mp 146–152°. A portion was recrystallized from acetone–water. It melted at 141–146° and had $[\alpha]_D$ +132° (H_2O).

Anal. Calcd for $C_{17}H_{32}N_2O_7S \cdot HCl$: C, 45.88; H, 7.48; N, 6.30; S, 7.21. Found: C, 45.39; H, 7.60; N, 6.41; S, 7.09 (corrected for 4.64% water, Karl-Fischer titration).

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Bis(pyrimidine nucleoside) Phosphates[1]

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6-Mercaptopurine must be metabolized to its ribonucleotide before it can inhibit the growth of cells.[2] Cells that lack the IMP–GMP pyrophosphorylase necessary for this conversion are resistant to 6-mercaptopurine,[2] and to its ribonucleotide,[3] which is unable to enter cells intact.[4] In an effort to overcome this problem of resistance, a number of derivatives of 6-mercaptopurine ribonucleotide that might enter cells were synthesized.[5] One of these, thioinosinyl-(5'→5')-thioinosine,[5] was found to be cytotoxic to a subline of HEp-2 cells resistant to 6-mercaptopurine.[3] This observation led us to synthesize the same type of derivative of the biologically active pyrimidine nucleoside analogs (1a–c and 6-azauridine). The same general approach used in our previous work[5] was employed. 5-Bromo-2'-deoxyuridine (1b) and 2'-deoxy-5-iodouridine (1c) were converted to their 5'-*O*-trityl derivatives (2b and c) for acetylation. Removal of the trityl group from the acetylated nucleosides (3b and c) gave nucleosides (4b and c) suitably blocked for conversion to the desired phosphates. The preparation of 3'-*O*-acetyl-2'-deoxy-5-fluorouridine (4a) was described previously.[5] 6-Azauridine was converted to its 2',3'-*O*-isopropylidene derivative (7).[6] These blocked nucleosides (4 and 7) were then allowed to react with *p*-nitrophenylphosphorodichloridate[7] and the blocked nucleotides (5) were treated with base to remove the acetyl group and the *p*-nitrophenyl group to give the desired 2'-deoxy-5-halouridylyl-(5'→5')-2'-deoxy-5-halouridines (6).[8,9] Treatment of the azauridine derivative (8) with base removed the *p*-nitrophenyl group, but removal of the 2',3'-*O*-isopropylidene group was easily accomplished by simply refluxing an aqueous solution of 9 which, in itself, is acidic enough to effect the hydrolysis to 10 (see Scheme I).

(1) This investigation was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

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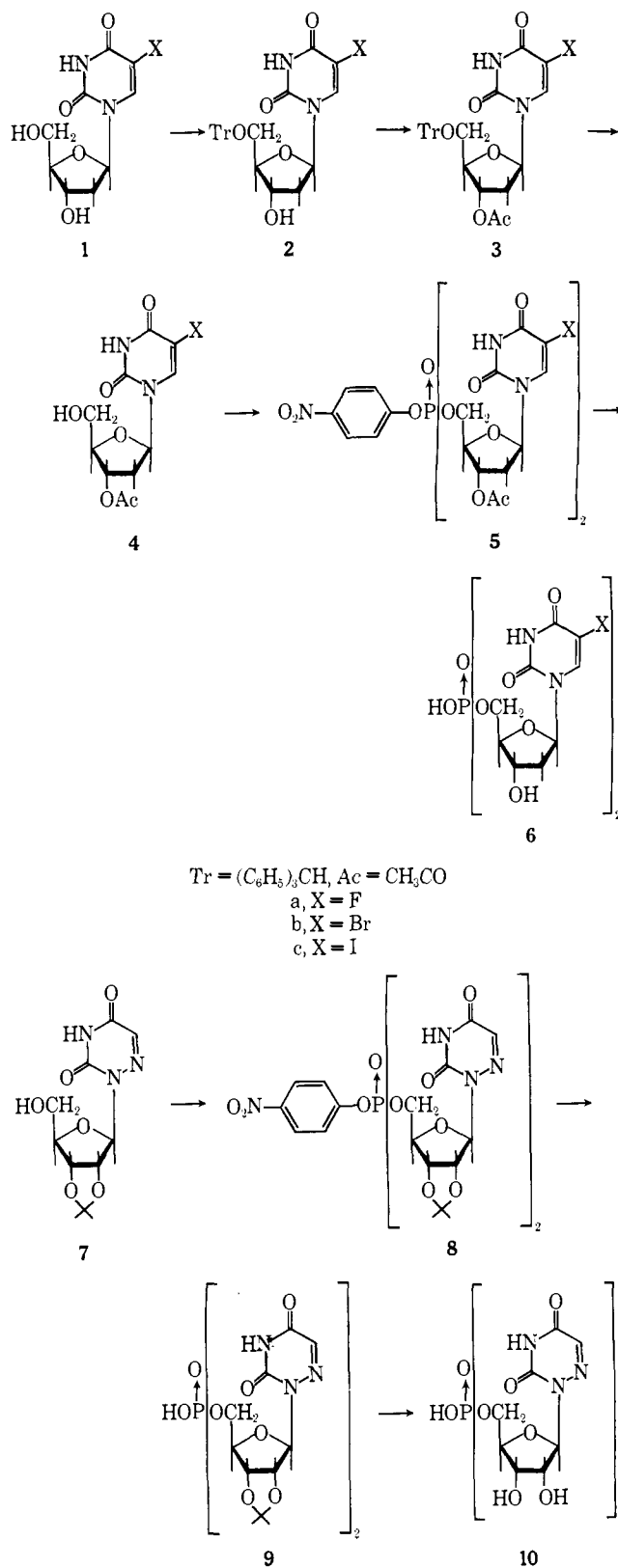(5) H. J. Thomas and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 24 (1962).

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(7) A. F. Turner and H. G. Khorana. *J. Am. Chem. Soc.*, **81**, 4651 (1959).

(8) The synthesis of 2'-deoxy-5-fluorouridylyl-(5'→5')-2'-deoxy-5-fluorouridine by another method has been reported.[9]

(9) D. G. Parsons and C. Heidelberger, *J. Med. Chem.*, **9**, 159 (1966).

$Tr = (C_6H_5)_3CH, Ac = CH_3CO$
a, X = F
b, X = Br
c, X = I

Biologic Data.—The cytotoxic effect of these dinucleoside phosphates on KB cells is compared to the effect of the corresponding nucleosides in Table I. The cells were grown on glass and growth was measured by determination of protein content.[10]

(10) V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956); *Cancer Chemotherapy Rept.*, **25**, 57 (1962).

TABLE I
CYTOTOXICITY TO KB CELLS

Compd	ED$_{50}$, μmoles l.[a]	Ratio[b]
6a	4.5	
5-FUdR	0.07	64
6b	23	
5-BUdR	11	2.1
6c	140	
5-IUdR	34	4.1
10	1.9	
6-Azauridine	3.2	0.6

[a] The concentration of material required to inhibit the growth of cells to 50% of the growth of untreated controls run in the same experiment. [b] Ratio of ED$_{50}$ of bisnucleoside phosphate to corresponding nucleoside.

It can be seen from the ED$_{50}$ ratios that only the bisnucleoside phosphate (**10**) from 6-azauridine appears to be more toxic to cells than the parent nucleoside, but the difference in this case is too small to indicate a clear superiority of **10** over 6-azauridine.

2'-Deoxy-5-fluorouridylyl-(5'→5')-2'-deoxy-5-flurouridine (**6a**) showed only slight and erratic activity against the ascites form of leukemia P815 (in BDF$_1$ mice) that had become resistant to the action of 5-fluorouracil (P815/FU-A). 6-Azauridylyl-(5'→5')-6-azauridine was not toxic to *Streptococcus faecalis* that had become resistant to 6-azauridine.[11] The results with these pyrimidine derivatives are in contrast to the activity of the purine derivative, thioinosinyl-(5'→5')-thioinosine, which was cytotoxic to HEp-2 cells resistant to 6-mercaptopurine.[3] This difference in activity can only be speculated on at this time, since the mechanism by which thioinosinyl-(5'→5')-thioinosine is cytotoxic has not been fully elucidated.

Experimental Section

The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer. The infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 221 spectrophotometer. The melting points, determined on a Kofler Heizbank, are corrected. Paper electrophoresis was carried out on Whatman 3 MM paper in 0.05 M sodium hydrogen citrate buffer (pH 4.4) and 0.05 M Na$_2$HPO$_4$ and NaH$_2$PO$_4$ buffer (pH 7.2) at a potential gradient of 15 v/cm for 1.5 hr. Inosinic acid was used as a standard on all electrophoresis strips, and the distance it migrated was assigned a value of 1.00; the migration of the bis(pyrimidine nucleoside) phosphates is expressed relative to this value (M_{Ia}).

5-Bromo-2'-deoxy-5'-O-trityluridine (2b).—A solution of 4.20 g (13.7 mmoles) of **1b**, 4.22 g (15.1 mmoles) of triphenylchloromethane, and 103 ml of dry pyridine was heated at 60° for 3 days. The solution was evaporated to 30 ml and poured slowly into 2.45 g of NaHCO$_3$ in 800 ml of ice-water. The solution was decanted from the gum that formed, and the gum was triturated in 800 ml of ice-water. The water was decanted, and the residue was dissolved in 200 ml of CHCl$_3$. The CHCl$_3$ solution was dried (MgSO$_4$), evaporated to 50 ml, and seeded. A white, crystalline solid was obtained: yield 4.28 g (69%); mp 144–146°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 279 (9.40), 0.1 N NaOH 275 (6.80); ν (in cm^{-1}) 3450 (OH), 3170, 3060, 2930, 2840 (CH), 1705, 1680 (C=O), 1620 (C=C). The analytical sample (from CHCl$_3$) was dried for 18 hr at 100° (0.07 mm) over P$_2$O$_5$; mp 144–146°.

Anal. Calcd for C$_{28}$H$_{25}$BrN$_2$O$_5$·$^1/_3$CHCl$_3$: C, 55.10; H, 4.14; N, 4.49. Found: C, 55.04; H, 4.04; N, 4.72.

2'-Deoxy-5-iodo-5'-O-trityluridine (2c).—Exactly as described for the preparation of **2b**, 1.42 g (4.0 mmoles) of **1c** and 1.23 g

(4.4 mmoles) of triphenylchloromethane gave a crystalline solid: yield 1.80 g (73%); mp 220°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 286 (6.61), 0.1 N NaOH 278 (4.84); ν (in cm^{-1}) 3440 (OH), 3340, 3170, 3055, 2960, 2870 (CH), 1705, 1665 (C=O), 1610, 1600 (C=C). The analytical sample (from ethanol) was dried for 18 hr at 100° (0.07 mm) over P$_2$O$_5$.

Anal. Calcd for C$_{28}$H$_{25}$IN$_2$O$_5$: C, 56.38; H, 4.23; N, 4.69. Found: C, 56.15; H, 4.68; N, 4.39.

3'-O-Acetyl-5-bromo-2'-deoxyuridine (4b).—A solution of 4.28 g (6.88 mmoles) of **2b**, 128 ml of pyridine, and 7.7 ml of Ac$_2$O was heated for 15 min at 100°, left at room temperature for 24 hr, and poured over 1500 ml of cracked ice. The white precipitate was extracted with two 100-ml portions of CHCl$_3$. The CHCl$_3$ was dried (MgSO$_4$) and evaporated *in vacuo*. A solution of the residue in 250 ml of 80% AcOH (v/v) was heated for 40 min in a 100° oil bath, then evaporated to dryness *in vacuo*. The residue was triturated in benzene, and the benzene was removed by evaporation. The residue was then triturated in ether, and the ether-insoluble material was collected and crystallized from EtOH (white solid) in two crops: total yield 1.82 g (76%); mp 188–190°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 278 (9.52), 0.1 N NaOH 276 (6.84); ν (in cm^{-1}) 3510 (OH), 3195, 3105, 3045, 2935, 2805 (CH), 1710, 1670 (C=O), 1615 (C=C). The analytical sample was dried for 18 hr at 100° (0.07 mm) over P$_2$O$_5$.

Anal. Calcd for C$_{11}$H$_{13}$BrN$_2$O$_6$: C, 37.85; H, 3.75; N, 8.02. Found: C, 37.87; H, 3.64; N, 8.04.

3'-O-Acetyl-2'-deoxy-5-iodouridine (4c).—As described for the preparation of **4b**, 1.60 g (2.68 mmoles) of **2c** gave **4c** as a white, crystalline solid: 754 mg (71%); mp 202–204°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 287 (7.76), 0.1 N NaOH 279 (5.78); ν (in cm^{-1}) 3525 (OH), 3200, 3105, 3050, 2930, 2810 (CH), 1715, 1665 (C=O), 1610 (C=C). The analytical sample (from ethanol) was dried for 18 hr at 78° (0.07 mm) over P$_2$O$_5$; mp 202–204°.

Anal. Calcd for C$_{11}$H$_{13}$IN$_2$O$_6$: C, 33.35; H, 3.31; N, 7.07. Found: C, 33.53; H, 3.44; N, 7.15.

2'-Deoxy-5-fluorouridylyl-(5'→5')-2'-deoxy-5-fluorouridine (6a).—To 2.31 g (8.03 mmoles) of **4a**[5] in 43 ml of dry dioxane was added 1.03 g (4.02 mmoles) of p-nitrophenylphosphorodichloridate[5] followed by 8.6 ml of dry pyridine. The reaction solution was worked up as described for the preparation of **6b** (see below). The thin layer chromatography purification of the product was also run the same way using 1:1 CHCl$_3$-MeOH as the developing solvent. The product was obtained as a white solid, 466 mg (21%). The analytical sample, purified by cellulose column using a 1-BuOH-AcOH-H$_2$O (5:2:3) solvent system, was dried for 20 hr at 100° (0.07 mm) over P$_2$O$_5$; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 268 (16.6), 0.1 N NaOH 267 (13.4); ν (in cm^{-1}) 3410 (OH), 3070, 2940, 2800 (CH), 1705 (C=O), 1570, 1495, 1445 (C=C, C=N), 1050 (P-O-C); M_{Ia}, (pH 7.2) 0.57.

Anal. Calcd for C$_{18}$H$_{21}$F$_2$N$_4$O$_{13}$P·0.25H$_2$O: C, 38.68; H, 3.87; N, 10.02; P, 5.54. Found: C, 38.94; H, 3.67; N, 9.64; P, 5.1.

5-Bromo-2'-deoxyuridylyl-(5'→5')-5-bromo-2'-deoxyuridine (6b).—To 626 mg (1.80 mmoles) of **4b** in 10 ml of anhydrous dioxane was added 231 mg (0.90 mmole) of p-nitrophenylphosphorodichloridate followed by 1.95 ml of dry pyridine. The reaction mixture was stirred at room temperature for 18 hr, diluted with 105 ml of H$_2$O, and extracted with four 100-ml portions of CHCl$_3$. The CHCl$_3$ extracts were dried (MgSO$_4$) and evaporated to dryness *in vacuo*. The p-nitrophenylbis(3-O-acetyl-2'-deoxy-5-bromouridine) 5',5''-phosphate (**5b**) was obtained as a colorless glass: yield 550 mg (69.5%). It was dissolved in 7.6 ml of dioxane. Then, 19 ml of 0.3 N NaOH was added and the resulting yellow solution was left at room temperature for 2 hr, then stirred with enough Amberlite IR-120 (H) ion-exchange resin to give pH 2. The resin was removed by filtration and washed (H$_2$O). Filtrate and washings were combined and evaporated to dryness below 40° *in vacuo*. A solution of the residue in 50 ml of H$_2$O was washed with enough ether to remove all the p-nitrophenol and evaporated to dryness below 40°. A solution of the residue in MeOH was filtered and evaporated to dryness to give a powdery glass (324 mg). This glass in 3 ml of MeOH was applied by streaking to a 20 cm, thin layer chromatogram plate of Mallinckrodt (neutral) silica gel of 1-mm thickness. The plate was developed with 1:1 CHCl$_3$-MeOH solvent. The band containing pure product was scraped from the plate and extracted (hot MeOH). Evaporation of the MeOH gave a white solid (76 mg). The remaining bands were scraped from the plate and extracted with hot MeOH. The fractions from the MeOH extractions were combined and rechromatographed. Another 112 mg of pure product was obtained: total yield 188

(11) R. E. Handschumacher, personal communication.

mg (31%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 278 (15.4), 0.1 N NaOH 276 (11.5); ν (in cm^{-1}) 3410 (OH), 3070, 2930, 2810 (CH), 1690 (C=O), 1615, 1445 (C=C, C=N), 1055 (P-O-C); M_{in} (pH 4.4) 0.68, (pH 7.2) 0.41.

Anal. Calcd for $C_{18}H_{21}Br_2N_4O_{12}P$: C, 31.97; H, 3.13; N, 8.29; P, 4.58. Found: C, 31.89; H, 3.23; N, 8.13; P, 4.39.

2'-Deoxy-5-iodouridylyl-(5'→5')-2'-deoxy-5-iodouridine (6c). —To 711 mg (1.80 mmoles) of **4c** in 10 ml of anhydrous dioxane was added 231 mg (0.90 mmole) of *p*-nitrophenylphosphorodichloridate followed by 1.95 ml of dry pyridine. The solution was worked up as described above for **6b**. The thin layer chromatography purification of the product was also run in the same way using 3:1 CHCl$_3$-MeOH as the developing solvent to give a white solid: 188 mg (27%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 287 (12.9), 0.1 N NaOH 280 (10.1); ν (in cm^{-1}) 3410 (OH), 3070, 2940, 2810 (CH), 1680 (C=O), 1610, 1445 (C=C, C=N), 1055 (P-O-C); M_{in} (pH 4.4) 0.62, (pH 7.2) 0.33.

Anal. Calcd for $C_{18}H_{21}I_2N_4O_{12}P$: C, 28.07; H, 2.75; N, 7.28; P, 4.02. Found: C, 28.05; H, 2.89; N, 7.08; P, 3.91.

1-(2,3,-O-Isopropylidene-β-D-ribofuranosyl)-6-azauracil (7).[6] —To 13.5 ml of 2,2-dimethoxypropane in 500 ml of dry acetone was added 18.4 ml of 70% HClO$_4$.[12] After 5 min, 10.0 g (40.8 mmoles) of 6-azauridine was added. The resulting mixture was stirred for 50 min at room temperature, neutralized by the addition of 21 ml of pyridine, and evaporated to dryness *in vacuo.* The residue was shaken with a mixture of 100 ml of CHCl$_3$ and 30 ml of H$_2$O. The aqueous layer was again extracted with 100 ml of CHCl$_3$. The CHCl$_3$ solutions were combined, dried (MgSO$_4$), and evaporated to dryness *in vacuo.* An acetone solution of the residue was also evaporated to dryness. This residue was dissolved in 25 ml of acetone. The addition of 150 ml of cyclohexane produced an oil that crystallized upon seeding; yield 5.45 g. The aqueous layer from above was diluted with enough concentrated NH$_4$OH to give pH 2 and again extracted with CHCl$_3$ (two 100-ml portions). The CHCl$_3$ extracts were worked up as described above. A second crop of crystalline material weighing 2.30 g was obtained: total yield 7.75 g (66.5%); mp 141–142° (lit.[6] 141–142°); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 260 (6.48), 0.1 N NaOH 254 (7.25); ν (in cm^{-1}) 3540 (OH), 3140, 3100, 2990, 2820 (CH), 1725 (C=O), 1670 (NH), 1585 (sh) (C=N).

6-Azauridylyl-(5'→5')-6-azauridine (10). —To 268 mg (0.94 mmole) of **7** in 5 ml of anhydrous dioxane was added 132 mg (0.52 mmole) of *p*-nitrophenylphosphorodichloridate followed by 1 ml of dry pyridine. The mixture was stirred at room temperature for 2 days. Examination of an aliquot by thin layer chromatography indicated incomplete reaction. Therefore, another 10 mg of *p*-nitrophenylphosphorodichloridate was added. After another 24 hr, the solution was diluted with 50 ml of H$_2$O and extracted with three 150-ml portions of CHCl$_3$. The CHCl$_3$ was dried (MgSO$_4$) and evaporated *in vacuo.* The *p*-nitrophenylbis(2,3-O-isopropylidene-6-azauridine) 5',5'''-phosphate (**8**) was thus obtained as a white, powdery glass; yield 262 mg (74%).

Without further purification, the glass in 10 ml of 0.3 N NaOH was left 2 hr at room temperature. The solution was neutralized by stirring it with Rexyn RG 50 (H) ion-exchange resin. The resin was removed by filtration, and the filtrate was washed several times with ether to remove *p*-nitrophenol. Evaporation of the aqueous solution to dryness *in vacuo* gave a gummy residue which became a white solid upon trituration in EtOH. The EtOH was removed by evaporation. The process was repeated several times giving 167 mg of bis(2,3-O-isopropylidene-6-azauridine) 5',5'''-phosphate (**9**) as a white solid which was dissolved in 50 ml of H$_2$O. The solution (pH 2) was refluxed for 1.5 hr, then evaporated to dryness *in vacuo.* The residue was triturated in EtOH, and the EtOH was removed by evaporation. The process was repeated several times. The white, powdery glass was dried for 18 hr at 100° (0.07 mm) over P$_2$O$_5$: yield 144 mg (49%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 N HCl 261 (12.6), 0.1 N NaOH 253 (14.2); ν (in cm^{-1}) 3430 (broad) (OH), 1740 (sh) and 1690 (C=O), 1025 (P-O-C); M_{in} (pH 4.4) 0.80, (pH 7.2) 1.07.

The analytical sample was obtained by chromatography on a Mallinckrodt silica gel no. SG7 plate using BuOH-AcOH-H$_2$O (5:2:3) as the eluent. The band obtained was removed from the silica gel with hot MeOH. The solid was dried at 100° (0.07 mm) over P$_2$O$_5$ for 18 hr.

(12) J. A. Zderic, J. G. Moffatt, D. Kau, K. Gerzon, and W. E. Fitzgibbon, *J. Med. Chem.,* **8**, 275 (1965).

Anal. Calcd for $C_{16}H_{21}N_8O_{14}P\cdot0.6H_2O$: C, 34.12; H, 3.97; N, 14.92; P, 5.50. Found: C, 34.37; H, 4.31; N, 14.83; P, 5.41.


3'-Deoxynucleosides. V.
3'-Deoxy-2-fluoroadenosine

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The effectiveness of 3'-deoxyadenosine (I) (cordycepin) as a cytotoxic agent is diminished by its rapid conversion into inactive 3'-deoxyinosine (II) through the action of adenosine deaminase.[1] On the other hand, 2-fluoroadenosine (III),[2] a potent cytotoxic
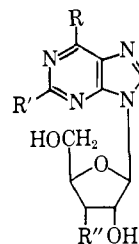
I, R = NH$_2$; R' = H; R'' = H
II, R = OH; R' = H; R'' = H
III, R = NH$_2$; R' = F; R'' = OH
IV, R = NH$_2$; R' = F; R'' = H
V, R = R' = NH$_2$; R'' = H

agent,[2] is relatively inert to the action of adenosine deaminase.[3] It appeared that incorporation of a fluorine atom at the 2 position of 3'-deoxyadenosine would give a derivative, 3'-deoxy-2-fluoroadenosine (IV), which would be stable to adenosine deaminase and might retain the biological properties of I.[4] For the synthesis of IV, 2-amino-3'-deoxyadenosine (V)[5] was subjected to a modification of the procedure[2] used for the preparation of III, which involved the selective diazotization of the 2-amino group in the presence of fluoroboric acid. Crystalline 3'-deoxy-2-fluoroadenosine (IV) was obtained in 18% yield.

In the presence of calf intestine adenosine deaminase, IV was not measurably deaminated under conditions which accomplished complete deamination of adenosine. 3'-Deoxy-2-fluoroadenosine did not inhibit the deamina-

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(2) (a) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.,* **79**, 4559 (1957); (b) *ibid.,* **82**, 463 (1960).

(3) (a) O. P. Chilson and J. R. Fisher, *Arch. Biochem. Biophys.,* **102**, 77 (1963); H. P. Barr and G. I. Drummond, *Biochem. Biophys. Res. Commun.,* **24**, 584 (1966).

(4) S. Fredericksen, *Biochim. Biophys. Acta,* **76**, 366 (1963), has reported that cordycepin 1-oxide is resistant to enzymic deamination and that enzymic reduction provides for *in situ* release of cordycepin.

(5) E. Walton, F. W. Holly, G. E. Boxer, R. F. Nutt, and S. R. Jenkins, *J. Med. Chem.,* **8**, 659 (1965).