4b as described in the synthesis of **5** gave a yellow semisolid: nmr (D_2O) δ 6.02 (t, 1 proton) (the remainder of the assignments were as expected); uv λ_{max} (H_2O) 263 mμ (ϵ 15,800); uv λ_{max} (0.1 *M* HCl) 243 mμ (ϵ 11,800); uv λ_{max} (0.1 *M* KOH) 263 mμ (ϵ 18,500); ORD (*c* 0.00810, H_2O, 22°) Φ_{225} −4488°, Φ_{245} 0°, Φ_{250} +3481°, Φ_{262} +847°. *Anal.* ($C_{10}H_{13}NO_4 \cdot 2H_2O$) C, H, N.

1-(*N*-4-Pyridone)-2-α-deoxyribose 5-Phosphate (7). This was prepared according to the procedure of Mitsunobu and coworkers[7] from 211 mg of **5**, 393 mg of triphenylphosphine, and 417 mg of dibenzyl phosphate in 1 ml of dimethoxyethane. After stirring for 5 min at 25°, 261 mg of diethyl azodicarboxylate in 1 ml of dimethoxyethane was added and stirring continued for 6 hr at 50°. After evaporation of the solvent the residue was dissolved in 50 ml of 75% ethanol containing palladium on carbon. Hydrogenolysis was slow and required several additions of fresh catalyst. The solution was filtered and the volume reduced to 10 ml; the product (195 mg, 50%) was isolated as the barium salt. *Anal.* ($C_{10}H_{12}NO_7PBa$) N, P.

1-(*N*-4-Pyridone)-2-β-deoxyribose 5-Phosphate (8). This was prepared using 169 mg of **6**, 377 mg of $POCl_3$, and 16 mg of water in 2.1 ml of trimethyl phosphate according to the procedure of Yoshikawa, *et al.*[8] Preparative chromatography was performed on Whatman 3MM paper using an isopropyl alcohol–concentrated NH_4OH–water (7:1:2) mixture as eluent. The monophosphate band was eluted with water; the resulting solution was lyophilized to yield 103 mg of **8** as the ammonium salt (61% yield).

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A Single-Step Phosphorylation of 5-Fluoro-2′-deoxyuridine to 5-Fluoro-2′-deoxyuridine 5′-Phosphate

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Thymidylate synthetase, which plays a vital role in the biosynthesis of DNA as a catalyst for the methylene tetrahydrofolate dependent conversion of 2′-deoxyuridine 5′-phosphate to thymidine 5′-phosphate, is strongly inhibited by 5-fluoro-2′-deoxyuridine 5′-phosphate (5-fluoro-dUMP).[1,2] However, the mode of substrate, product, and inhibitor binding to the enzyme is still a controversial matter. Santi and coworkers[3,4] and Heidelberger and collaborators[5,6] have presented evidence supporting the formation of a ternary covalent complex involving 5-fluoro-dUMP, methylene tetrahydrofolate, and thymidylate synthetase. However, the two groups of investigators proposed different covalent structures for the ternary complex. Recently, gel electrophoresis has been employed to detect two types of ternary complexes of thymidylate synthetase, differing in stoichiometry of inhibitor and coenzyme interaction with the enzyme.[7] In order to study the properties of the ternary complexes in detail, it was important to have a readily available source of moderate quantities (50 mg) of both 5-fluoro-dUMP and its labeled analogs.

This report deals with the direct syntheses of 5-fluoro-2′-deoxyuridine 5′-phosphate and [6-^{3}H]-5-fluoro-dUMP by a selective phosphorylation of the deoxynucleoside with phosphorus oxychloride in triethyl phosphate in a one-step procedure.[8,9] Careful control of the stoichiometry of the phosphorylating agent to the deoxynucleoside makes it possible to effect selective phosphorylation at the 5′ position in a reasonable yield. Pure 5-fluoro-dUMP is then readily isolated by preparative tlc, which also permits the recovery and recycling of unreacted starting material.

Experimental Section

5-Fluoro-2′-deoxyuridine and unlabeled 5-fluoro-dUMP were obtained from Terra-Marine Bioresearch Co. [6-^{3}H]-5-Fluoro-2′-deoxyuridine (790 mCi/mmol) was obtained from the Radiochemical Center, Amersham. Thin-layer chromatography was performed on a cellulose plate and developed with the following systems: A, 2-propanol–28% aqueous ammonia (50:50 v/v); B, 2-propanol–saturated ammonium sulfate–0.5 *M* sodium acetate (2:79:19 v/v). Paper chromatograms were developed by the ascending technique on Whatman No. 1 paper with solvent A. The separated materials were detected with uv light or by spraying the chromatogram with the molybdate perchloric acid reagent [60% v/v perchloric acid–1 *N* HCl–4% ammonium molybdate–H_2O (5:10:25:60)]. They were also identified by a comparison of the experimentally determined R_f values with those of authentic samples. Both the product obtained and the starting material used were recovered by eluting the appropriate samples of cellulose from the preparative thin-layer chromatography system with water, followed by evaporation. Evaporations were carried out *in vacuo* with bath temperatures kept below 40°.

[6-^{3}H]-5-Fluoro-2′-deoxyuridine 5′-Phosphate Disodium Salt. Unlabeled 5-fluoro-2′-deoxyuridine (35.7 mg, 0.156 mmol) was added to an aqueous solution of [6-^{3}H]-5-fluoro-2′-deoxyuridine (500 μl, 250 μCi). The resulting solution was evaporated and then azeotropically evaporated three times with ethanol–toluene to dryness. The white powder was added to a cold (0°) solution of triethyl phosphate (0.7 ml) and phosphorus oxychloride (63.0 mg, 0.41 mmol) with stirring for 3 hr. The reaction mixture, containing a trace of water, was then held at −5° for 2 days. After this time period, another portion of phosphorus oxychloride (35.0 mg, 0.23 mmol) was added. The mixture was vigorously agitated with ice (3 g) and ether (5 ml). The ether layer was separated and discarded, and the aqueous layer was further extracted with three portions of ether (10 ml). The aqueous solution was carefully neutralized with 1% NaOH solution and concentrated *in vacuo* to a small volume and then applied to four thin-layer cellulose plates (20 × 20 cm). Bands in the chromatograms were removed and eluted with water and evaporated to give 24.0 mg of product (70% based on the amount of starting material consumed) and 14.5 mg of starting material was recovered. Spectral data (uv and ir) and R_f values from paper chromatograms and thin-layer chromatograms were identical with that of the authentic sample of 5-fluoro-2′-deoxyuridine 5′-phosphate. The synthetically prepared nucleotide inhibited thymidylate synthetase to an extent identical with that of an authentic sample of 5-fluoro-dUMP (Aull, Lyon, and Dunlap, unpublished results).

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Synthesis and Biological Properties of 6′-Amino-6′-deoxygentamicin A

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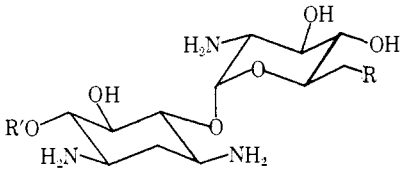
Structure-activity relationship studies on aminoglycoside antibiotics possessing a paromamine moiety have shown that replacement of the 6′ primary hydroxyl group by an amino group leads to compounds with enhanced antibacterial properties. For example, neamine (**1**) is biologically more active than paromamine (**2**)[1] and kanamycin B (**3**) is more potent than kanamycin C (**4**).[2] It was of interest, therefore, to convert the relatively weakly active gentamicin A (**5**)[3] to its 6′-amino-6′-deoxy derivative **6** to examine its biological properties.



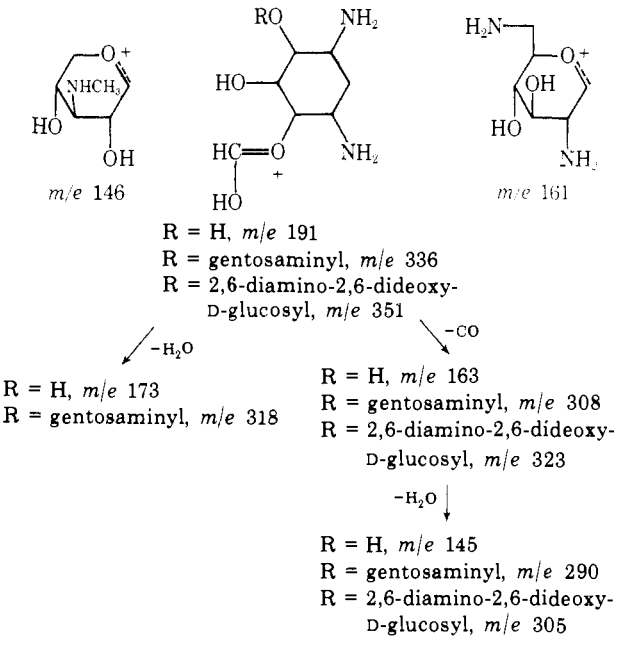
1. R = NH_2; R′ = H
2. R = OH; R′ = H
3. R = NH_2; R′ = 3-amino-3-deoxy-α-D-glucopyranosyl
4. R = OH; R′ = 3-amino-3-deoxy-α-D-glucopyranosyl
5. R = OH; R′ = 3-methylamino-3-deoxy-α-D-xylopyranosyl
6. R = NH_2; R′ = 3-methylamino-3-deoxy-α-D-xylopyranosyl

The synthesis of 6′-amino-6′-deoxygentamicin A (**6**) was accomplished by standard procedures as follows. Gentamicin A (**5**) was converted to tetra-*N*-benzyloxycarbonylgentamicin A (**7**) and then to the corresponding penta-*O*-acetyl-6′-*O*-triphenylmethyl derivative **8** in a overall yield of 73%. The triphenylmethyl group was removed with acid and the *p*-toluenesulfonyl group introduced to give penta-*O*-acetyltetra-*N*-benzyloxycarbonyl-6′-*O*-*p*-toluenesulfonylgentamicin A (**9**) in 91% yield. The *p*-toluenesulfonyl group was displaced by azide ion in near quantitative yield and the resulting compound **10** subjected to O-deacetylation (70% yield) followed by catalytic hydrogenation to give crude 6′-amino-6′-deoxygentamicin A (**6**). The compound thus obtained was purified by column chromatography on silica gel using chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase (40% yield) followed by rechromatography on Dowex 1-X2 in the hydroxyl cycle using water as the eluent to provide an analytically pure sample in 21% yield. The structure of **6** was established as follows.

Elemental analysis of **6** was consistent with the formula $C_{18}H_{37}N_5O_4 \cdot CH_3OH$ and the mass spectrum showed the $(MH)^+$ ion at *m/e* 468 in agreement with the composition. Prominent fragment ions in the mass spectrum[4] are shown in Scheme I.

The pmr spectrum of **6** showed the *N*-methyl protons at

Scheme I. Prominent Mass Spectral Fragment Ions of 6′-Amino-6′-deoxygentamicin A (**6**)



δ 2.5 ppm as a sharp singlet and the two anomeric signals at δ 5.33 and 5.05 ppm, each with a coupling constant of 3.5 Hz. The low-field signal is assigned to H-1′ and the doublet at δ 5.05 ppm to H-1″ by analogy with the assignments made by Lemieux and coworkers[5] for structurally closely related kanamycin B. Furthermore, the pmr spectrum of gentamicin A (**5**) was similar to that of **6** and exhibited two doublets at δ 5.22 and 5.02 ppm with coupling constants of 3.5 and 3.75 Hz, respectively. The doublet at δ 5.22 ppm was attributed to H-1′ by the INDOR response of the H-2′ resonances (quartet at δ 2.75 ppm in the normal spectrum, $J_{2',3'}$ = 9.0 Hz) obtained by monitoring the left-hand peak of the anomeric doublet centered at δ 5.22 ppm. Similarly, the doublet at δ 5.02 ppm could be assigned to H-1″ (quartet at δ 3.60 ppm for H-2″, $J_{2'',3''}$ = 9.0 Hz in the normal spectrum).

The biological properties of gentamicin A (**5**) have been reported.[3] Although both **5** and its 6′-amino-6′-deoxy congener **6** have some broad spectrum activity, neither has potent inhibitory activity *vs. Escherichia coli, Klebsiella pneumoniae*, or *Pseudomonas aeruginosa*. In particular, neither compound has activity against strains carrying gentamicin adenylylating or acetylating *R* factors or against strains carrying kanamycin phosphorylating *R* factors. However, as shown in Table I, the *in vitro* biological activity of **6** against some sensitive strain is superior to that of **5**, gentamicin C complex (Garamycin), and the structurally related kanamycin B. The most striking property of **6** is its potent *in vitro* inhibitory activity against *Staphylococcus aureus* and *Streptococcus pyogenes*.

Experimental Section

Thin-layer chromatography was performed on silica gel GF (Analtech, Inc., Newark, Del.) using chloroform–methanol–ammonium hydroxide (3:4:2) as the developing phase. Column chromatography was carried out on silica gel (60–200 mesh, J. T. Baker Chemical Co., Phillipsburg, N. J.) using the same solvent system and on Dowex 1-X2 (200–400 mesh, hydroxide form, Sigma Chemical Co., St. Louis, Mo.) with water as the eluent.

The pmr spectra were recorded using a Varian Associates XL-100 nmr spectrometer. Chemical shifts are given in δ values for solution in deuterium oxide using DSS as the internal standard.