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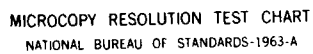
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SYNTHESIS OF C-5 SUBSTITUTED TUBERCIDIN DERIVATIVES

Annual Progress Report

Donald E. Bergstrom

(for the period 1 February 1979 to 31 January 1980)

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University of California
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Abstract

C-5 substituted pyrrolo(2,3-d)pyrimidine nucleosides were synthesized via reactions of 5-mercuritubercidin (2). Palladium catalyzed carbonylation of 2 in methanol gave 5-methoxycarbonyltubercidin (5) which could be converted to the nucleoside antibiotic sangivamycin (6) by reaction with ammonia. Reduction of 5 with LiBH_4 in tetrahydrofuran gave 5-hydroxymethyltubercidin (7). Longer carbon chains were introduced by the palladium catalyzed coupling of olefins with mercuritubercidin. 5-Mercuritubercidin and methyl acrylate in 0.1 M Li_2PdCl_4 in methanol gave (E)-5-(2-methoxycarbonylethenyl)tubercidin (3) which on treatment with aqueous sodium hydroxide was hydrolyzed to (E)-5-(2-carboxyethenyl)tubercidin (8). Nucleoside 8 was converted to (E)-5-(2-bromoethenyl)tubercidin 9 by N-bromosuccinimide in N,N-dimethylformamide.

3-Chloro-1-butene reacted with 2 and Li_2PdCl_4 to give a mixture of E and Z 5-(2-buten-1-yl)tubercidin (13,14). Styrene coupled with 2 to give 5-styryltubercidin 12. Finally the mercury could be replaced directly by iodine in DMF to give 5-iodotubercidin (15).

Characterization of the C-5 substituted tubercidin derivatives by ^{13}C NMR is discussed.

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TABLE OF CONTENTS

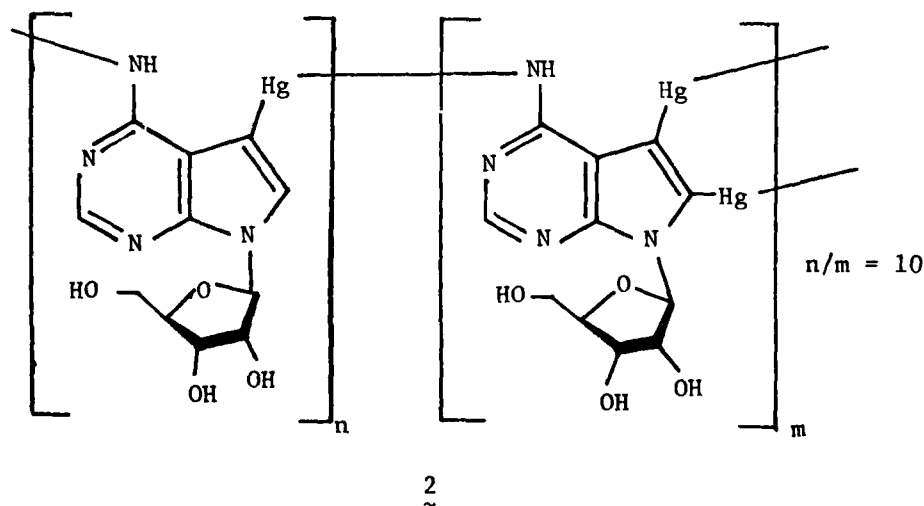
	Page
I. Introduction	4
II. Synthetic Studies	7
A. Synthesis and Reactions of 5-Methoxycarbonyl-tubercidin	7
B. Synthesis of 5-(2-Bromoethenyl)tubercidin.	9
C. Reduction of (E)-5-(2-Cyanoethenyl)tubercidin.	10
D. (E)-5-(2-Phenylethenyl)tubercidin.	10
E. Reaction of 5-Mercuritubercidin with 3-Chloro-1-butene.	10
F. Iodination of 5-Mercuritubercidin.	10
III. Spectroscopic Characterization	12
IV. List of Compounds Submitted for Biological Testing (Table III)	15
V. Experimental Section	16
References	20

I. Introduction

The major objective of our research program is the synthesis of a series of tubercidin derivatives which vary structurally in the C-5 side chain. The parent compound, tubercidin (1), is an effective agent against Schistosoma mansoni and S. japonium, but is too toxic to man unless administered intraerythrocytically.^{1,2} On the basis of the biochemical properties and biological activity of tubercidin and the few known examples of C-5 modified derivatives of tubercidin, there is reason to be optimistic about finding significant biological activity from other examples of this class of compounds. Selective activity toward protozoan and helminthic parasites may possibly be achieved on the basis of certain biochemical differences between the parasites and mammalian cells. The former require exogenous purine and purine nucleosides while most mammalian cells can synthesize the purine ring de novo. Consequently adenosine metabolizing enzymes and the transport system in parasites may differ enough from the corresponding enzymes and transport system in mammalian cells to provide selective targets for nucleoside antagonists.

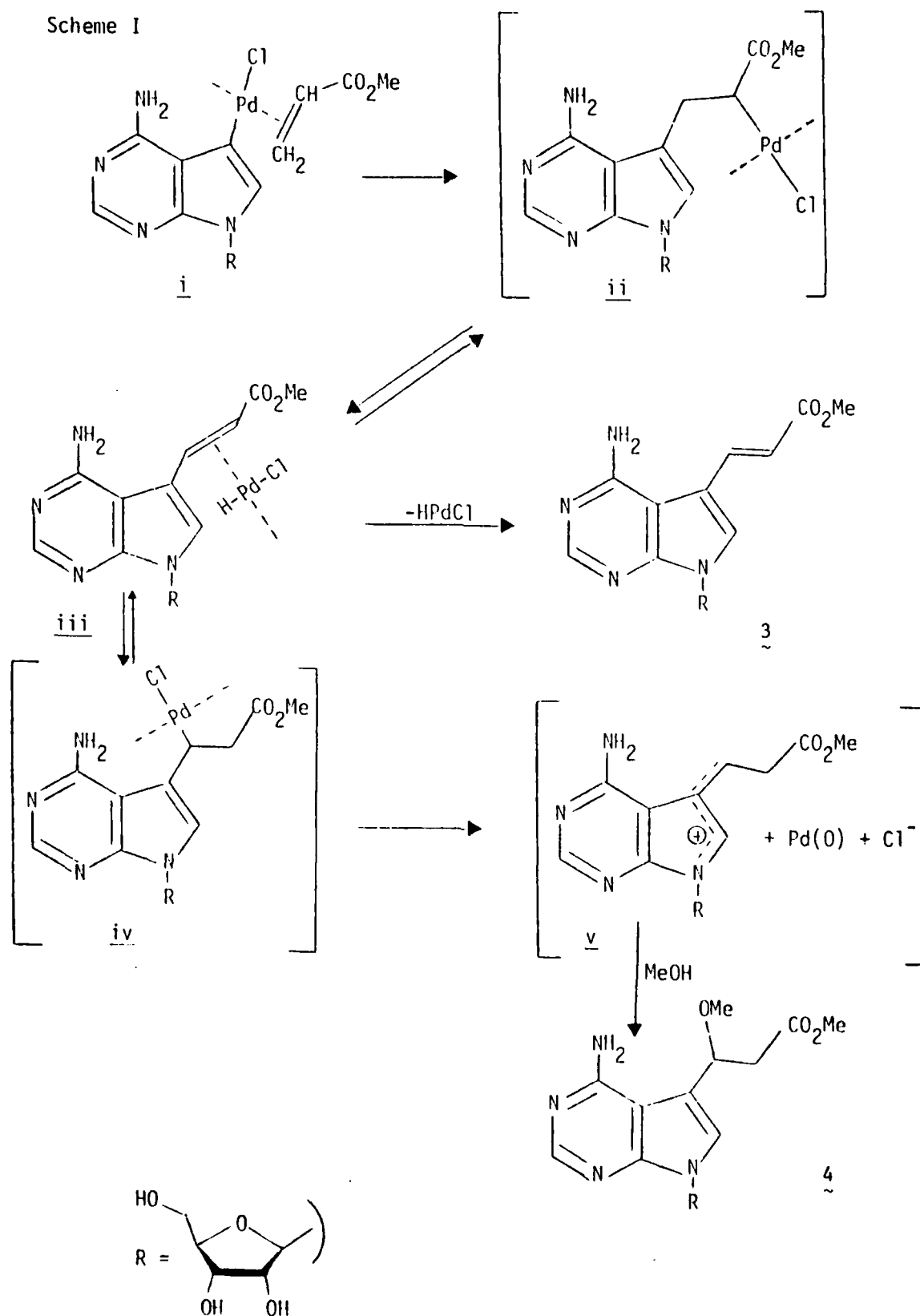
In our previous annual report (for the period 1 February 1978 to 31 January 1979) we reported development of new methodology for the synthesis of C-5 substituted derivatives of tubercidin (1).

Mercuriation of tubercidin by mercuric acetate in aqueous solution gave a white polymeric product which on the basis of ¹H NMR spectroscopy and elemental analysis was proposed to have structure 2.³



Mercuritubercidin (2) reacts with olefins and lithium palladium chloride in methanol solution leading to a C-5 coupled product. For example the organopalladium intermediate (1) derived from 2 couples with methyl acrylate to give principally 5-(2-methoxycarbonyl-1-ethenyl)tubercidin (Scheme I).

Scheme I



Although no mechanistic studies were done in this instance the schemes proposed by Heck⁴ for the reaction of phenylmercuric acetate with propylene and Pd(II) in methanol and acetonitrile and the results of our studies⁵ on the reaction of 5-chloromercuri-2'-deoxyuridine with propylene and Pd(II) lead us to propose the pathway shown in Scheme I. The mercuritubercidin complex 2 must undergo metal-metal exchange with palladium chloride or the methyl acrylate-palladium chloride π -complex to give intermediate i. Insertion of methyl acrylate into the Pd-C σ bond proceeds regioselectively to give ii which through cis elimination of Pd-H leads to the π -complex iii.

The predominant pathway involves dissociation of π -complex iii to give Pd(0), HCl, and nucleoside 3. However iii may be in equilibrium with both ii and iv. Intermediate iv, unlike ii, could dissociate with loss of Pd(0) to a stabilized carbocation, v, which would be expected react with the most readily available source of nucleophile, methanol, to give nucleoside 4. The reaction of ethylene with 5-chloromercuriuridine and Li_2PdCl_4 in MeOD previously established the occurrence of a palladium-mediated intramolecular hydride shift⁵ as postulated here for the interconversion of intermediates ii and iv.

The trans stereochemistry of 3 is presumed on the basis of the 16 Hz coupling constant between the olefinic protons. In this and all other coupling reactions to olefins of structure $\text{CH}_2=\text{CHY}$ where Y is a group capable of conjugation, the isolated products have shown only trans stereochemistry.

All of the coupling reactions previously discussed probably follow this same pathway. C-5 substituted tubercidin derivatives for which experimental details were given in the progress report for 1978 are listed in Table I.

Table I. Tubercidin Derivatives Synthesized in 1978

5-(1-Methoxyethyl)tubercidin
5-(1-Hydroxyethyl)tubercidin
5-(1-Isopropoxyethyl)tubercidin
5-Allyltubercidin
5-Mercuri-2',3'-O-isopropylidene tubercidin
5-(2-Cyanoethenyl)tubercidin
5-(2-Carboxamidoethenyl)tubercidin
5-(2-Carbomethoxyethenyl)tubercidin
5-(2-Carbomethoxyethyl)tubercidin

Not all of these derivatives could be completely characterized. For example 5-allyltubercidin was obtained from **2**, Li_2PdCl_4 , and allyl chloride in low yield along with many side products. It was never purified to an acceptable extent. Consequently during 1979, the reaction between **2** and 3-chloro-1-butene was investigated more fully to finally establish whether allylic chlorides could be coupled successfully to tubercidin.

Finally, one major advance in the research project during 1979, was the discovery that carbon monoxide could be coupled to mercuritubercidin (**2**) in methanol to give the versatile synthetic intermediate 5-methoxycarbonyltubercidin. This now appears to be the most straightforward route to introduce the single carbon unit at C-5.

The results of biological testing have arrived sporadically and will not be discussed in the present report but in a final report to appear in July, 1980.

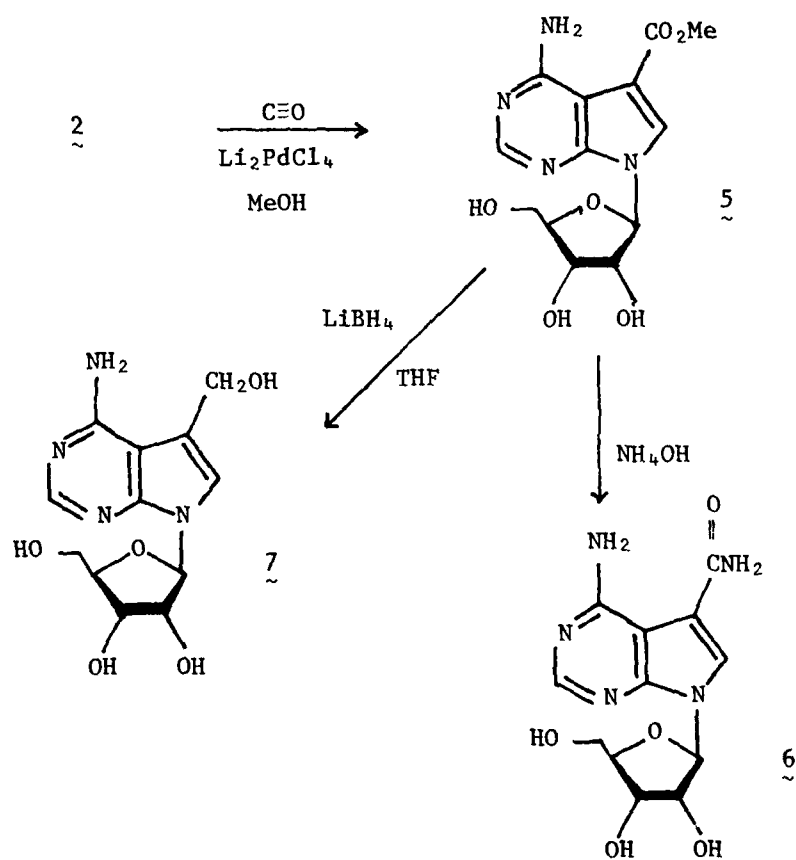
II. Synthetic Studies

A. Synthesis and Reactions of 5-Methoxycarbonyltubercidin

A straightforward route for the introduction of a single carbon unit at C-5 of tubercidin is particularly desirable. Not only do the nucleoside antibiotics, sangivamycin and toyocamycin, have a one carbon functional group at this position, but the hypermodified t-RNA bases, Nucleoside Q and Q*, have a methylene group at C-5 of a pyrrolo[2,3-d]pyrimidine nucleus.^{6,7} We have found it possible to introduce a one carbon functional group at C-5 in oxidation states characteristic of both classes of compounds. The key reaction is the palladium catalyzed carbonylation reaction first developed by P. M. Henry.⁸ Aryl mercuric chlorides react at room temperature with carbon monoxide and palladium chloride in acetic acid to give carboxylic acids. In methanol solvent, the product is a methyl ester. R. F. Heck⁹ and R. C. Laroch¹⁰ have explored and greatly expanded the scope of the reaction.

When mercuritubercidin and lithium palladium chloride were combined in methanol under a carbon monoxide atmosphere slow conversion to 5-methoxycarbonyltubercidin (**5**) occurred (Scheme II). The yield was not high (24% after purification) but the method is both direct and simple. The methoxycarbonyl group can be further converted to other functional groups without protection of the 4-amino or sugar hydroxyls. Ammonolysis in aqueous ammonium chloride-ammonium hydroxide gave the nucleoside antibiotic sangivamycin (**6**). The only previous synthesis of **6** involved reaction of 5-cyano-6-bromotubercidin with ammonium hydroxide in 30% H_2O_2 followed by catalytic hydrogenolysis of the 6-bromo group.¹⁵ Reduction of **5** with lithium borohydride in refluxing THF gave 5-hydroxymethyltubercidin (**7**).

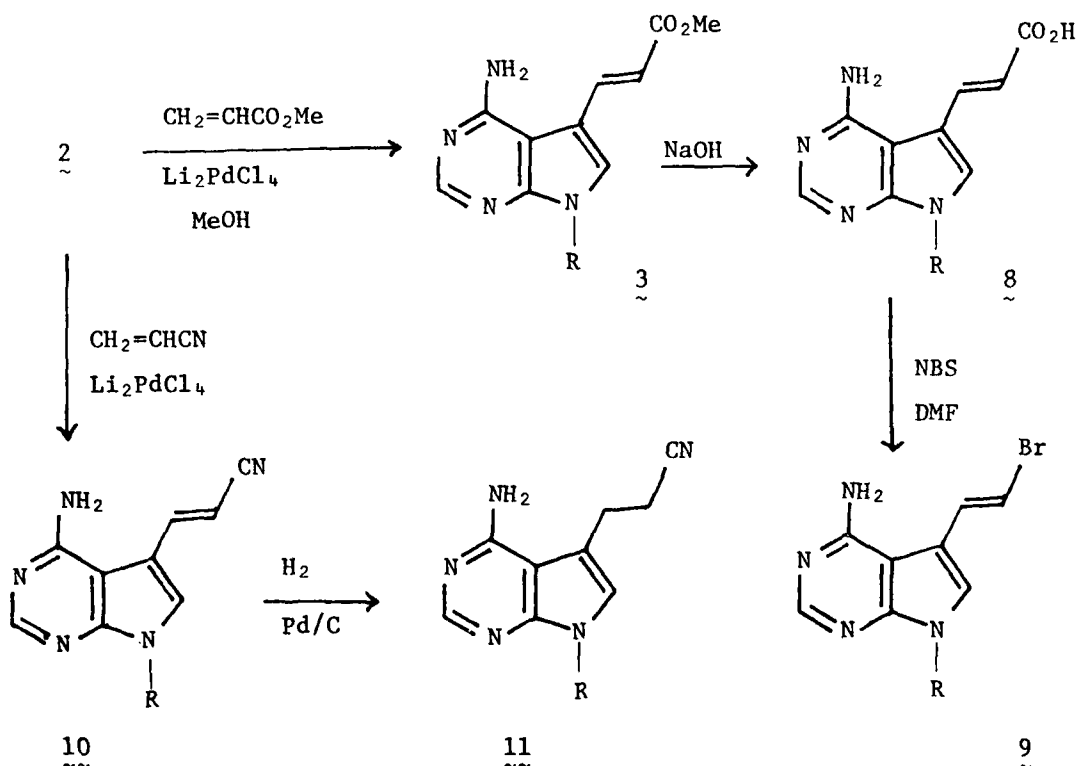
Scheme II



B. Synthesis of 5-(2-Bromoethenyl)tubercidin

Nucleoside **3** has proved to be a versatile intermediate. We previously demonstrated that the carbon-carbon double bond could be selectively reduced. We also have been able to reduce the side chain completely to give 5-(3-hydroxypropyl)tubercidin, but experimental details have not yet been completely worked out so this reaction will be reported at a later date. Perhaps more interesting from the synthetic chemist's viewpoint is the transformation of **3** to (E)-5-(2-bromoethenyl)-tubercidin (Scheme III). Jones et al.¹¹ demonstrated that (E)-5-(2-carboxyvinyl)pyrimidine nucleosides could be decarboxylated and brominated by NBS in hot aqueous solution to give (E)-5-(2-bromovinyl)nucleosides in good yield. (E)-5-(2-Carboxyethenyl)tubercidin (**8**), prepared by hydroxide catalyzed hydrolysis of **3**, did not react to give identifiable products with NBS in water but in DMF gave (E)-5-(2-bromoethenyl)tubercidin (**9**) in 26% yield. The 15 Hz coupling constant between the olefinic protons confirmed that the trans stereochemistry was retained.

Scheme III



C. Reduction of (E)-5-(2-Cyanoethenyl)tubercidin

A number of attempts have been made to reduce the cyanoethenyl group of 10 (Scheme III) to a 3-aminopropyl side chain. Reducing conditions known to transform the cyano function to amines have given anomalous results with either nucleosides 10 or 11. Reduction of 10 by H₂ at low pressure in neutral solution over 10% Pd/C cleanly reduces 10 to 5-(2-cyanoethyl)tubercidin (11). When the reduction was carried out in dilute HCl, further reduction was evident but 5-(3-aminopropyl)tubercidin could not be isolated. Neither could this reduction be achieved by NaBH₄-CoCl₂ or by AlH₃.

D. (E)-5-(2-Phenylethenyl)tubercidin

The reaction of mercuritubercidin with styrene and Li₂PdCl₄ has only recently given definitive results. Like the reaction with mercuripyrimidine nucleosides the coupling proceeds regioselectively. However the tubercidin reaction gives many minor side products and a significantly lower yield of the desired product.

Coupling in methanolic 0.1 M Li₂PdCl₄ gave (E)-5-(2-phenylethenyl)tubercidin (12) in 25% yield (Scheme IV). Unlike (E)-5-(2-phenylethenyl)-uridine⁵, 12 was not appreciably fluorescent. The trans stereochemistry was established by ¹H NMR at 360 MHz. At this high field the olefinic protons were clearly separated (7.01 and 7.54 ppm) and showed a 16.05 Hz coupling constant.

E. Reaction of 5-Mercuritubercidin with 3-Chloro-1-butene

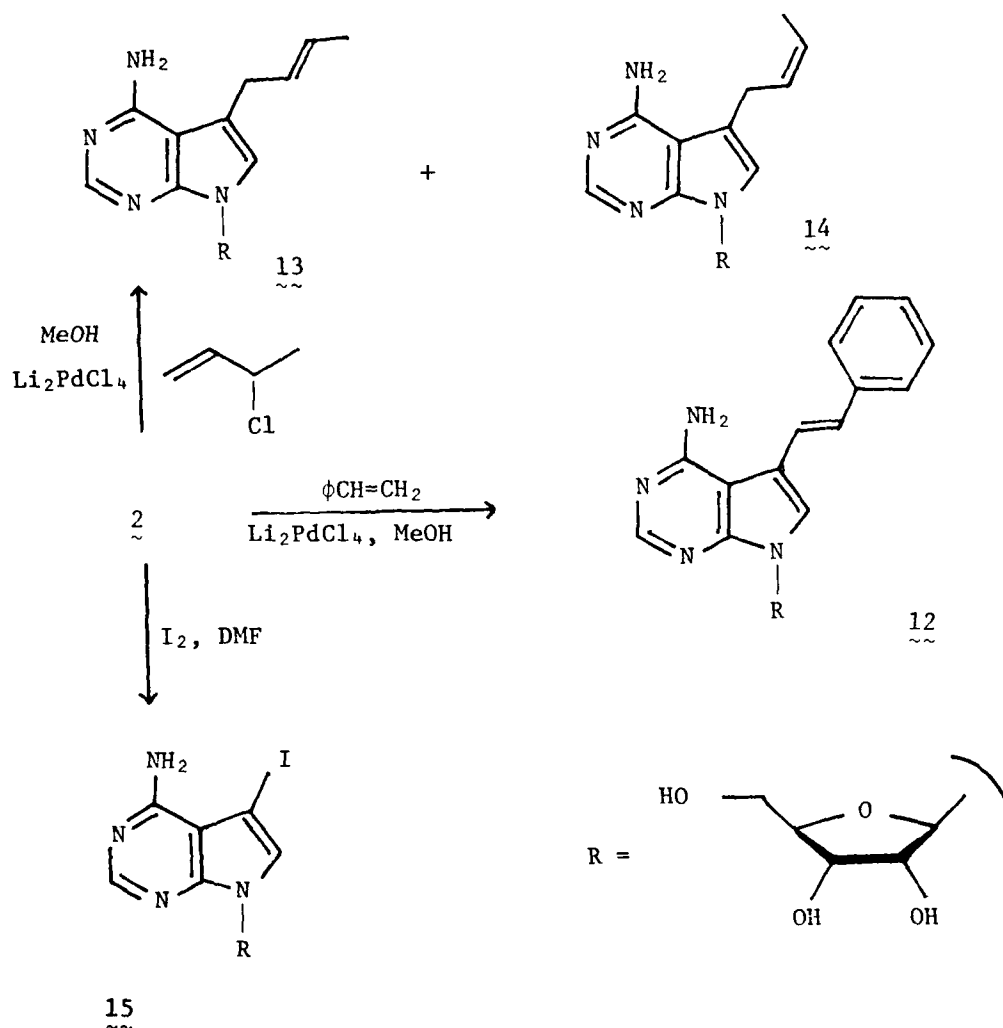
Like 2'-deoxyuridine¹², mercuritubercidin, 2, gave both (E) and (Z) isomers on coupling with 3-chloro-1-butene (Scheme IV). The isomers, 13 and 14, could not be separated by either TLC or HPLC on an analytical C-18 reverse phase column. However, their identity and relative yield was clearly established by ¹³C NMR of the mixture. Perhaps most encouraging is the contrast between the coupling reactions of 3-chloro-1-butene and allyl chloride. The former proceeds significantly more cleanly.

Studies on 5-mercuri-2'-deoxyuridine clearly show¹² that longer chain allylic chlorides couple as rapidly as 3-chloro-1-butene and give only a single product, the (E) isomer. Consequently, in the future this may prove the most efficient pathway for introducing aliphatic side chains at C-5 of tubercidin.

F. Iodination of 5-Mercuritubercidin

Finally the halogenation of mercuritubercidin was investigated in the interest of developing a short route to 5-chloro-, 5-bromo- and 5-iodotubercidin. All exhibit significant biological activity, but in particular 5-bromotubercidin was demonstrated to be an *in vivo* reversible inhibitor of RNA synthesis¹³ and 5-iodotubercidin (15) a potent inhibitor of adenosine kinase.¹⁴ The halogenation of mercuritubercidin (2) was not anticipated to be a problem in light of the successful halogenation of

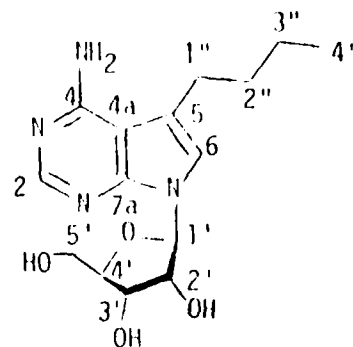
Scheme IV



5-mercuripyrimidine nucleotides.¹⁵ Iodination by I₂ in methanol-water, DMF, or HMPA proceeded smoothly but purification of the product was difficult because of the insolubility of 15 in water (neutral, acidic, or basic) and organic solvents with the exception of HMPA and methanol (sparingly soluble). Elimination of all traces of mercuric iodide was especially tedious. Column chromatography on silica gel eluting with methanol-chloroform resulted in substantial loss of material but the purity was now sufficient for recrystallization from methanol. A minor product which separated but could never be purified was assigned the structure 5,6-diiodotubercidin on the basis of the resemblance of its ¹H NMR spectrum to that for 5,6-dibromotubercidin. Approximately ten percent diiodination was anticipated on the basis of the structure of 2. Bromination of 2 with either Br₂, NBS, or CuBr₂ in methanol, water, or DMF gave complex mixtures of products of which 5-bromotubercidin was a major (but overall low yield) constituent.

III. Spectroscopic Characterization

The majority of synthetic compounds described here and in previous reports were characterized by ¹H NMR, ¹³C NMR, and elemental analysis. ¹³C NMR has provided the most reliable data for making structure assignments (Table II). Chenon et al¹⁶ have established carbon chemical-shifts for the naturally occurring pyrrolo[2,3-d]pyrimidine nucleosides. Like these authors we have determined ¹³C NMR spectra with complete proton decoupling. Our data on a large number of C-5 substituted compounds (Table II) confirms the observations made by Chenon et al. The chemical shifts of the ribosyl carbons are for the most part invariant and unaffected by the structure of the C-5 substituent. The only heterocycle carbon shifts significantly dependent on C-5 substitution are C-5 and C-6. Comparison of nucleosides 1, 5-7, and toyocamycin (5-cyanotubercidin) are illustrative. The carbon signals for C-2, C-4, C-4a, and C-7a fall within a 2 ppm range while those at C-5 vary from 83.2 ppm¹⁶ for toyocamycin to 115.5 ppm for 5-hydroxymethyltubercidin (7), and at C-6 from 119.1 for 5 to 132.6 for toyocamycin. The relative magnitude and direction of the shifts parallels those observed in derivatives of benzene.¹⁷ For example, both C-1 and C-2 of methyl benzoate are shifted downfield (2 ppm and 1.2 ppm respectively) from the carbon signal for benzene (128.5 ppm). The C-5 and C-6 resonances of 5-methoxycarbonyltubercidin (5) are shifted 6.5 and 7.0 ppm downfield respectively from the signals observed in tubercidin. In contrast, the C-1 and C-2 signals of benzyl alcohol fall at 141.0 and 126.9 ppm respectively. These values represent shifts of 12.5 ppm downfield for C-1 and 1.6 ppm upfield for C-2. The corresponding carbons in 5-hydroxymethyltubercidin show parallel shifts with respect to tubercidin. The C-5 resonance of 7 is shifted 16 ppm downfield while the C-6 resonance falls 3.2 ppm further upfield. Other tubercidin derivatives have also shown parallel and consistent results in comparison to ¹³C NMR data on simpler model compounds.

Table II. ^{13}C NMR Spectra ^a

Nucleoside	C-5 Substituent	C-2	C-4	C-4a	C-5	C-6	C-7a
<u>1</u>	H	151.5	157.5	103.1	99.5	122.3	149.9
<u>6</u>	CONH ₂	152.69	157.95	—	110.85	125.90	150.82
<u>5</u>	CO ₂ Me	153.75	157.49	100.48	106.06	129.26	151.08
<u>7</u>	CH ₂ OH	151.26	157.23	102.62	115.54	119.06	151.06
<u>3</u>	CH=CHCO ₂ Me	151.50	157.38	—	110.49	123.65	151.10
<u>4</u>	$\begin{array}{c} \text{OMe} \\ \\ \text{CHCH}_2\text{CO}_2\text{Me} \end{array}$	152.23	154.84	102.05	115.40	122.17	151.06
	CH=CHCONH ₂	151.57	157.27	100.79	111.47	122.78	150.90
<u>10</u>	CH=CHCN	152.35	157.94	100.11	92.52	122.94	151.32
<u>8</u>	CH=CHCO ₂ H	150.05	154.04	100.55	112.16	124.74	147.04
<u>9</u>	CH=CHBr	151.01	156.86	100.12	111.22	119.74	150.88
<u>11</u>	CH ₂ CH ₂ CO ₂ Me	150.48	156.67	101.24	112.62	118.69	149.82
	CH ₂ CH ₂ CN	151.26	157.26	101.82	111.78	120.10	150.55
<u>12</u>	$\begin{array}{c} \text{OMe} \\ \\ \text{CHCH}_3 \end{array}$ ^a	152.43	158.35	103.56	120.20	123.03	151.98
	$\begin{array}{c} \text{OH} \\ \\ \text{CHCH}_3 \end{array}$	150.27	156.03	101.71	119.24	121.71	149.26
<u>12</u>	CH=CH-	151.40	157.61	100.88	113.83	120.26	150.79
<u>13,14</u>	(E)&(Z) CH ₂ CH=CHCH ₃	151.73	157.74	102.92	114.32	120.53 120.05	151.06
<u>15</u>	I	151.76	156.91	—	—	127.05	150.02

^a All spectra were run in 6-d DMSO and are referenced to external TMS.

C-1'	C-2'	C-3'	C-4'	C-5'	C-1''	C-2''	C-3''	C-4''	C-5''
87.6	73.7	70.8	85.1	61.9					
86.96	72.32	70.50	85.25	61.72	166.17				
87.48	74.03	70.23	85.84	61.12	165.17		-OCH ₃	51.80	
87.14	73.73	70.81	85.13	61.97	56.43				
86.97	73.78	70.29	85.03	61.49	136.39	115.06	166.27	-OCH ₃	51.15
87.60	73.95	71.08	85.41	62.0	73.95	42.37	174.96	-OCH ₃	55.90 52.73
86.96	73.85	70.57	85.20	61.66	130.64	121.11	167.16		
87.00	73.72	70.29	85.07	61.52	142.58	110.72	119.00		
87.88	74.47	70.44	85.68	61.49	135.53	118.95	167.82		
86.83	73.55	70.26	84.95	61.49	128.58	104.93			
86.17	72.63	69.74	84.00	60.93	20.45	33.86	171.94	-OCH ₃	50.44
87.03	73.41	70.48	84.81	61.70	21.80	18.26	—		
89.39	75.65	72.79	86.90	63.92	74.71	23.58	-OCH ₃	56.88	
87.20	74.19	70.99	85.47	61.97	62.73	25.34			
86.90	73.64	70.47	84.98	61.67	unassigned				
87.51	73.98	71.11	85.43	62.28	29.49 24.33	130.53 128.70	126.51 125.90	18.22 13.35	
86.62	73.67	70.26	85.01	61.30					

IV.

Table III. Compounds Submitted for Biological Testing

<u>Structure No.</u>	<u>C-5 Substituent</u>	<u>Code No.</u>	<u>WRAIR No.</u>	<u>Date Rcd.</u>
11 ~	-CH ₂ CH ₂ CN	DEB-5	BJ30609	79/07/20
4 ~	$\begin{array}{c} \text{OMe} \\ \\ -\text{CHCH}_2\text{CO}_2\text{Me} \end{array}$	DEB-6	BJ30618	79/07/20
—	3-β-D-ribofuranosyl-2,7-dioxypyrido[2,3-d]pyrimidine	DEB-7	BJ30627	79/07/20
—	-CH ₂ CH ₂ CO ₂ Me	DEB-8	BJ36610	79/08/31
12 ~	-CH=CHPh	DEB-9	BJ45351	79/12/26
7 ~	-CH ₂ OH	DEB-10	BJ45360	79/12/26
5 ~	-CO ₂ Me	DEB-11	BJ45379	79/12/26

V. Experimental Section

Proton magnetic resonance spectra were taken on either a Varian EM360 60Mz instrument or a Fourier Transform NMR, Jeol Model PS100. Sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionate (TSP) was employed as the internal standard for spectra run in D₂O or d⁶-DMSO. ¹³C NMR spectra were obtained on the latter instrument. Infrared spectra were obtained on a Beckman IR-8 in solid KBr with a polystyrene standard. Ultraviolet spectra were measured on either a Cary 15 or Cary 17 spectrometer. Melting points were taken on a Büchi 510 M.P. apparatus and are uncorrected. Elemental analyses were performed by Galbraith Labs or the Microanalytical Lab of UC Berkeley. Column chromatography was done on Bio-Gel P2 and E. Merck Silica Gel 60. Analytical thin layer chromatography (TLC) was carried out on E. Merck Precoated Silica Gel F-254 (0.25 mm) plastic-backed TLC sheets cut to 30 x 110 mm. The sheets were developed in the specified solvent systems in 12 cm high wide-mouth jars lined with filter paper. Solvent systems were: A, MeOH-CHCl₃ (1:3, v/v); B, AcCN-nBuOH-0.1 M NH₄OAc-conc. NH₄OH (10:60:20:10, v/v); C, MeOH-EtOAc (3:2, v/v); and others which will be specified. All solvents and reagents were reagent grade. Tubercidin was purchased from the Upjohn Company Fine Chemicals Division. Water was deionized and then distilled through glass. Coupling reactions with ethylene and hydrogenations were carried out in Parr bottles using an apparatus similar to that described by Barefield.¹⁸ The apparatus was modified from the one described by the addition of a separate permanent connection adapted for easy exchange of lecture bottles.

For purposes of reference, tubercidin has the following properties: MP 247°C (dec); UV spectrum: λ_{max} 272 ($\epsilon = 12,200$) in .01 M HCl, λ_{max} 270 (12,100) in .01 M NaOH.¹² IR (KBr): 3200 (br), 1600 (br), 1450, 1362, 1260, 1140, 1051, 1012, 912, 878 cm⁻¹; TLC (solvent system/Rf value): A/.28, B/.43, C/.54; ¹H NMR (d⁶-DMSO): δ 8.18 (1H, s, H2), 7.45 (1H, d, J = 4Hz, H6), 7.17 (2H, s, NH₂), 6.70 (1H, d, J = 4Hz, H5), 6.11 (1H, d, J = 6Hz, H1'), 4.55 (1H, mult., H4'), 4.1 (2H, mult., H2' and H3'), 3.6 (2H, mult., H5').

5-(Methoxycarbonyl)tubercidin (5). To a 250 ml Parr bottle were added 525 mg (0.946 mM) 5-acetoxymercuritubercidin and 20 ml of a methanolic 0.1 M Li₂PdCl₄ solution (2 mM). The mixture was stirred under 35 psi carbon monoxide for three days at 25°C. The reaction mixture was filtered, saturated with hydrogen sulfide and refiltered. Neutralization with conc. NH₄OH evaporation onto 3 g of silica gel and chromatography on 200 g silica gel using a chloroform-methanol gradient gave 77 mg (0.23 mM, 24%) of white crystalline product, mp 214.5-216°. The product exhibited a single component on TLC (silica gel; system A) and HPLC (C-18 reverse phase; 0.01 M NH₄H₂PO₄, acetonitrile; 85:15, v/v). ¹H NMR (DMSO-d₆) δ 8.31 (s, 1H), 8.17 (s, 1H), 6.12 (d, 1H, J = 6Hz), 4.44 (m, 1H), 4.0 (broad m, 2H), 3.83 (s, 3H), 3.62 (m, 2H). UV $\lambda_{\text{max}}^{\text{MeOH}}$ 276 nm, 229 nm.

Anal. Calcd. for •C₁₃H₁₆N₄O₆: C, 48.15; H, 4.97; N, 17.28.
Found: C, 48.02; H, 5.10; N, 17.10.

5-Carboxamidotubercidin (Sangivamycin) (6). 5-Methoxycarbonyl-tubercidin (5) (0.092 g, 0.284 mM) and ammonium chloride (0.82 g) dissolved in conc. ammonium hydroxide (15 mL) with warming. Once 5 was completely dissolved the mixture was cooled and allowed to stir at room temperature for seven hr. Evaporation to dryness in vacuo gave a white solid. Chromatography on silica gel eluting with a gradient of 15% to 40% methanol-chloroform gave 52 mg of 3 (Rf 0.15, system A). The ^{13}C NMR spectrum was virtually identical to that reported for sangivamycin by Chen et al.¹⁹

5-Hydroxymethyltubercidin (7). 5-(Methoxycarbonyl)tubercidin (1.246 g, 3.84 mM) in 70 ml dry THF was combined with 0.836 g (38 mM) LiBH_4 and refluxed under an argon atmosphere for 20 hours. The reaction mixture was then evaporated four times with 10 ml portions of methanol containing about 5% HCl. The reaction mass was then taken up in methanol and neutralized with conc. NH_4OH and evaporated with 7 g of silica gel. Chromatography on 300 g silica gel using a chloroform-methanol gradient afforded, after recrystallization from methanol, 518 mg (1.74 mM, 45%) of white crystalline product, mp 219-220.5°C. HPLC C-18 reverse phase; 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$, acetonitrile; 92:8, v/v) and TLC (silica gel, system A) indicated a pure product. ^1H NMR ($\text{DMSO}-d_6$) δ 8.13 (s, 1H), 7.32 (s, 1H), 6.05 (d, 1H, $J = 6\text{Hz}$), 4.63 (s, 2H), 4.47 (m, 1H), 4.12 (m, 1H), 3.93 (m, 1H), 3.59 (m, 2H); $\lambda_{\text{MeOH}}^{\text{max}}$ 270 nm.

Anal. Calcd. for $\text{C}_{12}\text{H}_{16}\text{N}_4\text{O}_5$: C, 48.65; H, 5.44; N, 18.91.
Found: C, 48.83; H, 5.54; N, 18.83.

(E)-5-(2-Carboxyethenyl)tubercidin (11). To 20 mL of 0.5 M NaOH solution was added 1.227 g (3.51 mM) 5-(2-methoxycarbonyl-ethenyl)tubercidin (7). After 3 hours at room temperature, the reaction mixture was acidified with 15% HCl to give, after filtering and drying at high vacuum, 1.030 g (3.06 mM, 87%) of a white powder: mp > 300°. HPLC (C-18 reverse phase, 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$, acetonitrile, 95:5, v/v) and TLC (silica gel; system A) showed a single component. ^1H NMR ($\text{DMSO}-d_6$) δ 8.38 (s, 1H), 8.32 (s, 1H), 7.98 (d, 1H, $J = 16\text{Hz}$), 6.46 (d, 1H, $J = 16\text{Hz}$), 6.20 (d, 1H, $J = 6\text{Hz}$), 4.47 (m, 1H), 4.18 (m, 1H), 4.00 (m, 1H), 3.71 (m, 2H). $\lambda_{\text{MeOH}}^{\text{max}}$: 313 nm, 261 nm.

Anal. Calcd. for $\text{C}_{14}\text{H}_{16}\text{N}_4\text{O}_6 \cdot \text{H}_2\text{O}$: C, 47.45; H, 5.12; N, 15.82.
Found: C, 47.09; H, 4.94; N, 15.67.

(E)-5-(2-Bromoethenyl)tubercidin (12). A mixture of 170 mg (0.50 mM) 5-(2-carboxyethenyl)tubercidin (11) and 100 mg (1.0 mM) potassium acetate in 7 mL dry DMF was heated on the steam bath to give a cloudy solution. The stirred mixture was cooled to room temperature and 90 mg (0.50 mM) N-bromosuccinimide in 1 mL dry DMF added dropwise and then stirred for 10 min. The reaction mixture was evaporated, taken up in methanol and evaporated with 3 g silica gel and chromatographed in a 2 cm diameter column of 200 g silica

gel giving, after recrystallization from methanol, 50 mg (0.13 mM, 26%) of light-orange crystals: mp 164.5–167°C. HPLC (C-18 reverse phase; $\text{NH}_4\text{H}_2\text{PO}_4$, acetonitrile, 83:17, v/v), and TLC (silica gel; system A) showed a single component. ^1H NMR ($\text{DMSO}-d_6$) δ 8.38 (s, 1H), 7.96 (s, 1H), 7.87 (d, 1H, $J = 15\text{Hz}$), 7.09 (d, 1H, $J = 15\text{Hz}$), 6.26 (d, 1H, $J = 6\text{Hz}$), 4.58 (m, 1H), 4.28 (m, 1H), 4.06 (m, 1H), 3.78 (m, 2H). $\lambda_{\text{max}}^{\text{MeOH}}$: 282 nm, 243 nm.

Anal. Calcd. for $\text{C}_{13}\text{H}_{15}\text{BrN}_4\text{O}_4$: C, 42.06; H, 4.07; Br, 21.53; N, 15.09. Found: C, 42.04; H, 4.17; Br, 21.56; N, 15.07.

5-(2-Cyanoethyl)tubercidin (14). 5-(2-Cyanoethenyl)tubercidin (800 mg, 3.78 mmole) was stirred at room temperature under 30 psig H_2 in 100 mL MeOH with 80 mg 10% Pd/C for six hours. Chromatography of the crude product, obtained from the filtrate after filtration and evaporation, on silica gel eluting with MeOH/ CHCl_3 gave 5-(2-cyanoethyl)tubercidin (610 mg, 76%) mp. 223–224.5° (dec): ^1H NMR (d_6 -DMSO) δ 2.75 (t, 2H, $J = 7\text{Hz}$), 3.13 (t, 2H, $J = 7\text{Hz}$), 3.59 (m, 4H), 3.90 (m, 1H), 4.10 (m, 1H), 4.40 (m, 1H), 5.50 (m, 2H), 6.01 (d, 1H, $J = 6\text{Hz}$), 6.76 (s, 1H), 7.27 (s, 1H), 8.07 (s, 1H). TLC. Rf 0.41 (system B); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ 276 nm (pH 5), 278 nm (pH 1.5), and 274 nm (pH 12).

Anal. Calcd. for $\text{C}_{14}\text{H}_{17}\text{N}_5\text{O}_4$: C, 52.66; H, 5.36; N, 21.93. Found: C, 52.43; H, 5.44; N, 21.71.

5-(2-Phenylethenyl)tubercidin (18). 5-Mercuritubercidin (4) (5.25 g, 10.5 mM) was combined with styrene (5.7 mL, 50 mM) and 200 mL of methanolic 0.1 M Li_2PdCl_4 solution (20 mM) and refluxed with stirring for 18 hours. The reaction mixture was then filtered, saturated with hydrogen sulfide, and refiltered and neutralized with conc. NH_4OH . The solution was evaporated with 10 g of silica gel and chromatographed on a 4 cm diameter column containing 300 g of silica gel eluting with a chloroform-methanol gradient. The chromatographic fractions containing the product were evaporated to a yellow oil, dissolved on 20 mL methanol and cooled overnight at 0° yielding 830 mg of yellow crystalline product. The mother liquor was reduced and yielded a second crop of 145 mg. Total yield: 975 mg (2.65 mM, 25.3%): mp 220.5–224°C. HPLC (C-18 reverse phase, 0.01 M $\text{NH}_4\text{H}_2\text{PO}_4$, acetonitrile, 7:3, v/v) and TLC (silica gel, system A) indicated a homogeneous product. ^1H NMR ($\text{DMSO}-d_6$) (100MHz) δ 8.12 (s, 1H), 7.90 (s, 1H), 7.72–7.22 (m, 5H), 7.57 (d, 1H, $J = 16.5\text{Hz}$), 7.04 (d, 1H, $J = 16.5\text{Hz}$), 6.12 (d, 1H, $J = 6\text{Hz}$), 4.47 (m, 1H), 4.14 (m, 1H), 3.95 (m, 1H), 3.64 (m, 2H). ^1H NMR ($\text{DMSO}-d_6$) (360 MHz) δ 8.08 (s, 1H, H-2), 7.85 (s, 1H, H-6), 7.67 (d, 2H, $J = 7.45\text{Hz}$, ortho-H), 7.36 (unsym., 2H, meta-H), 7.23 (t, 1H, $J = 7.32\text{Hz}$, para-H), 7.54 (d, 1H, $J = 16.06\text{Hz}$, H-1''[Table II]), 7.01 (d, 1H, $J = 16.04\text{Hz}$, H-2''), 6.08 (d, 1H, $J = 6.2\text{Hz}$), 4.44 (m, 1H, H-2'), 4.12 (m, 1H, H-3'), 3.91 (m, 1H, H-4'), 3.53–3.70 (m, 2H, H-5'). $\lambda_{\text{max}}^{\text{MeOH}}$: 310 nm, 268 nm.

Anal. Calcd. for $\text{C}_{19}\text{H}_{19}\text{N}_4\text{O}_2$: C, 62.12; H, 5.21; N, 15.25. Found: C, 61.85; H, 5.42; N, 15.09.

(E) and (Z)-5-(2-Buten-1-yl)tubercidin (13, 14). 5-Mercuritubercidin (0.525 g, 1.0 mM), 1.01 mL (10 mM) 3-chloro-1-butene and 10 mL (1.0 mM) of a methanolic 0.1 M LiPdCl₄ solution were combined and stirred 48 hours at room temperature. The reaction mixture was saturated with H₂S, filtered, neutralized with conc. NH₄OH and evaporated with 5 g of silica gel. Chromatography on 200 g of silica gel eluting with a chloroform-methanol gradient gave, after recrystallization from methanol, 65 mg (0.20 mM, 20%) of white crystals: mp 187.5-190°. HPLC (C-18 reverse phase; 0.01 M NH₄H₂PO₄; 85:15, v/v) and TLC (silica gel; system A) showed a single component. ¹H NMR (DMSO-d₆) δ 8.11 (s, 1H), 7.18 (s, 1H), 6.05 (d, 2H, J = 6Hz), 5.65 (narrow m, 2H), 4.49 (m, 1H), 4.15 (m, 2H), 3.68 (narrow m, 2H), 3.49 (narrow m, 2H), 1.69 (narrow m, 3H). The ratio of E to Z isomer was estimated to be 2:1 on the basis of the ¹³C resonances. λ^{MeOH}_{mas}: 275 nm.

Anal. Calcd. for C₁₅H₂₀N₄O₂: C, 56.24; H, 6.29; N, 17.49.
Found: C, 56.52; H, 6.36; N, 17.62.

5-Iodotubercidin (15). 5-Mercuritubercidin (2) (1.575 g, 3.0 mM) and iodine (1.53 g) were dissolved with stirring in 50 mL DMF. After 18 hrs the DMF was removed by lyophilization and the brown oil chromatographed on silica gel (100 g) eluting with 16% MeOH/CHCl₃. Fifteen mL fractions were collected. Fractions 8-45 were combined and evaporated to give a mixture of 23 and mercuric iodide. Most of the mercuric iodide could be eliminated by washing the solid first with acetone and then with methanol. Recchromatography on silica gel gave 23 (0.5486 g, 47%). Analytically pure 5-iodotubercidin, mp 190-191° (dec), could be obtained by recrystallization from methanol: ¹H NMR (6d-DMSO-D₂O) δ 3.57 (narrow m, 2H), 3.91 (m, 1H), 4.09 (m, 1H), 4.36 (m, 1H), 6.03 (d, 1H, J = 6.3Hz), 7.68 (s, 1H), 8.11 (s, 1H); λ^{MeOH}_{max}: 282 nm (ε 9,030).

Anal. Calcd. for C₁₁H₁₃N₄O₄I: C, 33.69; H, 3.34; N, 14.29.
Found: C, 33.85; H, 3.40; N, 14.18.

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