Nucleoside Peptides. I. The Synthesis of 5′-Deoxy-5′-amino-5′-*N*-aminoacyl Peptide Derivatives of Guanosine, Adenosine, and 2′-Deoxyadenosine and Their Effect on Cell-Free Protein Synthesis

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Abstract: Several 5′-*N*-aminoacyl-5′-amino-5′-deoxy- and 5′-amino-2′,5′-dideoxy-9-β-D-ribofuranosylpurine nucleoside peptides have been synthesized which represent a new class of peptide nucleosides. Appropriately blocked amino acids and peptides have been coupled to the corresponding purine 5′-amino-5′-deoxynucleoside derivative by the active ester and DCC methods of peptide formation. These compounds have been studied to determine their effect on poly-U directed polyphenylalanine synthesis. In instances where the aminoacyl moiety was L-phenylalanine and the nucleoside was either 5′-amino-5′-deoxyadenosine or 5′-amino-2′,5′-dideoxyadenosine, inhibition of poly-U directed polyphenylalanine synthesis was observed at high concentrations and significant stimulation was observed at lower concentrations. Chemical and biological properties of this new type of nucleoside peptide are discussed. Pmr data indicate that 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**) exists in solution in a folded conformation with phenyl and adenine ring stacking.

Akashi and Ishihara[1] first reported that acid-insoluble yeast RNA contains peptides in a bound form. Ingram and Sullivan[2] and Goto and coworkers[3,4] have verified the presence of amino acids covalently bound to RNA which are not removed by the usual procedures for deproteinization. Although it has been shown that the ribose is probably involved in the linkage of this type of peptide in the case of guanosine,[3] the precise point of attachment and the nature of the linkage have not been determined. Similarly the presence of amino acids in highly purified DNA has been reported by Balis, *et al.*,[5a,b] and Olenick and Hahn[6] and have been verified by Champagne[7] and Webb.[8] The proposal has been made[9] that seryl or threonyl residues may serve as "punctuation" in the genetic code of DNA. Indeed the possibility has been considered that small peptides bound to DNA may play a role in protein synthesis by acting as "derepressors" of structural genes.[5a] Although the biochemical role of these amino acids firmly bound to DNA is as yet undetermined, Salser and Balis[10] have shown that the quantity of amino acids so bound to DNA was greater in tumors than in the nonmalignant tissues of reference. Soluble RNA has been shown[11,12] to possess peptidyl components at positions other than at the 3′-hydroxyl of the specific tRNAs. Efforts in our laboratory have been directed toward the chemical synthesis of unusual nucleoside peptides, particularly as model compounds to aid in the identification and study of the nucleoside peptides of DNA and RNA. The first work along these lines reported from our laboratories was the synthesis of *N*-(9-β-D-ribofuranosylpurin-6-on-2-yl)alanine[13] or "guanosine propionic acid," a naturally occurring nucleoside derivative of alanine isolated from a *fusarium* species.[14] In the present work the 5′ position of adenosine, 2′-deoxyadenosine, and guanosine was selected for study. The 5′-amino group was chosen as a site of attachment of the peptidyl moiety. This assured a nucleoside peptide of superior stability, similar to the aminoacyl function found in various nucleoside antibiotics[15-17] such as puromycin, gougerotin, amicetin, and blasticidin S, which are known to be inhibitors of protein synthesis.[15] It is of significant interest that the polyoxins, a new group of antibiotics,[18] are pyrimidine nucleoside peptide derivatives with an aminoacyl moiety attached *via* the 5′-amino group of the sugar. Thus the present work is the first reported successful synthesis of peptide derivatives of 5′-amino-5′-deoxy-D-furanosyl nucleosides related to this interesting group of antibiotics. The syntheses of the requisite purine 5′-amino-5′-deoxynucleosides have recently been reported[19] from this laboratory.

Chemical Syntheses. The active ester method of Bodanszky[20] was chosen since selective reactivity with

(1) S. Akashi and H. Ishihara, *J. Biochem.* (*Tokyo*), **45**, 927 (1960).
(2) V. M. Ingram and E. Sullivan, *Biochim. Biophys. Acta*, **61**, 583 (1962).
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(4) S. Akashi and H. Ishihara, *ibid.*, **49**, 481 (1961).
(5) (a) M. E. Balis, J. S. Salser, and A. Elder, *Nature* (*London*), **203**, 1170 (1964); (b) J. S. Salser and M. E. Balis, *Biochim. Biophys. Acta*, **149**, 220 (1967).
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(8) S. J. Webb, *Can. J. Microbiol.*, 13, 57 (1967).
(9) A. Bendick and H. Rosenkranz, *Progr. Nucl. Acid Res.*, 1, 219 (1963).
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(13) J. F. Gerster and R. K. Robins, *J. Amer. Chem. Soc.*, **87**, 3752, (1965).
(14) A. Ballio, S. Russi, and G. Serlupi-Crescenzi, *Gazz. Chim. Ital.*, **94**, 156 (1964).
(15) J. J. Fox, K. A. Watanabe, and A. Bloch, *Progr. Nucl. Acid Res. Mol. Biol.*, **5**, 251 (1966).
(16) L. B. Townsend and R. K. Robins, "Handbook of Biochemistry and Molecular Biology," 2nd ed, Chemical Rubber Publishing Co., Cleveland, Ohio, 1970, G 115.
(17) R. J. Suhadolnik, "Nucleoside Antibiotics," Wiley-Interscience, New York, N. Y., 1970.
(18) K. Isono, K. Asahi, and S. Suzuki, *J. Amer. Chem. Soc.*, **91**, 7490 (1969).
(19) M. G. Stout, M. J. Robins, R. K. Olsen, and R. K. Robins, *J. Med. Chem.*, 12, 658 (1969).
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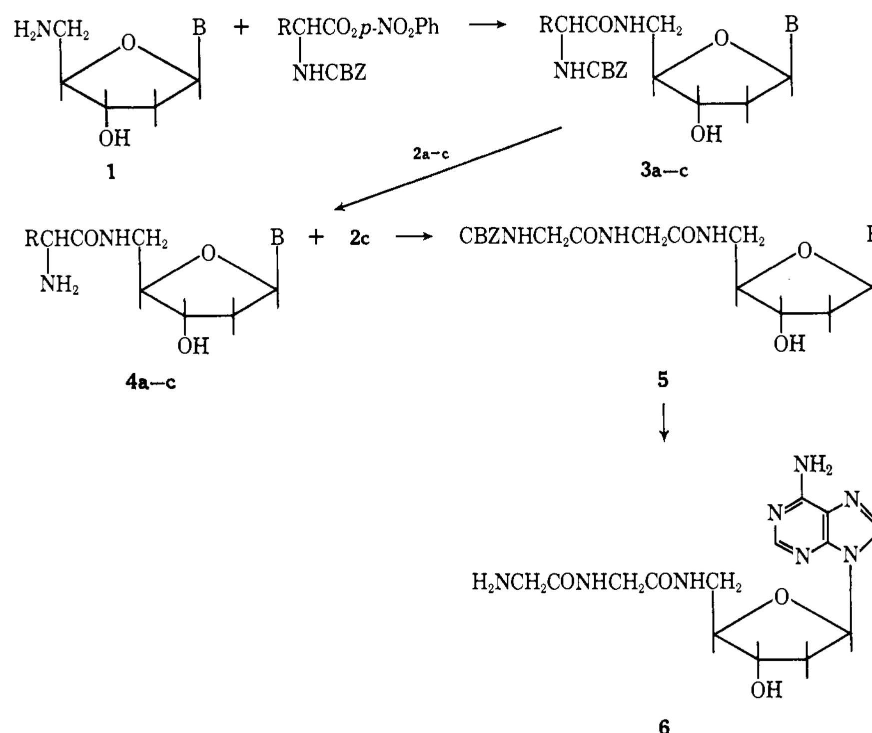
Scheme I



B = adenine-9
a, R = $(CH_3)_2CHCH_2$–
b, R = $PhCH_2$–
c, R = H–

the 5′-amino group of unblocked 5′-amino-2′,5′-dideoxyadenosine (**1**)[19] was desired. Reaction of the *N*-carbobenzyloxy-*p*-nitrophenyl ester of L-phenylalanine (**2b**) with 5′-amino-2′,5′-dideoxyadenosine (**1**) in ethanol gave after deblocking a 93% overall yield of 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**). Similarly, 5′-*N*-(L-leucyl)-5′-amino-2′,5′-dideoxyadenosine (**4a**) and 5′-*N*-glycyl-5′-amino-2′,5′-dideoxyadenosine (**4c**) were obtained in excellent yield from **1** and **2a** or **2c** (Scheme I).

The nucleoside peptide 5′-*N*-glycylglycyl-5′-amino-2′,5′-dideoxyadenosine (**6**) was prepared in a stepwise fashion from 5′-*N*-glycyl-5′-amino-2′,5′-dideoxyadenosine (**4c**) by treatment with the *N*-carbobenzyloxy-*p*-nitrophenyl ester of glycine (**2c**) to yield the carbobenzyloxyglycylglycyl derivative **5**. Treatment of **5** with palladium/carbon in the presence of hydrogen resulted in quantitative removal of the carbobenzyloxy group to yield 5′-*N*-glycylglycyl-5′-amino-2′,5′-dideoxyadenosine (**6**) in an overall yield of 74% from **4c** (Scheme I). Extension of this work to the adenosine and guanosine series was accomplished by treatment of the readily available 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneadenosine (**7a**)[19,21] or 5′-amino-5′-deoxy-2′,3′-*O*-isopropylideneguanosine (**7b**)[19,21] with the *N*-CBZ *p*-nitrophenyl esters of L-leucine (**2a**) and L-phenylalanine (**2b**) giving the *N*-CBZ isopropylidene blocked nucleoside derivatives **8a**, **8b**, and **8c**, respectively. Removal of the 2′,3′-*O*-isopropylidene groups from **8a–c** was accomplished with 50% aqueous formic acid to yield the corresponding 5′-*N*-(*N*-CBZ-L-phenylalanyl)-5′-amino-5′-deoxyadenosine (**9a**) and the 5′-*N*-(*N*-CBZ-L-aminoacyl)-5′-amino-5′-deoxyguanosines (**9b** and **9c**) (Scheme II). Removal of the CBZ blocking groups by catalytic hydrogenation gave the required aminoacyl purine nucleosides, 5′-*N*-(L-phenylalanyl)-5′-amino-5′-deoxyadenosine (**10a**), 5′-*N*-(L-leucyl)-5′-amino-5′-deoxyguanosine (**10b**), and 5′-*N*-(L-phenylalanyl)-5′-amino-5′-deoxyguanosine (**10c**) (Scheme II).

Investigation of the introduction of a dibasic amino acid such as L-lysine was studied utilizing the symmetrical anhydride method.[22,23] The symmetrical anhydride of N_α,N_ϵ-di-CBZ-L-lysine (**12d**) and 5′-amino-5′-deoxy-2′,3′-*O*-isopropylidene adenosine (**7a**) gave the intermediate **13d** which was not isolated but treated with 50% aqueous formic acid to remove the isopropylidene group and give 5′-*N*-(N_α,N_ϵ-di-CBZ-L-lysyl)-5′-amino-5′-deoxyadenosine (**14d**). Removal of the CBZ blocking groups gave an overall yield of 47% 5′-*N*-(L-lysyl)-5′-amino-5′-deoxyadenosine (**15d**) (Scheme II).

An alternative procedure for introduction of the peptidyl function consists in the direct coupling of a preformed peptide to the nucleoside 5′-amino group. This approach was investigated for the synthesis of 5′-*N*-(L-lysylglycylglycyl)-5′-amino-5′-deoxyadenosine. Since it is known that the DCC coupling between glycine and lysine leads to a high amount of "acyl urea" side product,[24] and CBZ peptide esters containing glycine as the second amino acid from the amino

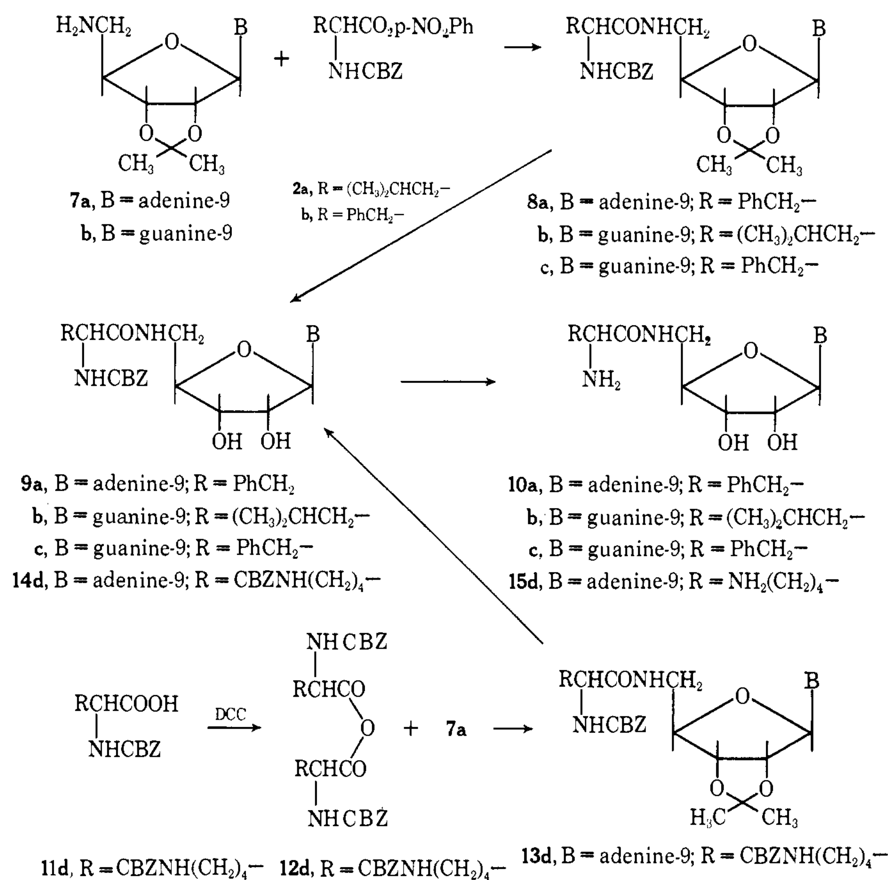
(21) W. Jahn, *Chem. Ber.*, **98**, 1705 (1965).

(22) H. Schüssler and H. Zahn, *ibid.*, **95**, 1076 (1962).

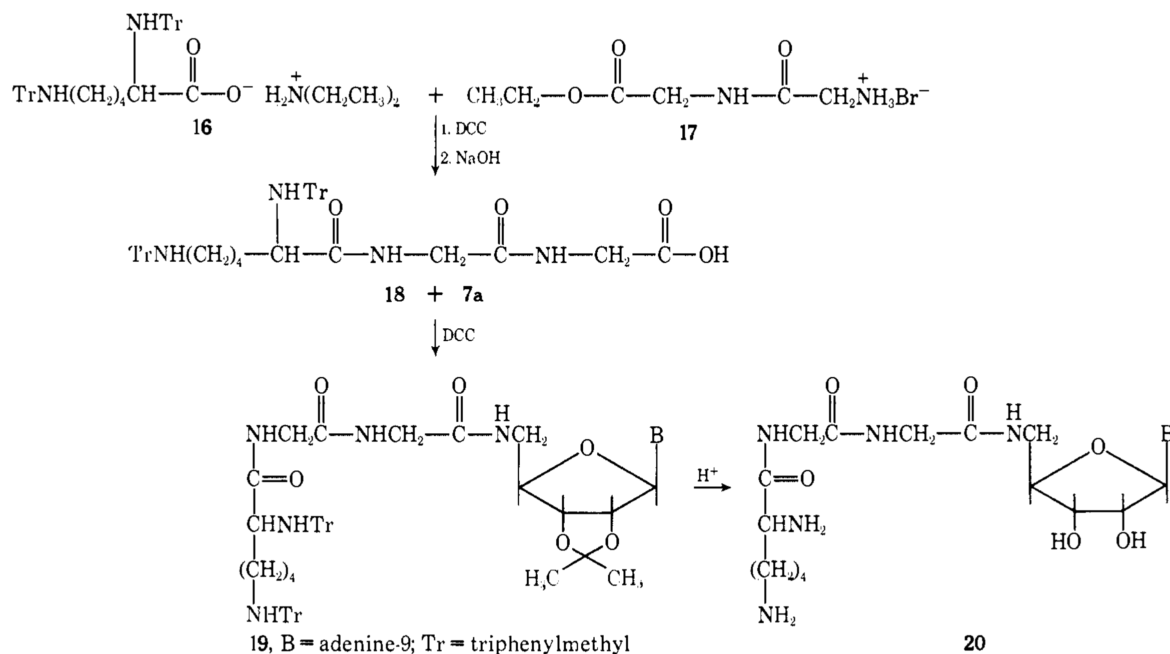
(23) D. H. Rammler and H. G. Khorana, *J. Amer. Chem. Soc.*, **85**, 1997 (1963).

(24) S. Banjusz and K. Medzihradsky, "Peptides," North Holland Publishing Co., Amsterdam, 1967, p 209.

Scheme II



Scheme III



terminal end may form hydantoins,[25] careful selection of a blocking function was made to minimize these problems. Use was made of the trityl blocking group according to the general procedure of Amiard and Goffind[26] to give N_α,N_ϵ-ditrityl-L-lysine diethylammonium salt[26] (**16**). Coupling of the glycylglycine ethyl ester hydrobromide[27] (**17**) and **16** *via* DCC, followed by saponification of the ethyl ester with 1 *N* sodium hydroxide, gave N_α,N_ϵ-ditrityl-L-lysylglycylglycine (**18**) (Scheme III). Coupling of **18** with **7a** and DCC gave, after the simultaneous removal of the isopropylidene and trityl groups with 55% aqueous formic acid, the desired nucleoside peptide 5′-*N*-(L-lysylglycylglycyl)-5′-amino-5′-deoxyadenosine (**20**) isolated as the hydrochloride.

(25) E. Schroder and K. Lubke, "The Peptides," Academic Press, New York, N. Y., 1965, p 25.
(26) G. Amiard and B. Goffind, *Bull. Soc. Chim. Fr.*, 1133 (1957).
(27) J. Greenstein and M. P. Winitz, "Chemistry of the Amino Acids," Vol. 2, Wiley, New York, N. Y., 1961, p 803.

Since these amino acids and peptide nucleosides were found to be chromatographically homogeneous in several solvent systems, it was assumed that the coupling reactions occurred with little or no racemization. This assumption was verified in that acid hydrolysis of compounds **4a**, **4b**, and **10a** followed by treatment with L-amino acid oxidase[28] yielded products which gave negative ninhydrin tests after separation by paper chromatography.

Results and Discussion

The inhibitory effects of the various 5′-*N*-aminoacyl-5′-amino-5′-deoxy- and 5′-*N*-aminoacyl-5′-amino-2′,5′-dideoxynucleosides on poly-U directed polyphenylalanine synthesis are shown in Table I.

Table I. Effects of Some 5′-*N*-Aminoacyl-5′-amino-5′-deoxy- and 5′-*N*-Aminoacyl-5′-amino-2′,5′-dideoxypurine Nucleosides on Poly-U Directed Polyphenylalanine Synthesis

Additions	No.	Concn, μmol/ml	nmol of [^{14}C]-phenylalanine incorp	% inhibition
None			6.2	0.0
5′-*N*-(L-Leucyl)-5′-amino-2′,5′-dideoxyadenosine	**4a**	12.0	4.5	23.0
5′-*N*-(L-Phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine	**4b**	2.3	1.86	70.0
5′-*N*-(Glycylglycyl)-5′-amino-2′,5′-dideoxyadenosine	**6**	7.6	4.70	25.0
5′-*N*-(L-Phenylalanyl)-5′-amino-5′-deoxyadenosine	**10a**	7.0	1.24	80.0
5′-*N*-(L-Leucyl)-5′-amino-5′-deoxyguanosine	**10b**	4.3	6.2	0.0
5′-*N*-(L-Phenylalanyl)-5-amino 5′-deoxyguanosine	**10c**	3.0	4.7	20.0
5′-*N*-(L-Lysyl)-5′-amino-5′-deoxyadenosine	**15d**	13.0	6.0	0.3
5′-*N*-(L-Lysylglycylglycyl)-5′-amino-5′-deoxyadenosine	**20**	5.12	4.65	25.9

Of the compounds tested, **4b** and **10a** showed the greatest inhibitory activity on incorporation of phenylalanine into polyphenylalanine. Specificity for the aminoacyl moiety at the 5′-position was nearly absolute for the phenylalanine group, since the lysyl derivative and the glycylglycyl derivative exhibited very little inhibitory activity as compared with the phenylalanyl derivatives. These compounds (**4b** and **10a**) may be considered as structural analogs of puromycin in which the 3′-*N*-aminoacyl moiety of puromycin (*p*-methoxyphenylalanine) is changed to a phenylalanine group and transferred to the 5′ position. It has previously been shown that the L-phenylalanyl analog (3′) of puromycin is nearly as active as the *p*-methoxy derivative.[29] Further, the N^6-dimethylamino moiety is not necessary for biological activity.[30] These compounds could also be considered as analogs of the activated amino acids (5′-adenylates) and therefore both **4b** and **10a** were tested for their ability to effect aminoacyl- (phenylalanyl)-sRNA synthesis. At concentrations of **4b** and **10a** which caused nearly 100% inhibition of protein

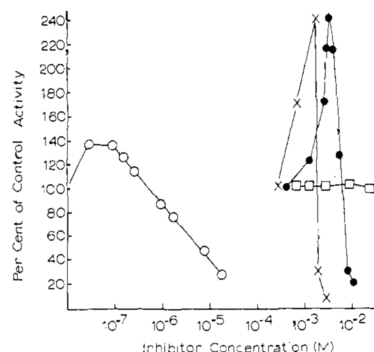


Figure 1. Effect of puromycin, 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxy-5′-aminoadenosine (**4b**), 5′-*N*-(L-phenylalanyl)-5′-amino-5′-deoxyadenosine (**10a**), and 5′-*N*-(L-lysyl)-5′-amino-5′-deoxyadenosine (**15**) on protein synthesis: O—O—O, puromycin; ×—×—×, **4b**; ●—●—●, **10a**; □—□—□, **15**.

synthesis, phenylalanyl-sRNA formation was inhibited to an extent of only 20%. Thus, these compounds do not exert their main inhibitory effect on protein synthesis by acting as analogs of the 5′-activated amino acids in the cell-free system. In order to determine the (I_{50}) values for compounds **4b** and **10a**, the incorporation of phenylalanine was measured in the presence of varying amounts of both inhibitors. The experimental results are presented in Figure 1. As can be seen, (I_{50}) values were obtained for both compounds, with **4b** being relatively more potent (3.65 times) than compound **10a**; surprisingly, a rather substantial stimulation of incorporation of phenylalanine into polyphenylalanine was noticed.

It has recently been reported that gougerotin[31] causes a maximum enhancement (about 80%) of polyphenylalanine synthesis in a cell-free system which has been shown to be dependent upon salt concentration. The possibility of stimulation due to salt concentration[31] is now under investigation in our laboratories.

Stenesh and Shen[32] have demonstrated that puromycin also stimulated polyphenylalanine synthesis in a cell-free system derived from two Bacilli. The extent of this stimulation was approximately 60%. The results of the latter authors have been confirmed in our system and we show that puromycin caused a stimulation of protein synthesis no greater than 50%.

It appears that puromycin inhibited protein synthesis 178 times greater than compound **4b** and 650 times greater than **10a** (based on I_{50} values). When stimulation of protein synthesis was based on the concentration of compound which gave maximum stimulation, puromycin was a more effective stimulator, *i.e.*, 180 times greater than compound **4b** and 660 times greater than **10a**. However, if the degree of stimulation was based on the *magnitude* of the effect, both **4b** and **10a** were nearly 3 times more effective in stimulating protein synthesis (puromycin caused 50% maximum stimulation while **4b** or **10a** caused 140% stimulation). As can be seen from Figure 1, the 5′-*N*-L-lysyl derivative **15** caused neither stimulation nor inhibition of incorporation.

In order to gain some insight into the mechanism of this stimulatory action, the incorporation of phenyl-

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(29) D. Nathans and A. Neidle, *Nature (London)*, **197**, 1076 (1963).
(30) B. R. Baker, R. E. Schaub, and H. M. Kissman, *J. Amer. Chem. Soc.*, **77**, 5911 (1955).
(31) M. Yukioka and S. Morisawa, *J. Biochem.*, **66**, 225, 233, 241 (1969).
(32) J. Stenesh and P. Y. Shen, *Biochem. Biophys. Res. Commun.*, **37**, 873 (1969).

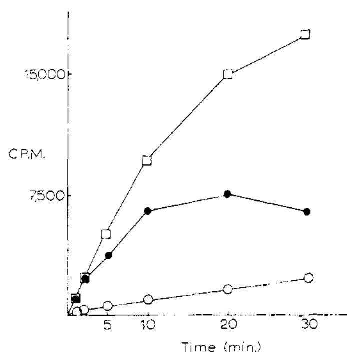

Figure 2. Effect of concentration of 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**) on rate of protein synthesis: □—□—□, 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′dideoxyadenosine (1.3 μmol/ml); ○—○—○, 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (2.34 μmol/ml); ●—●—●, no additions.

alanine into polypeptide was measured with time, in the presence of varying amounts of **4b**. As can be seen from Figure 2, the incorporation of phenylalanine was the same in the absence or the presence of stimulatory concentrations of **4b** during the first 2 min of the reaction, but the incorporation in the control mixture began to fall off after 5 min and at the end of 30 min was somewhat less than that observed after 20 min. In the presence of stimulatory amounts of **4b**, the incorporation of phenylalanine remained nearly linear for 20 min and increased substantially even after 30 min of incubation.

Puromycin is known to have a secondary effect on protein synthesizing systems, *i.e.*, to cause a breakdown of polysomes to monomer subunits.[33,34] It has been suggested by Stenesh and Shen[32] that this could be one explanation for the stimulation seen at low concentrations of puromycin. If this were the case, with puromycin or compounds **4b** and **10a**, then one would expect that as the monomer units were released, they would become available for binding to mRNA and aminoacyl-sRNA to form the active polysome unit. The results presented in Figure 2 for these 5′-*N*-aminoacyl derivatives **4b** and **10a** are consistent with such an interpretation.

In a recent paper, Sundaralingam and Arora[35] have examined the single-crystal X-ray diffraction patterns of puromycin. The suggestion was made[35] that the aromatic nucleus of the amino acid is stacked above the purine moiety of the nucleoside. These authors suggest that part of the inhibitory effect of this compound may be due to this unique conformation in solution and the ability of the amide proton on the 3′-amino moiety to hydrogen bond to the ribosomal peptide synthetase. Space filling models (Coulter) of puromycin and **4b** show that it is possible to form a conformation where the L-phenylalanyl moiety is stacked over the purine nucleus just as it is possible with a model of 3′-*O*-phenylalanyladenosine.

Pmr techniques have been fruitfully employed to elicit the solution conformations of diribonucleoside monophosphates[36-38] and coenzymes such as NAD.[39]

(33) S. Villa Trevino, E. Farber, T. Staeheln, F. O. Wettstein, and H. Noll, *J. Biol. Chem.*, **239**, 3826 (1964).
(34) A. R. Williamson and R. Schweet, *J. Mol. Biol.*, 11, 358 (1965).
(35) M. Sundaralingam and S. K. Arora, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1021 (1969).

These dinucleotides were shown to exist in folded conformations, involving stacking interaction between the bases. Evidence for this type of intramolecular base-base interaction includes the high-field shifts noticed for base protons in the dinucleotides compared with the monomer mixtures.

The pmr spectra of 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**) has been compared to a spectra of a mixture of 5′-amino-2′,5′-dideoxyadenosine (**1**) and L-phenylalanine (Table II). The 0.15–0.3-ppm

Table II. Nmr Evidence that 5′-*N*-(L-Phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**) Exists in a Folded, Ring-Stacked Conformation in Aqueous Solution

	Chemical shifts from DSS, ppm		
	H-8	H-2	φ
L-Phenylalanine and 5′-amino-2′,5′-dideoxyadenosine mixture[a]	8.28	8.23	7.36
5′-*N*-(L-Phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**)[a]	8.13	8.02	7.05
Δδ	0.15	0.21	0.31

[a] Each 0.02 *M* in D_2O.

high-field shifts in **4b** compare closely with the 0.1–0.25-ppm shifts found for the comparisons of ApA *vs.* the mixture of 5′-AMP and 3′-AMP.[36] These data provide evidence for phenyl and adenine ring stacking. This lends strong support to the suggestion that 5′-*N*-(L-phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (**4b**) exists in a folded conformation in solution.

The precise mechanism of protein synthesis inhibition and stimulation by this new class of 5′-aminoacyl-5′-aminopurine nucleosides is currently under further study.

Experimental Section

E. coli, (Q-13) cells, harvested in mid-log, were obtained from General Biochemicals, Chagrin Falls, Ohio. The S-30 fraction was prepared, using well-described procedures,[40] and was preincubated against Tris (0.01 *M*, pH 7.6)–Mg (0.038 *M*) buffer and dialyzed overnight against 100 vol of cold Tris (0.01 *M*, pH 7.6)–KCl (0.05 *M*)–Mg (0.01 *M*) buffer. Puromycin dihydrochloride and L-amino acid oxidase were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, and all of the compounds utilized were analytically pure crystalline solids.

Incubation time was 20 min at 37° unless otherwise specified. The standard incubation mixture contained (amounts in micromoles): ATP, 0.34; GTP, 0.08; PEP, 2.5; [^{14}C]amino acid, 25 nmol, specific activity *ca.* 2.0 × 10^4 cpm/nmol; 2-mercaptoethanol 4.2; Tris, pH 7.8, 16; ammonium chloride, 17; sRNA, *E. coli* B, 0.25 mg; pyruvate kinase, 10 μg, in a final volume of 0.25 ml. Assay mixtures were worked up in the usual manner[41] and the reaction was stopped by the addition of 6% TCA. The precipitates, after heating at 90° for 20 min, were collected on Millipore filters and counted, using a liquid scintillation spectrometer (Packard Model 3320).

Melting points were determined on a Fisher-Johns block and are uncorrected. Uv spectra were determined on a Beckman DK-2 instrument. Optical rotations were determined on a Perkin-Elmer

(36) P. O. P. Tso'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997 (1969).
(37) S. I. Chan and J. H. Nelson, *J. Amer. Chem. Soc.*, 91, 168 (1969).
(38) B. W. Bangerter and S. I. Chan, *ibid.*, 91, 3910 (1969).
(39) O. Jardetzky and N. G. Wade-Jardetzky, *J. Biol. Chem.*, 241, 85 (1966).
(40) K. Iwasaki, S. Sabol, A. Wahba, and S. Ochoa, *Arch. Biochem. Biophys.*, 125, 542 (1968).
(41) O. W. Jones, E. E. Townsend, H. H. Sober, and L. A. Heppel, *Biochemistry*, 3, 238 (1964).

Model 141 polarimeter. Hydrogenations were effected using a Parr hydrogenation apparatus at room temperature when a specified hydrogen gas pressure was reported. Evaporations were accomplished using a Büchler rotating evaporator under reduced pressure (aspirator or vacuum pump). Paper chromatography was run on Whatman No. 1 chromatography paper by the descending technique in the following solvent systems: (1) BuOH–H_2O (83:17), (2) EtOH–1 *N* NH_4OAc (7:3), (3) BuOH–HCOOH–H_2O (15:3:2), (4) *n*-PrOH–NH_4OH (concentrated)–H_2O (6:3:1) (v/v). Thin-layer chromatography (tlc) was run on glass plates coated with SilicAR-7GF (Mallinckrodt Chemical Works) using the upper phase of EtOAc–*n*-PrOH–H_2O (4:1:2) unless otherwise specified. Silica gel for column chromatography was J. T. Baker No. 3405. Where indicated by elemental analysis, solvation was verified by nmr spectroscopy in absolute DMSO-d_6 and, in the case of hydration, by exchange with addition of D_2O and reintegration of the spectral area where the H_2O peak had occurred. Uv spectral data are given only for MeOH solutions. Spectra of all compounds were determined also in pH 1 and 11 buffers and found to correspond with those of the parent nucleosides. *N*-CBZ amino acid *p*-nitrophenyl esters were purchased from Sigma Chemical Co., St. Louis, Mo.

5′-*N*-(*N*-CBZ-L-Leucyl)-5′-amino-2′,5′-dideoxyadenosine (3a). To 80 ml of boiling EtOH was added 1 g (0.004 mol) of 5′-amino-2′,5′-dideoxyadenosine (**1**)[19] and the mixture was heated at reflux until solution was complete and then cooled to room temperature. *N*-CBZ-L-Leucine *p*-nitrophenyl ester (**2a**) (1.9 g, 0.0049 mol) was added and the solution was stirred at room temperature for 15 hr. Product began to crystallize from solution after about 20 min. The mixture was evaporated to dryness and the residue was dissolved in 20 ml of $CHCl_3$–MeOH (1:1). This solution was applied to a column (1 × 26 in., 150 g) of silica gel and the column was washed with 500 ml of $CHCl_3$–Me_2CO (8:2). This wash containing *p*-nitrophenol and unreacted amino acid was discarded and elution with absolute EtOH was begun; 50-ml fractions were collected. Fractions 3–7 were chromatographically homogeneous (tlc) and were evaporated slowly to about 150 ml. The product **3a** (1.72 g, 87%) crystallized in fine white needle clumps and a second crop (0.18 g) raised the yield to 96%. A small sample dried at 100° for 24 hr at 0.01 mm had mp 205–208°; uv max (MeOH) 259 mμ (ε 14,900).

Anal. Calcd for $C_{24}H_{31}N_7O_5$: C, 57.93; H, 6.28; N, 19.71. Found: C, 57.91; H, 6.38; N, 19.87.

5′-*N*-(L-Leucyl)-5′-amino-2′,5′-dideoxyadenosine (4a). A solution of 1.78 g (0.0036 mol) of **3a** in 75 ml of EtOH and 75 ml of H_2O was hydrogenated for 9 hr at 48 psi in the presence of 0.55 g of 5% Pd/C. The mixture was filtered and the filtrate was evaporated to yield 1.3 g (100%) of **4a** as a crisp solid foam. This material was crystallized from EtOAc–MeOH to give 1.17 g (90%) of **4a**, mp 98–103°, with prior softening; $[\alpha]^{29}D$ −32.8° (*c* 1, DMF); uv max (MeOH) 25 mμ (ε 14,100).

Anal. Calcd for $C_{16}H_{25}N_7O_3$: C, 52.88; H, 6.93; N, 26.98. Found: C, 52.92; H, 6.99; N, 26.97.

5′-*N*-(*N*-CBZ-L-Phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (3b). A solution of 1 g (0.004 mol) of **1** in 80 ml of hot EtOH was cooled to room temperature and treated with 2.02 g (0.0048 mol) of **2b**. This mixture was stirred and product began to crystallize from the resulting solution after about 15 min. The reaction was complete as judged by tlc after about 1.5 hr. The mixture was filtered and the filter cake washed with cold EtOH, yield 1.78 g (84% of crystalline **3b**. The combined filtrate was evaporated to dryness and chromatographed on a silica gel column (0.5 × 22 in., 50 g) as in the preparation of **3a** above. The appropriate fractions were combined and evaporated and the residue was crystallized from EtOH to give an additional 0.2 g (total yield 1.98 g, 93%) of crystalline **3b**; mp 113–117°; uv max (MeOH) 259 mμ (ε 14,400).

Anal. Calcd for $C_{27}H_{29}N_7O_5 \cdot 0.5C_2H_5OH$: C, 60.63; H, 5.82; N, 17.68. Found: C, 60.30; H, 5.80; N, 17.64.

5′-*N*-(L-Phenylalanyl)-5′-amino-2′,5′-dideoxyadenosine (4b). A solution of 1.78 g (0.0034 mol) of **3b** in 50 ml of EtOH and 50 ml of H_2O was hydrogenated for 18 hr at 47 psi in the presence of 0.6 g of 5% Pd/C. The mixture was filtered and the filtrate was evaporated to dryness to give 1.33 g (100%) of **4b** as a crisp white solid foam. This material was crystallized from EtOAc–MeOH to give flat crystals; mp 114–125°; $[\alpha]^{30}D$ −37.0° (*c* 1, DMF); uv max (MeOH) 259 mμ (ε 14,000).

Anal. Calcd for $C_{19}H_{23}N_7O_3$: C, 57.42; H, 5.83; N, 24.67. Found: C, 57.40; H, 5.79; N, 24.60.

5′-*N*-(*N*-CBZ-Glycyl)-5′-amino-2′,5′-dideoxyadenosine (3c). A solution of 1.1 g (0.0044 mol) of **1** in 80 ml of hot EtOH was cooled to room temperature and treated with 1.60 g (0.0048 mol) of **2c.** The resulting solution was stirred for 18 hr, evaporated to dryness, and purified on a column (1 × 26 in., 150 g) of silica gel as in the preparation of **3a** above. The appropriate fractions were evaporated to dryness to give 1.73 g (89%) of **3c** as a white solid foam. A sample of this material for analysis was crystallized from EtOAc–MeOH and dried at 78° for 24 hr and then at 100° for 24 hr at 0.01 mm; mp 97–104°; uv max (MeOH) 259 mμ (ε 14,800).

Anal. Calcd for $C_{20}H_{23}N_7O_5$: C, 54.41; H, 5.25; N, 22.21. Found: C, 54.16; H, 5.16; N, 22.26.

5′-*N*-Glycyl-5′-amino-2′-5′-dideoxyadenosine (4c). A solution of 1.48 g (0.0034 mol) of **3c** (solid foam) in 25 ml of EtOH and 75 ml of H_2O was hydrogenated for 36 hr at 50 psi in the presence of 0.5 g of 5% Pd/C. The mixture was filtered and the filtrate evaporated to give 1.03 g (100%) of **4c** as a solid foam. This product was crystallized from EtOH to yield 0.97 g (94%) of crystals of **4c**; mp 192–196°; $[\alpha]^{30}D$ −36.0° (*c* 1, DMF); uv max (MeOH) 259 mμ (ε 14,600).

Anal. Calcd for $C_{12}H_{17}N_7O_3$: C, 46.90; H, 5.58; N, 31.91. Found: C, 46.90; H, 5.79; N, 32.05.

5′-*N*-(*N*-CBZ-Glycylglycyl)-5′-amino-2′,5′-dideoxyadenosine (5). A solution of 2.3 g (0.0075 mol) of **4c** in 100 ml of hot EtOH–H_2O (4:1) was cooled to 20° and 2.75 g (0.0083 mol) of **2c** in 25 ml of EtOH was added. The resulting solution was stirred for 15 hr and then evaporated to dryness. The residue was purified on a column (1 × 26 in., 150 g) of silica gel as in the preparation of **3a** above. The appropriate fractions were pooled and evaporated to give a stiff syrup which crystallized upon standing, yield 2.80 g (75%) of hard needle clusters; mp 145–146°; uv max (MeOH) 259 mμ (ε 16,400).

Anal. Calcd for $C_{22}H_{26}N_8O_6$: C, 53.00; H, 5.26; N, 22.48. Found: C, 53.04; H, 5.29; N, 22.31.

5′-*N*-Glycylglycyl-5′-amino-2′,5′-dideoxyadenosine (6). A solution of 2.5 g (0.005 mol) of **5** in 60 ml of H_2O and 30 ml of EtOH was hydrogenated for 42 hr at 50 psi in the presence of 0.8 g of 5% Pd/C. The mixture was filtered and the filtrate evaporated to dryness to give 1.8 g (99%) of **6** as a solid foam. A sample of this material was dissolved in hot EtOH and the solution was evaporated slowly. Colorless crystals of **6** deposited, were collected by filtration, and dried at 100° for 24 hr at 0.01 mm; mp 154–157°; $[\alpha]^{30}D$ −41.4° (*c* 1, DMF); uv max (MeOH) 259 mμ (ε 13,900).

Anal. Calcd for $C_{14}H_{20}N_8O_4$: C, 46.15; H, 5.53; N, 30.76. Found: C, 46.30; H, 5.80; N, 30.60.

5′-*N*-(*N*-CBZ-L-Phenylalanyl)-5′-amino-5′-deoxyadenosine (9a). A solution of 1.6 g (0.0052 mol) of **7a**[19] and 2.2 g (0.0053 mol) of **2b** in 150 ml of THF was allowed to stand for 27 hr at room temperature. The solution was evaporated to dryness, the residue was dissolved in 25 ml of EtOAc, and 250 ml of Et_2O was added. The resulting precipitate of **8a** (2.6 g, 85%) was collected by filtration and air dried. A solution of 2.25 g (0.0038 mol) of this product (crude **8a**) in 80 ml of 50% aqueous HCO_2H was allowed to stand for 26 hr at room temperature and evaporated to dryness and the residue was coevaporated to dryness twice with 75-ml portions of EtOH. A sample of the resulting solid (1.94 g, 91%) was crystallized from EtOH–EtOAc to give crystals of **9a**; mp 187–189°; uv max (MeOH) 258 mμ (ε 16,000).

Anal. Calcd for $C_{27}H_{29}N_7O_6 \cdot 0.5H_2O$: C, 58.26; H, 5.43; N, 17.62. Found: C, 58.44; H, 5.29; N, 17.90.

5′-*N*-(L-Phenylalanyl)-5′-amino-5′-deoxyadenosine (10a). A solution of 2.1 g (0.0038 mol) of **9a** in 150 ml of DMF was hydrogenated for 26 hr at 40 psi in the presence of 1.0 g of 10% Pd/C. The mixture was filtered and the filtrate evaporated to dryness. The residue was dissolved in 25 ml of EtOH and 50 ml of EtOAc was added slowly with stirring followed by 120 ml of Et_2O. The precipitated product (1.39 g, 87%) was recrystallized from MeOH to provide crystals of **10a**; mp 208–209°; $[\alpha]^{32}D$ −54.8° (*c* 1, DMF–H_2O (1:3)); uv max (MeOH) 258 mμ (ε 15,600).

Anal. Calcd for $C_{19}H_{23}N_7O_4$: C, 55.20; H, 5.61; N, 23.72. Found: C, 55.23; H, 5.66; N, 23.76.

5′-*N*-(*N*-CBZ-L-Leucyl)-5′-amino-2′,3′-*O*-isopropylidene-5′-deoxyguanosine (8b). A mixture of 1.60 g (0.005 mol) of **7b**[19] and 2.10 g (0.0054 mol) of **2a** in 200 ml of THF was stirred at room temperature. Solution was complete in about 20 hr and a precipitate separated from solution thereafter. After 48 hr, 200 ml of EtOAc was added and the resulting precipitate (2.75 g, 98%) was filtered and air dried. A sample was crystallized from MeOH–EtOAc to give solid **8b**; mp 145–155°; uv max (MeOH) 253 mμ (ε 15,800).

Anal. Calcd for $C_{27}H_{35}N_7O_7 \cdot 0.25H_2O$: C, 56.48; H, 6.23; N, 17.08. Found: C, 56.32; H, 5.93; N, 16.86.

5′-*N*-(*N*-CBZ-L-Leucyl)-5′-amino-5′-deoxyguanosine (9b). A solution of 2.35 g (0.0041 mol) of **8b** in 80 ml of 50% aqueous HCO_2H was allowed to stand for 20 hr at room temperature. The solution was evaporated to dryness and the residue co-evaporated with two 75-ml portions of EtOH to yield 1.6 g (73%) of colorless solid product. A sample was recrystallized from MeOH–EtOAc to give microneedles of **9b**; mp 244–245°; uv max (MeOH) 253 mμ (ε 15,300).

Anal. Calcd for $C_{24}H_{31}N_7O_7$: C, 54.43; H, 5.90; N, 18.52. Found: C, 53.85; H, 5.84; N, 18.86.

5′-*N*-(L-Leucyl)-5′-amino-5′-deoxyguanosine (10b). A solution of 1.8 g (0.0034 mol) of **9b** in 150 ml of DMF was hydrogenated for 3.5 hr at 40 psi in the presence of 0.7 g of 10% Pd C. The mixture was filtered and the filtrate evaporated to about 3 ml and then treated with 30 ml of EtOH followed by 20 ml of Et_2O. The resulting solid (1.33 g, 99%) was filtered and a sample was purified by slow addition of a DMF solution of this material to a rapidly stirred solution of EtOH–Et_2O. The resulting precipitate of **10b** had mp 236–237°; $[\alpha]^{29}D$ −7.0° (*c* 1, H_2O); uv max (MeOH) 253 mμ (ε 13,900).

Anal. Calcd for $C_{16}H_{25}N_7O_5 \cdot 0.25H_2O$: C, 48.05; H, 6.43; N, 24.52. Found: C, 48.14; H, 6.57; N, 24.71.

5′-*N*-(*N*-CBZ-L-Phenylalanyl)-5′-amino-2′,3′-*O*-isopropylidene-5′-deoxyguanosine (8c). A mixture of 1.61 g (0.005 mol) of **7b** and 2.30 g (0.0055 mol) of **2b** in 150 ml of THF was stirred for 26 hr at room temperature. The resulting solution was evaporated to dryness and the residue was stirred with 100 ml of EtOAc. The yellow crystalline solid (3 g, 100%) was filtered and a sample was recrystallized from *i*-PrOH–Me_2CO to give fine crystals of **8c**; mp 255–257° dec; uv max (MeOH) 252 mμ (ε 15,100).

Anal. Calcd for $C_{30}H_{33}N_7O_7$: C, 56.69; H, 5.51; N, 16.24. Found: C, 59.36; H, 5.81; N, 15.80.

5′-*N*-(*N*-CBZ-L-Phenylalanyl)-5′-amino-5′-deoxyguanosine (9c). A mixture of 2.25 g (0.0037 mol) of **8c** in 80 ml of 50% aqueous HCO_2H was stirred for 23 hr at room temperature. Reaction was shown to be complete by tlc ($CHCl_3$–Me_2CO (1:3)) and the mixture was evaporated to dryness. The residue was coevaporated with two 75-ml portions of EtOH and dried to give 2.0 g (94%) of solid product. A sample was purified by adding a DMF solution of the material to a rapidly stirred portion of Et_2O. The resulting precipitate of **9c** had mp 156–157°; uv max (MeOH) 253 mμ (ε 15,900).

Anal. Calcd for $C_{27}H_{29}N_7O_7 \cdot 0.5H_2O$: C, 56.63; H, 5.28; N, 17.13. Found: C, 56.62; H, 5.16; N, 17.31.

5′-*N*-(L-Phenylalanyl)-5′-amino-5′-deoxyguanosine (10c). A solution of 1.85 g (0.0032 mol) of **9c** in 150 ml of DMF was hydrogenated for 6 hr at 40 psi in the presence of 0.7 g of 10% Pd/C. The mixture was filtered and the filtrate evaporated to dryness. The solid residue was stirred with 40 ml of EtOH–Et_2O (3:1) and filtered to give 1.37 g (97%) of a crystalline solid, mp 232–234°. A sample was recrystallized from EtOH–H_2O to give crystals of **10c**; mp 236–238°; $[\alpha]^{30}D$ −11.2° (*c* 1, H_2O); uv max (MeOH) 253 mμ (ε 15,6000).

Anal. Calcd for $C_{19}H_{23}N_7O_5 \cdot 0.5H_2O$: C, 52.05; H, 5.52; N, 22.36. Found: C, 52.19; H, 5.50; N, 22.42.

5′-*N*-(N_α,N_ϵ-CBZ-L-Lysyl)-5′-amino-5′-deoxyadenosine (14d). A solution of 5.5 g (0.00132 mol) of N_α,N_ϵ-diCBZ-L-lysine (**11d**) and 1.6 g (0.00778 mol) of DCC in 150 ml of dry EtOAc was stirred at room temperature for 20 min and then filtered directly into a flask containing 2.0 g (0.0065 mol) of **7a**. The reaction mixture was stirred for 6 hr at room temperature and then evaporated to dryness. The resulting solid was treated with 100 ml of 50% HCOOH for 24 hr, the residual amino acid was removed *via* filtration with a Celite pad, and the filtrate was evaporated to dryness *in vacuo*. The resulting solid was evaporated to dryness with ethanol twice, then crystallized from MeOH–*i*-PrOH to give 2.64 g (60%) of **14d**.

Anal. Calcd for $C_{32}H_{38}N_8O_8 \cdot H_2O$: C, 56.45; H, 5.92; N, 16.47. Found: C, 56.10; H, 5.73; N, 16.26.

5′-(*N*-L-Lysyl)-5′-amino-5′-deoxyadenosine (15d). A mixture of 2.60 g of **14d** in 75 ml of ethanol, 75 ml of water, and 0.9 g of 5% Pd/C was hydrogenated at 47 psi for 6.75 hr. The catalyst was removed *via* a Celite pad and the filtrate was evaporated to dryness. The resulting solid was triturated with 50 ml of EtOH and cooled to give 1.24 g (78%) of **15d**. This was recrystallized from MeOH–*i*-PrOH for analysis.

Anal. Calcd for $C_{16}H_{26}N_8O_4 \cdot 2.5H_2O$: C, 43.72; H, 7.10; N, 25.50. Found: C, 43.79; H, 7.58; N, 25.40.

N_α,N_ϵ-Ditrityl-L-lysylglycylglycine (18). To 50 ml of methylene chloride cooled with an ice bath and stirred with a magnetic stirrer were added 7.40 g (0.010 mol) of N_α,N_ϵ-ditrityl-L-lysine diethylammonium salt (**16**), 2.41 g (0.010 mol) of glycylglycine ethyl ester hydrobromide (**17**), and 2.20 g (0.010 mol) of DCC. The solution which resulted was stirred for 14 hr at 10°, cooled with an ice bath, treated with 0.5 ml of glacial acetic acid, and then stirred for 10 min longer. The precipitate of *N*,*N*-dicyclohexylurea (2.0 g) was collected and washed with 10 ml of methylene chloride and the filtrate was washed with 5% $NaHCO_3$ solution and water and then dried over anhydrous sodium sulfate. After the drying agent was removed, 3 ml of diethylamine was added and the solution was evaporated *in vacuo* to dryness. The residue was stirred with ethyl ether and the ditrityllysine diethylammonium salt was collected. The filtrate was once again evaporated *in vacuo* to dryness to yield a colorless chromatographically homogeneous glass, 7.4 g (98%). This glass was dissolved in 100 ml of boiling absolute EtOH and 40 ml of 1 *N* sodium hydroxide was added dropwise over a period of 8 min to the refluxing solution. The hot mixture was poured into 300 ml of ice–water which was vigorously stirred and a fine white powder precipitated. To this suspension was added 8 ml of glacial acetic acid and the mixture was stirred in an ice bath for 1 hr. The product was collected by filtration, washed with water, and dried *in vacuo* at 50°. This product contained impurities and was taken up in 40 ml of methyl ethyl ketone and cooled to −70° with an acetone–Dry Ice bath. The precipitate (0.60 g) which resulted was collected and discarded. The filtrate was treated with 200 ml of *n*-heptane and an oil was deposited; the supernatant was decanted and the oil was dissolved in 40 ml of benzene. This solution was slowly added to 300 ml of *n*-heptane which was vigorously stirred and the pure product precipitated as a white powder; 4.72 g (64%); mp 122–125°.

Anal. Calcd for $C_{48}H_{48}N_4O_4 \cdot 2H_2O$: C, 73.82; H, 6.71; N, 7.18. Found: C, 73.98; H, 6.54; N, 7.17.

5′-*N*-(L-Lysylglycylglycyl)-5′-amino-5′-deoxyadenosine dihydrochloride (20). A solution of 3.50 g (0.00472 mol) of **7a**, 1.37 g (0.00450 mol) of **18**, and 15 ml of CH_2Cl_2 was cooled on an ice bath and 4.24 g (0.006 mol) of DCC was added. This mixture was stirred at 15° for 20 hr, 0.13 ml of glacial acetic acid was added, and the reaction mixture was stirred on an ice bath for 5 min; then the mixture was evaporated *in vacuo* to dryness. The residue was recrystallized from 20 ml of EtOH to give 3.08 g of chromatographically homogeneous material which was found by pmr to be a mixture of the desired product and *N*,*N*′-dicyclohexylurea in a ratio of 2:1. This product (1.14 g) was stirred in 30 ml of 55% HCOOH at 20° for 48 hr and the *N*,*N*′-dicyclohexylurea and the triphenylcarbinol were removed by filtration. The filtrate was evaporated *in vacuo* to dryness, taken up in EtOH, and again evaporated *in vacuo* to dryness. The residual glass was dissolved in 3 ml of water, the pH was adjusted to 3.5 with 10% HCl, and the solution was lyophilized to give 0.650 g of chromatographically homogeneous material; mp 151–153° (181–184° dec); pmr showed 4 mol of water.

Anal. Calcd for $C_{20}H_{32}N_{10}O_6 \cdot 2HCl \cdot 4H_2O$: C, 36.75; H, 6.47; N, 21.43. Found: C, 36.77; H, 6.66; N, 21.19.