EtOH, and then Et_2O, and air-dried; 249 g (66%) of crude 1-benzylideneamino-3-(4-chlorobutyl)hydantoin was obtained. Recrystallization from 1 l. of $MeNO_2$ gave 87 g of product, mp 148–150°. Concentration of the filtrate gave an additional 71 g: mp 146–148°; total yield 158 g.

A solution of 50 g (0.17 mol) of 1-benzylideneamino-3-(4-chlorobutyl)hydantoin in 200 ml of piperidine was heated on the steam bath for 2 hr and evaporated to dryness *in vacuo*. The residue was triturated with H_2O, filtered, washed with H_2O, and dissolved in 10% aqueous HCl. The solution was filtered through Celite and steam distilled until no more PhCHO was evolved. A solution of 24 g of V in MeOH was added. The solution was then evaporated to dryness *in vacuo*. The residue was crystallized from EtOH giving 40 g of 18.

1-[(5-Nitrofurfurylidene)amino]-3-[2-(2-pyridyl)ethyl]-hydantoin (35, Method C). A mixture of 157 g (0.77 mol) of II, 500 ml of pyridine, and 90 g of distilled 2-vinylpyridine was heated under reflux for 30 hr and then poured into 3.5 l. of H_2O. The white crystalline product was filtered, washed with H_2O, and dried at 110° to give 225 g of 1-benzylideneamino-3-[2-(2-pyridyl)ethyl]hydantoin. This was steam distilled with 400 ml of H_2O and 80 ml of concentrated H_2SO_4 to remove all of the PhCHO. After treatment with charcoal and filtering, 100 g of V in MeOH was added. The solution was extracted with Et_2O to remove any unreacted V. After cooling, the product was filtered, dissolved in warm dilute aqueous HCl, treated with charcoal, filtered, and made alkaline with NH_4OH. The crude product was filtered and washed with H_2O and EtOH. Recrystallization from DMF gave 185 g of 35.

3-(5-Aminopentyl)-1-[(5-nitrofurfurylidene)amino]hydantoin Hydrochloride (39, Method D). To a solution of 177 g (0.87 mol) of II in 1.5 l. of DMF was added 38 g (0.87 mol) of a 52.6% dispersion of NaH in mineral oil in portions. After heating on the steam bath with stirring for 25 min, 103 g (0.87 mol) of 5-chlorovaleronitrile was added during 10 min. The mixture was then heated under reflux for 44 hr. The insoluble NaCl was filtered and the filtrate was concentrated nearly to dryness. The residue was triturated with 500 ml of hot EtOH, cooled, and filtered. Recrystallization from 250 ml of EtOH and 700 ml of $MeNO_2$ gave 130 g of 1-benzylideneamino-3-(4-cyanobutyl)hydantoin, mp 164–167°.

A mixture of 12 g (0.042 mol) of this nitrile, 5.7 g of NaOAc, 165 ml of Ac_2O, and *ca.* 2 g of Raney nickel catalyst No. 28 (W. R. Grace Co.) was shaken with H_2 in a Parr apparatus at 40 psig and 50°. The reduction stopped after a pressure drop of 6 psig (calcd 7). After cooling in an ice bath, the product (with the catalyst) was filtered and washed with H_2O. The above procedure was repeated three times and the combined products were recyrstallized from 1.2 l. of EtOH to give 33 g of 3-(5-acetamidobutyl)-1-benzylideneaminohydantoin, mp 182–184°.

A mixture of 79 g (0.24 mol) of the above amide and 1.2 l. of 10% HCl was steam distilled for 8 hr. The solution was then concentrated to *ca.* 400 ml and cooled, and a solution of 34 g (0.24 mol) of V in 100 ml of EtOH was added. After thorough cooling the product was collected, washed with cold EtOH, and recrystallized from EtOH to give 76 g of 39.

3-Chlorobutyl-*N*,*N*-dimethylamine. A solution of 71 g (0.8 mol) of 4-amino-2-butanol, 220 g of HCOOH, and 160 ml of 37% HCHO solution was heated under reflux for 21 hr and allowed to stand overnight. Then 80 ml of concentrated HCl was added and the solution was evaporated under reduced pressure to a yellow viscous residue which was dissolved in water and made alkaline with aqueous NaOH. This solution was extracted with several portions of $CHCl_3$. Distillation of the $CHCl_3$ under reduced pressure left 62 g of 4-dimethylamino-2-butanol as a pale yellow liquid.

A solution of this product in 500 ml of $CHCl_3$ was cooled in an ice bath and 54 ml (40% excess) of $SOCl_2$ was added dropwise with stirring at a rate such that the temperature never exceeded 10°. The ice bath was removed and stirring was continued until room temperature was reached. The solution was then heated under reflux until evolution of HCl ceased (*ca.* 6 hr). After distillation of most of the $CHCl_3$ under reduced pressure, the solid was filtered and washed with Et_2O. Recrystallization from EtOH gave 50 g of white solid. A solution of this hydrochloride in water was made alkaline with aqueous NaOH and extracted with several portions of Et_2O. After drying over $MgSO_4$ and removal of the Et_2O, distillation of the residue gave 32 g of 3-chloro-1-dimethylaminobutane, bp 34–36° (5 mm).‡

3-Chloropropyl-*N*-isopropyl-*N*-methylamine. A solution of 58 g (0.5 mol) of 3-isopropylaminopropanol, 90 ml of 88% HCO_2H, and 52 ml of 37% HCHO was heated under reflux for 17 hr and cooled, and 30 ml of concentrated HCl was added. The solvent was distilled at atmospheric pressure until a temperature of 110° was reached. The remaining solution was poured into 50 ml of ice–water, made strongly alkaline with aqueous KOH, and extracted several times with Et_2O. The combined extracts were dried over K_2CO_3, the Et_2O was distilled, and the residue was distilled under reduced pressure giving 56 g of 3-(isopropylmethylamino)-1-propanol, bp 95–99° (25–27 mm).

This material was treated with $SOCl_2$ as in the previous example. Recrystallization of the product from EtOAc gave 63 g of 3-chloropropyl-*N*-isopropyl-*N*-methylamine·HCl, mp 58–61°.

The free base was liberated as above; 10 g of the salt gave 6 g of amine, bp 40–41° (4 mm).

Ethyl *N*-[5-[1-(5-Nitrofurfurylideneamino)-2,4-dioxo-3-imidazolyl]pentyl]carbamate (30). A solution of 7.8 g (0.068 mol) of $Na_2CO_3 \cdot H_2O$ in 65 ml of H_2O was added dropwise to a mixture of 46.8 g (0.13 mol) of 39 and 500 ml of H_2O at 4–6°. Then 12.5 ml (0.13 mol) of $ClCO_2Et$ was added dropwise at 4–6°. After stirring for 10 min, an additional 5.1 g (0.041 mol) of $Na_2CO_3 \cdot H_2O$ in 40 ml of H_2O was added at 5°. Then 400 ml of water was added and the mixture was stirred and cooled for 40 min. The product was filtered, washed with water, air-dried, and recrystallized from 1100 ml of EtOH to give 38 g (74%) of 30.

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References

(1) (a) M. Abrams and B. Prophete, *Mo. Med.*, 51, 280 (1954); (b) K. J. Hayes, U. S. Patent 2,610,181 (1952); *Chem. Abstr.*, 47, 6980*i* (1953).
(2) W. Traube and E. Hoffa, *Ber.*, 31, 168 (1898).
(3) F. E. Gould, G. S. Johnson, and A. F. Ferris, *J. Org. Chem.*, 25, 1658 (1960).
(4) R. Freedman and R. E. Chamberlain, *Antimicrob. Ag. Chemother.*, 502 (1967).
(5) A. T. Babayan, A. A. Grigoryan, and G. T. Martirosyan, *Dokl. Akad. Nauk Arm. SSR*, 26, 153 (1958); *Chem. Abstr.*, 52, 19906*i* (1958).

‡Reference 5 gives bp 38–39° (10 mm).

Synthesis of 5′-Phosphates of the Naturally Occurring 6-Ureidopurine Ribonucleosides and Their Analogs†

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N-(Purin-6-ylcarbamoyl)-L-threonine ribonucleoside (PCTR,‡ **1a**), a hyper-modified nucleoside, has been isolated and characterized from tRNA of many organisms.[1,2] It also occurs as a free nucleoside in human and rat urine.[3] It has been shown to be an anticodon adjacent nucleotide in yeast $tRNA^{Ile}$[4] and in *Escherichia coli* $tRNA^{Ser}$, $tRNA^{Met}$, $tRNA^{Lys}$,[5] and $tRNA^{Asn}$.[6] A glycine analog, *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside (PCGR, **1b**), has been isolated from yeast tRNA.[7] Recently the syntheses of PCTR, PCGR, and their analogs have been reported.[8,9]

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‡Abbreviations used are as follows: PCTR, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside; PCGR, *N*-(purin-6-ylcarbamoyl)-glycine ribonucleoside; PCTRP, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside 5′-phosphate; PCGRP, *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside 5′-phosphate.

Some of the ureido analogs of PCTR showed excellent cytokinin activity,[10,11] and some exhibited growth inhibitory activity in cultured mammalian cells.[9]

PCTR is attached to the anticodon IAU through its 5′-phosphate in $tRNA^{Ile}$. In order to make available sufficient quantities of this naturally occurring nucleotide for biochemical and biosynthetic studies, the synthesis of PCTR 5′-phosphate and some of its analogs was undertaken. This paper describes the synthetic methods and properties of the *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside 5′-phosphate (**2a**) and its comparison with the natural material.§ The syntheses of *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside 5′-phosphate (**2b**) and the analogs containing isopropyl (**2c**), allyl (**2d**), and isopentyl (**2e**) side chains are also reported (see Scheme I).

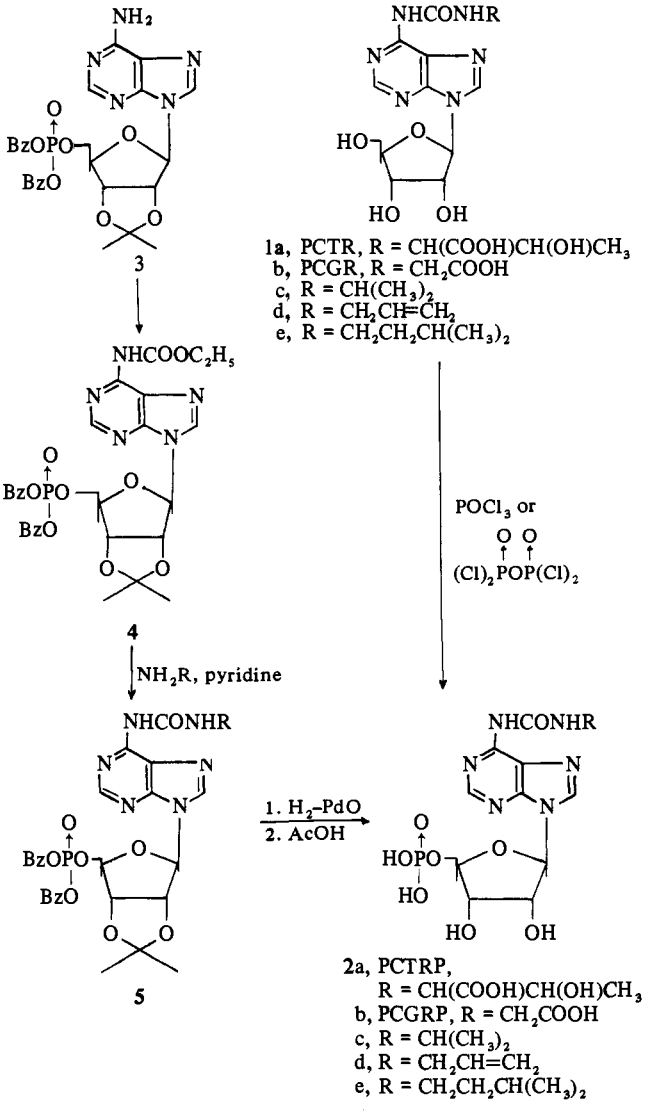
Ethyl 9-(2′,3′-isopropylidene-5′-dibenzylphosphoryl-β-D-ribofuranosyl)purine-6-carbamate (**4**) was synthesized in 45% yield by a reaction of 2′,3′-isopropylideneadenosine 5′-dibenzylphosphate[12] (**3**) with EtO_2CCl in cold pyridine at −10° (Scheme I). The displacement of the ethoxy group of the urethane (**4**) by L-threonine, glycine, and isopropylamine in pyridine at 120° for 6 hr gave the corresponding protected ureidopurine nucleotides **5**. The removal of benzyl groups by hydrogenation over palladium oxide and the isopropylidene group by heating at 100° in 70% AcOH gave the nucleotides **2a–c** in good yield.

In the case of the availability of the properly substituted 6-ureidopurine ribonucleosides, a direct phosphorylation procedure using $POCl_3$ in triethyl phosphate[13] was most convenient. The nucleosides **1a–e** were converted in one step into their 5′-phosphates by this procedure (Scheme I). Small amounts of nucleoside diphosphate and a trace of starting material were removed by purification on a column of AG1-x8 (formate). The yields of the purified nucleotides were in the range of 40–50%. Pyrophosphoryl chloride in *m*-cresol was also utilized for the direct phosphorylation of 6-ureidopurine ribonucleosides.[14] PCTR, PCGR, and the isopropyl analog **1c** were phosphorylated by this reagent. By this method, the isopropyl ureido nucleotide **2c** was obtained in 65%; however, PCTRP and PCGRP were formed in less than 20% yield.

The nucleotides PCTRP, PCGRP, and **2c** were also prepared by a reaction of 2′,3′-isopropylideneadenosine 5′-dibenzylphosphate (**3**) with isocyanates derived from *O*-benzyl-L-threonine benzyl ester and glycine benzyl ester[8,9] and with isopropyl isocyanate followed by deblocking. The yields in these reactions were unsatisfactory (13–20%). Phosphorylation with 2-cyanoethyl phosphate and DCC[15] was used successfully in the case of the 2′,3′-isopropylidene 6-ureidopurine nucleosides which did not have COOH or OH groups in the side chain. Yields in this procedure were in the range of 20%.

In evaluating the merits of the procedures to prepare the 5′-nucleotides, it is clear that in the case of the available 6-ureidopurine nucleosides, the direct phosphorylation by $POCl_3$ in triethyl phosphate should be the method of choice since sugar hydroxyls or amino acid carboxyls need not be protected. Direct phosphorylation by pyrophosphoryl chloride in *m*-cresol, however, gave poor results in the case of 6-ureidopurine nucleosides with amino acid side chains but was quite satisfactory in case of the alkyl side chains. The urethane route is a clean unambiguous method of preparing PCTRP, PCGRP, and other amino acid analogs; however, it requires the preparation of protected urethane **4**.

Scheme I



Enzymatic hydrolysis of the 5′-nucleotides **2a–e** with 5′-nucleotidase gave the corresponding ribonucleosides **1a–e**. These results in conjunction with the electrophoresis data further confirm the compounds to be the desired 5′-mononucleotides. Alkaline phosphatase, acid phosphatase, and crude venom phosphodiesterase also hydrolyzed these 5′-phosphates to the parent ribonucleosides. The latter enzyme contains enough 5′-nucleotidase to hydrolyze the nucleotides.

The paper chromatography and electrophoretic mobilities of 5′-phosphates prepared by different methods were identical with those prepared by the urethane route and were in agreement with one another (Table I). Electrophoretic and chromatographic mobilities of natural *N*-(purin-6-ylcarbamoyl)-threonine ribonucleoside 5′-phosphate isolated from yeast tRNA§ were identical with those of the synthetic PCTRP (**2a**, Table I).

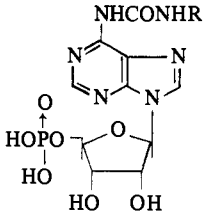
Experimental Section#

Ethyl 9-(2′,3′-Isopropylidene-5′-dibenzylphosphoryl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (4). To a stirred solution of 2.84

§ The natural *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside 5′-phosphate was provided to us by Dr. M. P. Schweizer of ICN Corp., Irvine, Calif. It was isolated from the venom phosphodiesterase (purified) hydrolysate of yeast total tRNA.

Melting points and ir, uv, and nmr (Me_4Si as an internal reference) spectra were recorded as reported previously.[8,9] Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

Table I. 6-Ureidopurine 5'-Nucleotides



Compd	R	Uv, λ max, nm (ε × 10⁻³) 0.1 *N* HCl	H_2O	0.1 *N* NaOH	Electrophoresis[a]	Tlc (R_f × 100) in solvent[b] A	B	Paper chromatography (R_f × 100) in solvent[b] A	B
2a, PCTRP	$CH(COOH)CH(OH)CH_3$	277 (19.2)	269 (24.2), 276 (20.5)	269 (22.5), 276 (19.8), 297 (8.0)	+2.87	0	13.6	1.8	1.9
Natural PCTRP	$CH(COOH)CH(OH)CH_3$	277	269, 276	269, 276, 297	+2.90		13.8		1.9
2b, PCGRP	CH_2COOH	277 (22.7)	269 (23.3), 276 (19.9)	270 (17.9), 277.5 (17.3), 295 (9.6)	+2.57	0	11.7	1.8	1.9
2c	$CH(CH_3)_2$	277 (26.5)	269 (25.1), 275 (21.5)	270 (18.6), 277 (18.0), 297 (11.6)	+1.92	49.3	29.8	11.1	22.0
2d	$CH_2CH{=}CH_2$	277 (22.4)	269 (21.9), 275 (18.7)	269 (16.7), 277 (16.4), 297 (10.1)	+2.10	47.2	28.5	9.3	17.2
2e	$CH_2CH_2CH(CH_3)_2$	277 (23.2)	269 (21.7), 275 (18.5)	269 (18.3), 277 (16.8), 297 (7.5)	+1.85	62.7	35.0	20.9	31.1
AMP		257 (15.1)	259 (15.4)	259 (15.4)	+1	0	36.2	1.3	6.2

[a]Mobilities are relative to AMP. Performed with Whatman 3MM paper at 2000 V using 0.025 *M* citric acid and 0.01 *M* potassium citrate buffer (pH 3.5) for 2 hr. [b]Solvent A: EtOAc–2-ethoxyethanol–16% HCO_2H (4:1:2); solvent B: *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1).

g (5 mmol) of 2',3'-isopropylideneadenosine 5'-dibenzylphosphate (3)[12] at −10° in 50 ml of anhydrous pyridine was added, dropwise, 2 ml (*ca.* 15 mmol) of EtO_2CCl over a period of 10 min. The mixture was then stirred at ambient temperature for 6 hr and then evaporated to dryness (at 30°). The residue was triturated with 10 ml of toluene and then reevaporated to dryness. This material was dissolved in a minimal amount of $CHCl_3$ and streaked on seven silica gel tlc plates (0.15 × 20 × 100 cm) which were developed in EtOH–EtOAc (1:49). The bands corresponding to R_f 0.40 were scraped off the plate and the product was extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated to dryness and the residue was dried over P_2O_5 at room temperature *in vacuo*. A light brown resin-like material was obtained (1.45 g, 45.5%). In a larger batch, the material was also purified on a silica gel column: uv λ 20% EtOH max 268 nm (ε 17,100), λ H_2O (0.1 *N* HCl) 276 nm (ε 16,500), λ H_2O max (0.1 *N* NaOH) 292 nm (ε 22,800); ir max 1750 (urethane, C=O), 1260 (P=O), 1150 and 1070 cm^{-1} (POC). *Anal.* ($C_{30}H_{34}N_5O_9P \cdot 4H_2O$) C, H, N.

***N*-(Purin-6-ylcarbamoyl)-L-threonine Ribonucleoside 5'-Phosphate (2a). Method A.** A stirred mixture of the urethane **4** [prepared from 1.7 g (3 mmol) of **3** and purified by column chromatography] and 714 mg (6 mmol) of L-threonine in 50 ml of anhydrous pyridine was heated in a glass bomb at 120° for 6 hr. After cooling to room temperature, the excess threonine was removed by filtration. The filtrate was evaporated to dryness and the residue was dissolved in 110 ml of 50% EtOH. The ethanolic solution was hydrogenated over PdO (300 mg, Matheson Coleman and Bell) at atmospheric pressure for 12 hr. After removing Pd, the filtrate was evaporated to dryness and the residue was heated in 100 ml of 70% AcOH at 100° for 1 hr. The mixture was evaporated to dryness (at 30°) and the residue was dissolved in H_2O (70 ml). The aqueous solution was applied to an AG1-x8 (formate) column (2.0 × 11 cm) prepacked in H_2O. The column was washed with 700 ml of H_2O and then eluted with 0.1 *N* HCO_2H (500 ml), 1.0 *N* HCO_2H (500 ml), and 2.0 *N* HCO_2H (1,000 ml). The 2.0 *N* HCO_2H eluate (1000 ml) was evaporated to dryness at 30° and the residue was lyophilized: yield 1.0 g (68%); ir max 1680 (ureido, C=O), 1260 (P=O), and 1110–1080 cm^{-1} (POC); nmr δ 1.20 (d, 3, *J* = 6 Hz, CH_3), 4.82–3.90 (m, 7, 3'-H, =CH, 2'-H, 4'-H, 5'-H), 6.16 (d, 1, *J* = 5.5 Hz, 1'-H), 8.72 (s, 1, 2-H), 8.78 (s, 1, 8-H), 9.72 (s, 1, NH), and 9.87 ppm (s, 1, NH). The product was converted to the monoBa salt (mp 215–220° dec) by adjusting the aqueous solution to pH 4.0 (Ba salt with $-P(O)(OH)_2$) and to the diBa salt (mp 270–280° dec) followed by adjusting to pH 7.5 (Ba salt with both $-P(O)(OH)_2$ and COOH) with saturated $Ba(OH)_2$, by precipitation with acetone. *Anal.* ($C_{15}H_{19}N_6O_{11}P \cdot Ba \cdot 3H_2O$) C, H, N, P. *Anal.* ($C_{15}H_{19}N_6O_{11}P \cdot Ba_{1.5} \cdot 5H_2O$, pH 7.5 compound) C, H, N, P. This compound was also prepared by method B (yield 33.6%) and method C (yield 8.2%).

***N*-(Purin-6-ylcarbamoyl)glycine Ribonucleoside 5'-Phosphate (2b). Method B.** To a cooled mixture of 0.2 ml (*ca.* 1.5 mmol) of $POCl_3$ and 5 ml of $(EtO)_3PO$ was added 368 mg (1 mmol) of PCGR (1b) at 0°. The mixture was stirred at 0° for 18 hr and diluted with ice water (20 ml). The aqueous solution was adjusted to pH 2 with 6 *N* NaOH and then adsorbed on a charcoal–Celite** (8 g each) column. The column was washed with H_2O (500 ml) and then eluted with 200 ml of EtOH–concentrated NH_4OH–H_2O (10:1:9). A paper chromatography by elution and uv estimation showed that the ammonia eluate contained the product (A_{269}, 11,248, yield 48.3%) and a trace of the diphosphate (A_{269}, 742). The eluate was evaporated to dryness and the residue was purified with an AG1-x8 (formate) column as described above in method A. The lyophilized product weighed 212 mg (47.5%): mp 125–130° dec; ir max 1710 (ureido, C=O), 1210 (P=O), and 1050 cm^{-1} (POC); nmr δ 4.82–3.88 (m, 7, 3'-H, 2'-H, 4'-H, 5'-H, CH_2), 6.17 (d, 1, *J* = 5.5 Hz, 1'-H), 8.77 (s, 1, 2-H), 8.85 (s, 1, 8-H), and 9.50 (br, s, NH). *Anal.* ($C_{13}H_{17}N_6O_{10}P \cdot 2.5H_2O$) C, H, N, P. This compound was also prepared by method A (44.7%) and method C (17.6%).

***N*-(Purin-6-ylcarbamoyl)isopropylamine Ribonucleoside 5' Phosphate (2c). Method C.** To a cooled mixture of 415 mg (1.18 mmol) of 1c[9] and 30 ml of *m*-cresol was added 1 ml (7.2 mmol) of pyrophosphoryl chloride[16] at 0–5°. After stirring at 0–5° for 4 hr, the mixture was diluted with ice water (100 ml) and extracted with ether (2 × 20 ml). The aqueous portion was adjusted to pH 2 with 6 *N* NaOH. The solution was adsorbed on a charcoal–Celite** (8 g each) column and the column was washed with H_2O (500 ml). The charcoal-bound materials were eluted with 300 ml of EtOH–concentrated NH_4OH–H_2O (10:1:9). A paper chromatography by elution and uv estimation showed that the ammonia eluate contained the product (A_{269}, 17,000, yield 68.9%). The eluate was evaporated to dryness and the residue was purified with an AG1-x8 (formate) column as described in method A. Evaporation of 1 *N* HCO_2H frac-

**Charcoal–Celite column was prepared by first mixing 8 g of each with 8 ml of water and then packing into a precision bore glass column (5 cm diameter) with a plunger.

tions followed by lyophilization gave 330 mg (64.7%) of the product: mp 135–140° dec; ir max 1710 (ureido, C=O), 1220 (P=O), and 1060 cm^{-1} (POC); nmr δ 1.18 (d, 6, J = 6 Hz, CH_3), 4.78–3.78 (m, 6, 3′-H, 2′-H, 4′-H, 5′-H, =CH–), 6.10 (d, 1, J = 5.5 Hz, 1′-H), 8.67 (s, 1, 2-H), 8.73 (s, 1, 8-H), 9.18 (s, NH), and 9.33 ppm (s, NH). For analysis, the product was converted to the Ba salt by adjusting the aqueous solution to pH 7.5 with saturated $Ba(OH)_2$ and by precipitating with acetone, mp 245–250° dec. *Anal.* ($C_{14}H_{19}N_6O_8PBa \cdot 3H_2O$) C, H, N, P. This compound was also prepared by method A (22.3%) and method B (47.5%).

N-(**Purin-6-ylcarbamoyl**)**allylamine Ribonucleoside 5′-Phosphate** (**2d**). This compound was prepared by method B in 47.8% yield: mp 133–134° dec; ir max 1710 (ureido, C=O), 1220 (P=O), and 1040 cm^{-1} (POC); nmr δ 3.93–4.20 (m, 5, CH_2, 4′-H, 5′-H), 4.70 (m, 1, 3′-H), 5.13–5.47 (m, 3, NCH_2, –CH=), 6.15 (d, 1, J = 6 Hz, 1′-H), 8.77 (s, 1, 2-H), and 8.87 ppm (s, 1, 8-H). *Anal.* ($C_{14}H_{19}N_6O_8P \cdot 1.5H_2O$) C, H, N, P.

N-(**Purin-6-ylcarbamoyl**)**isoamylamine Ribonucleoside 5′-Phosphate** (**2e**). This compound was prepared by method B in 47.5% yield: ir max 1710 (ureido, C=O), 1230 (P=O), and 1030 cm^{-1} (POC); nmr δ 0.92 (d, 6, J = 6 Hz, CH_3), 1.45 (m, 3, CH_2, –CH=), 3.29 (h, 2, J = 6 Hz, J = 3 Hz, NCH_2), 4.23–3.92 (m, 4, 5′-H, 4′-H, 2′-H), 4.65 (q, 1, J = 5.5 Hz, J = 3 Hz, 3′-H), 6.16 (d, 1, J = 5.5 Hz, 1′-H), 8.8 (s, 1, 2-H), and 8.92 ppm (s, 1, 8-H). The analytical sample was purified from a cellulose column (neutral, Whatman) using *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1), mp 151–155° dec. *Anal.* ($C_{16}H_{23}N_5O_8P \cdot 2NH_4$) C, H, P; N: calcd, 22.66; found, 20.45.

Enzymatic Hydrolysis. (a) 5′-Nucleotidase. The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris–HCl, pH 9.0 (20 μl), and 5′-nucleotidase (25 μl, 10 mg/ml, Sigma Chemical Co.) at 37° for 3 hr.

(b) Intestinal **Alkaline Phosphatase.** The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris–HCl, pH 8.0 (20 μl), and intestinal alkaline phosphatase (25 μl, 10 mg/ml, Worthington Biochemical Corp.) at 25° overnight.

(c) **Acid Phosphatase.** The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 0.15 *M* NaOAc buffer, pH 5.5 (20 μl), and wheat germ acid phosphatase (25 μl, 10 mg/ml, Worthington Biochemical Corp.) at 25° overnight.

(d) Venom **Phosphodiesterase.** The substrate (3.0 μmol) in H_2O (20 μl) was incubated with 1 *M* Tris–HCl and 0.01 *M* $MgSO_4$, pH 8.8 (20 μl), and venom phosphodiesterase (25 μl, 10 mg/ml, Ross Allen's Reptile Institute) at 37° for 3 hr.

In all cases, the incubation mixtures, analyzed by paper electrophresis and paper chromatography, revealed that the nucleotides were converted into the nucleosides. There was no unchanged nucleotide.

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References

(1) G. B. Chheda, R. H. Hall, D. I. Magrath, J. Mozejko, M. P. Schweizer, L. Stasiuk, and P. R. Taylor, *Biochemistry*, 8, 3278 (1969).
(2) M. P. Schweizer, G. B. Chheda, R. H. Hall, and L. Baczynskyj, *ibid.*, 8, 3283 (1969).
(3) G. B. Chheda, *Life Sci.*, 8, 979 (1969).
(4) S. Takemura, M. Murakami, and M. Miyazaki, *J. Biochem. (Tokyo)*, 65, 553 (1969).
(5) H. Ishikura, Y. Yamada, K. Murao, M. Saneyoshi, and S. Nishimura, *Biochem. Biophys. Res. Commun.*, 37, 990 (1969).
(6) F. Harada and S. Nishimura, *Biochemistry*, 11, 301 (1972).
(7) M. P. Schweizer, K. McGrath, and L. Baczynskyj, *Biochem. Biophys. Res. Commun.*, 40, 1046 (1970).
(8) G. B. Chheda and C. I. Hong, *J. Med. Chem.*, 14, 748 (1971).
(9) C. I. Hong, G. B. Chheda, S. P. Dutta, E. O. Curtis, and G. L. Tritsch, *ibid.*, 16, 139 (1973).
(10) W. H. Dyson, C. M. Chen, S. M. Alam, R. H. Hall, C. I. Hong, and G. B. Chheda, *Science*, 170, 328 (1970).
(11) W. H. Dyson, R. H. Hall, C. I. Hong, S. P. Dutta, and G. B. Chheda, *Can. J. Biochem.*, 50, 237 (1972).
(12) J. Baddiley and A. R. Todd, *J. Chem. Soc. (London)*, 648 (1947).
(13) M. Yoshikawa, T. Kato, and T. Tanenishi, *Tetrahedron Lett.*, 50, 5065 (1967).
(14) K. Imai, S. Fujii, K. Takanohashi, Y. Furukawa, T. Masuda, and M. Honjo, *J. Org. Chem.*, 34, 1547 (1969).
(15) G. M. Tener, *J. Amer. Chem. Soc.*, 83, 159 (1961).
(16) P. C. Crofts, I. M. Downie, and R. B. Heslop, *J. Chem. Soc. (London)*, 3673 (1960).

Guanylhydrazones with Potential Antileukemic Activity. 1. Aza Analogs of 4,4′-Diacetyldiphenylurea Bis(guanylhydrazone)

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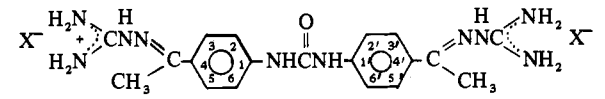
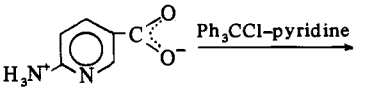
The finding of significant antitumor effect for methylglyoxal bis(guanylhydrazone) (Me-G)[1,2] and the subsequent use of the compound in the clinic were followed by considerable research efforts in which a number of closely analogous compounds were synthesized, none of which proved to be as active as the parent compound. More recently, it has been found that 4,4′-diacetyldiphenylurea bis(guanylhydrazone) (DDUG, **1**) has a marked inhibitory activity against a wide spectrum of tumors and is the most potent bis(guanylhydrazone) against L1210 tumors.[3,4] This inhibitor was first synthesized by Marxer, who has also prepared several closely related compounds.[4] In contrast to Me-G congeners, these analogs also exhibit marked antitumor effects. The requirement for less stringent structural specificity in this series[5,6] may be related to passive diffusion as the mechanism of cellular transport in the case of DDUG and its derivatives.[7]

We have undertaken the synthesis of two aza analogs of DDUG, **6b** and **7b**, which differ from **1** by replacement of carbon atoms in the phenyl ring (both 2 and 2′ in **6b**, but



1



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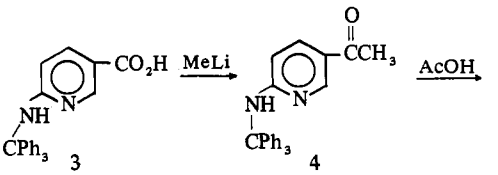
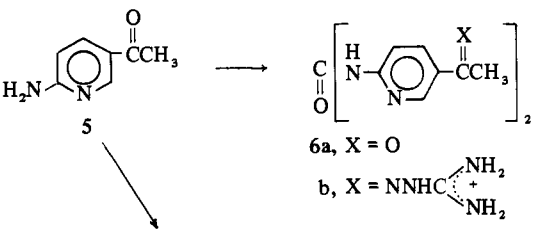
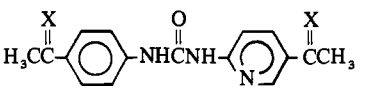


3 4

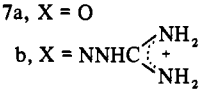


5 6a, X = O

b, X = NNHC(NH2)(+)NH2



7a, X = O



b, X = NNHC(NH2)(+)NH2