# Synthesis and Biological Activity of Analogs of Naturally Occurring 6-Ureidopurines and Their Nucleosides[1,†]

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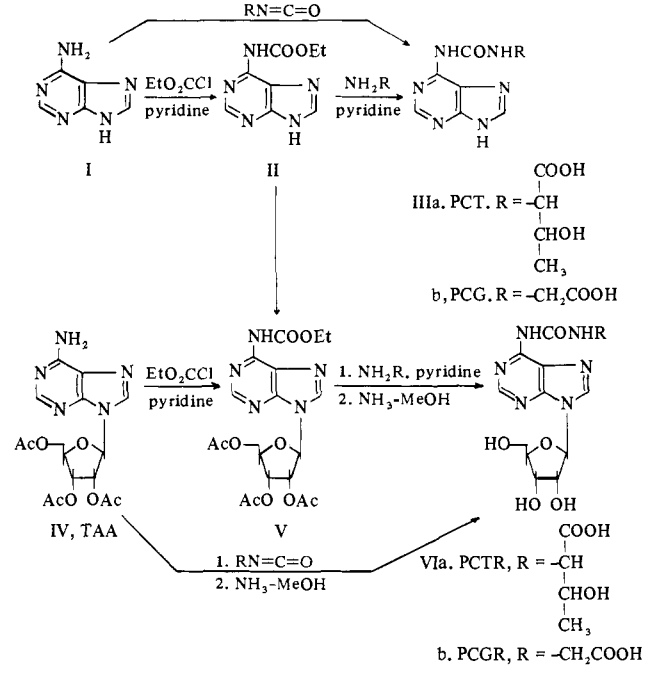
Syntheses and biological activities of 86 analogs of the naturally occurring 6-ureidopurines, *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, IIIa), *N*-(purin-6-ylcarbamoyl)glycine (PCG, IIIb), and their ribonucleoside PCTR and PCGR, are described. These compounds were prepared by displacement of the ethoxy group of ethyl purine-6-carbamate (II) and its tri-*O*-acetyl ribonucleoside (V), by many of the natural amino acids, and by a variety of aliphatic and aromatic amines. Some of the analogs were prepared by reaction of isocyanates with adenine and tri-*O*-acetyladenosine. The mass spectral fragmentation pattern, the ultraviolet spectral characteristics, and the stability properties of these compounds are described. Cytokinin activity in soybean tissue and growth inhibitory activity in cultured normal and leukemic human cells are also discussed. Comparison of the cytokinin activity with the antineoplastic activity of these compounds revealed no direct relationship between the two types of activities. Incubation of the naturally occurring ureidopurines, their ribonucleosides, and some of their analogs with urease, protease, peptidase, acylase, and similar other enzymes did not lead to a cleavage of the ureido bond. The latter data suggest that the 6-ureido side chain of these analogs should be metabolically stable *in vivo*.

Natural occurrence, isolation,[2-4] and synthesis[5] of the ureidopurine nucleosides, *N*-(purin-6-ylcarbamoyl)-L-threonine ribonucleoside (PCTR, VIa) and *N*-(purin-6-ylcarbamoyl)glycine ribonucleoside[6] (PCGR, VIb), have recently been reported. PCTR, along with other modified nucleosides, has been isolated from human and rat urine.[4,7] The finding of several other amino acids in nucleoside hydrolysates suggests that analogs of PCTR, differing only in amino acid side chain, may occur in nucleic acids.[8]

The anticodon adjacent modified nucleosides like $N^6$-($\Delta^2$-isopentenyl)adenosine (IPA),[9] PCTR,[10] compound Y ribonucleoside,[11,12] besides participating in the acceptor and transfer function of tRNA at a macromolecular level, appear to have interesting biological activities as free nucleosides. IPA has shown growth inhibitory and stimulatory,[13] antineoplastic,[14] and cytokinin[15,16] activities. At the enzyme level, it is a competitive inhibitor of tRNA methylase,[17] adenosine kinase,[18] and adenosine aminohydrolyase.[19] Some of the analogs of PCTR have shown excellent cytokinin activity.[20-22] Compound Y also seems to have cytokinin activity.[23] Because of these biological activities of modified nucleosides, and because of a possibility of the natural occurrence of compounds similar to PCTR, a program was undertaken to synthesize analogs of PCT (IIIa) and PCTR derived from other essential and nonessential amino acids. The analogs were also prepared from aryl- and alkylamines of biological importance. This paper describes the synthesis, chemical properties, and biological activities of the ureidopurines and their nucleosides. Attempts are also made to investigate if there is a relationship between the cytokinin activity and antineoplastic activity of these compounds.

**Chemistry.** The key intermediates, ethyl purine-6-carbamate (II) and ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (V), were prepared by the reaction of $EtO_2CCl$ with adenine and tri-*O*-acetyladenosine in pyridine, respectively[5] (Scheme I). Displacement of the EtO group of urethane base II with 2 molar equiv of amino acids and amines in pyridine at 120° for 6 hr gave the desired ureidopurine, generally, in good yields. Analogous displacement of the ethyl purine-6-carbamate ribonucleoside (V) yielded the tri-*O*-acetyl derivative of 6-ureidopurine nucleosides, which upon treatment with 4 *N* $NH_3$-MeOH at room temperature for 8 hr gave the desired nucleosides in good yields.

Scheme I




Side chains of the analogs of 6-ureidopurine and their ribonucleosides were derived from the following essential amino acids: L-threonine (IIIa, VIa, Scheme I), L-valine (**3**, **52**), L-phenylalanine (**1**, **50**), L-leucine (**7**, **59**), L-isoleucine (**2**, **51**), L-methionine (**4**, **54**), and L-tryptophan (**58**) (Tables I and II). Other naturally occurring nonessential amino acids utilized were: glycine (IIIb, VIb, Scheme I), L-serine (**6**, **57**), L-alanine (**8**, **60**), L-tyrosine (**5**, **55**), L-aspartic acid (**56**), D-threonine (**53**), and β-aminoisobutyric acid (**28**, **79**) (Tables I and II). The following amino acid amides were also incorporated: DL-threonine amide (**11**, **63**), L-threonine amide (**62**), glycine amide (**67**), L-phenylalanine amide (**61**), L-isoleucine amide (**65**), L-valine amide (**64**), and L-leucine amide (**66**). 6-Ureidopurines containing amino acid esters side chains were prepared from DL-threonine methyl ester

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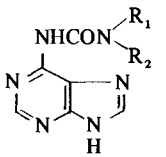
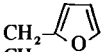
Table I. 6-Ureidopurines

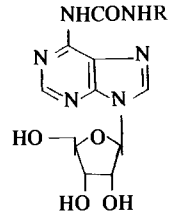


| Compd no. | $R_1$ | $R_2$ | Method | Yield, % | Mp,[a] °C | Formula[b] | Uv max, nm ($\epsilon \times 10^{-3}$) pH 1–2 | pH 5–7[c] | pH 11.5–12.5 | Biological activity: Cytokinin, $M$[d] | Mammalian cells[e] Nc37 | 6410 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $CH(COOH)CH_2C_6H_5$ | H | A | 58.0 | 197–199 ef | $C_{15}H_{14}N_6O_3$ | 276.5 (19.5) | 270 (18.9) | 278 (17.1) | NA | | |
| 2 | $CH(COOH)CH(CH_3)CH_2CH_3$ | H | A | 59.2 | 195–197 ef | $C_{12}H_{16}N_6O_3$ | 277 (19.9) | 269.5 (18.8) | 278 (16.5) | NA | | |
| 3 | $CH(COOH)CH(CH_3)_2$ | H | A | 50.4 | 200–202 ef | $C_{11}H_{14}N_6O_3$ | 277.5 (20.0) | 270 (18.9) | 278 (17.5) | NA | | |
| 4 | $CH(COOH)CH_2CH_2SCH_3$ | H | A | 69.4 | 209–210 ef | $C_{11}H_{14}N_6O_3S$ | 277.5 (20.2) | 270 (18.8) | 278 (17.2) | NA | NA | NA |
| 5 | $CH(COOH)CH_2$-$C_6H_4$-OH | H | A | 27.0 | 188–190 ef | $C_{15}H_{14}N_6O_4 \cdot 0.5H_2O$ | 277.5 (20.2) | 270 (18.9) | 278 (17.5) | NA | | |
| 6 | $CH(COOH)CH_2OH$ | H | A | 7.0 | 195–196 ef | $C_9H_{10}N_6O_4$ | 277.5 (18.3) | 269.5 (17.0) | 278 (16.9) | NA | | |
| 7 | $CH(COOH)CH_2CH(CH_3)_2$ | H | A | 57.4 | 200–202 ef | $C_{12}H_{16}N_6O_3$ | 277 (20.6) | 270 (19.3) | 278 (18.0) | NA | | |
| 8 | $CH(COOH)CH_3$ | H | A | 47.6 | 215–216 ef | $C_9H_{10}N_6O_3 \cdot 0.5H_2O$ | 277 (21.4) | 269 (20.0) | 277 (18.5) | NA | | |
| 9 | $CH(COOCH_3)CH(OH)$-$CH_3$ (DL)[f] | H | | 30.0 | 183–185 ef | $C_{11}H_{14}N_6O_4$ | 277 (19.7) | 268 (18.3) | 277 (16.1) | NA | NA | NA |
| 10 | $CH_2COOC_2H_5$ | H | A | 76.0 | 235–237 s, 250–270 dec | $C_{10}H_{12}N_6O_3$ | 276 (21.2) | 268.5 (19.9) | 277.5 (18.0) | NA | ± | NA |
| 11 | $CH(CONH_2)CH(OH)CH_3$[g] | H | A | 4.0 | 183–184 ef | $C_{10}H_{13}N_7O_3 \cdot 0.5H_2O$ | 277 (18.8) | 269 (17.8) | 278 (15.6) | NA | | |
| 12 | $CH_2CH_2COOH$ | H | A | 83.6 | 242–243 ef | $C_9H_{10}N_6O_3$ | 278 (19.0) | 270 (17.2) | 278 (16.0) | NA | | |
| 13 | $CH(COOCH_2C_6H_5)CH$-$(OCH_2C_6H_5)CH_3$ | H | B | 32.6 | 140–142 | $C_{24}H_{24}N_6O_4$ | 277 (20.0) | 269 (18.4) | 280 (15.5) | NA | + | + |
| 14 | $CH(COOCH_3)CH_2C_6H_5$ | H | B | 20.0 | 208–210 | $C_{17}H_{18}N_6O_3$[h] | 277 (19.9) | 267 (18.4) | 278 (15.2) | NA | | |
| 15 | $CH(COOCH_2C_6H_5)CH(CH_3)_2$ | H | B | 32.6 | 212–214 | $C_{18}H_{20}N_6O_3$ | 275 (18.5) | 267 (18.4) | 276 (15.1) | NA | NA | NA |
| 16 | $CH_3$ | H | A | 40.0 | >300 dec | $C_7H_8N_6O$ | 277 (21.2) | 269 (19.1) | 277 (17.2) | | | |
| 17 | $CH_2CH_2CH_3$[i] | H | A | 79.0 | 348–350 dec | $C_9H_{12}N_6O$ | 278 (20.9) | 269 (18.7) | 278 (16.9) | $5 \times 10^{-5}$ | NA | NA |
| 18 | $CH(CH_3)_2$[i] | H | A | 69.2 | 231–232 s, >300 dec | $C_9H_{12}N_6O$ | 278 (19.6) | 269 (17.0) | 277 (15.5) | $1 \times 10^{-5}$ | NA | NA |
| 19 | $CH_2CH(CH_3)_2$ | H | A | 73.0 | 250–255 s, >300 dec | $C_{10}H_{14}N_6O$[j] | 277.5 (20.8) | 268.5 (18.4) | 278 (16.6) | | + | NA |
| 20 | $CH_2CH_2CH_2CH_2CH_3$ | H | A | 67.0 | 227–228 s, >300 dec | $C_{11}H_{16}N_6O$ | 278.5 (19.7) | 269 (17.6) | 278 (15.9) | $5 \times 10^{-5}$ | + | + |
| 21 | $CH_2CH_2CH(CH_3)_2$ | H | A | 88.0 | >300 dec | $C_{11}H_{16}N_6O$ | 277 (21.3) | 269.5 (19.0) | 277.5 (17.3) | $1 \times 10^{-5}$ | ++ | ++ |
| 22 | $CH_2C(CH_3)_3$ | H | A | 75.8 | 350–352 dec | $C_{11}H_{16}N_6O$ | 278 (24.0) | 269 (21.0) | 277 (18.5) | | ± | ± |
| 23 | $CH_2CH_2CH_2OH$ | H | A | 54.0 | 207–208 | $C_9H_{12}N_6O_2$ | 277.5 (20.8) | 269.5 (17.8) | 277.5 (16.9) | NA | | |
| 24 | $CH_2CH(OH)CH_3$ | H | A | 77.0 | 230–232 | $C_9H_{12}N_6O_2$ | 277 (21.3) | 269 (18.8) | 277.5 (16.9) | NA | | |
| 25 | $CH_2CH_2CH_2CH_2OH$ | H | A | 53.5 | 206–208 | $C_{10}H_{14}N_6O_2$ | 278 (19.8) | 269.5 (17.1) | 278 (15.9) | NA | | |
| 26 | $CH_2CH_2NH_2$ | H | A | 61.0 | 245–250 s, 270–290 dec | $C_8H_{11}N_7O$ | 276.5 (20.1) | 269 (18.5) | 277.5 (17.3) | | NA | NA |
| 27 | $CH_2CH_2OCH_2CH_3$ | H | A | 77.2 | >300 dec | $C_{10}H_{14}N_6O_2$ | 277 (18.9) | 269 (17.2) | 277.5 (15.4) | | + | + |
| 28 | $CH_2CH(CH_3)COOH$ | H | A | 91.0 | 225–227 cf, 290–300 dec | $C_{10}H_{12}N_6O_3$ | 277 (19.0) | 269 (17.3) | 278 (15.9) | NA | | |
| 29 | $CH_2CH_2N(CH_3)_2$ | H | A | 43.3 | 200–202 s, 240–250 dec | $C_{10}H_{15}N_7O$ | 276 (17.3) | 268.5 (16.5) | 277 (15.1) | | NA | NA |
| 30 | $CH_2CH_2CH_2N(CH_3)_2$ | H | A | 50.3 | 209–210 s, 275–280 dec | $C_{11}H_{17}N_7O$ | 278 (17.6) | 269 (16.0) | 277 (14.6) | | ± | ± |

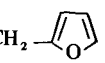
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | $CH_2CH_2CH_2N(C_2H_5)_2$ | H | A | 35.4 | 206–207 | $C_{13}H_{21}N_7O$ | 277 (19.8) | 269 (17.7) | 277.5 (16.3) | | NA | NA |
| 32 | $C_6H_4F(2)$ | H | B | 68.0 | >300 dec | $C_{12}H_9FN_6O$ | 283 (25) | 278 (25.8) | 284 (22.8) | $1 \times 10^{-7}$ | NA | ± |
| 33 | $C_6H_3F_2(2,4)$ | H | A | 47.5 | >300 dec | $C_{12}H_8F_2N_6O$ | 279 (28.6) | 277.5 (29.6) | 285 (29.2) | | ± | NA |
| 34 | $C_6H_3F(2)CH_3(4)$ | H | A | 19.3 | >300 dec | $C_{13}H_{11}FN_6O \cdot 0.5H_2O$ | 285 (26.5) | 283 (33.1) | 287.5 (36.0) | | ± | ± |
| 35 | $C_6H_4Cl(2)$ | H | A | 38.0 | >300 dec | $C_{12}H_9ClN_6O \cdot 0.5H_2O$ | 282.5 (22.2) | 278 (21.9) | 284 (23.0) | $1 \times 10^{-7}$ | + | + |
| | | | B | 92.0 | >300 dec | $C_{12}H_9ClN_6O$[k] | | | | | | |
| 36 | $C_6H_4Cl(3)$[i] | H | A | 42.3 | >300 dec | $C_{12}H_9ClN_6O$ | 286 (25.7) | 279 (27.3) | 284 (25.1) | $1 \times 10^{-5}$ | ++ | ++ |
| | | | B | 60.0 | >300 dec | $C_{12}H_9ClN_6O$ | | | | | | |
| 37 | $C_6H_4Cl(4)$ | H | A | 47.5 | >300 dec | $C_{12}H_9ClN_6O$ | 288 (16.6) | 281 (23.5) | 286 (25.0) | | | |
| | | | B | 87.3 | >300 dec | $C_{12}H_9ClN_6O$ | | | | NA | NA | NA |
| 38 | $C_6H_3Cl_2(2,5)$ | H | A | 20.0 | >300 dec | $C_{12}H_8Cl_2N_6O$ | 280 (14.8) | 287 (23.3) | 295 (20.4) | $1 \times 10^{-4}$ | | |
| 39 | $C_6H_3Cl(2)CH_3(4)$ | H | A | 46.5 | >300 dec | $C_{13}H_{11}ClN_6O \cdot 0.5H_2O$ | 281 (29.5) | 280.5 (31.6) | 286 (32.8) | NA | + | ++ |
| 40 | $C_6H_4CH_3(2)$ | H | A | 58.0 | >300 dec | $C_{13}H_{12}N_6O \cdot H_2O$ | 280 (23.9) | 279 (26.5) | 286 (29.5) | $2 \times 10^{-5}$ | | |
| 41 | $CH_2$–$C_6H_5$ | H | A | 82.5 | >300 dec | $C_{13}H_{12}N_6O \cdot 0.5H_2O$ | 277 (21.6) | 269 (19.5) | 278 (17.3) | NA | + | ± |
| 42 | $CH_2CH_2$–$C_6H_5$ | H | A | 77.4 | 256–258 s, 290–300 dec | $C_{14}H_{14}N_6O$ | 277 (19.9) | 268 (17.7) | 277 (18.0) | NA | + | + |
| 43 | $CH_2CH_2CH_2$–$C_6H_5$ | H | A | 69.0 | 220–222 s, 290–300 dec | $C_{15}H_{16}N_6O$ | 278.5 (19.9) | 269 (17.6) | 278 (15.9) | NA | ++ | ++ |
| 44 | $CH_2CH_2CH_2CH_2$–$C_6H_5$ | H | A | 66.5 | 220–222 s, 280–285 dec | $C_{16}H_{18}N_6O$ | 278 (19.0) | 268.5 (16.8) | 287.5 (14.9) | NA | ++ | ++ |
| 45 | $CH_2C_6H_3Cl(2)$ | H | A | 64.6 | 260 s, 305–310 dec | $C_{13}H_{11}ClN_6O$[l] | 278 (19.7) | 269 (17.7) | 278 (16.0) | NA | | |
| 46 |  | H | A | 81.5 | 260–261 dec | $C_{11}H_{10}N_6O_2$ | 277.5 (21.7) | 269.5 (19.9) | 277.5 (17.6) | NA | | |
| 47 | $CH_3$ | $CH_3$ | A | 51.0 | 209–210 s, 250–260 dec | $C_8H_{10}N_6O$ | 280 (21.5) | 277.5 (18.6) | 278 (14.9) | | NA | NA |
| 48 | $CH_2CH{=}CH_2$ | $CH_3$ | A | 47.4 | 153–155 s, 250–260 dec | $C_{10}H_{12}N_6O$ | 276.5 (18.1) | 276 (15.7) | 281 (13.8) | | + | + |
| 49 | $CH_2CH_2{=}CH_2$ | $C_2H_5$ | A | 28.5 | 142–143 | $C_{11}H_{14}N_6O$ | 280 (20.3) | 277.5 (17.5) | 279 (14.3) | | NA | ± |

[a] ef = melts with effervescence; s = soften; dec = melts with decomposition. [b] All compounds were analyzed for C, H, and N. The analytical results were within ±0.4% of the theoretical values. [c] At neutral pH pronounced shoulder at 276–277 nm except 32–40 and 47–49. [d] Activity is expressed as the lowest molar concentration giving growth in the soybean assay described in the text. NA, not active at highest concentration tested ($10^{-4}$). [e] The notation represents the viable cell number relative to the controls after 72 hr of incubation: ++, 30–60%; +. 60–80%; ±, 80–90%; NA, 90–110%. [f] Method of preparation is described in the Experimental Section. [g] Purified by a silica gel column with solvent A. [h] C: calcd, 56.47; found, 55.65. [i] This compound was reported by McDonald, *et al.*[22] [j] N: calcd, 35.87; found, 37.89. [k] C: calcd, 49.92; found, 49.19. [l] C: calcd, 27.76; found, 28.48.

Table II. 6-Ureidopurine Ribonucleosides




| Compd no. | R | Method | Yield, % | Mp,[a] °C | Formula[b] | Uv max, nm (ε × $10^{-3}$) pH 1-2[c] | pH 5-7[d] | pH 11.5-12.5[e] | Biological activity: Cytokinin, *Mf* | Mammalian cells[g] Nc37 | 6410 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **50** | $CH(COOH)CH_2C_6H_5$ | C | 54.6 | 161–163 dec | $C_{20}H_{22}N_6O_7$ | 277 (18.7) | 269.5 (22.0) | 271 (17.5), 300 (8.0) | NA | NA | NA |
| 51 | $CH(COOH)CH(CH_3)CH_2CH_3$ | C | 72.5 | 135 s, 150–153 ef | $C_{17}H_{24}N_6O_7$ | 277 (21.8) | 270 (23.8) | 270.5 (20.0), 298 (6.5) | NA | NA | NA |
| 52 | $CH(COOH)CH(CH_3)_2$ | C | 35.4 | 148–150 ef | $C_{16}H_{22}N_6O_7$ | 277 (21.1) | 270 (22.1) | 270 (19.2), 299 (5.0) | NA | NA | NA |
| 53 | $CH(COOH)CH(OH)CH_3$(D)[h] | | 20.0 | 205–207 dec | $C_{15}H_{20}N_6O_8 \cdot 2H_2O$ | 277 (20.2) | 270 (23.3) | 271 (16.8), 299 (11.4) | NA | NA | NA |
| 54 | $CH(COOH)CH_2CH_2SCH_3$ | C | 21.0 | 127–129 s, 138–141 | $C_{16}H_{22}N_6O_7S$ | 276.5 (23.0) | 270.5 (23.4) | 271.5 (18.3), 297 (9.2) | NA | NA | NA |
| 55 | $CH(COOH)CH_2$-$C_6H_4$-OH | C | 47.0 | 206–208 dec | $C_{20}H_{22}N_6O_8$ | 277 (22.6) | 270 (22.7) | 271.5 (19.8), 297 (7.0) | NA | NA | NA |
| 56 | $CH(COOH)CH_2COOH$ | C | 44.0 | 160–170 s, ef, 206–208 dec | $C_{15}H_{18}N_6O_9 \cdot 1.5H_2O$ | 276 (24.4) | 269 (24.7) | 270 (20.3), 298 (8.8) | NA | | |
| 57 | $CH(COOH)CH_2OH$ | C | 26.4 | 140 s, 158–160 ef | $C_{14}H_{18}N_6O_8 \cdot 0.5H_2O$ | 276 (20.1) | 269 (20.5) | 271 (14.5), 295 (10.8) | NA | NA | NA |
| 58 | $CH(COOH)CH_2$-(3-indolyl) | C | 52.0 | 203–204 dec | $C_{22}H_{23}N_7O_7 \cdot 0.5H_2O$[i] | 276.5 (23.8) | 269.5 (23.3) | 270 (21.9), 300 (3.4) | NA | | |
| 59 | $CH(COOH)CH_2CH(CH_3)_2$ | C | 64.5 | 130–132 s, 145–147 ef | $C_{16}H_{22}N_6O_7 \cdot H_2O$ | 277 (20.0) | 269.5 (21.0) | 270 (18.0), 299 (5.2) | NA | NA | NA |
| 60 | $CH(COOH)CH_3$ | C | 50.0 | 203–204 ef | $C_{14}H_{18}N_6O_7$ | 276.5 (22.7) | 269.5 (23.2) | 270 (17.9), 300 (9.1) | NA | NA | NA |
| 61 | $CH(CONH_2)CH_2C_6H_5$ | C | 70.0 | 205–206 | $C_{20}H_{23}N_7O_6 \cdot 0.25H_2O$ | 277 (20.8) | 269 (21.5) | 270.5 (12.1), 301 (17.2) | NA | | |
| 62 | $CH(CONH_2)CH(OH)CH_3$(L) | C | 30.0 | 205–205.5 | $C_{15}H_{21}N_7O_7 \cdot H_2O$ | 276.5 (23.5) | 269 (25.4) | 271 (12.7), 300 (23.7) | NA | NA | NA |
| 63 | $CH(CONH_2)CH(OH)CH_3$(DL)[g] | | 38.2 | 163–165 | $C_{15}H_{21}N_7O_7 \cdot H_2O$ | 277 (22.7) | 269.5 (24.1) | 270 (11.5), 300 (22.8) | NA | | |
| 64 | $CH(CONH_2)CH(CH_3)_2$ | C | 40.5 | 220–221 | $C_{16}H_{23}N_7O_6 \cdot H_2O$ | 277 (23.2) | 268.5 (23.0) | 270 (13.3), 248 (17.9) | NA | NA | NA |
| 65 | $CH(CONH_2)CH(CH_3)CH_2CH_3$ | C | 81.6 | 221–224 dec | $C_{17}H_{25}N_7O_6$ | 276.5 (23.0) | 269 (23.5) | 270 (13.3), 298 (18.6) | NA | NA | NA |
| 66 | $CH(CONH_2)CH_2CH(CH_3)_2$ | C | 60.0 | 203–204 | $C_{17}H_{25}N_7O_6 \cdot H_2O$[j] | 277 (22.6) | 269 (22.9) | 270 (11.3), 299 (20.7) | NA | NA | NA |
| 67 | $CH_2CONH_2$ | C<br>D | 65.5<br>62[k] | 201–202, 225–227 | $C_{13}H_{17}N_7O_6$<br>$C_{13}H_{17}N_7O_6 \cdot 0.5H_2O$ | 276 (19.9) | 268.5 (21.5) | 270 (10.4), 297 (19.5) | NA | ± | + |
| 68 | $CH_2CH_2COOH$ | C | 57.0 | 143–145 | $C_{14}H_{18}N_6O_7 \cdot H_2O$ | 277 (22.3) | 269 (21.9) | 269 (17.0), 297 (8.4) | NA | NA | NA |
| 69 | $CH_2CH_2$-(4-imidazolyl) | C | 78.5 | 200–202 | $C_{16}H_{20}N_8O_5 \cdot 0.5H_2O$ | 276.5 (22.3) | 269 (22.7) | 277.5 (15.8), 299 (11.1) | | NA | NA |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | $CH_3$ | C | 73.0 | 199–201 | $C_{12}H_{16}N_6O_5$ | 276.5 (25.2) | 269 (24.0) | 277.5 (18.3), 298 (18.5) | | | |
| 71 | $CH(CH_3)_2$ | C | 59.0 | 191–193 | $C_{14}H_{20}N_6O_5$ | 277 (23.6) | 269 (23.4) | 277.5 (15.3), 298 (14.1) | | | |
| 72 | $C(CH_3)_3$ | D | 63.0 | 123–125 | $C_{15}H_{22}N_6O_5 \cdot 2H_2O$ | 277 (24.2) | 270 (24.7) | 278 (18.0), 302 (5.4) | | + | NA |
| 73 | $CH_2CH_2CH(CH_3)_2$ | C | 37.4 | 170–172 | $C_{16}H_{24}N_6O_5$ | 278 (25.2) | 270 (24.6) | 277.5 (17.3), 298 (11.6) | $1 \times 10^{-5}$ | ++ | ++ |
| 74 | $CH_2CH{=}CH_2$ | C | 51.5 | 171–172 | $C_{14}H_{18}N_6O_5 \cdot 0.5H_2O$ | 277 (22.7) | 269 (22.2) | 277.5 (15.0), 298 (13.3) | $7 \times 10^{-5}$ | ± | ± |
| 75 |  | C | 37.0 | 157–159 | $C_{21}H_{28}N_6O_5$ | 278 (19.2) | 270 (16.3) | 278 (11.6), 300 (3.9) | | | |
| 76 | $CH_2CH_2CH_2OH$ | C | 43.5 | 132–133 | $C_{14}H_{20}N_6O_6 \cdot 1.5H_2O$ | 277 (23.8) | 269 (23.3) | 277 (16.7), 297.5 (9.3) | NA | NA | NA |
| 77 | $CH_2CH(OH)CH_3$ | C | 70.7 | 154–156 | $C_{14}H_{20}N_6O_6$ | 277 (23.1) | 269 (24.3) | 278 (15.9), 297 (15.2) | NA | | |
| 78 | $CH_2CH_2CH_2CH_2OH$ | C | 56.2 | 154–155 | $C_{15}H_{22}N_6O_6$ | 277.5 (23.3) | 269 (22.9) | 277.5 (16.0), 298 (10.0) | NA | NA | NA |
| 79 | $CH_2CH(CH)_3COOH$ | C | 55.6 | 135–137 ef | $C_{15}H_{20}N_6O_7$[l] | 277 (21.2) | 269 (20.8) | 277 (16.0), 299 (7.5) | NA | | |
| 80 | $CH_2$–  | C | 36.3 | 164–166 | $C_{16}H_{18}N_6O_6$ | 277 (21.2) | 269.5 (22.0) | 278 (13.7), 297 (16.8) | NA | NA | NA |
| 81 | $C_6H_5$[m] | C | 67.0 | 189–190 | $C_{17}H_{18}N_6O_5$ | 283 (25.9) | 279 (27.0) | 308 (32.3), 313 (44.8)[n] | | NA | ± |
| 82 | $C_6H_4Cl(2)$ | C | 42.0 | 193–194 | $C_{17}H_{17}ClN_6O_5 \cdot 0.5H_2O$ | | | | | | |
| | | D | 37.0 | 186–188 | $C_{17}H_{17}ClN_6O_5 \cdot 0.5H_2O$ | 280 (28.2) | 279.5 (27.4) | 309 (34.3), 315 (45.0)[n] | | ± | NA |
| 83 | $CH_2$-$C_6H_5$ | C | 78.3 | 150 s, 169–170 | $C_{18}H_{20}N_6O_5 \cdot 0.5H_2O$[o] | 277 (25.8) | 269 (25.7) | 299 (14.0) | | NA | NA |
| 84 | $CH_2CH_2$-$C_6H_5$ | C | 83.0 | 112–114 | $C_{19}H_{22}N_6O_5 \cdot 0.5H_2O$ | 278 (23.7) | 269.5 (22.6) | 277 (16.0), 299 (9.8) | | NA | NA |
| 85 | $CH_2CH_2CH_2$-$C_6H_5$ | C | 86.5 | 132–134 | $C_{20}H_{24}N_6O_5$ | 276.5 (22.5) | 270 (20.8) | 276 (15.8), 299 (7.3) | | ± | ± |
| 86 | $CH_2CH_2CH_2CH_2$-$C_6H_5$ | C | 82.3 | 87–89 | $C_{21}H_{26}N_6O_5 \cdot H_2O$ | 277.5 (22.6) | 269 (21.7) | 278 (14.8), 299 (10.8) | | ± | NA |

[a]ef = melts with effervescence ; s = soften; dec = melts with decomposition. [b]All compounds were analyzed for C, H, and N. The analytical results were within ±0.4% of the theoretical values. [c]Shoulder at 270 mm. [d]At neutral pH pronounced shoulder at 276–277 nm except 81 and 82. [e]Shoulder (or peak) at 270 or 278 nm except 81 and 82 and $\epsilon$ at 298–308 nm varies very much according to pH. [f]Activity is expressed as the lowest molar concentration giving growth in the soybean assay described in the text. NA, not active at highest concentration tested ($10^{-4}$). [g]The notation represents the viable cell number relative to the controls after 72 hr of incubation: ++, 30–60%; +, 60–80%; ±, 80–90%; NA, 90–110%. [h]The method of preparation is described in the Experimental Section. [i]C; calcd, 52.17; found, 49.81. [j]H: calcd, 6.61; found, 6.14. [k]Isocyanate of glycine methyl ester was allowed to react with tri-*O*-acetyladenosine. [l]C: calcd, 45.45; found, 43.05. [m]This compound was reported by McDonald, *et al.*[21] [n]In 95% EtOH solution (0.1 *N* NaOH). [o]N: calcd, 20.53; found, 21.48.

(9) and glycine ethyl ester (10). 6-Ureidopurines carrying the side chains of amino alcohols were prepared from 4-aminobutanol (25, 78), 3-aminopropanol (23, 76), and 1-amino-2-propanol (24, 77). The following alkyl-, aryl-, and aralkylamines were also incorporated into the side chains of the 6-ureidopurines: methylamine (16, 70), propylamine (17), isopropylamine (18, 71), isobutylamine (19), *tert*-butylamine (72), amylamine (20), isoamylamine (21, 73), neopentylamine (22), allylamine (74), 1-adamantanamine (75), ethylenediamine (26), 2-ethoxyethylamine (27), 2-dimethylaminoethylamine (29), 3-dimethylaminopropylamine (30), 3-diethylaminopropylamine (31), furfurylamine (46, 80), aniline derivatives (32–40, 81, 82), benzylamine (41, 83), 2-phenylethylamine (42, 84), 3-phenylpropylamine (43, 85), and 4-phenylbutylamine (44, 86). The secondary amines, dimethylamine, methylallylamine, and ethylallylamine, were also incorporated into the 6-ureidopurines (47, 48, and 49, respectively).

Amines, in general, reacted with the urethane II with ease and gave greater yields than the amino acids. The poor yields shown by compounds derived from serine (6), tyrosine (5), and threonine amide (11) appear to be due to the interference of the hydroxy groups in the reaction and also due to the problems in purification. The prolonged reaction time or higher temperature did not help to improve the yield but led to the degradation of the urethane II to adenine. The $NH_2$ groups attached directly to purine, pyrimidine, and pyrazine nuclei did not react well with II under our usual conditions to give bis heterocyclic ureas. Yields, in general, in the case of nucleosides were somewhat lower than in the case of the corresponding compounds with free bases.

Some of the 6-ureidopurines and their nucleosides were prepared by the reactions of various isocyanates with the amino group of adenine and tri-*O*-acetyladenosines[5,22,24,25] (Scheme I). *O*-Benzyl-L-threonine benzyl ester, L-phenylalanine methyl ester, and L-valine benzyl ester were converted to the corresponding isocyanates by reacting with $COCl_2$ in PhMe at 85–90°.[5] Adenine was then allowed to react with an appropriate isocyanate to give 6-ureidopurines along with some 6-ureido-9-carbamoylpurines and 9-carbamoylpurines. The benzyl-protecting groups were smoothly removed by catalytic hydrogenation with PdO to give good yields of the deblocked products. PCTR reported earlier[5] in lower yield by the isocyanate route could now be made in excellent yield using PdO for the removal of benzyl groups. The naturally occurring compounds PCT (IIIa) and PCG (IIIb) were also obtained in fairly good yields.[5] The reaction of isocyanates with TAA (IV) gave the desired 6-ureidopurine ribonucleosides.

In terms of yields and ease of isolation of the products, the urethane method proved better than the isocyanate procedure. Amines and amino acids with additional functional groups like COOH or OH reacted without protection. By this procedure, there appears to be no racemization of amino acids incorporated into the ureidopurines. (Several of the amino acid analogs prepared were checked for the racemization by the method given in ref 26.) The isocyanate route was useful in cases of amines from which the isocyanates could be easily prepared or were readily available.

The uv spectra of 6-ureidopurine and their ribonucleosides are unique‡ and deserve some special comments. The presence of two maxima (at 269 and 276 nm) in neutral media appears to be characteristic of the 6-ureidopurine moiety (purine-6-NH-CO-NHR where R = H or alkyl group) since $N^6$-alkyl[27] and $N^6$-acyladenines[28] show only one peak at 268 and 279–280 nm, respectively. The typical spectra of 6-ureidopurine‡ with a side chain derived from an amino acid or primary alkyl- or aralkylamine do not show bathochromic or hyperchromic shift in alkaline solution. The uv spectra of 6-arylureidopurines (purine-6-NH-CO-NH-*Ph*), however, are quite different due to the conjugation and electron-withdrawing ability of phenyl groups. They do not exhibit the two peaks but show a maximum at 279 nm which upon basification shifts to 284 nm. The substituent on the phenyl ring also causes a small shift in the uv spectra (see Table I). The ureidopurines derived from secondary alkylamines give the distinctive uv spectra, though somewhat similar to $N^6$-acyladenines.[28] These compounds in basic and acidic solution give smooth maxima at 278 and 280 nm, respectively; however, in neutral solution, a maximum at 277 nm with two shoulders at 273 and 286 nm is observed.‡ These data suggest that the hydrogen of the NH group adjacent to the side chain R (purine-6-NH-CO-NH-R, where R = H or alkyl) is essential for the maximum at 269 nm. The spectra of 6-ureidopurine ribonucleosides derived from amino acids and primary alkylamines also show two maxima at 269 and 276 nm which are closely analogous to those of PCTR (VIa). The differences of $\epsilon_{max}$ between 269 and 276 nm for the ribonucleoside is much greater than that for the free base. The ratio of the $\epsilon_{max}$ (269/276) for 6-alkylureidopurines was found to be 1.025, while that for their ribonucleosides was 1.162. This ratio is consistent enough to be used for identification of the 6-alkylureidopurines and their ribonucleosides (purine-6-NH-CO-NH-R, R = H or alkyl); however, it cannot be used in the case of the compounds derived from the arylamines. In basic solution, the ribonucleosides have three maxima at 270, 277, and 298 nm which are highly pH dependent. In aqueous solution the $\epsilon_{max}$ at 298 nm for PCTR in 1 *N* NaOH is 2.3 times larger as compared to the $\epsilon_{max}$ in 0.1 *N* NaOH. In alcoholic NaOH solution, the hyperchromicity is much greater than that in the aqueous NaOH solution. The acidic ureido nucleosides derived from amino acids usually give a weaker peak at 298 nm than the compounds derived from alkyl- and aralkylamines and amides. For example, in a molecule of PCTR amide (62), the $\epsilon_{max}$ in alkaline solution is almost twice as large as that for PCTR. The arylureidopurine ribonucleosides, like their free bases, exhibit only one maximum (at 279 nm) which upon basification shifts to 308 nm with 18% hyperchromicity. The same compound in 95% ethanolic NaOH (0.1 N) exhibited a shift from 279 to 314 nm with 56% hyperchromicity.[22]

The mass spectra of 6-ureidopurines having an amino acid side chain did not exhibit the molecular ion peak. Generally, the $M^+ - 18$ peak due to loss of $H_2O$ was found to be the largest peak (in mass number) in the spectrum. This was in agreement with the mass spectra of the naturally occurring compounds PCT and PCG.[3,5,29] The ester-

‡The actual ultraviolet spectra in acid, neutral, and alkaline media for the following 6-ureidopurines and their ribonucleosides—*N*-(purin-6-ylcarbamoyl)phenylalanine (1), 6-(*O*-chlorophenylureido)purine (35), *N,N*-dimethylureidopurine (47), PCTR (VIa), PCTR amide (62), and 6-(*O*-chlorophenylureido)purine ribonucleoside (82)—will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-139.

ified derivative PCG ethyl ester (**10**) gave a weak molecular ion *m/e* 264 and a strong peak at *m/e* 218 due to the loss of $C_2H_5OH$. PCT methyl ester (**9**), however, did not exhibit molecular ion ($M^+$) but gave a peak at 244 due to the loss of $CH_3OH$ and $H_2O$ from $M^+$. All the compounds derived from the alkylamines gave molecular ions. For example, 6-methylureidopurine (**16**) and 6-dimethylureidopurine (**47**) gave fairly strong molecular ions at *m/e* 192 and 206, respectively. In the case of the other 6-alkylureidopurines, the intensity of a $M^+$ ion, however, depended on the type of the side chains. The straight-chain analog, like *n*-butyl derivative **20**, gave stronger $M^+$ ion than the branched-chain compounds **21** and **22**. The peak at *m/e* 191, represented as purine-6-NH-CO-NH-$CH_2^+$, was found to be generally present in all the compounds except the *tert*-butyl derivative. The 6-arylureidopurines gave somewhat weaker molecular ion peaks.[29] The compounds derived from amino alcohols did not give molecular ion, the largest peak being observed at $M^+ - H_2O$. The following ions were common to all 6-alkyl- and 6-arylureidopurines: *m/e* 162 (purine-6-NH-$CO^+$), 161 (purine-6-$NCO^+$), 135 (adenine$^+$), 119 (purine$^+$), and 108 (adenine-HCN, $C_4H_4N_4^+$). The 161 was the base peak in all of these compounds.

The 6-ureidopurines and their ribonucleosides are stable in acidic and neutral solutions at least for a period of 1 month. This is in contrast to the lability of $N^6$-acyladenosines and $N^6$-(α-aminoacyl)adenosines.[30] Under physiological conditions, $N^6$-glycyladenine and $N^6$-glycyl-9-methyladenine undergo rapid hydrolysis and rearrangement; however, *N*-(purin-6-ylcarbamoyl)-L-threonine and *N*-(purin-6-ylcarbamoyl)glycine are stable and do not labilize the purine ring system. In aqueous 0.1 *N* NaOH the 6-ureidopurines are quite stable at room temperature (for more than 1 week). Compounds derived from L-threonine, serine, and 1-amino-2-propanol undergo complete hydrolysis in aqueous 0.1 *N* NaOH at 100° for 1 hr while the compounds without the hydroxyl groups are stable.

**Biological Activity.** The 6-ureidopurines and their ribonucleosides were tested for growth inhibitory activity for mammalian cells in axenic culture derived from normal and leukemic buffy coats. In order to shed some light on the possible relationship between the cytokinin and antitumor activity of the ureidopurines, these activities were investigated and compared (see Tables I and II). PCT has been found in tobacco tRNA;[20] however, it had no cytokinin activity in soybean as well as in tobacco tissue test systems.[21] The PCTR, PCG, PCGR, and their analogs containing other natural amino acids were also inactive as cytokinins.[21] These compounds containing free acidic groups were also inactive in mammalian cell lines when tested for their growth inhibitory activity.[5] 6-Allylureidopurine[21] and its ribonucleoside **74**, which can be looked upon as a metabolite of PCTR, exhibited good cytokinin activity; however, they showed only marginal growth inhibitory activity in the mammalian cell lines. 6-*n*-Butylureidopurine[21] and the 6-isopropylureidopurine (**18**) were also good as cytokinins but were inactive as mammalian cell growth inhibitors. The isoamyl derivatives **21** and **73** and the *n*-pentyl derivative **20** were active both as cytokinins as well as mammalian cell growth inhibitors. In the case of the 6-arylureidopurines, the parent compound, phenylureidopurine, was weakly active as a cytokinin.[21] The *o*-chlorophenylureidopurine (**35**) and the *o*-fluorophenylureidopurine (**32**) were the two most active cytokinins of the lot (active at $10^{-7}$ *M*). However, their activity in the mammalian tissue cultures was poor. Though the compounds with the additional substituents on the *o*-halophenyl ring (**34**, **39**) showed a loss of cytokinin activity, their growth inhibitory activity in the tissue cultures did not decrease. The phenylpropyl (**43**) and phenylbutyl (**44**) analogs were the two most active compounds in the tissue cultures; however, they were inactive as cytokinins. The benzylureidopurine **41** and the phenylethylureido analog **42** were also active in leukemic and normal cells but were inactive as cytokinins. The potent compounds with a varying degree of cytokinin activity, *i.e.*, *o*-fluoro (**32**) = *o*-chloro (**35**) > isoamyl (**21**) > allyl (**74**), did not possess growth inhibitory activity in the same order; *i.e.*, isoamyl (**21**) > *o*-chloro (**35**) > *o*-fluoro (**32**) > allyl (**74**). From the present results, there appears to be no direct correlation between the cytokinin and growth inhibitory activities of the 6-ureidopurines. However, it is of interest to note that the potent ureido cytokinins do show some growth inhibitory activity in mammalian cell lines. Since the growth medium used for cell culture contains 10% fetal calf serum, binding of some of the compounds by serum proteins would reduce the concentration available to the cells and, in this manner, affect the rate of uptake, possibly even reducing the drug concentration below an effective threshold. No serum proteins are used in the cytokinin assay medium. Furthermore, differences in metabolism *per se* in plant and animal cell systems might account for the observed lack of correlation between results from cytokinin and cell culture systems. The poor cytokinin and growth inhibitory activity in the tissue cultures by the natural substances, PCT, PCG, and their nucleosides, could be due to the presence of a negatively charged carboxyl group which could prevent the entry through the negatively charged mammalian cell membrane. To determine if this membrane charge might account for the findings, esters and amides (**9**, **10**, **13**, **15**, **62**, **64-67**) of some of the amino acid analogs were synthesized and tested. The slight activity of some of these in mammalian cells but inactivity as cytokinins indicates that the natural compounds are at best only marginally active and that the negative charge on PCT and PCG and other analogs is not in itself responsible for the lack of the biological activities herein investigated. Ribosylation of the 6-ureidopurines did not enhance the cytokinin activity of potent analogs.

The 6-ureidopurines differ chemically in that the 6 position of purines has a NH-CO-NH-R group as compared to the usual cytokinins which have -NH-$CH_2$-R. Since there is very little known about the mechanism of cytokininesis, the activity of the ureidopurines raises a question whether these compounds act at a receptor site similar to the usual purine cytokinins like $N^6$-($\Delta^2$-isopentenyl)adenine and kinetin or whether they bind at a site different from the above.

PCT, PCTR, PCG, PCGR, and some of their analogs were incubated with the following enzymes: urease (jack bean), pronase, protease, acylase (hog kidney), peptidases, arginase, snake venom, rat liver cell extract, yeast cell extract, and *Escherichia coli* cell extract. None of the enzymes cleaved the ureido bond, indicating that these compounds should be quite stable *in vivo*.

## Experimental Section

**General.** Melting points were determined in capillary tubes on Mel-Temp apparatus and are corrected. Ir spectra were recorded generally in KBr disks with a Perkin-Elmer 137B Infracord spec-

trophotometer. Uv spectra were recorded on a Cary Model 14 and a Beckman Acta V spectrophotometer. Nmr spectra were determined in DMSO-$d_6$ on a Varian A-60A spectrometer, using $Me_4Si$ in a capillary as an external reference unless specified otherwise. Mass spectra were determined by Du Pont/CEC 21-491 spectrometer at 70 eV, and in the case of ir and nmr spectral data only the important peaks are reported. Optical rotations were determined on a Jasco Model ORD/UV-5 at 584.4 nm (sodium D line). Tlc was carried out on Bakerflex silica gel 1B-F (J. T. Baker) using the following solvent systems: (A) EtOAc-*n*-PrOH-$H_2O$ (4:1:2); (B) EtOAc-2-ethoxyethanol-16% HCOOH (4:1:2); (C) *i*-PrOH-$H_2O$-concentrated $NH_4OH$ (7:2:1). C, H, and N analyses were carried out by Galbraith Laboratories, Knoxville, Tenn. 37921, and by the Heterocyclic Chemical Corp., Harrisonville, Mo. 64701.

Ethyl Purine-6-carbamate (II). This compound was prepared by a reaction of adenine with $EtO_2CCl$, using the method of Chheda and Hong.[5]

**Ethyl 9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (V).** This compound was prepared by a reaction of TAA (IV) with $EtO_2CCl$.[5]

**Amino Acid Isocyanates.** These compounds were prepared in excellent yields by passing $COCl_2$ into a stirred suspension of the protected amino acids (L-phenylalanine methyl ester HCl and L-valine benzyl ester HCl) in anhydrous PhMe at 85–90° for 4 hr.[5] The isocyanates showed a strong absorption at 2273–2300 $cm^{-1}$ (N=C=O) in ir spectra. Generally, the crude isocyanates were used for the next reaction.

***N*-(Purin-6-ylcarbamoyl)-L-phenylalanine (1).** Method A. A stirred mixture of 1.04 g (5.0 mmol) of II and 1.65 g (10 mmol) of L-phenylalanine in 50 ml of anhydrous pyridine was heated in a glass bomb at 115–120° for 6 hr. After cooling to room temperature, the excess phenylalanine was removed on a filter, and the filtrate was evaporated to dryness.§ The residue was dissolved in 100 ml of boiling EtOH, treated with charcoal, and filtered. Upon cooling to −5° the filtrate gave amorphous white product (collected on a filter), 795 mg. An additional 470 mg of the product was obtained from the filtrate: total 1.22 g (78%); mp 183–185° effervescent. The crude product was dissolved in 150 ml of cold aqueous 0.3 *N* NaOH and filtered. The filtrate was acidified to pH 2 with 2 *N* HCl and the resulting white precipitate was collected on a filter and washed with cold $H_2O$ and EtOH: yield 945 mg (58%); mp 197–199° effervescent; tlc ($R_f$ × 100) solvent A 25.0, solvent B 87.7, solvent C 77.6; ir max ($cm^{-1}$) 1680 (ureido, C=O), 1603, 1530, and 1480 (NH, C=C, C=N); nmr δ 3.49 (d, 2, $J$ = 7 Hz, $CH_2$), 4.95 (q, 1, $J$ = 7 Hz, CH), 7.63 (s, 5, phenyl H), 8.74 (s, 1, 8-H), 8.80 (s, 1, 2-H), 9.56 (d, 1, $J$ = 8 Hz, NH), and 10.1 ppm (s, 1, adenine 6-NH); $[\alpha]^{25}D$ +34.14° (*c* 1.23, DMSO). Table I lists the compounds prepared in an analogous manner. Some of the compounds were crystallized from EtOH and petroleum ether (bp 30–60°); compound 6 was crystallized from $H_2O$. The last part of purification (base–acid precipitation) was done only when a product contained a considerable amount of adenine as an impurity.

***N*-(Purin-6-ylcarbamoyl)-DL-threonine Methyl Ester (9).** This compound was prepared by method A with some modification as described below. A stirred mixture of 518 mg (2.5 mmol) of II, 848 mg (5 mmol) of DL-threonine methyl ester HCl, and 50 ml of anhydrous pyridine was heated in a glass bomb at 120° for 5 hr. The reaction mixture was evaporated to dryness§ and the residue dissolved in 20 ml of EtOAc was then applied to a silica gel column (100–200 mesh, 2.5 × 100 cm, prepacked in EtOAc) and elution was carried out with solvent B. Fractions between 240 and 300 ml were combined and evaporated to give a white residue. This was dissolved in hot EtOH (20 ml) and petroleum ether (bp 30–60°) was added to turbidity. After cooling at 0° for several hours, the product was collected on a filter and washed with petroleum ether (bp 30–60°) and MeCN: yield 140 mg; mp 183–185° effervescent. An additional 79 mg was obtained from the filtrate: total 219 mg (30%); tlc ($R_f$ × 100), solvent A 59.9, solvent B 77.7, solvent C 84.8; ir max ($cm^{-1}$) 1730 (ester, COO), 1670 (ureido C=O), 1600 and 1500 (NH, C=C, C=N); nmr δ 1.49 (d, 3, $J$ = 6.5 Hz, C-$CH_3$), 3.98 (s, 3, O-$CH_3$), 4.59 (m, 2, -CH-), 8.70 (s, 1, 8-H), 8.82 (s, 1, 2-H), 9.42 (d, 1, $J$ = 8 Hz, NH), and 10.1 ppm (broad, 1, adenine 6-NH).

***N*-(Purin-6-ylcarbamoyl)-*O*-benzyl-L-threonine Benzyl Ester (13).** Method B. A stirred suspension mixture of 680 mg (5 mmol) of adenine, 3.5 g (11 mmol) of isocyanate of *O*-benzyl-L-threonine benzyl ester,[5] and 10 ml of anhydrous DMSO was heated in a glass bomb at 88 ± 2° for 5 hr and then stirred at room temperature overnight. The reaction mixture was then evaporated to dryness, the residue was dissolved in a minimum amount of $CHCl_3$ and applied to a silica gel column (175 g, 100–200 mesh, dry packed), and the column was then eluted with PhH, $CHCl_3$, and $CHCl_3$-EtOH (19:1). The first 500 ml of $CHCl_3$-EtOH (19:1) gave a 9-substituted product *N*-(6-aminopurin-9-ylcarbamoyl)-*O*-benzyl-L-threonine benzyl ester. Fractions between 500 and 900 ml were evaporated to dryness to give crude product. This was crystallized twice from $CHCl_3$-petroleum ether (bp 30–60°): yield 750 mg (32.6%); mp 140–142°; tlc ($R_f$ × 100) solvent A 82, solvent B 93, solvent C 85; ir max ($cm^{-1}$) 1750 (ester, COO), 1680 (ureido C=O), 1625, 1600, 1540 (NH, C=C, C=N), and 735 ($C_6H_5$); nmr δ 1.60 (d, 3, $J$ = 6 Hz, $CH_3$), 5.08–4.50 (m, 4, -$OCH_2$-, -CH-), 5.52 (s, 2, -$COOCH_2$-), 7.72 (s, 10, $C_6H_5$), 8.80 (s, 2, 2-H, 8-H), 9.73 (broad, 1, NH), and 10.33 ppm (s, 1, adenine 6-NH). Table I lists the compounds prepared in an analogous manner. The compounds with an aryl side chain were precipitated out at the end of the reaction and the crude products were recrystallized from DMSO–MeOH.

***N*-[9-(β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-D-threonine (53).** Method C. This compound was prepared by a same method of preparation of PCTR (VIa)[5] with some modification in the purification procedure. A stirred mixture of 930 mg (2 mmol) of V, 476 mg (4 mmol) of D-threonine, and 30 ml of anhydrous pyridine was heated in a glass bomb at 120° for 6 hr. After bringing to room temperature, the unreacted threonine (231 mg) was removed by filtration. The filtrate was evaporated to dryness§ and the residue was stirred in 100 ml of 4 *N* $NH_3$-MeOH at room temperature for 8 hr. After evaporating the solvent, the residue was dissolved in 2 ml of $H_2O$. The aqueous solution was applied to a silica gel column (100–200 mesh, 5.0 × 30 cm, dry packed) and dried. The column was eluted with solvent A (2 l.) which removed all the impurities except the product. The column-bound product was washed with 2 *N* $NH_4OH$ in 50% EtOH (300 ml). After evaporating the solvent to dryness, the residue was dissolved in $H_2O$, treated with charcoal, and filtered. The aqueous solution was evaporated to dryness and the residue was triturated twice with 50 ml of hot EtOH: yield 165 mg (20%); mp 205–207° effervescent; tlc ($R_f$ × 100) solvent A 5.3, solvent B 60.0, solvent C 72.6; ir max ($cm^{-1}$) 1690 (ureido, C=O), 1620 and 1530 (NH, C=C, C=N); nmr δ 1.43 (d, 3, $J$ = 5 Hz, $CH_3$), 4.08 (d, 2, $J$ = 3 Hz, 5'-$CH_2$), 4.66–4.26 (m, 4, 2'-H, 4'-H α-CH, β-CH), 5.0 (m, 1, 3'-H), 6.43 (d, 1, $J$ = 6 Hz, 1'-H), 8.95 (s, 1, 2-H), and 9.05 ppm (s, 1, 8-H); $[\alpha]^{25}D$ −89.1° (*c* 1.055, DMSO). Table II lists the compounds prepared in an analogous manner. Most of the compounds were crystallized from hot EtOH, EtOH–EtOAc, EtOH–petroleum ether (bp 30–60°), EtOH–MeCN, or $H_2O$ after evaporating $NH_3$-MeOH.

***N*-[9-(β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-DL-threonine Amide (63).** A stirred mixture of 930 mg (2 mmol) of V and 678 mg (4 mmol) of DL-threonine methyl ester HCl in 50 ml of anhydrous pyridine was heated in a glass bomb at 120° for 6 hr. The light brown solution was then evaporated to dryness§ and the residue was stirred in 4.5 *N* $NH_3$-MeOH for 1 day. The solution was evaporated to dryness and the residue was dissolved in 50 ml of solvent A and 10 ml of EtOH. The solution was then applied to a silica gel column (100–200 mesh, 2.5 × 80 cm, prepacked in EtOAc) and the column was eluted with solvent A. The fractions between 1960 and 3120 ml were evaporated to dryness and the residue was crystallized from EtOH–MeCN (1:2): yield 306 mg (38.2%); mp 150°, soften 163–165°; tlc ($R_f$ × 100) solvent A 21.7, solvent B 46.9, solvent C 72.6; ir max ($cm^{-1}$) 1680 (ureido, C=O), 1610, and 1540 (NH, C=C, C=N); nmr δ 1.5 (d, 3, $J$ = 6 Hz, $CH_3$), 4.07 (m, 2, 5'-$CH_2$), 4.67–4.35 (m, 4, 2'-H, 4'-H, α-CH, β-CH), 5.12–4.90 (m, 1, 3'-H), 6.41 (d, 1, $J$ = 5.5 Hz, 1'-H), 7.46 (s, 1, amide NH), 7.70 (s, 1, amide NH), 8.93 (s, 1, 2-H), 9.05 (s, 1, 8-H), and 10.0 ppm (broad s, NH).

***N*-[9-(β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-*o*-chloroaniline (82).** Method D. A stirred mixture of 1.97 g (5 mmol) of TAA (IV) and 1.54 g (10 mmol) of *o*-chlorophenyl isocyanate in 10 ml of anhydrous pyridine was heated in a glass bomb at 85–90° for 5 hr and then stirred at room temperature overnight. After evaporating the solvent to dryness,§ the residue was crystallized twice from MeOH to give the granular material, *N*-[9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-*o*-chloroaniline. The crude yield gave 1.42 g (52.0%), mp 146–148°. *Anal.* ($C_{23}H_{23}ClN_6O_8$) C, H, N. This compound (1 g, 1.8 mmol) was stirred in 4 *N* $NH_3$-MeOH at 4° for 16 hr. The precipitated product was collected on a filter: yield 546 mg (71%); mp 186–188° (yield based on TAA, 37%); tlc ($R_f$ × 100) solvent A 49.3, solvent B 65.4, solvent C 78.0; ir max ($cm^{-1}$) 1700 (ureido, C=O), 1600, 1540 (NH, C=C, C=N), and 750

§The residue was triturated with 10 ml of $PhCH_3$ and evaporated to dryness to remove the residual pyridine.

(Ph); nmr (internal TMS) δ 6.15 (d, 1, $J$ = 8 Hz, 1'-H), 7.13–7.60 (m, 3, Ph), 8.50 (m, 1, H next to Cl on Ph), 8.82 (s, 1, 2-H), 8.87 (s, 1, 8-H), 10.37 (broad, 1, adenine 6-NH), and 12.27 ppm (broad, 1, NH next to Ph). Table II lists the compounds prepared in an analogous manner.

**$N$-[9-(β-D-Ribofuranosyl-9$H$-purin-6-yl)carbamoyl]-L-threonine (PCTR, VIa).** A mixture of 33 mg (0.046 mmol) of $N$-[9-(2,3,5-tri-$O$-acetyl-β-D-ribofuranosyl-9$H$-purin-6-yl)carbamoyl]-$O$-benzyl-L-threonine benzyl ester,[5] 50 mg of PdO, and 10 ml of 50% EtOH was hydrogenated at atmospheric pressure for 1 hr. After filtering Pd, the filtrate was evaporated to dryness and the residue stirred in 20 ml of 4 $N$ $NH_3$–MeOH at room temperature for 6 hr. The solvent was evaporated to dryness and the residue was dissolved in 5 ml of $H_2O$ which was lyophilized. Uv quantitation showed 100% yield. Uv and chromatographic mobilities in various solvent systems were identical with those of the authentic PCTR.

**Growth Inhibition.** The compounds were tested with cultured cells derived from the buffy coat of a normal individual (Nc 37) and a patient with myeloblastic leukemia (RPMI 6410). Compounds were dissolved in 0.5% DMSO in growth medium (RPMI 1640 + 10% fetal calf serum) at $10^{-4}$ $M$ (except for 30, 32, and 35 which were tested at $5 \times 10^{-5}$ $M$ because of their limited solubility) and results expressed as per cent of viable cell number relative to control containing 0.5% DMSO after 72 hr of incubation. This DMSO concentration did not alter growth. Tables I and II show the results.

**Cytokinin Activity.** The compounds were tested for cytokinin activity using a soybean assay previously described.[20,21] The test compounds were dissolved in a minimum quantity of DMSO and then diluted with sterile $H_2O$. Just prior to solidification of the agar, portions of serial dilutions were added aseptically to 25-ml flasks containing 9 ml of a medium previously described,[20,21] complete except cytokinin. Pieces of soybean tissue (0.5-mg pieces, three per flask) were incubated for 30 days in the dark at 24°. Fresh weights were determined and compared to controls. The lowest concentration of tested compounds that gave a response is used as the criterion for comparing the activities of the analogs. The assays were carried out by Dr. W. H. Dyson in the laboratory of Dr. Ross H. Hall of McMaster University, Hamilton, Ontario, Canada.

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# Potential Anti-Parkinson Drugs Designed by Receptor Mapping

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A series of methoxy-1- and -2-aminoindans and aminotetralins as well as 2-oxo-5-amino-1,2,5,6,7,8-hexahydroquinoline was synthesized as potential anti-Parkinson agents. The design of the molecules was based on Kier's receptor mapping technique in that the distances between the heteroatoms of oxotremorine (OT) and dopamine (DA) in reported preferred conformations are similar. None of the compounds antagonized oxotremorine tremors or showed dopaminergic properties. Some of the molecules are analgesics and some are monoamine oxidase inhibitors.

Kier has proposed that drug receptors may be mapped by a comparison of the preferred conformation of several structurally different agonists and/or antagonists to the same receptor. The specific receptor is considered to have sites which interact with those heteroatoms which form a pattern common to all molecules which react with it.[1] Kier also suggested that this technique should be useful in drug design.[1] Since the discovery of novel "leads" remains an important problem in medicinal chemistry, we were eager to evaluate the usefulness of Kier's approach to the design of new drug molecules.

**Development of the Model.** It has recently been demonstrated that if one increases the body levels of dopamine by administration of its metabolic precursor L-DOPA (3,4-dihydroxyphenylalanine), there is an alleviation of the symptoms of Parkinsonism.[2] Hornykiewicz had previously established that the basal ganglia of Parkinson patients are deficient in dopamine.[3]