was added dropwise a solution of 0.487 g of **1a** (2.33 mmol) in 5 mL of benzene. The reaction was carried out, monitored, and worked up as described above for racemic **7**. The crude product **7a** was purified by column chromatography as above to yield 0.228 g (38%) of pure **7a**.

(*S*)-2-(2,5-Dimethoxy-4-methylphenyl)-2-[(benzoyloxy)-amino]propane (7b). To a solution of 1.26 g of benzoyl peroxide (5.20 mmol) in 15 mL of benzene in a 100-mL round-bottomed flask was added dropwise a solution of 2.17 g of **1b** (10.3 mmol) in 15 mL of benzene. The crude product was purified by column chromatography as above to yield 1.32 g (39%) of pure **7b**.

(*RS*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-(hydroxy-amino)propane (2ab). To 0.515 g of racemic **7** (3.58 mmol) was added 3.6 mL of 95% ethanol and 7.2 mL of concentrated HCl, both of which had been purged with nitrogen, in a 10-mL round-bottomed flask. The solution was magnetically stirred and heated under reflux under nitrogen. The reaction was complete after 135 min. The ethanol was removed under vacuum and the residue was taken up in 5 mL of deionized water. The solution was then lyophyllized overnight to remove water and HCl. This left a dark purple powder containing crude **2** as the hydrochloride salt. Recrystallization from ethanol/ether gave pure 2 (41%), mp 128–130 °C (lit.[5] mp 126–128 °C).

(*R*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-(hydroxy-amino)propane (2a). To 0.29 g (0.882 mmol) of **7a** in a 10-mL round-bottomed flask was added 1 mL of 95% ethanol and 2 mL of concentrated HCl, both of which had been purged with nitrogen. The solution was magnetically stirred and heated under reflux and nitrogen. The reaction was allowed to proceed for 180 min and was worked up as described above. The crude product **2a** was recrystallized from ethanol/ether to give 0.049 g (21%) of pure **2a**: mp 137.5–139 °C.

(*S*)-1-(2,5-Dimethoxy-4-methylphenyl)-2-(hydroxy-amino)propane (2b). To 1.18 g of **7b** (3.58 mmol) was added 3.6 mL of 95% ethanol and 7.2 mL of concentrated HCl, both of which had been purged with nitrogen, in a 25-mL round-bottom flask. The solution was magnetically stirred and heated under reflux under nitrogen. The reaction was allowed to proceed for 150 min and was worked up as described above. The crude **2b** was recrystallized from ethanol/ether to give 0.352 g (38% of pure **2b**: mp 137.5–139 °C. anal. ($C_{12}H_{20}NO_3Cl$) C, H, N.

Acknowledgment. This research was supported by Public Health Service Research Grant MH 21219.

Puromycin Analogues. Effect of Aryl-Substituted Puromycin Analogues on the Ribosomal Peptidyltransferase Reaction

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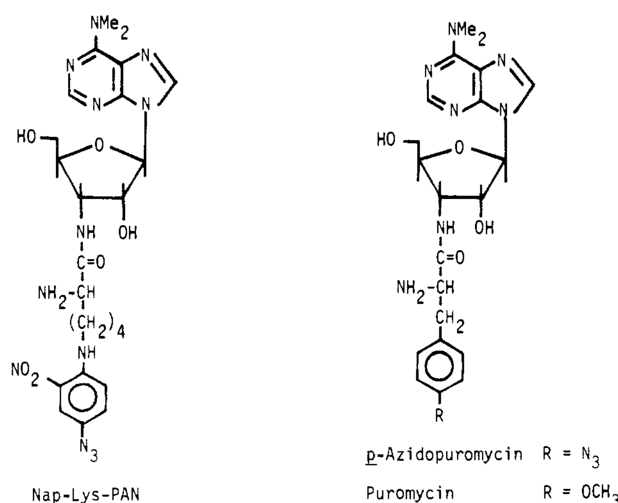
A series of ortho- and para-substituted L-phenylalanylpuromycin analogues were synthesized and evaluated as substrates for the peptidyltransferase reaction of *Escherichia coli* ribosomes. Kinetic results reveal that substitution of the *p*-methoxy group of the puromycin molecule alters the peptidyltransferase activity of the molecule with the following decreasing order of substrate efficiencies: p-NH_2 > p-$NHCOCH_3$ > p-NO_2 = p-$NHCO(CH_2)_2CH_3$ > p-$NHCOCH_2Br$. However, the inability of the ribosome to tolerate a nitro group at the ortho position of the phenylalanine ring precluded the use of the photosensitive puromycin analogue, 2-nitro-4-azidophenylalanylpuromycin aminonucleoside (**7a**), as a photoaffinity label for the peptidyltransferase site.

Numerous antibiotics have been shown to bind to bacterial and/or eucaryotic ribosomes and inhibit protein synthesis. The peptide chains of proteins are synthesized on ribosomes by a series of reactions culminating in peptide bond formation catalyzed by peptidyltransferase.[1,2] Current models of the active center of peptidyltransferase invoke a P site which binds the CCA terminus of peptidyl-tRNA and an A site which binds the CCA terminus of aminoacyl-tRNA.[3] The antitumor antibiotic puromycin inhibits protein synthesis by substituting for the incoming coded aminoacyl-tRNA at the A site and serving as an acceptor of the nascent peptide chain of ribosome-bound peptidyl-tRNA.[3] For this reason, puromycin has been used extensively in the investigation of the peptidyltransferase site.[4,5]

Chart I



Several approaches have recently been developed to label the A-site protein(s) of peptidyltransferase.[5a,6-10] In

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(4) R. J. Harris and S. Pestka, in "Molecular Mechanisms of Protein Biosynthesis", H. Weissbach and S. Pestka, Eds., Academic Press, New York, 1977, pp 413–442.
(5) (a) R. Vince, J. Brownell, and K. L. L. Fong, *Biochemistry*, 17, 5489 (1978); (b) K. L. L. Fong and R. Vince, *J. Med. Chem.*, 21, 792 (1978); (c) R. Vince and K. L. L. Fong, *Biochem. Biophys. Res. Commun.*, 81, 559 (1978).

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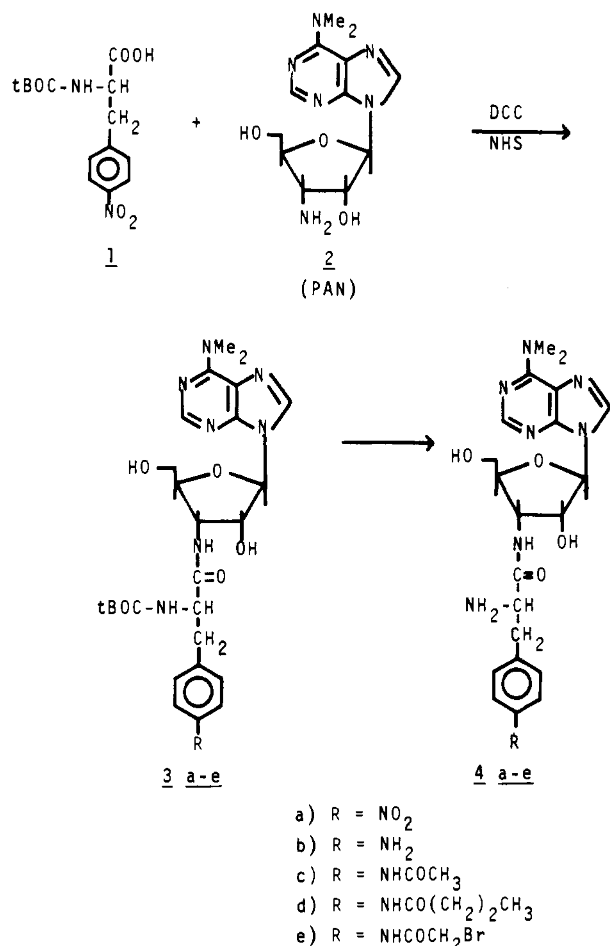
Scheme I



our approach, the photosensitive puromycin analogue N^{ϵ}-(2-nitro-4-azidophenyl)-L-lysinylpuromycin aminonucleoside (Nap-Lys-PAN) was synthesized[5a] (Chart I). The nitroaryl azide was used because it could be photolyzed with visible radiation, eliminating photochemical damage to the ribosome which usually occurs at lower wavelengths. Consequently, Nap-Lys-PAN was covalently bound to the A site of ribosomes in such a manner that it subsequently participated in peptide transfer with Ac-[^{14}C]Phe-tRNA positioned at the P site.[5a] In similar experiments, *p*-azido[^{3}H]puromycin (Chart I) was used in an attempt to label the puromycin binding site by placing the photosensitive moiety closer to the amino acid binding area.[9,10] Although azidopuromycin exhibited covalent binding to ribosomal proteins when irradiated at 254 nm, there was no indication that the bound material could participate in transpeptidation. These experiments initiated the study of aryl-substituted phenylalanine derivatives of puromycin as potential A-site labeling reagents which avoid low-wavelength activation. A logical choice for this approach included the 2-nitro-4-azidophenyl analogue **7a**. This compound combines the desirable features of Nap-Lys-PAN with those of azidopuromycin; i.e., photoactivation with visible light and closer proximity of the affinity group to the amino acid binding site. In addition, a chemically reactive bromoacetamido analogue, **4e**, was prepared as a potential labeling reagent. The present article reports the studies on binding of **4e**, **7a**, and related model compounds with *Escherichia coli* ribosomes.

Table I. Physical Data for Compounds in Scheme I

no.	R_1	R_2	% yield	mp, °C	mol formula
3a	*t*-Boc	NO_2	71	214-215	$C_{26}H_{34}N_8O_8 \cdot H_2O$
4a	H	NO_2	67	201-202	$C_{21}H_{26}N_8O_6$
3b	*t*-Boc	NH_2	88	98[a]	$C_{26}H_{36}N_8O_6$[b]
4b	H	NH_2	57	110-113	$C_{21}H_{29}N_8O_4 \cdot 3H_2O$
3c	*t*-Boc	$NHCOCH_3$	69	241-242	$C_{28}H_{38}N_8O_7$
4c	H	$NHCOCH_3$	88	162-164	$C_{23}H_{30}N_8O_5$[b]
3d	*t*-Boc	$NHCO(CH_2)_2CH_3$	64	219-221	$C_{30}H_{40}N_8O_7$
4d	H	$NHCO(CH_2)_2CH_3$	78	218-220	$C_{25}H_{34}N_8O_5$
3e	*t*-Boc	$NHCOCH_2Br$	80	180 dep	$C_{28}H_{37}N_8O_7Br \cdot 2H_2O$
4e	H	$NHCOCH_2Br$	75	155 dep	*c*

[a] Effervesced at 98 °C and then melted slowly. [b] After heating at 100 °C (0.1 mm) for 2 h. [c] Isolated as the trifluoroacetic acid salt.

Chemistry. The synthetic route for the monosubstituted aryl derivatives of puromycin is outlined in Scheme I. *N*-(*tert*-Butyloxycarbonyl)-*p*-nitro-L-phenylalanine (**1**) was condensed with puromycin aminonucleoside (PAN, **2**) using dicyclohexylcarbodiimide/*N*-hydroxysuccinimide by a method previously described[11] and gave the N-protected *p*-nitro-L-phenylalanylpuromycin aminonucleoside (**3a**). The nitro group of **3a** was reduced catalytically and gave α-*N*-(*tert*-butyloxycarbonyl)-*p*-amino-L-phenylalanylpuromycin aminonucleoside (**3b**). The coupling reaction of **3b** with the *N*-hydroxysuccinimide ester of the appropriate acid in dry dioxane or dimethylformamide gave the corresponding amides **3c-e**. In the case of the bromoacetamido derivative **3e** and its 5′-(bromoacetyl) ester were obtained in a 2:1 ratio. The ester group was selectively hydrolyzed in 1% ammonium hydroxide in methanol, and **3e** was subsequently isolated in 80% yield. Removal of the *N*-(*tert*-butyloxycarbonyl) protecting group from **3e** was accomplished with anhydrous trifluoroacetic acid at ambient temperature for 5 min. The bromoacetamido derivative **4e** was isolated as a trifluoroacetic acid salt. The remaining compounds in this series were deblocked in a similar manner and subsequently passed through IRA-400 (OH^-) resin to remove the remaining

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(9) W. Nicholson and B. S. Cooperman, *FEBS Lett.*, **90**, 203 (1978).
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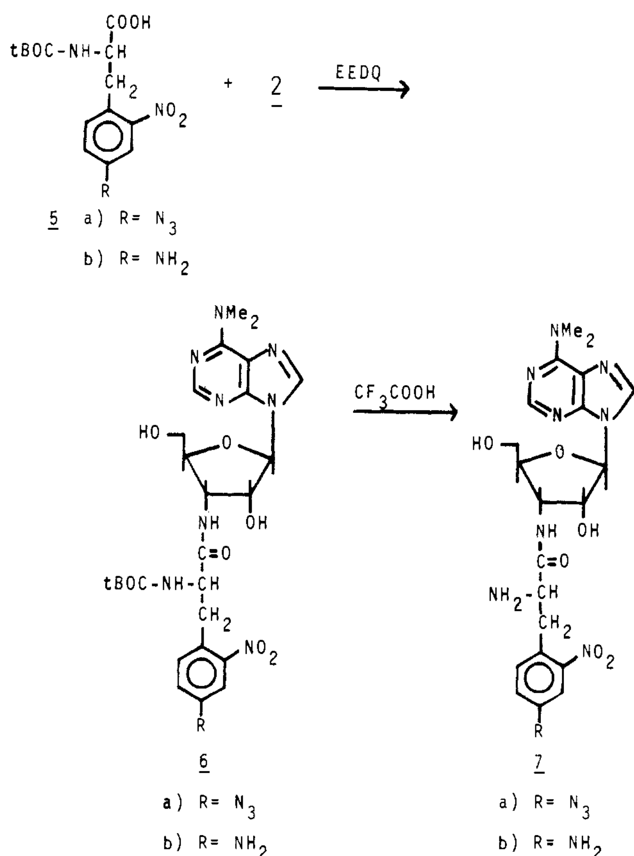
Scheme II



trifluoroacetic acid (Table I).

The 4-amino- and 4-azido-2-nitro-L-phenylalanine derivatives were prepared from 5[12,13] as outlined in Scheme II. All reactions with the 4-azido-2-nitrophenyl compounds were carried out in the absence of direct light. Condensation of 5 with PAN in the presence of *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) gave the N-protected disubstituted Phe-PAN, **6**. Removal of the *tert*-butyloxycarbonyl group with trifluoroacetic acid and subsequent passage through Dowex-1 (OH^-) resin gave the desired products **7a** and **7b**. The final compounds were purified by chromatography on silica gel plates and subsequently recrystallized.

Biology. The para-substituted L-phenylalanylpuromycin analogues, **4a–e**, were evaluated as substrates for the peptidyltransferase reaction. The kinetic constants, K_m and V_{max}, were determined by double-reciprocal plots of initial velocity of product formation vs. substrate concentration. The ratio of V_{max}/K_m appears as a rate constant in the Michaelis–Menton equation at low substrate concentrations. Thus, V_{max}/K_m was used as a measure of substrate efficiency for each analogue. The kinetic results presented in Table II reveal that substitution of the *p*-methoxy group with a nitro group in the puromycin molecule causes a decrease in both K_m and V_{max}, resulting in a relatively low substrate efficiency (21%). However, placement of an amino group in the same position increases the substrate efficiency to 89%. It appears that the amide side chain interferes with the binding as shown by the drop-off in activities of **4c** and **4d**. In fact, the chemical affinity labeling analogue **4e** demonstrated very little ability to act as a substrate in this assay. It was also found that preincubation of 70S *E. coli* ribosomes with **4e** (10^{-4} M) at 4 °C for 20 h gave only 10% irreversible inactivation of peptidyltransferase. These results indicate that the bromoacetylpuromycin analogue has a poor binding affinity for the active site and is not a suitable affinity probe for investigating the peptidyltransferase site.

Since recognition by the peptidyltransferase center is a prerequisite to selective affinity labeling of the A site, the substrate efficiency of **7a** was investigated. The course of reaction of Ac[^{14}C]Phe-tRNA as a donor substrate with puromycin (0.1 mM) is presented in Figure 1. The rate of product formation is rapid and reaction is complete in approximately 5 min. However, the 4-azido-2-nitro-L-phenylalanine derivative, **7a**, did not act as a substrate in this assay.

Table II. Kinetic Constants and Substrate Efficiencies for Puromycin Analogues in the Peptidyltransferase Reaction[a]

compd	K_m, mM	V_{max}, pmol/min	V_{max}/K_m	substrate efficiency, % of puromycin
4a	0.255	0.39	1.53	20.7
4b	0.120	0.79	6.58	89.0
4c	0.256	0.45	1.76	23.7
4d	0.367	0.53	1.44	19.5
4e	inact[b]			
puromycin	0.134	0.99	7.39	100

[a] The Ac[^{14}C]Phe-tRNA was bound to the ribosomes in a reaction mixture containing 100 mM Tris-Cl (pH 7.5), 100 mM NH_4Cl (pH 7.6), 15 mM $Mg(OAc)_2$, 0.65 mM dithiothreitol, 2.78 A_{260} units of washed *E. coli* ribosomes, 1.2 mM GTP, 63 μg of FWR, 0.35 A_{260} units of poly(U), and 21 pmol of Ac[^{14}C]Phe-tRNA (464 pCi/pmol). The binding mixture was incubated at 28 °C for 8 min, and the peptidyltransferase reaction was initiated by the addition of 80 μL of incubation cocktail at 20 μL of puromycin analogue. Reactions were incubated for a specified time, and product formation was measured as described in ref 14. Counting efficiency was approximately 90%. All counts were corrected by blanks in which substrate was absent. The kinetic parameters, K_m and V_{max}, were determined from double-reciprocal plots using a Wilkinson analysis.[17] All plotted points represented an average of duplicate determinations at five substrate concentrations. The standard deviation of the obtained values averaged ±5%. [b] No significant product formation was detected at the highest concentration tested (2×10^{-4} M).

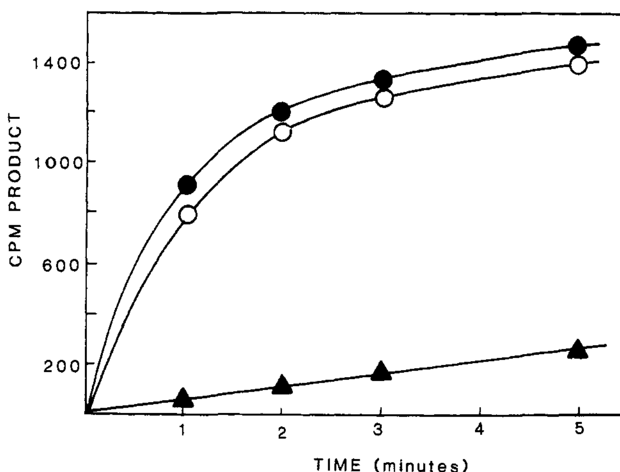


Figure 1. The course of reaction at 28 °C of Ac[^{14}C]Phe-tRNA as a donor substrate with 1×10^{-4} M puromycin (●), 1×10^{-4} M puromycin and 2×10^{-4} M compound **7a** (○), and 2×10^{-4} M compound **7b** (▲) with *E. coli* ribosomes. Compound **7a** did not exhibit product formation at a 2×10^{-4} M concentration over a 10-min period. Reaction conditions and measurement of product formation were as described in Table II.

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The low affinity of **7a** toward the peptidyltransferase site was also demonstrated by its inability to inhibit the puromycin reaction (Figure 1). The inability of **7a** to interact with the ribosome suggests that the binding site cannot tolerate a nitro group in the ortho position of the phenyl ring. This suggestion is supported by the observation that the *p*-amino derivative **4b** is an excellent substrate (Table II), while the 4-amino-2-nitro derivative **7b** is relatively inactive (Figure 1). In addition, it has previously been demonstrated that *p*-azidopuromycin (Chart I) binds to the A site of ribosomes. Thus, the unexpected inability of **7a** to interact with ribosomes precludes its use as a photoaffinity label for the peptidyltransferase A site.

Experimental Section

Puromycin dihydrochloride and PAN were obtained from ICN Pharmaceuticals Inc., L-[^{14}C]Phenylalanine was obtained from New England Nuclear, and *E. coli* paste (B, midlog) was purchased from General Biochemicals. The polynucleotides were obtained from Miles Laboratories, and ATP, GTP, phosphoenolpyruvate, and pyruvate kinase were purchased from Sigma. Preparation of ribosomes, S-100, factors washable from ribosomes (FWR), and Ac-L-[^{14}C]Phe-tRNA were as previously described.[14]

Elemental analyses were performed by M-H-W Laboratories, Garden City, MI. Melting points were determined on a Mel-Temp and are not corrected. NMR spectra were obtained with a Varian T-60A spectrometer, IR with a Perkin-Elmer 237B spectrophotometer, and UV with a Beckman 25 recording spectrophotometer. Compounds were purified by preparative thin-layer chromatography on glass plates (20 × 20 cm) coated with 2 mm of silica gel 254 (E. Merck, Darmstadt) or with column chromatography on silica gel 60, 70–230 mesh (E. Merck, Darmstadt). Since several compounds were prepared by similar procedures, only one representative example is described. Satisfactory elemental analyses (±0.4% of calculated values) were obtained.

***N*-(*tert*-Butyloxycarbonyl)-*p*-nitro-L-phenylalanine (1).** Following the general procedure of Schweyzer et al.,[15] *p*-nitro-L-phenylalanine[16] (5.51 g, 26.0 mmol) and magnesium hydroxide (3.04 g, 52.1 mmol) in water (55 mL) were mixed with *tert*-butyloxycarbonyl azide (7.50 g, 52.1 mmol) in dioxane (90 mL). The reaction was stirred at 40 °C for 20 h and then poured into a 1:1 mixture of ethyl acetate and water (200 mL). The aqueous layer was separated, acidified with citric acid, and extracted with ethyl acetate (3 × 200 mL). The combined extracts were washed with saturated sodium chloride solution (2 × 50 mL), dried (Drierite), and evaporated to a brown syrup (7.8 g). Crystallization from ethyl acetate–petroleum ether (bp 30–60 °C) gave pure **1** as a white solid: yield 3.52 g (44%); mp 108–109 °C; IR (KBr) 1350, 1525 (NO_2), 1690 (carbamate), 1725 cm^{-1} (COOH); NMR ($CDCl_3$) δ 1.37 (s, 9 H, CMe_3), 3.17 (br, 2 H, CH_2), 4.5 (br, 1 H, CH), 5.07 (br, 0.5 H, NH), 7.63 (A_2B_2 quartet, 4 H, *p*-nitrophenyl), 11.2 (s, 1 H, COOH). Anal. ($C_{14}H_{18}N_2O_6$) C, H, N.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-*p*-nitro-L-phenylalanyl]amino]-3′-deoxy-β-D-ribofuranosyl]purine (3a). To a solution of **1** (111 mg, 0.36 mmol), PAN (100 mg, 0.34 mmol), and *N*-hydroxysuccinimide (42 mg, 0.36 mmol) in dried dimethylformamide (5 mL) was added sublimed dicyclohexylcarbodiimide (73.9 mg, 0.36 mmol). The solution was stirred at ambient temperature for 20 h under anhydrous conditions. The precipitated dicyclohexylurea was removed by filtration and washed with ethyl acetate (25 mL). The combined filtrate and wash were evaporated in vacuo, dissolved in hot ethyl acetate (7 mL), chilled to 0 °C, and filtered. The filtrate was diluted with ethyl acetate to 20 mL and washed with saturated sodium bicarbonate (5 mL) and water (2 × 5 mL). The ethyl acetate layer was then dried (Drierite) and evaporated to **3a** as a pale yellow solid (174 mg). This crude product was purified by chromatography on two preparative chromatography plates developed with methanol–chloroform (1.5:10) and gave the analytical sample of **3a** as a white solid (140 mg, 70.5%): mg 214–215 °C; TLC (methanol–chloroform, 1.5:10) R_s 0.65; IR (KBr) 3400 (br, OH, NH), 1690 (carbamate), 1660 (amide), 1600 (purine), 1540, 1340 cm^{-1} (–NO_2); NMR (Me_2SO-d_6) δ 1.33 (s, 9 H, CMe_3), 3.0 (br, 2 H, C_β H_2), 6.13 (d, 1 H, $J_{1',2'}$ = 2 Hz, H-1′, overlapped by 1 exchangeable H), 7.83 (A_2B_2 quartet, 4 H, *p*-nitrophenyl), 8.12 and 8.3 (2 s, 2 × 1 H, H-2 and H-8), remainder of spectrum exhibited overlapping with a discernable singlet at 3.4 (NMe_2). Anal. ($C_{26}H_{34}N_8O_8{\cdot}H_2O$) C, H, N.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-*p*-amino-L-phenylalanyl]amino]-3′-deoxy-β-D-ribofuranosyl]purine (3b). A mixture of 10% palladium on carbon and glacial acetic acid (70 mL) was shaken under hydrogen (1 atm, Parr shaker) at ambient temperature for 1 h. Compound **3a** (1.7 g, 2.0 mmol) dissolved in glacial acetic acid (50 mL) was added to the mixture, and shaking was continued under the same conditions for an additional 4 h. During this period, fresh hydrogen was replaced three times. The mixture was filtered through Celite and the Celite was washed with acetic acid (50 mL). The filtrate and wash were evaporated in vacuo (40 °C, 0.5 mm) to a white solid residue, which had a lower R_f value than **3a** on TLC. This crude product was applied to a silica gel column (145 g) packed in methanol–chloroform (1:30). Pure **3b** was eluted from the column with methanol–chloroform (1:15) and gave a white solid (1.43 g, 88.3%): mp foamed at 98 °C, then melted slowly; IR (KBr) 3400 (br, OH, NH, NH_2), 1650 (amide), 1600 cm^{-1} (purine); NMR (MeOH-d_4) δ 1.4 (s, 9 H, CMe_3), 2.82 (d, 2 H, J = 7 Hz, C_β H_2), 3.43 (s, 6 H, NMe_2), 5.93 (d, 2 H, $J_{1',2'}$ = 2 Hz, H-1′), 6.77 (A_2B_2 quartet, 4 H, *p*-aminophenyl), 8.08 and 8.22 (2 s, 2 × 1 H, H-2 and H-8). Anal. ($C_{26}H_{36}N_8O_6$) C, H, N.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-*p*-acetamido-L-phenylalanyl]amino]-3′-deoxy-β-D-ribofuranosyl]purine (3c). A solution of **3b** (354 mg, 0.59 mmol) and the *N*-hydroxysuccinimide ester of acetic acid (200 mg, 1.27 mmol) in dried dioxane (9 mL) was stirred at ambient temperature for 3 days. The product precipitated from the reaction mixture and was collected by centrifugation (5000 rpm), washed with ether, and dried in air to yield a very fine yellow solid (383 mg). The yellow solid was purified on a silica gel column (65 g) using methanol–chloroform (1:15) as eluent and gave pure **3c** as a white solid (241 mg, 69.3%): mp 241–242 °C; IR (KBr) 3410 (NH, OH), 1690 (carbamate), 1660 (amide), 1600 (purine); NMR (Me_2SO-d_6) δ 1.3 (s, 9 H, CMe_3), 2.0 (s, 3 H, $COCH_3$), 5.87 (br s, 1 H, H-1′, overlapped by 1 exchangeable H), 7.22 (A_2B_2 quartet, 4, *p*-acetamidophenyl), 8.1 and 8.3 (2 s, 2 × 1 H, H-2 and H-8), remainder of spectrum appearing as multiplets from 3.2 to 4.6, overlapping with a singlet at 3.42 (NMe_2). Anal. ($C_{28}H_{38}N_8O_7$) C, H, N.

6-(Dimethylamino)-9-[3′-[(*p*-nitro-L-phenylalanyl)amino]-3′-deoxy-β-D-ribofuranosyl]purine (4a). A solution of **3a** (150 mg, 0.26 mmol) in anhydrous trifluoroacetic acid (3 mL) was incubated at ambient temperature for 8 min with protection from moisture. The excess trifluoroacetic acid was removed in vacuo [25 °C (1 mm)] by azeotroping with dried acetonitrile (8 × 3 mL) to a white solid foam. The solid foam was dissolved in methanol and passed slowly through a column of IRA-400 (OH^-) resin (20 mL) packed in methanol. The basic eluent was collected and evaporated to dryness to give a crude product of **4a**. The crude product was applied to a silica gel column (20 g) packed in methanol–chloroform (1:10). Pure material was removed from the column using methanol–chloroform (1.5:10) as eluent and 81.6 mg (67%) of **4a** was obtained as a white solid. An analytical sample was prepared by crystallization from methanol as yellow crystals: mp 201–202 °C; IR (KBr) 1655 (amide), 1520 and 1350 cm^{-1} (NO_2); UV λ_{max}, in nm ($\epsilon \times 10^{-4}$), 268 (3.06) at pH 1, 272 (3.05) at pH 7, 272 (3.06) at pH 13. Anal. ($C_{21}H_{26}N_8O_6$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-4-azido-2-nitro-L-phenylalanine (5a).** To a cold (0–4 °C) solution of 4-amino-2-nitro-L-phenylalanine[12,13] (0.50 g, 2.22 mmol) in 6 N HCl (10 mL) was added dropwise a solution of sodium nitrite (0.172 g, 2.50 mmol) in water (2 mL). The reaction mixture was stirred for 1 h. All of the following reactions were carried out in the dark or under red light. To the above solution was added sodium azide (0.162

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(15) R. Schweyzer, P. Sieber, and H. Kuppeler, *Helv. Chim. Acta*, 42, 2622 (1959).
(16) (a) F. Bergel and J. Stock, *J. Chem. Soc.*, **2409** (1954); (b) R. A. Houghton and H. Rapaport, *J. Med. Chem.*, 17, 566 (1974).
(17) G. N. Wilkinson, *Biochem. J.*, **80**, 325 (1961).

g, 2.50 mmol) in water (2 mL), and the mixture was stirred for 1 h at 0–4 °C. The solvent was removed in vacuo, and the residue was dissolved in 0.5 N NaOH (10 mL) and dioxane (10 mL). The solution was chilled and di-*tert*-butyl dicarbonate (0.560 g, 2.60 mmol) in dioxane (1 mL) was slowly added. The mixture was stirred at room temperature for 1 h and evaporated to dryness. The solid residue was dissolved in water (50 mL), and the aqueous solution was washed with ether (to remove excess di-*tert*-butyl carbonate) and adjusted to pH 2–3 with citric acid. The aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried with sodium sulfate, and evaporated to an oily residue (**5a**, 0.71 g) which slowly solidified: mp 43–45 °C; IR (KBr) 2120 (N_3), 1720 (C=O), 1535, 1350 (NO_2), 1155 cm^{-1} (C–O); NMR ($CDCl_3$) δ 1.3 (s, 9 H, CMe_3), 3.4–3.8 (m, 2 H, CH_2), 4.4–4.9 (m, 1 H, CH), 5.3 (br s, 1 H, NH), 7.1–7.7 (m, 3 H, aromatic), 10.4 (s, 1 H, COOH). Because **5a** was unstable, it was used immediately for the next reaction.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-4-azido-2-nitro-L-phenylalanyl]amino]-3′-deoxy-β-D-ribofuranosyl]purine (6e). A mixture of **5a** (0.68 g, 1.8 mmol), PAN (0.53 g, 1.8 mmol), and EEDQ (0.49 g, 2.0 mmol) in 95% ethanol (30 mL) and chloroform (10 mL) was stirred for 4 h at 45 °C. After complete removal of the solvent, the solid residue was washed with ethyl acetate to yield a light yellow powder (1.33 g). Crystallization from methanol–methylene chloride gave an analytical sample of **6a** (0.875 g, 77%) as a light yellow powder: mp 197–199 °C dec; IR (KBr) 3320 (NH), 2120 (N_3), 1690, 1660 (C=O), 1600, 1540 cm^{-1} (NO_2); NMR ($CDCl_3$–MeOH-d_4) δ 1.33 (s, 9 H, CMe_3), 3.50 (s, 6 H, NMe_2), 5.86 (s, 1 H, H-1′), 7.1–7.9 (m, 3 H, aromatic), 8.20 (s, 2 H, H-2 and H-8). Anal. ($C_{26}H_{33}N_{11}O_8$) C, H, N.

6-(Dimethylamino)-9-[3′-[(4-azido-2-nitro-L-phenylalanyl)amino]-3′-deoxy-β-D-ribofuranosyl]purine (7a). Compound **6a** (0.50 g, 0.80 mmol) was stirred in anhydrous trifluoroacetic acid (6 mL) at room temperature for 13 min. The trifluoroacetic acid was evaporated in vacuo, and the residue was dissolved in methanol and passed through a column of Dowex-1 resin (OH$^-$) (20 mL) packed in methanol. Evaporation of the eluate left an oily residue (0.4 g), which was applied to two preparative silica gel plates and developed with methanol–methylene chloride (1:10) containing 0.5% ammonium hydroxide. The band containing **7a** was extracted with methanol–methylene chloride (1.5:10). The extract was evaporated to a solid, which was recrystallized from ethyl acetate–hexane and gave a light yellow powder (0.151 g, 36%): mp 180–182 °C dec; IR (KBr) 2120 (N_3), 1650, 1600 (purine), 1530 cm^{-1} (NO_2); NMR ($CDCl_3$–MeOH-d_4) δ 3.60 (s, 6 H, NMe_2), 5.87 (s, 1 H, H-1′), 7.0–7.8 (m, 3 H, aromatic), 8.10 (s, 2 H, H-2 and H-8); UV (ethanol) λ_{max}, in nm ($\epsilon \times 10^{-4}$), 210 (2.88), 263 (2.85). Anal. ($C_{21}H_{25}N_{11}O_6$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-4-amino-2-nitro-L-phenylalanine (5b).** The N-blocked amino acid **5b** was prepared from the sodium salt of 4-amino-2-nitro-L-phenylalanine[12,13] with di-*tert*-butyl dicarbonate as described for **5a**. The oily product was characterized by IR and NMR and used in the next reaction without further purification.

6-(Dimethylamino)-9-[3′-[[*N*-(*tert*-butyloxycarbonyl)-4-amino-2-nitro-L-phenylalanyl]amino]-3′-deoxy-β-D-ribofuranosyl]purine (6b). A mixture of PAN (179 mg, 0.61 mmol), **5b** (200 mg, 0.61 mmol), and EEDQ (157 mg, 0.63 mmol) in 95% ethanol (10 mL) was stirred at 45 °C for 4 h. The solvent was removed in vacuo, and the residue was applied to two preparative silica gel plates and developed with methanol–methylene chloride (1.5:10). The band containing **6b** (yellow band) was extracted with methanol–methylene chloride (1:10) and evaporated to a yellow solid (148 mg, 40.4%). Recrystallization from methylene chloride–hexane afforded an analytical sample of **6b**: mp 145 °C dec; NMR ($CDCl_3$–MeOH-d_4) δ 1.33 (s, 9 H, CMe_3), 3.47 (s, 6 H, NMe_2), 5.77 (br s, 1 H, H-1′), 6.9–7.3 (m, 3 H, aromatic), 8.12 (s, 2 H, H-2 and H-8). Anal. ($C_{26}H_{35}N_9O_8$) C, H, N.

6-(Dimethylamino)-9-[3′-[(4-amino-2-nitro-L-phenylalanyl)amino]-3′-deoxy-β-D-ribofuranosyl]purine (7b). The N-blocked compound **6b** (150 mg, 0.25 mmol) was stirred in anhydrous trifluoroacetic acid (2.5 mL) at room temperature for 5 min. The volatile materials were removed in vacuo, and the oily product was dissolved in methanol–water and stirred with Dowex-1 resin (OH$^-$). The solvent was removed in vacuo, and the yellow solid residue was applied to a preparative silica gel plate and developed with methanol–methylene chloride (1.5:10) containing 2% ammonium hydroxide. The band corresponding to **7b** was extracted with methanol–methylene chloride (1.5:10) and gave a yellow solid (50 mg, 40%). Crystallization from ethyl acetate–hexane gave the analytical sample of **7b** as a yellow powder: mp 135 °C softens, 152–153 °C dec; NMR ($CDCl_3$–MeOH-d_4) δ 3.50 (s, 6 H, NMe_2), 5.83 (br s, 1 H, H-1′), 6.97–7.23 (m, 3 H, aromatic), 8.13 and 8.20 (2 s, 2 H, H-2 and H-8); UV (H_2O) λ_{max}, in nm ($\epsilon \times 10^{-4}$), 272 (2.04). Anal. ($C_{21}H_{27}N_9O_6 \cdot 1.5H_2O$) C, H, N.

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