Synthetic Design, Stereochemistry, and Enzymatic Activity of a Reversed Aminoacyl Nucleoside: An Analogue of Puromycin[1]

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Abstract: The aminoacyl nucleoside puromycin (**1**), produced by *Streptomyces albo-niger*, is a broad-spectrum antibiotic with antitumor activity. It inhibits protein synthesis by accepting the nascent peptide chain of ribosome-bound peptidyl tRNA. A molecular system which incorporates all the features of puromycin necessary for biological activity but which is devoid of structural components detrimental to its use in humans or animals is the reversed aminoacyl nucleoside, 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (**2**). The stereospecific synthesis of **2** starting from D-xylose is described. Structural confirmation came from spectroscopic studies, particularly natural abundance ^{13}C NMR. A conformation for **2** based on spin-lattice relaxation (T_1) data and biological precedent is proposed. The aminoacyl nucleoside **2** is not a substrate for adenosine deaminase, and the aminonucleoside derived from **2** is not expected to be a substrate for adenosine kinase.

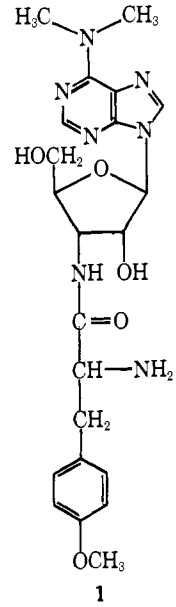
Introduction

The aminoacyl nucleoside puromycin (**1**), produced by *Streptomyces albo-niger*, is a broad-spectrum antibiotic with antitumor activity.[2,3] Its structure bears a close resemblance to the aminoacyl-adenyl terminus of aminoacyl tRNA,[3] and it can therefore act as an acceptor of the peptide chain of ribosome-bound peptidyl tRNA. Puromycin therefore inhibits protein synthesis by substituting for the incoming coded aminoacyl tRNA. As puromycin acts as a codon-independent functional analogue of aminoacyl tRNA, it is likely that there is a binding site on an enzyme such as peptidyl transferase for the adenine system.[4] Investigation has also shown that there are certain structural requirements of puromycin that are necessary for biological activity. The aminonucleoside and an aromatic L-amino acid are required for maximum inhibition of protein biosynthesis. However, the methyls of the dimethyl group, the methoxyl group, the furanosyl oxygen, and the 5′-OH appear to be unnecessary for biological activity.[5-10] Additionally, the removal of the 5′-OH group from the puromycin structure appears to be desirable from the standpoint of toxicity. The nephrotoxicity of puromycin[10] has been ascribed to the enzymatic release of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)purine (PAN) by hydrolysis of the *p*-methoxyphenylalanyl group.[11] Metabolic studies have demonstrated that PAN can be monodemethylated by liver enzymes both in vitro and in vivo and subsequently converted to the 5′-nucleotide. Since PAN itself is not a substrate for adenosine kinase, it has been suggested that the demethylated PAN from liver is made available to the kidney where nucleotide formation can occur, and that this nucleotide may be the active metabolite of PAN which induces kidney toxicity.[11]

A molecular system which incorporates all the features of puromycin necessary for biological activity but which is devoid of structural components detrimental to its use in mammalian systems is the "reversed" aminoacyl nucleoside,[14] 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (**2**). We wish to report on the synthesis and enzymatic activity of **2** and on the establishment of its stereochemistry by natural abundance PFT carbon-13 NMR techniques.

Results and Discussion

Central to any of the several chemical strategies for obtaining **2** is the synthesis of the appropriate 3-amino sugar. A convenient and readily available starting material is xylose (**3**)



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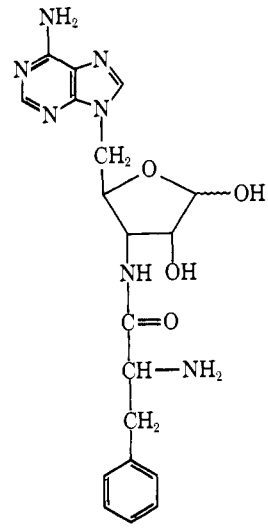

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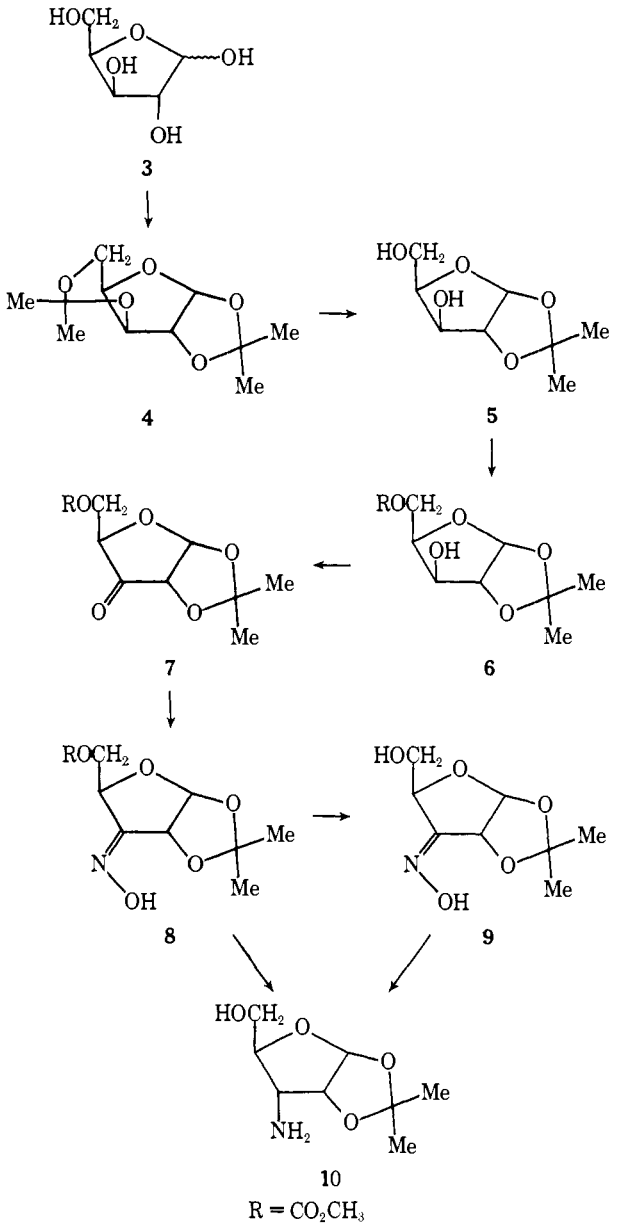
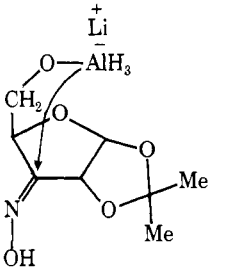
(represented in the furanose form for convenience). Its initial modification at carbon-3, the transformation of a hydroxyl group to an amino group, requires protection of the other hydroxy groups. This was done by first converting xylose to its 1,2:3,5-di-*O*-isopropylidene derivative **4** with acetone/concentrated H_2SO_4/anhydrous $CuSO_4$ and subsequently selectively and quantitatively hydrolyzing the 1,3-dioxane ring with 0.2% aqueous HCl at room temperature.[15] The selective hydrolysis of the 1,3-dioxane ring is the result of a more favorable stereoelectronic arrangement for attack of water and subsequent ring cleavage in the case of the 1,3-dioxane ring as compared with the 1,3-dioxolane ring. Although D-xylose exists almost predominantly in the pyranose form in solution, the furanose form has been detected by ^1H and ^{13}C NMR techniques.[12,13] Both the di- and monoisopropylidene derivatives of D-xylose exist in the furanose form. The hydrolysis of **4** to **5** can be conveniently followed by monitoring the disappearance of two of the singlets (due to the methyl groups) in the δ 1.0–1.5 ppm region. Additionally, the doublet at δ 5.99 ($J_{1,2}$ = 4 Hz) for the anomeric hydrogen remained. The 5-hydroxyl group was then selectively protected by taking advantage of the greater reactivity of the primary 5-OH compared with the secondary 3-OH toward methyl chloroformate

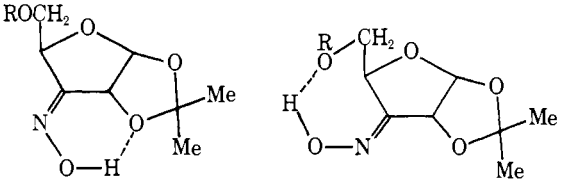


in pyridine at 0 °C.[16] It was anticipated that later in the synthetic scheme (after the 3 position had been modified) it would be necessary to remove selectively the 5-protecting group for further elaboration at that position. Since the isopropylidene group is acid labile, a base labile blocking group such as the carbonate was chosen.

In earlier studies we had examined the possibility of converting the 3β-hydroxyl group to the 3α-amino group via initial displacement of the 3β-tosylate with azide ions under a variety of conditions. However, only very low yields of product were obtained because of the difficulty in the S_N2 displacement reaction arising from adverse steric and dipolar effects.[17,18] An alternative approach involved stereospecific reduction of the 3-oxime. Oxidation of **6** with $Me_2SO/DCC/H_3PO_4$[19,20] gave the 3-keto compound **7** in almost quantitative yield. The keto group of **7** undergoes facile hydration, a feature which made structural verification of the oxidation product difficult. However, careful preparative layer chromatography gave unhydrated ketone which showed carbonyl absorptions in the infrared at 1755 cm^{-1} (3-keto) and 1735 cm^{-1} (carbonate). Two peaks were also observed in the ^{13}C NMR spectrum for these carbons and were unequivocally identified as 5-carbonate carbon (155.1 ppm) and 3-keto carbon (207.4 ppm). Conversion of the ketone **7** to the oxime **8** occurred smoothly in the presence of hydroxylamine hydrochloride and pyridine at 55 °C. Reduction of oxime **8** with $LiAlH_4$ in THF occurred stereospecifically to give the α-amino sugar **10**. It should be noted that the conversion of **8** to **10** involves not only the reduction of the oxime but also the removal of the carbonate protecting group. Carbonates are reductively cleaved to alcohols by $LiAlH_4$ at a much faster rate than oximes are reduced to amines, and it was anticipated that after its formation, the 5-CH_2OH would direct approach of the reducing agent stereospecifically to the 3 position. That this induced asymmetric



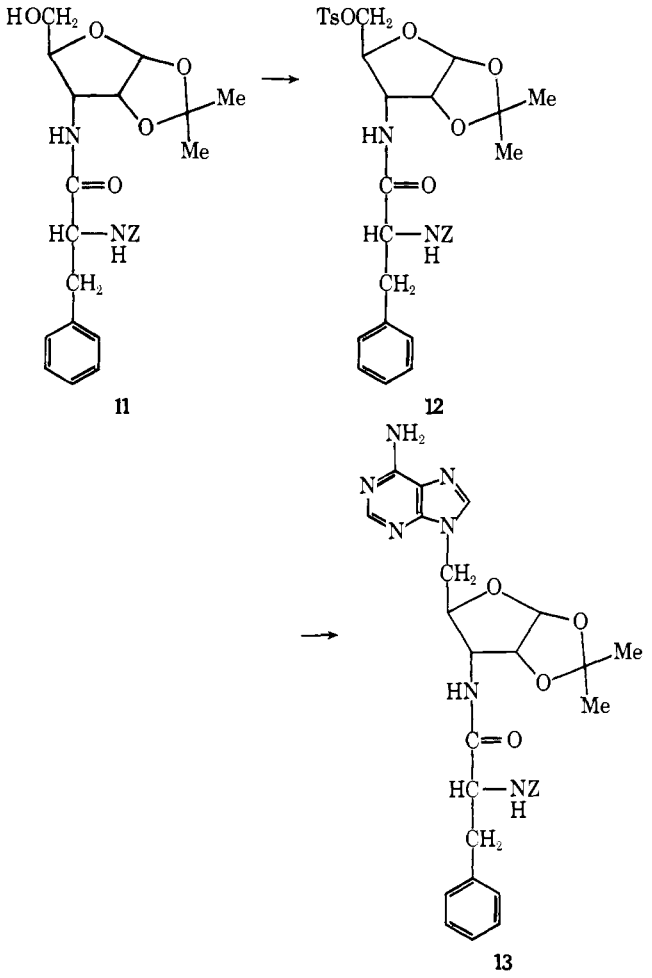
conversion produced one isomer almost exclusively was shown unequivocally by chromatographic analysis and NMR studies. The ^1H NMR spectrum of **10** exhibited coupling constants ($J_{1,2} = J_{2,3} \simeq 4.0$ Hz) consistent with the α-stereochemistry of the amino group.[21] Its ^1H noise-decoupled PFT ^{13}C NMR spectrum showed *only* eight resonances for the eight carbons of the α-amino sugar **10** with carbon-3 showing a single resonance at δ 54.6. Compound **10** can also be obtained by initial cleavage of the carbonate protecting group with $NaOCH_3/CH_3OH$, followed by reduction of the oximino alcohol **9** with $LiAlH_4$. An interesting observation, hitherto undetected in the sugar series, arose from our efforts to establish unequivocally the complete structures of all intermediates in this synthesis. The oximes **8** and **9** exist in two forms, syn and anti as evi-



9, R = H
8, R = CO_2CH_3

denced by ^{13}C NMR spectral studies which showed two peaks for almost all of the carbons in these compounds. The syn:anti ratio was about 2:1.

The synthetic plan then involved further modification at C-3 and subsequent tailoring at C-5 for the attachment of the purine base. Selective peptide bond formation at the 3-NH_2, without esterification of the 5-OH, and racemization of the amino acid was achieved by reaction of the amino sugar **10** with *N*-benzyloxycarbonyl-L-phenylalanine in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ).[22] The relatively slow formation and rapid consumption of the intermediate carbonic anhydride ensures against its accumulation which minimizes side reactions such as esterification and racemization. Under our reaction conditions this conversion is almost quantitative if unreacted starting material is taken into account. The aminoacyl sugar **11** is smoothly converted to its tosylate **12** by reaction with *p*-toluenesulfonyl chloride and pyridine. Introduction of the purine ring at the 5 position was achieved through nucleophilic displacement of the tosyloxy group with sodium adenine (prepared by reaction of adenine with sodium hydride) to give the protected reversed nucleoside **13** in 62% purified yield. That no racemization was occurring under the conditions of this coupling reaction was confirmed by the observation that at the



11 12 13

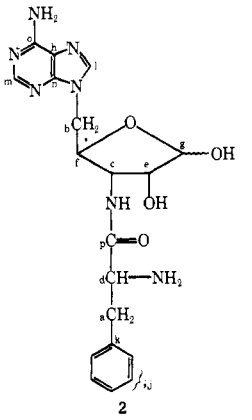
termination of the reaction the unreacted starting material (about 8%) recovered from the reaction showed a rotation almost identical with that found at the commencement of the run. Interestingly also, little change in optical rotation was noted in going from **12** ($[\alpha]^{25}D$ +47° (*c* 0.49, $CHCl_3$)) to **13** ($[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH)). The structure of **13** was confirmed by infrared, ^1H NMR, and ^{13}C NMR spectral data. Restricted rotation of the peptide bond to the 3′-amino group was clearly evident in the ^{13}C NMR spectrum which showed coalescence of the cis and trans forms at 125 °C. That attachment of the purine ring involved the 9 position was substantiated by correlation of the UV absorption maxima in ethanol at 265 nm (ε 14 700) with those known for 9-substituted adenines.[21,23-27] Removal of the isopropylidene group was achieved almost quantitatively by careful treatment with HCl. Treatment of the resulting product under conditions of hydrogenolysis with 10% Pd on charcoal and 2 atm of H_2 gave the reversed aminoacyl nucleoside **2** as its dihydrochloride salt, which when lyophilized appeared as a white powder, mp 172–175 °C dec. Like puromycin, the reversed nucleoside is also unstable at room temperature.

The structure of **2** was confirmed by ^1H NMR, ^{13}C NMR, and UV data. The ultraviolet spectrum showed λ_{max} at 263 nm (ε 15 300). The optical rotation $[\alpha]^{25}D$ +50° (*c* 0.21, H_2O) was quite similar to the protected reversed nucleoside $[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH). The carbon-13 spectrum clearly showed all the carbons of **2** and that the ribose ring had the two anomeric forms α and β in the ratio of 1:3 (see Table I).

Chemical shift assignments were aided by ^{13}C spin-lattice relaxation (T_1) measurements. Further, as relaxation data relate closely to overall and segmental motions, bonded and nonbonded interactions, and related factors controlling molecular motions, some correlations between T_1 data and conformation in solution can be made. Spin-lattice relaxation

Table I. ^{13}C NMR Data for the Dihydrochloride of **2** in D_2O

Carbon	Ppm from Me_4Si	T_1, s[a]
a	37.8	0.23
b	46.6	0.12
c	53.4, 53.8	0.28
d	55.2	0.18
e	74.1, 74.6	0.24
f	78.0, 78.5	0.23
g	97.6, 102.2	0.25
h	118.5	2.60
i	128.8	0.21
j	130.2	0.29
k	134.7	1.89
l	145.2	0.22
m	145.9	0.29
n	149.7	3.32
o	150.4	3.32
p	170.3	2.65



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[a] The estimated maximum errors in the T_1 values vary from ±10 to ±20%.

measurements of 0.2 M **2** in D_2O were made using the inversion-recovery technique[28-30] with the pulse sequence $(T\text{-}180°\text{-}\tau\text{-}90°)_x$. The similarity of T_1 values for the ribosyl carbons and their magnitudes are consistent with a relatively rigid ring undergoing some anisotropic rotation. These values are close to those reported for adenosine-5′-monophosphate (AMP) at a concentration of 1.0 M.[31,36] The relatively short T_1 value for the 5′-CH_2 suggests that the relaxation is dominated by ^{13}C-^1H dipole-dipole interactions with the directly bonded hydrogens. The nonprotonated purine ring carbons which are directly bonded to nitrogens (and therefore strongly affected by ^{13}C-^{14}N dipolar interactions)[33] have T_1 values close to those observed for these carbons in 1.0 M AMP. As expected, the protonated purine carbons C(2) and C(8) show much shorter T_1 values and again similar to those observed for 1.0 M AMP where it has been suggested[31,32] that the AMP molecules are aggregated with stacking of the bases. This arrangement allows for a greater degree of freedom for the ribosyl ring through segmental motion about the glycosyl bond. The phenlalanyl methine carbon undergoes relaxation faster than the methylene carbon which may imply contribution from the attached NH_2 to the relaxation of the methine carbon. The magnitude of both T_1 values, however, suggests that segmental motion is not significant here. The protonated aromatic carbons show T_1 values of 0.21 and 0.29 s implying restriction to spin rotation of the phenyl ring. As expected, the nonprotonated carbon has a long T_1 value.

The reversed aminoacyl nucleoside is a structural analogue of puromycin. It has been suggested[4] that the mechanism of action of puromycin requires it to assume a U-shaped conformation where the nucleic acid base and the aromatic ring are stacked. The x-ray structure of puromycin[34] reveals the association of the methylated base and the aromatic ring. The two rings are stacked at an interplanar spacing of 3.4 Å. Measurement of ^{13}C spin-lattice relaxation times for 0.2 M puromycin dihydrochloride suggests[35] that in solution the puromycin molecule acquires a preferred conformation where segmental and internal rotational motion is limited. This conformation may indeed be similar to that found in the crystalline state of puromycin and also that found in general with relatively concentrated solutions of nucleic acid components such as AMP. The ^{13}C T_1 values observed for the reversed puromycin analogue are close to those observed for puromycin, and it is suggested that the analogue may also take up a preferred conformation in solution with association and stacking of the purine and aromatic rings. When this confor-

mation is assumed during inhibition of protein biosynthesis, the free amino group of the phenylalanyl moiety of the analogue would be sterically well positioned to make a nucleophilic attack on the carbonyl of the peptide. An arrangement such as this would also involve a continuous stack of four hydrophobic rings.

For the puromycin analogue **2** to be an effective inhibitor of protein biosynthesis, it is important that it not undergo in vivo degradation by enzymes such as adenosine deaminase. Mammalian adenosine deaminase reacts with adenosine and structural analogues of adenosine converting them to the corresponding inosines.[37,38] In general, substrate binding and significant substrate activity requires the presence of a 9-substituted unhindered adenine ring and the 5′-hydroxyl of the carbohydrate moiety.[37-40] Dramatic changes occur with respect to substrate activity when the 5′-OH is altered as in the reversed aminoacyl analogue **2** of puromycin, where the 5′-OH has been replaced by the purine ring. Indeed when **2** was treated with adenosine deaminase (calf doudenal mucosa) in 0.05 M phosphate buffer and the reaction assayed spectrophotometrically, no deamination was observed even after 20 h.

The 5′-OH of puromycin has been implicated in its toxicity. Toxic manifestations, including renal lesions, apparently result from small amounts of aminonucleoside produced by the hydrolytic removal of the amino acid moiety from administered puromycin. The aminonucleoside is first monodemethylated and then converted to the 5′-nucleotide by adenosine kinase, and evidence suggests that it is the 5′-nucleotide that is responsible for the cytotoxicity associated with puromycin.[9,41] In the reversed nucleoside **2**, hydrolytic cleavage of the phenylalanine would release an aminonucleoside which would not be a substrate for adenosine kinase.

Biological evaluation of **2** as an inhibitor of protein biosynthesis is currently in progress.

Experimental Section

The melting points reported are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. The infrared spectrometer used was a Beckman IR 20A. The ^{1}H NMR spectra were recorded on a Varian A-60 spectrometer. The ^{13}C NMR spectra were obtained using a Bruker HX-90E pulse Fourier transform NMR spectrometer interfaced with a Nicolet 1080 computer and disk unit. The mass spectrometer employed was a Hitachi RMU-6E instrument with direct inlet capability at an ionizing energy of 70 eV. The optical rotations were measured with a Perkin-Elmer 141 polarimeter. The ultraviolet data were taken with a Beckman Model 24 ultraviolet spectrometer. Lyophilizations were done with a Virtis Automatic Freeze-Dryer Model 10-010. The catalytic hydrogenation was done on a Parr Model 3911 low-pressure hydrogenation apparatus. Solvents were evaporated under reduced pressure using a Buchi Model R rotary evaporator. Reactions requiring constant shaking were run on an Eberbach Shaker Bath Model 6250. Elemental analyses were performed by the University of Iowa Microanalytical Service on a Hewlett-Packard F and M Scientific 185 C, H, and N analyzer.

Preparative layer chromatography plates were prepared by coating seven 20 × 20 cm glass plates with a slurry made from 150 g of E. Merck PF 254 or PF 254 + 366 silica gel in 390 ml of water. The plates were air dried overnight, baked for 4 h at 110 °C, and then allowed to equilibrate to room temperature. The plates were then stored in a desiccator. Separations were accomplished in glass developing tanks and were followed by ultraviolet light using a Chromato-Vue light box equipped with long and short wavelength ultraviolet lamps.

1,2:3,5-Di-*O*-isopropylidene-α-D-xylofuranose (4) was prepared in 90% yield as an oil from xylose: bp 120-125 °C (1 mm) [lit.[15] bp 90-92 °C (0.2 mm)]; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.28 (s, 3 H), 1.36 (s, 3 H), 1.42 (s, 3 H), 1.47 (s, 3 H), 3.95-4.10 (m, 3 H), 4.28 (d, 1 H), 4.50 (d, $J_{2,1}$ = 4 Hz, 1 H), 5.99 (d, $J_{1,2}$ = 4 Hz, 1 H).

1,2-*O*-Isopropylidene-α-D-xylofuranose (5)[15] was prepared from **4** in 97% yield by controlled hydrolysis with 0.2% aqueous HCl: ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 3.98-4.40 (m, 6 H), 4.52 (d, $J_{2,1}$ = 4 Hz, 1 H); mass spectrum *m/e* 175 ($M^+ - CH_3$), 159 ($M^+ - CH_2OH$), 129, 127 ($M^+ - CH_3, - CH_2OH, - OH$), 115 ($M^+ - CH_3, - CH_3COOH$).

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose (6) was prepared from **5** by reaction with methyl chloroformate and pyridine.[16] The carbonate crystallized from 1:1 benzene-hexane as white crystals (70%): mp 133-134 °C; $[\alpha]^{25}D$ −12.5° (*c* 2, CH_3OH); IR ν_{max} (Nujol) 3420 (OH), 1730 (carbonate) cm^{-1}; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 2.98 (s, br, 1 H), 3.82 (s, 3 H), 4.28-4.48 (m, 4 H), 4.57 (d, $J_{2,1}$ = 4.0 Hz, 1 H), 5.97 (d, $J_{1,2}$ = 4.0 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.2, 26.8, 55.2, 64.7, 74.6, 78.1, 85.2, 104.8, 112.0, 156.2.

Anal. Calcd for $C_{10}H_{16}O_7$: C, 48.4; H, 6.5. Found: C, 48.5; H, 6.7.

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (7). To a stirred solution of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-xylofuranose (4.96 g, 20.0 mmol) and dicyclohexylcarbodiimide (12.17 g, 59.1 mmol) in 25 ml of Me_2SO and 30 ml of ethyl acetate was added 1 ml of pyridine, followed by a solution of phosphoric acid (0.98 g, 10.0 mmol) in 5 ml of Me_2SO. The reaction mixture was stoppered and cooled in an ice bath for 20 min. The ice bath was removed and the reaction mixture stirred at room temperature for 18 h. Ethyl acetate was added (50 ml) followed by a solution of oxalic acid (5 g) in methanol (10 ml). After gas evolution has ceased (about 30 min), 75 ml of a saturated aqueous solution of NaCl was added and the solution was filtered. The filtrate layers were separated, and the aqueous phase was extracted with ethyl acetate (50 ml) and CH_2Cl_2 (50 ml). The organic layers were combined, washed with 5% aqueous $NaHCO_3$ (50 ml) and saturated aqueous NaCl (50 ml), and dried over Na_2SO_4. The solvent was removed in vacuo. Ethyl acetate (25 ml) was added to the residue and the solution filtered. The filtrate was chromatographed on a column (2.5 × 20 cm) of silica gel. The column was eluted with 200 ml of $CHCl_3$, leaving, after evaporation of the solvent, 4.42 g of crude yellow oil. This oil was chromatographed on preparative layer silica gel plates. The plates were developed with 1:1 ether/CH_2Cl_2, and the band with R_f 0.8 was cut out and eluted with CH_2Cl_2, giving 4.03 g (16.4 mmol, 82%) of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose as a pale-yellow oil. An analytical sample was prepared by crystallization. The purified sugar was dissolved in a small amount of chloroform. Ether was then slowly added dropwise until the solution became turbid. The solution was then allowed to stand several days in the refrigerator. The solution was filtered, and the white crystals were collected and dried. An alternate method of crystallization involved dissolving the purified sugar in methylene chloride, followed by freeze drying. The residue was washed with ether and the product isolated as white crystals: mp 57-58 °C; $[\alpha]^{25}D$ +62° (*c* 1.55, CH_3OH); IR ν_{max} (Nujol) 1755 (C=O, ring), 1735 (carbonate) cm^{-1}; ^{1}H NMR δ Me_4Si ($CDCl_3$) 1.41 (s, 3 H), 1.48 (s, 3 H), 3.78 (s, 3 H), 4.12-4.70 (m, 4 H), 6.10 (d, $J_{1,2}$ = 4 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 27.1, 27.4, 55.2, 66.2, 76.2, 77.0, 103.3, 114.5, 155.1, 207.4.

Anal. Calcd for $C_{10}H_{14}O_7$: C, 48.8; H, 5.7. Found: C, 48.6; H, 5.9.

1,2-*O*-Isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose Oxime (8). Hydroxylamine hydrochloride (27.76 g, 399.0 mmol) and 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose (15.12 g, 61.46 mmol) were dissolved in 75 ml of dry pyridine. The reaction mixture was protected from moisture and heated in an oil bath at 55 °C for 12 h with stirring. The pyridine was then removed under reduced pressure at a temperature of 30-35 °C. Water (50 ml) and CH_2Cl_2 (50 ml) were added to the residue, and the mixture was stirred until all the solid dissolved. The phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 50 ml). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and the solvent evaporated in vacuo (bath temperature, 30 °C). After drying 1-2 h on a vacuum pump, a gummy residue remained. This gum was dissolved in a few milliliters of $CHCl_3$ and chromatographed on a column of silica gel (40 × 3.5 cm). The column was first washed with 200 ml of Skelly B. Then 600 ml of ether was passed through the column and the ether fraction was evaporated to dryness to give 12.20 g (46.74 mmol, 76%) of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose oxime as a white solid. This was recrystallized from ether/pentane to yield the product as white prisms: mp 90-91 °C; $[\alpha]^{25}D$ +120° (*c* 2.0,

CH_3OH); IR ν_{max} (Nujol) 3390 (N-OH), 1735 (C=O), 1640 (C=N, weak) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.41 (s, 3 H), 1.49 (s, 3 H), 3.76 (s, 3 H), 4.25-4.81 (m, 2 H), 5.00 (2 doublets, syn, anti forms, $J_{2,1}$ = 4 Hz, 1 H), 5.15-5.39 (m, 1 H), 5.96 and 5.98 (2 doublets, syn, anti forms, $J_{1,2}$ = 4 Hz, 1 H), 10.30 (s, br, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 27.5, 27.7, 55.1, 67.1, 67.9, 73.5, 75.3, 77.6, 78.4, 105.1, 105.4, 113.8, 114.3, 155.5, 156.7, 157.6.

Anal. Calcd for $C_{10}H_{15}NO_7$: C, 46.0; H, 5.8; N, 5.4. Found: C, 45.6; H, 6.0; N, 5.5.

1,2-*O*-Isopropylidene-α-D-*erythro*-3-pentosulofuranose Oxime (9). To a stirred solution of 1,2-*O*-isopropylidene-5-*O*-methoxycarbonyl-α-D-*erythro*-3-pentosulofuranose oxime (6.10 g, 23.4 mmol) in 20 ml of CH_3OH and cooled in an ice bath was added a solution of CH_3ONa (1.36 g, 25.2 mmol) in 100 ml of cold CH_3OH. The reaction flask was sealed and stirred an additional 15 min in the cooling bath. The flask was then placed in the refrigerator for 48 h. Then 12 ml of 2 N HCl was added and the solution stirred at room temperature for 15 min. The solvent was removed in vacuo (bath temperature, 25 °C) to give 4.30 g (91%) of crude product. Recrystallization from $CHCl_3$/pentane yielded 3.94 g (19.4 mmol, 83%) of 1,2-*O*-isopropylidene-α-D-*erythro*-3-pentosulofuranose oxime as white crystals: mp 139-140 °C; $[\alpha]^{25}D$ +231° (*c* 1.0, CH_3OH); IR ν_{max} (Nujol) 3450 (N-OH), 3260 (CH_2-OH) cm^{-1}; ^1H NMR δ Me_4Si (acetone-d_6) 1.33 (s, 3 H), 1.39 (s, 3 H), 3.41-4.12 (m, br, 3 H), 4.86-5.08 (m, 2 H), 5.96 and 5.98 (2 doublets, syn, anti forms, $J_{1,2}$ = 3.9 Hz, 1 H), 10.41 (s, br, 1 H); ^{13}C NMR δ Me_4Si (CH_3OD) 27.6, 27.8, 28.0, 62.4, 64.2, 75.1, 79.8, 80.1, 80.8, 106.1, 106.4, 114.0, 114.5, 157.7, 159.2.

Anal. Calcd for $C_8H_{13}NO$: C, 47.3; H, 6.5; N, 6.9. Found: C, 47.1; H, 6.5; N, 6.9.

1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-ribofuranose (10). A solution of 1,2-*O*-isopropylidene-α-D-*erythro*-3-pentosulofuranose oxime (**8**) (2.33 g, 11.6 mmol) in 20 ml of dry THF was added dropwise to a stirred suspension of lithium aluminum hydride (1.09 g, 28.7 mmol) in 30 ml of THF cooled in an ice bath. After the addition was complete, the reaction mixture was refluxed for 3 h and then stirred at room temperature 12 h. The excess lithium aluminum hydride was decomposed by the slow, dropwise addition of 15 ml of a 1:1 THF/water solution while the reaction vessel was cooled in an ice bath. The reaction mixture was filtered and the filter cake was washed with additional THF/water solution (20 ml) and then refluxed in CH_2Cl_2 to remove any occluded product. The CH_2Cl_2 solution was combined with the THF filtrate, and the solvents were removed in vacuo (bath temperature, 25 °C), giving a crude yellow oil. This oil was chromatographed on preparative layer silica gel plates. The plates were developed with 5% CH_3OH/CH_2Cl_2. The band with R_f 0.25 was cut out and eluted with 15% CH_3OH/CH_2Cl_2. 1,2-*O*-Isopropylidene-3-amino-3-deoxy-α-D-ribofuranose was isolated (1.19 g, 6.30 mmol, 54%) as a pale-yellow oil: $[\alpha]^{25}D$ +41° (*c* 1.15, CH_3OH); IR ν_{max} (Nujol) 3100-3500 (br, NH_2, OH) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.52 (s, 3 H), 2.32-2.59 (br, 3 H), 3.02-3.81 (m, 4 H), 4.52 (t, $J_{2,1} = J_{2,3}$ = 3.95 Hz, 1 H), 5.80 (d, $J_{1,2}$ = 3.95 Hz, 1 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.5, 26.6, 54.6, 62.2 80.7, 81.4, 104.2, 112.0; mass spectrum *m/e* 174 ($M^+ - CH_3$), 157 ($M^+ - CH_3, - NH_3$), 127 ($M^+ - CH_3, - CH_2OH, - NH_2$), 114 ($M^+ - CH_3, - CH_3COOH$), 100, 85, 71, 57, 43, 28.

Anal. Calcd for $C_8H_{15}NO_4$: C, 50.8; H, 8.0; N, 7.4. Found: C, 50.6; H, 8.2; N, 7.1.

A second band with R_f 0.35 was also cut out from the preparative layer plates. After elution with 15% CH_3OH/CH_2Cl_2 44 mg (0.23 mmol, 2%) of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-xylofuranose was recovered: ^1H NMR δ Me_4Si ($CDCl_3$) 1.32 (s, 3 H), 1.50 (s, 3 H), 2.30-2.65 (br, 3 H), 3.00-4.00 (m, 4 H), 4.26 (d, $J_{2,1}$ = 4 Hz, 1 H), 5.91 (d, $J_{1,2}$ = 4 Hz, 1 H).

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose (11). *N*-benzyloxycarbonyl-L-phenylalanine (3.30 g, 11.0 mmol)[42] and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (2.87 g, 11.6 mmol) were dissolved in 95 ml of dry benzene. The solution was stirred and heated at 40 °C for an hour. Then a solution of 1,2-*O*-isopropylidene-3-amino-3-deoxy-α-D-ribofuranose (2.09 g, 11.0 mmol) in 25 ml of dry methanol was added to the benzene reaction mixture which was then stirred and heated at 40 °C for 20 h. After cooling to room temperature, the solvent was removed in vacuo (bath temperature, 25 °C). The residue was a yellow oil. This oil was chromatographed on a column of silica gel (3.5 × 35 cm). The column was washed with 900 ml of ether to remove unreacted starting materials and quinoline, a by-product of the reaction. The column was then eluted with 400 ml of ether, followed by 750 ml of 1:1 ether/CH_2Cl_2, yielding 2.95 g (6.28 mmol, 57%) of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose as a white foam that flowed into a liquid at 62-64 °C: $[\alpha]^{25}D$ +43° (*c* 0.78, CH_3OH); IR ν_{max} (KBr) 3420 (OH), 3325 (NH), 1705 (C=O, urethane), 1660 (C=O, amide) cm^{-1}; ^1H NMR δ Me_4Si (CD_3OD) 1.29 (s, 3 H), 1.44 (s, 3 H), 3.01 (d, br, 2 H), 3.45-4.20 (m, 8 H), 5.08 (s, 2 H), 5.82 (d, 1 H), 5.95 (d, 1 H), 7.30 (s, 5 H), 7.34 (s, 5 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 26.3, 26.8, 39.1, 51.1, 56.5, 60.4, 60.7, 67.1, 76.2, 79.5, 80.1, 85.7, 104.1, 104.9, 111.6, 112.5, 126.8-129.2, 136.2, 136.4, 155.9, 171.9.

Anal. Calcd for $C_{25}H_{30}N_2O_7 \cdot CH_3OH$: C, 62.1; H, 6.8; N, 5.6. Found: C, 62.4; H, 6.9; N, 5.4.

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose (12). 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-α-D-ribofuranose (4.20 g, 8.95 mmol) was dissolved in pyridine (30 ml) and cooled in an ice bath. Then a solution of *p*-toluenesulfonyl chloride (1.86 g, 9.77 mmol) in 10 ml of pyridine was added dropwise with stirring. The reaction flask was sealed, and the solution was stirred 10 min in the ice bath. The reaction mixture was then allowed to stand at room temperature for 4 days. The solution was then stirred in an ice bath and 100 ml of cold 2 N HCl was slowly added. The aqueous layer was extracted with CH_2Cl_2 (3 × 75 ml). The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and evaporated to dryness in vacuo (bath temperature, 30 °C). The residue was a pale-yellow foam. It was chromatographed on preparative layer silica gel plates. The plates were developed with ether. The band with R_f 0.6 was cut out and eluted with 10% CH_2Cl_2/ether. 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose (4.25 g, 6.81 mmol, 76.1%) was isolated as a brittle white foam that flowed into a liquid at 56-57 °C: $[\alpha]^{25}D$ +47° (*c* 0.49, $CHCl_3$); IR ν_{max} (Nujol) 3310 (NH), 1700 (C=O, urethane), 1665 (C=O, amide), 1250 and 1170 (SO_2) cm^{-1}; ^1H NMR δ Me_4Si ($CDCl_3$) 1.23 (s, 3 H), 1.32 (s, 3 H), 2.42 (s, 3 H), 3.09 (d, 2 H), 3.20-4.55 (m, 6 H), 5.12 (s, 2 H), 5.58-5.64 (br, 1 H), 5.70 (d, $J_{1,2}$ = 4 Hz, 1 H), 5.80-6.05 (br, 1 H), 7.15-7.50 (m, 12 H), 7.87 (d, 2 H); ^{13}C NMR δ Me_4Si ($CDCl_3$) 21.6, 26.3, 26.5, 39.4, 51.7, 56.6, 67.0, 69.1, 74.3, 77.7, 78.1, 78.5, 104.4, 105.1, 111.9, 112.8, 127.3-129.9, 132.9, 136.3, 136.5, 144.9, 155.9, 171.0.

Anal. Calcd for $C_{32}H_{36}N_2O_9S$: C, 61.5; H, 5.8; N, 4.5. Found: C, 61.4; H, 6.2; N, 4.6.

1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose (13). Sodium hydride (343 mg, 7.15 mmol of a 50% oil dispersion) was placed in a flask and rinsed with a few milliliters of Skelly B. The liquid was removed and the process repeated a second time. The sodium hydride was then dried on a vacuum pump. Adenine (965 mg, 7.15 mmol) was then added to the flask, followed by 15 ml of DMF. The suspension was stirred for 1 h at room temperature and subsequently for 1 h at 50 °C. A solution of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-3-deoxy-5-*O*-*p*-toluenesulfonyl-α-D-ribofuranose in 10 ml of DMF was then added to the stirring suspension of the sodium salt of adenine. The reaction mixture was then heated 10 h at 95-100 °C. The DMF was then evaporated under reduced pressure at 30 °C. The gummy residue was washed with 100 ml of a hot solution of 10% CH_3OH/CH_2Cl_2 and filtered. The filtrate was evaporated in vacuo at 25 °C and the resulting yellow/orange gum was purified on preparative layer silica gel plates. The plates were developed with 10% CH_3OH/CH_2Cl_2. The band at R_f 0.6 was cut out and eluted with 20% CH_3OH/CH_2Cl_2 yielding 2.47 g (4.22 mmol, 62%) of 1,2-*O*-isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose as a white foam that liquefied at 109-110 °C: $[\alpha]^{25}D$ +44° (*c* 1.6, CH_3OH); IR ν_{max} (neat) 3320 (br, NH), 1705 (C=O, urethane), 1650 (C=O, amide) cm^{-1}; uv λ_{max} (95% EtOH) 265 nm (ϵ 14 700); ^1H NMR δ Me_4Si (CD_3OD) 1.30 (s, 3 H), 1.47 (s, 3 H), 3.05 (d, 2 H), 3.50-4.90 (m, 7 H), 5.08 (s, 2 H), 5.75-5.82 (br, 1 H), 5.95 (d, *J* = 4 Hz, 1 H), 6.80-7.15 (br, 2 H), 7.30 (s, 5 H), 7.32 (s, 5 H), 8.14 (s, 1 H), 8.26 (s, 1 H); ^{13}C NMR δ Me_4Si (CH_3OD), 26.1, 26.5, 26.8, 27.0, 38.3, 39.5, 45.6, 55.0, 55.4, 58.8, 61.0, 67.6, 74.7, 77.9, 79.4, 80.1, 105.3, 105.7, 113.6, 113.8, 119.7, 127.8-130.9, 136.2, 136.5, 138.1, 138.5, 143.2, 150.8, 153.7, 157.2, 158.0, 158.6, 175.5, 175.8.

Anal. Calcd for $C_{30}H_{33}N_7O_6$: C, 61.3; H, 5.7; N, 16.7. Found: C, 60.3; H, 5.9; N, 15.8.

3-L-Phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose (2) Dihydrochloride. 1,2-*O*-Isopropylidene-3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-α-D-ribofuranose (755 mg, 1.29 mmol) was dissolved in 3 ml of concentrated HCl and left to stand at room temperature for 2.5 h. Then 12 ml of water were added, and the solution was evaporated in vacuo (bath temperature, 22 °C). A tan gummy compound was isolated: mp 178–181 °C dec; $[\alpha]^{25}D$ +32° (*c* 0.41, H_2O); IR ν_{max} (Nujol) 3410 (br, OH), 3300 (br, NH), 1690 (C=O, urethane), 1660 (C=O, amide); ^1H NMR δ Me_4Si external (D_2O) 3.20–4.00 (m, br, 8 H), 4.20–5.00 (m, br, 8 H), 5.65–5.85 (br, 1 H), 7.75 (s, 5 H), 7.89 (s, 5 H), 8.84 (s, 1 H), 8.97 (s, 1 H). This compound was identified as 3-*N*-benzyloxycarbonyl-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose and used directly without further purification. This sugar (302 mg, 0.55 mmol) was dissolved in 40 ml of water. Then 205 mg of 10% Pd on charcoal was added and the mixture subjected to catalytic hydrogenation for 2 h at 28 psi and room temperature. The catalyst was removed by filtration through a column of cotton. The cotton was washed with an additional 30 ml of water. The filtrate was poured into a 500-ml, round-bottom flask and frozen with liquid nitrogen. The solution was then lyophilized and 215 mg (0.44 mmol, 80%) of 3-L-phenylalanylamino-5-(6-aminopurin-9-yl)-3,5-dideoxy-D-ribofuranose dihydrochloride was isolated as a white powder. An analytical sample was prepared by crystallization from cold water/ethanol: mp 172–175 °C dec; $[\alpha]^{25}D$ +50° (*c* 0.21, H_2O); IR ν_{max} (Nujol) 3240–3550 (br, NH and OH), 1665 (C=O) cm^{-1}; UV λ_{max} (H_2O, pH 7), 263 nm (ε 15 300); ^1H NMR δ Me_4Si external (D_2O) 3.10–4.00 (m, 4 H), 4.10–5.00 (br, 7 H), 5.10–5.40 (br, 4 H), 5.64–5.74 (s, br, 1 H), 7.88 (s, 5 H), 8.87 (s, 1 H), 9.00 (s, 1 H); ^{13}C NMR δ Me_4Si (D_2O, dioxane internal standard) 37.8, 46.6, 53.4, 53.8, 55.2, 70.1, 74.6, 78.0, 78.5, 97.6, 102.2, 118.5, 128.8, 130.2, 134.7, 145.2, 145.9, 149.7, 150.4, 170.3.

Anal. Calcd for $C_{19}H_{25}Cl_2N_7O_4 \cdot 5H_2O$: C, 39.6; H, 6.1; N, 17.0. Found: C, 40.0; H, 6.2; N, 17.0.

Enzymatic Evaluation. Adenosine deaminase (E.C. 2.5.4.4) Type I from calf intestinal mucosa was purchased from Sigma Chemical Co. Substrate activity of **2** was monitored spectrophotometrically by observation of the absorbancy at 263 nm. The enzymatic reaction was carried out in 0.05 M phosphate buffer (pH 7.4) at 25 °C.[37,38] No deamination occurred even after 20 h. Under similar conditions adenosine was rapidly converted to inosine.

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