD_3 + D_2O) 8.20 (H_8, s, 1), 8.14 (H_2, s, 1), 6.12 ($H_{1'}$, d, $J_{1',2'}$ = 4 Hz, 1).

3′-O-(N-Benzyloxycarbonyl-L-phenylalanyl)-2′-O-methyladenosine (IVb). Compound IIIb (0.60 g, 1.09 mmol) was dissolved in dimethylformamide (1.5 ml) and dimethylformamide dimethyl acetal (0.8 ml) with stirring. After 1 hr, the uv spectrum showed complete conversion to the dimethylaminomethylene derivative. The solution was evaporated to an oil, coevaporated with pyridine (twice) under reduced pressure, and finally dissolved in dry pyridine (2 ml). With stirring and ice cooling, Z-Phe (0.60 g, 2 mmol) and DCC (0.62 g, 3 mmol) were added to the pyridine solution and the mixture was kept at ambient temperature until silica gel tlc (S_5) showed no further change (1–2 days). The reaction was then quenched with ice, petroleum ether (bp 30–60°) was added, and the mixture was filtered. The filtrate was washed with petroleum ether (three times), and the resulting pyridine–water solution was evaporated under reduced pressure to an oil that was co-freeze-dried with dioxane (twice) to give a yellow powder. The powder was dissolved in solvent S_2 and left at ambient temperature for 15 hr, then evaporated and co-freeze-dried with dioxane. The residue was dissolved in 80% acetic acid (15 ml) and allowed to react for 15 hr, freeze-dried, dissolved in dioxane, and freeze-dried (twice). The product obtained was purified on two silica gel plates (S_5). Attempts to precipitate the resulting compound were unsuccessful, but freeze-drying from dioxane gave a white powder (0.39 g, 64%), chromatographically uniform (tlc, S_5, R_F 0.18): uv (95% ethanol) λ_{max} (nm) 260 (ϵ 13,800); λ_{min} (nm) 229; nmr (CD_3SOCD_3) 8.34 (H_8, s, 1), 8.10 (H_2, s, 1), 7.22 (phenyls, s, 10), 5.91 ($H_{1'}$, d, $J_{1',2'}$ = 7 Hz, 1), 4.95 (CH_2 of benzyloxycarbonyl, s, 2), 3.15 (2′-*O*-methyl, s, 3).

2′-O-(N-Benzyloxycarbonyl-L-phenylalanyl)-3′-O-methyladenosine (IVa). The title compound was prepared by the same method as its isomer IVb in 69% yield. The product was chromatographically uniform (tlc, S_5, R_F 0.17): uv (95% ethanol) λ_{max} (nm) 260 (ϵ 14,400); λ_{min} (nm) 229; nmr (CD_3SOCD_3) 8.25 (H_8, s, 1), 8.10 (H_2, s, 1), 7.20, 7.11 (phenyls, 2 s, 10), 6.14 ($H_{1'}$, d, $J_{1',2'}$ = 5 Hz, 1), 4.87 (CH_2 of benzyloxycarbonyl, s, 2), 3.23 (3′-*O*-methyl, s, 3).

2′,5′-Di-O-tetrahydropyranylcytidine 3′-Phosphate (Ib). The tetrabutylammonium salt of N^4-dimethylaminomethylenecytidine 3′-phosphate (Holý *et al.*, 1969) (12 mmol) was dissolved in dimethylformamide (120 ml) and dihydropyran (36 ml) and cooled to −70°. Trifluoroacetic acid (12 ml) was added with stirring, after which the mixture was allowed to warm to ambient temperature. The reaction was allowed to proceed (1–2 days) until paper chromatography (S_1) showed complete conversion. The reaction mixture was again cooled to −70° and triethylamine (24 ml) was added; the mixture was allowed to warm to ambient temperature and evaporated to a thick oil under reduced pressure. This oil was dissolved in ammonia-saturated methanol (0°) (100 ml) and kept at room temperature (1–2 days) until the uv spectrum showed complete removal of the dimethylaminomethylene group. The methanol solution was evaporated to an oil under reduced pressure, coevaporated with ethanol–1% triethylamine (twice), and dissolved in ethanol (36 ml) containing 10% triethylamine. Barium acetate (3.09 g, 12.1 mmol) was added to the ethanol solution that was then added dropwise to acetone (1.5 l.) to precipitate the compound, and the mixture was kept overnight at 4°. The compound was collected by centrifugation, washed by trituration–centrifugation with ether–acetone (1:1) containing a few drops of triethylamine (twice), and with absolute ether containing a few drops of triethylamine (twice), and dried *in vacuo*. The yield of chromatographically uniform (S_1) Ba^{2+} salt of Ib was 7.4 g (88%), mol wt 697 [measured by uv, pH 2.0, λ_{max} (nm) 279, using ϵ_{279} 13,000]. For $C_{28}H_{30}N_6O_7 \cdot 3H_2O$: Calcd N/P = 3.00. Found N/P = 3.36. The title compound is quantitatively hydrolyzed by treatment with 0.1 N HCl–dioxane (1:1) for 25 hr at ambient temperature.

Condensation of N^4-Dimethylaminomethylene-2′,5′-di-O-tetrahydropyranylcytidine 3′-Phosphate (Ia) with the Protected Aminoacyl Nucleosides IVa, IVb, VIII, and XVI. The barium salt of 2′,5′-di-*O*-tetrahydropyranylcytidine 3′-phosphate (Ib) (0.07 g, 0.1 mmol) was dissolved in 50% pyridine (1–2 ml) and the solution was applied to a Dowex 50 (Py^+) column (5 ml). The column was eluted with 50% pyridine (20 ml), and the eluate was made alkaline with triethylamine and then evaporated to dryness *in vacuo*. The residue was coevaporated three times with dimethylformamide and stirred with dimethylformamide (2 ml) and dimethylformamide dineopentyl acetal (0.2 ml) for 17 hr at room temperature. The solution was evaporated *in vacuo* and its uv spectrum (95% ethanol) showed quantitative conversion to the *N*-dimethylaminomethylene derivative Ia (sharp maximum at 317 nm). The residue was dissolved in cold 50% pyridine (2 ml), the solution was applied to a Dowex 50 (Py^+) column (5 ml), and the column was eluted with 50% pyridine (*ca.* 20 ml). All operations were performed at +4°. The eluate was evaporated *in vacuo* to a small volume, and the residue was repeatedly coevaporated with anhydrous pyridine. The nucleoside (IVa, IVb, VIII, or XVI) (0.2 mmol) was added, and the mixture was evaporated with pyridine. The residue was dissolved in anhydrous pyridine (1 ml), dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) was added, and the mixture was kept for 6 days at room temperature. The reaction was quenched with ice, diluted with 50% pyridine to *ca.* 10 ml, and extracted (3 × 10 ml) with petroleum ether (bp 30–60°). The precipitated dicyclohexylurea was removed by filtration, washed with 50% pyridine, and the solution was evaporated to dryness *in vacuo*. The residue was codistilled (three times) with dioxane to remove the pyridine and then dissolved in system S_2 (15 ml). The solution was kept for 17 hr at ambient temperature, evaporated *in vacuo*, and the residue dissolved in 0.1 N HCl–dioxane (10 ml, 1:1). After 17 hr at room temperature, the solution was lyophilized and the residue was colyophilized with dioxane (three times). The residue was dissolved in methanol–water–acetic acid (2:1:trace) and applied to two thin plates of cellulose. The layers were developed in system S_3. Usually, three main bands were detected (in the order of increasing R_F): nucleoside 3′-phosphate, product (Xa, Xb, XI, or XIV), and starting nucleoside (IVa, IVb, VIII, or XVI). The bands of product were collected and eluted with system S_2. The eluate was evaporated *in vacuo* and the residue was co-freeze-dried with water. The final residue was dissolved in methanol–water (3:1) containing a trace of acetic acid, and the solution was filtered through a small cotton plug and diluted with methanol to 10 ml. The yield was determined spectrophotometrically (Table III). Compounds Xa, Xb, XI, and XIV were characterized by uv spectra, paper chromatography, tlc, paper electrophoresis, and by alkaline hydrolysis to the parent dinucleoside phosphates.

2′- and 3′-O-L-Phenylalanyl Dinucleoside Phosphates (XIIa, XIIb, XIII, and XV). The *N*-benzyloxycarbonyl derivative (Xa, Xb, XI, or XIV) (*ca.* 10 μmol) was dissolved in cold 80% acetic acid (5–8 ml), 5% PdO–$BaSO_4$ (100 mg) was added, and a gentle stream of hydrogen was bubbled through the solution with stirring; the reaction mixture was kept cold by means of an ice bath. After 90 min, the hydrogenation was stopped, the catalyst was filtered off through a glass fiber filter (Reeve Angel), and the filtrate was lyophilized. The residue was diluted to 10 ml with 80% acetic acid (volumetric flask) and the yield was determined spectrophotometrically after dilution of the stock solution (Table IV). Aliquots were freeze-dried and used for chromatographic analysis, electrophoresis, enzymatic degradation (Table IV), and alkaline hydrolysis, which gave phenylalanine and the parent dinucleoside phos-

TABLE III: 2′- and 3′-*O*-(*N*-Benzyloxycarbonyl-L-phenylalanyl) Dinucleoside Phosphates Xa, Xb, XI, and XIV.[a]

		Uv Spectrum				
Compound	Yield (%)	λ_{max}	λ_{min}	250/260	280/260	290/260
C-A(Z-Phe) XIV	36	265	237	0.73	0.74	0.48
C-A(2′Me)(Z-Phe) XI	32	266	238	0.73	0.74	0.48
C-A(2′Z-Phe)Me Xa	35	266	237	0.74	0.76	0.46
C-A(2′Z-Phe)H Xb	34	266	238	0.74	0.71	0.46

[a] Dinucleotide condensations were run on a 0.1 mM scale with the exception of the preparation of Xb, which was run on a 0.03 mM scale.

phates. Samples of compounds for biochemical experiments were also prepared by freeze-drying aliquots.

2′- and 3′-O-L-Phenylalanyl Nucleosides (*Va*, *Vb*, *IX*). The hydrogenation was performed as described for the oligonucleotide derivatives. The samples were treated in the same manner as the oligonucleotide samples and stored at −20°. The yields and uv properties are given in Table IV.

Preparation of Ribosomes and the Soluble Enzyme Fraction. Ribosomes were prepared from late log phase *E. coli* MRE-600 (RNase I$^-$) cells (General Biochemicals) essentially as described by Belitsina and Spirin (1970). Cells (50 g) were lysed by grinding at 4° for 20 min with bacterial grade alumina in a ratio of 2.5 g of alumina to 1.0 g of cell paste. The lysed cells were then extracted with a volume of buffer [0.1 M Tris-HCl (pH 7.4), 0.022 M NH_4Cl, 0.01 M $MgCl_2$, 0.001 M mercaptoethanol] equivalent to 3 ml/g of the total cell paste used. Electrophoretically pure DNase (Worthington Biochemicals) was added to a final concentration of 3 μg/1 ml. Following two low-speed clarification centrifugations of the lysate (10,000*g* for 20 min and 20,000*g* for 40 min), the ribosomes were pelleted by centrifugation at 150,000*g* for 2.5 hr. The upper two-thirds of the supernatant in each centrifuge tube was removed and stored at −80° and designated the S-150 soluble enzyme fraction. The remainder of the supernatant was removed and the ribosomal pellets were washed three times by resuspension and pelleting, once through 0.01 M Tris-HCl (pH 7.4)–0.5 M NH_4Cl–0.001 M $MgCl_2$, and twice through 0.01 M Tris-HCl (pH 7.4)–0.5 M NH_4Cl–0.01 M $MgCl_2$ (Ravel and Shorey, 1971). The washed ribosomal pellets were then resuspended in a buffer containing 0.01 M Tris-HCl (pH 7.4)–0.05 M NH_4Cl–0.01 M $MgCl_2$ to give a final concentration of about 350 A_{260} units/ml and stored at −80°. These ribosomes showed no requirement for preactivation (Miskin *et al.*, 1968) to give maximum activity in the assay systems used.

Preparation of N-Acetylphenylalanyl-tRNA. E. coli tRNA (General Biochemicals) was charged with either [^{14}C]phenylalanine (specific activity 460 Ci/mol) or [^{3}H]phenylalanine (specific activity 5100 Ci/mol) under the conditions given by Rychlík *et al.* (1969). A fraction of the S-150 soluble enzyme eluted from DEAE-cellulose with 0.25 M KH_2PO_4 (pH 6.9) was used as the source of AA-tRNA synthetase (Muench, 1971) and charging was 0.4 nmol of phenylalanine/mg of tRNA. The AA-tRNA was isolated by the phenol extraction method as described by Ravel and Shorey (1971). AA-tRNA was N-acetylated by the method of Haenni and Chapeville (1966) and stored at −80°.

Assay of Peptidyltransferase Activity. Peptidyltransferase activity was measured essentially as described earlier (Chládek *et al.*, 1973). Each reaction mixture of 0.1 ml contained: 0.05 M Tris-HCl (pH 7.4), 0.10 M NH_4Cl, 0.01 M $MgCl_2$, 3.7 A_{260} units of NH_4Cl-washed ribosomes, 10 μg of poly(U), and 0.12 A_{260} unit of *N*-acetyl-[^{3}H]phenylalanyl-tRNA (2000 cpm). The reaction was initiated by addition of synthetic acceptor at concentrations given in the text and allowed to incubate 30 min at 37° before termination of the reaction by the addition of cold 2.5% CCl_3COOH solution (2 ml). After 15 min at 4°, each reaction mixture was filtered through a HAWP-Millipore membrane followed by three washes with 2-ml aliquots of cold 2.5% CCl_3COOH. The membranes were dried and radioactivity was determined by liquid scintillation counting in a 4.5 g of 2,5-diphenyloxazole/100 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)] per 1 l. of toluene scintillation mixture. The amount of Ac-[^{3}H]Phe residue transferred from Ac-[^{3}H]Phe-tRNA to the acceptor was determined as the difference between radioactivity retained on the filter after incubation without acceptor and that retained after incubation with an acceptor. It was expressed as the percentage of the radioactivity of Ac-[^{3}H]Phe-tRNA added to the experimental mixture (Figures 2, 4, and 5).

TABLE IV: 2′- and 3′-*O*-L-Phenylalanyl Dinucleoside Phosphates and 2′- and 3′-*O*-L-Phenylalanyl Nucleosides.

		Uv Spectrum						
Compound	Yield (%)	λ_{max}	λ_{min}	250/260	280/260	290/260	2′–5′[b] (%)	Cp/N[b]
C-A-Phe XV[a]	99	265	236	0.74	0.71	0.47	4	0.87
C-A(2′Me)Phe XIII	85	264	236	0.77	0.71	0.45	1.5	0.85
C-A(2′Phe)Me XIIa	72	264	241	0.76	0.70	0.46	0	0.89
C-A(2′Phe)H XIIb	70	265	240	0.77	0.74	0.45	3	0.86
A(2′Phe)Me Va	76	258	243	0.83	0.28			
A(2′Me)Phe Vb	74	257	242	0.93	0.25			
A(2′Phe)H IX	85	258		0.83	0.24			

[a] No 3′→3′ or 3′→2′ isomer present, as determined by the quantitative degradation by snake venom diesterase (Chládek and Žemlička, 1974). [b] Determined by digestion with pancreatic RNase.

Analysis of Peptidyltransferase Reaction Products. The peptidyltransferase reaction products formed by the reaction of Ac-[^{14}C]PhetRNA with the acceptor substrates were identified by paper electrophoresis. Peptidyltransferase reactions were run as described above at acceptor concentrations giving maximum release. The reactions were terminated by the addition of 0.1 ml of 1% CCl_3COOH and allowed to stand 15 min at 4°. The precipitated material was then pelleted by centrifugation 15,000*g* for 10 min. The supernatants were removed from the pellets and two 0.08-ml aliquots of each were taken. One aliquot was used for direct application to the electrophoresis paper and the other aliquot was saponified with 0.01 ml of 6 N KOH at 37° for 30 min and then subjected to electrophoresis. Radioactive Phe, Ac-Phe, and Ac-Phe-Phe were also spotted on the paper as well as uv-detectable amounts of deacylated and aminoacylated acceptor compounds as markers. High-voltage paper electrophoresis on Whatman No. 1 paper was then performed at 40 V/cm for 2.5 hr in a buffer of 5% acetic acid–0.5% pyridine (pH 3.5). Strips for each sample were cut from the paper and divided into 0.5-cm segments for the analysis of radioactivity content by liquid scintillation counting. Data are shown for the assay with C-A-Phe (XV); analogous results were obtained with other acceptor substrates (not shown) (Figure 3).

Acknowledgments

Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill. The authors are indebted to Dr. E. Hills and to Mr. J. Owens for measuring of the nmr spectra. The authors are grateful to Dr. J. G. Moffat (Institute of Molecular Biology, Syntex Research) for his kind advice with the synthesis of cordycepin and to Drs. K. Philips, J. P. Horwitz (Michigan Cancer Foundation), and H. McAllister (Wayne State University) for their critical evaluation of the manuscript.

Added in Proof

After this paper was submitted, the report of Robins *et al.* (1974) appeared describing the synthesis of several aminoacyl derivatives of 2′-deoxyadenosine and 3′-deoxyadenosine.

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