[4,5-d]pyrimidin-7(6H)-one. Swirling gave a solution and after about 5 min. a precipitate began to form. After standing in a tightly sealed flask at room temperature for 2 days, the reaction mixture was diluted with 17.5 ml. of water and left at room temperature for 1 hr. The precipitate of 1,3-dicyclohexylurea (5.88 g.) was removed by filtration. The filtrate was evaporated to dryness *in vacuo*, the residue dissolved in 100 ml. of water, and the resulting solution washed with chloroform (5 × 100 ml.). The aqueous solution of 5-amino-3-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-3H-v-triazolo[4,5-d]pyrimidin-7(6H)-one 5′-(2-cyanoethyl)phosphate (IIIb) was diluted with enough 1N sulfuric acid to give 170 ml. of a 0.1N solution. The resulting solution was left at room temperature for 2 days and then neutralized by the addition of 1.46 g. (8.5 mmoles) of barium hydroxide in 150 ml. of water. The precipitate of barium sulfate was removed by filtration. The aqueous solution of 5-amino-3-β-D-ribofuranosyl-3H-v-triazolo[4,5-d]pyrimidin-7(6H)-one 5′-(2-cyanoethyl)phosphate (IVb) was diluted with 66.7 ml. of 3N lithium hydroxide and enough water to give 400 ml. of a 0.5N solution, which was heated for 15 min. in a 100° oil bath. After removal of the precipitate which formed, the solution was stirred for 30 min. with 315 ml. of Amberlite IR-120(H) ion-exchange resin. The resin was filtered off and washed thoroughly. The combined filtrate and washings (800 ml.) were diluted with an equal volume of ethanol, and the precipitate which formed was collected by filtration; yield, 3.19 g. (73%). To prepare the

analytical sample, this material was washed with boiling water and dried at 110°/0.07 mm. over phosphorus pentoxide for 8 hr.

Spectral data. λ max in mμ (ε × 10⁻³): pH 1-255 (12.9); pH 7-255 (12.6); pH 13-222 (23.8) and 279 (11.7). ν in cm.⁻¹: 3410 (OH); 3300–3100 (NH); 2930 (CH); 1700 (C=O); 1640 (NH); 1600, 1530 (shoulder), and 1540 (C=C, C=N); 1090 (P—O—C).

Anal. Calcd. for $C_9H_{11}BaN_6O_8P$: C, 21.64; H, 2.22; N, 16.82; P, 6.20. Found: C, 21.40; H, 2.56; N, 16.47; P, 6.06.

Synthesis of Potential Anticancer Agents. XXVIII. Simple Esters of 6-Mercaptopurine Ribonucleotide[2]

JOHN A. MONTGOMERY, H. JEANETTE THOMAS, AND HOWARD J. SCHAEFFER

6-Mercaptopurine ribonucleotide and six simple ester derivatives have been prepared from 9-(2′,3′-O-isopropylidene-β-D ribofuranosyl)-9H-purine-6(1H)-thione by reaction with diphenyl, dibutyl, and diethyl phosphorochloridates followed by appropriate hydrolysis reactions.

It has now been firmly established that neoplasms susceptible to the action of either 6-mercaptopurine (purine-6(1H)-thione) or 8-azaguanine (5-amino-v-triazolo[4,5-d]pyrimidin-7(6H)-one) convert these compounds to their respective ribonucleotides. Neoplasms that are resistant to these two compounds (whether the resistance is natural or acquired) do not have the pyrophosphorylase necessary to carry out this conversion.[3,4] It is questionable whether this resistance can be overcome by treatment with synthetically prepared ribonucleo-

tides since it is well known that the nucleotides of the naturally occurring purines are poorly incorporated into cell nucleic acids[5] and, indeed, it has been shown that they are not incorporated intact.[6,7] These findings raise serious doubts that nucleotides, as such, can penetrate the cell membrane. This difficulty might be overcome if one could prepare an ester of a nucleotide which could penetrate the cell wall and then be metabolized to the nucleotide itself.[8] Toward this end, some simple esters of 6-

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(2) Chemical Abstracts name: 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione 5′-phosphate. For paper XXVII of this series see *J. Org. Chem.*, **26**, 1926 (1961).

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(8) O. C. Touster found that, whereas sodium D-glucuronate is not metabolized by humans, the nonionized glucuronic acid lactone is. He postulated that this difference may be due to the ability of the lactone to penetrate the cell membrane and then be hydrolyzed to the acid which is metabolized.[9]

(9) O. C. Touster, R. M. Hutchison, and L. Rice, *J. Biol. Chem.*, **215**, 677 (1955).

R =

a. R' = C₆H₅
b. R' = C₄H₉
c. R' = C₂H₅
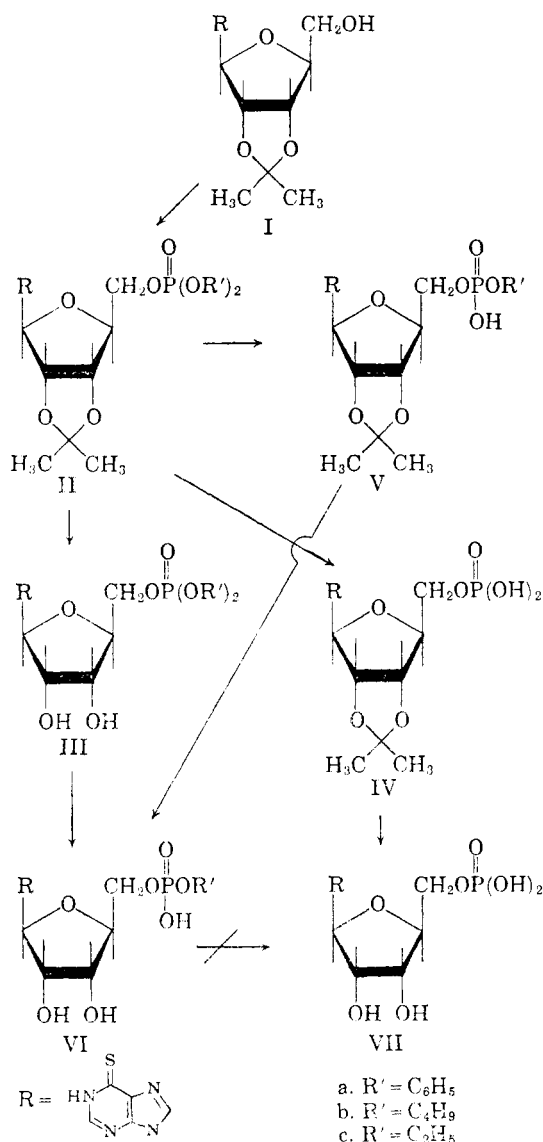
mercaptopurine ribonucleotide have now been prepared.

9 - (2',3' - O - Isopropylidene - β - D - ribofuranosyl) - 9H - purine - 6(1H) - thione (I)[10] was allowed to react with diphenyl,[11] dibutyl, and diethyl phosphorochloridates to give the corresponding derivative of 9-(2',3'-O-isopropylidene-β - D - ribofuranosyl) - 9H - purine - 6(1H) - thione disubstituted 5'-phosphates (IIa, b, and c). Treatment of 9-(2',3'-O-isopropylidene-β-D-ribofuranosyl) - 9H - purine - 6(1H) - thione 5' - diphenylphosphate (IIa) in dioxane solution with 1N lithium hydroxide solution caused hydrolysis of one of the phenyl groups giving 9-(2',3'-O-isopropylidene - β - D - ribofuranosyl) - 9H - purine-6(1H) - thione 5' - phenylphosphate (Va), and treatment of this material (Va) in dioxane with 0.1N hydrochloric acid hydrolytically removed the

the isopropylidene group of Va to give 9-β-D-ribofuranosyl - 9H - purine - 6(1H) - thione 5'-phenylphosphate (VIa). This route to VIa, probably largely because of solubility difficulties, proved to be inferior to the second procedure we employed in which the isopropylidene group of IIa was first removed in a 1:1 mixture of methanol and 0.1 N hydrochloric acid followed by hydrolysis of one phenyl group of IIIa by heating it with 3N lithium hydroxide solution at 100° for fifteen minutes. In an attempt to remove both phenyl groups of IIIa to prepare 6-mercaptopurine ribonucleotide[10,12] itself (VII), IIIa was subjected to more drastic conditions of basic hydrolysis. Complex reaction mixtures resulted from which none of the desired ribonucleotide (VII) could be isolated, although its presence was established by paper chromatography. This compound (VII) was prepared, in low yield, by treatment of IIa with sodium in liquid ammonia followed by acid hydrolysis of the isopropylidene group of the intermediate IV. Later the ribonucleotides of both 6-mercaptopurine and 8-azaguanine were prepared in good yield by an adaptation[10] of the method of Gilham and Tener.[13]

The mono- and dibutyl and ethyl esters of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione 5'-phosphate were prepared by the second route described above for the phenyl esters.

Yield data, physical constants, elemental analyses, spectral data, and electrophoretic and chromatographic data are all summarized in Tables I, II, and III. Typical procedures are described in the Experimental Section.

EXPERIMENTAL

The melting points were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined with a Beckman Model DK-2 spectrophotometer, but the optical densities at the maxima were measured with a Beckman DU. The infrared spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 21 spectrophotometer. Electrophoresis and chromatographic studies were carried out as previously described.[10]

9 - (2',3' - O - Isopropylidene - β - D-ribofuranosyl-9H-purine-6(1H)-thione 5'-diphenylphosphate (IIa). To a chilled solution of 2.00 g. (6.15 mmoles) of 9-(2',3'-O-isopropylidene-β-D-ribofuranosyl)9H-purine-6(1H)-thione[10] in 40 ml. of anhydrous pyridine was slowly added 4.97 g. (18.4 mmoles) of diphenyl phosphorochloridate. The resulting solution, protected by a calcium chloride tube, was stirred for 1 hr. in an ice bath and left 16 hr. at room temperature. It was then chilled and 3.25 g. (30.7 mmoles) of solid sodium carbonate was added, followed by the slow addition of 72 ml. of cold water. The resulting solution was evaporated in vacuo at 35° to a thick sludge. The residue was extracted with 100 ml. of chloroform. The chloroform solution was washed twice with saturated bicarbonate solution (65 ml.), then water (65 ml.), dried with magnesium sulfate, and evaporated in vacuo to dryness. From the residue, on

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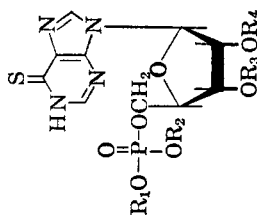(11) H. Bredereck, E. Berger, and J. E. Ehrenberg, Ber., 73, 269 (1940).

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TABLE I

PHYSICAL PROPERTIES OF

Compd. No.	R_1	R_2	R_3	R_4	Recrystn. Solvent[a]	Yield, %	M.P.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Phosphorus, % Calcd.	Phosphorus, % Found
IIa	C_6H_5	C_6H_5	Isopropylidene	Isopropylidene	A	68	200–201 dec.	53.95	53.69	4.53	4.56	10.07	10.26	5.57	5.69
b	C_6H_5	C_6H_5	Isopropylidene	Isopropylidene	A	54	179–180 dec.	48.83	48.84	6.44	6.47	10.85	10.81	—	—
c	C_6H_5	C_6H_5	Isopropylidene	Isopropylidene	A	64	160–161	44.34	44.32	5.47	5.48	12.17	12.23	6.73	6.70
IIIa	C_6H_5	C_4H_9	H	H	A	78	—[b]	51.16	50.85	4.10	4.43	10.85	10.86	6.00	5.97
b	C_4H_9	C_4H_9	H	H	B	80	—[b]	45.37	45.29	6.13	5.96	11.76	11.72	6.50	6.55
c	C_2H_5	C_2H_5	H	H	C	53	—[b]	40.00	39.86	5.04	5.20	13.33	13.62	7.37	7.30
VIa	C_6H_5	H	H	H	A	20	208–210 dec.	42.76[c]	42.40	4.04[c]	4.35	12.47[c]	12.53	6.90[c]	6.65
b	C_4H_9	H	H	H	C	38	206 dec.	39.20[c]	39.22	4.93[c]	5.05	13.05[c]	12.91	—	—
c	C_2H_5	H	H	H	D	25	159 dec.	36.73[d]	36.93	4.37	4.72	—	—	7.90	7.69
VII	H	H	H	H		9.3		31.41[d]	31.06	3.95[d]	4.27	14.65[d]	14.50	—	—

[a] A—methanol; B—1:1 methanol-water; C—isopropyl alcohol; D—ethanol. [b] Indefinite. [c] Calcd. as a hemihydrate. [d] Calcd. as a monohydrate.

TABLE II

Compd. No.	U.V. Spectra pH 13		Solvent Systems[a]						
	λ_{max}	$\epsilon_{max} \times 10^{-3}$	R_{Ad} Values[b]				Relative Migration[c]	
			A	B	C	D	E	F
IIa	312	23.4	2.20	1.44	1.79	0	—	—
b	312	22.7	2.04	1.36	1.73	0	—	—
c	311	22.7	1.81	1.42	1.79	1.73	—	—
IIIa	312	21.3	1.59[d]	1.31[d]	1.62[d]	—	0	62
b	312	21.9	1.71[d]	1.47[d]	1.75[d]	1.28[d]	0	71
c	312	21.7	1.13[d]	1.18[d]	1.33[d]	2.21[a]	0	70
VIa	311	21.8	0.17[d,e]	0.59[d,e]	0.81[d,e]	2.11[d,e]	92	96
b	311	21.1	0.19[d,e]	0.71[d,e]	0.90[d,e]	2.38[d,e]	90	99
c	310	21.4	0.05[d,e]	0.50[d,e]	0.64[d,e]	2.35[d,e]	100	97
VII	311	21.3	0[d,e]	0.23[d,e]	0.05[d,e]	2.26[d,e]	97	107

[a] A—water-saturated butyl alcohol; B—butyl alcohol–acetic acid–water (5/2/3); C—isopropyl alcohol–ammonium hydroxide–water (70/5/25); D—5% disodium hydrogen phosphate; E—0.05M ammonium formate buffer (pH 3.5), 20 volts/cm. for 2 hr.; F—0.05M sodium tetraborate (pH 9), 15 volts/cm. for 1.5 hr. [b] R_f of adenine = 1.00. [c] Migration of inosinic acid = 100. [d] Positive Schiff's test. [e] Positive phosphate test.

TABLE III

INFRARED SPECTRA, IMPORTANT MAXIMA, CM.$^{-1}$ (KBr)

Group Assignment	Compound Number								
	II			III			VI		
	a	b	c	a	b	c	a	b	c
OH				3400	3400	3400	3400	3425	3420
CH	3150	3160	3130	3150	3180	3190	3150	3150	3160
	3050	3100	3050	3070	2970	3000	3090	2950	2900
	3000	2950	2990	2930	2890	2930			
	2850	2850	2920						
Acidic H	2750–2400	2750–2400	2750–2400	2800–2500	2750–2500	2750–2500	2800–2400	2800–2600	2800–2500
C=C,	1605	1595	1605	1595	1600	1595	1620	1600	1600
C=N	1590[a]	1580	1580	1550	1550[a]	1580[a]	1595	1565	1560
	1550[a]	1535	1540			1540[a]	1570	1555	1540
Phenyl	1495			1490			1495		
CH	1475[a]	1480	1480	1465	1480	1480	1480	1480	1480
C=S	1420	1420	1415	1415	1420	1420	1480	1480	1480
CH	1340	1340	1340	1340	1340	1335	1350	1345	1340
OH				1110	1120	1120[a]	1115	1110[a]	1110[a]
COC	1080	1075	1080						
POC	1030	1060	1050	1050	1050	1030	1090	1080	1060[a]
	1010	1030	1035	1020	1030	1010	1080	1040[a]	1040
	960			960			1035		
Purine ring	910[a]	930[a]	910[a]	900	900	900	920	980	970
	860	870	860	870	870	870	870	880	870

[a] Shoulder.

crystallization from 50 ml. of methanol, there was obtained a white solid: yield, 2.11 g. (68%); m.p., 198–200° dec.

The analytical sample, obtained by recrystallization from methanol, was dried for 24 hr. over phosphorus pentoxide at 100°/0.07 mm.

9 - β - D - Ribofuranosyl - 9H - purine-6(1H)-thione 5'-dibutylphosphate (IIIb). A solution of 8.62 g. (16.7 mmoles) of 9-(2',3'-O-isopropylidene-β-D-ribofuranosyl)-9H-purine-6(1H)-thione 5'-dibutylphosphate in 604 ml. of methanol and 325 ml. of 0.3 N hydrochloric acid was heated in a hot water bath for 45 min., then chilled in an ice bath, and finally carefully neutralized to pH 7 with sodium hydroxide solution. On evaporation of the neutral solution to about 50 ml., a white solid was obtained: yield, 7.26 g.; m.p., indefinite.

The analytical sample was obtained by dissolving the product in 25 ml. of methanol, diluting the solution with 25 ml. of water, heating the resulting cloudy suspension until it was clear, and allowing it to cool down slowly. The resulting crystalline product was dried at 100°/0.07 mm. over phosphorus pentoxide for 8 hr.: yield, 6.38 g. (80%).

9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione 5'-ethylphosphate (VIc). A solution of 2.46 g. (5.85 mmoles) of 9-β-D-ribofuranosylpurine-6(1H)-thione 5'-diethylphosphate in 24.6 ml. of 6 N sodium hydroxide was kept at room temperature for 5 hr. It was then stirred with 150 ml. of Amberlite IR-120(H) ion exchange resin until the pH was 2.3. The resin was filtered off and the filtrate evaporated *in vacuo* to dryness. The residue was dissolved in 200 ml. of hot ethanol, which on cooling deposited a crystalline solid: yield, 567 mg. (25%); m.p., 159° dec.

The analytical sample was obtained by recrystallization from ethanol and was dried at 110°/0.07 mm. over phosphorus pentoxide for 8 hr.

9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione 5'-phosphate (VII).[10,11] To a solution of 557 mg. (1.00 mmole) of 9-(2',3'-O-isopropylidene-β-D-ribofuranosyl)9H-purine-6(1H)-thione 5'-diphenylphosphate in 50 ml. of liquid ammonia was added slowly 250 mg. (10.9 mmoles) of sodium. The blue color that formed was allowed to remain for 15 min. before it was discharged by the addition of a few drops of methyl alcohol. After evaporation of the ammonia, the

remaining residue was slowly diluted with 30 ml. of ice water. The resulting solution, which was kept cold, was adjusted to pH 6 by stirring it with Amberlite IR-120(H) resin. After filtration of the resin, the solution was taken to pH 2.7 with hydrochloric acid and placed in a boiling water bath for 2 hr. The solution, which had darkened, was adjusted to pH 7 with ammonium hydroxide, filtered to remove a black precipitate, and further adjusted to pH 8.2. To the solution was then added 1.05 mmoles of barium acetate in 2 ml. of water. When the addition of two volumes of ethanol did not yield a precipitate, the solution was evaporated *in vacuo* at room temperature to 10 ml., diluted with four volumes of ethanol, and finally chilled. The solid that formed was collected by centrifugation: yield, 318 mg. This material contained about 35% 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione 5'-phosphate identified by its ultraviolet spectrum and chromatographic behavior.

The crude product was purified by absorption from aqueous solution on a Dowex 1-X2 (formate) ion exchange resin column (1 cm. × 18 cm.). The product was obtained when the column was eluted with 5 N formic acid. The formic acid was removed by freeze drying, and a yellow solid was obtained: yield, 34 mg. (9.3%).

# Potential Anticancer Agents.[1] LIII. Alkylating Agents Derived from Some Folic Reductase Inhibitors

JOSEPH I. DeGRAW, LEONARD O. ROSS, LEON GOODMAN, AND B. R. BAKER

Condensation of 2,2'-(p-aminophenylimino)diethanol dihydrochloride (VIII) with cyanoguanidine and acetone afforded the bis(2-hydroxyethyl)amine (X) which, with thionyl chloride, gave the nitrogen mustard (XI) that is related to the folic reductase inhibitor (I). The bis(2-hydroxyethyl)amine hydrochloride (XX) was synthesized by two routes. The preferred path involved the condensation of methyl propionate with {p-[bis(2-hydroxyethyl)amino]phenyl}acetonitrile (XIII), or with the blocked derivative (XIV), to give the nitriles (XXII and XVIII), which were converted to the enol ethers (XVII and XVI). Condensation of XVII and XVI with guanidine afforded the pyrimidine bases (XIX and XV) as precursors of XX. Alternatively, the known 2,4-diamino-6-ethyl-5-(p-nitrophenyl)pyrimidine (XXIX) was converted to XX by the successive treatments of acetylation, reduction, hydroxyethylation and hydrolysis. Careful treatment of XX with thionyl chloride gave the nitrogen mustard (XXI) that is related to the folic reductase inhibitor, "Daraprim" (II).

A number of the 2,4-diamino-5-aryl-6-alkylpyrimidines[2] and of the 4,6-diamino-1-aryl-1,2-dihydro-2,2-dimethyl-s-triazines[3] have shown exceptional activity in the antimalarial field. Typical active compounds of the groups are the 1-p-chlorophenyl-s-triazine (I) and "Daraprim" (II). There is an obvious structural similarity between I and II so that the marked parallelism of their physiological activities is not surprising. Both I and II are related structurally to the 4-amino derivatives of folic acid [*e.g.*, Amethopterin (III)]; the latter are clinically useful anticancer drugs and function as folic acid antagonists. In certain microbiological systems the groups exemplified by I and II act as inhibitors in the folic acid area; the pyrimidine

compounds act as irreversible inhibitors of folic reductase[4] and their action can be reversed by the
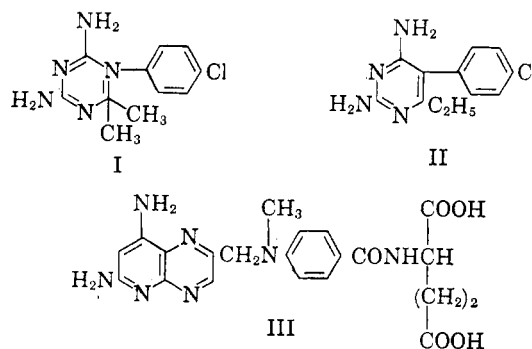
addition of citrovorum factor[5]; the triazines appear to act as irreversible inhibitors of both folic acid and citrovorum factor.[6] Doctor[7] has observed that

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series *cf.* E. J. Reist, I. G. Junga, M. E. Wain, O. P. Crews, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2139 (1961).

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