Synthesis of Naturally Occurring 6-Ureidopurines and Their Nucleosides[1]

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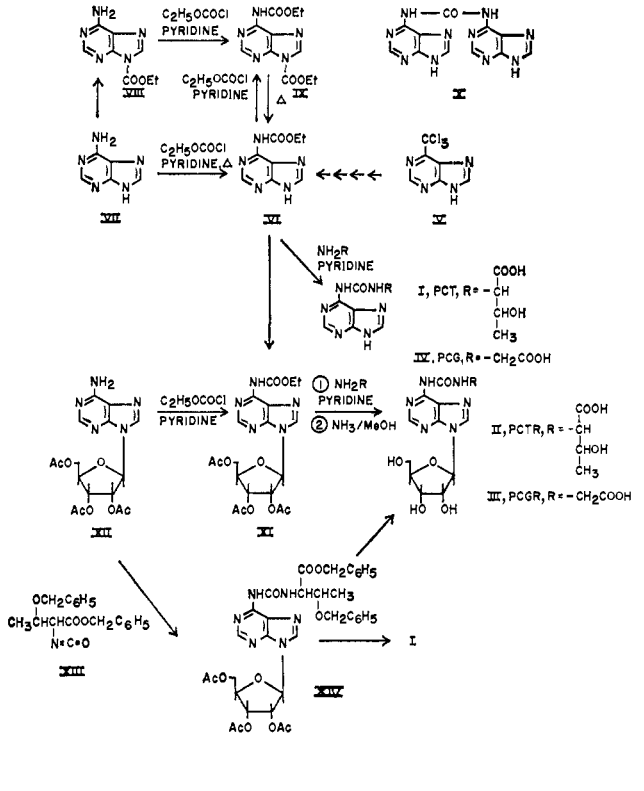
The naturally occurring modified purines, *N*-(purin-6-ylcarbamoyl)threonine (PCT, I) and *N*-(purin-6-ylcarbamoyl)glycine (PCG, IV), and their ribosides, PCTR (II) and PCGR (III), have been synthesized. Facile syntheses of the key intermediates, ethyl purine-6-carbamate (VI) and its riboside (XI), were achieved by new methods. Displacement of the EtO group of urethanes VI and XI with L-threonine and glycine gave PCT (I), PCTR (II), PCG (IV), and PCGR (III). Compounds I and II were also prepared through reaction of 2′,3′,5′-tri-*O*-acetyladenosine with a suitably blocked threonine isocyanate, followed by deblocking. These synthetic studies confirm the earlier suggestion that the threonine of natural compound I has the L configuration.

A novel modified purine in which an amino acid, threonine, is attached to the NH_2 of adenine through a ureidocarbonyl group, has recently been isolated and characterized.[2a] Structurally, it was shown to be *N*-(purin-6-ylcarbamoyl)threonine[2b] (PCT, I, Scheme I). This ureidopurine is of general occurrence in

SCHEME I



tRNA[2c] of yeast, *Escherichia coli*,[2a] tobacco tissue,[2d] and the human spleen.[2e] Its riboside, *N*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)carbamoyl]threonine (PCTR, II), has been identified in human and rat urine.[3a,3b] Sequence determination of $tRNA^{Ile}$ by Takemura and coworkers[4] revealed that PCTR occupied a position adjacent to an anticodon, I-A-U. Ishikura, *et al.*,[5] have recently reported that PCTR is also present at the same anticodon adjacent position in lysyl, methionyl, and seryl tRNAs which respond to A-beginning codons. Despite knowledge of its unique location in several species of tRNA the exact function of this threonine-containing nucleoside still remains unknown. Recently, Schweizer, *et al.*,[6a] have isolated a glycine-containing ureido nucleoside, *N*-(purin-6-ylcarbamoyl)-glycine riboside (PCGR, III), from yeast tRNA.

In order to make available sufficient quantities of this class of compounds for pharmacological and biochemical studies and for the structure proof of the natural compounds, this synthetic work was undertaken. The present paper describes the synthetic methods and chemical properties of PCT (I), PCG (IV), and their nucleosides, PCTR (II) and PCGR[6b] (III). The acylation of adenine by ethyl chloroformate was also fully investigated, and a one-step synthesis of the required key intermediates, ethyl purine-6-carbamate (VI) and its riboside, has been achieved.

Initially, this urethane VI was prepared in 4 steps starting from 6-trichloromethylpurine (V), according to the method of Giner-Sorrolla and Bendich.[7a] The modifications in the conversion of 6-trichloromethylpurine (V) to purine-6-carboxamide resulted in an 88% yield, as compared with the reported yield of 30%.[7b] In spite of improvements in the yields to nearly quantitative in each step,[7a] this procedure for preparing the urethane VI, still proved to be very laborious and expensive, thus necessitating a search for a more direct method of synthesis.

For this purpose, the reaction of adenine with ethyl chloroformate was studied under various conditions.[1,3b] Equimolar amounts of adenine and EtO_2CCl in pyridine at 35° for 3 hr gave a modest yield of ethyl 6-aminopurine-9-carboxylate (VIII). With a threefold

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Table I
Ultraviolet Spectra of Products

Compd	pH	λ_{max}, mμ	ε × 10⁻³
N-(Purin-6-ylcarbamoyl)-L-threonine (PCT, I)	1.8	277	20.2
	7.0	269, 277	19.1, 18.8
	12.4	278	17.7
PCT, I (urethane method)	1.6	277	20.6
	5.0	269, 276	19.2, 18.9
	12.2	278	18.1
N-(Purin-6-ylcarbamoyl)-D-threonine (I)	1.6	277	20.3
	4.7	270, 277	19.2, 18.9
	12.1	278	17.7
N-(Purin-6-ylcarbamoyl)-L-threonine riboside (PCTR, II)	1.6	272, 277	20.4, 21.6
	6.5	270, 277	22.9, 19.4
	12.4	270, 277, 299	16.3, 16.0, 11.1[b]
N-[(9-β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-glycine (PCGR, III)	1.2	276, 271	19.1, 18.2
	5.0	269, 276	20.9, 17.1
	12.1	270, 277, 298	13.5, 13.8, 12.8[b]
N-(Purin-6-ylcarbamoyl)glycine (PCG, IV)	1.4	276.5	18.6
	6.2	270, 276.5	17.4, 16.9
	[illegible].3	278	16.2
Ethyl purine-6-carbamate (VI) (from adenine)	1.4	276	14.6
	6.4	275	12.5
	12.1	282	11.5
Chloromercuri derivative of ethyl purine-6-carbamate	2	275	
	6.6	275, 270 sh[a]	
	11.5	279	
Ethyl 6-aminopurine-9-carboxylate (VIII) (from thallium salt)	1.5	258	12.0
	6.1	259	11.6
	12	269[c]	11.0
Ethyl 6-aminopurine-9-carboxylate (VIII) (by direct acylation)	1.5	257	
	6.8	258	
	11.6	268[c]	
9-Carboxy-9*H*-purine-6-carbamic acid diethyl ester (IX)	1.5	272	
	4.5	264.5	
	12.0	280	
1,3-Dipurin-6-ylurea (X)	2	293	33.6
	6.6	292, 305 sh[a]	12.7, 8.55
	12.2	298, 280 sh	23.8, 17.7
Ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (XI)	1.2	275	18.6
	6.6	267	17.5
	12.1	291	22.1
N-[9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-*O*-benzyl-L-threonine benzyl ester (XIV)	2.2	269, 276	21.4, 20.0
	7.5	268, 276	22.2, 18.8
	13	271, 278, 296	17.3, 17.2, 11.8[b]

[a] Sh, shoulder. [b] In alkaline solution, these peaks are highly pH dependent. [c] In alkaline solution, there is a rapid degradation to adenine, and hence the peak at 269 mμ and ε values are due to adenine.

excess of EtO_2CCl, the same reaction gave a 6-substituted product VI, in addition to the predominant 9-carboxylate VIII. A longer reaction time with an excess of EtO_2CCl led to the formation of a new product, the 6,9-dicarboxylate IX, in addition to VI and VIII. The same reaction mixture when heated in a bomb at 115°, gave the desired ethyl purine-6-carbamate (VI) in 52% yield in 1 step, starting from the readily available adenine. In a large-scale preparation of the ethyl purine-6-carbamate (VI), using refluxing conditions, an interesting by-product X was isolated as an insol precipitate. Pyridine served as a good solvent and a base in these reactions. Use of DMF and DMSO as solvents along with acid acceptors failed to give the desired product VI. Since these results were first presented,[1,3b] Leonard, *et al.*,[8] have, very recently, reported another facile synthesis of ethyl purine-6-carbamate (VI).

The structure of the urethane VI was confirmed by comparison of its ir and uv spectra (Table I) and chromatographic mobilities (Table II) with those of an authentic sample prepared from 6-trichloromethylpurine.[7a] Compound VIII was earlier suggested to be ethyl 6-aminopurine-9-carboxylate on the basis of uv and nmr spectral data.[9] We have now synthesized this compound from a reaction of adenine-9-thallium[10] with ethyl chloroformate, thus confirming the 9-substituted structure VIII for this product. The structure of the dicarboxylate IX was established by synthesis from both the carbamate VI and the carboxylate VIII, and by instantaneous conversion to ethyl purine-6-carbamate VI in dil alkali. The novel compound X was highly insol in most org solvents but dissolved readily in 1 *N* NaOH. When this sol was heated, 2 moles of adenine/mole of X was obtained. In its mass spectrum, X exhibited a molecular ion peak at 296

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TABLE II
PAPER CHROMATOGRAPHY DATA

	$R_f \times 100$ in various solvents						
	A	B	C	D	E	F	G
PCT, I (urethane route)	32	40	48	35	0.6	1.6	57
PCT, I (isocyanate route)	31	41	48	35	0.6	1.6	56
N-(Purin-6-ylcarbamoyl)-D-threonine (I)	32	40	48	35	0.6	1.6	57
PCTR, II (urethane route)	31	41	47	41	0	1.0	57
PCTR, II (isocyanate route)	31	41	48	41	0	1.1	57
N-(Purin-6-ylcarbamoyl)glycine (PCG, IV)	22	Streaked	42	47	0	5	52
N-(Purin-6-ylcarbamoyl)glycine riboside (PCGR, III)	20	46	20	41	0	4.9	43
Ethyl purine-6-carbamate (VI) (from 6-trichloromethylpurine)	70	50	75	69	49	81	
Ethyl purine-6-carbamate (VI) (from adenine)	70	50	75	69	49	81	
Ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (XI) (from VI)	77	62	88	82	72	90	83
Ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (XI) (from TAA)	77	62	88	81	71		
1,3-Dipurin-6-ylurea (X)	41				20	31	55

and the characteristic ions[2b] at 162 and 161. Its nmr spectrum had a single peak for each pair of C_2 and C_8 protons and an absorption at 1700 cm^{-1} for the ureido-carbonyl in ir. The elemental analysis and the uv spectral data also support the structure X, 1,3-dipurin-6-ylurea.

On the basis of acylation experiments under various conditions, it is suggested that the acylation of adenine with EtO_2CCl occurs first at N^9, and then at N^6. The major protion of ethyl purine-6-carbamate (VI) appears to be formed by degradation of the preformed 6,9-dicarboxylate IX when the reaction mixture is heated.

For synthesis of its nucleoside, ethyl purine-6-carbamate (VI) was converted into the ClHg salt,[11] which, when allowed to react with tri-*O*-acetylribofuranosyl bromide or chloride,[12] gave the desired urethane XI in 37% yield.[3a] Nevertheless, a direct and cleaner procedure was developed for synthesis of this urethane. Reaction of tri-*O*-acetyladenosine[13] (TAA, XII) with EtO_2CCl in pyridine gave a 58% yield of the desired ethyl 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purinecarbamate (XI). The structural identity of XI was established by comparison of the ir and uv spectra and the chromatographic mobilities (Table II) with XI prepd *via* the ClHg procedure. These studies show that TAA undergoes acylation at N^6 and that the ClHg derivative of VI gives β nucleoside.

Displacement of the EtO group of urethane VI with threonine and glycine in pyridine gave the desired PCT (I) and PCG (IV) in 38 and 51% yields, respectively. Analogous displacement of the urethane nucleoside XI yielded tri-*O*-acetyl derivatives of ureido nucleosides, which, upon treatment with NH_3–MeOH gave the nucleosides PCTR (II) and PCGR (III) in 45 and 52% yields, respectively. The PCGR has been synthesized by reaction of TAA with ethyl isocyanatoacetate.[6a]

PCT (I) and PCTR (II) were also prepared by an isocyanate route. L-Threonine was converted into *O*-benzyl-L-threonine benzyl ester with $PhCH_2OH$ according to the procedure of Mizoguchi, *et al.*[14] This dibenzyl threonine was converted into its isocyanate (XIII), which was then allowed to react with TAA (XII) in hot PhMe, giving the completely protected ureido nucleoside XIV in 48% yield. This nucleoside, when treated with Na in liq NH_3 gave the desired PCTR (II) in 20% yield. Treatment of XIV with $HBr–F_3CCO_2H$ resulted in removal of the sugar and benzyl groups giving PCT (I) in 30% yield. The PCT (I) and PCTR (II) obtained by this method were identical with the PCT and PCTR (in ir and uv spectra and paper chromatography) obtained *via* the urethane route as well as to the naturally occurring compounds.[3a] It is clear from these results that isocyanate reacts with the 6 position of TAA and that the natural materials have the 6-ureidopurine structure.

In evaluating the synthetic merits of the 2 routes, it is clear that when the amino acids have to be attached to the amino N of the adenosine in a ureido fashion, the urethane route[1] should be the method of choice, since protection of the COOH or OH groups is not necessary. The PCT and PCTR prepd by this method, when checked by the procedure of Manning and Moore,[15] exhibited no detectable racemization of L-threonine. The isocyanate route is quite useful in cases of amines, which could be converted readily to isocyanates. However, a requirement of blocking and deblocking of the functional groups like COOH, OH, and SH of amino acids may limit its applicability.

N^6-Ureidopurines and their nucleosides have two uv maxima at 269 and 277 mμ, with relatively high extinction coefficients, as compared to the other nucleosides.[3a] The nucleosides have 3 maxima in alk soln with a high degree of pH dependency.[1] The peak at 299 mμ is unique and can be used for quantitating the ureido nucleosides in a mixture containing adenosine and other nucleosides.

The β configuration at the anomeric carbon in the natural nucleoside PCTR was assigned on the basis of the isolation of β-adenosine from the alkaline hydrolysate of PCTR.[3a] Furthermore, synthesis of PCTR starting from TAA (derived from β-adenosine) further establishes that the natural product has β configuration at carbon-1. Configuration of the amino acid threonine was earlier suggested to be L on the basis of synthetic material.[2b] Using the above syn-

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thetic procedures, both the PCT and PCTR have been synthesized with D-threonine.[1] The optical rotation of the PCT containing D-threonine further supports the conclusion[2b] that the threonine of naturally occurring PCT has an L configuration.

PCT and PCTR were inactive as cytokinins in tobacco bioassay, but some of their analogs were quite potent cytokinins.[2d] The PCT and PCTR (at 10^{-7} *M*) exhibited slight growth-stimulatory activity in the human leukemic myeloblast cell line (RPMI 64-10) grown in culture. Further testing of these compounds and their analogs in other cell lines is in progress.

Experimental Section

General.—Melting points were detd in capillary tubes on Mel-Temp apparatus and are corrected. Ir spectra were recorded generally in KBr disks with a Perkin-Elmer 137B Infracord spectrophotometer. Uv spectra were recorded on a Cary Model 14 spectrophotometer. Nmr spectra were detd in DMSO-d_6 on a Varian A-60A spectrometer, using Me_4Si in a capillary as an external ref unless specified otherwise. The mass spectra were recorded on a CEC 21-491 double-focusing mass spectrometer, using an ionization voltage of 70 eV. In case of ir, nmr, and mass spectral data only the important peaks are reported. Optical rotations were detd on a Jasco Model ORD/UV-5 at 584.4 mμ (sodium D line). Tlc was carried out on glass plates coated with silica gel with PF 254 (E. Merck AG). C, H, N anal. were carried out by Galbraith Laboratories and by the Heterocyclic Chemical Corporation, Harrisonville, Mo. 64701. Where anal. are indicated only by symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theoretical value.

Paper Chromatography.—The following solvent systems, measured by vol, were used: (A) *i*-PrOH–H_2O–concd NH_4OH (7:2:1); (B) *i*-PrOH–concd HCl–H_2O (680:176:144); (C) EtOAc–2-ethoxyethanol–16% HCOOH (4:1:2); (D) *i*-PrOH–1% aq $(NH_4)_2SO_4$ (2:1); (E) *n*-BuOH–H_2O–concd NH_4OH (86:14:5); (F) EtOAc–*n*-PrOH–H_2O (4:1:2); (G) *n*-PrOH–concd NH_4OH–H_2O (55:10:35); and (H) EtOAc–2-ethoxyethanol–8% HCOOH (4:1:2).

The chromatograms were run in a descending manner on Whatman No. 1 paper for 16–20 hr in systems A, B, D, E, and G, and for 8 hr in systems C, F, and H. The spots were detected by viewing chromatograms in shortwave uv light.

Purine-6-carboxamide.—A suspension of 20.0 g of trichloromethylpurine (Cyclo Chemicals Corp.) in 309 ml of cold concd NH_4OH was stirred at 5 ± 1° for 1 hr, then, after being allowed to warm to room temp, was stirred for 23 hr. The brown suspension was heated in a bomb at 115–120° for 3 hr and then evapd to dryness. The resulting solid was heated to boiling in 600 ml of H_2O and filtered to remove insol material. The filtrate was concd to 300 ml, charcoaled hot, and cooled giving a white product (single spot in tlc, solvent A); 10.52 g (88.2%); mp 310–315°. Recrystn of this material from H_2O gave an anal. sample, mp 310–315°. The product was identical with the known purine-6-carboxamide.[7]

Ethyl Purine-6-carbamate (VI).—To a stirred suspension of 1.35 g (10 mmoles) of adenine in 40 ml of pyridine was added (over 10 min) 2.86 ml (3.25 g, 30 mmoles) of EtO_2CCl at −5°. After being stirred for 30 min at 0°, the mixt was stirred at ambient temp for 3 hr,[16a,b] and then heated in a glass bomb with stirring at 115° for an addnl 3 hr. After evapg the solvent[16c] *in vacuo*, the residue was triturated with 100 ml of hot H_2O, and the white cryst ppt was collected on a filter, and washed with warm H_2O (100 ml) and EtOH (20 ml); 0.79 g; mp 310–315° dec. Addnl product was obtained from the filtrate; 0.29 g (total 1.08 g, 52%); mp 310–315° dec. A sample for anal. was prepd by recrystn from hot EtOH, mp 310–315° dec. Ir and uv spectra and mobilities in paper chromatography were identical with those of the urethane VI prepd from purine-6-carboxamide *via* purine-6-carbohydrazide and purine-6-carboazide:[7] ir max in cm^{-1}, 1740 (C=O, urethane) and 1600 broad (C=C, C=N); nmr δ 1.67 (t, 3, *J* = 6 Hz, CH_3), 4.63 (q, 2, *J* = 7 Hz, CH_2), 8.8 (s, 1, 8-H), and 8.94 ppm (s, 1, 2-H); mass spectrum *m/e* 209 (P + 2), 208 (P + 1), 207 (parent peak), 162, 161, and 135. *Anal.* ($C_8H_9N_5O_2 \cdot 0.25H_2O$) C, H, N.

1,3-Dipurin-6-ylurea (X).—In prepg a large batch of VI, using 100 mmoles of adenine and keeping the proportions the same as before for the other reactants, we isolated the by-product X. In this instance, the light brown soln was refluxed for 3 hr instead of being heated in a bomb at 115°. Here the insol white by-product that pptd was collected on a filter and washed with pyridine and EtOH. The crude material was triturated with hot EtOH–H_2O (2:1): yield, 3.49 g (23.6%); mp 300° dec. The anal. sample was prepd by dissolving in 1 *N* NaOH and reptg with 1 *N* HCl. This compd is extremely insol in most org solvents: ir max in cm^{-1}, 1700 (ureido, NHCONH), 1620, and 1540 (C=N, C=C); nmr (CF_3COOD solvent) δ 9.19 (s, 2, 8-H) and 9.26 (s, 2, 2-H) ppm; these data suggested that the compd has 2 pairs of identical protons (2-H and 8-H); mass spectrum *m/e* 297 (P + 1), 296 (parent peak), 253 (P − 43), 162, 161, 136, and 135. Hydrolysis of the compd with 1 *N* NaOH at 100° for 60 min yielded 2 mole equiv of adenine. *Anal.* ($C_{11}H_8N_{10}O$) C, H, N.

Work-up of the initial filtrate, by the method already described gave the desired urethane VI in 47% yield, mp 310–315° dec.

Ethyl 6-Aminopurine-9-carboxylate (VIII). Method A.—To a suspension of 2.30 g (6.7 mmoles) of Tl salt of adenine[10] in 25 ml of DMF was added (dropwise) 1.5 ml (13.4 mmoles) of EtO_2CCl. The reaction mixt was stirred at room temp for 6 hr. The white ppt was removed on a filter, and washed with 20 ml of DMF. The combined DMF soln was evapd to dryness and the residue was dissolved in a mixt of 50 ml of solvent H and 10 ml of EtOH. The soln was adsorbed onto a silica gel column (100–200 mesh, 2.5 × 50 cm, prepacked in EtOAc). Fractions between 280 and 480 ml contg the pure desired product were evapd to dryness at 30°. The residue was dissolved in a min vol. of THF, and cooled to 5° for several hours. The ppt that formed was collected on a filter and washed with EtOAc: yield, 70 mg (5.1%); mp 157–158° with effervescence.

VIII was readily converted to adenine in alk soln (pH 11). Heating with H_2O or EtOH also caused rapid hydrolysis to adenine. VIII underwent hydrolysis in most of the solvents for chromatog, except in solvents C and H. It was formed as a major product in the above reaction; but during the isolation and purification, it degraded to adenine: tlc (R_f × 100), 84 (solvent C); ir max in cm^{-1}, 1750 (urethane), 1660, 1600, and 1580 (C=N, C=C); nmr δ 1.74 (t, 3, *J* = 7.5 Hz, CH_3), 4.84 (q, 2, *J* = 7 Hz, CH_2), 7.83 (s, 2, NH_2), 8.65 (s, 1, 2-H), and 8.88 ppm (s, 1, 8-H); mass spectrum *m/e* 209 (P + 2), 208 (P + 1), 207 (parent peak), 162, 161, 148, and 108. *Anal.* ($C_8H_9N_5O_2$) C, H, N.

Method B.—To a stirred suspension of 1.35 g (10 mmoles) of adenine in 20 ml of pyridine was added, dropwise, 0.96 ml (1.08 g, 10 mmoles) of EtO_2CCl at −5°. After 30 min at 0°,

(16) (a) The pink color that appeared during the first hour gradually faded away, and the light brown soln was obtained at the end of 3 hr. (b) At this point, fractionation of the reaction mixt with tlc and quantitation by uv revealed that ethyl purine-6-carbamate (VI) and its 9 isomer (VIII) were formed in equal amounts. Similar examn of the reaction mixt after stirring overnight at ambient temp, showed an addnl product, 9-carboxy-9*H*-purine-6-carbamic acid diethyl ester (IX). When this reaction mixt was heated at 115° for 1 hr, VIII and IX were converted to adenine and VI, respectively. (c) After evapn of the solvent, the residue was triturated with toluene, and the mixture was reevapd to dryness *in vacuo*. This process was repeated once more, in order to remove traces of pyridine. (d) In another batch, this ppt was treated once again, as before, with *n*-BuOH and pet ether, to ensure the complete extn of the product. (e) This compound is quite hygroscopic, and it should be dried *in vacuo* over P_2O_5 for 18 hr, immediately after filtering. (f) When the same reaction was carried out in hot DMSO containing Et_3N, the yield of the desired product was poor, and product isolation was difficult. Pyridine proved to be a far better solvent for this reaction. (g) PCT and PCTR have a tendency to gel during crystn from aq or alc solvents. The solution should be cooled gradually to prevent gel formation. PCTR is crystallizable with difficulty. MeCN should be allowed to conc at room temp by slow evapn. (h) In other batches involving this procedure, yields of the crude product varied from 70 to 90%. During distn fair amounts of this crude material decompd and polymd to brown tar. (i) Paper and thin-layer chromatography indicated that this material contained the desired product, TAA, and a fair amount of non-uv-absorbing material. When this reaction was carried out with equimolar amounts of TAA and isocyanate, it gave poor yields of the product, with a lot more non-uv-absorbing material. Heating the reaction to 100° instead of 80° also resulted in a poor yield of the product, and led to decompn. When DMF and DMSO were used as solvents in this reaction, only a trace of product could be detected; most of the TAA remained unchanged.

the mixt was stirred at ambient temp for 3 hr.[16a] The solvent was removed *in vacuo*; the residue was triturated with 20 ml of EtOAc and filtered to give 1.92 g of white solid. Examn of this material by tlc and quantitation by uv revealed that it contained 50% of the desired product, rest being mainly adenine. Ir and uv spectra and mobilities in tlc were identical with those of VIII prepd from the Tl salt (method A) as well as prepd according to the method of Dyer, *et al.*[9]

9-Carboxy-9*H*-purine-6-carbamic Acid Diethyl Ester (IX). Method A.—To a suspension of 2.07 g (10 mmoles) of VI in 200 ml of dry pyridine at −5° was added, dropwise, 2 ml (21 mmoles) of EtO_2CCl. The reaction mixt was then stirred overnight at ambient temp. After evapn of the solvent,[16c] examn of the residue with tlc (solvent C) revealed that the product had an R_f of 0.89 as compared to R_f 0.77, 0.34, and 0.84 for ethyl purine-6-carbamate, adenine, and VIII, resp. The material was isolated by prep tlc, in 65–70% yield.

Method B.—To a suspension of 30 mg (0.145 mmole) of VIII in 3 ml of dry pyridine at −5° was added 0.2 ml of EtO_2CCl The reaction mixt was stirred at ambient temp overnight. The solvent was evapd,[16c] and the product was sepd by prep tlc, in about 60% yield. It was identical in tlc and in uv spectra with the product IX obtained by method A.

Because of the very labile nature of IX, no satisfactory anal. could be obtained.

Chloromercuri Derivative of Ethyl Purine-6-carbamate.—To a suspension of 1.50 g (7.25 mmoles) of VI and 1.99 g (7.33 mmoles) of $HgCl_2$ in 145 ml of EtOH–H_2O (1:1) was added 7.25 ml of 1 *N* NaOH. The reaction mixt was stirred for 2 hr at room temp, then cooled to 5°. The pptd cream-colored product was collected on a filter, washed with EtOH and Et_2O (10 ml each), and dried over P_2O_5; 2.55 g (80%); mp 300° dec; ir max in cm^{-1}; 1735 (C=O urethane).

Ethyl 9-(2,3,5,-Tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (XI) *via* the ClHg Salt.—To a suspension of 5.1 g (11.6 mmoles) of chloromercuri derivative of VI in 120 ml of dry toluene was added a soln of 1-bromo-2,3,5-tri-*O*-acetyl-β-D-ribofuranose (12.0 mmoles)[11] in 10 ml of anhyd PhMe. The reaction mixt was refluxed for 2 hr, then stirred at room temp overnight. PhMe was evapd to about 30 ml, and pet ether (bp 30–60°) (150 ml) was added. The pptd brown material (unreacted Hg salt) was collected on a filter, and extd with 3 portions of 100 ml of warm $CHCl_3$. The filtrate, together with with the combined ext was washed with 100 ml of aq KI (30%) and 100 ml of H_2O, dried, and then evapd to a syrupy residue.

The syrup was decolored (charcoal–MeOH) and dissolved in 100 ml of *n*-BuOH, then 100 ml of pet ether (bp 30–60°) was added. After standing at room temp for 30 min, the ppt[16d] was removed on a filter, and the filtrate was evapd to dryness. The residue was dissolved in *n*-BuOH (100 ml). This soln was dild with 200 ml of pet ether (30–60°) and cooled at 4°. The pale brown product[16e] was filtered, yield 2.55 g (47.3%).

This material was purified on a silica gel column (170 g, 70–325 mesh, 2.54 × 75 cm), using 3% EtOH in EtOAc as the eluting solvent. The desired urethane XI was eluted in fractions between 300 and 450 ml. Evapn of the solvent gave a pure dull white powder, yield 1.99 g (37.0%). The ir and uv spectra and the chromatographic mobilities were identical with those of the urethane XI prepared from TAA as described below.

Ethyl 9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine-6-carbamate (XI) from 2′,3′,5′-Tri-*O*-acetyladenosine.—To a stirred soln of 4.20 g (10.7 mmoles) of TAA[13] at −5° in 80 ml of pyridine was added, dropwise (over a period of 10 min), 3.05 ml (3.48 g, 32.1 mmoles) of EtO_2CCl. The pink reaction mixt was slowly brought to room temp (over a period of 30 min) and stirred for 18 hr. The light brown soln was evapd to dryness.[16c] The residue was dissolved in $CHCl_3$ (20 ml), and applied to a silica gel column (100–200 mesh, 2.5 × 100 cm, prepacked in $CHCl_3$). Elution was carried out with EtOAc. Fractions between 650 and 3600 ml were combined and evapd, giving a shiny white powder; yield 2.71 g (58.2%); mp 60–65°; ir max im cm^{-1}, 1740 shoulder (urethane, C=O) and 1610 (C=C, C=N, C=O); nmr δ 1.51 (t, 3, *J* = 5 Hz, CH_3), 2.20 (s, 3, CH_3CO), 2.23 (s, 3, CH_3CO), 2.32 (s, 3, CH_3CO), 4.50 (q, 2, *J* = 7 Hz, CH_2), 4.67 (s, 3, 5′-H, 4′-H), 6.67–5.87 (m, 3, 1′-H, 2′-H, 3′-H), 8.95 (s, 2, 2-H, 8-H), and 10.75 ppm (s, 1, NH). *Anal.* ($C_{19}H_{23}N_5O_9$) C, H, N.

***N*-(Purin-6-ylcarbamoyl)-L-threonine (PCT, I).**—A stirred mixt of 2.07 g (10 mmoles) of VI and 1.79 g (15 mmoles) of L-threonine in 60 ml of anhyd pyridine was heated in a glass bomb at 120° for 6 hr. After cooling to room temp overnight, the unreacted threonine and adenine were removed on a filter, and the filtrate was evapd to dryness.[16c] The residue was triturated with 200 ml of hot EtOH and filtered to collect the insol product; yield 785 mg; mp 202–203° with effervescence. The filtrate gave an addnl 290 mg of product (total 1.075 g, 38.4%), mp 204–205° with effervescence. The anal. sample was prepd by dissolving the product in solvent H at room temp, followed by cooling the soln at 0° for several days: mp 213–214°; ir max in cm^{-1}, 3250 (OH), 1710 (ureido NHCONH), 1610, and 1540 (C=C, C=N); nmr δ 1.53 (d, 3, *J* = 6 Hz, CH_3), 4.57 (m, 1, α-CH), 4.68 (m, 1, βCH), 8.75 (s, 1, 8-H), and 8.89 ppm (s, 1, 2-H); $[\alpha]^{25}D$ + 30.0° (*c* 0.4, H_2O) and +18.0° (*c* 0.5, DMSO); mass spectrum *m/e* 208 (parent peak), 244, 162, 161, 135, 119, and 108. *Anal.* ($C_{10}H_{12}N_6O_4$) C, H, N.

PCT has a tendency to gel when crystd from aq or alc solns. Thus, some batches of crude product were purified by silica gel column chromatog (100–200 mesh, 2.5 × 120 cm, prepacked in EtOAc, and eluted with solvent H). The fractions between 720 and 1100 ml (containing PCT) were combined, and evapd to dryness. The residue was triturated with pet ether (30–60°) giving pure white product.

***N*-(Purin-6-ylcarbamoyl)-D-threonine.**—A stirred mixt of 1.035 g (5 mmoles) of VI and 1.19 g (10 mmoles) of D-threonine in 50 ml of anhyd pyridine was heated in a glass bomb at 120° for 6 hr. After cooling to room temp overnight, the unreacted threonine and adenine were removed on a filter, and the filtrate was evapd to dryness.[16c] The residue was triturated with a mixt of 30 ml of EtOH and 30 ml of solvent H and filtered to collect the insol product: yield 448 mg; mp 197–198° with effervescence. The filtrate gave an addnl 160 mg of product (total 608 mg, 43.5%); mp 195–196° with effervescence; ir max in cm^{-1}, 3200 (OH), 1710 (ureido NHCONH), 1610, and 1540 (C=C, C=N); nmr δ 1.43 (d, 3, *J* = 6 Hz, CH_3), 4.56 (m, 2, α-CH and β-CH), 9.06 (s, 1, 8-H), and 9.18 ppm (s, 1, 2-H); $[\alpha]^{25}D$ −37.2° (*c* 0.160, H_2O) and −18.8° (*c* 0.311, DMSO). *Anal.* ($C_{10}H_{12}N_6O_4$) C, H, N.

***N*-[9-(β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-L-threonine (PCTR, II).**—A stirred mixt of 930 mg (2 mmoles) of XI, 476 mg (4 mmoles) of L-threonine, and 30 ml of pyridine[16f] was heated in a glass bomb at 100° for 5 hr. After bringing to room temp, the unreacted threonine (250 mg) was removed by filtration. The filtrate was evapd *in vacuo*.[16c] The residue was stirred in 100 ml of 4.5 *N* NH_3–MeOH at room temp for 20 hr, and the solvent was evapd to dryness. The oily residue was dissolved in 100 ml of hot EtOH. The soln was diluted with 100 ml of EtOAc, and cooled to 4° for several hr. The white amorphous powder[16c] was collected on a filter, and washed with cold EtOAc (30 ml) and MeCN (20 ml): yield 315 mg; mp 182–188°. An addnl 59 mg (mp 185–189°) of the product was obtained from the filtrate; total 374 mg (45.4%). The anal. sample was prepd by crystn[16g] first from 1:3 EtOH–pet ether (bp 30–60°), and then from a large excess of MeCN, mp 204–207°. The mp of the anal. samples varied from 190° to 207°: ir max in cm^{-1}, 3300 (OH), 1700, 1680 (ureido, NHCONH), 1620, and 1590 (C=C, C=N, C=O); nmr δ 1.56 (d, 3, *J* = 6 Hz, CH_3), 5.0–4.3 (m, 3′-H, =CH, 2′-H, 4′-H,5′-H), 6.48 (d, 1, *J* = 5.5 Hz, 1′-H), 8.93 (s, 1, 2-H), 9.05 (s, 1, 8-H), and 10.18 ppm (s, 2, NH); $[\alpha]^{25}D$ −13.9° (*c* 1.005, DMSO); mass spectrum *m/e* 206 (P-106), 164, 136, and 108. *Anal.* ($C_{15}H_{20}N_6O_8$) C, H, N.

***N*-(Purin-6-ylcarbamoyl)glycine (PCG, IV).**—A mixture of 1.04 g (5.0 mmoles) of VI and 0.75 g (10 mmoles) of glycine in 50 ml of pyridine[16f] was heated with stirring in a glass bomb at 115–120° for 7 hr. After cooling to room temp, the white, pptd material (1.27 g) was collected on a filter and washed with pyridine and EtOH. The crude product was dissolved in 200 ml of cold aq 0.1 *N* NaOH, treated with charcoal, and filtered. The filtrate was acidified to pH 4.5 with 2 *N* HCl, and the unreacted urethane VI (50 mg) was removed by filtration. When the filtrate was further acidified to pH 2, the white product pptd; it was collected on a filter and washed with cold H_2O and EtOH; yield 475 mg; mp 233–234° dec. An addnl 131 mg of the product (mp 230–232° dec) was obtained from the filtrate; total 606 mg (51.5%). The anal. sample was prepd by crystn from a large excess of boiling EtOH: tlc (R_f × 100), solvent A, 51.0; ir max cm^{-1}, 1660 (ureido, NHCONH) and 1603 (C=C, C=N, C=O); nmr δ 4.33 (d, 2, *J* = 5.5 Hz, CH_2), 8.73 (s, 1, 8-H), 8.86 (s, 1, 2-H), 9.34 (broad NH), and 10.01 ppm (s, 1, NH); mass spectrum *m/e* 218 (P-18), 162, 161, 135, 119, and 108. *Anal.* ($C_8H_8N_6O_3$) C, H, N.

***N*-[9-(β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]glycine**

(**PCGR, III**).—A stirred mixt of 930 mg (2.0 mmoles) of XI and 300 mg (4 mmoles) of glycine in 40 ml of pyridine was heated in a glass bomb at 115° for 6 hr. After cooling to room temp, the excess glycine was removed on a filter, and the filtrate was evapd *in vacuo*.[16e] The residue was stirred in 100 ml of 4.5 *N* NH_3–MeOH for 20 hr, and the solvent was evapd to dryness. The residue was crystd from 300 ml of hot EtOH, cooled at 5° for 2 days: yield 380 mg (52%); mp 214–216° with effervescence; tlc ($R_f \times 100$) in solvent C, 41; ir max in cm^{-1}, 3300 (OH), 1680 (ureido, NHCONH), 1620, 1590 (C=C, C=N, C=O), 1240 (OH), and 1120–1040 (COC); nmr (internal TMS) δ 3.80–3.64 (m, 4, CH_2 and 5′-H), 4.62–4.00 (m, 3, 2′-H, 3′-H, 4′-H) 6.02 (d, 1, $J_{1',2'}$ = 5.5 Hz, 1′-H), 8.55 (s, 1, 2-H), 8.67 (s, 1, 8-H), 263, 218, 190, and 9.54 ppm (broad NH); mass spectrum *m/e* 294 (P-74), 293, 178, 164, 162, 161, and 135. *Anal.* ($C_{13}H_{16}N_6O_7$) C, H, N.

Isocyanate of *O*-Benzyl-L-threonine Benzyl Ester (XIII).—$COCl_2$ was bubbled into a stirred suspension of 1.72 g (5.0 mmoles) of *O*-benzyl-L-threonine benzyl ester oxalate (1:1) in 38 ml of anhyd PhMe. The suspension was brought to 80° during the first hour, then stirred at this temp for an addnl 4 hr while $COCl_2$ was bubbled in. N_2 was passed through the reaction mixt at room temp till the $COCl_2$ test was neg. The soln was then evapd to dryness, giving a crude, yellow, syrupy isocyanate; yield 1.38 g (84.7%). The crude material,[16h] when distd, gave a colorless product: yield 39.2%, bp 172–174° (0.1 mm); ir (film) max in cm^{-1}, 2300 (N=C=O), and 1765 (C=O ester); $[\alpha]^{25}D$ −20.1° (*c* 1.14, toluene). *Anal.* ($C_{19}H_{19}NO_4$) C, H, N.

***N*-[9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl-9*H*-purin-6-yl)-carbamoyl]-*O*-benzyl-L-threonine Benzyl Ester (XIV).**—To a soln of 5.78 g (17.6 mmoles) of the crude isocyanate XIII in 150 ml of anhyd PhMe was added 2.88 g (7.32 mmoles) of TAA (XII). The mixt was stirred at 85 ± 2° for 18 hr, then cooled. Unreacted TAA (216 mg) was removed by filtration, and the filtrate was evapd to a pale brown syrup[16i] (10.4 g). The crude material was applied to a silica gel column (2.54 × 72 cm, 177 g, Brinkman silica gel, particle size <0.08 mm). The material was eluted with 2% EtOH in EtOAc (1180 ml). Fractions between 260 and 420 ml were combined and evapd to dryness (6.26 g). This material was repurified on a second silica gel column (same size as before), using EtOAc (neat) for elution. The fractions between 300 and 620 ml contained pure material, which upon evapn of the solvent, gave white foam, 2.31 g (48%). Fractions between 600 and 2000 ml contained 0.89 g of addnl material, which, however, was contaminated with non-uv-absorbing material: ir (film) max cm^{-1}, 1765 (C=O ester), 1720 (C=O urea); $[\alpha]^{25}D$ +5° (*c* 1.0, DMSO). *Anal.* ($C_{35}H_{33}N_6O_{11}$) C, H, N.

***N*-(Purin-6-ylcarbamoyl)-L-threonine (PCT, I).**—Into a soln of 816 mg (1.14 mmoles) of the blocked nucleoside XIV in CF_3COOH (20 ml) was bubbled HBr at 25° for 1.5 hr. The soln was allowed to stir at room temp for an addnl hr, then evapd to dryness. The resulting dark brown syrup was triturated with THF–Et_2O (1:1), and the insol fine product (288 mg) was collected by centrifugation and dried *in vacuo* for 6 hr at room temp. The crude material (288 mg) was treated with charcoal in H_2O at pH 4, then evapd to dryness. The residue was crystd from 20 ml of hot MeOH: yield 130 mg (39.6%) of powder; mp 195–210°; one spot, tlc, solvent A. The material was recrystd twice from 8 ml of MeOH–H_2O (5:3), giving 51 mg of white product, mp 218–220°. The filtrate yielded an addnl 50 mg of product: mp 210–213° (total 101 mg, 30.8%); $[\alpha]^{25}D$ +30° (*c* 0.12, H_2O). The ir and uv spectra and the mobilities in paper chromatog for PCT obtained by this procedure were identical with those for PCT synthesized *via* the urethane, ethyl purine-6-carbamate. *Anal.* ($C_{10}H_{12}N_6O_4 \cdot 0.5H_2O$) C, H, N.

PCT is somewhat hygroscopic, and usually the mp varied between 190 and 213°. The anhyd amorphous product melted at 215–220°.

Another batch of PCT was purified on a silica gel column, since crystn from hydroxylic solvents often led to gel formation. In this column purification, initial elution was carried out with MeOH–$CHCl_3$ (1:9) in order to remove fast-moving impurities. The pure PCT was eluted with MeOH–$CHCl_3$ (1:1). The yield was 45%.

***N*-[(9-β-D-Ribofuranosyl-9*H*-purin-6-yl)carbamoyl]-L-threonine (PCTR, II).**—Into a stirred soln of 419 mg (0.58 mmole) of the blocked nucleoside XIV (anhyd condns maintained throughout) in 200 ml of liq NH_3, sodium was added from a glass tube just until a deep blue color persisted in the soln for 1 min. Solid $(NH_4)_2SO_4$ was added gradually until the blue color was discharged. The mixt was allowed to boil to a brownish white residue. This material was dissolved in H_2O, and adsorbed onto a charcoal–Celite column (2.54 cm diam, dry-packed with a mixt of 20 g of Celite and 5 g of charcoal). Elution with 800 ml of H_2O removed most of the salt from the product. Uv-absorbing material was eluted in 1.7 l. of 10% NH_3 in 50% aq EtOH. Evapn of the solvent gave 101 mg (40.7%) of product. This desalted material was purified by Celite partition column chromatog (2 × 49 cm), using 800 ml of solvent H for elution. The product was eluted in fractions between 300 and 420 ml. Evapn of the solvent gave 50 mg (20.2%) of white product. This material was triturated with 3 ml of EtOH–MeCN (1:2), and was filtered: yield 30 mg (12.0%); mp 170–190°. (It starts to foam at 145–170°). The material was identical with the PCTR (II) obtained by urethane route in ir and uv spectra and in paper chromatog in 6 solvents.

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