Inhibition of Ribosomal Peptidyltransferase with 2′(3′)-O-Acetyl-2″(3″)-O-glycyl-1,2-di(adenosin-N^6-yl)ethane and -1,4-di(adenosin-N^6-yl)butane. Effect of Alkyl Chain Length[1-4]

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The chemical synthesis of the title compounds XIIIa and XIIIb is described. The reaction of N^6-(2-aminoethyl)adenosine (Ia) with methyl orthoacetate (II) in the presence of HCl in DMF gave the 2′,3′-cyclic orthoester of the pyrazoline nucleoside III which, on hydrolysis, afforded compound VI. A similar transformation of Ia effected with triethyl N-(benzyloxycarbonyl)orthoglycinate (IV) and CF_3COOH in DMF gave the cyclic derivative V, whereas chloronucleoside VII was converted to VIII. The reaction of VII with orthoacetate II using HCl in DMF afforded the dioxolane IX which, in turn, was transformed to the corresponding N^6-(ω-aminoalkyl) derivative X employing an excess of 1,4-diaminobutane. A similar reaction of VIII gave intermediate Vb. Coupling of Va with IX effected with triethylamine in DMF led to "bridged" cyclic orthoester XIa. Condensation of Vb with IX or VIII with X afforded a similar intermediate, XIb. Hydrolysis of XIa and XIb with 80% formic acid at −15 °C led to the corresponding bridged nucleosides XIIa and XIIb. Hydrogenolysis of the latter using PdO–$BaSO_4$ in cold 80% acetic acid gave the title models XIIIa and XIIIb. Compounds XIIIa and XIIIb inhibited the ribosome-catalyzed puromycin reaction to a greater extent than A-Gly. The inhibition is dependent on the length of the alkyl chain in the "bridge". Thus, XIIIb is a better inhibitor than XIIIa. Inhibition of the puromycin reaction with XIIIb is strictly competitive. The results indicate that XIIIb and possibly also XIIIa are not multisubstrate (transition state) analogues of the ribosomal peptidyltransferase-catalyzed protein synthesis. Instead, they simulate the 3′ terminal of the aminoacyl (acceptor) tRNA. The implications of these findings for the mechanisms of action of peptidyltransferase are discussed.

It is generally assumed that peptide bond formation is catalyzed by ribosomal peptidyltransferase and involves a direct reaction of peptidyl- and aminoacyl-tRNA.[5] Although the substrate requirements of the process are now well documented,[6a,b] the intimate details, i.e., nature of the intermediate(s) or transition state(s) involved and topochemistry of both tRNAs, remain elusive. In order to shed more light on these questions, we have prepared several 2′(3′)-O-aminoacyl derivatives of a "bridged" adenosine, 1,2-di(adenosin-N^6-yl)ethane,[7a,b] including a "bifunctional" derivative comprising the features of both peptidyl- and aminoacyl-tRNA.[2b] The compounds all proved to be good substrates for peptidyltransferase acting at the ribosomal A site. It was concluded that the above compounds do not resemble the transition state of peptide bond formation catalyzed by peptidyltransferase but rather the 3′ terminal of aminoacyl (acceptor) tRNA.[2b,7b] Comparison of space-filling models of bridged adenosine nucleosides [1,2-di(adenosin-N^6-yl)ethane] with the corresponding 3′ terminal segment of tRNA (C-A) revealed a striking similarity in a stacked form.[7b,8] Further model studies, supported by hypochromism measurements,[10] have shown that with an increasing chain length of the alkyl bridge the distance between the base ("adenine") residues would simulate even more closely the situation found in C-A (Figure 1).

It was therefore of interest to examine the influence of the alkyl chain length in aminoacyl bridged nucleosides on their acceptor or inhibitory activity in the peptidyltransferase-catalyzed peptide bond formation. The chemical synthesis of previously described models containing phenylalanyl or leucyl and N-acetylleucyl moieties at their respective ribofuranose portions[2b] is tedious and time consuming. We have therefore sought a simpler approach to prepare suitable models that retain the ability to function as substrates or inhibitors of peptidyltransferase. The present study describes the chemical synthesis and peptidyltransferase assay of the title compounds, XIIIa and XIIIb, which differ by the presence of two methylene groups in the alkyl chain joining both purine moieties.

Synthesis. The target bridged derivatives XIIIa and XIIIb contain different but relatively simple substituents in their ribose moieties—an acetyl and glycyl group. One approach to the aminoacyladenine bridged nucleosides with selectively functionalized sugar portions is a stepwise acylation (aminoacylation) of a suitably protected derivative.[2b] This rather lengthy sequence includes separation of complex mixtures by TLC or column chromatography in several steps. We have therefore used the "orthoester[3b] method", which shortened the entire procedure i.a. by

(1) This paper is no. 5 in the series "Simple Models of Nucleic Acid Interactions" and no. 31 of the series "Aminoacyl Derivatives of Nucleosides, Nucleotides and Polynucleotides". For preceding reports, see ref 2a,b; for a preliminary account, see ref 3a,b.

(2) (a) J. Zemlička. *Biochemistry*, 19, 163 (1980); (b) C. Li, P. Bhuta, and J. Zemlička, *ibid.*, 17, 2537 (1978).

(3) (a) J. Zemlička, P. Bhuta, C. Li, M. Murata, and J. Owens, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 37, 1306 (1978); (b) J. Zemlička, M. Murata, and J. Owens, *ACS Symp. Ser.*, no. 73, 69 (1978).

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(5) Abbreviations used are: N-AcPhe-tRNA, N-acetyl-L-phenylalanyl transfer ribonucleic acid; AcPhe, N-acetyl-L-phenylalanyl; CPK models, Corey–Pauling–Koltun models; A-Gly, 2′(3′)-O-glycyladenosine; A-Ac, 2′(3′)-O-acetyladenosine; DMF, dimethylformamide; C-A, cytidylyl-(3′–5′)-adenosine; Gly, glycyl; Ac, acetyl; Phe, L-phenylalanyl; AcLeu, N-acetyl-L-leucyl; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; CD, circular dichroism; TLC, thin-layer chromatography.

(6) (a) R. J. Harris and R. H. Symons, *Bioorg. Chem.*, 2, 286 (1973); (b) A. A. Krayevsky, M. K. Kukhanova, and B. P. Gottikh, *Nucleic Acids Res.*, 2, 2233 (1975).

(7) (a) C. Li and J. Zemlička, *J. Org. Chem.*, 42, 706 (1977); (b) P. Bhuta, C. Li, and J. Zemlička, *Biochem. Biophys. Res. Commun.*, 77, 1237 (1977).

(8) It is of interest to note that stacking of cytosine and adenine residues in C-A is well established from hypochromism, circular dichroism,[9a] and NMR[9b] stuides. By contrast, X-ray diffraction of $tRNA^{Phe}$ from yeast at 3-Å resolution has shown that the 3′ terminal adenosine is destacked.[9c] The biological significance of the latter finding, if any, is not yet clear.

(9) (a) M. M. Warshaw and I. Tinoco, Jr., *J. Mol. Biol.*, 20, 29 (1966); (b) B. W. Bangerter and S. I. Chan, *J. Am. Chem. Soc.*, 91, 3910 (1969); (c) A. Rich, *Acc. Chem. Res.*, 10, 388 (1977).

(10) J. Zemlička and J. Owens, *J. Org. Chem.*, 42, 517 (1977).

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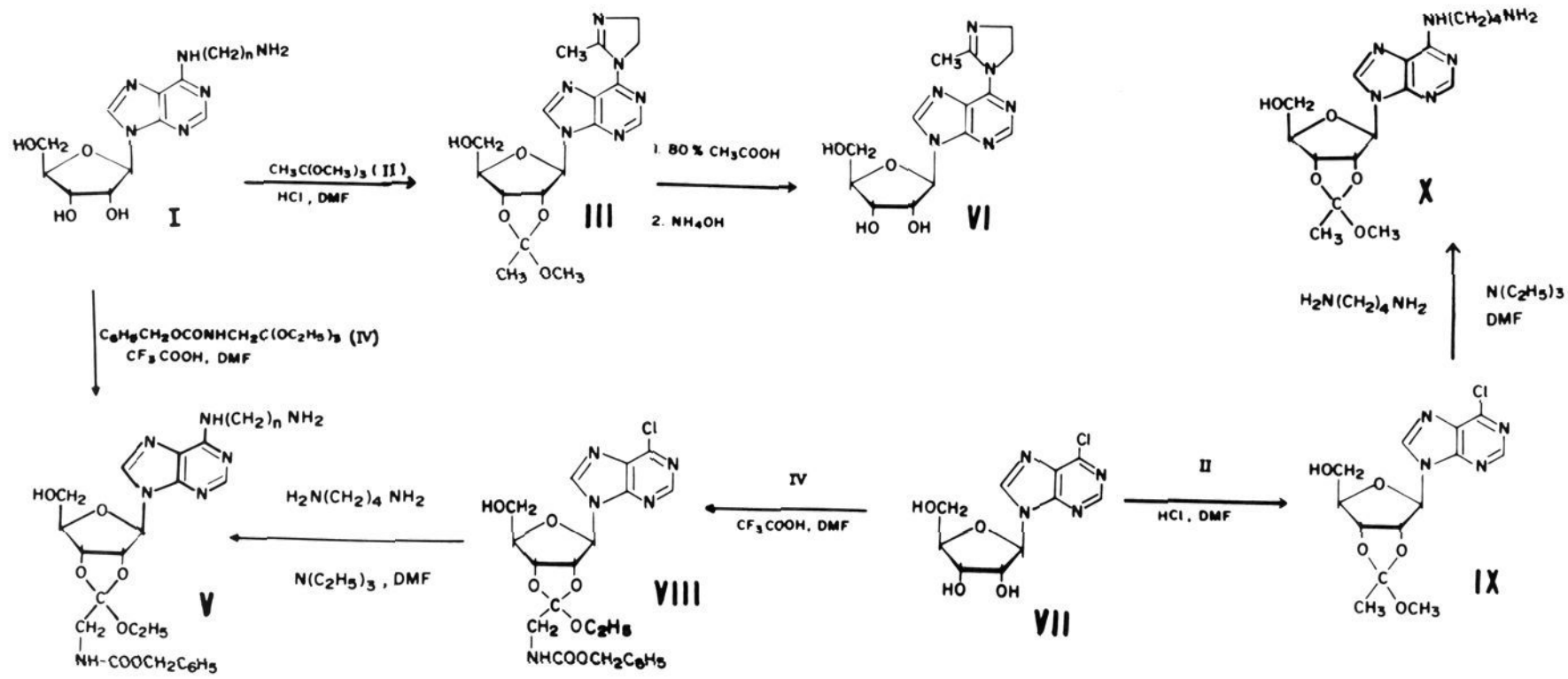
Scheme I



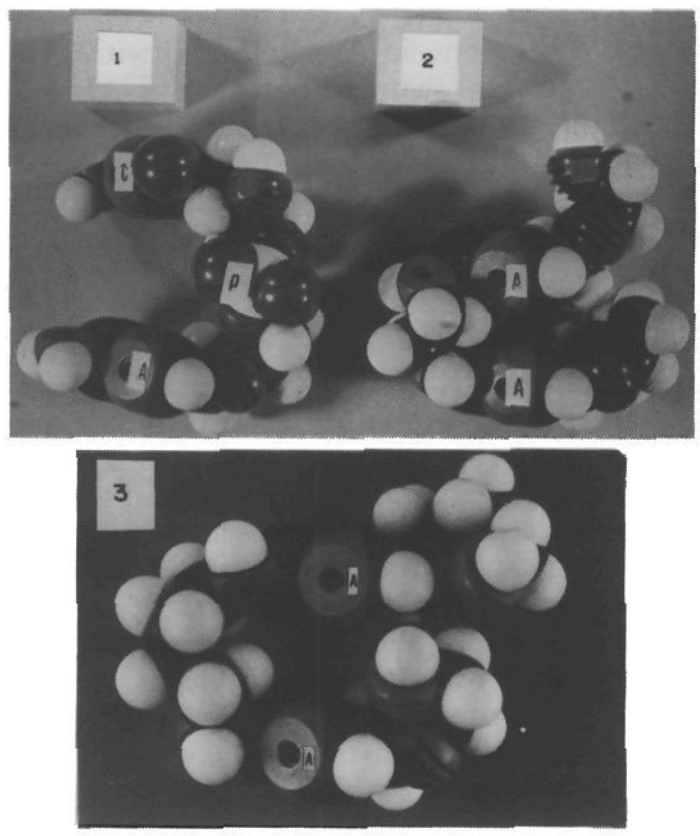


Figure 1. Comparison of space-filling models of C-A and "bridged" adenosines XIVa and XIVb. For corresponding structural formulas of XIVa and XIVb, cf. Scheme II: 1 = C-A; 2 = XIVa; 3 = XIVb; p = phosphodiester bridge; C = cytosine; A = adenine residue. Note that the distance between bases in XIVb resembles more closely that in C-A than XIVa.

eliminating some laborious chromatographic separations.

The preparation of orthoester precursors is usually simple and straightforward. However, the reaction of N^6-(2-aminoethyl)adenosine[10] (Ia) with orthoester II, catalyzed with HCl in DMF, proved to be an exception. Thus, the 2-aminoethyl moiety of Ia underwent a cyclization in addition to the formation of 2′,3′-cyclic orthoester giving the imidazoline derivative III in 45% yield (Scheme I). Hydrolysis of III with 80% acetic acid, followed by ammonolysis, gave the free nucleoside VI in almost quantitative yield. By contrast, the reaction of Ia with triethyl *N*-(benzyloxycarbonyl)orthoglycinate (IV), catalyzed with CF_3COOH in DMF, readily afforded compound V as a single product in 70% yield. Likewise, 6-chloro-9-β-D-ribofuranosylpurine (VII) and methyl orthoacetate

Table I. Diastereoisomeric Composition of Some Ribonucleoside 2′,3′-Cyclic Orthoesters Determined by NMR Spectroscopy

compd	NMR signal	% endo[a]	% exo[a]
III	H_8	37.3	62.7
	$H_{1'}$	36.5	63.5
	OCH_3	38.3	61.7
	$CH_3C\leftarrow$	38.5	61.5
IX	$H_{1'}$	36.2	63.8
	OCH_3	36.6	63.4
	$CH_3C\leftarrow$	38.2	61.8
X	H_1[b]	40	60
	OCH_3	37.9	62.1
2′,3′-*O*-(methoxymethylidene)uridine[c]	$H_{1'}$	37	63
2′,3′-*O*-[ethoxy(2-phenylethyl)methylidene]adenosine	H_8	36.6[d]	63.4[d]
	$H_{1'}$	35.4	64.6

[a] Determined from the height of the corresponding signal.[16] [b] The signal was not well resolved. Calculated from the corresponding integration curve. [c] Calculated from the published NMR spectrum.[11a] [d] CD_3COCD_3 + D_2O.[11c]

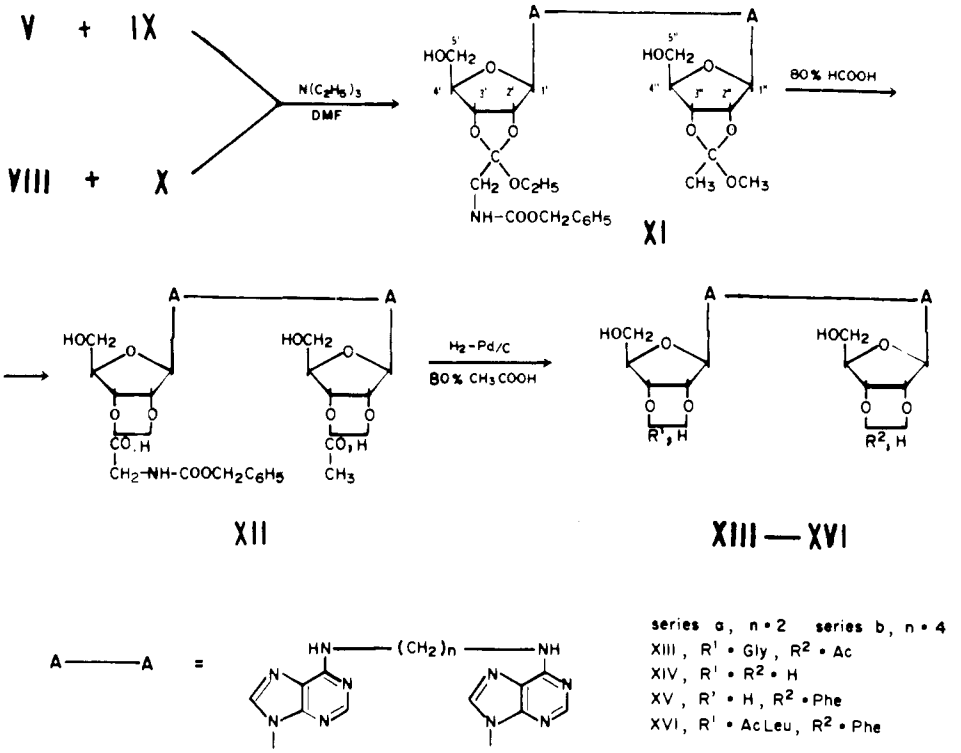
(II) in the presence of HCl in DMF afforded the desired cyclic orthoester IX as a syrup in 80% yield. A similar orthoester exchange with IV gave compound VIII (84%).

The precursors of the series b (Scheme I) were obtained in a similar fashion. Thus, N^6-(4-aminobutyl)adenosine[10] (Ib) reacted with orthoester IV in the presence of CF_3COOH as catalyst to give the corresponding dioxolane derivative Vb in almost 40% yield. The cyclic orthoacetate X was obtained from the chloronucleoside IX and excess 1,4-diaminobutane using triethylamine in DMF[10] in about 70% yield.

NMR spectroscopy confirmed the structures of orthoester precursors. As previously demonstrated,[11a-c] the 2′,3′-cyclic orthoesters of ribonucleosides are mixtures of exo and endo stereoisomers formed in a ratio of 3:2. This

(11) (a) H. P. M. Fromageot, B. E. Griffin, C. B. Reese, and J. E. Sulston, *Tetrahedron*, **23**, 2315 (1967); (b) J. Žemlička, *J. Am. Chem. Soc.*, **97**, 5896 (1975); (c) J. Žemlička, S. Chládek, D. Ringer, and K. Quiggle, *Biochemistry*, **14**, 5239 (1975).

Scheme II



was further confirmed in the present study. Thus, the 2′,3′-cyclic orthoesters listed in Table I contain 60–65% of the exo and 35–40% of the endo form. In most cases, it was possible to determine, with a good accuracy, the exo/endo ratio based on more than one signal of the corresponding NMR spectrum.

The coupling of orthoester intermediates was performed according to the procedure employed for a similar reaction of free nucleosides.[10] Thus, N^6-(2-aminoalkyl) derivative Va and chloronucleoside IX afforded, in the presence of triethylamine in DMF, the bridged intermediate XIa in 20% yield after purification by preparative TLC (Scheme II). Similarily, the coupling of Vb and IX gave the tetramethylene compound XIb in 40% yield. In an alternate coupling mode, precursors VIII and X afforded intermediate XIb in 64% yield. The structures of XIa and XIb were confirmed by NMR spectra, which were rather complex. This is not surprising in view of the fact that XIa and XIb are mixtures of four endo/exo stereoisomers. Hydrolysis of cyclic orthoesters XIa and XIb with 80% formic acid at −15 °C for 1 h gave, after preparative TLC, the acetylglycyl derivatives XIIa and XIIb in 51 and 81% yield, respectively. Both products, which are probably mixtures of four possible positional isomers, were homogeneous on TLC. Finally, hydrogenolysis of both *N*-(benzyloxycarbonyl) derivatives, XIIa and XIIb, in cold 80% acetic acid for 4 h using $Pd/BaSO_4$ as catalyst afforded the target derivatives XIIIa and XIIIb (71 and 100% yield). Both products were characterized by paper electrophoresis and yellow coloration with ninhydrin typical for similar glycine esters.[12]

Biological Activity. Glycyl derivatives XIIIa and XIIIb did not exhibit any acceptor activity in the reaction with *N*-AcPhe-tRNA catalyzed by 70S ribosomes (data not shown). This was hardly surprising in view of a similar inactivity of A-Gly.[13] However, the results of the inhibition of peptidyltransferase-catalyzed puromycin reaction with compounds XIIIa and XIIIb summarized in Figure 2 are more interesting. It is clear that both XIIIa and XIIIb are substantially better inhibitors than A-Gly, although even marginal inhibitory activity of the latter is

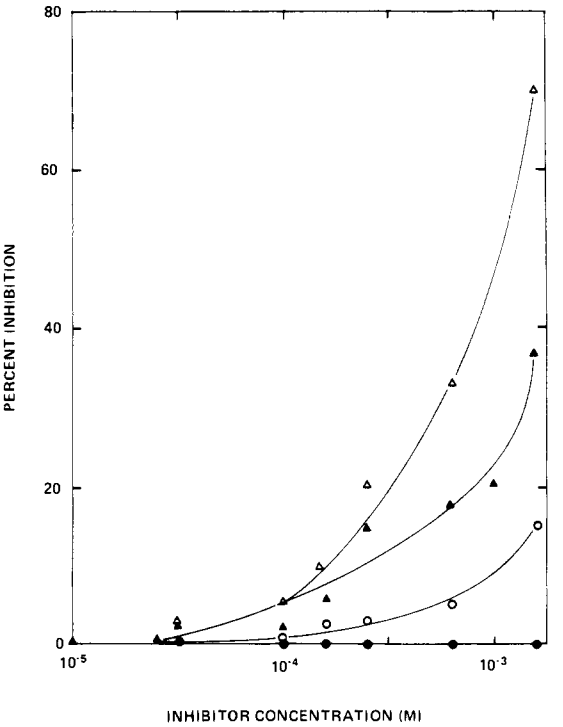


Figure 2. Inhibition of *N*-acetyl-L-[^{14}C]phenylalanylpuromycin formation at 37 °C. Reactions were performed as described under Experimental Section. Puromycin concentration was kept at 1 × 10^{-4} M with the varied concentrations of inhibitors. Incubation was for 30 min. Percent inhibition represents the difference in ethyl acetate extracted *N*-acetyl-L-[^{14}C]phenylalanylpuromycin counts in the absence and in the presence of inhibitors. Inhibitors added were: (a) XIIIb, △; (b) XIIIa, ▲; (c) A-Gly, ○; (d) A-Ac,

(12) J. Žemlička and S. Chládek, *Biochemistry*, 10, 1521 (1971).
(13) I. Rychlík, S. Chládek, and J. Žemlička, *Biochim. Biophys. Acta*, 138, 640 (1967).

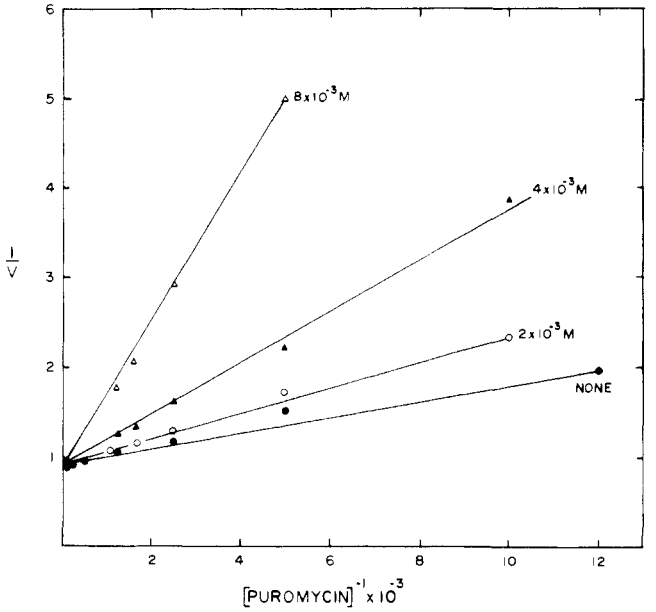


Figure 3. Lineweaver–Burk plot of the effect of XIIIb on the rate of *N*-acetyl-L-[^{14}C]phenylalanylpuromycin formation. The puromycin concentration was varied as indicated on the abscissa and the concentration of XIIIb is shown in the figure. The initial velocity, *V*, was defined as the amount of *N*-acetyl-L-[^{14}C]-phenylalanylpuromycin formed per milliliter of ethyl acetate in the first 5 min of reaction at 32 °C. The experimental conditions were the same as those described in a previous communication.[2b]

of interest in view of its complete lack of acceptor properties. The other model, A-Ac, was used in addition to A-Gly to determine the extent of inhibition of peptidyltransferease with the corresponding "halves" of molecules of XIIIa and XIIIb, respectively. However, A-Ac was totally inactive.

It is also apparent that the inhibition of peptidyltransferase strongly depends on the length of the bridge between both purine residues. Thus, the tetramethylene derivative XIIIb is more effective than compound XIIIa with only two methylene units in the bridge. Lack of acceptor properties precluded a determination of the site specificity of XIIIa and XIIIb on the basis of peptide transfer itself. However, kinetic studies have shown that the most active derivative, XIIIb, acts as a competitive inhibitor in the peptidyltransferase-catalyzed transfer of AcPhe from *N*-AcPhe-tRNA to puromycin (Figure 3). It seems reasonable, therefore, to conclude that the high inhibitory activity of XIIIb reflects its resemblance to the 3′ terminus of aminoacyl-tRNA at the ribosomal A site. As can be seen from Figure 1, the time-averaged distance between bases in C-A corresponds more closely to that of compound XIVb than that of XIVa. This is further supported by UV spectral studies[10] at pH 7, which showed a higher hypochromism (*H*) and, hence, a shorter distance between the bases for XIVa (*H* = 19.2) than XIVb (*H* = 8.2).

It is also of interest to compare the above results with those obtained using 2′(3′)-*O*-L-phenylalanyl and 2′(3′)-*O*-(*N*-acetyl)-L-leucyl-2″(3″)-*O*-L-phenylalanyl esters XVa and XVIa derived from a nucleoside with two methylene units in the bridge.[2b] The latter were excellent acceptors of AcPhe from *N*-AcPhe-tRNA in a ribosomal system and inhibited competitively the puromycin reaction. Although the corresponding pair of compounds derived from a tetramethylene-bridged nucleoside, XVa and XVIb, is not yet available, the results obtained with XIIIa and XIIIb suggest that such models would possess even higher acceptor activity than XVa and XVIa. It is also reasonable to

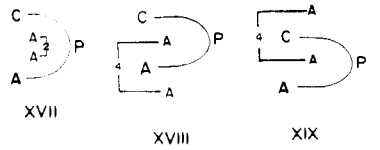


Figure 4. Self-intercalation of bridged nucleosides XIVa and XIVb with a C-A unit. For the corresponding situation of two C-A units including CPK models, cf. ref 2b. Abbreviated notation[9b] is used: A = adenine or purine residue of XIVa (-2-) or XIVb (-4-); P = phosphodiester bridge.

assume that compounds XIIIa and XIIIb bind to the A site of peptidyltransferase in a stacked form.[7b] One possibility of such an interaction can be visualized in the case of a more tightly stacked derivative, XVa, as an intercalation between the adenine and cytosine rings of the C-A terminus of peptidyl-tRNA (Figure 4, formula XVII). Compound XIVb with base residues more distal could then intercalate by inserting one of them between the C-A unit (Figure 4, formulas XVIII and XIX). Such an interaction then closely resembles the previously suggested[2b] intercalation of both C-A termini of aminoacyl- and peptidyl-tRNA.

Our results have provided evidence that the length of an aliphatic chain between both purine residues is important for the inhibition of the peptidyltransferase-catalyzed puromycin reaction with models XIIIa and XIIIb. We have found that compound XIIIb is not a transition-state analogue of the AcPhe transfer from *N*-AcPhe-tRNA; rather, it simulates the 3′ terminus of aminoacyl-tRNA. In this respect, conclusions drawn on the basis of compound XIIIb (tetramethylene bridge) are similar to those based on a set of models with two methylene units in the bridge[2b] (XVa and XVIa).

Experimental Section

General Methods.[7a] TLC, including preparative TLC, was performed as described[10] in the following solvents: S_1, dichloromethane (chloroform)–methanol (9:1); S_2, dichloromethane (chloroform)–methanol (4:1); S_3, dichloromethane (chloroform)–methanol (1:1). Descending paper chromatography on Whatman No. 1 paper and TLC on microcrystalline (Avicel) cellulose plates was conducted in the following solvents: S_4, 2-propanol–NH_4OH–H_2O (7:1:2); S_5, 1-butanol saturated with 1 N NH_4OH; S_6, 1-butanol–acetic acid–H_2O (5:2:3). NMR spectra were determined using Varian A60-A instrument unless specified otherwise. Tetramethylsilane was used as an internal reference in $CDCl_3$ and CD_3COCD_3 and DSS as an external reference in CD_3SOCD_3.

Starting Materials. 6-Chloro-9-β-D-ribofuranosylpurine[14] and triethyl *N*-(benzyloxycarbonyl)orthoglycinate[15] were prepared as described. N^6-(2-Aminoethyl)- and N^6-(4-aminobutyl)adenosine were obtained as reported previously.[10] A-Gly[15] and A-Ac[11a] were prepared as described.

9-β-D-[2,3-*O*-(Methoxyethylidene)ribofuranosyl]-6-(2-methyl-4,5-dihydroimidazolyl)purine (III). N^6-(2-Aminoethyl)adenosine (Ia; 0.31 g, 1 mmol) was stirred with trimethyl orthoacetate (1 mL) and 6 M HCl in DMF (0.55 mL, 3.3 mmol) in DMF (10 mL) at room temperature for 24 h. The progress of the reaction was checked by TLC (S_2) along with the pH, which was kept at ca. 1 by adding a new portion(s) of 6 M HCl in DMF when necessary. Triethylamine (2 mL) was then added and the solution was evaporated. The residue was partitioned between aqueous saturated $NaHCO_3$ (10 mL) and dichloromethane (3 × 20 mL). The dried ($MgSO_4$) organic phase was evaporated and

(14) J. Žemlička and J. Owens, in "Nucleic Acid Chemistry—Improved and New Synthetic Procedures, Methods, and Techniques", Part 2, L. B. Townsend and R. S. Tipson, Eds, Wiley, New York, 1978, p 611.
(15) J. Žemlička and S. Chládek, *Collect. Czech. Chem. Commun.*, 31, 3775 (1966).

the crude product III chromatographed (loose layer of silica gel) in solvent S_1 containing 0.2% of triethylamine (double development). The major UV-absorbing band was eluted with the solvent, the eluate was evaporated, and the residue was dissolved in dichloromethane (5 mL). Addition of petroleum ether (100 mL) precipitated product III as an amorphous powder, which was collected by filtration; it was washed with petroleum ether and dried in vacuo over P_2O_5: yield 0.175 g (45%); homogeneous on TLC (S_1); UV max (ethanol) 267 nm (ϵ 18 200), min 230 (ϵ 2100); NMR (CD_3COCD_3) δ 8.28 (s, H_8, endo[16]), 8.27 (s, total 1, H_8, exo[16]), 8.22 (s, 1, H_2), 6.34 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$, endo), 6.17 (d, $J_{1',2'}$ = 3.5 Hz, total 1, $H_{1'}$, exo), 3.43 (s, OCH_3, endo), 3.23 (s, OCH_3, exo, total 3), 1.83 (s, 3, CH_3 of imidazoline), 1.68 (s, $CH_3C\leq$, exo), 1.56 (s, $CH_3\leq$, endo, total 3). For diastereoisomeric composition, cf. Table I. Anal. ($C_{17}H_{22}N_6O_5\cdot1.75H_2O$) C, H, N.

6-(2-Methyl-4,5-dihydroimidazolyl)-9-β-D-ribofuranosylpurine (VI). A solution of compound III (60 mg, 0.154 mmol) in 80% acetic acid (2 mL) was kept at room temperature for 80 min, whereupon it was lyophilized. TLC (S_1) showed a complete hydrolysis after 1 h. The residue was lyophilized from water (2 × 2 mL) and then it was dissolved in methanol saturated with NH_3 at 0 °C. The solution was kept for 140 min at room temperature (the crystalline VI separated) and the solvents were evaporated. Methanol (1 mL) was added and the product VI was collected by filtration: yield 50 mg (97%); mp 157–160 °C (transition point). Crystalization from methanol (8 mL) afforded 40 mg (78%) of III: mp 153–155 °C (transition point); UV max (H_2O) 268, 211 nm (ϵ 21 500, 22 400), min 232 (ϵ 2700); CD max (H_2O) 270 nm ([θ] −3500); NMR (CD_3SOCD_3 + D_2O) δ 8.75 (s, 1, H_8), 8.63 (s, 1, H_2), 6.34 (s, $J_{1',2'}$ = 6 Hz, 1, $H_{1'}$), 2.23 (s, 3, CH_3). Anal. ($C_{14}H_{18}N_6O_4\cdot2.25H_2O$) C, H, N.

2',3'-O-[[[N-(Benzyloxycarbonyl)amino]methyl]ethoxymethylidene]-N^6-(4-aminobutyl)adenosine (Vb). A mixture of N^6-(4-aminobutyl)adenosine (Ib; 1 g, 3 mmol), orthoester IV (2 g, 6.4 mmol), and trifluoroacetic acid (0.66 mL, 8.8 mmol) in DMF (30 mL) was stirred for 21 h at room temperature. The reaction was quenched by the addition of triethylamine to pH 8–9 (pH paper), and the solution was evaporated. The residue was partioned between dichloromethane (80 mL) and saturated aqueous $NaHCO_3$ (30 mL). The organic phase was washed with 1% aqueous $NaHCO_3$ (2 × 20 mL), dried ($MgSO_4$), and evaporated to give a syrup (0.87 g), which was chromatographed on two loose layers of silica gel in solvent S_3 containing 0.1% of triethylamine. The major UV absorbing band was eluted with the same solvent, and the eluate was evaporated to give the amorphous powder Vb: yield 0.64 g (39%); homogenous on TLC (S_3 and S_4); UV max (ethanol) 267 nm; NMR ($CDCl_3$) δ 7.95 (s, 1, H_8), 7.47 (s, 1, H_2), 7.02 (m, 5, phenyl), 5.92 (d, $J_{1',2'}$ = 2 Hz, 1, $H_{1'}$), 5.25 (s, 1, CH_2 of benzyl), 3.28–3.80 (m, CH_2 of C_2H_5O overlapped with glycine and ribofuranose CH_2), 1.18 (t, 3, CH_3 of C_2H_5O). Anal. ($C_{26}H_{35}N_7O_7\cdot1.5H_2O$) C, H, N.

9-[2',3'-O-[[[N-(Benzyloxycarbonyl)amino]methyl]ethoxymethylidene]-β-D-ribofuranosyl]-6-chloropurine (VIII). A mixture of nucleoside VII (1 g, 3.5 mmol), orthoester IV (1.41 g, 4.5 mmol), trifluoroacetic acid (0.63 mL, 8.4 mmol), and DMF (30 mL) was kept overnight at room temperature. TLC (S_1) indicated that the reaction was complete after 3 h. The workup followed the procedure described in the previous preparation (compound Vb). Compound VIII was obtained as an amorphous powder after precipitation from dichloromethane solution (20 mL) with petroleum ether (200 mL): yield 1.48 g (84%). It moves on TLC (S_1) as a double spot (mixture of diastereoisomers). A sample for analysis was purified by loose-layer TLC (S_1): UV max (ethanol) 264 nm (ϵ 6900), min 227 (ϵ 1900); NMR (CD_3COCD_3) δ 8.64 and 8.57 (2 s, 2, H_8 + H_2), 7.26 (m, 5, phenyl), 6.50 and 6.37 (poorly resolved m, $H_{1'}$ of endo and exo stereoisomers), 5.13 (s, overlapped with ribose signals, CH_2 of benzyl). Anal. ($C_{22}H_{24}ClN_5O_7\cdot0.25H_2O$) C, H, N.

2',3'-O-[[[N-(Benzyloxycarbonyl)amino]methyl]ethoxymethylidene]-N^6-(2-aminoethyl)adenosine (Va). A mixture of N^6-(2-aminoethyl)adenosine (Ia; 0.62 g, 2 mmol), orthoester IV (1.38 g, 4 mmol), trifluoroacetic acid (0.45 mL, 6 mmol), and DMF (20 mL) was stirred for 6.5 h at room temperature. TLC (S_5) showed a single UV absorbing component. Triethylamine was then added to pH 8–9 and the solution was evaporated. The residue was partitioned between dichloromethane (50 mL) and saturated aqueous $NaHCO_3$ (20 mL). The organic layer was dried ($MgSO_4$) and evaporated to give a syrupous Va: homogenous on TLC (S_5); yield 71%, as determined by UV spectrophotometry, which after trituration with ether–petroleum ether afforded a yellow foamy solid (purity 68%). This product was used in condensation with IX. An analytical sample was obtained by preparative TLC (S_5) of the above material (0.1 g), which removed the faster moving, colored impurities to give pure Va: yield 60 mg; ninhydrin-positive; UV max (ethanol) 273 nm (ϵ 17 200); NMR (CD_3COCD_3, FT 100) δ 8.26, 8.23, 8.21, and 8.13 (4 s, 2, H_8 + H_2), 7.31 (s, 5, C_6H_5), 6.37 (d, $H_{1'}$, endo), 6.27 (d, $H_{1'}$, exo, total 1), 5.15 (s, CH_2 of $C_6H_5CH_2$, endo), 5.07 (s, CH_2 of $C_6H_5CH_2$, exo), 1.12 (m, 3, CH_3 of C_2H_5). Anal. ($C_{24}H_{31}N_7O_7\cdot H_2CO_3\cdot1.25H_2O$) C, H, N.

6-Chloro-9-[2,3-O-(methoxyethylidene)-β-D-ribofuranosyl]purine (IX). A mixture of nucleoside VII (0.57 g, 2 mmol), orthoester II (1 mL, 8 mmol), 6 M HCl in DMF (1 mL, 9.6 mmol), and DMF (20 mL) was kept overnight at room temperature. TLC (S_1) showed the reaction was essentially complete. Triethylamine was added to pH 8–9, the solution was evaporated, and the residue was partitioned between dichloromethane (50 mL) and saturated aqueous $NaHCO_3$ (20 mL). The dried ($MgSO_4$) organic phase was evaporated to give syrup IX in 80% yield, as determined by UV spectrophotometry, containing 2 mol of DMF (NMR) whose purity was sufficient to be used in the next step (preparation of X). Preparative TLC of this product in S_1 afforded a TLC-homogeneous (S_1) syrup, IX: UV max (ethanol) 264 nm, 250 (sh), min 225; NMR (CD_3COCD_3) δ 8.74 (s, 1, H_8), 8.68 (s, 1, H_2), 6.53 (d, $J_{1',2'}$ = 3 Hz, $H_{1'}$, endo), 6.38 (d, $J_{1',2'}$ = 2.5 Hz, $H_{1'}$, exo, total 1), 3.48 (s, OCH_3, endo), 3.30 (s, OCH_3, exo, total 3), 1.74 (s, $CH_3C\leq$, exo), 1.62 (s, $CH_3C\leq$, endo, total 3). For diastereoisomeric composition, cf. Table I.

N^6-(4-Aminobutyl)-2',3'-O-(methoxyethylidene)adenosine (X). A mixture of orthoester derivative IX (1.2 g, 3.5 mmol), 1,4-diaminobutane (1.35 g, 15 mmol), and triethylamine (0.4 g, 4 mmol) in DMF (50 mL) was stirred at room temperature for 16 h. The precipitated triethylamine hydrochloride was filtered off, the filtrate was evaporated, and the residue was partitioned between dichloromethane (150 mL) and aqueous $NaHCO_3$ (30 mL). The organic phase was dried ($MgSO_4$) and evaporated to a syrup, which was dried at 0.01 mm overnight. It was then chromatographed on two loose layers of silica gel in solvent S_3 containing 0.1% of triethylamine. The major UV-absorbing band was eluted with the same solvent (1 L) to give the hygroscopic powder X: yield 0.94 g (68%); homogeneous on TLC (S_3) and paper chromatography (S_4); ninhydrin positive; UV max (ethanol) 267 nm (ϵ 16 000); NMR ($CDCl_3$) δ 8.71 (s, 1, H_8), 8.26 (s, 1, H_2), 6.70 (poorly resolved d, $H_{1'}$, endo), 6.24 (poorly resolved d, $H_{1'}$, exo, total 1), 3.62 (s, OCH_3, endo), 3.44 (s, OCH_3, exo, total 3), 1.82 (s, $CH_3C\leq$, exo), 1.70 (s, $CH_3C\leq$, endo, total 3). For diastereoisomeric composition, cf. Table I.

2',3'-O-[[[N-(Benzyloxycarbonyl)amino]methyl]ethoxymethylidene]-2'',3''-O-(methoxyethylidene)-1,4-di(adenosin-N^6-yl)butane (XIb). Method A. Coupling of Vb and IX. A mixture of orthoester Vb (1.4 g, 2.5 mmol), IX (0.87 g, 2.5 mmol), and triethylamine (1 mL, 7.2 mmol) in DMF (15 mL) was stirred for 18 h at room temperature. The resultant solution was evaporated to a syrup, which was chromatographed on two loose layers of silica gel in solvent S_1 containing 0.2% of triethylamine. The major UV-absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was triturated with ether to give 0.88 g (40%) of XIb as an amorphous powder. TLC (S_1) showed a doubled spot (mixture of diastereoisomers). An analytical sample was obtained by rechromatography of this material on Stahl's layer of silica gel in S_1: UV max (ethanol) 267 nm (ϵ 32 400), min 230 (ϵ 3300); NMR ($CDCl_3$) δ 8.15 (s, H_8, 2), 7.74 (s, H_2, 2), 7.22 (s, C_6H_5, 5), 6.06 (br s, $H_{1'}$, 2), 3.37 (s, CH_3O, endo), 3.23 (s, CH_3O, exo, total 3), 2.72 (q, CH_2 of C_2H_5, 2), 1.72 (s, CH_3-C$\leq$, exo), 1.60 (s, CH_3-C$\leq$, endo, the CH_3 signals are overlapped with N-CH_2 bands of tetramethylene bridge), 1.15 (t, CH_3 of C_2H_5O, 3). Anal. ($C_{39}H_{49}N_{11}O_{12}\cdot1.5H_2O$) C, H, N.

(16) Endo and exo assignments are tentative throughout the paper. The less abundant stereoisomer with the more deshielded $H_{1'}$ is assumed to be endo.[11b]

Method B. Coupling of VIII and **X.** A solution of orthoester VIII (0.58 g, 1.14 mmol), compound X (0.45 g, 1.14 mmol), triethylamine (0.23 g, 2.3 mmol), and DMF (10 mL) was stirred for 18 h at room temperature. The reaction mixture was worked up as in method A to give 0.48 g (64%) of XIb identical (TLC, UV) with the material obtained above.

2′(3′)-*O*-Acetyl-2″(3″)-*O*-[*N*-(benzyloxycarbonyl)-glycyl]-1,4-di(adenosin-N^6-yl)butane (XIIb). A solution of orthoester XIb (0.15 g, 0.17 mmol) in 80% HCOOH (5 mL) was kept at −15 to −20 °C for 1 h; then it was diluted with water (25 mL) and lyophilized. The residue was chromatographed on two loose layers of silica gel in solvent S_1 (developed twice). The major UV-absorbing band was eluted with the same solvent, and the eluate was evaporated to give 115 mg (81%) of XIIIb. An analytical sample was obtained by rechromatography of this material on Stahl's layer of silica gel in $CHCl_3$–CH_3OH–CH_3COOH (9:0.5:0.5): UV max (0.01 HCl) 263 nm (ε 34 000), min 232 (ε 6800); NMR ($CDCl_3$) δ 7.87 (br s, 4, $H_8 + H_2$), 7.40 (s, 5, C_6H_5), 5.22 (s, 2, CH_2 of $C_6H_5CH_2$), 1.18 (s, 3, CH_3 of CH_3CO), the rest of the signals were poorly resolved. Anal. ($C_{36}H_{43}N_{11}O_{12}\cdot 2CH_3COOH\cdot 0.5H_2O$) C, H, N.

2′,3′-*O*-[[[*N*-(Benzyloxycarbonyl)amino]methyl]ethoxymethylidene]-2″,3″-*O*-(methoxyethylidene)-1,2-di(adenosin-N^6-yl)ethane (XIa). Coupling of Va and IX was performed as described for compound XIb (method A) on a 1-mmol scale. The derivative XIa was obtained in 20% yield after purification by preparative TLC (cf. XIb): UV max (0.01 N HCl) 263 nm (ε 24 900), shoulder ~274 (22 400), min (ε 6100); NMR (CD_3SOCD_3) δ 8.25 (2 s, 2, $H_8 + H_2$), 7.32 (m, 5, C_6H_5), 6.25 (poorly resolved m, 2, $H_{1'}$), 5.10 (s, 2, CH_2 of $C_6H_5CH_2$), 3.37 (s, overlapped with ribose and glycine protons, OCH_3), 1.67 and 1.55 (2 s, $CH_3C<$), 1.14 (m, 3, CH_3 of C_2H_5O). Anal. ($C_{37}H_{45}N_{11}O_{12}\cdot CH_3OH$) H; C: calcd, 52.49; found, 53.05; N: calcd, 17.75; found, 17.12.

2′(3′)-*O*-Acetyl-2″(3″)-*O*-[*N*-(benzyloxycarbonyl)-glycyl]-1,2-di(adenosin-N^6-yl)ethane (XIIa). The reaction was performed as in the case of compound XIIb. The crude XIIa was purified by TLC in solvent S_1 and then in dichloromethane–methanol (85:15): yield 51%; UV max (ethanol) 272 nm, min 233; NMR (CD_3SOCD_3) δ 8.23 and 8.12 (2 s, $H_8 + H_2$), 7.25 (s, C_6H_5), 5.88 and 5.78 (2 m, poorly resolved $H_{1'}$), 5.00 (s, CH_2 of $C_6H_5CH_2$), 2.07 (s, CH_3CO). Anal. ($C_{34}H_{39}N_{11}O_{12}\cdot 2HCOOH\cdot H_2O$) C, H, N.

2′(3′)-*O*-Acetyl-2″(3″)-*O*-glycyl-1,2-di(adenosin-N^6-yl)ethane (XIIIa). A moderate stream of hydrogen was bubbled through a magnetically stirred solution of compound XIIa (14 μmol) in 80% acetic acid (2 mL) containing 5% PdO–$BaSO_4$ (28 mg) at 0 °C for 4 h. The catalyst was filtered using a Celite bed and it was washed with cold 80% acetic acid (3 mL). The clear filtrate was lyophilized to give a quantitative yield of XIIIa as determined spectrophotometrically using[10] ϵ_{263} 26 300; UV max (0.01 N HCl) 264 nm. Paper electrophoresis of XIIIa in 1 M acetic acid (Whatman No. 1 paper, 40 V/cm, 1 h, mobility 1.52 of glycine) showed a trace of XIVa as the only impurity. Compound XIIIa gives a yellow coloration with ninhydrin.

2′(3′)-*O*-Acetyl-2″(3″)-*O*-glycyl-1,4-di(adenosin-N^6-yl)butane (XIIIb). The hydrogenolysis was performed as above to give XIIIb in 71% yield as determined spectrophometrically using[10] ϵ_{264} 38 100; UV max (0.01 N HCl) 265 nm; electrophoretic mobility 1.77 of glycine; yellow coloration with ninhydrin.

Assay of Peptidyltransferase Activity. The ability of XIIIa and XIIIb to participate in the peptidyltransferase-catalyzed peptide bond formation and to inhibit the puromycin reaction was measured as described previously.[2b] Samples of XIIIa, XIIIb, A-Gly and A-Ac were prepared as before[7b] as 0.5–1 μmol aliquots. A typical reaction mixture contained in 0.1 mL of 0.05 M Tris-HCl (pH 7.4), 0.1 M NH_4Cl, 0.01 M $MgCl_2$, 4.0 A_{260} of NH_4Cl-washed ribosomes from *Escherichia coli* MRE-600 cells, 10 μg of poly(U), 0.20 A_{260} unit (2000 cpm) of *N*-Ac[^{14}C]Phe-tRNA, puromycin, and inhibitor at desired concentrations. For further details, compare 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 acetate buffer (pH 5.5) saturated with $MgSO_4$, and the products were extracted with 1.5 mL of ethyl acetate. 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-methoxyethanol/1 L of toluene scintillation mixture in a Packard Tri-Carb liquid scintillation spectrometer at 73% counting efficiency.

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