Analogues of 2′(3′)-*O*-L-Phenylalanyladenosine as Substrates and Inhibitors of Ribosomal Peptidyltransferase[1]

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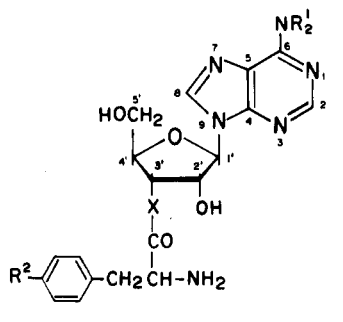
The chemical syntheses of 2′(3′)-*O*-(L-3-amino-3-phenylpropionyl)adenosine (**2e**), the corresponding D stereoisomer **2f**, 2′(3′)-*O*-(DL-phenylglycyl)adenosine (**2g**), 2′(3′)-*O*-(*N*-benzylglycyl)adenosine (**2h**), and 9-(2-*O*-L-phenylalanyl-β-D-xylofuranosyl)adenine (**3b**) are described. Compounds **2e–h** were obtained by acylation of 5′-*O*-(4-methoxytrityl)adenosine with the appropriate *N*-benzyloxycarbonyl or *N*-*tert*-butoxycarbonyl amino acids with dicyclohexylcarbodiimide in pyridine. The corresponding reaction of *N*-(benzyloxycarbonyl)-D-phenylglycine led to an almost complete racemization of the aminoacyl residue (compounds **2c** and **2g**). Subsequent chromatographic separation and deprotection of intermediates **2a–d** afforded the desired target derivatives **2e–h**. Product **3b** was obtained by a similar acylation of 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)adenine with *N*-(benzyloxycarbonyl)-L-phenylalanine, followed by deblocking. The NMR spectra of 2′ and 3′ isomers of stereoisomers **2a** and **2b** are discussed. Compounds **2g** and **3b** are both substrates and inhibitors of *Escherichia coli* ribosomal peptidyltransferase, although the activity of **3b** is low. Derivatives **2e,f,h** do not accept AcPhe from *N*-AcPhe-tRNA in a peptidyltransferase-catalyzed reaction, but they inhibit the puromycin reaction in the same system. The order of inhibitory activity is **2e > 2f > 2h**. The implications of these findings for the mechanism of peptidyltransferase and comparison of the latter with the action of chymotrypsin are discussed.

2′(3′)-*O*-Aminoacyl nucleosides, which are related to the 3′-terminal units of aminoacyl tRNAs,[3] are important models for studying the mechanism of ribosomal protein synthesis[4] and, particularly, the peptide bond formation step.[5] One of the most biologically active 2′(3′)-*O*-aminoacyl nucleosides found to date is 2′(3′)-*O*-L-phenylalanyladenosine (**1a**).[6,7] The latter can also be



1a: R′ = R² = H, X = O (+ 2′-ISOMER)

1b: R′ = CH_3, R² = CH_3O, X = NH

regarded as an analogue of the antibiotic puromycin (**1b**), a strong inhibitor of ribosomal protein synthesis.[8] There is a reasonably close parallel between the inhibition of protein synthesis by 2′(3′)-*O*-aminoacyl nucleosides and the corresponding puromycin analogues.[9–11] Therefore, 2′(3′)-*O*-aminoacyl nucleosides, which are readily available from inexpensive starting materials,[4,12] can be used as valuable leads for the design of puromycin analogues of therapeutic interest, accessible only from expensive puromycin aminonucleoside[13,14] or respective precursors. 2′(3′)-*O*-L-Phenylalanyladenosine and some related 2′(3′)-*O*-aminoacyl nucleosides have also been used as probes of protein synthesis steps, other than peptide bond formation, such as the binding site requirements of elongation factor T_u (EF-T_u) and EF-T_u-mediated hydrolysis of GTP.[15–18] Our prior studies also included an examination of a series of N-protected 2′(3′)-*O*-aminoacyl nucleosides that were found to inhibit cell growth in the murine leukemia L1210 in vitro system.[19] All these reasons have led us to the synthesis and biochemical evaluation of new 2′(3′)-*O*-aminoacyl nucleosides—analogues of 2′(3′)-*O*-phenylalanyladenosine.

Our selection centered on five derivatives, **2e–h** and **3b**,

(1) This paper is no. 40 in the series "Aminoacyl Derivatives of Nucleosides, Nucleotides and Polynucleotides". For a preceding report, see ref 2.
(2) Bhuta, P.; Chládek, S. *Biochim. Biophys. Acta* **1982**, *698*, 167.
(3) Abbreviations used are: tRNA, transfer ribonucleic acid; *N*-AcPhe-tRNA; *N*-acetyl-L-phenylalanyl tRNA; AcPhe, *N*-acetyl-L-phenylalanyl; AcPheOH, *N*-acetyl-L-phenylalanine; PheOH, L-phenylalanine; AcPhePhe-OH, *N*-acetyl-L-phenylalanyl-L-phenylalanine; A-(AcPhePhe), 2′(3′)-*O*-(*N*-acetyl-L-phenylalanyl)-L-phenylalanyladenosine; Cbz, benzyloxycarbonyl; Boc, *tert*-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; TLC, thin-layer chromatography, MeOTr, 4-methoxytriphenylmethyl.
(4) Žemlička, J.; Chládek, S.; Ringer, D.; Quiggle, K. *Biochemistry* **1975**, *14*, 5239.
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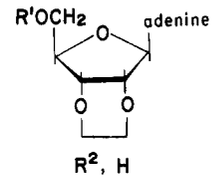


2a: R^1=H, R^2= L-$C_6H_5CH(NHCbzo)CH_2CO$

2b: R^1=H, R^2= D-$C_6H_5CH(NHCbzo)CH_2CO$

2c: R^1=H, R^2= D,L-$C_6H_5CH(NHCbzo)CO$

2d: R^1=MeOTr, R^2= $C_6H_5CH_2NCH_2CO$ (BOC)

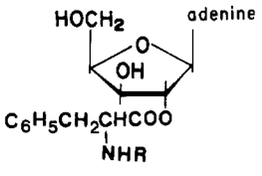
2e: R^1=H, R^2= L-$C_6H_5CH(NH_2)CH_2CO$

2f: R^1=H, R^2= D-$C_6H_5CH(NH_2)CH_2CO$

2g: R^1=H, R^2= D,L-$C_6H_5CH(NH_2)CO$

2h: R^1=H, R^2=$C_6H_5CH_2NHCH_2CO$

2i: R^1=H, R^2= L-C_6H_5CH(AcPheNH)CO



3a: R = Cbzo
3b: R = H
3c: R = AcPhe

four of which are isomeric with 2′(3′)-*O*-phenylalanyladenosine (compounds **2e,f,h** and **3b**), whereas analogue **2g** is a lower homologue of **1a**. The view has been expressed[20] that β-amino acid derivatives are unlikely to exhibit acceptor activity in a peptidyltransferase-catalyzed reaction. By contrast, the puromycin analogue of compound **2e + 2f** has been suggested for study.[21] However, the major reason for selection of stereoisomeric β-amino esters **2e** and **2f** was the fact that hydrolysis of the corresponding *N*-acetyl-3-amino-3-phenylpropionates catalyzed by chymotrypsin exhibited a reversed stereoselectivity.[22] Compounds **2e** and **2f** could, thus, provide additional basis for comparison of ribosomal peptidyltransferase and proteolytic enzymes (chymotrypsin). Furthermore, it was anticipated that analogue **2g** derived from phenylglycine, whose more simple *N*-acetyl-L-ester derivatives are also substrates for chymotrypsin,[23] might complement information on the hydrophobic locus of the peptidyltransferase A site. Similar reasoning led to the selection of compound **2h**. An inspection of space-filling models has indicated (data not shown) a similar orientation of the relevant[24] functional groups (aromatic moiety,

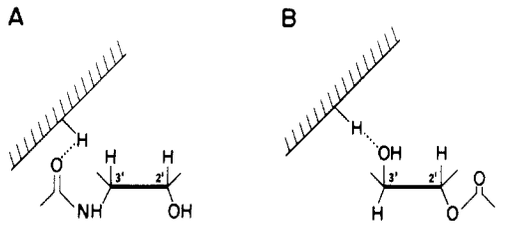


Figure 1. (A) Partial structure of puromycin (**1b**) in the vicinity of the CONH group. Note that the carbonyl function and H-3′ are eclipsed as found[29] in the crystal structure of **1b**. (B) Partial structure of 9-(2-*O*-L-phenylalanyl-β-D-xylofuranosyl)adenine (**3b**). Note that the 3′-hydroxy group can interact with the same portion of the ribosome (peptidyltransferase) as the carbonyl function of **1b**.

Table I. 2′(3′)-*O*-Aminoacyl Nucleosides

compd	λ_{max} (0.01 N HCl), nm	A_{250}/A_{260}	A_{280}/A_{260}	A_{290}/A_{260} [a]	mobility [b]	yield, %
2e + 2f	257	0.86	0.21	0.05	3.3	79 [c]
2g	258	0.89	0.28	0.08	3.5	79
2h	257	0.89	0.21	0.05	3.1 [d]	59
3b	258	0.83	0.19	0.03	3.9	71

[a] 2′(3′)-*O*-L-Phenylalanyladenosine had A_{250}/A_{260} = 0.88 and A_{280}/A_{260} = 0.17 (ref 34). [b] Toward the cathode, paper electrophoresis on Whatman No. 1 in 1 M acetic acid (see General Methods). [c] The yields and appropriate constants were similar for pure stereoisomers **2e** and **2f**. [d] Yellow coloration with ninhydrin.

carbonyl and amino functions) of both **1a** and **2h**. Analogue **3b** is derived from a biologically active[25] xylonucleoside. The ability of **3b** to accept a peptidyl (*N*-acylaminoacyl) residue from the corresponding peptidyl (*N*-acylaminoacyl) tRNA remains to be established.[26] However, the presence of the 3′-(up)-xylo hydroxy group could, in a favorable case, substitute, at least to some extent, for binding of the CONH group into the appropriate peptidyltransferase locus at the A site (Figure 1), and it was, therefore, of interest to examine the acceptor properties of analogue **3b**.

Synthesis. The preparation of compounds **2e–h** followed methods that were elaborated previously for the synthesis of 2′(3′)-*O*-aminoacyl nucleosides.[4,12] Thus, the appropriate *N*-benzyloxycarbonyl or *N*-*tert*-butoxycarbonyl amino acids were condensed with 5′-*O*-(4-methoxytrityl)adenosine by the action of dicyclohexylcarbodiimide (DCC) in pyridine. The resulting mixtures were resolved by preparative TLC, and intermediates **2a–c** were deprotected by hydrogenolysis[4,12] (Table I). In the case of **2d**, the risk of removal of the *N*-benzyl group[30] by hydrogenolysis during the final deprotection step prompted us to employ *tert*-butoxycarbonyl as a blocking group, which was removed by treatment[4] with 90% CF_3COOH.

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(26) Unlike 2′-*O*-aminoacyl nucleosides, several 2′-*O*-aminoacyl derivatives of cytidylyl-(3′–5′)-3′-deoxyadenosine[27] or the 3′-deoxy-2′-*O*-L-phenylalanyl tRNA[28] were shown to exhibit weak or moderate acceptor activity.
(27) Bhuta, A.; Quiggle, K.; Ott, T.; Ringer, D.; Chládek, S. *Biochemistry* **1981**, *20*, 8.
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Our strategy was also modified for the synthesis of xylofuranosyl derivative **3a**, which was obtained by condensation of *N*-(benzyloxycarbonyl)-L-phenylalanine with the corresponding 3′,5′-di-*O*-isopropylidene nucleoside. The isopropylidene group was then removed by treatment with Dowex 50 (H^+) in 70% ethanol[31] to give intermediate **3a**, which was then converted to **3b** by the usual procedure of hydrogenolysis.

It was not possible to obtain optically pure 2′(3′)-*O*-(D-phenylglycyl)adenosine by the above procedure. The amino acid portion in the resultant 2′(3′)-*O*-aminoacyl nucleoside was racemized to a large extent as shown by comparison of NMR and optical rotation data with those of the respective mixture of stereoisomers **2c** derived from racemic phenylglycine. This was further confirmed by activation of *N*-(benzyloxycarbonyl)-D-phenylglycine with DCC in the absence of the nucleoside component under otherwise identical conditions. The starting amino acid was completely racemized. The behavior of *N*-(benzyloxycarbonyl)-D-phenylglycine, thus, contrasts with that of *N*-benzyloxycarbonyl derivatives of natural amino acids that are resistant to racemization[32] even under conditions employed for the synthesis of 2′(3′)-*O*-aminoacyl nucleosides (DCC and pyridine).[33] However, active esters of L-phenylglycine are known to racemize quite easily in the presence of a strong base[32] because of a double activation of α-hydrogen atom.

NMR Spectra. Compounds **2e–h** and the corresponding intermediates **2a–d** were characterized along lines similar to those employed for other 2′(3′)-*O*-aminoacyl nucleosides.[4,12,34] Once again, NMR spectroscopy proved to be an especially valuable method. Differentiation of 2′ and 3′ positional isomers is now regarded to be routine in distinguishing 2′(3′)-*O*-aminoacyl nucleosides,[4] but NMR spectra of the corresponding D and L stereoisomeric pairs have, to our knowledge, not yet been studied.

As expected, the NMR spectrum of the mixture of **2a** and **2b**, which consists of all four possible isomers (Figure 2A), can be derived by superposition of the spectra of both optically, but not isomerically, pure stereoisomers **2a** and **2b** (Figure 2B,C). The spectrum of the mixture is well resolved despite the fact that four different compounds are present—two diastereoisomers, **2a** and **2b**, each of which is a mixture of the 2′ and 3′ isomer. The isomeric composition of both **2a** and **2b** is virtually identical; therefore, changes in the NMR pattern, as seen in Figure 2B,C, result from differences between stereoisomers **2a** and **2b**. It is clear that signals of the phenyl groups, amino function, and most of the ribose and amino acid protons are little influenced by stereoisomerism. By contrast, significant differences are observed in the region of purine ($H_8 + H_2$) and anomeric ($H_{1'}$) protons. Thus, the heterocyclic signals in the L stereoisomer **2a** are better resolved than in the D stereoisomer **2b** (cf. Figure 2B,C), whereas the $H_{1'}$ protons show an opposite trend. The low-amplitude signals at δ 8.05 and 8.13 (Figure 2B,C) are tentatively assigned to H_8 or H_2 of the 2′ isomers of **2a** and **2b**, respectively. This is in agreement with the ratio of the more resolved signal of **2a** at δ 8.05 (Figure 2B) to the rest of $H_8 + H_2$ determined from the integration curve (1:7.3).

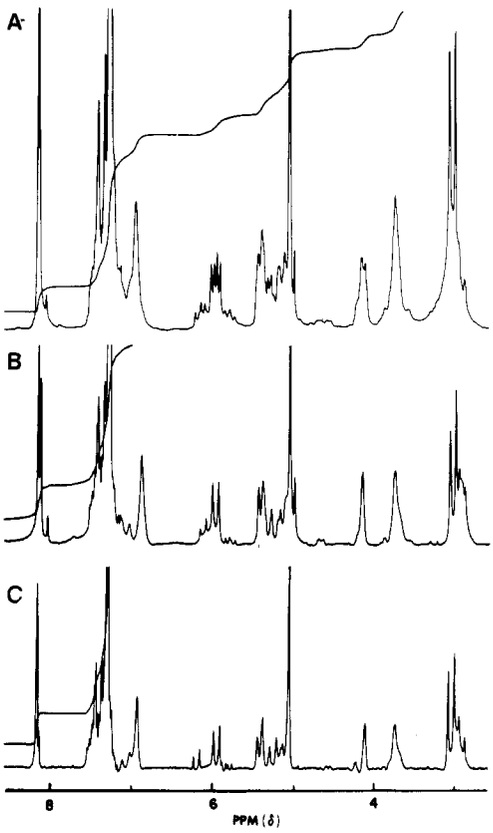

Figure 2. (A) NMR spectrum of the mixture of diastereoisomers **2a** and **2b** prepared from *N*-(benzyloxycarbonyl)-DL-3-amino-3-phenylpropionic acid (see Experimental Section). All spectra were measured in CD_3COCD_3. The 2′/3′ isomer ratio was calculated from the heights of the corresponding $H_{1'}$ signals. (B) NMR spectrum of the L stereoisomer **2a**: δ 8.17, 8.14, and 8.05 (3 s, 2, $H_8 + H_2$), 7.31 (m + d, 11, C_6H_5 partially overlapped with NH or OH), 6.89 (s, 2, NH_2), 6.13 (d, $H_{1'}$, 2′ isomer, $J_{1',2'}$ = 6.8 Hz), 5.99 (d, $H_{1'}$, 3′ isomer, $J_{1',2'}$ = 7.3 Hz, total integration 1), 5.81 (t, $H_{2'}$, 2′ isomer), 5.07 (s, CH_2 of benzyl, overlapped with ribose protons). Isomeric composition: 75% 3′ isomer, 25% 2′ isomer. (C) NMR spectrum of D stereoisomer **2b**: δ 8.17 and 8.13 (2 s, 2, $H_2 + H_8$), 7.36 (m, 10, C_6H_5), 6.93 (d, 3, NH_2 partially overlapped with another d, 1, NH or OH), 6.19 (d, $H_{1'}$, 2′ isomer, $J_{1',2'}$ = 7.3 Hz), 5.95 (d, $H_{1'}$, 3′ isomer, $J_{1',2'}$ = 7.6 Hz, total integration 1), 5.80 (t, $H_{2'}$, 2′ isomer), 5.06 (s, CH_2 of benzyl, overlapped with ribose protons). Isomeric composition: 73% 3′ isomer, 27% 2′ isomer.

The calculated ratio based on the 25% 2′-isomer content in the mixture is 1:7. Similarly, triplets at δ 5.8 (figure 2B,C) probably belong to the $H_{2'}$ protons of the respective 2′ isomers. This assignment is supported by the fact that 2′-O-acylation causes a significant low-field shift of the $H_{2'}$ in ribonucleosides.[35] The 2′ and 3′ isomer assignment is based on the ratio of 2′ and 3′ isomers, which, in cases of isomerizable derivatives such as *O*-acyl,[36] phosphoryl,[36] and silyl[37] ribonucleosides, always show a preponderance of the 3′ isomer in a thermodynamically controlled equilibrium mixture. In addition, according to a general rule,[36] the $H_{1'}$ signal of the 2′ isomer is at lower field and has a lower $J_{1',2'}$ coupling constant than the corresponding 3′ isomer. One of the few exceptions found to date are 2′- and 3′-*O*-*tert*-butyldimethylsilyl derivatives,[37] which showed a reversed trend of the respective chemical shifts but not $J_{1',2'}$ constants. However, it is clear that both $\delta_{H_{1'}}$ of the 2′ and 3′

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(32) Bodanszky, M.; Bodanszky, A. *Chem. Commun.* **1967**, 591.
(33) Thus, 2′(3′)-*O*-D-phenylalanyladenosine prepared by a similar method[12] did not display any acceptor activity.[9] Admixture of as little as 0.2% of the L stereoisomer is readily detectable by the peptidyltransferase assay (see Biological Activity section).
(34) Chládek, S.; Pulkrábek, P.; Sonnenbichler, J.; Žemlička, J.; Rychlík, I. *Collect. Czech. Chem. Commun.* **1970**, *35*, 2296.
(35) Endo, T.; Žemlička, J. *J. Org. Chem.* **1979**, *44*, 3652, Table II.
(36) Fromageot, H. P. M.; Griffin, B. E.; Reese, C. B.; Sulston, J. E.; Trentham, D. R. *Tetrahedron* **1966**, *22*, 705.
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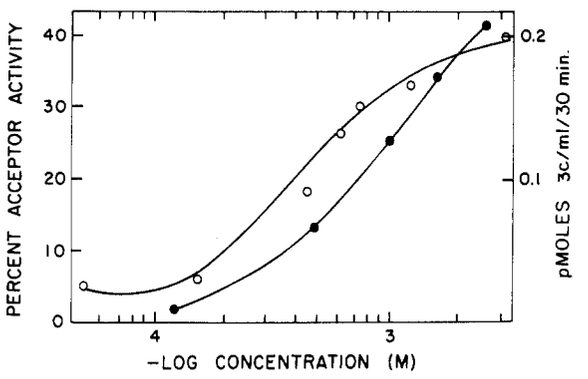

Figure 3. Acceptor activity of 2′(3′)-*O*-(DL-phenylglycyl)adenosine (**2g**) and 9-(2-*O*-L-phenylalanyl-β-D-xylofuranosyl)adenine (**3b**). For the corresponding assay, see Experimental Section. Percent acceptor activity refers to the amount of Ac[^{14}C]Phe residue transferred from *N*-Ac[^{14}C]Phe-tRNA to the acceptor **2g**. It was determined as the difference of radioactivity retained on the filter after incubation without acceptor and that remaining after incubation with **2g**. In the case of **3b**, the actual amounts of product **3c** formed are given. Compounds **2g** (○) and **3b** (●).

isomers and the corresponding $J_{1',2'}$ values of compounds **2a** and **2b** (Figure 2B,C) exhibit the pattern predicted by the rule.[36]

Compound **2c** was obtained only as a diastereoisomeric mixture of 2′ and 3′ isomers; therefore, the NMR spectrum was not subjected to a detailed study. The $H_{1'}$ protons appeared as two partially overlapped doublets, but it was not possible to discern whether they belong to diastereoisomers or the corresponding positional isomers. A similar complex pattern was recognized in the purine H_8 and H_2 protons of **2c**. The *N*-benzylglycyl derivative **2d**, where the possibility of diastereoisomerism is precluded, was predominantly the 3′ isomer.

In some cases, the 2′,3′ isomerism was also reflected in the behavior of the respective derivatives on TLC. Thus, compound **2b** moved as a double spot in dichloromethane–methanol (9:1, S_1), but no isomer separation was observed in the case of stereoisomer **2c**. Both compounds appeared as a single spot in dichloromethane–methanol (95:5, S_2). By contrast, compounds **2c** and **2d** exhibited a double spot in solvent S_2. Partial or complete separation of isomeric 2′(3′)-*O*-aminoacyl nucleosides and their corresponding precursors was observed before in various chromatographic[27,31,38,39] or electrophoretic systems.[40]

Biological Activity. A. Acceptor Properties. All target compounds **2e–h** and **3b** were tested for acceptor activity, i.e., the ability to accept an AcPhe residue from the *N*-AcPhe-tRNA–poly(U)–70S ribosome complex. Only derivatives **2g** and **3b** exhibited measurable activity—nucleoside **2g** being a moderate acceptor and compound **3b** a weak acceptor (Figure 3). Previous preliminary results[41] indicated that the racemic mixture **2e** + **2f** exhibited a moderate acceptor activity. By contrast, both stereoisomers **2e** and **2f**, including their equimolar mixture, were inactive in the acceptor assay. This discrepancy was resolved by finding traces of a strong acceptor, isomeric 2′(3′)-*O*-L-phenylalanyladenosine, in the mixture of **2e** + **2f** prepared from a commercial sample of DL-3-amino-3-phenylpropionic acid. It was calculated, based on the activity of 2′(3′)-*O*-L-phenylalanyladenosine,[42] that as little as 0.2% of the latter in the mixture of **2e** + **2f** could have been responsible for the previous result. Paper electrophoresis of the commercial DL-3-amino-3-phenylpropionic acid in 1 M acetic acid (ninhydrin detection) showed only a single spot present at a level that would have readily revealed 0.1% of phenylalanine. However, the presence of a minute amount of a ninhydrin-negative phenylalanine derivative, which would give rise to the respective *N*-benzyloxycarbonyl intermediate and, thus, be incorporated into the nucleoside during subsequent synthetic steps, cannot be excluded. The impurity was removed by repeated crystallization employed in the resolution of the starting DL-3-amino-3-phenylpropionic acid. Thus, the virtually complete lack of acceptor properties of **2e** and **2f** is in sharp contrast to the corresponding D- and L-esters of *N*-acetyl-3-amino-3-phenylpropionic acid, which proved to be substrates for chymotrypsin but with a reversed stereoselectivity.[22] It appears that although peptidyltransferase is, by necessity, less specific for the type of natural amino acid, it is much more sensitive toward other structural factors, such as the position of the amino group, than chymotrypsin.

The phenylglycyl derivative **2g** (Figure 3) was the strongest acceptor of the series **2e–h** and **3b**. The L stereoisomer of compound **2g** is a lower homologue of an excellent substrate, 2′(3′)-*O*-L-phenylalanyladenosine,[7,42] and it is therefore somewhat surprising that nucleoside **2g** exhibited only moderate activity. Apparently, the removal of the methylene group located between the hydrophobic group (phenyl) and asymmetric center of an amino acid is more detrimental to activity than lengthening of the chain.[43]

Compound **2g** is a stereoisomeric mixture derived from an unnatural amino acid, and it was therefore of interest to determine the stereospecificity of the peptidyltransferase-catalyzed peptidation. Although aminoacyl nucleoside analogues derived from racemic carboxylic acids have been used[44–46] for structure–activity studies of peptidyltransferase, their application for determination of the stereochemical course of the reaction has not yet been described. Thus, product **2i** obtained from the peptidyltransferase-catalyzed peptidation of **2g** was subjected to alkaline hydrolysis, and the resultant dipeptide was degraded with carboxypeptidase A, which is specific for peptides with a C-terminal aromatic amino acid residue of the L configuration.[47] Total digestion of the dipeptide to AcPheOH then proved that the original product **2i** resulted exclusively from the L stereoisomer of **2g**.

The last example of an active acceptor, albeit weak, is the xylofuranosyl derivative **3b**. This is the first 2′-*O*-aminoacyl nucleoside with established acceptor activity that corresponds roughly to that of 2′-deoxy-3′-*O*-L-phenylalanyladenosine.[7] It is of interest to note that the sugar conformation (3′-endo) of 9-β-D-xylofuranosyladenine[25] is the same as in puromycin.[29] Again, product **3c** of the peptidyltransferase-catalyzed reaction was isolated and hydrolyzed with alkali to the corresponding

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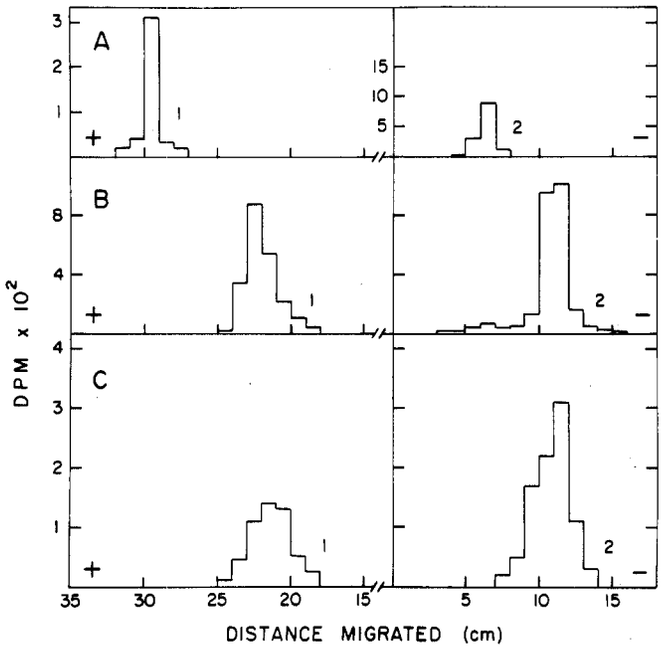

Figure 4. Paper electrophoresis of the products obtained from the peptidyltransferase-catalyzed reaction of **3b** with *N*-AcPhe-tRNA–poly(U)–70S ribosome complex. For details of the assay and analysis of the reaction mixture, see Experimental Section. Panel A. Reference compounds: 1, AcPheOH, and 2, PheOH. Panel B. Control reaction of 2′(3′)-*O*-L-phenylalanyladenosine with the ribosomal complex: 1, product after alkaline hydrolysis (AcPhePheOH); 2, product before hydrolysis [A-(AcPhePhe)]. Panel C. Reaction of compound **3b**: 1, product after alkaline hydrolysis (AcPhePheOH); 2, product before alkaline hydrolysis (nucleoside **3c**).

dipeptide (Figure 4). Our observation lends some credence to the hypothesis (Figure 1) that the 3′-(up)-xylo hydroxy group is able to substitute, at least in part, for other binding sites such as 3′-ester or amide function. The *N*-benzylglycyl derivative **2h** did not exhibit any acceptor activity.

B. Inhibition Studies. All target compounds **2e–h** and **3b** were investigated as inhibitors of the puromycin reaction with *N*-AcPhe-tRNA–poly(U)–70S ribosome complex. The results are summarized in Figure 5. It is apparent that compounds **2e** and **2f**, which do not exhibit acceptor activity, are inhibitors of peptidyltransferase. Interestingly, the L stereoisomer **2e** is significantly more effective than the D stereoisomer **2f** following the pattern found in the corresponding stereoisomers of α-aminoacyl derivatives.[9,48] An equimolar mixture of **2e** and **2f** exhibited the expected intermediate activity. It is noteworthy that the antibiotics blasticidin S and streptothricin F, which are inhibitors of protein synthesis but not substrates for peptidyltransferase, also incorporate β-L-amino acid moieties.[49,50]

Inhibitory activity of compounds **2g** and **3b** followed their efficiency as substrates in the peptidyltransferase-catalyzed reaction. Thus, derivative **2g** was considerably more active than **3b**. The effectiveness of the *N*-benzylglycyl analogue **2h** was very poor, corresponding roughly to that of 2′(3′)-*O*-glycyladenosine both as a substrate[7,9] or inhibitor.[51] It is apparent then that the presence of an aromatic moiety in **2h** has little influence on activity.

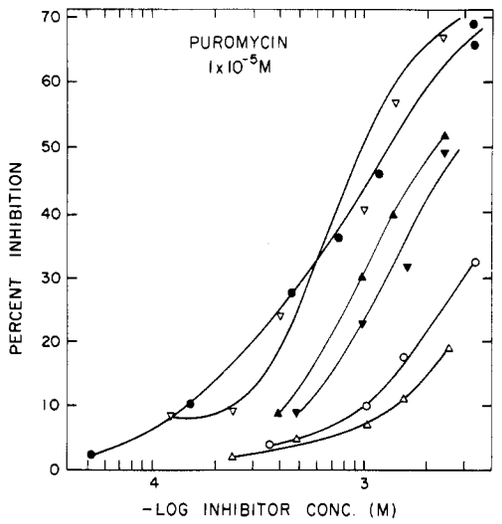

Figure 5. Inhibition of the puromycin reaction with compounds **2e–h** and **3b**. For details of the assay, see Experimental Section: compound **2e** (▽), **2f** (▼), equimolar mixture of **2e** + **2f** (▲), **2g** (●), **2h** (○), **3b** (△).

Experimental Section

General Methods. TLC, including preparative TLC on loose layers, was performed as described[4] in the following solvents: S_1, dichloromethane–methanol (9:1); S_2, dichloromethane–methanol (95:5).

Paper electrophoresis was also conducted according to the literature procedure[4] in 1 M acetic acid at 10 °C on Whatman No. 1 at 40 V/cm for 1 h or on 3MM paper for 3 h. NMR spectra were determined with an FX 100 Fourier transform NMR spectrometer (JEOL Ltd., Tokyo, Japan) with CD_3COCD_3 or $CDCl_3$ as solvents and $Si(CH_3)_4$ as an internal reference unless stated otherwise.

Starting Materials. *N*-(Benzyloxycarbonyl)-L-phenylalanine, ethyl *N*-benzylglycinate, 5′-*O*-(4-methoxytrityl)adenosine, and DL- and D-phenylglycine were commercial products. Racemic 3-amino-3-phenylpropionic acid was either obtained from Aldrich Chemical Co., Milwaukee, WI (lot no. 052567), or it was prepared as described.[52] 9-β-D-Xylofuranosyladenine was obtained through the courtesy of Leonard H. Kedda, Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD.

***N*-Benzylglycine Hydrochloride.** Ethyl *N*-benzylglycinate (0.02 mol) was hydrolyzed according to the literature[53] to give *N*-benzylglycine hydrochloride (60%), mp 223–224 °C. Crystallization from ethanol (17 mL) afforded a sample (25%): mp 226–228 °C (lit.[53] mp 214–215 °C); NMR [D_2O, external $Si(CH_3)_4$] δ 7.48 (s, 5, C_6H_5), 4.29 (s, 2, CH_2 of benzyl), 3.88 (s, 2, CH_2 of glycine).

Resolution of DL-3-Amino-3-phenylpropionic Acid. The mixture of D- and L-3-(formylamino)-3-phenylpropionic acid was resolved via the corresponding quinine and quinidine salt with minor modifications of the described procedure.[54] The quinidine salt of L-3-(*N*-formylamino)-3-phenylpropionic acid was crystallized three times from methanol to a constant $[\alpha]^{25}_D$ +184.9° (*c* 5, methanol) and mp 189–190 °C [lit.[52] $[\alpha]^{25}_D$ +181° (*c* 5, ethanol); mp 192–193 °C]. The free *N*-formyl-L-acid was obtained as described,[54] but it was further purified by passing through a Dowex 50 (WX 2, H^+ form, 200–400 mesh) column in 50% aqueous dioxane, and, finally, it was crystallized from water: mp 139–142 °C; $[\alpha]^{25}_D$ +112.8° (*c* 8, ethanol) [lit.[54] mp 142–143 °C; $[\alpha]^{20}_D$ +116.4°]; NMR δ 8.17 (s, 1, NCHO), 7.38 (m, 5, C_6H_5), 5.48 (t, 1, CH), 2.85 (m of d, 2, CH_2). This compound was hydrolyzed to the hydrochoride of L-3-amino-3-phenylpropionic acid as described.[54]

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The crude D-acid was obtained from the mother liquors after the first crystallization of the above quinidine salt of the L-acid.[54] The D-acid was converted to the quinine salt[54] obtained as an oil, which crystallized after addition of acetone. It was recrystallized four times from 20% aqueous ethanol to a constant $[\alpha]^{25}_D$ −159.7° (c 3.5, ethanol) and mp 143–145 °C [lit.[55] $[\alpha]^{22}_D$ −164.2°]. The free *N*-formyl-D-acid was obtained as described: mp 133–135 °C; $[\alpha]^{25}_D$ −110.5° [lit.[51] 134–135 °C; $[\alpha]^{22}_D$ −118.2° (c 1, 2, ethanol)]. NMR was identical with that of the L-acid. Both *N*-formyl-D- and -L-acids contained traces of the respective free amino acids as evidenced by NMR and ninhydrin test. This compound was hydrolyzed to the hydrochloride of D-3-amino-3-phenylpropionic acid.[54]

***N*-(Benzyloxycarbonyl)-DL-3-amino-3-phenylpropionic Acid.** The known[56] procedure was modified as follows: A solution of 2 N NaOH (5.5 mL, 0.011 mol) and benzyloxycarbonyl chloride (1.54 mL, 0.011 mol) were added simultaneously dropwise from two separate syringes into DL-3-amino-3-phenylpropionic acid (Aldrich Chemical Co.; 1.65 g, 0.01 mol) in 2 N NaOH (5 mL, 0.01 mol) with magnetic stirring and ice cooling. After approximately 10 min, a thick white precipitate appeared (pH 12), which dissolved on addition of water (50 mL). The solution was extracted with ether (2 × 30 mL) and acidified with 1 N HCl (25 mL), and the product was taken into ether (2 × 40 mL). The latter ether portions were combined, dried ($MgSO_4$), and evaporated in vacuo to give the title compound as a white solid: yield 1.98 g (66%); homogeneous on TLC (S_1). The product was purified for analysis by precipitation from dichloromethane with petroleum ether: 62% yield; NMR (CD_3COCD_3 + D_2O) δ 7.32 (m, 10, C_6H_5), 5.17 (t, 1, CH), 5.05 (s, 2, CH_2 of benzyl), 2.84 (d of d, 2, CH_2). Anal. ($C_{17}H_{17}NO_4$) C, H, N.

***N*-(Benzyloxycarbonyl)-L-3-amino-3-phenylpropionic Acid.** This compound was prepared by the procedure described above from the hydrochloride of L-3-amino-3-phenylpropionic acid. The amount of 2 N NaOH used for dissolving the starting amino acid was doubled: yield 65%; mp 120–130 °C; $[\alpha]^{25}_D$ +20° (c 0.5, ethanol); NMR was identical with that of the corresponding racemic product.

***N*-(Benzyloxycarbonyl)-D-3-amino-3-phenylpropionic Acid.** This compound was prepared according to the procedure described above from the hydrochloride of D-3-amino-3-phenylpropionic acid: yield 42%; $[\alpha]^{25}_D$ −22.2° (c 0.5, ethanol); NMR was identical with that of the L enantiomer and racemic compound.

***N*-(Benzyloxycarbonyl)-DL-phenylglycine.** This product was obtained by the procedure described above. Thus, DL-phenylglycine (0.02 mol) afforded the title derivative in 88% yield: mp 128–130 °C; NMR (CD_3COCD_3) δ 7.34 (m, 10, C_6H_5), 5.36 (apparent t, 1, CH, collapsed to a singlet after addition of D_2O), 5.08 (s, 2, CH_2). Anal. ($C_{16}H_{15}NO_4$) C, H, N.

***N*-(Benzyloxycarbonyl)-D-phenylglycine** was prepared according to the procedure outlined above: yield 92%; mp 131–132 °C; $[\alpha]^{25}_D$ −100.8° (c 0.5, ethanol); NMR was identical with that of racemic compound, except that the CH signal appeared as a doublet at δ 5.38, which collapsed to a singlet after additon of D_2O.

***N*-Benzyl-*N*-(*tert*-butoxycarbonyl)glycine.** The general literature procedure[57] was followed. 2-[[(*tert*-Butoxycarbonyl)-oxy]imino]-2-phenylacetonitrile (BOC-ON, Aldrich Chemical Co.; 1.35 g, 5.5 mmol) was added at room temperature with magnetic stirring to a solution of *N*-benzylglycine hydrochloride (1 g, 5 mmol) and triethylamine (1.74 mL, 12.5 mmol) in 50% aqueous dioxane. The mixture containing an oily product was stirred for 3.5 h (the oil dissolved in ca. 30 min.). Water (8 mL) was then added, followed by ethyl acetate (10 mL), and the layers were separated. The aqueous portion was extracted once more with ethyl acetate (10 mL), and it was acidified with citric acid (1.9 g, 10 mmol). The product was extracted with ether (2 × 15 mL), and the extracts were dried ($MgSO_4$) and evaporated in vacuo to give a colorless syrup. Adding petroleum ether (10 mL) and then cooling the mixture to −15 °C gave the crystalline *N*-benzyl-*N*-(*tert*-butoxycarbonyl)glycine: yield 0.87 g (65%); mp 98–101 °C; homogeneous on TLC (S_1); NMR (CD_3COCD_3) δ 7.30 (s, 5, C_6H_5), 4.51 (s, 2, CH_2 of benzyl), 3.88 (d, 2, CH_2), 1.43 (s, 9, CH_3). Anal. ($C_{14}H_{19}NO_4.0.25H_2O$) C, H, N.

9-(3,5-*O*-Isopropylidene-β-D-xylofuranosyl)adenine. This intermediate was prepared by the procedure used for 2′,3′-*O*-isopropylideneribonucleosides.[58] 9-β-D-Xylofuranosyladenine (0.27 g, 1 mmol), dried by coevaporation with dimethylformamide (DMF) in vacuo, was dissolved in DMF (6 mL). Triethyl orthoformate (0.3 mL, 1 mmol), acetone (0.1 mL, 1 mmol), and 6 M HCl in DMF (0.2 mL, 1.2 mmol) were added, and the solution was magnetically stirred at room temperature. TLC in S_1, after 22 h, showed that the reaction was not complete. Therefore, new portions of triethyl orthoformate (1 mmol), acetone (0.5 mmol), and HCl in DMF (0.6 mmol) were added, and the mixture was kept for 5 days at room temperature. To improve further the conversion, we added triethyl orthoformate (2 × 1 mmol) and acetone (2 × 1 mmol) over the next 2 days. NH_4OH (0.5 mL) was then added, the mixture was evaporated in vacuo, and the residue was chromatographed on a 3-mm thick 35 × 15 cm loose layer of silica gel in solvent S_1. The faster moving, strongly UV-absorbing band was eluted with the same solvent, and the eluate was evaporated to a syrup, which partly crystallized during drying in vacuo. An addition of methanol (4 mL) and ether (20 mL) completed the crystallization to give the title compound: yield 0.16 g (52%); mp 202–204 °C; homogeneous on TLC (S_1) [lit.[59,60] mp 206–208 °C]; NMR (CD_3SOCD_3) δ 8.31 (s, 1, H_8), 8.17 (s, 1, H_2), 7.26 (s, 2, NH_2), 6.13 (d, 1, OH), 5.98 (s, 1, H_1'), 4.29 (apparent m, 5, ribose protons), 1.44 and 1.26 (2 s, 6, CH_3).

Aminoacylation of 5′-*O*-(4-Methoxytrityl)adenosine. The general procedure[4,12] was followed with minor modifications. A mixture of 5′-*O*-(4-methoxytrityl)adenosine (1 mmol) and N-protected amino acid (1 mmol) was made anhydrous by evaporation with pyridine (2 × 5 mL). The residue was dissolved in pyridine (5 mL), the solution was cooled in an ice bath, and dicyclohexylcarbodiimide (1 mmol) was added with magnetic stirring, which continued for 1 h at 0 °C and for 20 h at room temperature. Ice was then added, dicyclohexylurea was filtered off and washed with pyridine (5 mL), and the filtrate was extracted with petroleum ether (3 × 10 mL). The aqueous pyridine layer was evaporated in vacuo, and the residue was lyophilized from dioxane (2 × 10 mL). The residue was dissolved in dichloromethane, any insoluble portion[61] was filtered off, and the solution was applied on two 3-mm thick 35 × 15 cm loose layers of silica gel, which were developed in solvent S_2. Three major UV-absorbing bands were invariably obtained; starting material, 2′-(3′)-*O*-aminoacyl derivative and 2′,3′-*O*-diaminoacyl derivative in the order of increasing mobility. The intermediate band was eluted with solvent S_1, the eluate was evaporated, the residue was dissolved in 80% acetic acid (20 mL), and the solution was allowed to stand for 4 h at room temperature. After lyophilization, the residue was chromatographed on a single layer of silica gel as above in S_1. The major UV-absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was converted to a solid by precipitation from dichloromethane solution with petroleum ether. Compounds **2a** + **2b**, **2a**, and **2b** (yield 36, 46, and 26%, respectively) were homogeneous on TLC in S_2, whereas **2a** + **2b** and **2b** moved as double spots in S_1, but a single spot was observed in the case of **2a**. Conversely, compound **2c** (yield 36%) was homogeneous in S_1, but it formed a double spot in S_2. For NMR of **2a** + **2b**, **2a**, and **2b**, see Figure 2. Anal. for compound **2a** + **2b** ($C_{27}H_{28}N_6O_7$) C, H, N. Compound **2c**, obtained from racemic phenylglycine, had $[\alpha]^{25}_D$ −46° (c 0.5, dichloromethane), whereas the product obtained from the corresponding D enantiomer had $[\alpha]^{25}_D$ −59°. NMR spectra (CD_3COCD_3) of both

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(60) Magnani, A.; Mikuriya, Y. *Carbohydr. Res.* **1973**, *28*, 158.
(61) Occasionally, the starting 5′-*O*-(4-methoxytrityl)adenosine crystallized out when the solution was allowed to stand for 30 min or longer at room temperature prior to chromatography.

products were very similar: δ 8.33, 8.29, and 8.23 (3 s, 2, H_8 + H_2), 7.37 (m, 10, C_6H_5), 6.02 and 5.95 (2 d, 1, $H_{1'}$), 5.52 (m, 2, $H_{2'}$ + $H_{3'}$), 5.15 (s, 2, CH_2 of benzyl). Anal. ($C_{26}H_{26}N_6O_7$) C, H, N.

2′(3′)-O-[N-Benzyl-N-(*tert*-butoxycarbonyl)glycyl]-5′-O-(4-methoxytrityl)adenosine (2d). The condensation of 5′-O-(4-methoxytrityl)adenosine with N-benzyl-N-(*tert*-butoxycarbonyl)glycine was performed as described above. Compound **2d** was isolated by chromatography in solvent S_2: yield 39%. It moves as a double spot on TLC in solvent S_2: NMR (CD_3SOCD_3) δ 8.28 (s, 1, H_8), 8.08 (s, 1, H_2), 7.30 and 6.86 (s + d, 19, C_6H_5 + p-methoxyphenyl), 6.13 (d, $H_{1'}$, 2′ isomer), 5.91 (d, $H_{1'}$, 3′ isomer, $J_{1'2'}$ = 5 Hz, total integration 1), 4.45 (s, 3, CH_3O), 1.36 (s, 9, CH_3C). Anal. ($C_{44}H_{46}N_6O_8$) C, H, N.

2′-O-[N-(Benzyloxycarbonyl)-L-phenylalanyl]-9-β-D-xylofuranosyladenine (3a). The condensation of 9-(3,5-O-isopropylidene-β-D-xylofuranosyl)adenine with N-(benzyloxycarbonyl)-L-phenylalanine was performed as described above on a 0.4-mmol scale. The procedure led to only ca. 40% conversion as judged by TLC in solvent S_2. Therfore, new portions of N-(benzyloxycarbonyl)-L-phenylalanine and dicyclohexylcarbodiimide (0.4 mmol each) were added, and the reaction was continued for 4 days at room temperature. The workup followed the general procedure: the crude product was partioned between dichloromethane (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was washed with water (10 mL), dried ($MgSO_4$), and evaporated. 9-[2-O-[N-(Benzyloxycarbonyl)-L-phenylalanyl]-3,5-O-isopropylidene-β-D-xylofuranosyl]adenine, obtained as a foam homogeneous on TLC (S_1), was dissolved in 70% ethanol (50 mL), Dowex 50 WX2 (H^+ form, 100–200 mesh, prewashed with 70% ethanol, 5 g wet weight) was added, and the mixture was magnetically stirred for 1 h at room temperature. The resin was filtered off and washed successively with 50% aqueous pyridine (50 mL) and pyridine (25 mL). The combined filtrate and washings were evaporated in vacuo, and the resultant crude **3a** was chromatographeed on a single 3-mm thick loose layer of silica gel in solvent S_1. The major UV-absorbing band was then rechromatographed on two silica gel GF 2-mm thick 20 × 20 cm plates (Analtech, Newark, DE) in the same solvent to give compound **3a**: 80 mg (37%); NMR (CD_3COCD_3) δ 8.21 (s, 1, H_8), 8.10 (s, 1, H_2), 7.33 and 7.29 (2 s, 10, C_6H_5), 6.82 (poorly resolved apparent t, 3, NH_2 and OH), 5.99 (s, 1, $H_{1'}$), 5.44 (s, 1, $H_{2'}$), 5.06 (s, CH_2 of benzyl). Anal. ($C_{27}H_{28}N_6O_7.0.5H_2O$) C, H, N.

Racemization of N-(Benzyloxycarbonyl)-D-phenylglycine with Dicyclohexylcarbodiimide in Pyridine. The experiment was essentially performed according to the general aminoacylation procedure described above, only the nucleoside component was omitted. Thus, N-(benzyloxycarbonyl)-D-phenylglycine (0.29 g, 1 mmol) was magnetically stirred with dicyclohexylcarbodiimide (0.21 g, 1 mmol) in pyridine (5 mL) at 0 °C for 1 h and then at room temperature for 24 h. Ice was added, the mixture was evaporated, and the residue was partitioned between 3% aqueous $NaHCO_3$ (20 mL) and ether (2 × 20 mL). The aqueous portion was filtered and acidified with 1 N HCl (20 mL) to give a solid, which was filtered off and washed with water (10 mL): yield 0.16 g (55%). Precipitation from dichloromethane (5 mL) with petroleum ether (25 mL) gave 0.15 g (52%) of racemic N-(benzyloxycarbonyl)phenylglycine: $[\alpha]^{25}_D$ 0 (c 0.5, ethanol), whose mobility on TLC (S_1) corresponded to that of the D enantiomer.

2′(3′)-O-Aminoacyl Nucleosides 2e–g and 3b. The general procedure was followed.[4,12] Compound **2a**, **2b**, **2c**, or **3a** (40–50 μmol) was dissolved in cold 80% acetic acid (3 mL), $PdO/BaSO_4$ (5%, 20–30 mg) was added, and a slow stream of hydrogen was introduced beneath the surface of the liquid with magnetic stirring and ice cooling for 2–3 h. The catalyst was filtered off with a Millipore HAWP filter (0.45 μm). Aliquots from the clear filtrate were removed for spectrophotometrical determination of yield using ϵ_{260} (pH 2) 14 300 for adenosine, purity (paper electrophoresis), and UV spectra (0.01 N HCl). The results are summarized in Table I. Frozen aliquots of 2′(3′)-O-aminoacyl nucleosides in 80% acetic acid were kept at −70 °C for many months without change, as confirmed by paper electrophoresis and biological activity (ribosomal peptidyltransferase assay). Compounds **2e–g** and **3b** were ninhydrin positive. All products were homogeneous on electrophoresis except occasional traces of hydrolytic products: adenosine and the corresponding amino acids. Compound **2e + 2f**, prepared from a commercial sample of DL-3-amino-3-phenylpropionic acid, contained a trace of 2′(3′)-O-L-phenylalanyladenosine as found by hydrolysis of a 10-μmol aliquot in aqueous triethylamine and subsequent electrophoresis on Whatman 3 MM paper. The mobility of DL-3-amino-3-phenylpropionic acid in 1 M acetic acid was 2.3 of phenylalanine. A sample of the commercial acid, used in the preparation, did not contain free phenylalanine as shown by electrophoresis at the 10-μmol level, which would readily detect 0.1% of the latter. The peptidyltransferase assay acceptor activity of this preparation of **2e + 2f** corresponded to ca. 0.2% of 2′(3′)-O-L-phenylalanyladenosine in the sample.

2′(3′)-O-(N-Benzylglycyl)adenosine (2h). Intermediate **2d** was deprotected as described before for 8-bromo-2′(3′)-O-L-phenylalanyladenosine.[4] Compound **2d** (39 mg, 50 μmol) was dissolved in 90% CF_3COOH (1.3 mL), and the dark red solution was kept for 35 min at room temperature. After dilution with water (1 mL), the mixture was lyophilized, the residue was dissolved in dioxane (0.5 mL), ether (5 mL) was added, and the white precipitate was isolated by decanting the solvent. The precipitation was repeated, and the solid **2h** was filtered off, washed with ether, dried and dissolved in 80% acetic acid. Further characterization followed the procedure outlined above (Table I). Compound **2h** gives a yellow coloration with ninhydrin similar to the parent N-benzylglycine.

Assay of Peptidyltransferase Activity. A. Acceptor Activity. The ability of compounds **2e–h** and **3b** to participate in the peptidyltransferase-catalyzed peptide bond formation was measured as described previously.[42,62] We prepared samples of **2e–h** and **3b** for assays by lyophilizing 1–2 μmol aliquots, dissolving in water, and adjusting the pH immediately before the assay to pH 6.5. When a precipitate appeared at this point, it was removed by centrifugation, and the UV absorbancy of the filtrate was rechecked. 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 (5200 cpm) of N-Ac[^{14}C]Phe-tRNA, a specific activity of 0.84 nmol of [^{14}C]phenylalanine per milligram tRNA, and substrate at desired concentrations. The reaction was stopped by the addition of 0.1 M $Be(NO_3)_2$ (0.1 mL) in 0.3 M acetate buffer (pH 5.5) saturated with $MgSO_4$. The products were extracted with ethyl acetate (1.5 mL). The ethyl acetate layer (1 mL) was transferred into a scintillation vial, and the radioactivity was determined in Scinti Verse scintillation mixture (10 mL, Fisher Scientific Co., Fair Lawn, NJ) in a Packard Tri-Carb liquid scintillation spectrometer at 73% counting efficiency. The acceptor activity of 2′(3′)-O-phenylglycyl derivative **2g** was determined by the trichloroacetic acid method.[42] The reaction was stopped by the addition of 2.5% trichloroacetic acid (3 mL) at 4 °C. After 15 min at 4 °C, the entire reaction mixture was filtered through a HAWP-Millipore filter (pore size 0.45 μm), which was washed with 2.5% trichloroacetic acid (3 × 3 mL) at 4 °C. The membranes were dried in a hot air oven, and the radioactivity was determined as specified above.

B. Inhibition of the Peptidyltransferase-Catalyzed Puromycin Reaction. In inhibition assays, the substrate was replaced by 1×10^{-5} M puromycin, and the corresponding inhibitor was added at desired concentrations. Inhibition with 2′(3′)-O-aminoacyl nucleosides exhibiting an acceptor activity (compounds **2g** and **3b**) was performed as follows:[62] The reaction was stopped by adding 0.1 N NaOH (0.1 mL), and the resultant mixture was incubated for 5 min at 37 °C to hydrolyze any 2′ or 3′ ester bond present. The ethyl acetate extraction was then carried out as described above. For futher details, compare the figures and the corresponding legends, which give a typical example of this procedure.

C. Identification of the Reaction Products Obtained from the Peptidyltransferase-Catalyzed Peptidation and Their Hydrolysis Products. The assay was performed as described above (section A), and the products were isolated by ethyl acetate extraction. One aliquot of the extract was subjected to paper electrophoresis[42] (see General Methods) on Whatman 3MM paper in 0.5% pyridine–5% acetic acid (v/v, pH 3.5) at 50 V/cm for 2.5 h. Another aliquot of the same extract was evaporated, and

(62) Li, C.; Bhuta, P.; Žemlička, J. *Biochemistry* **1978**, *17*, 2537.

the product was hydrolyzed in 0.2 N KOH (25 μL) at 37 °C for 30 min. It was then subjected to electrophoresis as described above. Ac[^{14}C]PheOH and [^{14}C]PheOH were used as reference compounds. The radioactive peaks were located with a Packard radiochromatogram scanner Model 7201. The mobilities of **2i** and the corresponding hydrolysis product, Ac[^{14}C]Phe-L-phenylglycine were virtually identical with those of A-(AcPhePhe) and AcPheOH. The peak areas were cut into 1-cm wide strips, and the radioactivity was measured as described in section A. Further details are given in the legend of Figure 4. An aliquot of Ac[^{14}]Phe-L-phenylglycine, obtained by hydrolysis of 2′(3′)-*O*-peptidyl nucleoside **2i** and subsequent electrophoresis, as described above, was eluted from the paper with 0.1 M NH_4HCO_3 (0.1 mL), and the eluate was lyophilized. Carboxypeptidase A (Worthington Biochemical Corp., Freehold, NJ, 10 μg, 0.1 unit per 20 nmol of peptide) in 0.1 M NH_4HCO_3 (0.1 mL) was added, and the mixture was incubated at 37 °C for 6 h and then subjected to paper electrophoresis as specified above. Only a single peak corresponding to that of AcPheOH was observed.

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Registry No. **2a**, 83649-50-7; **2b**, 83649-51-8; **2c**, 83649-52-9; **2d**, 83649-53-0; **2e**, 83649-54-1; **2f**, 83649-55-2; **2g**, 83649-57-4; **2h**, 83649-56-3; **3a**, 83649-43-8; **3b**, 83649-44-9; *N*-benzylglycine hydrochloride, 7689-50-1; ethyl *N*-benzylglycinate, 6436-90-4; DL-3-amino-3-phenylpropionic acid, 3646-50-2; L-3-(*N*-formylamino)-3-phenylpropionic acid quinidine, 83649-45-0; L-3-(*N*-formylamino)-3-phenylpropionic acid, 3082-67-5; D-3-(*N*-formylamino)-3-phenylpropionic acid quinine, 83649-46-1; D-3-(*N*-formylamino)-3-phenylpropionic acid, 40856-45-9; D-3-amino-3-phenylpropionic acid, 83649-47-2; *N*-(benzyloxycarbonyl)DL-3-amino-3-phenylpropionic acid, 14440-98-3; benzyloxycarbonyl chloride, 501-53-1; *N*-(benzyloxycarbonyl)-L-3-amino-3-phenylpropionic acid, 14441-07-7; L-3-amino-3-phenylpropionic acid hydrochloride, 83649-48-3; *N*-(benzyloxycarbonyl)-D-3-amino-3-phenylpropionic acid, 14441-08-8; *N*-(benzyloxycarbonyl)-DL-phenylglycine, 5491-18-9; DL-phenylglycine, 2835-06-5; *N*-(benzyloxycarbonyl)-D-phenylglycine, 17609-52-8; D-phenylglycine, 875-74-1; *N*-benzyl-*N*-(*tert*-butoxycarbonyl)glycine, 76315-01-0; 2-[[(*tert*-butoxycarbonyl)oxy]-imino]-2-phenylacetonitrile, 58632-95-4; *N*-benzylglycine hydrochloride, 7689-50-1; 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)-adenine, 7687-49-2; 9-β-D-xylofuranosyladenine, 524-69-6; 5′-*O*-(4-methoxytrityl)adenosine, 51600-11-4; *N*-(benzyloxycarbonyl)-L-phenylalanine, 1161-13-3; 9-[2-*O*-[[*N*-(benzyloxycarbonyl)-L-phenylalanyl]-3,5-*O*-isopropylidene-β-D-xylofuranosyl]adenine, 83649-49-4; peptidyltransferase, 9059-29-4.

1-(4-Aminobenzyl)- and 1-(4-Aminophenyl)isoquinoline Derivatives: Synthesis and Evaluation as Potential Irreversible Cyclic Nucleotide Phosphodiesterase Inhibitors

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In an effort to increase the specificity of the potent phosphodiesterase inhibitor papaverine, we synthesized two series of novel 1-(4-aminobenzyl)- and 1-(4-aminophenyl)isoquinoline derivatives, incorporating alkylating moieties on the amine substituents. These compounds were evaluated for their inhibitory action on phosphodiesterase preparations from bovine heart and rat cerebral cortex. Studies were also conducted to determine whether these compounds were reacting with the enzymes in an irreversible manner. The compounds were potent inhibitors of the phosphodiesterases; however, no evidence was found for an irreversible inhibition.

The role of cyclic nucleotides as intracellular mediators of the action of numerous physiological and pharmacological agents is well recognized. Certain defects in the metabolism of cyclic nucleotides may be involved in a wide variety of diseases, including cancer and cardiovascular disorders.[2] Intracellular concentrations of adenosine 3,5-monophosphate (cAMP) and guanosine 3,5-monophosphate (cGMP) are regulated, in part, by hydrolysis to their corresponding 5′-nucleotides by cyclic nucleotide phosphodiesterases. Multiple forms of phosphodiesterases differing in both structural and kinetic properties have been isolated from various tissues.[3] Enzymes with a relative substrate specificity for hydrolyzing either cAMP or cGMP have been described,[3,4] and previous work has also demonstrated the differential effects of various agents on the activity of these enzymes.[5] Thus, it should be possible to develop pharmacological agents that selectively alter intracellular levels of a specific cyclic nucleotide

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