ml), protected with a drying tube, was gradually heated with stirring to 70° and maintained at this temperature for 3 hr. The reaction mixture was then diluted with water (200 ml) and extracted with three 100-ml portions of petroleum ether (bp 30–60°). The combined extracts were washed (H_2O, two 100-ml portions), dried ($MgSO_4$), and evaporated to dryness under reduced pressure. The resulting residue solidified (scratching) and was recrystallized from the minimum amount of hot petroleum ether (bp 30–60°) to give the pure product. The residue containing **2** and **6** did not solidify and was distilled *in vacuo*. The yields and properties are summarized in Table V.

Acknowledgment.—The authors wish to express their appreciation to Dr. W. J. Barrett and members of the Analytical and Physical Chemistry Division for the microanalytical results reported and to Dr. W. R. Laster and members of the Cancer Screening Division for the screening data reported.

Derivatives and Analogs of 6-Mercaptopurine Ribonucleotide[1]

H. JEANETTE THOMAS AND JOHN A. MONTGOMERY

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received July 18, 1967

A number of derivatives of 6-mercaptopurine ribonucleotide have been prepared and evaluated for cytotoxicity in normal and 6-mercaptopurine-resistant cell lines.

In earlier papers we described the synthesis of 6-mercaptopurine ribonucleotide (**8**)[2] and a number of ester derivatives of it.[3,4] One of these derivatives, thioinosinyl-(5′→5′)-thioinosine, was found to inhibit the growth of human epidermoid carcinoma cells resistant to 6-mercaptopurine (HEp-2/MP).[5] Later the monophenyl ester of 6-mercaptopurine ribonucleotide was also found to inhibit this cell line.[6] In pursuit of this activity of phosphate esters, a number of other derivatives of 6-mercaptopurine ribonucleotide have been prepared and evaluated for their cytotoxicity.

9-(5-*O*-Trityl-β-D-ribofuransoyl)-9*H*-purine-6(1*H*)-thione[4] was acetylated with acetic anhydride in pyridine and the trityl group of the resultant 9-(2,3-di-*O*-acetyl-5-*O*-trityl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione (**1**) was removed by treatment with aqueous acetic acid to give 9-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione (**2**) (Scheme I). Treatment of **2** with di-*o*-tolylphosphorochloridate, di-*p*-tolylphosphorochloridate, and di-3,5-xylylphosphorochloridate gave the corresponding phosphate esters (**3b–d**). The diphenyl ester (**3a**) was prepared by acetylation of the diphenyl ester of 6-mercaptopurine ribonucleotide (**4a**).[3] The di-*p*-nitrophenyl ester was prepared by the reaction of di-*p*-nitrophenyl phosphate with **2** using *N*,*N*-di-*p*-tolylcarbodiimide to effect the esterification. 9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-di-*p*-nitrophenyl phosphate (**3e**) was converted by basic hydrolysis to the mono-*p*-nitrophenyl ester (**6**) of 6-mercaptopurine ribonucleotide for comparison of its activity with that of the monophenyl ester.[6]

Since there is evidence that the 3′,5′-cyclic phosphate of adenosine can penetrate cells, intact,[7–9] and that it is enzymatically cleaved to the 5′-phosphate,[10] the 3′,5′-cyclic phosphate of 6-mercaptopurine ribonucleoside (**10**) was prepared by the reaction of its *N*,*N*′-dicyclohexylcarboxamidinium salt with dicyclohexylcarbodiimide in pyridine solution.[10]

The biologic activity of 6-methylthiopurine ribonucleoside has been shown to result from its enzymic conversion to the ribonucleotide (**9**) by adenosine kinase.[11] We synthesized **9** for comparison with the biosynthetic material and this synthesis by the methylation of 6-mercaptopurine ribonucleotide is described below.

Reaction of 9-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione or 9-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione (**2**) with 5′-*O*-trityl-5-fluorouridine 3′-phosphate followed by the appropriate deblocking procedures gave 5-fluorouridylyl-(3′→5′)-thioinosine (**5**), an isomer of an ester previously prepared.[4]

Reaction of an analog of 6-mercaptopurine ribonucleoside, *cis*-3-(1,6-dihydro-6-thioxopurin-9-yl)cyclopentanemethanol (**11**),[12] with *p*-nitrophenylphosphorodichloridate gave the phosphate ester **12** which was converted to bis[*cis*-3-(1,6-dihydro-6-thioxopurin-9-yl)-cyclopentanemethyl] phosphate (**13**) by treatment with aqueous sodium hydroxide.

In order to compare the activity of some inosine phosphates (**7a** and **b**) with the corresponding thioinosine compounds, these latter compounds (**4a** and **b**) were converted to the S-(2-hydroxyethyl) derivatives which are hydrolyzed readily by aqueous base to **7a** and **7b**. This approach to the conversion of derivatives of 6-mercaptopurine to the corresponding derivatives of hypoxanthine was suggested by the observation of the

(1) This work 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. PH-43-64-51.

(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, *J. Org. Chem.*, **26**, 1929 (1961).

(4) H. J. Thomas and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 20 (1962).

(5) J. A. Montgomery, G. J. Dixon, E. A. Dulmadge, H. J. Thomas, R. W. Brockman, and H. E. Skipper, *Nature*, **199**, 769 (1963).

(6) F. M. Schabel, Jr., and G. J. Dixon, personal communication.

(7) E. W. Sutherland and T. W. Rall, *Pharmacol. Rev.*, **12**, 265 (1960).

(8) T. Posternak, E. W. Sutherland, and W. F. Henion, *Biochim. Biophys. Acta*, **65**, 558 (1962).

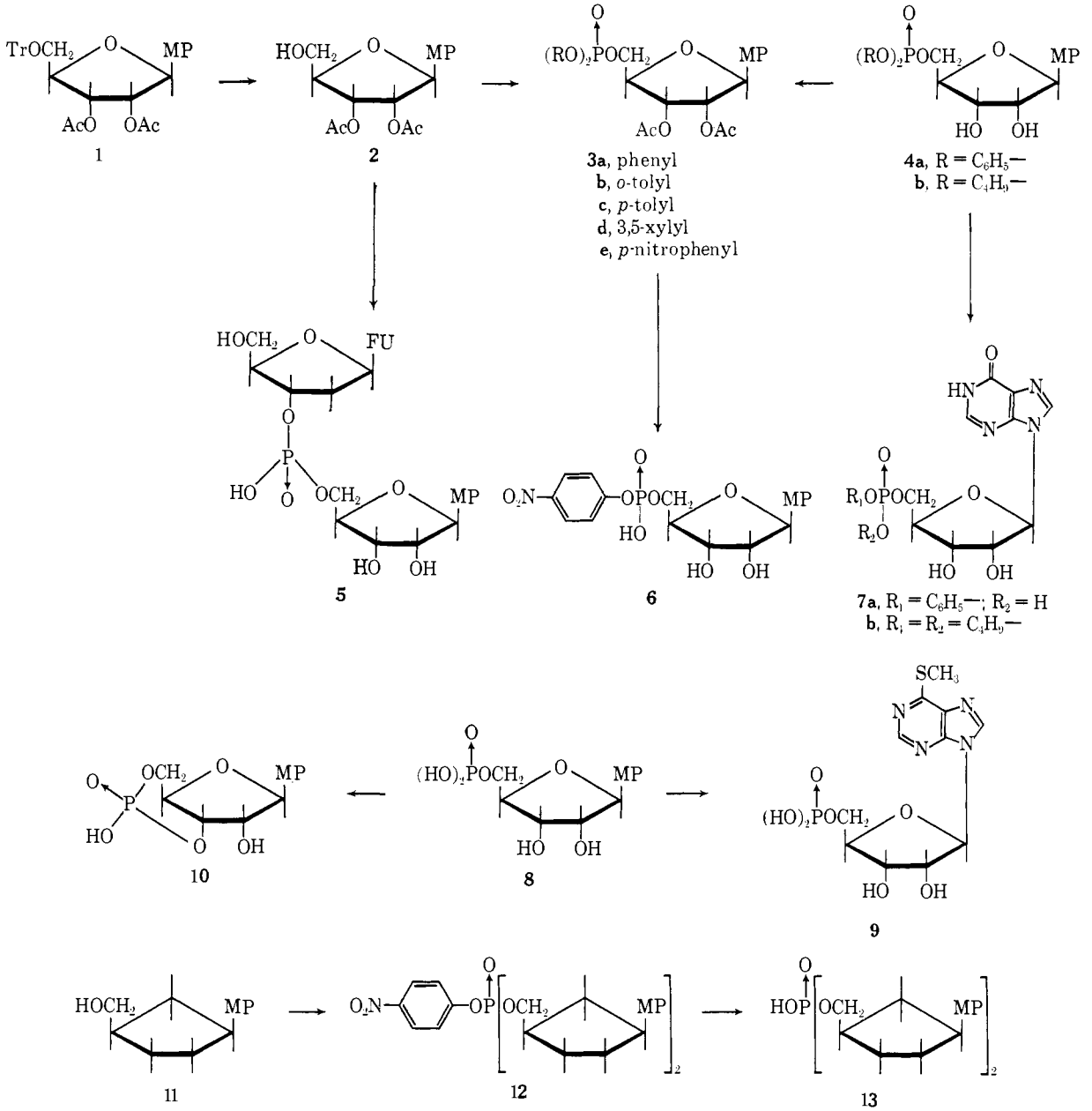
(9) G. Northrop and R. E. Pailes, Jr., *J. Pharmacol. Exptl. Therap.*, **145**, 135 (1964).

(10) M. Smith, G. I. Drummond, and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 698 (1961).

(11) L. L. Bennett, Jr., R. W. Brockman, H. P. Schnebli, S. Chumley, G. J. Dixon, F. M. Schabel, Jr., E. A. Dulmadge, H. E. Skipper, J. A. Montgomery, and H. J. Thomas, *Nature*, **205**, 1276 (1965).

(12) H. J. Schaeffer, D. D. Godse, and G. Liu, *J. Pharm. Sci.*, **53**, 1510 (1964).

SCHEME I



ease of acid or base hydrolysis of 6-(2-hydroxyethylthio)purine to hypoxanthine.[13]

Biologic Data.—The cytotoxicity[14] of the analytical samples of these nucleotide derivatives to KB and HEp-2 cells in culture and to a subline of HEp-2 cells resistant to 6-mercaptopurine (HEp-2/MP) is compared to that of 6-mercaptopurine in these lines (Table I). The acetylated derivatives of substituted phenyl esters of 6-mercaptopurine ribonucleotide (**3a–d**) and **13** show only moderate cytotoxicity and the inosinic acid derivatives (**7a**, **b**) even less. The bis nucleoside phosphate **5**, the *p*-nitrophenyl ester **6**, the cyclic phosphate **10**, and 6-methylthiopurine ribonucleotide (**9**) show the same order of cytotoxicity as 6-mercaptopurine. Furthermore, the HEp-2/MP cell line is sensitive to **5** and **6**, but not to **10**. The results with **5** and **6** are in agreement with those previously obtained with 5-fluorouridylyl-(5′→5′)-thioinosine[5] and the monophenyl ester of 6-mercaptopurine ribonucleotide.[6] The failure of the cyclic phosphate **10** to affect the 6-MP-

TABLE I
CYTOTOXICITY

Compd	ED_{50}, μmoles/l.[a] KB	HEp-2/0	HEp-2/MP
3a	43		
3b	54		
3d	43		
5	0.06	<1.6	<1.6
6		1.4	76
7a	>92		
7b	92		
9	0.06		
10		1.6	>240
13	64	>17	
6-MP	1.2	0.76	2060

[a] The concentration of agent necessary to inhibit the growth of treated cells to 50% of that of untreated control cells as determined by a measure of total protein.[14]

(13) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

(14) *Cancer Chemotherapy Rept.*, **25**, 57 (1962).

resistant cell line may be due to facile extracellular cleavage to 6-mercaptopurine ribonucleotide.

Experimental Section

Uv spectra were determined in aqueous solution with a Carey Model 14 spectrophotometer. Ir spectra were determined in KBr with a Perkin-Elmer Model 221 spectrophotometer. The melting points were determined on a Kofler Heizbank and are corrected. Paper electrophoresis was carried out on Whatman 3 MM paper in pH 7.2 buffer (2 parts of 0.05 *M* NaH_2PO_4 to 3 parts of 0.05 *M* Na_2HPO_4) at a potential gradient of 15 v/cm for 1.5 hr. Thioinosinic 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 other phosphates is expressed relative to this value (M_{Tin}).

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione (2).—To a solution of 2.11 g (4.0 mmoles) of 9-(5-*O*-trityl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione[15] in 96 ml of dry pyridine was added 4.08 g (40.0 mmoles) of Ac_2O. The solution was heated for 15 min in a boiling-water bath, left at room temperature for 24 hr, and then slowly poured over 800 ml of cracked ice. The product, 9-(2,3-di-*O*-acetyl-5-*O*-trityl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione (1), immediately precipitated as a white solid. When the ice melted, the solid was collected by filtration: yield 2.11 g, mp 167°.

A suspension of this material in 211 ml of 80% AcOH (v/v) was stoppered and stirred for 24 hr at room temperature. The resulting solution was evaporated to dryness *in vacuo*, the residue was triturated with ethanol, and the ethanol was removed by evaporation. Trituration of the residue with ether produced a white solid that was crystallized from EtOH (300 ml): yield 1.08 g (84%), mp 237° dec.

In another run the analytical sample was obtained. It was dried for 2 hr at 100° (0.07 mm) (P_2O_5); mp 244° dec; λ_{max} [in mμ (ε × 10^{-3})] 0.1 *N* HCl—322 (23.9), pH 7—317 (20.6), and 0.1 *N* NaOH—311 (22.6); $\bar{\nu}$ (in cm^{-1}) 3400 (broad) (OH), 1745 (C=O), 1600, 1560, 1520 (C=C, C=N), 1135, 1105, 1080, 1045 (CO–).

Anal. Calcd for $C_{14}H_{16}N_4O_6S$: C, 45.64; H, 4.38; N, 15.21. Found: C, 45.66; H, 4.57; N, 15.14.

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-Diphenyl Phosphate (3a).—A solution of 2.06 g (4.00 mmoles) of 9-β-D-ribofuranosyl-9*H*-purine-6(1*H*)-thione 5′-diphenyl phosphate[8] in 20 ml of anhydrous pyridine and 20 ml of Ac_2O was heated in a boiling-water bath for 15 min, then sealed tightly, left for 20 hr at room temperature, and finally evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml of 50% pyridine–water and left for 2 hr at room temperature before the solution was evaporated to dryness *in vacuo*. The residue was triturated with H_2O (25 ml), the water was removed by evaporation *in vacuo*, and the residue precipitated as a gel from 25 ml of EtOH: yield 1.74 g (72%); λ_{max} [in mμ (ε × 10^{-3})] 0.1 *N* HCl—323 (22.8), pH 7—320 (19.0), 0.1 *N* NaOH—311 (22.7); $\bar{\nu}$ (in cm^{-1}) 3150 (sh), 3110, 3060, and 3000 (OH), 1755 (C=O), 1600 and 1540 (C=C, C=N), 1490 (phenyl), 1460 (CH), 1415 (C=S), 1050, 1025, and 1010 (POC). The analytical sample was obtained by precipitation from EtOH. It was dried for 20 hr at 78° (0.07 mm) (P_2O_5).

Anal. Calcd for $C_{24}H_{25}N_4O_8PS$: C, 51.98; H, 4.20; N, 9.33; P, 5.16. Found: C, 51.92; H, 4.32; N, 9.30; P, 5.10.

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-Di-*O*-tolyl Phosphate (3b).—To a cold solution of 368 mg (1.00 mmole) of **2** in 30 ml of dry pyridine was added with stirring 0.8 ml (3.00 mmoles) of di-*O*-tolylphosphorochloridate. The resulting solution was kept in the cold for 1 hr and then left at room temperature for 18 hr. It was then chilled in an ice bath and 527 mg of solid $NaHCO_3$ was added, followed by the slow addition of H_2O (12 ml). The resulting solution was evaporated to dryness *in vacuo* and the residue was taken up in $CHCl_3$ (30 ml). The $CHCl_3$ solution was washed (30 ml of $NaHCO_3$, 30 ml of H_2O), dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of MeOH and precipitated as a white solid by the addition of an equal volume of water; yield 427 mg (68%).

The analytical sample was obtained by precipitation from MeOH–H_2O. It was dried for 20 hr at 100° (0.07 mm) (P_2O_5); λ_{max} [in mμ (ε × 10^{-3})] 0.1 *N* HCl—323 (22.9), pH 7—320 (21.4), 0.1 *N* NaOH—233 (14.1) and 311 (22.7); $\bar{\nu}$ (in cm^{-1}) 3150 (sh), 3110, 3050, 2990, 2925, 2855 (CH), 1755 (C=O), 1600, 1540 (C=C, C=N), 1495 (phenyl), 1465 (CH), 1415 (C=S), 1045, 960 (POC).

Anal. Calcd for $C_{38}H_{39}N_4O_9PS$: C, 53.50; H, 4.65; N, 8.91; P, 4.93. Found: C, 53.20; H, 4.65; N, 9.07; P, 4.70.

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-Di-*p*-tolyl Phosphate (3c).—This reaction was carried out in exactly the same way as described for the preparation of **3b** using 271 mg (0.74 mmole) of 9-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione and 0.56 ml (2.21 mmoles) of di-*p*-tolylphosphorochloridate. The product was obtained as a white solid; yield 54 mg (17%).

The analytical sample was obtained by precipitation from MeOH–H_2O. It was dried for 20 hr at 100° (0.07 mm, P_2O_5); λ_{max} [in mμ (ε × 10^{-3})] 0.1 *N* HCl—322 (16.1), pH 7—319 (14.1), 0.1 *N* NaOH—313 (16.9); $\bar{\nu}$ (in cm^{-1}) 3100, 3040, 2920, 2850 (CH), 1745 (C=O), 1595, 1555 (C=C, C=N), 1500 (phenyl), 1470 (CH), 1430 (C=S), 1035, 955 (POC).

Anal. Calcd for $C_{38}H_{39}N_4O_9PS$: C, 53.50; H, 4.65; N, 8.94; P, 4.93. Found: C, 53.45; H, 4.93; N, 8.74; P, 4.87.

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-Di(3,5-xylyl) Phosphate (3d).—To a chilled solution of 368 mg (1.00 mmole) of **2** in 30 ml of anhydrous pyridine was added 1.08 g (3.00 mmoles) of di(3,5-xylyl)phosphorochloridate and the resulting solution was stirred for 1 hr in an ice bath and left 16 hr at room temperature. The solution was then chilled and 527 mg of solid Na_2CO_3 was added, followed by the slow addition of H_2O (12 ml). The resulting solution was evaporated *in vacuo* to a thick sludge. The residue was extracted with $CHCl_3$ (30 ml). The $CHCl_3$ solution was washed (30 ml of $NaHCO_3$, 30 ml of H_2O), dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue was precipitated from MeOH–H_2O (1:1) as a white solid; yield 241 mg (37%), melting point indefinite.

The analytical sample was obtained by precipitation from EtOH–H_2O (1:1). It was dried at 78° (0.07 mm, P_2O_5) for 20 hr; λ_{max} [in mμ (ε × 10^{-3})] 0.1 *N* HCl—323 (20.3), pH 7—321 (18.2), 0.1 *N* NaOH—233 (sh) (14.9) and 311 (21.1); $\bar{\nu}$ (in cm^{-1}) 3110, 3050, 2925, 2860 (CH), 1755 (C=O), 1610, 1595, 1540 (C=C, C=N), 1475 (CH), 1410 (C=S), 1030, 995 (sh), 970 (POC).

Anal. Calcd for $C_{36}H_{39}N_4O_9PS$: C, 54.88; H, 5.06; N, 8.53; P, 4.72. Found: C, 55.01; H, 5.12; N, 8.37; P, 4.60.

9-(2,3-Di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-Di-*p*-nitrophenyl Phosphate (3e).—A solution of 3.06 g (9.00 mmoles) of di-*p*-nitrophenyl phosphate in 15 ml of anhydrous dioxane was obtained by gentle heating. The solution was cooled quickly to room temperature, whereupon 725 mg (3.26 mmoles) of *N,N*-di-*p*-tolylcarbodiimide was added. After stirring the resulting suspension for 15 min, 470 mg (1.28 mmoles) of **2** was added and stirring continued for 3 hr. The mixture was then stored in a desiccator (P_2O_5) at room temperature for 40 hr. Upon filtration, the reaction mixture yielded 650 mg of di-*p*-tolylurea. The filtrate was evaporated to dryness *in vacuo*. The residue, a yellow glass, was shaken with a mixture of 14 ml of $CHCl_3$ and 8 ml of 1 *M* LiOAc and refrigerated for 20 hr. The precipitate that had formed was collected by filtration as a cream-colored solid; yield 819 mg (92.5%). This material was used without further purification to prepare **6** as described below.

2′-Deoxy-5-fluorouridylyl-(3′→5′)-thioinosine (5).—To a solution of 4.0 mmoles of 2-cyanoethyl phosphate (from 1.15 g of its Ba salt) in 100 ml of anhydrous pyridine was added 1.13 g (2.0 mmoles) 2′-deoxy-5′-*O*-trityl-5-fluorouridine.[4] The resulting solution was evaporated to dryness *in vacuo* and the residue was redissolved in 75 ml of pyridine. To the solution was added 3.30 g (16.0 mmoles) of dicyclohexylcarbodiimide. The resulting solution, in a tightly sealed flask, was allowed to stand for 2 days at room temperature. Upon filtration, the reaction mixture yielded 1.06 g of 1,3-dicyclohexylurea. The filtrate was diluted with H_2O (4 ml) and the solution was set aside for 1 hr. A second precipitate of the urea (2.14 g) was obtained. The filtrate was evaporated to dryness *in vacuo*, the residue was dissolved in 50 ml of 0.5 *N* LiOH, and the resulting solution was heated for 1 hr at 100°. After cooling, the solution was filtered to remove some insoluble material. The filtrate was chilled in an ice bath and stirred with 52 ml of Amberlite IR-120 (H) resin. The resin was removed by filtration, the acidic filtrate

(15) H. J. Thomas, K. Hewson, and J. A. Montgomery, *J. Org. Chem.*, **27**, 192 (1962).

was evaporated to dryness *in vacuo*, and the residue was dissolved in EtOH. The ethanol solution was evaporated to dryness and the white residue was dissolved in 50 ml of anhydrous pyridine.

To this solution was added 615 mg (1.90 mmoles) of 2′,3′-*O*-isopropylidenethioinosine[2] followed by 1.95 g (9.45 mmoles) of dicyclohexylcarbodiimide. The resulting solution was kept at room temperature with the exclusion of moisture for 2 days. After the solution was diluted with 4 ml of H_2O and allowed to stand for 1 hr at room temperature, the precipitate of 1,3-dicyclohexylurea was removed by filtration. The filtrate was evaporated to dryness *in vacuo*. A solution of the residue in 50 ml of 80% AcOH (v/v) was heated in a 100° oil bath for 15 min. The crystalline triphenylcarbinol that formed upon cooling was removed by filtration. The filtrate was evaporated to dryness *in vacuo*. The residue of 2′-deoxy-5-fluorouridylyl-(3′→5′)-2′,3′-*O*-isopropylidenethioinosine was dissolved in 200 ml of H_2O and refluxed for 2.5 hr and then filtered. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 25 ml of H_2O and placed on a column (1.75 × 15 cm) of Dowex 1-X2 (formate) resin. The column was eluted with increasing strengths of formic acid from 0.1 to 5.0 *N*. The product was obtained with 2.5 *N* HCO_2H. Evaporation of the HCO_2H and trituration of the residue with EtOH produced a yellow solid: yield 38 mg (6.5%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] pH 7—273 (9.45) and 323 (18.6), 0.1 *N* NaOH—231 (19.1), 270 (7.75), and 311 (19.0); $\bar{\nu}$ (in cm^{-1}) 3400 (broad) (OH), 1705 (C=O), 1595, 1540, 1475 (C=C, C=N), 1050 (POC); M_{Tin} 0.70. The analytical sample was dried for 20 hr at 78° (0.07 mm, P_2O_5).

Anal. Calcd for $C_{19}H_{22}FN_6O_{11}PS \cdot 1.5H_2O$: C, 36.83; H, 4.07; P, 5.00. Found: C, 37.14; H, 4.42; P, 4.64.

Reaction of 5′-*O*-trityl-2′-deoxy-5-fluorouridylic acid with **2** followed by removal of the blocking groups gave **5** in 6% yield.

9-β-D-Ribofuranosyl-9*H*-purine-6(1*H*)-thione 5′-*p*-Nitrophenyl Phosphate (6).—To a suspension of 193 mg (0.28 mmole) of 9-(2,3-di-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6(1*H*)-thione 5′-di-*p*-nitrophenyl phosphate in Me_2CO (6 ml) was slowly added with stirring 16 ml of 0.1 *N* $Ba(OH)_2$. The resulting yellow, cloudy solution was stirred for 30 min at room temperature and then filtered. The filtrate was stirred with enough Amberlite IR-120 (H) resin to give pH 2. The resin was removed by filtration and the aqueous filtrate was washed with ether until the ether layer no longer gave a yellow color when stirred with dilute NaOH. The aqueous solution was evaporated to dryness *in vacuo*. The residue was triturated with EtOH and the EtOH was evaporated. The process was repeated. A yellow solid was obtained; yield 88 mg (65%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 *N* HCl—320 (21.7), pH 7—316 (21.8), 0.1 *N* NaOH—308 (25.2); $\bar{\nu}$ (in cm^{-1}) 3425 (OH), 3130 and 2920 (CH), 2800–2400 (acidic H), 1610, 1590, 1560, and 1515 (C=C, C=N), 1485 (C=S), 1335 (CH), 1080 (POC); M_{Tin} 0.51. The analytical sample was dried for 18 hr at 78° (0.07 mm, P_2O_5).

Anal. Calcd for $C_{16}H_{16}N_5O_9PS$: C, 39.59; H, 3.32; N, 14.43; P, 6.38. Found: C, 39.81; H, 2.73; N, 14.02; P, 6.50.

Inosine 5′-Phenyl Phosphate (7a).—To a solution of 516 mg (1.00 mmole) of 9-β-D-ribofuranosyl-9*H*-purine-6(1*H*)-thione 5′-diphenyl phosphate (**4a**)[3] in 50 ml of DMF was added 154 mg (1.04 mmoles) of anhydrous K_2CO_3 and 0.14 ml (2.00 mmoles) of 2-bromoethanol. The resulting mixture was stirred and heated at 70° for 1 hr, then cooled to room temperature, diluted with H_2O (50 ml), and evaporated to dryness *in vacuo*. The residue was dissolved in 8 ml of 3 *N* LiOH, heated for 15 min at 100°, and filtered. The filtrate was stirred with Amberlite IR-120 (H) resin until pH 1 was reached. The resin was removed by filtration and washed with three 50-ml portions of ether to remove the phenol that had formed. The aqueous solution was taken to pH 7.8 with dilute NaOH and then evaporated to dryness *in vacuo*. The residue was purified by placing it on a column (1 × 15 cm) of Dowex 1-X2 (formate) resin. The column was eluted with increasing strengths of HCO_2H from 0.1 to 5.0 *N*. The product was obtained when the column was eluted with 2.5 *N* HCO_2H. Evaporation of the solution *in vacuo* and trituration with EtOH produced a crystalline solid: yield 63 mg (14%); mp 156°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] pH 7—249 (10.3), 0.1 *N* NaOH—254 (11.2); $\bar{\nu}$ (in cm^{-1}) 3400 (broad) (OH), 3100 (broad) (CH), 2800–2400 (acidic H), 1710 (C=O), 1590 and 1570 (C=C, C=N), 1490 (phenyl), 1070 (POC); M_{Tin} 0.53. The analytical sample was dried for 20 hr at 100° (0.07 mm, P_2O_5).

Anal. Calcd for $C_{16}H_{17}N_4O_8P$: C, 42.58; H, 4.47; N, 12.42. Found: C, 42.76; H, 4.50; N, 12.23.

Inosine 5′-Dibutyl Phosphate (7b).—A solution of 1.00 g (2.12 mmoles) of **4a**[3] and 0.3 ml (4.25 mmoles) of 2-bromoethanol in 107 ml of DMF containing a suspension of 309 mg (2.23 mmoles) of anhydrous K_2CO_3 was heated and stirred at 73° for 1 hr and then diluted with enough water to give a complete solution (about 100 ml). The solution was evaporated to about 30 ml *in vacuo* and then was diluted with enough 50% EtOH–dilute NaOH to give a total volume of 200 ml of 0.1 *N* NaOH. The solution was left for 20 min at room temperature and then stirred with 43 ml of Amberlite IR-120 (H) ion-exchange resin until neutral. The resin was removed and the solution was evaporated to dryness *in vacuo*. A solution of the residue in 100 ml of $CHCl_3$ was washed (two 100-ml portions of $NaHCO_3$ solution, 100 ml of H_2O), dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The crude product, which weighed 417 mg, was purified by passing it through a Whatman cellulose column (4 × 45 cm) using water-saturated BuOH as the eluent. The peak fractions were examined by paper chromatography. The fractions containing product were combined and evaporated to dryness *in vacuo*. The residue, upon trituration with ether, crystallized. The crystalline material was dissolved ($CHCl_3$) and precipitated as a gel upon addition of ether. When it was dried for 18 hr at 78° (0.07 mm, P_2O_5), a clear melt was obtained; yield 158 mg (16%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] pH 7—248 (10.2), 0.1 *N* NaOH—253 (11.2); $\bar{\nu}$ (in cm^{-1}) 3370 (OH), 3100, 2965, 2930, 2870 (CH), 1700 (C=O), 1590, 1545, 1155 (C=C, C=N), 1245 (unassigned), 1120 (COC), 1020 (POC).

Anal. Calcd for $C_{18}H_{29}N_4O_8P$: C, 46.94; H, 6.35; P, 6.73. Found: C, 46.68; H, 6.38; P, 6.71.

6-Methylthiopurine Ribonucleotide (9) Barium Salt.—To a solution of 1.33 g (3.00 mmoles) of the disodium salt of 6-mercaptopurine ribonucleotide (**8**)[2] in H_2O (9 ml) and 3 ml of 1 *N* NaOH was slowly added with stirring 0.75 ml (12.0 mmoles) of MeI. The resulting mixture was stirred at room temperature for 20 hr before evaporation to dryness *in vacuo*. The pH of a solution of the residue in 15 ml of H_2O was adjusted to 2 with Amberlite IR-120 (H) ion-exchange resin. The resin was removed by filtration and the solution was adjusted to pH 8 with concentrated $Ba(OH)_2$. The solution was then filtered and diluted with 2 vol. of absolute EtOH. The white precipitate that formed was collected by filtration, washed (EtOH), and dried at 78° (0.07 mm, P_2O_5); yield of Ba salt 1.10 g (69%).

In another run, the analytical sample was obtained by purification of the crude reaction product on a column of Dowex 1-X2 (formate) resin using 2.5 *N* HCO_2H as the eluent. The product was again obtained as its Ba salt: λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 *N* HCl—293 (16.6), 0.1 *N* NaOH—291 (19.1); $\bar{\nu}$ (in cm^{-1}) 3400 (broad) (OH), 3100 (sh), 2925 (CH), 1570 (C=C, C=N), 1090 (broad) (POC); M_{Tin} 0.97.

Anal. Calcd for $C_{11}H_{13}BaN_4O_7PS \cdot H_2O$: C, 24.84; H, 2.84; P, 5.83. Found: C, 24.97; H, 3.04; P, 5.75.

9-β-D-Ribofuranosylpurine-6(1*H*)-thione 3′,5′-Cyclic Phosphate (10).—The 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt[10] of 9-β-D-ribofuranosyl-9*H*-purine-6(1*H*)-thione 5′-phosphate[2] was prepared by the addition of 879 mg (3.00 mmoles) of 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine[16] to a solution of 1.09 g (3.00 mmoles) of the ribonucleotide **8** in 75 ml of dry pyridine. With heat, all but a small amount of the material dissolved. The resulting solution was filtered and the filtrate evaporated to dryness *in vacuo*. A solution of the residue in 300 ml of pyridine was added dropwise over a 2-hr period to a refluxing solution of 1.24 g (6.00 mmoles) of dicyclohexylcarbodiimide in 300 ml of pyridine. The resulting solution was refluxed for another 2 hr and then evaporated to dryness *in vacuo*. A solution of the residue in H_2O (300 ml) was kept at room temperature for 2 hr, filtered to remove some insoluble solid, and evaporated to dryness *in vacuo* at less than 40°. The residue was dissolved in 50 ml of *i*-PrOH–NH_4OH–H_2O (10:1:25) and the solution was applied to a column (4 × 90 cm) of Whatman cellulose powder. The column was eluted with the same solvent system at a rate of 5 ml/8 min. The first 775 ml of solvent eluted mostly purine-6(1*H*)-thione and a small amount of product. The next 130 ml of solvent eluted pure product. These fractions were combined and evaporated to dryness *in vacuo*. The residue was triturated with EtOH and the EtOH was removed by evaporation. The process was repeated and the product was obtained as a yellow solid. It was dried for 20 hr at 78° (0.07 mm, P_2O_5); yield 101 mg (8%); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] pH 7—320 (18.8), 0.1 *N*

(16) J. G. Moffat and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 649 (1961).

NaOH—310 (19.6); $\bar{\nu}$ (in cm^{-1}) 3400 (OH), 3140 (broad) (CH), 1590, 1545, 1475 (C=C, C=N), 1075, 1015 (POC); M_{Tlc} 0.67.

Anal. Calcd for $C_{19}H_{14}N_5O_6PS \cdot C_2H_5OH$: C, 35.21; H, 4.92; N, 17.11; P, 7.57. Found: C, 35.40; H, 4.95; N, 17.00; P, 7.50.

Bis[*cis*-3-(1,6-dihydro-6-thioxopurin-9-yl)cyclopentanemethyl] Phosphate (13).—To a cold solution of 221 mg (0.885 mmole) of *cis*-3-[6(1*H*)-thio-9*H*-purine]cyclopentanemethanol[12] in 20 ml of dry pyridine was added 113 mg (0.442 mmole) of *p*-nitrophenylphosphorodichloridate.[2] The resulting solution was stirred in the cold for 15 min and then left at room temperature for 20 hr. It was then poured into 25 ml of ice water and the resulting solution was evaporated to dryness *in vacuo*. The residue was dissolved in 10 ml of 0.3 *N* NaOH and the solution was left at room temperature for 2 hr. Upon neutralization (HCl), the solution deposited a gelatinous precipitate that was collected by filtration and purified by dissolving it in 50% $NaHCO_3$ followed by precipitation as a gel with AcOH; yield 72 mg (25%). The analytical sample was obtained in another run in which the crude material was purified by solution in 0.3 *N* NaOH and a precipitation by addition of concentrated HCl. It was dried at 100° (0.07 mm, P_2O_5, 20 hr); λ_{max} [in mμ ($\epsilon \times 10^{-3}$)] 0.1 *N* HCl—325 (35.9), pH 7—321 (38.7), 0.1 *N* NaOH—310 (40.8); $\bar{\nu}$ (in cm^{-1}) 3420 (OH), 1585, 1525, 1465 (C=C, C=N), 1000 (POC); M_{Tlc} 0.58.

Anal. Calcd for $C_{22}H_{27}N_5O_4PS_2 \cdot 0.75H_2O$: C, 45.87; H, 4.99; N, 19.49; P, 5.36. Found: C, 46.02; H, 5.06; N, 19.60; P, 4.80.

Acknowledgments.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical and Physical Chemistry Division of Southern Research Institute who performed most of the microanalytical and spectral determinations reported and to Dr. G. J. Dixon and Miss E. A. Dulmadge for the biological results reported.

Analogs of 6-Methyl-9-β-D-ribofuranosylpurine[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205

Received July 18, 1967

6-Methyl-9-β-D-ribofuranosylpurine, 9-(2-deoxy-β-D-ribofuranosyl)-6-methylpurine, 6-methyl-9-β-D-xylofuranosylpurine, 2-fluoro-6-methyl-9-β-D-ribofuranosylpurine, and 6-ethyl-9-β-D-ribofuranosylpurine were prepared by fusion of the appropriate O-acetyl sugars and purines. The assignment of the anomeric configuration to the nucleosides thus obtained was based on an analysis of their nmr spectra. Evidence is presented that the cytotoxicity of these nucleosides, determined using human epidermoid carcinoma cells no. 2 in culture, may correlate with the efficiency with which they are converted to nucleotides by adenosine kinase.

The toxicity[2] and antitumor activity[3] of 6-methylpurine caused Gordon, *et al.*,[4] to synthesize its ribonucleoside, a compound that is more than 200-fold as cytotoxic to HEp-2 cells as 6-methylpurine itself.[5] In addition, a HEp-2 cell line that has lost AMP pyrophosphorylase and is resistant to 2-fluoroadenine (HEp-2/FA) and cross-resistant to 6-methylpurine is sensitive to 6-methylpurine ribonucleoside.[5] This activity can be explained by the fact that the ribonucleoside is an excellent substrate for adenosine kinase[6] and therefore can be converted to its cytotoxic form, the ribonucleotide, in cells lacking the pyrophosphorylase that normally converts the purine base to its ribonucleotide.

In pursuit of compounds with greater cytotoxic specificity for cancer cells than 6-methylpurine ribonucleoside, it seemed logical to select other nucleosides that should be substrates for adenosine kinase. Consequently, we selected the deoxyribonucleoside and the xylonucleoside of 6-methylpurine and the ribonucleosides of 2-fluoro-6-methylpurine[7] and 6-ethylpurine.[8]

These nucleosides were all prepared by the fusion method of Sato, *et al.*[11] Although an excellent preparative procedure, the fusion method is known to give rise to anomeric mixtures[12-18] even with sugars that exert steric control by orthoester ion formation in the halosugar–heavy metal purine derivative condensation.[19]

In all of the fusion reactions of 6-methylpurine (**1**), we observed formation of the *cis*(α anomer) as well as the *trans* (β anomer) nucleoside. However, in contrast to the results of Lee, *et al.*,[12] who obtained approximately equal amounts of α and β anomers from the fusion of tetra-*O*-acetyl-D-xylofuranose and *N*-nonanoyladenine, we obtained roughly 10β to 1α in the

(1) This work 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.

(2) F. S. Phillips, S. S. Sternberg, L. Hamilton, and D. A. Clark, *Ann. N. Y. Acad. Sci.*, **60**, 283 (1954).

(3) D. A. Clark, F. S. Phillips, S. S. Sternberg, and C. C. Stock, *ibid.*, **60**, 235 (1954).

(4) M. P. Gordon, V. S. Welsky, and G. B. Brown, *J. Am. Chem. Soc.*, **79**, 3245 (1957).

(5) L. L. Bennett, Jr., M. H. Vail, S. Chumley, and J. A. Montgomery, *Biochem. Pharmacol.*, **15**, 1719 (1966).

(6) H. P. Schnebli, D. L. Hill, and L. L. Bennett, Jr., *J. Biol. Chem.*, **242**, 1997 (1967).

(7) J. A. Montgomery and K. Hewson, *J. Am. Chem. Soc.*, **82**, 463 (1960).

(8) Although these compounds might be poorer substrates for adenosine kinase than 6-methyl-9-β-D-ribofuranosylpurine, they would be expected, by analogy with known substrates,[6,9] to be phosphorylated to some extent to the cytotoxic form. One factor in the cytotoxicity of purine nucleosides appears to be the ease of phosphorylation,[10] and selective action could result from differences in the kinase from normal and neoplastic cells. Such a difference might be more evident with poorer substrates.

(9) B. Lindberg, H. Klenow, and K. Hansen, *J. Biol. Chem.*, **242**, 350 (1967).

(10) L. L. Bennett, Jr., and J. A. Montgomery, unpublished observations.

(11) T. Sato, T. Simodate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1140 (1960).

(12) W. W. Lee, A. P. Martinez, G. L. Tong, and L. Goodman, *Chem. Ind.* (London), **52**, 2007 (1963).

(13) L. Pichat, P. DuFay, and Y. Lamorre, *Compt. Rend.*, **259**, 2453 (1964).

(14) R. J. Rousseau, L. B. Townsend, and R. K. Robins, *Biochemistry*, **5**, 756 (1966).

(15) J. A. Montgomery and K. Hewson, *J. Med. Chem.*, **9**, 354 (1966).

(16) K. Imai, A. Nohara, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **14**, 1377 (1966).

(17) K. Onodera, S. Hirano, H. Fukunoi, and F. Masuda, *Carbohydrate Res.*, **1**, 254 (1965).

(18) K. Onodera and H. Fukunoi, *Agr. Biol. Chem.* (Tokyo), **27**, 864 (1963).

(19) B. R. Baker, *Ciba Found. Symp. Chem. Biol. Purines*, 120 (1957).