Aminoacyl Derivatives of "Bridged" Nucleosides: Chemical Synthesis and Substrate Activity of 2′(3′)-*O*-(*N*-Acetyl)-L-leucyl-2″(3″)-*O*-L-phenylalanyl-(1,2-diadenosin-N^6-yl)ethane, a Spacer Probe for Ribosomal Peptidyltransferase†

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ABSTRACT: The chemical synthesis of the title compound XIVa is described. 2′(3′)-*O*-(*N*-Benzyloxycarbonyl)-L-leucyl-1,2-di(adenosine-N^6-yl)ethane (Ia) gave on reaction with 4-methoxytrityl chloride in pyridine di- and tritrityl derivatives II and III. Condensation of II with *N*-*tert*-butoxycarbonyl-L-phenylalanine using dicyclohexylcarbodiimide in pyridine led to compound IV accompanied by a product of diaminoacylation on the same ribose portion V and the triaminoacyl derivative VI or VII. Detritylation of IV and V in 80% acetic acid afforded intermediates VIII and IX which gave on hydrolysis in 12 N HCl leucine and phenylalanine. Hydrogenolysis of IX using PdO–$BaSO_4$ in cold 80% acetic acid led to compound X. Further hydrolysis with CF_3COOH gave the diaminoacyl derivative XI which was hydrolyzed in NH_4OH to afford bridged nucleoside XII, leucine, and phenylalanine. Acetylation of X using *N*-hydroxysuccinimide acetate in dimethylformamide led to the intermediate XIII which was converted to the title compound XIVa on subsequent treatment with CF_3COOH. Hydrolysis of XIII in 6 N HCl at 110 °C afforded leucine, phenylalanine, and glycine. The latter resulted from the hydrolytic cleavage of the purine residues of XIII. Compounds Ib and XIVa were active in the release of AcPhe from *N*-AcPhe-tRNA·poly(U)·70S ribosome complex: the release of AcPhe caused by XIVa was slightly lower than that of Ib. Preincubation of XIVa with ribosomes did not decrease the acceptor activity, indicating the absence of the intramolecular transpeptidation (XIVa → XV). The reaction products of the ribosome-catalyzed release of AcPhe—compounds Id and XIVb—were characterized by thin-layer chromatography before and after alkaline hydrolysis. Derivatives Ib and XIVa inhibit the ribosome-catalyzed puromycin reaction: both are strictly competitive inhibitors. The results indicate that neither Ib nor XIVa are multisubstrate (transition state) analogues of the ribosome-catalyzed peptide bond formation step of protein synthesis. Instead, they mimic the 3′ terminal (nucleoside or oligonucleotide) of the aminoacyl (acceptor) tRNA. The implications of these findings for the function of ribosomal peptidyltransferase are discussed.

The topochemistry of the peptide bond formation step of protein synthesis is an intriguing problem of molecular biology. According to the current view (Lengyel and Söll, 1969), peptide bond formation, as mediated by the ribosomal peptidyltransferase, takes place between peptidyl- and aminoacyl-tRNA.[1] It is generally assumed that this process involves a direct reaction between these two species of tRNA and results in the formation of the peptide bond. A considerable body of information has been gathered relative to the structural (substrate) requirements of the process (Harris and Symons, 1973; Krayevsky et al., 1975). However, the details of the mechanism of peptide bond formation, the nature of the intermediate (transition state), and the mutual orientation (topochemistry) of both tRNA molecules all remain to be fully elaborated.

It is conceivable that in the process of peptide bond formation, the 3′-terminal adenosine units of both tRNAs are stacked (Žemlička et al., 1975). In such an arrangement, space-filling (CPK) models indicate (Žemlička et al., 1977) that both peptidyl and aminoacyl residues can achieve the stereoelectronic alignment necessary for reaction. A similar situation has been postulated for the reaction of peptidyl-tRNA with antibiotic puromycin catalyzed by ribosomes (Raacke, 1971). In addition, 2′(3′)-*O*-aminoacyl bridged nucleosides such as Ib and Ic (Li and Žemlička, 1977) can also be considered as models of the 3′ terminus of aminoacyl-tRNA in accord with their capability of releasing an AcPhe residue from *N*-AcPhe-tRNA in a ribosomal system (Bhuta et al., 1977).

It was therefore of interest to study adenosine derivatives that incorporate the structural features of the terminal units of both tRNAs, including the possibility of stacking of adenine residues of both peptidyl (*N*-acylaminoacyl) and aminoacyl moieties (transition state or multisubstrate analogues). Additional impetus for the study is derived from the fact that compound A (Chart I), which comprises two adenosine units covalently joined through their respective carbohydrate resi-

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[1] Abbreviations used are: *N*-AcPhe-tRNA, *N*-acetyl-L-phenylalanyl transfer ribonucleic acid; CPK models, Corey-Pauling-Koltun models; AcLeu, *N*-acetyl-L-leucyl; LeuOH, L-leucine; PheOH, L-phenylalanine; GlyOH, glycine; MeOTr, 4-methoxytrityl (*p*-anisyldiphenylmethyl); ZLeu, *N*-benzyloxycarbonyl-L-leucyl; Boc, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; UV, ultraviolet; NMR, nuclear magnetic resonance; AcHSI, *N*-hydroxysuccinimide acetate; DMF, dimethylformamide; TLC, thin-layer chromatography; AcPhePheOH, *N*-acetyl-L-phenylalanyl-L-phenylalanine; AcLeuPhe, *N*-acetyl-L-leucyl-L-phenylalanyl; A-(AcLeu), 2′(3′)-*O*-(*N*-acetyl)-L-leucyladenosine; A-(AcPhe), 2′(3′)-*O*-(*N*-acetyl)-L-phenylalanyladenosine; C-A, cytidylyl-3′→5′-adenosine; Ado, adenosine; Cyd, cytidine; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole.

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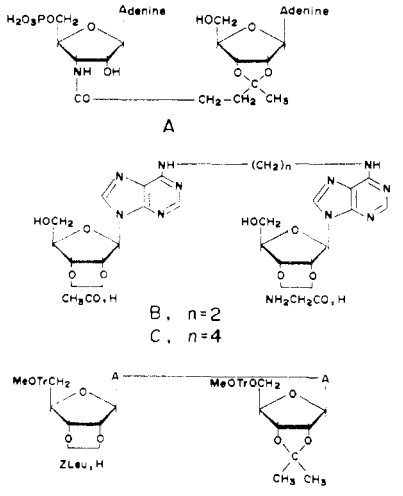
CHART I.



dues (Seela and Cramer, 1975; Seela and Erdmann, 1976), inhibited poly(phenylalanine) synthesis in a cell-free ribosomal system.[2] It is, however, not yet clear whether derivative A functions at the A or P site or a combination of both.

Materials and Methods

General Methods (See Li and Žemlička, 1977). Descending paper chromatography was performed on Whatman no. 1 paper in the following solvents: S_1, 2-propanol–concentrated NH_4OH–water (7:1:2); S_2, 1-butanol–acetic acid–water (5:2:3). TLC, including preparative TLC, was conducted as described by Žemlička and Owens (1977) in solvents: S_3, chloroform–methanol (95:5); S_4, chloroform–methanol (9:1); S_5, chloroform–methanol (4:1), S_6, chloroform–methanol–acetic acid (8:1:1); S_7, phenol–water [3:1 (Merrifield, 1958)]; and S_8, 1-butanol saturated with 1% acetic acid. For a detailed description of the column chromatography procedure, see Li and Žemlička (1977). Paper electrophoresis was performed in 1 M acetic acid at 40 V/cm for 1–2 h in an apparatus described before (Žemlička and Owens, 1977). NMR spectra were determined using Varian A60-A instrument. Tetramethylsilane was used as an internal reference in $CDCl_3$ and an external reference in CD_3SOCD_3.

Starting Materials (See Li and Žemlička, 1977). BocPheOH was purchased from Fluka AG, Buchs, Switzerland. Compound Ia was prepared in 1-g batches according to the described procedure (Li and Žemlička, 1977). For the preparation of AcHSI, see Lapidot et al. (1967). Compound Ib was prepared as described (Li and Žemlička, 1977).

2′(3′)-O-(N-Acetyl)-L-leucyladenosine, A-(AcLeu). 2′(3′)-*O*-L-Leucyladenosine (8.6 μmol) prepared by lyophilization of an aliquot of solution in 80% acetic acid (Chládek et al., 1970; Žemlička et al., 1969) was dissolved in DMF (0.1 mL), AcHSI (2.5 mg, 18 μmol; Lapidot et al., 1967) was added, and the solution was kept for 19 h at room temperature. After evaporation in vacuo at room temperature, the residue was chromatographed on 15 × 5 cm precoated TLC sheets of silica gel F-254 (0.25-mm thick, Merck, Darmstadt, Germany) in solvent S_4. A major UV-absorbing band was eluted with solvent S_5 to give A-(AcLeu), homogeneous on TLC (S_4) whose UV spectrum and TLC mobility corresponded to the compound prepared by a different route (Chládek, 1972). The yield, as determined spectrophotometrically, was 5.5 μmol (64%).

[2] There is a distinct possibility of stacking of both adenine residues in compound A.

Tritylation of 2′(3′)-O-(N-Benzyloxycarbonyl)-L-leucyl-1,2-di(adenosin-N⁶-yl)ethane (Ia). A solution of compound Ia (Li and Žemlička, 1977) (4 g, 4.95 mmol) and 4-methoxytrityl chloride (9.27 g, 30 mmol) in pyridine (100 mL) was stirred for 15 h at room temperature, whereupon it was poured onto ice and partitioned between water (200 mL) and chloroform (3 × 200 mL). The organic layers were dried ($MgSO_4$), evaporated to a syrup which was dissolved in chloroform (30 mL), and petroleum ether (500 mL) was added. The precipitated solid was collected by filtration and purified further by column chromatography on 500 g of silica gel (Figure 1). Peaks B and C were evaporated; the residues were dissolved in chloroform and precipitated by the addition of petroleum ether as above. Peak B afforded tritrityl derivative III (0.5 g, 7%): homogeneous on TLC (S_3); UV λ_{max} (ethanol) 274 (ε 34 600), λ_{min} 249 nm (ε 24 500); NMR ($CDCl_3$ + D_2O) δ 8.31 and 8.23 (2 s, 2, H_8), 7.81 (s, 2, H_2), 7.22–6.77 (m, 47, MeOTr + phenyl), 6.19 and 6.02 (2, poorly resolved broad m, $H_{1'}$), 5.15 (s, 2, CH_2 of benzyloxycarbonyl), 3.63 (poorly resolved 2 s, 9, CH_3O), 1.65 (poorly resolved m, CH and CH_2 of Leu), 0.96 (m, 6, CH_3 of Leu). Anal. Calcd for $C_{96}H_{93}N_{11}O_{14}\cdot H_2O$: C, 70.18; H, 5.83; N, 9.38. Found: C, 70.39; H, 5.74; N, 9.41. Peak C afforded ditrityl derivative *II* (4.11 g, 61%): homogeneous on TLC (S_3); UV λ_{max} (ethanol) 274 (ε 32 900), 248 nm λ_{min} (ε 19 500); NMR ($CDCl_3$ + D_2O) δ 8.24 (s, 2, H_8), 7.98 and 7.83 (2 s, 2, H_2), 7.30–6.74 (m, 33, MeOTr + phenyl), 6.23 and 6.07 (2 br s, 2, $H_{1'}$), 5.13 (s, 2, CH_2 of benzyloxycarbonyl), 3.59 and 3.53 (2 s, 6, CH_3O), 1.60 (br s, 3, CH and CH_2 of Leu), 0.92 (br s, 6, CH_3 of Leu). Anal. Calcd for $C_{76}H_{77}N_{11}O_{13}$: C, 67.49; H, 5.74; N, 11.39. Found: C, 67.56; H, 5.85; N, 10.91.

Aminoacylation of Ditrityl Derivative II. A mixture of compound II (4.1 g, 3 mmol) and BocPheOH (0.97 g, 3.6 mmol) was made anhydrous by evaporation with pyridine (100 mL) at 0.04 mm and room temperature. The residue was dissolved in cold pyridine (60 mL), and a cooled solution of DCC (0.75 g, 3.6 mmol) in pyridine (20 mL) was added. The reaction mixture was stirred for 1 h at 0 °C and 24 h at room temperature. Ice-cold water (30 mL) was then added and dicyclohexylurea was filtered off and washed with pyridine (20 mL). The filtrate was evaporated at 0.05 mm and room temperature to a syrup which was lyophilized from dioxane (100 mL). The resultant white solid was partitioned between chloroform and saturated aqueous $NaHCO_3$. The combined organic layers were washed with water (2 × 250 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on a silica gel column (40 × 5 cm, 420 g) using 1% methanol in chloroform as the eluent, and the obtained peaks were further purified by preparative thin-layer chromatography on a loose layer of silica gel (35 × 15 cm; Žemlička and Owens, 1977) in chloroform containing 6.5% methanol. The fastest band, A, afforded triaminoacyl derivatives(s) VI or VII: 0.6 g (12%); TLC (S_3) homogeneous; UV λ_{max} (ethanol) 274 (ε 35 200), λ_{min} 248 nm (ε 21 600); NMR ($CDCl_3$) δ 8.25 (br s, 2, H_8), 7.30–6.72 (m, 45, H_2 + MeOTr + phenyl), 6.37 (br s, 2, $H_{1'}$), 5.13 (s, 2, CH_2 of benzyloxycarbonyl), 3.62 and 3.56 (2 overlapped s, 6, CH_3O), 1.36 and 1.25 (2 overlapped s, 21, CH_3 of Boc, also overlapped with CH and CH_2 of Leu), 0.90 (br s, 6, CH_3 of Leu). Anal. Calcd for $C_{104}H_{111}N_{13}O_{19}$: C, 67.62; H, 6.06; N, 9.86. Found: C, 67.68; H, 6.15; N, 9.68. Band B afforded compound IV: 1.2 g (25%); TLC (S_3) homogeneous; UV λ_{max} (ethanol) 274 (ε 33 500), λ_{min} 248 nm (ε 20 300); NMR ($CDCl_3$) δ 8.28 (s, 2, H_8), 7.75 (s, 2, H_2), 7.25–6.77 (m, 38, MeOTr + phenyl), 6.01 and 5.95 (2 br, s, 2, $H_{1'}$), 5.15 (s, 2, CH_2 of benzyloxycarbonyl), 3.64 (s, 6, CH_3O), 1.67 (poorly resolved m, 3, CH and CH_2 of Leu), 1.40

(s, 9, CH_3 of Boc), 1.05 (br s, 6, CH_3 of Leu). Anal. Calcd for $C_{90}H_{94}N_{12}O_{16}$: C, 67.57; H, 5.92; N, 10.51. Found: C, 67.47; H, 6.03; N, 10.26. Band C (slowest) afforded compound V: 0.78 g (14%); TLC (S_3) homogeneous; UV λ_{max} (ethanol) 274 (ϵ 34 500), λ_{min} 248 nm (ϵ 21 500); NMR ($CDCl_3$) δ 8.30 (s, 2, H_8), 7.82 (s, 2, H_2), 7.28–6.73 (m, 38, MeOTr + phenyl), 6.25 and 6.13 (2 br s, 2, $H_{1'}$), 5.10 (s, 2, CH_2 of benzyloxycarbonyl), 3.57 (s, 6, CH_3O), 1.26 (s, 12, CH_3 of Boc overlapped with CH and CH_2 of Leu), 0.93 (poorly resolved d, 6, CH_3 of Leu). Anal. Calcd for $C_{90}H_{94}N_{12}O_{16}\cdot H_2O$: C, 66.81; H, 5.98; N, 10.39. Found: C, 66.61; H, 6.08; N, 10.36.

2′(3′)-O-(N-Benzyloxycarbonyl)-L-leucyl-2″(3″)-O-(N-tert-butoxycarbonyl)-L-phenylalanyl-1,2-di(adenosin-N⁶-yl)ethane (IX). A solution of compound IV (0.28 g, 0.17 mmol) in 80% acetic acid (15 mL) was kept for 5 h at room temperature. As judged from TLC (S_4), the detritylation was complete. The solution was evaporated at 0.05 mm and room temperature and the resultant syrup was coevaporated with ethanol to remove traces of acetic acid. The crude product was chromatographed on 4-mm-thick loose silica gel layer (35 × 15 cm) (Žemlička and Owens, 1977) in solvent S_4. The band of IX was eluted with the same solvent, the eluate was evaporated, and the residue was dissolved in a minimum amount of solvent S_4. Addition of a mixture of petroleum ether–ether (1:1) precipitated compound IX as a white solid: 0.16 g (85%); TLC (S_4) homogeneous; UV λ_{max} (ethanol) 273 (ϵ 34 400), λ_{min} 233 nm (ϵ 5800); NMR (CD_3SOCD_3) δ 8.30 (s, 4, H_8 + H_2), 7.30 (s, 10, phenyl), 6.01 (br s, 2, $H_{1'}$), 5.10 (s, 2, CH_2 of benzyloxycarbonyl), 1.43 (s, 9, CH_3 of Boc), 0.90 (br s, 6, CH_3 of Leu). Anal. Calcd for $C_{50}H_{62}N_{12}O_{14}\cdot 2H_2O$: C, 55.03; H, 6.09; N, 15.41. Found: C, 55.18; H, 5.86; N, 15.62. Compound IX did not react with periodate–benzidine reagent and on hydrolysis in 12 N HCl for 24 h at 37 °C gave, according to TLC in solvent S_7, LeuOH and PheOH as the only amino acids.

2′(3′)-O-(N-Benzyloxycarbonyl)-L-leucyl-(N-tert-butoxycarbonyl)-L-phenylalanyl-1,2-di(adenosin-N⁶-yl)ethane (VIII). The above procedure (compound IX) was followed using 0.2 g (0.12 mmol) of V to give 0.1 g (82%) of compound VIII: homogeneous on TLC (S_4); UV λ_{max} (ethanol) 273 (ϵ 35 100), λ_{min} 232 nm (ϵ 5300); NMR (CD_3SOCD_3) δ 8.30 (s, 4, H_8 + H_2), 7.45 (br s, 10, phenyl), 6.08 (s, 2, $H_{1'}$), 5.08 (s, 2, CH_2 of benzyloxycarbonyl), 1.50 (s, 9, CH_3 of Boc), 0.85 (br s, 6, CH_3 of Leu). Anal. Calcd for $C_{50}H_{62}N_{12}O_{14}\cdot 2H_2O$: C, 55.03; H, 6.09; N, 15.41. Found: C, 54.77; H, 5.89; N, 15.50. Compound VIII was positive with periodate–benzidine spray and on hydrolysis in 12 N HCl for 24 h at 37 °C afforded, according to TLC in solvent S_7, LeuOH and PheOH as the only amino acids.

2′(3′)-O-L-Leucyl-2″(3″)-O-(N-tert-butoxycarbonyl)-L-phenylalanyl-1,2-di(adenosin-N⁶-yl)ethane (X). A moderate stream of hydrogen was bubbled through a magnetically stirred solution of compound IX (11 mg, 10 μmol) in 80% acetic acid (3 mL) containing 5% PdO–$BaSO_4$ (100 mg) at 0 °C for 2 h. The catalyst was filtered off using a Celite bed and it was washed with cold 80% acetic acid (3 mL). TLC (S_4) showed a complete absence of the starting material IX and the presence of a single ninhydrin-positive spot of X; UV λ_{max} (0.01 N HCl) 263, shoulder ca. 276, λ_{min} 232 nm. Yield of X as determined spectrophotometrically using ϵ_{263} 26 300 (Žemlička and Owens, 1977) was 87%. Compound X was homogeneous on paper electrophoresis in 1 M acetic acid (mobility 1.62 of PheOH). On paper chromatography in S_1 it was hydrolyzed to nucleoside XII and LeuOH.

2′(3′)-O-(N-Acetyl)-L-leucyl-2″(3″)-O-(N-tert-butoxycarbonyl)-L-phenylalanyl-1,2-di(adenosin-N⁶-yl)-ethane (XIII). A solution of compound X (87 μmol) in 80% acetic acid was lyophilized and the residue coevaporated with freshly distilled DMF at 0.05 mm and room temperature. The residue was dissolved in DMF (5 mL), AcHSI (75 mg, 520 μmol) (Lapidot et al., 1967) was added, and the solution was stirred for 20 h at room temperature. After evaporation at 0.05 mm and room temperature, the residue was chromatographed on 2-mm-thick 20 × 20 cm Stahl's silica gel GF 254 layer (type 60, Brinkman Instruments, DesPlaines, Ill.) in solvent S_8. The major UV-absorbing band was eluted with solvent S_6 and the eluate was evaporated. The residue was dissolved in a minimum amount of solvent S_4, the solution was filtered, and to the filtrate a mixture of petroleum ether–ether (4:1) was added. The solid XIII was collected and dried in vacuo: 40 mg (48%); TLC (S_4 and S_8) homogeneous; UV λ_{max} (ethanol) 274 (ϵ 31 900), λ_{min} 233 nm (ϵ 5800); NMR (CD_3SOCD_3 + D_2O) δ 8.32 (m, 4, H_2 + H_8), 7.32 (br s, 5, phenyl), 5.98 (poorly resolved m, 2, $H_{1'}$), 1.9 (d, overlapped with CH_2 and CH of Leu, CH_3CO), 1.3 (d, Boc) and 0.88 (m, CH_3 of Leu). Total integration of last three signals was 21 protons. Anal. Calcd for $C_{44}H_{58}N_{12}O_{13}\cdot 1.25H_2O$: C, 53.63; H, 6.17; N, 17.00. Found: C, 53.83; H, 5.81; N, 16.62.

2′(3′)-O-(N-Acetyl)-L-leucyl-2″(3″)-O-L-phenylalanyl-1,2-di(adenosin-N⁶-yl)ethane (XIVa). A solution of compound XIII (2 mg, 2 μmol) in trifluoroacetic acid (50 μL) was kept at room temperature for 5 min, whereupon it was evaporated and the residue coevaporated with water. TLC (S_5) showed the complete disappearance of the starting material XIII: UV λ_{max} (0.01 N HCl) 263, shoulder ca. 276, λ_{min} 233 nm. Compound XIVa was homogeneous on paper electrophoresis in 1 M acetic acid (mobility 1.65 of PheOH). It was ninhydrin positive and in solvent S_1 it was hydrolyzed to nucleoside XII and PheOH as shown by paper chromatography (S_1). TLC in solvent S_7 showed the presence of PheOH as the only ninhydrin-positive material. Yield as determined spectrophotometrically using ϵ_{263} 26 300 (Žemlička and Owens, 1977) was 92%.

Acid Hydrolysis of Compound XIVa. A solution of compound XIVa (2 μmol) in 6 N HCl (0.1 mL) was heated in a sealed tube for 24 h at 110 °C. After evaporation at 0.05 mm and room temperature, the residue was coevaporated with water. The residue was chromatographed (TLC) in solvent S_7 to give GlyOH, LeuOH, and PheOH as the only amino acids.

2′(3′)-O-L-Leucyl-2″(3″)-O-L-phenylalanyl-1,2-di-(adenosin-N⁶-yl)ethane (XI). Compound X (2 μmol) in 80% acetic acid (2 mL) was lyophilized, the residue was dissolved in trifluoroacetic acid (50 μL), and the solution was kept at room temperature for 5 min. After evaporation, the residue was coevaporated with ethanol. TLC (S_4) showed the complete absence of the starting material X: UV λ_{max} (0.01 N HCl) 263, shoulder ca. 276, λ_{min} 232 nm. Yield as determined spectrophotometrically using ϵ_{263} 26 300 (Žemlička and Owens, 1977) was 91%. Compound XI was homogeneous on paper chromatography in S_2 (R_f 0.53) and electrophoresis in 1 M acetic acid (mobility 2.52 of PheOH). It was ninhydrin positive and in solvent S_1 it was hydrolyzed to nucleoside XII, PheOH and LeuOH (the amino acids were identified by TLC in solvent S_7).

Assay of Peptidyltransferase Activity. The ability of Ib and XIVa to participate in the peptidyltransferase reaction was measured as follows using *E. coli* MRE-600 ribosomes and *N*-Ac[^{14}C]Phe-tRNA prepared according to a previously described procedure (Chládek et al., 1973).

Samples of Ib, Ic, and XIVa for assays were prepared as before (Bhuta et al., 1977) usually as 0.5–1-μmol aliquots.

TABLE I: Comparative Kinetic Parameters for Aminoacyl Bridged Nucleosides and Puromycin.

Compd	K_M[a] × 10^3	K_I[b] × 10^3	V_{max}[c] × 10^3	Intercept[a]
Puromycin	0.22		0.39	0.77
XIVa	0.44	0.45	0.50	0.771 ± 0.007
Ib	0.24	0.35	0.43	0.773 ± 0.007

[a] Obtained from a Lineweaver–Burk plot. [b] Obtained from a Dixon plot and slope vs. inhibitor concentration plot as shown in Figure 7. [c] Picomoles of product formed per minute, calculated from a Lineweaver–Burk plot.

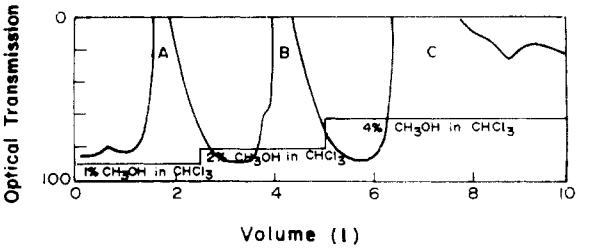


FIGURE 1: Chromatography of the products from the reaction of MeOTrCl with compound Ia in pyridine on a 50 × 5 cm silica gel column (see Materials and Methods). Identification of peaks: A, MeOTrOH; B, tritrityl derivative III; C, ditrityl derivative II. The tops of the main peaks were off scale.

Compound XIVa (trifluoroacetate) was lyophilized from 80% acetic acid (0.2 mL/μmol) just before the assay. It was ascertained that the presence of trifluoroacetate ions does not adversely influence the peptidyltransferase-catalyzed reaction (cf. also Waller et al., 1966).

A typical reaction mixture contained in 0.1 mL 0.05 M Tris-HCl (pH 7.4), 0.1 M NH_4Cl, 0.01 M $MgCl_2$, 4.0 A_{260} units of NH_4Cl-washed ribosomes, 10 μg of poly(U), and 0.20 A_{260} unit (2000 cpm) of *N*-Ac[^{14}C]Phe-tRNA and acceptor substrates at desired concentrations.

Reactions were initiated by the addition of acceptor compounds. Concentrations, temperatures, and incubation lengths are given in the figures and the corresponding legends. The reaction was stopped by the addition of 0.1 mL of 0.1 M $Be(NO_3)_2$ in 0.3 M sodium acetate buffer (pH 5.5), saturated with $MgSO_4$ unless stated otherwise, and the reaction products were extracted with 1.5 mL of ethyl acetate as described (Monro et al., 1968). One milliliter of the ethyl acetate layer was transferred into a scintillation vial and the radioactivity was determined in 10 mL of 4.5 g of 2,5-diphenyloxazole/100 mg of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene/0.25 L of 2-methoxy-ethanol/L of toluene scintillation mixture in a Packard Tri-Carb liquid scintillation spectrometer at 73% counting efficiency.

Lineweaver–Burk and Dixon plots were constructed using a linear-regression analysis. For further details, see the figures and Table I.

Results and Discussion

Synthesis. In principal, two approaches are possible for the introduction of different acyl (aminoacyl) residues into the 2′,3′ vicinal glycol functions of N^6–N^6 bridged adenosine. In the first, two orthoester derivatives derived from 6-chloro-9-(β-D-ribofuranosyl)purine and an N^6-(ω-aminoalkyl)adenosine, respectively, can be prepared and coupled to give the corresponding bis(orthoester) derivative of an N^6–N^6 bridged adenosine. In the next step, the latter can be hydrolyzed to give the desired 2′(3′),2″(3″)-*O*-diacyl derivative. This approach was successfully employed (Žemlička et al., 1977) for the synthesis of bridged nucleosides B and C (Chart I). However, access to the 2′(3′)-*O*-L-aminoacyl derivatives of bridged nucleosides is handicapped because the crorresponding optically active (L) amino acid orthoesters have to date not been described (Žemlička and Chládek, 1966; Graham, 1969; Žemlička and Murata, 1976). Therefore, an alternative approach was used for the synthesis of adenosine-bridged derivatives which carry two different aminoacyl residues in their respective 2′,3′ and 2″,3″ vicinal glycol functions. The synthesis of XIVa, a prototype of the approach, is the subject of this communication.

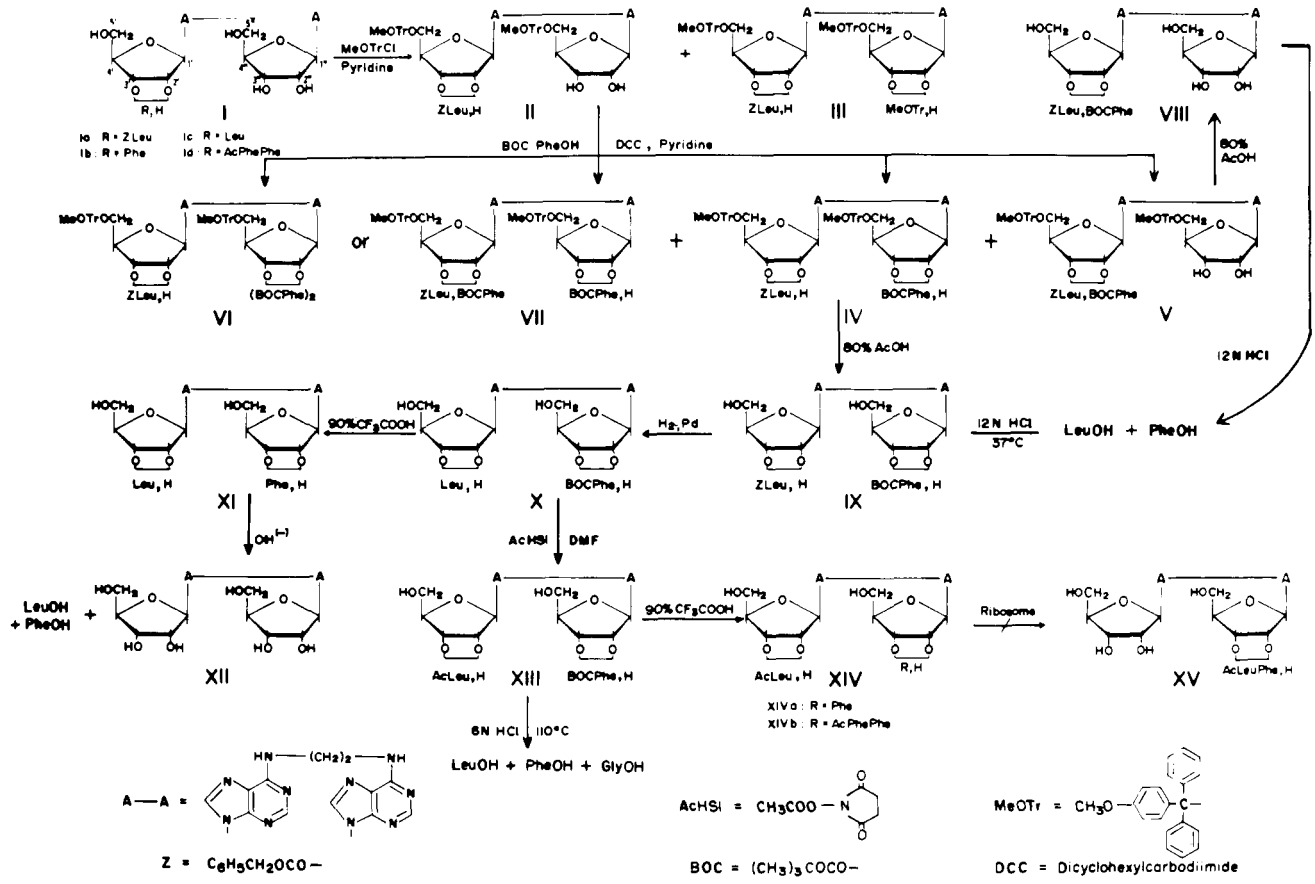
AcLeu was considered a reasonable choice of an *N*-acylaminoacyl residue because AcLeu oligonucleotides, derived from the appropriate tRNA, have a high donor activity in a ribosomal system (Monro et al., 1968). Similarly, Phe was selected as an aminoacyl (acceptor) moiety because of considerable acceptor activity of the corresponding adenosine derivative in a ribosome-catalyzed peptidation (Rychlík et al., 1969). These considerations prompted the synthesis of compound XIVa.

The selective introduction of a single amino acid residue into a bridged nucleoside has already been reported (Li and Žemlička, 1977). However, the attachment of two different amino acids into 2′(3′) and 2″(3″) respective positions of ribofuranose residues of 1,2-di(adenosine-N^6-yl)ethane (XII) posed a significant synthetic task.

The ZLeu derivative of bridged adenosine Ia (Li and Žemlička, 1977) served as a starting material for the synthesis of XIVa. Compound Ia was tritylated with 3 equiv of MeOTrCl in pyridine to give the 5′,5″-ditritylated derivative II in 61% yield after isolation by column chromatography on silica gel (Figure 1). In addition, a tritrityl derivative (III) was obtained in 7% yield. It was possible to suppress the 2″(3″)-O-tritylation in the case of the 2′,3′-*O*-isopropylidene derivative of XII by protection with a 2″,3″-*O*-dimethylaminomethylene function using the reaction with dimethylformamide dimethyl acetal (Li and Žemlička, 1977). The presence of alkali-labile ZLeu residue in I precluded the use of (strongly basic) dimethylformamide acetal for such purposes. The structures of II and III were confirmed by UV and NMR spectroscopy. Thus, UV spectra indicated the absence of N-tritylation of the purine residues and corresponded to those of similar derivatives described previously (Li and Žemlička, 1977). The NMR spectrum of II (integration of aromatic and methoxy group signals) indicated the presence of two MeOTr groups, whereas III exhibited three MeOTr functions. In accord with our previous observations (Li and Žemlička, 1977) on related MeOTr derivatives of bridged nucleoside XII, the methoxy signals in II and III appeared as two singlets (those of II were better resolved). Therefore, both MeOTr groups are not magnetically equivalent. Compound II contains a free *cis*-vicinal glycol grouping as shown by a positive periodate test (Li and Žemlička, 1977). A similar test with III was negative. The latter result also excluded the possibility of tritylation on 2′- or 3′-hydroxy groups adjacent to the ZLeu residue.

Compound II was then condensed with BocPheOH using DCC in pyridine to give after chromatography on silica gel (Li and Žemlička, 1977) three major products in addition to the recovery of 20% of starting material II (Scheme I). The desired diaminoacyl derivative IV, substituted with a different amino acid at each ribofuranose ring, was obtained in 25% yield, whereas the yield of the isomer V substituted with both amino acids at the same ribofuranose moiety was 14%. In addition, the corresponding triaminoacyl derivative VI or VII was also obtained in 12% yield. It is not possible, on the basis of available

SCHEME I.



spectral (UV and NMR) data, to distinguish between structures VI and VII. The formation of a substantial amount of V is surprising considering the bulkiness of the 2′(3′)-*O*-ZLeu residue. It is likely that this unfavorable steric effect is offset to a considerable extent by an inductive influence of the ZLeu function at the neighboring 2′- or 3′-hydroxy groups which are thus rendered more nucleophilic.

To avoid formation of by-product V, we have attempted to block the remaining 2′- or 3′-hydroxy group in the intermediate XVI (Chart I) (Li and Žemlička, 1977) with a 2,2,2-trichloroethoxycarbonyl group (Cook, 1968). The latter is stable in acid but it is readily removable by treatment with zinc in acetic acid. It was, therefore, anticipated that both MeOTr and 2′,3′-*O*-isopropylidene functions could be selectively removed and the resultant 2′(3′)-*O*-trichloroethoxycarbonyl derivative subjected to tritylation and the subsequent transformations outlined in Scheme I. However, the reaction of XVI with 2,2,2-trichloroethoxycarbonyl chloride in pyridine (Cook, 1968) gave a complex mixture of products, and this approach was therefore abandoned.

The choice of the protecting group (Boc) for PheOH also deserves some comment. The primary requirement of N-protecting groups for both Leu and Phe is that it be sufficiently stable in acid to ensure the integrity of alkali-labile aminoacyl (ester) linkages. At the same time, one of the amino acid N-protecting groups should be capable of selective removal. The combination of Boc and benzyloxycarbonyl groups meets these criteria.

The NMR spectra of IV and V were in accord with the presence of ZLeu and BocPhe residues in the ratio of 1:1. Although two pairs of signals belonging to $H_{1'}$ and $H_{1''}$ were discernible, it was not possible to distinguish between both isomers IV and V on the basis of NMR alone. It should also be pointed out that IV can exist as a mixture of four positional isomers, whereas only two isomers are possible with V. Compounds IV and V were detritylated in 80% acetic acid to give derivatives IX and VIII in 82 and 85% yield, respectively. Again, the complex splitting pattern of $H_{1'}$ and $H_{1''}$ signals prevented isomer assignment from the NMR spectra. However, only compound VIII gave a positive periodate–benzidine reaction [presence of vicinal *cis*-glycol grouping (cf. Li and Žemlička, 1977)]. Thus, the structures of both isomeric derivatives were established as VIII and IX, respectively. Compound VIII is the first example of a ribonucleoside substituted with different acyl (aminoacyl) groups at the vicinal 2′- and 3′-hydroxy groups. Both compounds VIII and IX, on treatment with 12 N HCl at 37 °C for 24 h and subsequent TLC in solvent S_7 (simultaneous removal of Boc and benzyloxycarbonyl groups accompanied by hydrolysis of aminoacyl ester linkages), gave LeuOH and PheOH as the only amino acids.

The deblocking of IX was performed with the aim of maintaining the integrity of aminoacyl linkages. This was accomplished by hydrogenolysis in 80% acetic acid at 0 °C using $PdO–BaSO_4$ as catalyst to give intermediate X (87% yield) followed by treatment with CF_3COOH for 5 min at room temperature (removal of Boc) to afford diaminoacyl derivative XI in 91% yield. The latter was characterized in terms of its UV spectrum, paper chromatography, electrophoresis and by hydrolysis in solvent S_1 to bridged nucleoside XII, LeuOH and PheOH. The amino acids were identified by TLC in solvent S_7. Acetylation of X with AcHSI (Lapidot et al., 1967) in DMF gave compound XIII in 48% yield after preparative TLC. Although the NMR spectrum of XIII was not well resolved, it showed the presence of the expected functional groups (phenyl of Phe, Boc group, acetyl function, and Leu).

The UV spectrum corresponded to those of similar derivatives. Because of the stability of intermediates with free ami-

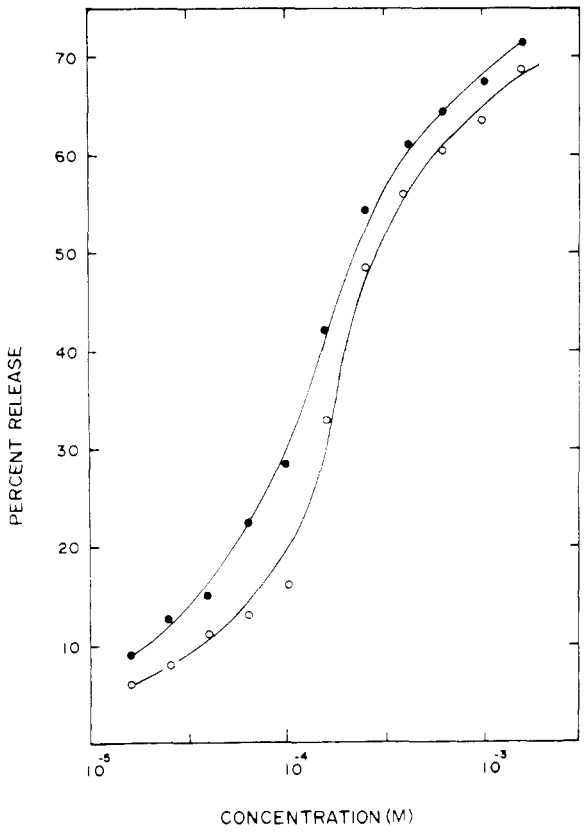


FIGURE 2: Extent of AcPhe release from *N*-Ac[^{14}C]Phe-tRNA by acceptor substrates at 32 °C in 30 min (for experimental conditions, see Materials and Methods). The percent release represents the acceptor-dependent increase in radioactivity in the ethyl acetate layer vs. the total amount of radioactivity added to the assay mixture: Ib (●); XIVa (○).

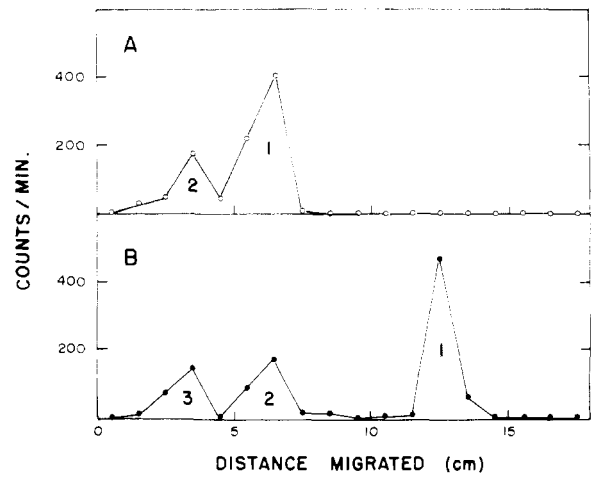


FIGURE 3: Thin-layer chromatography of products formed with Ib and XIVa as acceptor substrates. The reaction conditions were the same as those described in Figure 2. The ethyl acetate layer was concentrated by evaporation in a stream of air at room temperature and immediately applied on a precoated silica gel F-254 TLC sheet (20 × 5 cm, EM Laboratories, Inc., Elmsford, N.Y.). The chromatogram was developed three times with solvent S_4 and once with S_5 and dried, and the radioactivity peaks were located with a Packard radio-chromatogram scanner Model 7201. The radioactivity-containing areas were then cut into 1-cm-wide strips, and the radioactivity was determined using PPO–POPOP toluene scintillation mix as described under the Materials and Methods. Panel A: Peak 1, compound Id; peak 2, unidentified. Panel B: Peak 1, compound XIVb; peak 2, compound Id; peak 3, unidentified.

noacyl moieties, compound X should be used immediately after preparation for the synthesis of XIII. Hydrolysis of XIII in 6 N HCl at 110 °C for 24 h gave three amino acids: LeuOH, PheOH, and GlyOH, all of which were separated and identified in solvent S_7. The presence of GlyOH in the hydrolysis mixture may appear at first glance surprising. However, it is known (Lindsay et al., 1962; Chládek and Žemlička, 1968) that purine derivatives, including adenosine, give a substantial amount of GlyOH when subjected to conditions of peptide bond hydrolysis, e.g., 6 N HCl, 105 °C.

Deprotection of XIII was achieved with CF_3COOH for 5 min at room temperature and the target, compound XIVa, obtained in 92% yield was adequately characterized. On hydrolysis with solvent S_1 compound XIVa gives PheOH as the only ninhydrin-positive amino acid.

Biochemical Results. Acceptor activity of monoaminoacyl derivatives Ib and Ic and hence their ability to react at the ribosomal A site were established previously (Bhuta et al., 1977). It was therefore of interest to determine how the presence of a "donor" group (AcLeu) would influence the substrate properties of the resultant model—compound XIVa. The reasons for choosing an AcLeu derivative were discussed in the preceding section. Surprisingly, the attachment of a very bulky AcLeu function to Ib has little effect on the ability of the latter to act as a substrate for ribosomal peptidyltransferase. This can be clearly seen from the concentration curve of substrate activity for Ib and XIVa (Figure 2). The extent of acceptor activity was unchanged after preincubation of XIVa with ribosomes for 30 min without *N*-AcPhe-tRNA (data not shown). This showed the absence of ribosome-catalyzed intramolecular peptidation (XIVa → XV, Scheme I). It was possible to characterize the products of peptidyltransferase-catalyzed reaction, nucleoside peptides Id and XIVb, by TLC (Figure 3). Thus, compound Id which moved on paper electrophoresis as a single peak (Bhuta et al., 1977) gave one major (1) and one minor peak (2, Figure 3, panel A). In the case of XIVb (panel B), we have observed one major peak (1) distinctly separated from a minor peak (2, compound Id) and a third component (3) identical with that on panel A (peak 2). Compound Id resulted from the hydrolysis of an AcLeu residue of the derivative XIVb or XIVa followed by peptidyltransferase-catalyzed peptidation. In each case, after alkaline hydrolysis, AcPhePheOH was obtained as a single radioactivity-containing product after TLC in solvent S_4 (data not shown). Although minor peaks 2 (panel A) and 3 (panel B) were not rigorously identified, the possibility cannot be excluded that they represent the 2′ isomer of Id. Several cases of separation of 2′- and 3′-*O*-acyl isomers of bridged nucleosides on TLC were previously reported (Li and Žemlička, 1977).

The time course of the peptidyltransferase-catalyzed reaction of Ib and XIVa also suggested a decreased reactivity for XIVa. Thus, in order to obtain roughly identical rate profiles (Figure 4), the concentration of XIVa relative to Ib has to be doubled. The corresponding K_M values are obtained from a Lineweaver–Burk plot (Table I). The K_M of Ib (0.24 mM) is very close to that of puromycin (0.22 mM). Our K_M value for puromycin at 32 °C agrees well with that obtained recently at 28 °C (0.22 mM, Duquette et al., 1974). The K_M of AcLeu derivative XIVa was appreciably higher (0.44 mM), which again indicates a lower affinity for the particular site (A site) on peptidyltransferase.

Although the above data provided ample evidence for the function of Ib and XIVa as substrates for peptidyltransferase, it did not exclude a possibility of additional participation of Ib and XIVa in other binding modes, particularly those involving a simultaneous bridging of A and P site (Bhuta et al., 1977). The structural similarity of XIVa with the 3′ terminals of both donor and acceptor tRNA and also a decreased reactivity in

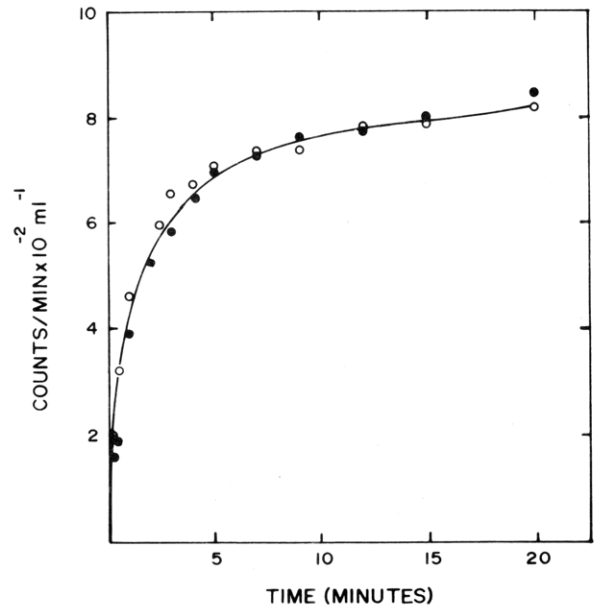


FIGURE 4: Time course of the acceptor activity at 32 °C: Ib (●) 10^{-4} M; XIVa (○) 2×10^{-4} M. For experimental conditions, see Materials and Methods.

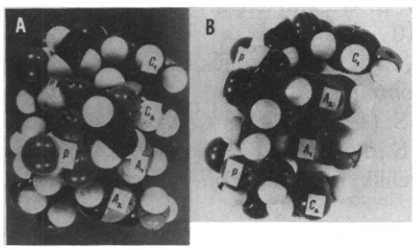
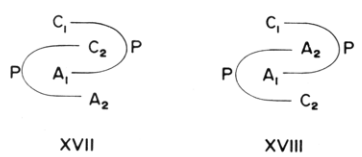


FIGURE 5: Self-intercalation of two C-A units: C_1, C_2, cytosine residues; A_1, A_2, adenine residues; p, phosphodiester bridges. Numbers 1 and 2 designate different molecules of C-A, all stacking of bases is intermolecular. In A, C_2 is inserted between C_1 and A_1; in B, A_2 is inserted between C_1 and A_1. Note the possibility of bridging both the adenine amino groups (A). In an abbreviated form (for a similar notation cf. Bangerter and Chan, 1969), both models can be described by XVII (panel A) and XVIII (B) (Chart II).

peptidyltransferase-catalyzed peptide bond formation relative to Ib (Figure 2) indicated at least a possibility for such a mode of action. In addition, studies with CPK models of C–A terminals of donor and acceptor tRNA showed that a peptide bond can be formed in arrangements where both adenosine units are stacked. Stacking of adenine residues in parent nucleoside XII was shown earlier (Žemlička and Owens, 1977). In this respect, two structures with both C–A residues intermolecularly stacked (intercalated) are of particular interest (Figure 5 and Chart II). From Figure 5 (panel A) it can be seen that both adenine residues can be "bridged" at their respective amino groups (e.g., with an aliphatic chain), giving rise to a structure similar to compound XII. It should be kept in mind, however, that other arrangements are also possible including those not involving intercalation or stacking. It is also of interest that acceptor activity of 2′(3′)-*O*-L-phenylalanyl derivatives in a ribosome-catalyzed peptide bond formation

CHART II.



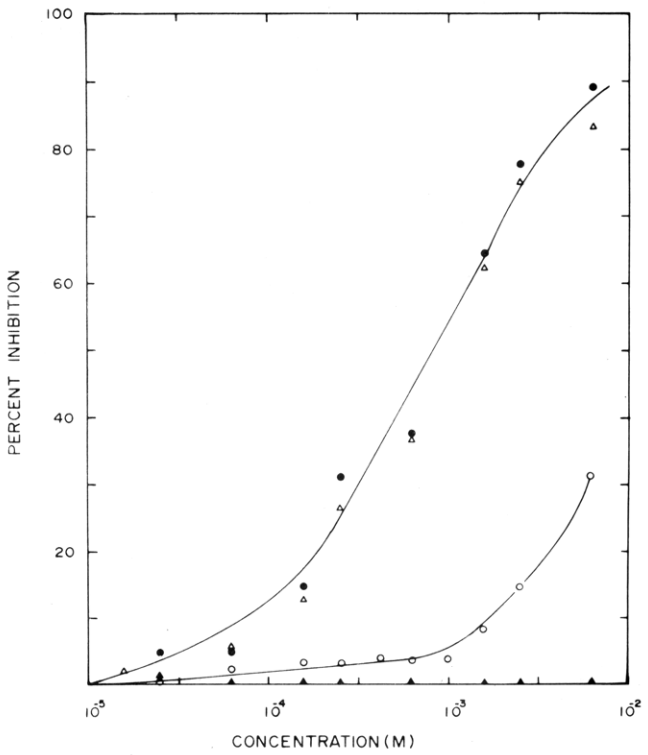


FIGURE 6: Inhibition of acetyl[^{14}C]phenylalanylpuromycin formation at 37 °C. Reactions were performed as described under Materials and Methods. Puromycin concentration was kept at 1×10^{-4} M with the varied concentrations of inhibitors. Incubation was for 30 min. The reaction was stopped by adding 0.1 mL of 1 N NaOH, and the tubes were further incubated at 37 °C for 5 min to hydrolyze any 2′(2″) or 3′(3″) ester bonds (Rychlík et al., 1970). Percent inhibition represents the difference in ethyl acetate extracted acetyl[^{14}C]phenylalanylpuromycin counts in the presence and in the absence of inhibitors. One-hundred percent acetyl[^{14}C]phenylalanylpuromycin formed corresponds to 1114 cpm. Inhibitors added were: (a) Ib (●); (b) Ic (○); (c) A-(AcLeu) (▲); (d) XIVa (△).

(Rychlík et al., 1969; Ringer and Chládek, 1974) follows after statistical correction the increase in self-association constants of the parent compounds: C-A > Ado ≫ Cyd (Bangerter and Chan, 1969). This may indicate the importance of donor tRNA–acceptor tRNA interactions, particularly of their respective CCA units, in the process of the ribosome-catalyzed peptide bond formation.

Compounds Ib, Ic, and XIVa were also examined as inhibitors of the puromycin reaction catalyzed by peptidyltransferase (Figure 6). Inhibition with derivatives Ib and Ic follows the order of their acceptor activities (Bhuta et al., 1977). By contrast, although the acceptor activity of XIVa is lower than that of Ib, the difference in their inhibitory activity is insignificant. A-(AcLeu) which was run for a comparison does not inhibit the puromycin reaction. This is in agreement with previous studies using A-(AcPhe) (Rychlík et al., 1970). In order to exclude the possibility that a portion of inhibitory activity of XIVa may be due to binding sites other than the A site, we have undertaken some kinetic experiments. A typical Dixon plot for the title AcLeu derivative XIVa is shown in Figure 7. The rest of the data obtained for Ib and XIVa from the corresponding Dixon and Lineweaver–Burk plots (figures not shown) was tabulated (Table I). The results are consistent with a competitive type of inhibition of puromycin reaction by both Ib and XIVa. It can therefore be concluded that Ib and XIVa act exclusively at the A site of ribosomal peptidyltransferase.

Comparison of CPK models of "bridged" nucleoside XII

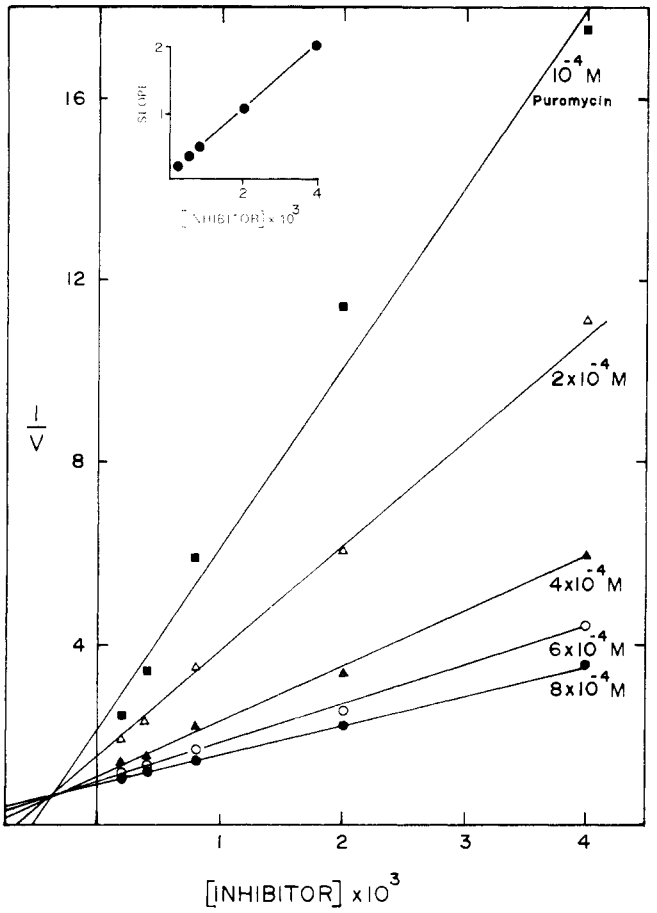
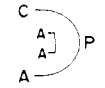


FIGURE 7: Dixon plot of the effect of XIVa on the rate of acetyl[^{14}C]-phenylalanylpuromycin formation at 32 °C. The initial velocity V was determined as the amount of radioactivity extracted in 1.0 mL of ethyl acetate in the first 5 min of the reaction. Experimental conditions were the same as described under Materials and Methods and in Figure 6. Inset shows the linear relationship between the inhibitor concentration and the slope obtained from the corresponding Lineweaver–Burk plot (figure not shown).

and C-A indicated a similarity, provided both adenine residues in XII are stacked (Bhuta et al., 1977). However, unlike C-A, compound XII has a plane of symmetry. Thus, compounds Ib, Ic, and XIVa can be regarded as analogues of 2′(3′)-*O*-aminoacyl oligonucleotides, some of which possess a significant acceptor activity (Krayevsky et al., 1975). On the other hand, CPK models also show that in a stacked form, Ib, Ic, and XIVa can intercalate between C and A units of the donor tRNA as shown in Chart III. It is evident that with an extended alkyl chain between both adenines such a situation will be much less likely, whereas the probability of interactions described in Figure 5 (aliphatic chain instead of a phospho diester bridge) should increase.

It is recognized that the surprising ability of Ib and particularly XIVa to mimic puromycin or the 3′ terminal oligonucleotide of aminoacyl-tRNA precludes their action as multisubstrate (transition state) analogues for peptidyltransferase. However, it would be premature to conclude that our experiments rule out the possibility of arrangement XVII in the transition state of peptide bond formation. The failure, for example, of the AcLeu moiety of XIVa to displace the corresponding 3′ terminal of donor tRNA may be due to other factors such as a lack of sufficient "donor" binding sites in XIVa. It is known that requirements of the P site of peptidyltransferase for an intact 3′-oligonucleotide chain are much more stringent than those for the A site (Monro et al., 1968).

CHART III: Association (Intercalation) of a Bridged Nucleoside with a C-A Unit.[a]



XIX

[a] For notation, cf. Figure 5. $\begin{matrix}A\\A\end{matrix}\rceil$ denotes bridged nucleoside Ib, Ic, or XIVa in a stacked form.

It can be concluded that further models including (1) varying the length of an aliphatic chain between adenines, (2) different modes of stacking (such as in XVIII) and (3) oligonucleotide derivatives thereof are needed to contribute to the elucidation of the problem of transition state of peptidyltransferase-mediated protein synthesis.

Acknowledgments

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Mode of Action of Saframycin A, a Novel Heterocyclic Quinone Antibiotic. Inhibition of RNA Synthesis in Vivo and in Vitro†

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ABSTRACT: Saframycin A, an antitumor antibiotic, was shown to preferentially block RNA synthesis in cultured L 1210 cells. The analysis of newly synthesized RNA of the nucleolar or nucleoplasmic fraction of saframycin A treated cells by polyacrylamide gel electrophoresis indicated saframycin A affected the synthesis of both pre-rRNA and heterogeneous nuclear RNA (hnRNA) to the same degree. Furthermore, this antibiotic was characterized as causing a shift of both RNAs toward lower molecular weight regions. Saframycin A alone, on the other hand, failed to affect the template activity of DNA with *Escherichia coli* RNA polymerase in vitro. But the drug showed an inhibitory activity in the presence of dithiothreitol (DTT). The effect was maximized by the pretreatment of DNA with the drug and DTT and related to the concentration of these two components. The requirement of DTT was slightly replaced by cysteamine, cysteine, and β-mercaptoethanol, but not by $NaBH_4$, NAD(P)H, ascorbate, and glutathione. Native conformation of DNA was found to be required for drug action, and the synthetic copolymer, poly(dG)·poly(dC), was more sensitive than poly(dA)·poly(dT). When ^3H-labeled DNA, whose template activity for RNA polymerase was lowered by 90% by the treatment of saframycin A, was subjected to neutral or alkaline sucrose gradient centrifugation, no degradation of DNA was detected. This result suggests that the decreased template activity was not due to strand scission of DNA.

A number of clinically useful antitumor antibiotics interfere with DNA through intercalation, cross-linkage, strand breakage, and other interactions. The inhibition of nucleic acid synthesis might occur preferentially in rapidly proliferating tissues such as tumors. During the course of screening for antitumor antibiotics, new antibiotics, saframycins (A, B, C, D, and E) produced by *Streptomyces lavendulae,* were discovered by the present authors (Arai et al., 1977). The structure of saframycin C (Figure 1) was determined by x-ray crystallography (Tamura et al., unpublished results). The saframycin complex belongs to the group of heterocyclic quinones such as mitomycin C and streptonigrin. However, the structure was characterized by the presence of twin heterocyclic quinones in its skeleton.

Among saframycins, the chemical composition of saframycin A differed from the others in that it possessed an extra nitrogen. Furthermore, saframycin A has been proven to exhibit an extreme cytotoxicity toward cultured cells and to exert an antitumor activity against several experimental tumors including leukemia L 1210 and P 388 and Ehrlich carcinoma, both in ascites and solid forms (Mikami et al., unpublished results). The compound was also active against gram-positive bacteria.

One of our aims is to elucidate the mode of action of saframycins and we chose saframycin A in our experiments because of its high biological activity. In this paper we present the effects of saframycin A on the synthesis of RNA in L 1210 cells and on the transcriptional system in vitro. The unique characteristics of this drug in regard to its requirement of reducing agents to interact with DNA will be presented.

Materials and Methods

Chemicals and Enzymes. Saframycins A and B were prepared in our laboratory, dissolved in methanol at a concentration of 10 mg/mL, and stored at −20 °C. None of the effects of the solvent was observed in any assay systems. *Escherichia coli* RNA polymerase (EC 2.7.7.6), poly(dG)·poly(dC), and poly(dA)·poly(dT) were obtained from Boehringer Mannheim Corp. [5-^3H]Uridine (26.7 Ci/mmol), [6-^3H]-thymidine (13.9 Ci/mmol), a uniformly, ^{14}C-labeled L-amino acid mixture (15 kinds of amino acids, 93–414 mCi/mmol), [5,6-^3H]UTP (41.4 Ci/mmol), [5-^3H]CTP (22 Ci/mmol), Aquasol-2, and Protosol were purchased from New England Nuclear, Boston, Mass. Agarose was "Seakem" obtained from Marine Colloids, Inc.

Cells. L 1210 cells were maintained in a suspension culture in Eagle's minimum essential medium supplemented with 10% fetal calf serum as described previously (Ishiguro & Arai, 1976). All experiments were performed with cells in the logarithmic phase of growth.

Macromolecular Synthesis of L 1210 Cells. To determine the effect of saframycin A on the synthesis of RNA, DNA, and protein, cells at a density of 9.45×10^5 cells/mL were treated

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