matographically homogeneous material; yield 410 mg. (79%); m.p. 117°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 271.5 (20.7); pH 7, 278 (20.2); pH 13, 278 (20.2).

A mixture of some of the 9-benzyl-6-dimethylamino-8-chloropurine (307 mg., 1.07 mmoles) whose preparation is described above, magnesium oxide (43 mg., 1.07 mmoles), and 5% palladium on charcoal (34 mg.) in absolute alcohol (30 ml.) was hydrogenated at atmospheric pressure. Reduction was complete in 6 hr. The catalyst was removed by filtration and the filtrate evaporated to dryness. Trituration of the resulting residue with ethyl alcohol gave a solid which was shown by its ultraviolet spectrum to be 9-benzyl-6-dimethylaminopurine; yield 135 mg. (50%); m.p. 128° (lit.[7] m.p. 131°); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (18.2); pH 7, 276 (18.5); pH 13, 277 (18.6).

Acknowledgments.—The authors are indebted to Dr. W. J. Barrett and the members of the Analytical Section of Southern Research Institute who performed the spectral and most of the microanalytical determinations reported. Some of the analyses were performed by the Galbraith Microanalytical Laboratories, Knoxville, Tennessee.

Complex Esters of Thioinosinic-(5′) Acid[1]

H. JEANETTE THOMAS AND JOHN A. MONTGOMERY

Kettering–Meyer Laboratory, Southern Research Institute, Birmingham, Alabama

Received July 20, 1961

Several dinucleoside phosphates, derivatives of thioinosinic-(5′) acid, have been prepared for evaluation of their biologic activity, particularly their ability to inhibit organisms resistant to the action of 6-mercaptopurine.

The basis for our program on the synthesis of derivatives of thioinosinic acid (I) has been adequately discussed.[2,3] Briefly, there is good reason to hope that a derivative of I can be synthesized that will inhibit 6-mercaptopurine-resistant neoplasms that are unable to perform the "lethal synthesis" that converts 6-mercaptopurine to its

(1) *Chemical Abstracts* nomenclature: 9-β-D-ribofuranosyl-9*H*-purine-6(1*H*)-thione 5′-phosphate. This work was supported by funds from the C. F. Kettering Foundation and from the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740.

(2) J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, **26**, 1926 (1961).

(3) J. A. Montgomery, H. J. Thomas, and H. J. Schaeffer, *ibid.*, **26**, 1929 (1961).

ribonucleotide.[4] The synthesis of a series of simple esters of I already has been described.[3] We have now undertaken the preparation of some dinucleoside phosphates.

The first of this type of derivative that we investigated was thioinosinyl (5′→5′)-thioinosine[5] (VII), a compound that would give one molecule of I regardless of which ribose phosphate linkage might be cleaved *in vivo*. This compound (VII) was prepared in two ways. In the first method, 2-cyanoethyl 2′,3′-*O*-isopropylidenethioinosine 5′-phosphate[2] was hydrolyzed in basic solution to 2′,3′-*O*-isopropylidenethioinosine 5′-phosphate (V), which was then allowed to react with 2′,3′-*O*-isopropylidenethioinosine (II)[2,8] and dicyclohexylcarbodiimide.[9] Removal of the isopropylidene groups gave the desired dinucleoside phosphate (VII). However, the reaction of II and V proceeded poorly so that this method proved not to be satisfactory for the preparation of any quantity of VII. We then developed a more satisfactory procedure which involves the reaction of *p*-nitrophenylphosphorodichloridate[10] with II, the basic hydrolytic removal of the *p*-nitrophenyl group[11] and the acidic removal of the isopropylidene groups. The order of removal of the blocking groups was reversed and, in fact, the intermediate *p*-nitrophenyl bis(thioinosine) 5′,5‴-phosphate (IV), because it is also of interest for biologic evaluation,[12] was isolated analytically pure. For the preparation of VII the route involving removal of the isopropylidene group first was found to be the preferred one.

3′-*O*-Acetylthymidylic-(5′) acid,[9] prepared from thymidylic-(5′) acid[13] by the method of Khorana,[9] was allowed to react with II and dicyclohexylcarbodiimide. In this case the phosphate esterification proceeded more satisfactorily than did the reaction of V with II, and after deblocking a 36% yield of the thymidylyl-(5′→5′)-thio-

(4) R. W. Brockman, *Clin. Pharm. Ther.*, **2**, 237 (1961).

(5) The nomenclature used in this paper was proposed by Gilham and Khorana[6] and has found wide acceptance.[7] By the accepted convention for other purine and pyrimidine ribonucleotides, inosinyl is the trivial radical name for inosinic acid.

(6) P. T. Gilham and H. G. Khorana, *J. Am. Chem. Soc.*, **80**, 6212 (1958).

(7) See "Use of Abbreviations," *J. Biol. Chem.*, **233**, 3 (1958).

(8) A. Hampton and M. H. Maguire, *J. Am. Chem. Soc.*, **83**, 150 (1961).

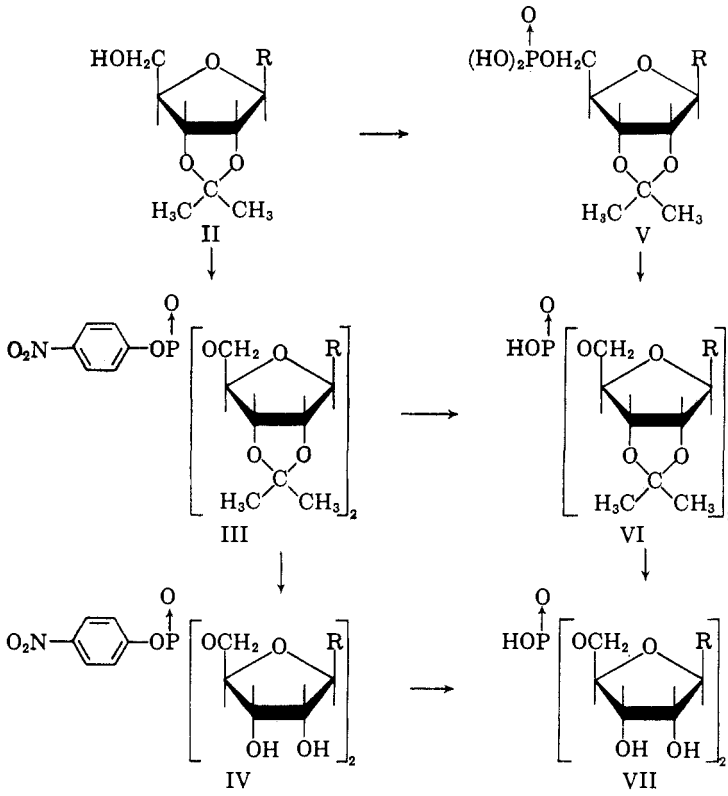
(9) P. T. Gilham and H. G. Khorana, *ibid.*, **80**, 6212 (1958).

(10) A. F. Turner and H. G. Khorana, *ibid.*, **81**, 4651 (1959).

(11) W. E. Razzell and H. G. Khorana, *J. Biol. Chem.*, **234**, 2105 (1959).

(12) It may well be that only the non-ionized tertiary phosphate will penetrate the cell membrane.

(13) California Corporation for Biochemical Research, 3625 Medford Street, Los Angeles 63, California.

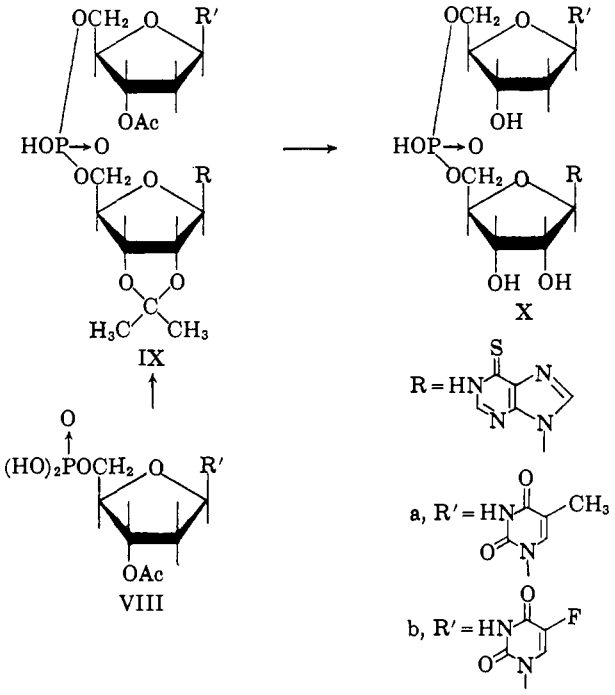


inosine (Xa) was obtained.

Reaction of 2′-deoxy-5-fluorouridine[14] with triphenylchloromethane followed by acetylation of the 2′-deoxy-5-fluoro-5′-*O*-trityluridine gave 3′-*O*-acetyl-2′-deoxy-5-fluoro-5′-*O*-trityluridine which was detritylated in refluxing 80% acetic acid to give 3′-*O*-acetyl-2′-deoxy-5-fluorouridine (XI). The 2-cyanoethyl ester of 3′-*O*-acetyl-2′-deoxy-5-fluorouridylic acid then was prepared from XI by the method of Tener.[15] Basic hydrolysis of the 2-cyanoethyl group also removed the 3′-*O*-acetyl group so that it was necessary to isolate 2′-deoxy-5-fluorouridylic acid as its barium salt, convert the barium salt to the pyridinium salt, and reacetylate[9] to obtain the desired 3′-*O*-

(14) C. Heldelberger, L. Griesbach, O. Crug, R. J. Schnitzer, and E. Greenberg, *Proc. Soc. Exp. Biol. Med.*, **97**, 470 (1958); M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4113 (1959).

(15) G. M. Tener, *ibid.*, **83**, 159 (1961).



acetyl-2′-deoxy-5-fluorouridylic acid (VIIIb). The reaction of VIIIb with II and the usual deblocking procedures gave 2′-deoxy-5-fluorouridylyl-(5′→5′)-thioinosine (Xb), a compound of particular interest because it is composed of two of the most active anticancer agents known—6-mercaptopurine and 5-fluorouracil.

Acknowledgments.—The authors are indebted to the members of the Analytical Section of Southern Research Institute, who, under the direction of Dr. W. J. Barrett, performed most of the microanalytical and spectral determinations reported, and to Dr. H. E. Skipper for his encouragement in this work. Some of the microanalytical determinations were performed by Galbraith Microanalytical Laboratories, Knoxville, Tennessee.

Experimental

The ultraviolet absorption spectra were determined in aqueous solution with a Cary model 14 spectrophotometer. The infrared spectra were determined in

pressed potassium bromide discs with a Perkin–Elmer model 221 spectrophotometer.

The chromatographic and electrophoretic migration values given in Table I were determined as previously described.[2]

TABLE I

CHROMATOGRAPHIC AND ELECTROPHORETIC MIGRATION DATA[a]

	R_f values — Solvent system[b]				Relative migration[a] Solvent system
Cpd.	A	B	C	D	E
II	0.66	0.78	0.60	Streaked	...
III	0.72	0.91	0.81, 0.67	0	...
IV	0.12	0.48	0.14	0	...
V	0	0.49	0.10	0.80	100
VI	0	0.64	0.72	..	...
VII	0	0.11	0	0.61	74
VIIIa	0.14	0.54	0.18	0.92	...
VIIIb	0	0.54	0	0.94	...
Xa	0	0.17	0.12	0.74	53
Xb	0	0.23	0.09	0.76	56
XI	0.70	0.82	0.59	0.84	...

[a] Migration of inosinic acid = 100. [b] A, Water saturated butyl alcohol; B, butyl alcohol–acetic acid–water (5:2:3); C, isopropyl alcohol–ammonium hydroxide–water (14:1:5); D, 0.1 *M* potassium acetate buffer, pH 6.1; E, 0.05 *M* sodium phosphate buffer, pH 7.2.

***p*-Nitrophenyl Bis-(2′,3′-*O*-isopropylidenethioinosine) 5′,5‴-Phosphate** (III).—To a stirred solution of 2.00 g. (6.17 mmoles) of 2′,3′-*O*-isopropylidenethioinosine[2] in 50 ml. of pyridine was added slowly, over a five-minute period, 1.97 g. (7.70 mmoles) of *p*-nitrophenylphosphorodichloridate.[10] The temperature of the solution rose to about 35°, and the solution became yellow. It was sealed tightly and left at room temperature for 18 hr. The solution, which had now turned dark brown, was evaporated *in vacuo* to 10 ml. and then slowly poured into 100 ml. of cracked ice. The precipitate, which formed immediately, was collected after the ice had melted. An orange solid was obtained; yield 1.88 g. (74%). This material was found by its ultraviolet spectrum to be 97% pure.

The analytical sample was obtained by crystallization from ethyl alcohol as a light-yellow solid. It was dried over phosphorus pentoxide at 110° (0.07 mm.) for 8 hr.; m.p. 217° (dec.); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 322 (45.8); pH 7, 318 (42.3); pH 13, 311 (47.0); $\bar{\nu}_{max}$ in cm.$^{-1}$: 2885 (CH); 1595, 1565, and 1510 (C=C, C=N); 1040 (P—O—C).

Anal. Calcd. for $C_{32}H_{34}N_9O_{12}PS_2$: C, 46.20; H, 4.12; N, 15.16; P, 3.72. Found: C, 46.31; H, 4.79; N, 15.08; P, 3.17.

***p*-Nitrophenyl Bis-(thioinosine) 5′,5‴-Phosphate** (IV).—A solution of 1.66 g. (2.00 mmoles) of *p*-nitrophenyl bis=(2′,3′-*O*-isopropylidenethioinosine] 5′,5‴-

phosphate in 598 ml. of methyl alcohol and 342 ml. of 0.3 *N* hydrochloric acid was heated in a boiling water bath for 45 min. After the solution was cooled and neutralized with sodium hydroxide, it was evaporated to 100 ml. The precipitate that formed was collected by filtration, washed thoroughly with water, and air-dried; yield 790 mg. (53%); m.p. 197° (dec.); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 322 (38.2); pH 7, 319 (34.8); pH 13, 311 (42.3); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3400 (OH); 1590, 1525, and 1490 (C=C, C=N); 1020 (broad) (P—O—C).

The analytical sample was obtained by recrystallization from water and was dried at 110° (0.07 mm.) over phosphorus pentoxide for 24 hr.

Anal. Calcd. for $C_{26}H_{26}N_9O_{12}PS_2$: C, 41.56; H, 3.49; P, 4.12. Found: C, 41.50; H, 3.63; P, 4.01.

Thioinosinyl-(5′ → 5′)-thioinosine (VII). Method A.—The intermediate used in the preparation of thioinosinic-(5′) acid, 2-cyanoethyl 2′,3′-*O*-isopropylidenethioinosine 5′-phosphate, was prepared as previously described.[3] The cyanoethyl group was removed by lithium hydroxide hydrolysis, and the product, 2′,3′-*O*-isopropylidenethioinosine 5′-phosphate (V), isolated as its barium salt.

A solution of 100 mg. (0.18 mmole) of this barium salt was freed from barium ions by stirring with Amberlite IR-120 (H) ion-exchange resin. The aqueous solution then was evaporated to dryness under reduced pressure and the residue dried by several evaporations of its pyridine suspensions. The residue then was dissolved in a mixture of 4 ml. of *N*,*N*-dimethylformamide and 25 ml. of dry pyridine, and 191 mg. (0.920 mmole) of dicyclohexylcarbodiimide was added. The resulting solution was sealed tightly and left for 4 days at room temperature. It then was diluted with 2 ml. of water and left for 1 hr. at room temperature; a crystalline precipitate of 1,3-dicyclohexylurea was removed by filtration and the filtrate evaporated to dryness *in vacuo*.

A solution of the residue (VI) in a mixture of 25 ml. of methyl alcohol and 14 ml. of 0.3 *N* hydrochloric acid was heated for 45 min. in a boiling water bath. After the addition of enough sodium hydroxide to raise the pH to 7, the solution was evaporated to dryness *in vacuo*. The residue was dried further by trituration with absolute ethyl alcohol and removal of the alcohol by evaporation *in vacuo*. The white, solid residue was purified by placing it on a column (1 × 12 cm.) of Dowex 1-X2 (formate) ion-exchange resin. The column was eluted with formic acid of increasing strengths from 0.1 to 5.0 *N*. The product was obtained on elution with 5.0 *N* formic acid. Evaporation of the formic acid solution gave a yellow solid: yield 15 mg. (13%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 323 (30.7); pH 7, 321 (30.9); pH 13, 310 (38.0); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3405 (broad) (OH); 1595, 1545, and 1475 (C=C, C=N); 1060 (P—O—C).

Method B.—A solution of 156 mg. (0.208 mmole) of *p*-nitrophenyl bis-(thioinosine) 5′,5‴-phosphate in 25 ml. of 0.25 *N* sodium hydroxide was allowed to remain at room temperature for 2 hr. before the sodium ions were removed by stirring the solution with 15 ml. of Amberlite IR-120 (H) ion-exchange resin. The solution was washed with ether until the ether layer no longer gave a yellow color when stirred with sodium hydroxide solution. The aqueous solution, on evaporation to dryness *in vacuo*, gave a light-yellow solid; yield 100 mg. This material was purified by passing it through an ion-exchange column (1 × 12 cm.) of Dowex 1-X2 (formate). The solid obtained was dried at 78° (0.07 mm.) over phosphorus

pentoxide for 18 hr.; yield 61 mg. (46%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 323 (34.0); pH 7, 321 (32.8); pH 13, 310 (40.5); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3400 (broad) (OH); 1590, 1540, and 1475 (C=C, C=N); 1055 (broad) (P—O—C).

The analytical sample, whose spectra were practically identical with those given above, was obtained by filtration and evaporation of an aqueous solution and trituration of the residue with ethyl alcohol.

Anal. Calcd. for $C_{20}H_{23}N_8O_{10}PS_2 \cdot 0.5\ H_2O$: C, 37.56; H, 3.78; P, 4.84. Found: C, 37.62; H, 4.27; P, 4.65.

2′-Deoxy-5-fluoro-5′-*O*-trityluridine.—To a solution of 1.00 g. (4.06 mmoles) of 2′-deoxy-5-fluorouridine[14] in 50 ml. of pyridine was added 2.46 (8.47 mmoles) of triphenylchloromethane. The resulting solution, protected by a calcium chloride tube, was heated at 56° for 3 days. The solution was evaporated to 25 ml. and poured slowly in a thin stream into 150 ml. of water and ice containing 720 mg. (8.56 mmoles) of sodium bicarbonate. The white gum that formed was stirred until the ice melted. The bicarbonate solution was filtered and the residue triturated with 150 ml. of water and ice. The combined filtrate and washings were chilled for 16 hr. The semi-solid thus obtained was collected by filtration and dissolved in 150 ml. of ethyl alcohol. The ethyl alcohol was evaporated to dryness *in vacuo* and, in order to remove the triphenylcarbinol, the residue was triturated with boiling hexane (3 × 100 ml.). The residue crystallized from 10 ml. of chloroform as a white solid; weight 558 mg.; m.p. 116°. Upon addition of 50 ml. of hexane to the chloroform filtrate, another crop was obtained; weight 55 mg., m.p. 116°; total yield 1.11 g. (49%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (8.75); pH 7, 268 (7.90); pH 13, 267 (7.00); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3430 (broad) (OH); 3040 (CH); 1710 (C=O).

The analytical sample was obtained by one recrystallization from chloroform. It was dried over phosphorus pentoxide at 100° (0.07 mm.) for 24 hr.; m. p. 116°.

Anal. Calcd. for $C_{28}H_{25}FN_2O_5 \cdot 0.625\ CHCl_3$: C, 61.05; H, 4.59; N, 4.98. Found: C, 61.12; H, 4.62; N, 5.11.

3′-*O*-Acetyl-2′-deoxy-5-fluorouridine.—To a solution of 2.20 g. (3.92 mmoles) of 2′-deoxy-5-fluoro-5′-*O*-trityluridine in 98 ml. of pyridine was added 4.00 g. (39.2 mmoles) of acetic anhydride and the resulting solution heated in a boiling water-bath for 15 min. and left at room temperature for 24 hr. When this solution was poured into 500 ml. of cracked ice, a gummy precipitate formed, which, after the ice melted, was extracted into chloroform (2 × 40 ml.). After being dried with magnesium sulfate, the chloroform solution was evaporated to dryness *in vacuo*. A solution of the residue in 100 ml. of 80% acetic acid was heated in an oil-bath at 100° for 20 min. and then evaporated to dryness *in vacuo*. The residue was dissolved in ethyl alcohol and this solution evaporated to remove traces of moisture. Ether trituration of the residue to remove triphenylcarbinol gave a white solid; yield 794 mg. (70%); m.p. 207°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (8.32), pH 7, 268 (8.21), pH 13, 268 (6.60); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3565 (OH); 3060 (CH); 1730 and 1705 (C=O); 1660 (C=N).

The analytical sample was obtained by recrystallization from methyl alcohol. It was dried over phosphorus pentoxide at 110° (0.07 mm.) for 5 hr.

Anal. Calcd. for $C_{11}H_{13}FN_2O_6$: C, 45.83; H, 4.54; N, 9.72. Found: C, 45.96; H, 4.59; N, 9.60.

3′-*O*-Acetyl-2′-deoxy-5-fluorouridylic-(5′) Acid (VIIIb).—To a solution of 4.28 mmoles of 2-cyanoethyl phosphate (from 1.23 g. of its barium salt) in 20 ml. of dry pyridine was added 617 mg. (2.14 mmoles) of 3′-*O*-acetyl-2′-deoxy-5-fluorouridine and 3.53 g. (17.1 mmoles) of dicyclohexylcarbodiimide. The resulting solution was kept at room temperature for 2 days. After diluting the solution with 4 ml. of water and then allowing it to stand for 1 hr., a precipitate of 1,3-dicyclohexylurea was filtered off. The residue, from evaporation of the filtrate to dryness *in vacuo* was dissolved in 50 ml. of 0.5 *N* lithium hydroxide and heated for 1 hr. in an oil-bath at 100°, and the resulting solution stirred with 53 ml. of Amberlite IR-120 (H) ion-exchange resin to remove the lithium ions. The resin was removed by filtration and thoroughly washed with water. The combined filtrate and washings were evaporated to 60 ml. and then the pH of the solution was raised to 7.5 with aqueous barium hydroxide. After chilling the solution for 12 hr., the precipitate of barium phosphate was removed by centrifugation. The solution (75 ml.) was diluted with 225 ml. of ethyl alcohol, and the precipitate that formed was collected by centrifugation. The barium salt of 2′-deoxy-5-fluorouridylic-(5′) acid was obtained as a white solid which, after drying, weighed 870 mg. (88%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 268 (6.48); pH 7, 268 (6.48); pH 13, 268 (5.18); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3400 (OH), 1670 (C=O), 1615 (C=N), 1100 (P—O—C).

The pyridinium salt of 2′-deoxy-5-fluorouridylic-(5′) acid was prepared by passing a solution of 870 mg. (1.89 mmoles) of its barium salt described above through a column (1.4 × 7 cm.) of Amberlite IR-120 (pyridinium) ion-exchange resin. The aqueous solution thus obtained was evaporated to dryness *in vacuo*. The residue was dissolved in pyridine and again evaporated to dryness. After the process was repeated several times to remove the last traces of moisture, a light-orange syrup was obtained which was dissolved in 40 ml. of dry pyridine and 40 ml. of acetic anhydride and left at room temperature for 24 hr. The resulting dark solution was evaporated to dryness *in vacuo*. To remove any mixed anhydrides present, the residue was dissolved in 40 ml. of 50% aqueous pyridine and left several hours at room temperature. After evaporation of the solution, the residue was freeze-dried several times from water. A light-orange glass was obtained. This material was used in the next reaction without further purification.

Thymidylyl-(5′ → 5′)-thioinosine (Xa).—To a solution of 2.00 mmoles of acetylthymidylic acid[6] (from 722 mg. of the calcium salt of thymidylic acid[9]) in 20 ml. of pyridine was added 2.06 g. (10.0 mmoles) of dicyclohexylcarbodiimide and 648 mg. (2.00 mmoles) of 2′,3′-*O*-isopropylidenethioinosine. The resulting solution was kept tightly sealed at room temperature for 2 days. It then was diluted with 4 ml. of water and left for 1 hr. at room temperature. The crystalline precipitate of 1,3-dicyclohexylurea was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was suspended in 100 ml. of water and brought to pH 13 with 4 ml. of 6 *N* sodium hydroxide. After the resulting yellow solution was stirred for 0.5 hr. at room temperature, it was filtered and then stirred with 50 ml. of Amberlite IR-120 (H) ion-exchange resin to remove the sodium ions. To the solution was added enough 1 *N* sulfuric acid to give 360 ml. of a 0.3 *N* solution which was left at room temperature for 24 hr. The solution was neutralized by the addition of an equivalent amount of barium hydroxide, and the barium sulfate that formed was removed by filtration through Celite. The aqueous solution

was evaporated to 20 ml. and diluted with 100 ml. of ethyl alcohol; the white precipitate that formed was collected by filtration and dried; yield 777 mg.

This material was purified by passing its aqueous solution (200 mg.) through a column (1 × 15 cm.) of Dowex 1-X2 (formate). The product was obtained upon elution with 1.0*N* formic acid. After the evaporation of the acid and several evaporations of the residue with ethyl alcohol, a yellow solid was obtained; yield 108 mg. (36%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 270 (11.0), 323 (20.1); pH 7, 272 (11.8), 321 (17.4); pH 13, 268 (9.44), 310 (20.7); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3400 (broad) (OH); 1675 (C=O); 1590, 1540, and 1475 (C=C, C=N); 1040 (P—O—C).

Anal. Calcd. for $C_{20}H_{26}N_6O_{11}PS$: C, 40.81; H, 4.28; P, 5.26. Found: C, 40.80; H, 4.74; P, 5.00.

2′-Deoxy-5-fluorouridylyl-(5′ → 5′)-thioinosine (Xb).—To a solution of 1.89 mmoles of 3′-*O*-acetyl-2′-deoxy-5-fluorouridylic-(5′) acid [from 870 mg. of the barium salt of 2′-deoxy-5-fluorouridylic-(5′) acid] in 20 ml. of dry pyridine was added 615 mg. (1.89 mmoles) of 2′,3′-*O*-isopropylidenethioinosine and 1.95 g. (9.45 mmoles) of dicyclohexylcarbodiimide. The resulting solution was kept tightly sealed at room temperature for 2 days. After the solution was diluted with 4 ml. of water and allowed to stand for 1 hr. at room temperature, the precipitate of 1,3-dicylohexylurea was removed by filtration. The filtrate was evaporated to dryness *in vacuo*. The residue was suspended in 9.3 ml. of water, and 3.7 ml. of 6 *N* sodium hydroxide was added to raise the pH to 13. The solution which resulted was stirred for 0.5 hr. at room temperature. After filtration, the solution was stirred with 55 ml. of Amberlite IR-120 (H) ion-exchange resin to remove sodium ions. The solution, now at pH 2.5, was refluxed for 1.5 hr. Evaporation to dryness gave a yellow solid; yield 695 mg.

The material was purified by placing it on a column (1.75 × 14 cm.) of Dowex 1-X2 (formate) resin. The column was eluted with increasing strengths of formic acid from 0.1 to 5.0 *N*. Formic acid (0.1 *N*) eluted 69 mg. of 6-mercaptopurine; formic acid (0.5 *N*) eluted 171 mg. of 2′-deoxy-5-fluorouridylic-(5′) acid. The product was obtained by eluting the column with 2.5*N* formic acid. Evaporation of the formic acid solution to dryness *in vacuo* and trituration with ethyl alcohol produced a yellow solid; yield 72 mg. (6.4%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): pH 1, 272 (10.5), 322 (20.0); pH 7, 272 (10.8), 320 (18.6); pH 13, 270 (9.2), 310 (20.7); $\bar{\nu}_{max}$ in cm.$^{-1}$: 3420 (broad) (OH): 1705 (C=O); 1620, 1595, and 1550 (C=C, C=N); 1050 (P—O—C).

The analytical sample was dried for 24 hr. at 78° (0.07 mm.) over phosphorus pentoxide.

Anal. Calcd. for $C_{19}H_{22}FN_6O_{11}PS \cdot 2.5H_2O$: C, 35.80; H, 4.27; N, 13.19; P, 4.86. Found: C, 36.27; H, 5.12; N, 12.90; P, 4.29.