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SYNTHESIS OF C-5 SUBSTITUTED TUBERCIDIN DERIVATIVES

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1 Feb 78 - 31 Jan 79

Donald E. Bergstrom and Michael J. Schweickert

(for the period 1 February 1978 to 31 January 1979)

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Methodology for the synthesis of tubercidin derivatives substituted by carbon chains at the C-5 position has been developed. 5-Mercuritubercidin, prepared by reaction of tubercidin with mercuric acetate in H_2O, undergoes coupling to olefins when treated with lithium palladium chloride in either methanol or N,N-dimethylformamide. In this way 5-(1-methoxyethyl)tubercidin was prepared from ethylene, 5-(2-cyanoethenyl)tubercidin from acrylonitrile, 5-(2-carboxamidoethenyl)tubercidin from acrylamide, and 5-(2-methoxycarbonylethenyl)tubercidin from methyl acrylate. Various synthetic transformations of these (over)

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20. ABSTRACT (continued)

tubercidin derivatives were investigated. 5-(1-Methoxyethyl)tubercidin was transformed to 5-(1-hydroxyethyl)tubercidin by refluxing in water and to 5-(1-isopropoxyethyl)tubercidin by warming with 2-propanol and sulfuric acid. Reduction of 5-(2-cyanoethenyl)tubercidin with H_2 over Pd/C gave 5-(2-cyanoethyl)tubercidin, while 5-(2-methoxycarbonylethenyl)tubercidin gave 5-(2-methoxycarbonylethyl)tubercidin under the same conditions.

The reaction of allyl chloride with 5-mercuritubercidin in 0.1 M Li_2PdCl_4 in methanol gave only a low yield of 5-allyltubercidin. Studies directed towards the use of other allylic chloride in the coupling reaction, and the potential of the cyanoethenyl and methoxycarbonylethenyl side chains for further synthetic transformations are discussed. The activity of the C-5 substituted tubercidin derivatives as antischistosomal, antitrypanosomal, and antileishmonial agents is under investigation.

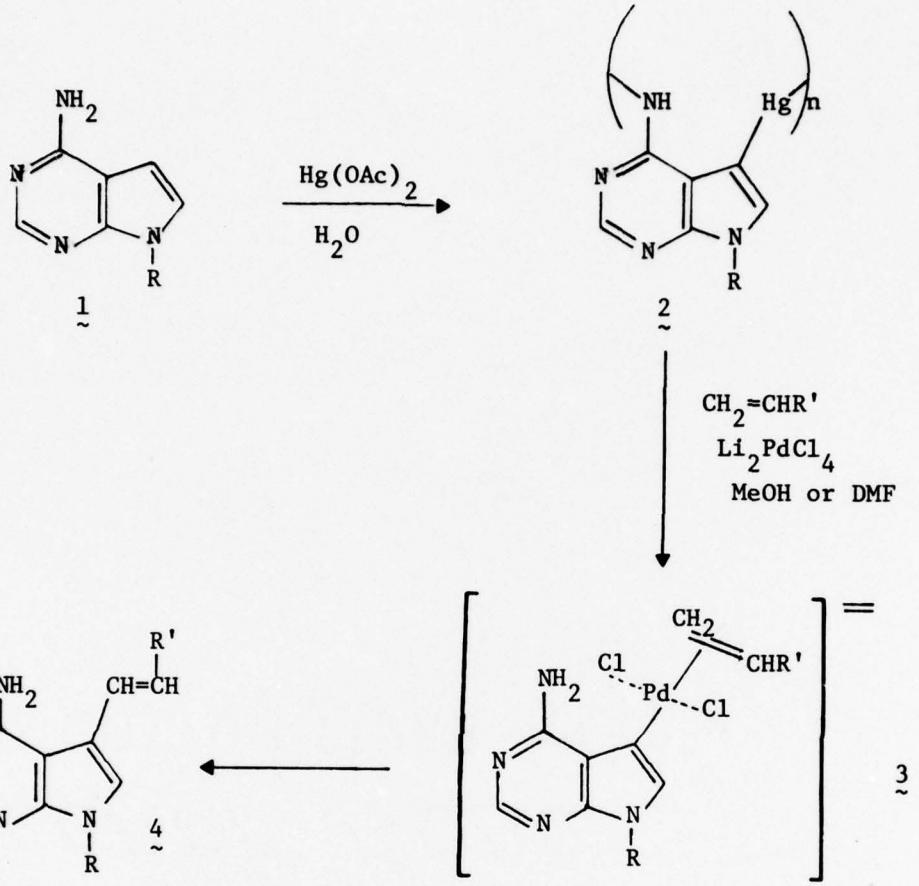
TABLE OF CONTENTS

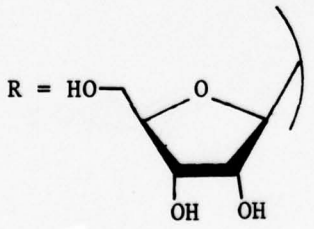
I. Introduction

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. japonĭum, 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.

Previously there were no simple routes to introduce substituents into the C-5 position. Now, methodology developed in our laboratory provides a straight forward route to derivatives of tubercidin substituted at C-5 by carbon chains. We previously demonstrated that the pyrimidine nucleosides can be substituted at the C-5 position by a two step procedure, mercuration by mercuric acetate, followed by olefin coupling via an organopalladium intermediate. [3,4] The same general route may be applied to tubercidin (Scheme I). 5-Mercuritubercidin (2) is prepared by heating an aqueous solution of tubercidin with an equivalence of mercuric acetate. [5] The reaction of 2 with olefins and lithium palladium chloride in methanol or N,N-dĭmethylformamide in order to obtain nucleosides of structure 4 has been the major thrust of this research effort.

Scheme I


NH2
N
N
N
R
1
Hg(OAc)2
H2O
NH
Hg
n
2
CH2=CHR'
Li2PdCl4
MeOH or DMF
CH2
CHR'
Cl
Pd
Cl
3
R'
CH=CH
4
+ Pd(O) + HCl


R = HO
O
OH
OH

II. Synthetic Goals

In order to fully probe the potential of C-5 substituted tubercidin derivatives as antiparasitic agents we planned to attach side chains which would exhibit different binding characteristics. These would include lipophilic side chains of variable length, and side chains to which groups capable of hydrogen bonding as either donors or acceptors would be attached. The original target molecules are listed in Table I.

The proposed target molecules were conceived not only on the basis of structure as related to potential activity but also on the basis of what we predicted could be done by the organopalladium coupling methodology. Since the chemistry is still largely exploratory it was anticipated that changes in synthetic goals would be inevitable. Consequently, working within the framework of our original goal (to obtain a series of compounds with significant structural variation in the C-5 side chain) we have modified targets to accomodate what we found to be possible chemically.

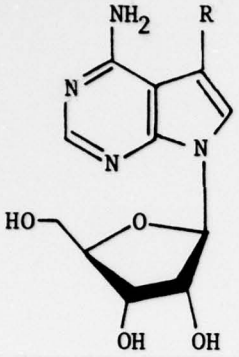
III. Synthesis and Reactions of 5-(1-Methoxyethyl)tubercidin

The reaction of ethylene at 30 psig with 2 and Li_2PdCl_4 in methanol gave 5-(1-methoxyethyl)tubercidin (6) in 73% yield after silica gel and Bio-gel P-2 chromatography (Scheme II). The reaction was also attempted in N,N-dimethylformamide (DMF) in anticipation of obtaining 5-vinyltubercidin (8) in analogy to the preparation of 5-vinylcytidine from 5-chloromercuricytidine.[6] However no isolable product could be obtained. The reduction of 6 to 5-ethyltubercidin (7) by H_2, Pd/C has also proven unsuccessful. Here also an analogous reaction, the successful reduction of 5-(1-methoxyethyl)uridine to 5-ethyluridine by H_2 Pd/C, provides a close model for the anticipated reduction. Variations in catalyst, solvent, and pH resulted in either no reduction or clear overreduction involving the pyrrolo[2,3-d]pyrimidine ring.

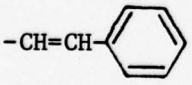
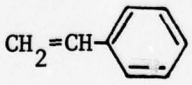
The methoxyl group of 6 is exchangeable. Refluxing 6 in neutral aqueous solution gave essentially quantitative conversion to 5-(1-hydroxyethyl)tubercidin (9). 5-(1-Isopropoxyethyl)tubercidin (23), a compound not originally designated as a target compound, was obtained in good yield when 6 was refluxed in 2-propanol with sulfuric acid added as a catalyst. Nucleoside 23 could also be obtained by carrying out the organopalladium coupling reaction of mercuritubercidin with ethylene in 2-propanol.

The problem of reducing the 1-methoxyethyl side chain to an ethyl group has discouraged attempts to add longer side chains via a coupling reaction with monosubstituted olefins. Unless the α-methoxy group can be eliminated by catalytic hydrogenolysis we can anticipate the problem of separating a minimum of four products. For example the reaction of propylene with 5-mercuri-2'-deoxyuridine resulted in the formation of a complex mixture that included 5-(1-methoxypropyl)-,

Table I

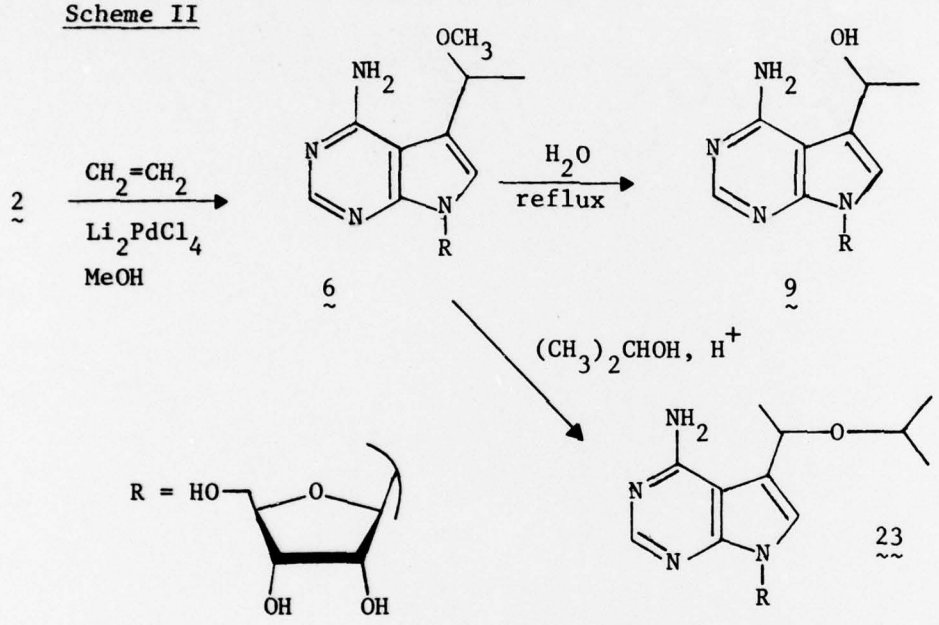


Target molecules predicted to be the products of the coupling reaction between 5-mercuritubercidin (2) and olefins (column 3) as catalyzed by lithium palladium chloride in methanol. Where subsequent steps are required, the reagents are given in column 4.

	R	Olefin	Additional Steps and Reagents
6	$-CH(OCH_3)CH_3$	$CH_2=CH_2$	
7	$-CH_2CH_3$	$CH_2=CH_2$	H_2, Pd/C
8	$-CH=CH_2$	$CH_2=CH_2$	Pd(II), DMF
9	$-CH(OH)CH_3$	$CH_2=CH_2$	H_2O
10	$-CH_2CH=CH_2$	$CH_2=CHCH_2Cl$	
11	$-CH_2CH_2CH_3$	$CH_2=CHCH_2Cl$	H_2, Pd/C
12	$-CH=CHCH_3$	$CH_2=CHCH_2Cl$	Rh(I), EtOH
13	$-CH=CHC\equiv N$	$CH_2=CHC\equiv N$	
14	$-CH=CHCO_2NH_2$	$CH_2=CHCO_2NH_2$	
15	$-CH=CHCO_2CH_3$	$CH_2=CHCO_2CH_3$	
16			
17	$-CH=CH(CH_2)_5CH_3$	$CH_2=CH(CH_2)_5CH_3$	
18	$-(CH_2)_7CH_3$	$CH_2=CH(CH_2)_5CH_3$	H_2, Pd/C
19	$-CH=CH(CH_2)_2N(CH_3)_2$	$CH_2=CH(CH_2)_2N(CH_3)_2$	
20	$-CH=CHCH_2CH(OH)CH_3$	$CH_2=CHCH_2CH(OH)CH_3$	
21	$-CH=CHCH_2C(=O)CH_3$	$CH_2=CHCH_2C(=O)CH_3$	
22	$-CH=CH-CH_2C\equiv N$	$CH_2=CH-CH_2CN$	

5-(1-methoxy-1-methylethyl)-, trans-5-(1-propenyl)-, and 5-(1-methylethenyl)-2'-deoxyuridine.[4] The maximum isolable yield of any one of these was 11%. Reduction of this complex mixture gave just two products, 5-propyl- and 5-isopropyl-2'-deoxyuridine, which could be separated and isolated in much higher yield. As a consequence no attempts have yet been made to synthesize nucleosides 16-22. A strategy to prepare these and related C-5 substituted tubercidin derivatives via allylic halide coupling is described below. Terminal olefins substituted by a conjugating group are much less of a problem since they usually give unsaturated products (4, R' = conjugating group).

Scheme II



IV. Preparation and Reactions of 5-(2-Cyanoethenyl)-, 5-(2-Carboxamidoethenyl)- and 5-(2-Methoxycarbonylethenyl)tubercidin.

Olefins conjugated with strong electron withdrawing groups couple to mercuritubercidin regioselectively and often with regeneration of the double bond. Thus acrylonitrile reacts with 2 in 0.1 M Li_2PdCl_4 in DMF to give trans-5-(2-cyanoethenyl)tubercidin (13) (Scheme III) as the sole isolable product. The yield of purified product seldom exceeds 25%, however no other products could be isolated and identified. The reaction conditions were varied extensively in an attempt to either improve the yield of 13 or at least allow the isolation of other side products that might give us a clue about the nature of the problem. Longer reaction times, higher temperature, or addition of hindered tertiary amine bases did not improve the yield. The vast majority of coupling reactions between nucleoside derived

organopalladium intermediates and olefins proceed faster, more cleanly, and in higher yield in methanol than in DMF. The reaction of acrylonitrile was an exception. Thin-layer chromatographic analysis of a reaction run in methanol showed very little 13. Instead the major product was tubercidin (1) resulting from protiodepalladation in the protic solvent.

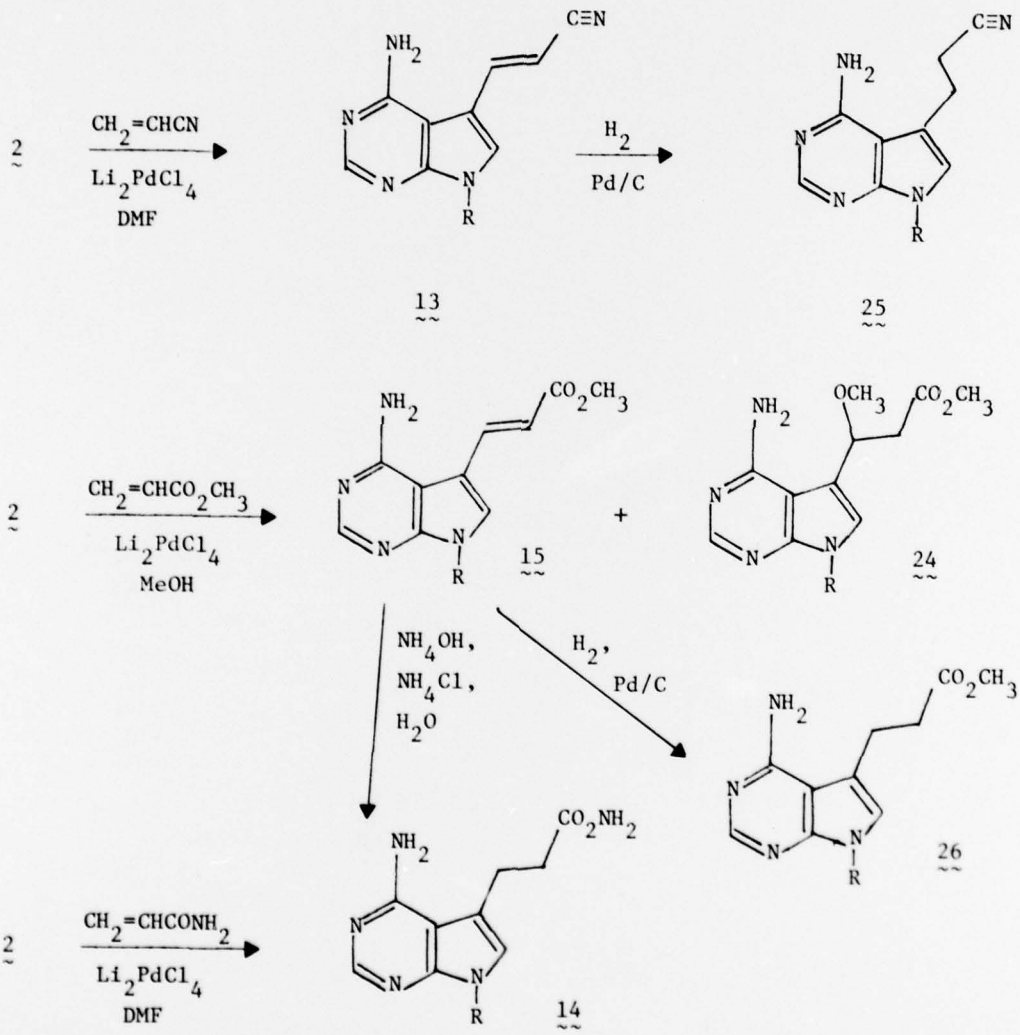
Methyl acrylate and 5-mercuritubercidin (2) react in methanolic Li_2PdCl_4 to give trans-5-(2-methoxycarbonylethenyl)tubercidin (15) (Scheme III) in yields as high as 57%. In one run a side product was separated (12% yield) and identified as 5-(1-methoxy-2-methoxycarbonylethyl)tubercidin (24). However this result could not be reproduced. Since the production of 15 in good yield is reproducible, this compound promises to be a key intermediate for building other C-5 side chains. Catalytic hydrogenation converts 15 quantitatively to 5-(2-methoxycarbonylethyl)tubercidin (26). The carbon-carbon double bond of nucleoside 13 may also be reduced by H_2 over Pd/C. Although 5-(2-cyanoethyl)tubercidin (25) is the major product there is a low level of over reduction, presumably at the cyano function. 5-(2-Carboxamidoethenyl)tubercidin (14) can be obtained in only very low yield (~7%) from the reaction of 2 with acrylamide and Li_2PdCl_4 in either methanol or DMF. A preferable route to 14 is through ammonolysis of 15 (95% yield).

V. Reaction of Allyl Chloride with 5-Mercuritubercidin

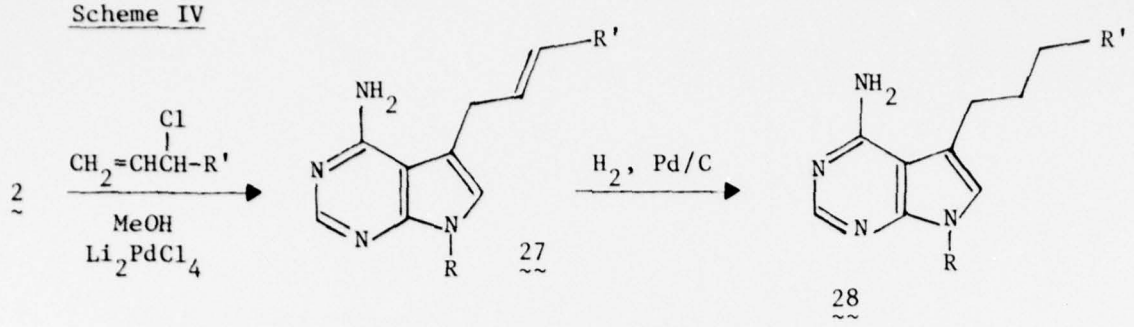
In order to link tubercidin regioselectively to the C-1 position of substituted olefins one must use either a conjugated olefin such as methyl acrylate, an olefin in which the C-2 position is considerably more sterically hindered than the C-1 carbon, or an olefin in which a nonconjugated polar function influences the transition state of the coupling reaction. Allylic chlorides fall into this latter category. Results from the coupling reaction between 5-chloromercuricytidine, Li_2PdCl_4 and allyl chloride may be contrasted to the previously discussed coupling reaction with propylene. The allyl chloride reaction gives a single isolable product, 5-allylcytidine, in greater than 75% yield after purification. [3] There are no traces of products resulting from coupling at C-2 of allyl chloride. Similarly 3-chloro-1-butene gives a high yield of trans-5-(2-buten-1-yl)cytidine even though in this case there was isolated a 10% yield of the isomeric compound 5-(1-buten-3-yl)cytosine which results because of isomerization of 3-chloro-1-butene to crotyl chloride prior to coupling.

As a result of the cytidine studies we envisaged preparing tubercidin derivatives substituted by long carbon side chains through the coupling reaction to allylic chlorides as outlined in Scheme IV. An additional important feature of the allylic halide coupling reaction, unlike the coupling reactions to other types of olefins, is that it is catalytic in palladium. Pd(II) is regenerated through elimination of $PdCl_2$ from the organopalladium intermediate which follows olefin insertion.[3] For these reasons we have put considerable effort into the coupling reaction between allyl chloride and 5-mercuritubercidin.

Scheme III


2
CH2=CHCN
Li2PdCl4
DMF
13
H2
Pd/C
25
CH2=CHCO2CH3
Li2PdCl4
MeOH
15
24
NH4OH,
NH4Cl,
H2O
H2,
Pd/C
26
CH2=CHCONH2
Li2PdCl4
DMF
14
R = HO
OH
OH

Scheme IV



2 → 27 → 28

R = β-D-ribofuranosyl

Under conditions that have proven successful for the preparation of 5-allylcytidine, 5-mercuritubercidin and allyl chloride gave a very low yield of 5-allyltubercidin (10). Purification has proven difficult and a pure analytical sample has yet to be obtained. Other products were separated from the reaction mixture but their complexity and impurity made identification impossible. No variation of reaction conditions improved the outcome of the reaction.

In order to ascertain whether the solubility of 2 was a factor, the 2',3'-O-isopropylidene derivative of 2 was synthesized (see experimental section), however its reaction with allyl chloride has not yet been examined in detail.

More recently a coupling reaction between 3-chloro-1-butene, 0.1 M Li_2PdCl_4 in methanol, and 2 was attempted. A single major product was produced which on the basis of thin-layer chromatographic behavior and the ^1H NMR may be the desired product 5-(2-buten-1-yl)-tubercidin (27, R' = CH_3).

VI. Compounds Submitted for Biological Testing

It would be desirable to submit at least two grams of each target compound to be tested for activity against schistosomes, trypanosomes, and leishmanias. Nevertheless, synthesis and purification of two grams of most of the nucleosides described here is such a time consuming chore that we have been willing to submit compound once greater than six hundred milligrams has been accumulated. Compounds submitted are listed in Table II below. No test results have yet been reported.

Table 2

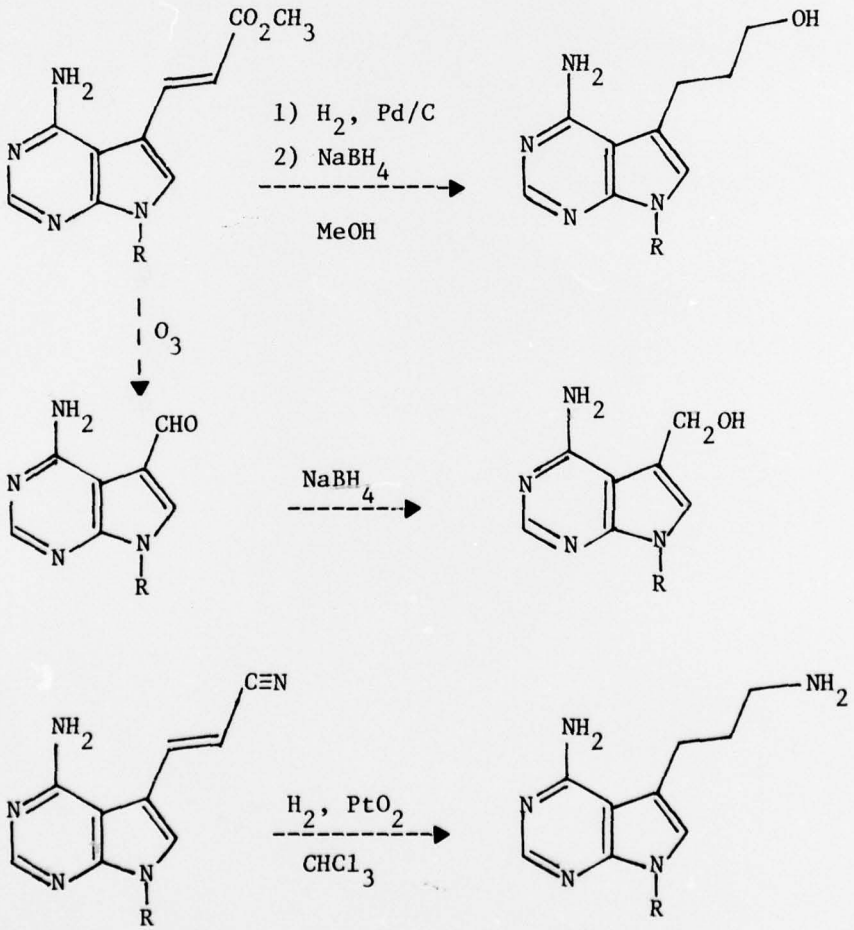
Compound	WRAIR No.	Date Received	Quantity
6	BH73823	8/3/78	676 mgs
6	BH86455	11/1/78	1,097 mgs
13	BH86464	11/1/78	600 mgs
15	BH86473	11/1/78	1,013 mgs
14	BH89536	12/1/78	650 mgs

VII. Conclusion

Current research effort is concentrated on synthetic transformations of nucleosides 13 - 15 as a path to diverse functional group introduction on to the C-5 side chain. The availability of nucleosides 13 and 15 in only two steps from tubercidin and the synthetic versatility of the cyano and methoxycarbonyl groups suggests that this is a viable strategy. Reactions currently under investigation are outlined in Scheme V. If the coupling reaction finally proves successful with 3-chloro-1-butene, then the sequence outlined in Scheme IV will be examined with longer chain allylic chlorides.

The reaction of 2 with styrene proceeds poorly to give low yields of two products that have yet to be identified. Further investigation of this reaction is awaiting new developments in organopalladium methodology.

Scheme V



R = β-D-ribofuranosyl

VIII. Experimental Section

Proton magnetic resonance spectra were taken on either a Varian EM360 60 Mz 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 D_2O or d^6-DMSO. ^{13}C-NMR spectra were obtained on the latter instrument by Paul Bruins. 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 spectometer. 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-$CHCl_3$ (1:3, v/v); B, AcCN-nBuOH-0.1 M NH_4OAc-conc. NH_4OH (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.[8] 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 (ε = 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; ^{1}H-NMR (d^6-DMSO): δ 8.18 (1H, s, H2), 7.45 (1H, d, J = 4 Hz, H6), 7.17 (2H, s, NH_2), 6.70 (1H, d, J = 4 Hz, H5), 6.11 (1H, d, J = 6 Hz, H1'), 4.55 (1H, mult, H4'), 4.1 (2H, mult, H2' and H3'), 3.6 (2H, mult, H5'); ^{13}C-NMR (d^6-DMSO): 157.5 (C4), 151.5 (C2), 149.9 (C7a), 122.3 (C6), 103.1 (C4a), 99.5 (C5), 87.6 (C1'), 85.1 (C4'), 73.7 (C2'), 70.8 (C3'), 61.9 (C5').

A. 5-(1-Methoxyethyl)tubercidin (6)

5-Mercuritubercidin[5] (1.767 g, 3.53 mmoles) was suspended in a soln of 0.1 M Li_2PdCl_4-MeOH (70 mL, 7.0 mmoles) in a 500 mL Parr bottle and the mixture stirred under 30 psig ethylene for 24 h. A black ppt. appeared during the course of the reaction. The mixture was gravity filtered and the ppt. washed with 70 mL methanol. H_2S was bubbled into the combined filtrates for 1 min, and the soln immediately re-filtered, the black metal sulfides then being washed with 50 mL methanol. The filtrate was evaporated to a yellow oil, which was dissolved in water and neutralized

with NH_4OH. The soln was evaporated to dryness, and the residue chromatographed on a silica gel column (150 g, 2.5 cm diam) using a MeOH-$CHCl_3$ gradient. The residue from evaporation of fractions containing a product with Rf of .50 (TLC sys A) was rechromatographed on a Bio-Gel P2 column (150 g, 2.3 cm diam) using H_2O as elutant. Fractions showing a single spot on TLC were combined and lyophilized to a fluffy white solid. Drying 24 h over P_2O_5 in vacuo gave pure 6 (0.837 g, 2.58 mmoles, 73% yield), MP 90°C (dec). ^{1}H-NMR (D_2O): δ 8.15 (1H, s, H-2), 7.36 (1H, s, H-6), 6.19 (1H, d, J = 6 Hz, H-1'), 4.9 to 4.2 (4H, multiplets, H-4', 3',2' and H-1"), 3.88 (2H, mult, H-5'), 3.27 (3H, s, $-OCH_3$), 1.48 (3H, d, J = 6 Hz, H-2"). UV: pH 1.9 (aq. HCl) λ_{max} 276 nm (ε 9420), λ_{min} 249 (4410); pH 6.7 (H_2O) λ_{max} 272 (10,100), λ_{min} 242 (3660); pH 13.0 (aq. NaOH) λ_{max} 272 (10,100), λ_{min} 242 (3810). IR (KBr): 3360 (br), 1630, 1585, 1470, 1298, 1213, 1085 cm^{-1}. ^{13}C-NMR (D_2O) δ 158.3 (C-4), 152.4 (C-2), 152.0 (C-7a), 123.0 (C-6), 120.2 (C-5), 103.6 (C-4a), 89.4 (C-1'), 86.9 (C-4'), 75.6, 74.7, 72.8 (C-3', C-2'c and C-1"), 63.9 (C-5'), 56.9 ($-OCH_3$), 23.6 ($-CH_3$). Elem. Anal. Calcd. for $C_{14}H_{20}N_4O_5 \cdot 1/2\ H_2O$: %C 50.44, %H 6.35, %N 16.81; Found: %C 50.88, %H 6.19, %N 16.76. TLC (solvent system/Rf value): A/.50, B/.49, C/.45

B. 5-(1-Hydroxyethyl)tubercidin (9)

5-(1-Methoxyethyl)tubercidin (6, 0.2021 g, 0.6230 mmoles) was refluxed in pH 7 water (25 mL) for 12 h. The soln was evaporated to dryness, and 25 mL water were again added, the reflux then being continued for 3 more days. At this time TLC indicated that only one product had formed: (solvent system/Rf value) A/.28, B/.43. The soln was lyophilized to a white solid, 9 (0.1876 g, 0.6043 mmoles, 97% yield). ^{1}H-NMR (D_2O) δ 7.94 (1H, s, H2), 7.25 (1H, s, H6), 6.17 (1H, d, J = 5.5 Hz, H1'), 4.9 to 4.2 (4H, mult, H4', H3', H2', H1"), 3.96 (2H, s, H5'), 1.53 (3H, d, J = 6 Hz, H2").

C. 5-(1-Isopropoxyethyl)tubercidin (23)

5-(1-Methoxyethyl)tubercidin (6, 0.105 mg, .324 mmoles), isopropanol (25 mL), conc. H_2SO_4 (1 mL), and 4A molecular sieves (2g) were refluxed (83°C) under argon for 14 h. The mixture was neutralized with NH_4OH and gravity filtered to remove $(NH_4)_2SO_4$ and the molecular seives. TLC of the filtrate indicated that it contained two products, one of which had Rf values corresponding to tubercidin, while the other ran faster than 6 in solvent systems A, B, and C. The filtrate was evaporated to a white residue, which was chromatographed on a silica gel column (80 g, 2.8 cm diam) using a MeOH-$CHCl_3$ gradient. Fractions containing one product with Rf = .54 (TLC Syst. A) were pooled and evaporated to a colorless oil. This oil was dissolved in water and lyophilized to a white solid (hydroscopic). Drying in vacuo over P_2O_5 overnight gave 23 (0.0981 g, 0.278 mmoles, 86% yield). UV (MeOH): λ_{max} 272 nm, λ_{min} 242 nm. TLC (solvent system/Rf value): A/.54,

B/.53, C/.63. ^{1}H-NMR (d^6-DMSO): δ 8.11 (1H, s, H2), 7.37 (1H, s, H6), 6.03 (1H, d, J = 6 Hz, H1'), 4.7 to 3.6 (multiplets, probably for H5', H4', H3', H2', H1"), 3.12 (1H, mult, -O-CH$(CH_3)_2$), 1.42 (3H, d, J = 6 Hz, H2"), 1.13 (6H, mult, -O-CH$(CH_3)_2$).

D. 5-Allyltubercidin (10)

5-Mercuritubercidin (8, .5013 g, 1.000 mmoles) and allyl chloride (.72 mL, 10 mmoles) were stirred in 0.1 M Li_2PdCl_4-MeOH (20.0 mL, 2.00 mmoles) for 48 h, during which time the white mercurial dissolved to give a clear dark orange soln. The soln was saturated with H_2S gas, and the black metal sulfides were removed by gravity filtration. The filtrate was evaporated to dryness, and the residue was chromatographed on a silica gel column (150 g, 2.5 cm diam) with a MeOH-$CHCl_3$ gradient. Fractions containing a product with Rf = .49 (TLC System A) were pooled and evaporated to dryness. The residue was then chromatographed on a column of Bio-Gel P6 (2.5 x 22 cm) using water as the elutant, and fractions containing one product Rf = .49 (TLC System A) were combined and evaporated to give a hygroscopic solid (the product may contain some LiCl). ^{1}H-NMR (D_2O; ext TMS): δ 7.75 (1H, s, H2), 6.87 (1H, s, H6), 5.92 (1H, d, J = 6 Hz, H1'), 6.15 to 5.6 (1H, mult, H2"), 5.15 to 4.8 (2H, mult, H3"), 4.7 to 4.1 (3H, mult, H4', H3', H2'), 3.73 (2H, mult, H5'), 3.14 (2H, d, J = 5 Hz, H1").

E. 5-Mercuri-2',3'-O-isopropylidene tubercidin

2',3'-O-Isopropylidene tubercidin, prepared in 97% yield according to the literature procedure,[7] (1.117 g, 3.65 mmoles), sodium acetate tri-hydrate (0.993 g, 7.2 mmoles), and mercuric acetate (1.162 g, 3.65 mmoles) were refluxed in 25 mL methanol under argon for 5 h. A white ppt. formed during the course of the rn, and this was collected by suction filtration on a Buchner funnel. The solid was washed with 75 mL methanol, 50 mL ether, and then dried overnight in vacuo over P_2O_5 to give 5-mercuri-2',3'-O-isopropylidene tubercidin (1.5008 g, 2.71 mmoles, 74% yield); MP 292°C (dec). UV (10^{-3} M KCN/H_2O): λ_{max} 270, λ_{min} 240. This material lacked sufficient solubility in KCN/D_2O (and other solvents) to allow a ^{1}H-NMR spectrum.

F. 5-(2-Cyanoethenyl)tubercidin (13)

5-Mercuritubercidin (1.0026 g, 2.000 mmoles) was stirred with acrylonitrile (1.33 mL, 20.0 mmoles) and 0.1 M Li_2PdCl_4-DMF (40.0 mL, 4.00 mmoles) for 8 days, diluted with 40 mL methanol, and the resulting soln saturated with H_2S. The mixture was neutralized, gravity filtered to remove the metal sulfides, and then the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (150 g, 2.5 cm diam) with a MeOH-$CHCl_3$ gradient, and all fractions containing a product with Rf = 0.34 (TLC System A) were combined. This pool was evaporated

to dryness and then chromatographed on a column of Bio-Gel P2 (100 g, 2.3 cm diam) with water as the elutant. Fractions showing one product with Rf = .34 (System A) were pooled, and the resulting soln concentrated to allow crystallization of a white product, which was eventually collected by filtration. The solid was dried overnight in vacuo over P_2O_5 to give pure 13 (0.1356 g, .428 mmoles, 22% yield); MP 168°C (dec). ^{1}H-NMR (d^6-DMSO): δ 8.20 (1H, s, H2 or H6), 8.17 (1H, s, H2 or H6), 8.14 (1H, d, J = 16.5 Hz, -CH=CH-CN), 7.13 (2H, s, NH_2), 6.17 (1H, d, J = 16.5 Hz, -CH=CH-CN), 6.12 (1H, d, J = 5 Hz, H1'), 4.44 (1H, mult, H4'), 4.08 (2H, mult, H2' and H3'), 3.65 (2H, mult, H5'). ^{13}C-NMR (d^6-DMSO): δ 157.9 (C4), 152.4 (C2), 151.3 (C7a), 142.6 (C1"), 122.9 (C6), 119.0 (CN), 110.7 (C2"), 100.1 (C4a), 92.5 (C5), 87.0 (C1'), 85.1 (C4'), 73.7 (C2'), 70.3 (C3'), 61.5 (C5'). IR (KBr): 3280 (br), 2230, 1614, 1575, 1444, 1310, 1203, 1050 cm^{-1}. UV: pH 1.2 (aq. HCl) λ_{max} 311 (16,810), λ_{min} 280 (10,410), λ_{max} 255 (19,430), λ_{min} 231 (10,530); MeOH ("neutral") λ_{max} 323 (14,790), λ_{min} 285 (10,190), λ_{max} 268 (15,540), λ_{min} 236 (6370); pH 12.5 (aq. NaOH) λ_{max} 319 (14,010), λ_{min} 286 (11,610), λ_{max} 268 (15,500), λ_{min} 238 (8493). Elem. Anal. Calcd. for $C_{14}H_{14}N_4O_4 \cdot 1/2\ H_2O$: %C 51.53, %H 4.94, %N 21.46; Found: %C 51.53, %H 5.08, %N 21.51. TLC (solvent system/Rf): A/.34, B/.48, C/.68.

G. 5-(2-Carboxamidoethenyl)tubercidin (14)

a. 5-Mercuritubercidin (0.5013 g, 1.000 mmoles) was stirred with acrylamide (0.710 g, 10.00 mmoles) in 0.1 M Li_2PdCl_4-DMF (14 mL, 1.4 mmoles) for two days, diluted with 14 mL methanol, and the resulting soln satd with H_2S gas. The mixture was gravity filtered to remove metal sulfides and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (100 g, 2 cm diam) using a MeOH-$CHCl_3$ gradient, and fractions containing a product with Rf = .33 (TLC system B) were pooled, evaporated to dryness, and rechromatographed on a column of Bio-Gel P2 (100 g, 2.5 cm diam) with water as elutant. Fractions containing one product with Rf = .33 (TLC system B) were pooled and concentrated to allow crystallization of a white product. The solid was collected by gravity filtration and then dried 24 h in vacuo over P_2O_5 to give pure 14 (0.0234 g, 0.070 mmoles, 7% yield); MP 220°C (dec). ^{1}H-NMR (d^6-DMSO): δ 8.17 (1H, s, H2), 7.98 (1H, s, H6), 7.71 (1H, d, J = 16 Hz, H1"), 6.89 (2H, s, NH_2), 6.36 (1H, d, J = 16 Hz, H2"), 6.12 (1H, d, J = 6 Hz, H1'), 4.47 (1H, mult, H4'), 4.05 (2H, mult, H3', H2'), 3.67 (2H, mult, H5'). ^{13}C-NMR (d^6-DMSO): δ 167.2 (-COHN$_2$), 157.3 (C4), 151.6 and 150.9 (C2 and C7a), 130.6 (C1"), 121.8 and 121.1 (C6 and C2"), 111.5 (C5), 100.8 (C4a), 86.9 (C1'), 85.2 (C4'), 73.8 (C2'), 70.6 (C3'), 61.7 (C5'). IR (KBr): 3290 (br), 1618, 1460, 1300, 1060 cm^{-1}. UV: pH 1.5 (aq. HCl) λ_{max} 306 (15,690), λ_{min} 275 (10,840), λ_{max} 246 (17,820), λ_{min} 228 (14,140); pH 5.9 (H_2O), λ_{max} 310 (14,120), λ_{min} 279 (11,470), λ_{max} 267 (12,520), λ_{min} 236 (9452); pH 12.6 (aq. NaOH) λ_{max} 312 (14,330), λ_{min} 279 (11,290), λ_{max} 268 (12,440), λ_{min} 238 (9653). Elem. Anal. Calcd. for $C_{14}H_{17}N_5O_5 \cdot 1/2\ H_2O$: %C 48.84, %H 5.27,

%N 20.34; Found: %C 48.61, %H 5.07, %N 20.09. TLC (solvent system/Rf value): A/.08, B/.33, C/.38.

b. 5-(2-Carbomethoxyethenyl)tubercidin (15, .029 g, .083 mmoles) and NH_4Cl (.021 g) were stirred in concentrated NH_4OH (4 mL) for 7 h at room temp. After 4 h of reaction a white ppt appeared in the viscous soln. After 7 h the crop of crystals was collected by filtration, washed, and dried overnight in vacuo over P_2O_5 to give 14 (.0261 g, .078 mmoles, 95% yield). This product was compared with 14 from the coupling rn by TLC (4 solvent systems), UV, IR and ^{1}H-NMR spectra, and MP.

H. 5-(2-Carbomethoxyethenyl)tubercidin (15)

5-Mercuritubercidin (2, 0.5013 g, 1.000 mmoles) and methyl acrylate (0.902 mL, 10.0 mmoles) were dissolved in 0.1 M Li_2PdCl_4-MeOH (20.0 mL, 2.00 mmoles) and stirred for 20 h. The mixture was treated with H_2S and then gravity filtered to remove metal sulfides. The filtrate was evaporated to dryness and chromatographed on a silica gel column (100 g. 2 cm diam) using a MeOH-$CHCl_3$ gradient. Fractions containing a product with Rf = .46 (TLC syst. A) were pooled; the resulting soln was evaporated to dryness, and then the residue was chromatographed on a column of Bio-Gel P2 (100 g, 2 cm diam) using H_2O as elutant. Fractions containing the product with Rf = .46 (TLC syst. A) were pooled and lyophilized to give a light yellow solid. Drying overnight in vacuo over P_2O_5 gave 15 (0.2013 g, 0.5746 mmoles, 57% yield), MP 120°C (dec). ^{1}H-NMR (d^6-DMSO) δ 8.20 (1H, s, H2 or H6), 8.18 (1H, s, H2 or H6), 8.02 (1H, d J = 16 Hz, H1"), 6.95 (2H, s, $-NH_2$), 6.45 (1H, d, J = 16 Hz, H2"), 6.12 (1H, d, J = 6 Hz, H-1'), 4.45 (1H, mult, H4'), 4.17 to 3.98 (2H, mult, H-2', H-3'), 3.76 (3H, s, $-OCH_3$), 3.65 (2H, mult, H5'). UV: pH 1.5 (aq. HCl) λ_{max} 308 nm (ε 13,460), λ_{min} 277 (10,170), λ_{max} 251 (13,970), λ_{min} 237 (13,470); pH 6.4 (water) λ_{max} 321 (11,610), λ_{min} 299 (10,390), λ_{max} 270 (11,840), λ_{min} 238 (8240); pH 12.6 (aq. NaOH), λ_{max} 296 (12,900), λ_{min} 275 (10,810), λ_{max} 267 (11,210), λ_{min} (10,150). IR (KBr): 3340 (br), 1620, 1585, 1440, 1300, 1190, 1080 cm^{-1}. ^{13}C-NMR (d^6-DMSO): 166.3 ($-\underset{\approx}{C}O_2CH_3$), 157.4 (C4), 151.5 and 151.1 (C2 and C7a), 136.9 (C1"), 123.6 (C6), 115.1 (C2"), 110.5 (C5), 100.7 (C4a), 86.9 (C1'), 85.0 (C4'), 73.8 (C2'), 70.3 (C3'), 61.5 (C5'), 51.1 ($-OCH_3$). Elem. Anal. Calcd. for $C_{15}H_{18}N_4O_6 \cdot 3/4H_2O$: %C 49.52, %H 5.40, %N 15.40; Found: %C 49.57, %H 5.31, %N 15.40. TLC (solvent system/Rf value): A/.45, B/.40, C/.56.

The 1"-methoxy adduct side product, 24, formed in 9% yield in this specific reaction. This compound is very hygroscopic. ^{1}H-NMR (D_2O): δ 8.36 (1H, s, H2), 7.67 (1H, s, H6), 6.24 (1H, d, J = 6 Hz, H1'), 5.3 to 3.8 (6H, multiplets, ribosyl and H1"), 3.73 (3H, s, $-CO_2\underset{\approx}{C}H_3$), 3.37 (3H, s, $-OCH_3$), 2.90 (2H, mult, H2"). TLC (solvent system/Rf value): A/.53, B/.47.

I. 5-(2-Carbomethoxyethyl)tubercidin (26)

5-(2-Carbomethoxyethenyl)tubercidin (15, 0.0360 g, 0.103 mmoles), 10% Pd/C catalyst (0.011 g, 10 mol % Pd/nucleoside) were stirred in methanol (20 mL) under 30 psig H_2 in a 200 mL Parr flask for 4 h. The Pd/C was removed from the mixture by gravity filtration, then the filtrate was evaporated to leave 26 as a cream-white solid; MP 93°C (dec). ^{1}H-NMR (D_2O): δ 7.80 (1H, s, H2), 6.87 (1H, s, H6), 5.98 (1H, d, J = 5.5 Hz, H1'), 4.7 to 4.1 (3H, complex mult., H4', H3', H2'), 3.9 (2H, mult, H5'), 3.69 (3H, s, OCH_3), 2.53 (4H, s, (broad), H1", H2"). UV (H_2O): λ_{max} 277, λ_{min} 248. TLC (solvent system/Rf value): A/.42, B/.46, C/.54.

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Abstract

Methodology for the synthesis of tubercidin derivatives substituted by carbon chains at the C-5 position has been developed. 5-Mercuritubercidin, prepared by reaction of tubercidin with mercuric acetate in H_2O, undergoes coupling to olefins when treated with lithium palladium chloride in either methanol or N,N-dimethylformamide. In this way 5-(1-methoxyethyl)-tubercidin was prepared from ethylene, 5-(2-cyanoethenyl)tubercidin from acrylonitrile, 5-(2-carboxamidoethenyl)tubercidin from acrylamide, and 5-(2-methoxycarbonylethenyl)tubercidin from methyl acrylate. Various synthetic transformations of these tubercidin derivatives were investigated. 5-(1-Methoxyethyl)tubercidin was transformed to 5-(1-hydroxyethyl)tubercidin by refluxing in water and to 5-(1-isopropoxyethyl)tubercidin by warming with 2-propanol and sulfuric acid. Reduction of 5-(2-cyanoethenyl)-tubercidin with H_2 over Pd/C gave 5-(2-cyanoethyl)tubercidin, while 5-(2-methoxycarbonylethenyl)tubercidin gave 5-(2-methoxycarbonylethyl)-tubercidin under the same conditions.

The reaction of allyl chloride with 5-mercuritubercidin in 0.1 M Li_2PdCl_4 in methanol gave only a low yield of 5-allyltubercidin. Studies directed towards the use of other allylic chloride in the coupling reaction, and the potential of the cyanoethenyl and methoxycarbonylethenyl side chains for further synthetic transformations are discussed. The activity of the C-5 substituted tubercidin derivatives as antischistosomal, antitrypanosomal, and antileishmonial agents is under investigation.

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