Synthesis and Evaluation of 5-Amino-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine and Certain Related Nucleosides as Inhibitors of Purine Nucleoside Phosphorylase

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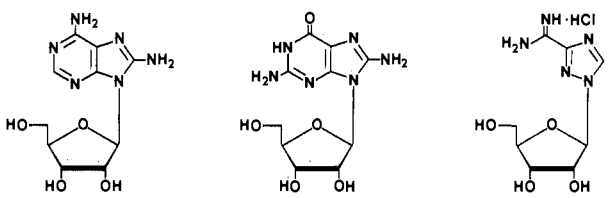
The 5-amino and certain related derivatives of the powerful purine nucleoside phosphorylase (PNPase) inhibitor 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (TCNR, **3**) have been prepared and evaluated for their PNPase activity. Acetylation followed by dehydration of 5-chloro-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (**4a**) gave 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (**5**). Ammonolysis of **5** furnished 5-amino-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine (5-amino-TCNR, **6**), the structure of which was assigned by single-crystal X-ray analysis. Acid-catalyzed fusion of methyl 5-chloro-1,2,4-triazole-3-carboxylate (**7a**) with 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose (**8**) gave methyl 5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**9a**) and the corresponding positional isomer **9b**. Transformation of the functional groups in **9a** afforded a route to 5′-deoxyribavirin (**9i**). Compound **9a** was converted in four steps to 5-amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine (5′-deoxy-5-amino-TCNR, **9g**). Similar acid-catalyzed fusion of 1,2,4-triazole-3-carbonitrile (**7b**) with **8** and ammonolysis of the reaction product **9h** gave yet another route to **9i**. Treatment of **9h** with NH_3/NH_4Cl furnished 1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine (5′-deoxy-TCNR, **9k**). The *C*-nucleoside congener of TCNR (3-β-D-ribofuranosyl-1,2,4-triazole-5-carboxamidine, **12**) was prepared in two steps from 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carbonitrile (**10**) by conventional procedure. 5-Amino-TCNR (**6**) displayed a more potent, high-affinity inhibition than TCNR, with a K_i of 10 μM. In contrast, 5′-deoxy-5-amino-TCNR (**9g**) was a significantly less potent inhibitor of PNPase, compared to 5′-deoxy-TCNR (K_i = 80 and 20 μM, respectively). Neither the *C*-nucleoside congener of TCNR (**12**) nor that of ribavirin were found to inhibit inosine phosphorolysis.

Human purine nucleoside phosphorylase (PNPase) is an essential enzyme of the purine salvage pathway, catalyzing the phosphorolysis of inosine, guanosine, and their 2′-deoxy derivatives to the respective purine bases. The genetic deficiency of PNPase leads to the selective loss of cellular immunity through severely depressed or lack of T-lymphocytic functions.[1] In addition, affected individuals do not excrete purine catabolites as urate but rather in much more water soluble nucleoside forms. We[2] and others[3-5] believe that very potent and specific inhibitors of PNPase may be useful agents in the treatment of T-cell leukemias, suppression of host-versus-graft response in organ transplant recipients, and potentiation of the cytotoxicity of nucleosides which serve as substrates for phosphorolysis, as well as in the treatment of some forms of gouty arthritis.

A number of inhibitors of PNPase have been identified, and most of these agents resemble purine bases[5,6] or their nucleosides. Comparison[7,8] of the inhibitory and substrate specificities of 8-aminoadenosine (**1**) and 8-aminoguanosine (**2**) to those of adenosine and guanosine, respectively, with both the bacterial (adenosine preferring) and mammalian (guanosine preferring) enzymes, recognized the beneficial effects of an amino group at C8 position toward PNPase function.[9,10] Previously, we reported[2] that 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (TCNR, **3**) was a potent inhibitor but poor substrate of mammalian PNPase (EC 2.4.2.1). Since TCNR, like the broad spectrum antiviral agent ribavirin[11] (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), is expected to structurally resemble inosine or guanosine in single-crystal X-ray analysis,[12,13] the synthesis of 5-amino-TCNR (**6**) is of particular interest. We feel that the introduction of an amino group at the 5-position of TCNR would provide a compound of similar structure to 8-aminoguanosine.



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We now report the synthesis and in vitro evaluation of 5-amino-TCNR (**6**), 5′-deoxy-TCNR (**9k**), 5′-deoxy-5-amino-TCNR (**9g**), and the *C*-nucleoside congener of TCNR (**12**) as inhibitors of human lymphoblast PNPase. Certain 5′-deoxyribonucleosides have been shown to be alternative substrates (inhibitors) of PNPase.[14,15] TCNR (**3**), like ribavirin, is converted to the 5′-monophosphate by adenosine kinase. TCNR-monophosphate exerts cytotoxic effects by inhibition of IMP dehydrogenase, which

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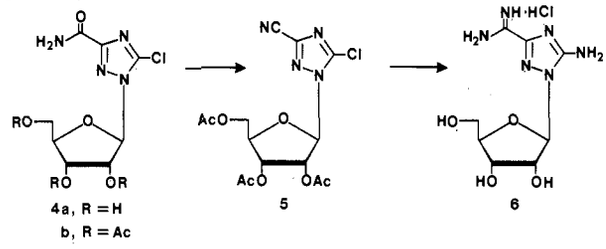
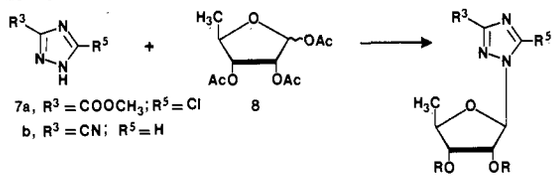
Scheme I



Scheme II



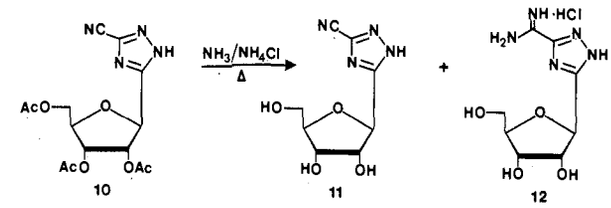
	R^3	R^5	R
9a	$COOCH_3$	Cl	Ac
b	Cl	$COOCH_3$	Ac
c	$CONH_2$	Cl	H
d	Cl	$CONH_2$	H
e	CN	Cl	Ac
f	CN	NH_2	H
g	$C(NH)NH_2$	NH_2	H
h	CN	H	Ac
i	$CONH_2$	H	H
j	$C(NOH)NH_2$	H	H
k	$C(NH)NH_2$	H	H

results in starvation for guanylates, elevation of rate of de novo purine synthesis, and increased rate of excretion of these newly formed purines to the culture medium. Furthermore, the 5′-deoxy congeners would not be expected to be substrates for adenosine kinase.

Chemistry. 5-Amino-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (5-amino-TCNR, **6**) was prepared from the readily available 5-chloro-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[16] (**4a**) (Scheme I). Compound **4a** was prepared as reported from our laboratory[16] and acetylated with acetic anhydride in pyridine to obtain the corresponding tri-*O*-acetyl derivative **4b**. Compound **4b** underwent dehydration with phosgene smoothly to furnish 5-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (**5**) in 95% yield. Transformation of the carbonitrile function of **5** to the amidine with concomitant nucleophilic displacement of the C5 chloro group was achieved in good yield by the treatment of **5** with liquid NH_3 in the presence of NH_4Cl at 110 °C to obtain the desired 5-amino-TCNR (**6**). The structure of **6** was confirmed by single-crystal X-ray analysis.

The synthesis of 5-amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine hydrochloride (5′-deoxy-5-amino-TCNR, **9g**) was accomplished via the acid-catalyzed fusion procedure by utilizing a 3,5-disubstituted 1,2,4-triazole (Scheme II). Fusion of methyl 5-chloro-1,2,4-triazole-3-carboxylate[17] (**7a**) with 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose[18] (**8**) at 120 °C in the presence of an acidic catalyst [bis(*p*-nitrophenyl) phosphate] provided a mixture of two isomeric nucleosides, which were separated by flash chromatography over silica gel. These blocked nucleosides were identified as methyl

Scheme III



5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**9a**) and methyl 3-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylate (**9b**) by conversion of each isomer to a nucleoside of known structure and anomeric configuration. Thus, catalytic (Pd/C) dehalogenation of **9a** followed by ammonolysis provided 5′-deoxyribavirin (**9i**), which was found to be identical with an authentic sample of **9i** prepared from ribavirin.[19] Treatment of the blocked nucleoside **9a** and **9b** with $MeOH/NH_3$ at room temperature provided 5-chloro-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (**9c**) and 3-chloro-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxamide (**9d**), respectively. Acetylation of **9c** in a manner analogous to that for the preparation of **4b** provided 5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, which on subsequent dehydration with phosgene gave the corresponding carbonitrile **9e**. Nucleophilic displacement of the chloro group in **9e** provided a convenient route to the preparation of 5′-deoxy-5-amino-TCNR (**9g**). Thus, treatment of **9e** with liquid NH_3 in the presence of NH_4Cl at 90 °C gave 5-amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine hydrochloride (**9g**) albeit in low yield, along with a 52.7% yield of 5-amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (**9f**).

This acid-catalyzed fusion procedure also proceeded readily with 1,2,4-triazole-3-carbonitrile[20] (**7b**) and **8**. A more than 84% yield of 1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (**9b**) was isolated from the reaction mixture by flash chromatography over silica gel. Compound **9h** was the only nucleoside product that could be detected by TLC or column chromatography procedures. The structure of **9h** was established by conversion of this product to the previously reported[19] carboxamide nucleoside **9i** on treatment with NH_4OH. The addition of NH_2OH to the carbonitrile group of **9h** with concomitant deacetylation of the carbohydrate moiety afforded 1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidoxime (**9j**) in good yield. Attempts to obtain the desired 5′-deoxy-TCNR (**9k**) by treatment of **9h** with NH_3 in a variety of solvents resulted in mixtures of products. However, a 54% yield of **9k**, isolated as the hydrochloride salt, was obtained by heating **9h** with an excess of liquid NH_3 and 1 molar equiv of NH_4Cl at 110 °C.

The *C*-nucleoside congener of TCNR (3-β-D-ribofuranosyl-1,2,4-triazole-5-carboxamidine hydrochloride, **12**) was prepared from the reported[21] 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-carbonitrile (**10**) (Scheme III). Treatment of **10** with NH_3/NH_4Cl in a manner

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Table I. Crystallographic Summary for Compound 6

empirical formula	$[C_8H_{15}N_6O_4]^+Cl^-\cdot 2H_2O$
fw	330.73
crystal system	orthorhombic
space group	$P2_12_12_1$
a, Å	7.0906 (10)
b, Å	12.658 (3)
c, Å	16.275 (3)
V, Å^3	1460.7 (4)
Z	4
F(000), electrons	696
temperature, K	295
ρ_{calcd}, g cm^{-3}	1.504
μ, cm^{-1}	27.027
2θ range, deg	3–152
R, R_w	0.0298, 0.0403
S (goodness of fit)	1.423
R (all data)	0.0365
extinction coefficient	1.40 (13) × 10^{-6}
max, min density in $\Delta\rho$ map, e Å^{-3}	0.19, −0.23
total reflections measured, unique	1757, 1757
reflections used ($F \geq 4\sigma_F$)	1612

analogous to that for the preparation of **9k** provided a mixture of 3-β-D-ribofuranosyl-1,2,4-triazole-5-carbonitrile (**11**) and **12**. The mixture was separated by preparative HPLC using a C-18 reverse-phase column.

Single-Crystal X-ray Diffraction Analysis of 6. A suitable crystal of **6** was grown by slow crystallization from EtOH. An Enraf-Nonius CAD4 diffractometer equipped with graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å) was employed for all work. Lattice parameters were determined by a least-squares refinement of the setting angles of 25 reflections (49.8° < 2θ < 57.6°). Data were measured by an ω-2θ scan technique in which backgrounds were measured for 25% of the scan range before and after the scan. Three check reflections were measured every hour to monitor crystal and instrument stability. Data were corrected for Lorentz, polarization, decay (range, 0.997–1.000), and absorption effects (transmission factor range, 0.570–0.835). Absorption correction was based on crystal shape and size (prism, 0.46 × 0.095 × 0.07 mm). Experimental and crystal data are summarized in Table I.[22]

Figure 1 (produced with ORTEPII)[23] illustrates the molecular conformation. The glycosidic torsion angle O1′–C1′–N1–C5 is −77.4 (3)°, which is considerably different from the 10.4° and 119.0° found in the two crystal forms of ribavirin.[13] The sugar is type N, $C_{3'}$-endo of form $^3T_2$ with a pseudorotation angle of 15.63° and amplitude of pucker of 39.67°,[24] similar to the V1 form of ribavirin. The side chain is *gg* (O1′ torsion angle, −63.6 (3)°; C3′, 54.1 (3)°). The bond lengths in the sugar are similar to those in ribavirin except that C1′–O1′ is 0.03 Å longer while C1′–N1 is 0.03 Å shorter than in the two forms of ribavirin. In all other aspects of geometry and pucker, the sugar resembles closely that of form V1 of ribavirin.[13] The base ring is planar (rms deviation = 0.004 Å); the ring geometry is similar in pattern to the base rings in both forms of ribavirin although the distances involving N1 are longer in the amidine. The amidine moiety including C3 is also planar (rms deviation = 0.001 Å) and is rotated 14.95 (11)° with respect to the triazole ring. The amidine C–N distances differ by 0.013 Å.

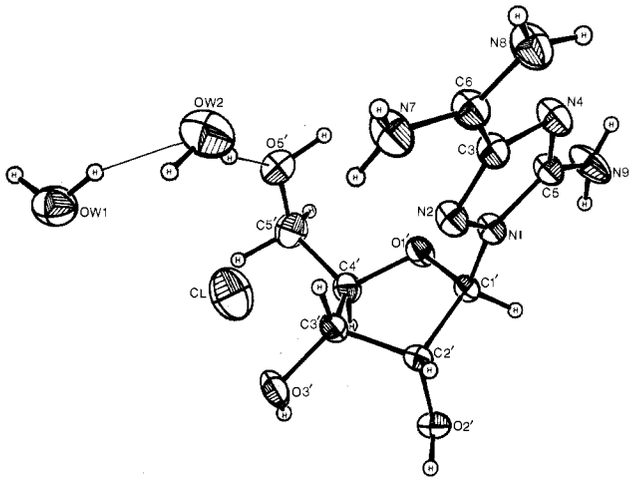

Figure 1. ORTEPII drawing of compound **6**.

Inhibition of Purine Nucleoside Phosphorylase Activity. In a preliminary screen of the 10 compounds studied, ribavirin, 5-aminoribavirin,[16] the *C*-nucleoside of ribavirin,[21] and the *C*-nucleoside of TCNR (**12**) did not provide significant inhibition of PNPase-dependent conversion of inosine to hypoxanthine. The inhibitory properties of the active compounds were evaluated with inosine as substrate at 10 and 20 μM and test compounds at 50, 100, 150, and 200 μM and the results analyzed by Dixon plots[25] are summarized in Table II. The apparent K_i value refers to the (−*I*) point of intercept of the slopes generated from the data with 10 and 20 μM inosine. The nature of the inhibition was determined from the pattern of the slopes, competitive inhibition indicated by linear slopes, complex inhibition (i.e., both competitive and noncompetitive components) by curvilinear slopes.

As previously described,[2,10] the slopes of the inhibition of PNPase by 8-aminoguanosine (**2**) and TCNR (**3**) were curvilinear. These compounds appeared to be potent inhibitors or competitors of inosine phosphorolysis at low inhibitor concentrations, but with increasing inhibitor concentration, the potency of the inhibition or competition apparently decreased to yield a low-affinity component. As noted previously,[2] complex inhibition may result from substrate or pseudosubstrate activation of the enzyme, or preparations of PNPase containing a mixture of isozymes with overlapping specificity but differing affinities for the substrates and inhibitors.[10,26,27] For 8-aminoguanosine and TCNR, the high-affinity component of the curves extrapolated to apparent K_i values of ≤5 μM (lower limit of sensitivity) and 30 μM, respectively. 5-Amino-TCNR (**6**) displayed a more potent, high-affinity inhibition than TCNR, with an apparent K_i value of 10 μM. Thus, the introduction of an amino group at the 5-position of TCNR resulted in an effect similar to the substitution of an amino group at the 8-position of guanosine.

The results with the 5′-deoxy congeners clearly showed competitive inhibition by Dixon plots. However, 5′-deoxy-5-amino-TCNR (**9g**) was a significantly less potent inhibitor than 5′-deoxy-TCNR (**9k**), for which the K_i values were 80 and 20 μM, respectively. A similar result was observed with 5-aminoribavirin. Ribavirin is both a weak

(22) Data reduction was performed with the Enraf-Nonius SDP-Plus program package (Frenz, B. A. *Enraf-Nonius SDP-Plus Structure Determination Package. Version 3.0*; Enraf-Nonius, Delft, 1985).
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Table II. Effects of Compounds on PNPase and Purine Nucleotide Synthesis

compd	PNPase inhibn, type	PNPase inhibn, K_i, μM	purine synthesis and distribn: total purine synthesized,[f] % of control culture	purine synthesis and distribn: medium purine accumulation,[f] % of total synthesized	cell A/G ratio[g]
8-aminoguanosine (**2**)	complex	<5	98	25	1.3
TCNR (**3**)	complex	30	98	18	2.1
ribavirin[a]	*e*		155	74	3.0
5-amino-TCNR (**6**)	complex	10	82	17	1.3
5-aminoribavirin[b]	*e*		88	11	1.3
5'-deoxy-TCNR (**9k**)	competitive	20	85	16	1.5
5'-deoxy-5-amino-TCNR (**9g**)	competitive	80	90	15	1.2
C-nucleoside of TCNR (**12**)	*e*		48	28	2.4
C-nucleoside of ribavirin[c]	*e*		95	10	1.3
formycin B[d]	competitive	110	12	30	–

[a] Reference 34. [b] Reference 16. [c] Reference 21. [d] Reference 35. [e] Not determined. [f] Samples from control cultures synthesized 25112 ± 1000 (N = 3) cpm/mL total purine, 2638 ± 300 or 10.5% of the total purine synthesized was contained in the media fraction. [g] A/G ratio of the cell fraction of control cultures = 1.3.

inhibitor of inosine phosphorolysis and a poor substrate for phosphorolysis; 5-aminoribavirin, as mentioned above, is neither an inhibitor nor a substrate for phosphorolysis. Formycin B, an example of a *C*-nucleoside congener, displayed linear inhibition patterns, and a K_i value of 110 μM was determined.

Substrate Activity. The compounds synthesized for this project had no activity as substrates for PNPase.

Cytotoxicity. Compounds were evaluated for their cytotoxic or cytostatic properties against the human B-lymphoblast line WI-L2. None of the compounds synthesized for this study were found to inhibit the growth of the WI-L2 line at 100 μM. As reported previously,[2] formycin B, ribavirin, and TCNR had ID_{50} values of 20, 50, and 100 μM, respectively.

Biological Activity. A compound may have considerable metabolic effects, but may not demonstrate growth inhibition or cytotoxicity. For example, the genetic deficiency of PNPase is usually neither cytotoxic nor cytostatic, thus an inhibitor of this enzyme may not be expected to display these properties. On the other hand, compounds that inhibit PNPase can be detected by measuring [^{14}C]formate incorporation into purines synthesized de novo.[28] PNPase deficiency is indicated by an increased accumulation of newly synthesized [^{14}C]purine in the culture medium. Further, compounds that interact with regulatory or substrate sites of enzymes such as IMP dehydrogenase or adenylosuccinate synthetase cause alterations in the distribution of purine nucleotides synthesized de novo which can be detected by changes in the adenylate to guanylate (A/G) ratios as well as elevated rate of accumulation of these newly synthesized purines in the culture medium.[29-31]

Our studies, summarized in Table II, were performed in vitro by employing WI-L2 cells.[2] No attempt was made to reduce the noncellular contribution of PNPase activity, i.e., the activity contributed by the fetal bovine serum of the culture media.[32,33] The well-established inhibitor[2,10] 8-aminoguanosine served as a positive control for inhibition of PNPase activity, and the inhibition is indicated by the increased accumulation of newly formed purine in the culture medium. As with the genetic deficiency of PNPase, the increased accumulation of medium purine with the inhibitor-dependent deficiency results from a normal rate of dephosphorylation of purine mononucleotides concomitant with a diminished rate of PNPase dependent purine salvage.[28] Control cultures accumulated 9% of the newly formed purine in the cell culture medium. Those cultures receiving 8-aminoguanosine accumulated 25% of their newly formed purine in the medium. TCNR (**3**), 5-amino-TCNR (**6**), and 5'-deoxy-TCNR (**9k**) each caused an increased accumulation of [^{14}C]purine in the medium, suggesting that each was a functional PNPase inhibitor.

After conversion to the nucleoside monophosphate by adenosine kinase, both ribavirin[30] and TCNR[2] inhibit IMP dehydrogenase, causing a decrease in the guanylate pools of cells and consequently a diminished feedback inhibition of the de novo purine biosynthetic pathway. With the accumulation of IMP in excess of the demands of adenylosuccinate synthetase, IMP is dephosphorylated to inosine by 5'-nucleotidase. This results in an increase (both medium and cellular) of inosine and hypoxanthine, the product of nucleoside phosphorylase activity. Ribavirin serves as the positive control for this mechanism of purine accumulation in the culture medium. Incubation of cultures with ribavirin results in a 55% increase (over control values) in the total amount of purine synthesized; 74% of the purine thus synthesized accumulates in the medium principally as hypoxanthine. Less than 10% of the purine accumulated as inosine, which indicates that a mechanism other than inhibition of PNPase is responsible for the increased accumulation. The adenylate to guanylate (A/G) ratio of 3 for the cell fraction of the ribavirin-containing cultures an A/G ratio of 1.2 for the control cells indicates that guanylate synthesis is preferentially inhibited and, consequently, excess purine nucleotide is synthesized and degraded to the nucleoside and the base. TCNR, likewise, causes an elevated A/G ratio consistent with its inhibition of IMP dehydrogenase. As observed in our previous work,[2] PNPase is also inhibited in these TCNR-containing cultures since inosine constitutes a major fraction of the pu-

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rine accumulated in the medium. The A/G ratios of the cell fraction of cultures containing 5-amino-TCNR (1.3), 5′-deoxy-TCNR (1.5), and 5-amino-5′-deoxy-TCNR (1.2) are not significantly different from the control culture value, suggesting that the purine accumulating in the medium of these cultures results from inhibition of PNPase.

The *C*-nucleoside congener of TCNR (**12**) displayed some interesting properties in this analysis. De novo purine synthesis is inhibited to 48% of the value found in the control cultures. The A/G ratio of 2.4 is markedly different from that of the control cultures, but the amount of purine accumulated in the medium is not different from that observed in the control cultures. In contrast, the *C*-nucleoside congener of ribavirin appears to have no effect on purine biosynthesis and distribution. An unidentified compound containing [^{14}C]formate label accumulates in the medium of cultures containing the *C*-nucleoside of TCNR. This [^{14}C]formate-labeled compound also accumulates in the culture media containing formycin B. The identity and mechanism responsible for the accumulation of this compound are currently under investigation.

Experimental Section

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting point apparatus. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ. Thin-layer chromatography (TLC) was conducted on plates of silica gel 60 F-254 (EM Reagents). Silica gel (E. Merck; 230–400 mesh) was used for flash column chromatography. All solvents used were reagent grade. Detection of nucleoside components in TLC was by UV light, and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were conducted under diminished pressure with the bath temperature below 30 °C. Infrared (IR) spectra were recorded with a Beckman Acculab 2 spectrophotometer. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz with an IBM NR/300 spectrometer. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H_2O as indicated by elemental analysis was verified by ^{1}H NMR spectroscopy.

5-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (4b). A mixture of 5-chloro-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide[16] (**4a**, 5.57 g, 20 mmol) and acetic anhydride (10 mL) in anhydrous pyridine (100 mL) was stirred for 18 h at ambient temperature. The reaction mixture was evaporated to dryness, and the residue was coevaporated with 50% aqueous ethanol (3 × 50 mL). The residual solid was triturated with absolute EtOH (50 mL) and left overnight at ambient temperature. The crystals that deposited were collected by filtration, washed with cold (0–5 °C) EtOH (2 × 10 mL), and recrystallized from EtOH to yield 4.0 g (50%) of **4b**: mp 183–185 °C; IR (KBr) ν_{max} 850 (CCl), 1700 (C=O of amide), 1735 (C=O of acetyl), and 3300–3450 (NH_2) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.96–2.08 (3 s, 9, 3 COCH_3), 6.20 (d, 1, $J_{1',2'}$ = 2.1 Hz, C$_{1'}H$), and 7.84 and 8.07 (2 **br** s, CONH_2). Anal. ($C_{14}H_{17}ClN_4O_8$, MW 404.76) C, H, N, Cl.

5-Chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (5). To an ice-cold solution of **4b** (1.01 g, 2.5 mmol) in CH_2Cl_2 (40 mL) and pyridine (5 mL) was added 20% phosgene in toluene (6 mL) dropwise with stirring. After the addition was complete (10 min), the reaction mixture was allowed to warm to room temperature. After the mixture was stirred at room temperature for 2 h, the resulting dark red solution was poured onto crushed ice (100 g) and extracted with CH_2Cl_2 (3 × 60 mL). The combined organic layers were washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified on a flash silica gel column (2 × 40 cm), with $CHCl_3$/MeOH (99:1, v/v) as eluent. The homogeneous product was crystallized from a mixture of ether/hexane to yield 0.92 g (95%) of **5** as needles: mp 76–78 °C; IR (KBr) ν_{max} 825 (CCl), 1735 (C=O of acetyl), and 2250 (C≡N) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.95–2.10 (3 s, 9, 3 COCH_3) and 6.28 (d, 1, $J_{1',2'}$ = 2.2 Hz, C$_{1'}H$). Anal. ($C_{14}H_{15}ClN_4O_7$, MW 386.75) C, H, N, Cl.

5-Amino-1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine Hydrochloride (5-Amino-TCNR, 6). A mixture of **5** (1.06 g, 2.75 mmol), NH_4Cl (0.15 g, 2.75 mmol), and liquid NH_3 (70 mL) was heated in a stainless steel reaction vessel (150 mL) at 110 °C for 24 h. After removal of excess NH_3, the residue was dissolved in MeOH and adsorbed onto silica gel (5 g). The silica gel was loaded on top of a flash silica gel column (3 × 30 cm) packed in $CHCl_3$ and eluted with $CHCl_3$/MeOH (9:1, 85:15, v/v). Fractions containing the pure product were pooled and evaporated to dryness. Crystallization of the residue from absolute EtOH gave 0.55 g (68%) of **6**: mp 222–224 °C dec; IR (KBr) ν_{max} 1665 (>C=NH) and 3200–3500 (OH, NH_2) cm^{-1}; UV (pH 1) λ_{max} 268 nm (ϵ 2700); (pH 7) UV λ_{max} 269 nm (ϵ 3100); UV (pH 11) λ_{max} 225 nm (sh) (ϵ 4200); ^{1}H NMR (Me_2SO-d_6) δ 5.73 (d, 1, $J_{1',2'}$ = 4.6 Hz, C$_{1'}H$), 7.09 (s, 2, C_5NH_2), and 9.22 [br s, 2, C(NH)NH_2]. Anal. ($C_8H_{15}ClN_6O_4$, MW 294.7) C, H, N, Cl.

Methyl 5-Chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (9a) and Methyl 3-Chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylate (9b). A mixture of methyl 5-chloro-1,2,4-triazole-3-carboxylate[17] (**7a**, 1.61 g, 10 mmol) and 5-deoxy-1,2,3-tri-*O*-acetyl-D-ribofuranose[18] (**8**, 2.60 g, 10 mmol) was heated in an oil bath maintained at 120 °C, until a clear melt was obtained. Bis(*p*-nitrophenyl) phosphate (50 mg) was added with stirring, and the heating was continued at 120 °C under diminished pressure for 45 min. The cooled reaction mixture was dissolved in $CHCl_3$ (200 mL), and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (2 × 50 mL), followed by water (2 × 50 mL). After drying (Na_2SO_4), the $CHCl_3$ was evaporated to dryness and the residue was purified on a flash silica gel column (2.5 × 40 cm) by using toluene/acetone (8:1, v/v), which provided two products in the following order. (i) Methyl 3-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxylate (**9b**; 0.91 g, 25.2%): R_f 0.44; mp 74 °C; IR (KBr) ν_{max} 845 (CCl), 1735 and 1755 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, 3, J = 6.4 Hz, C$_{5'}CH_3$), 2.14 and 2.15 (2 s, 6, 2 OCOCH_3), 4.04 (s, 3, OCH_3), 4.38 (m, 1, C$_{4'}H$), 5.40 (m, 1, C$_{3'}H$), 5.80 (m, 1, C$_{2'}H$), 6.87 (d, 1, J = 2.6 Hz, C$_{1'}H$). Anal. ($C_{13}H_{16}ClN_3O_7$, MW 361.73) C, H, N.

(ii) Methyl 5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxylate (**9a**; 1.21 g, 33.5%): R_f 0.38; mp 56 °C dec; IR (KBr) ν_{max} 840 (CCl), 1730 and 1760 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.44 (d, 3, J = 6.4 Hz, C$_{5'}CH_3$), 2.11 and 2.12 (2 s, 6, 2 OCOCH_3), 3.99 (s, 3, OCH_3), 4.36 (m, 1, C$_{4'}H$), 5.37 (t, 1, C$_{3'}H$), 5.83 (t, 1, C$_{2'}H$), 6.04 (d, 1, J = 3.7 Hz, C$_{1'}H$). Anal. ($C_{13}H_{16}ClN_3O_7$, MW 361.73) C, H, N, Cl.

5-Chloro-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (9c). A solution of **9a** (1.80 g, 5 mmol) in MeOH/NH_3 (150 mL, saturated at 0 °C) was kept in a pressure flask at 25 °C for 48 h. The solvent was removed, and the residue was chromatographed on a column of silica gel (2.5 × 35 cm) with EtOAc/MeOH (19:1, v/v) as the eluent to provide 1.20 g (92%) of **9c**: mp 155–157 °C; IR (KBr) ν_{max} 835 (CCl), 1680 (C=O of amide), and 3250–3420 (OH, NH_2) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.22 (d, 3, J = 5.8 Hz, C$_{5'}CH_3$), 4.04–4.49 (m, 3, C$_{2',3',4'}H$), 5.27, 5.63 (2 d, 2, J = 4.7 Hz, C$_{2',3'}OH$), 5.75 (d, 1, $J_{1',2'}$ = 3.5 Hz, C$_{1'}H$), and 7.78, 8.03 (2 br s, 2, CONH_2). Anal. ($C_8H_{11}ClN_4O_4$, MW 262.65) C, H, N, Cl.

3-Chloro-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-5-carboxamide (9d). The title compound was prepared in a similar manner as described for **9c**, by using **9b** (1.0 g, 2.76 mmol) and MeOH/NH_3 (50 mL). The product was isolated as a crystalline solid (EtOH/H_2O) to yield 0.62 g (85%): mp 203–205 °C; IR (KBr) ν_{max} 835 (CCl), 1670 (C=O of amide), and 3240–3470 (OH, NH_2) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.21 (d, 3, J = 5.8 Hz, C$_{5'}CH_3$), 3.92–4.36 (m, 3, C$_{2',3',4'}H$), 5.17, 5.50 (2 d, 2, J = 4.7 Hz, C$_{2',3'}OH$), 6.68 (d, 1, $J_{1',2'}$ = 2.8 Hz, C$_{1'}H$), and 8.19, 8.46 (2 **br** s, 2, CONH_2). Anal. ($C_8H_{11}ClN_4O_4$, MW 262.65) C, H, N, Cl.

5-Chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (9e). In the same manner as for **4b**, acetylation of **9c** (0.83 g, 3.16 mmol) with acetic anydride (2 mL) in pyridine (10 mL) gave syrupy 5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (0.97 g, 89%), dehydration of which as described for **5** using 20% phosgene in toluene (5 mL) gave the title compound as a crys-

talline solid: 0.55 g (59.8%); mp 84–85 °C; IR (KBr) ν_{max} 855 (CCl), 1750 (C=O of acetyl), and 2260 (C≡N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.30 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 2.07, 2.09 (2 s, 6, 2 $COCH_3$), 4.33, 5.23, 5.66 (m, 3, $C_{2',3',4'}H$), 6.20 (d, 1, $J_{1',2'}$ = 2.0 Hz, $C_{1'}H$). Anal. ($C_{12}H_{13}ClN_4O_5$, MW 328.70) C, H, N, Cl.

5-Amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (9f) and 5-Amino-1-(5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine Hydrochloride (5'-Deoxy-5-amino-TCNR, 9g). A mixture of **9e** (0.50 g, 1.6 mmol), NH_4Cl (85 mg, 1.6 mmol), and liquid NH_3 (20 mL) was heated in a stainless steel reaction vessel (50 mL) at 90 °C for 18 h. The vessel was cooled in a dry ice/acetone bath and opened, and NH_3 was allowed to evaporate. The residual solid was purified on a flash silica gel column (2 × 25 cm) by using EtOAc/H_2O/MeOH/acetone (3:1:1:1, v/v), which provided two products in the following order. (i) Compound **9f** (0.19 g, 52.7%): mp 174–175 °C; IR (KBr) ν_{max} 2250 (C≡N) and 3200–3380 (OH, NH_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.18 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 3.87–4.35 (m, 3, $C_{2',3',4'}H$), 5.16, 5.45 (2 d, 2, J = 5.0 Hz, $C_{2'}$ and $C_{3'}OH$), 5.70 (d, 1, $J_{1',2'}$ = 3.2 Hz, $C_{1'}H$), and 7.20 (br s, 2, C_5NH_2). Anal. ($C_8H_{11}N_5O_3$, MW 225.21) C, H, N.

(ii) Compound **9g** (60 mg, 13.6%): mp 74 °C; IR (KBr) ν_{max} 1640 (>C=NH) and 3150–3400 (OH, NH_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.19 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 3.81–4.39 (m, 3, $C_{2',3',4'}H$), 5.75 (d, 1, $J_{1',2'}$ = 2.9 Hz, $C_{1'}H$), 7.06 (br s, 2, C_5NH_2), and 9.15 [br s, 2, C(NH)NH_2]. Anal. ($C_8H_{15}ClN_6O_3 \cdot H_2O$, MW 278.70) C, H, N.

1-(2,3-Di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (9h). A mixture of 1,2,4-triazole-3-carbonitrile[20] (**7b**, 0.94 g, 10 mmol) and **8** (2.6 g, 10 mmol) was heated in an oil bath maintained at 150 °C. Bis(*p*-nitrophenyl) phosphate (15 mg) was added with stirring; heating at 150 °C under diminished pressure was continued for 30 min. The cooled reaction mixture was dissolved in $CHCl_3$ (100 mL), and the organic phase was washed with saturated aqueous $NaHCO_3$ solution (2 × 25 mL), followed by water (2 × 25 mL). After drying (Na_2SO_4), the $CHCl_3$ was evaporated to dryness and the residue was purified on a flash silica gel column (2 × 20 cm) with toluene/acetone (8:1, v/v) as the eluent to provide 2.48 g (84.3%) of **9h** as homogeneous foam: IR (KBr) ν_{max} 1690 (C=O) and 2210 (C≡N) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.46 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 2.13 (s, 6, 2 $COCH_3$), 4.39–5.67 (m, 3, $C_{2',3',4'}H$), 5.96 (d, 1, $J_{1',2'}$ = 3.2 Hz, $C_{1'}H$), and 8.34 (s, 1, C_5H). Anal. ($C_{12}H_{14}N_4O_5 \cdot {}^1/_2H_2O$, MW 294.26) C, H, N.

1-(5-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (5'-Deoxyribavirin, 9i). A mixture of **9h** (1.47 g, 5 mmol) and concentrated NH_4OH (20 mL) was heated on a steam bath for 1 h. The solvent was removed, and the residue was crystallized from aqueous EtOH as needles to give 0.90 g (79%) of **9i**: mp 132 °C; IR (KBr) ν_{max} 1725 (C=O) and 3150–3400 (NH_2, OH) cm^{-1}; UV (pH 1) λ_{max} 213 nm (ϵ 6500); UV (pH 7) λ_{max} 208 nm (ϵ 9000); UV (pH 11) λ_{max} 204 nm (ϵ 15 500); ^1H NMR (Me_2SO-d_6) δ 1.25 (d, 3, J = 5.6 Hz, $C_{5'}CH_3$), 3.98 (m, 2, $C_{3',4'}H$), 4.35 (m, 1, $C_{2'}H$), 5.20 and 5.56 (2 d, 2, J = 5.0 Hz, $C_{2',3'}OH$), 5.80 (d, 1, J = 2.9 Hz, $C_{1'}H$), 7.64 and 7.85 (2 br s, 2, $CONH_2$), and 8.83 (s, 1, C_5H). Anal. ($C_8H_{12}N_4O_4$, MW 228.21) C, H, N. Compound **9i** was identical with an authentic sample of 5'-deoxyribavirin prepared from ribavirin.[19]

1-(5-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidoxime (9j). A solution of **9h** (0.50 g, 1.7 mmol) and free NH_2OH (1 g) in EtOH (15 mL) was stirred at ambient temperature for 16 h. The solvent was removed, and the product was crystallized from water to provide 0.28 g (67.6%) of **9j**: mp 184–186 °C; IR (KBr) ν_{max} 3380–3460 (OH, NH_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.26 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 3.99–4.34 (m, 3, $C_{2',3',4'}H$), 5.21, 5.56 (br s, 2, $C_{2',3'}OH$), 5.64 [br s, 2, C(NOH)NH_2], 5.77 (d, 1, $J_{1',2'}$ = 3.2 Hz, $C_{1'}H$), 8.76 (s, 1, C_5H), and 9.81 [br s, 1, C(NOH)NH_2]. Anal. ($C_8H_{13}N_5O_4$, MW 243.22) C, H, N.

1-(5-Deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamidine Hydrochloride (5'-Deoxy-TCNR, 9k). 9k). In a similar manner as for **6**, the title compound was prepared by using **9h** (0.66 g, 2.24 mmol), liquid NH_3 (25 mL), and NH_4Cl (0.12 g, 2.25 mmol). The product was isolated as homogeneous foam: 0.32 g (54%); mp 85 °C (sinters); IR (KBr) ν_{max} 3300–3450 (OH, NH_2) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.29 (d, 3, J = 5.8 Hz, $C_{5'}CH_3$), 4.04–4.41 (m, 3, $C_{2',3',4'}H$), 5.0–6.0 (br s, 2, $C_{2',3'}OH$), 5.90 (d, 1, $J_{1',2'}$ = 3.2 Hz, $C_{1'}H$), 9.16 (s, 1, C_5H), 9.57 [br s, 2, C(NH)NH_2]. Anal. ($C_8H_{14}ClN_5O_3$, MW 263.68) C, H, N, Cl.

3-β-D-Ribofuranosyl-1,2,4-triazole-5-carbonitrile (11) and 3-β-D-Ribofuranosyl-1,2,4-triazole-5-carboxamidine Hydrochloride (12). A mixture of 3-(2,3,5-tri-*O*-acetyl-β-D-ribofuransoyl)-1,2,4-triazole-5-carbonitrile[21] (**10**, 1.48 g, 4.96 mmol), NH_4Cl (0.29 g, 5.5 mmol), and liquid NH_3 (30 mL) was heated in a stainless steel reaction vessel (75 mL) at 100 °C for 24 h. After removal of NH_3, the residue was purified by preparative HPLC using a C-18 reverse-phase column (1% AcOH), which provided two products. The appropriate homogeneous fractions were collected and lyophilized to provide 0.72 g (64%) of **11**: mp 138 °C (sinters); IR (KBr) ν_{max} 2260 (C≡N), 3280–3440 (OH, NH) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.57 (m, 2, $C_{5'}CH_2$), 3.87 (m, 1, $C_{4'}H$), 3.90, 4.13 (m, 2, $C_{2',3'}H$), 4.82 (d, 1, $J_{1',2'}$ = 5.7 Hz, $C_{1'}H$), 5.11, 5.40 (2 br s, 2, $C_{2',3'}OH$). Anal. ($C_8H_{10}N_4O_4$, MW 226.2) C, H, N.

Lyophilization of the subsequent fractions provided the desired **12** as foam: yield 39 mg (2.8%); ^1H NMR (Me_2SO-d_6) δ 3.47 (m, 2, $C_{5'}CH_2$), 3.73–4.13 (m, 3, $C_{2',3',4'}H$), 4.78 (d, 1, $J_{1',2'}$ = 3.7 Hz, $C_{1'}H$), 6.70, 7.33 [br s, 2, C(NH)NH_2]; FAB mass spectrum, 279 (M^+). Anal. ($C_8H_{14}ClN_5O_4$, MW 279.68) C, H, N, Cl.

Registry No. **4a**, 52327-03-4; **4b**, 111379-64-7; **5**, 111379-65-8; **6**, 111379-66-9; **7a**, 21733-05-1; **7b**, 3641-10-9; α-**8**, 76497-54-6; β-**8**, 62211-93-2; **9a**, 111379-68-1; **9b**, 111379-67-0; **9c**, 111379-69-2; **9d**, 111379-70-5; **9e**, 111379-72-7; **9f**, 111379-73-8; **9g**, 111379-74-9; **9h**, 111379-75-0; **9i**, 111379-76-1; **9j**, 111379-77-2; **9k**, 111379-78-3; **10**, 62404-66-4; **11**, 111379-79-4; **12**, 111379-80-7; PNPase, 9030-21-1; 5-chloro-1-(2,3-di-*O*-acetyl-5-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, 111379-71-6.

Supplementary Material Available: Tables of positional and equivalent isotropic thermal parameters for non-hydrogen atoms (Table I), bond lengths (Å) and bond angles (deg) (Table II), hydrogen bonding (Table III), anisotropic thermal parameters (Table IV), positions and isotropic thermal parameters for hydrogen atoms (Table V), bond lengths and bond angles involving hydrogen atoms (Table VI), torsion angles (Table VII), and least-squares planes (Table VIII) for compound **6** (8 pages). Ordering information is given on any current masthead page.