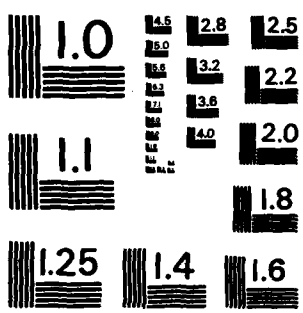
AD-A128 629 SYNTHESIS OF C-5 SUBSTITUTED TUBERCIDIN DERIVATIVES(U) 1/1
CALIFORNIA UNIV DAVIS D E BERGSTROM JUN 80
DAMD17-78-C-8014

UNCLASSIFIED F/G 6/15 NL

END
DATE FILMED
5 83
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS - 1963 - A

AD

SYNTHESIS OF C-5 SUBSTITUTED TUBERCIDIN DERIVATIVES

Final Report

Donald E. Bergstrom

(for the period 1 February 1978 to 30 June 1980)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD-17-78-C-8014

University of California
Davis, California 95616

Approved for public release; distribution unlimited

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER / 2. GOVT ACCESSION NO. AD-A126629	3. RECIPIENT'S CATALOG NUMBER
4. TITLE *(and Subtitle)* Synthesis of C-5 Substituted Tubercidin Derivatives	5. TYPE OF REPORT & PERIOD COVERED Final Report (February 1978-June 1980) 6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Donald E. Bergstrom, Ph.D.	8. CONTRACT OR GRANT NUMBER(s) DAMD17-78-C-8014
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of California Davis, California 95616	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62770A.3M162770A803.00.044
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research and Development Command Fort Detrick Frederick, Maryland 21701	12. REPORT DATE June 1980 13. NUMBER OF PAGES 26
14. MONITORING AGENCY NAME & ADDRESS(*if different from Controlling Office*)	15. SECURITY CLASS. *(of this report)* Unclassified 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT *(of this Report)* Approved for public release; distribution unlimited	
17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*	
18. SUPPLEMENTARY NOTES	
19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*	
20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*	

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

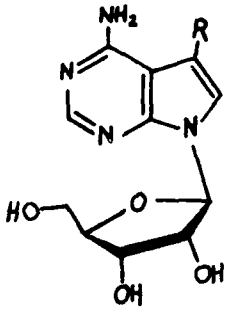
SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

TABLE OF CONTENTS

I. Introduction

The present report describes progress for the period Feb. 1, 1980 and summarizes the results of two and one-half years of synthetic studies. At the outset our major objective was to synthesize a series of C-5 substituted tubercidin derivatives to be tested as antiparasitic agents. As discussed in our proposal of August 1977 there is significant biochemical rational for biological activity in this series of compounds. However there was neither sufficient data to allow us to predict activity in this class with absolute certainty nor was there any clue to the type of C-5 substituent most likely to give the greatest activity. Consequently we have sought to develop synthetic methodology of broad enough scope to attach many kinds of functional groups at the C-5 position of tubercidin. The history of the development of this synthetic methodology is outlined in the two previous annual reports. Side chains are attached at C-5 by the reaction of 5-mercuritubercidin[1] with lithium palladium chloride and olefins or carbon monoxide. The majority of compounds synthesized for biological testing were made by this route. During the period February - June, 1980 (here after referred to as year 3) attempts were made both to expand the scope of the coupling reaction, and to develop other direct methods for attaching side chains. Table I outlines the target compounds whose syntheses we proposed to investigate by the end of the contract. The status of each compound is outlined in the Table.

Table I Synthetic Targets



Compound	R	Status[a]
1	CN	U
2	CH=CHPh	S2
3	CH=CHCF$_3$	U
4	CH$_2$CH$_2$CH$_2$NH$_2$	U
5	CH=CHBr	S2
6	CH$_2$OH	S2
7	CH$_2$OR'	R3
8	CH$_2$NHR'	U
9	CH$_2$CH=CHCH$_3$	S2

Table I continued.

[a]U = synthesis unsuccessful; details discussed in section following.
S2 = synthesis successful; discussed in 2nd annual report.
R3 = reaction successful, but not efficient enough for large scale preparation; details discussed in section following.

Direct halogenation or nitration of tubercidin were not previously proposed as possible reactions for investigation. The 5-halogenated derivatives are established as biologically active compounds, but synthetic work from another laboratory suggested that they would be obtained only by way of a lengthy circuitous route.[2] During the course of our studies on the bromination of 5-mercuritubercidin we discovered that N-bromosuccinimide could be used to successfully brominate tubercidin at either the C-5 or C-6 position. A major part of our effort in year three was devoted to investigation of this and related reactions. The results of the halogenation studies are summarized in Appendix A, which is a draft of a manuscript submitted for publication.

II. Synthetic Studies

A. 5-Cyanotubercidin (Toyocamycin) (1)

Three routes from 5-iodotubercidin were investigated. Cuprous cyanide in dimethyl sulfoxide or hexamethylphosphoramide (following established procedures) gave a mixture of products, but none that could be isolated and identified as 5-cyanotubercidin. Neither tris(triphenylphosphine) nickel (0) with sodium cyanide in ethanol nor DMF[3] gave detectable product. Although touted as an improved method for transforming arylhalides to nitriles, even with simple model compounds such as bromobenzene, we obtained poor results. Neither were we successful with cyanogenation by tetrakis(triphenylphosphine) palladium (0) in THF.[4] In each case the zero-valent transition metal complex was generated in a number of different ways, and the reaction attempted under variable conditions.

B. 5-(3,3,3-Trifluoropropenyl)tubercidin (3)

3,3,3-Trifluoropropene reacts with 5-chloromercuri-2'-deoxyuridine and lithium palladium chloride in methanol to give a good yield of 5-(3,3,3-trifluoropropenyl)-2'-deoxyuridine.. Under identical conditions 5-mercuritubercidin failed to give a detectable product. Other solvents, longer reaction times, or variations in catalyst composition still gave only negative results. The complete absence of isolable product has been particularly baffling.

C. 5-(3-Aminopropyl)tubercidin (4)

Reduction of 5-(Carboxamidoethyl)tubercidin by diborane in THF represented our last attempt to synthesize 4. Thin layer chromatography of the crude reaction mixture shows a new product, however difficulty was encountered during purification. In one instance the product could not be isolated after running on Bio-gel P-2 eluting with water.

D. 5-Alkoxymethyltubercidin (7)

One way to attach long alkyl side chains at C-5 would be via an ether linkage to a methylene group. Since previous research has established conditions for replacing the hydroxy of a hydroxymethyl arene with an alkoxy group, we anticipated that 5-hydroxymethyltubercidin (6) could be transformed to 7. Because of the labor required to obtain sizable amounts of 6 the reaction was attempted on less than 0.1 mmole of compound. In 0.3 M HCl in ethanol 6 was transformed to a new product in three days at 70 - 80°C. Because of the scale of the reaction the product was characterized only by thin layer chromatography. The RF was sufficiently high (0.47 in 25% MeOH/$CHCl_3$) to suggest that the ethoxy group had replaced the hydroxy group.

E. 5-Alkylaminomethyltubercidin (8)

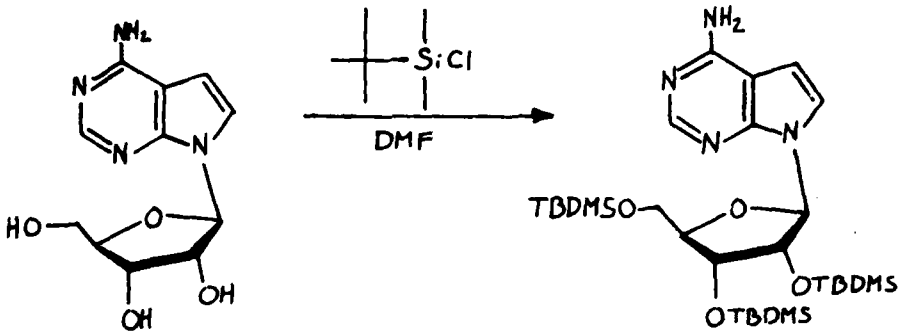
This reaction has been tested only on a small test scale (0.04 mmole) by heating 6 with cydohexylamine in 0.5 N HCl, and did not give any detectable product.

F. Coupling Reactions with Haloolefins

Isolable products could not be obtained from the reactions of either vinyl fluoride or 1,1-difluoroethene with 5-mercuritubercidin. However, vinyl bromide did give a low yield of two products. These were not separated, but characterized by ^1H NMR, thin layer chromatography and high pressure liquid chromatography. They were tentatively identified as 5-(1-methoxyethyl)tubercidin and 5-(2,2-dimethoxyethyl)tubercidin, on the basis of the ^1H NMR spectrum. NMR data for peak assignments of the 2,2-dimethoxyethyl side chain was available from a spectrum of 5-(2,2-dimethoxyethyl)-2'-deoxyuridine. The yields were far too low to warrent scale up and preparation for biological testing.

G. Nitration of Tubercidin

Success with the direct electrophilic halogenation of tubercidin (Appendix A) led us to investigate a mild nitration procedure. Of all the methods of nitration, probably the most mild uses the reagent nitronium tetrafluoborate in sulfolane. Reaction of tubercidin with a two-fold excess of nitronium tetrafluoborate in sulfolane resulted in the evolution of a brown gas (N_2O_4). Thin layer chromatographic analysis showed very little tubercidin but five new products. Since the nitronium ion is a strong oxidizing agent, it was reasonable to suppose that substantial sugar oxidation had occurred. To prevent oxidation at the ribosyl hydroxyl groups, the tert-butyldimethylsilyl group was investigated as a potential protecting group. Tubercidin is easily converted to 2', 3', 5'-O-tri(tert-butyldimethylsilyl)-tubercidin (10) by reaction with tert-butyldimethylsilyl chloride and imidazole in DMF (see Experimental Section). Reaction with nitronium tetrafluoborate gave a low yield of one new product which could be purified by chromatography but the yield of purified product was very low. The structure of the product has yet to be determined. None of the proton signals on the pyrrolo [2,3-d]pyrimidine changed position. The sugar region of the ^1H spectrum (3.4-5.0 ppm) is too complex to assign individual signals with certainty, but there may be extra protons in this region. One new peak (79.15 ppm) appeared in the ^{13}C spectrum leading us to believe that what



ever the reaction it may have occurred on one of the methyls of a protecting group. Recently Watanabe and Ueda found that tubercidin protected at the hydroxyl groups by acetyl could be nitrated.[5] 2', 3', 5'-Tri-O-acetyl-7-deazaadenosine (7-deazaadensoine and tubercidin are synonomous) reacted with fuming nitric and sulfuric acids in methylene chloride to give a mixture of 5-nitro- and 6-nitro-2', 3', 5'-tri-O-acetyltubercidin. The authors did not report whether the acetyl protecting groups could be removed without further decomposing the molecules.

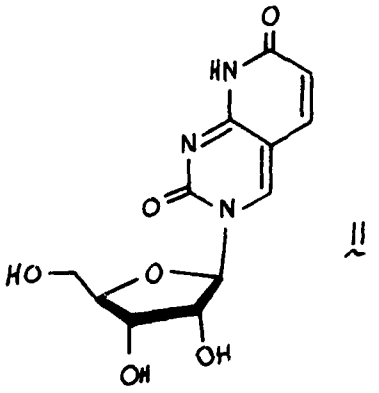
H. Halogenation of Tubercidin

N-Bromosuccinimide in DMF has been reported to be a particularly mild brominating reagent for polycyclic aromatic hydrocarbons susceptible to over oxidation. As discussed in Appendix A, under carefully controlled conditions it is possible to selectively synthesize either 5-bromo-or 6-bromo-tubercidin. N-Chlorosuccinimide gave only 5-chloro-or 5,6-dichloro-tubercidin. Direct iodination (by N-iodosuccinimide) was unsuccessful but as reported previously (2nd Annual Progress Report) 5-iodotubercidin can be obtained by iodination of 5-mercuritubercidin.

III. Tubercidin Derivatives Submitted for Biological Testing

Every new compound synthesized in amount over 0.3 grams since the inception of the contract has been submitted for biological testing. The compounds and the quantity submitted are listed in Table II. Some test results have been obtained on compounds submitted prior to the end of 1979. Where tests have been run the type of results are indicated. All compounds examined so far have shown no activity and some toxicity in tests against Leishmania donovani and Schistosoma mansoni. Some activity has been observed in antitrypanosomal tests. The highest activity was displayed by 5-hydroxymethyltubercidin (6). The results of these tests are summarized in Table III. Although a cure rate of 80% (4 of 5 mice) was found at a dose level of 212 mg per kg of body weight, at higher dose levels the compound proved to be toxic. Because of the relatively high dosage necessary for cure and the observed toxicity 6 has little promise as an effective anti-trypanosomal agent. Yet, it is worthwhile to point out the difference in activity

between 6 and the parent compound tubercidin. Tubercidin showed no activity at any dose level from 13.3 mg/kg to 424 mg/kg. At 53 mg/kg 60% of the mice died as a result of the toxicity. Higher doses led to 100% toxic deaths. Thus, there is a significant differance between the compounds and the differance is in line with our original predictions. C-5 substituted derivatives of tubercidin are more selective than the parent compound. A more definitive statement can be made once all of the submitted derivatives have been tested. One additional compound, 3-β-D-ribofuranosyl-2,7-dioxopyrido[2,3-d]pyrimidine (11), synthesized in conjunction with a National Cancer Institute project was submitted (1.0 gram) for biological testing in September 1979 as DEB-7 (WRAIR No. BJ 30627). No activity was found in the primary curative test for Schistosoma mansoni.



11

IV. Experimental

Data on instrumentation and general procedure may be found in the Experimental Section of the Annual Progress Report for the period 1 February 1979 to 31 January 1980. Experimental procedures for preparation of C-5 and C-6 halogenated tubercidin derivatives are given in Appendix A. Procedures for preparation of 2', 3', 5'-O-Tri(tert-butyldimethylsilyl)tubercidin and data on the reaction with nitronium tetrafluoborate are given below.

2', 3', 5'-O-Tri(tert-Butyldimethylsilyl)tubercidin (10)

To 266 mg (1.0 mmol) tubercidin and 817 mg (12.0 mmol) imidazole in 4.0 ml dry DMF was added 904 mg (6.0 mmol) tert-butyldimethylsilyl chloride and the mixture was stirred 20 hours at 24°. The reaction mixture was poured into water and extracted with ether, dried over anhydrous $MgSO_4$, filtered and evaporated. Chromatography on 100 g silica gel eluting with a methanol-chloroform gradient gave, after evaporation of the fractions containing product, 468 mg (77%) of white crystals. HPLC analysis on ultrasphere 5μ ODS using methanol solvent shows >99% purity monitoring at 270 mm.

^{1}H-NMR ($CDCl_3$, TMS): 8:32 (s,1H), 7.35 (d,1H), 6.36 (d,1H), 6.25 (d,1H), 4.55 (m,1H), 4.28 (m,1H), 4.08 (m,1H), 3.88 (m,2H), 0.90 (s,18H), 0.74 (s,9H), 0.11

(s,12H), -0.10 (s,3H), -0.28 (s,3H); ^{13}C-NMR (DMSO-d_6): 157.40, 151.72, 150.51, 121.09, 102.72, 99.91, 86.01, 84.85, 75.42, 74.92, 72.72, 62.97, 25.82, 25.72, 18.04, 17.80, 17.49, -5.48, -4.75.

Nitration of 2', 3', 5'-O-Tri(tert-Butyldimethylsilyl)tubercidin

2', 3', 5'-O-Tri(tert-butyldimethylsilyl)tubercidin (1.82 gm, 3.0 mmol) was stirred with 6 ml of 0.5 M nitronium tetrafluoborate in sulfolane solution (3.0 mmol) at room temperature for 16 hours. The reaction mixture was poured into water and extracted with ether. The organic phase was dried over anhydrous $MgSO_4$ and evaporated. The reaction product was purified by chromatography on silica gel eluting with a methanol-chloroform gradient, preparative reversephase chromatography on an RP-8 column eluting with 7% water in methanol, rechromatography on silica gel and recrystallization from ether-pentane. This product of about 85% purity by HPLC was again preparatively chromatographed on the RP-8 column using 10% water in methanol. Analytical HPLC showed 95% purity monitoring at 270 mm. Yield: 45mg. ^{1}H-NMR($CDCl_3$, TMS): 8.34 (s,1H), 7.08 (d,1H), 6.40 (d,1H), 5.74 (d,1H), 5.18 (m,1H), 4.5-3.7 (m,6H), 0.98 (s,16H), 0.78 (s,9H), 0.18 (s,12H), -0.14 (s,3H), -0.60 (s,3H).

^{13}C-NMR (DMSO-d_6): 157.56, 151.32, 149.76, 122.75, 103.34, 99.53, 87.63, 86.41, 79.15, 74.46, 73.29, 61.64, 25.75, 25.55, 17.84, 17.50, -4.87, -5.66.

Personnel Supported

Michael J. Schweichert M.S. Degree 1978

Mark K. Ogawa, Research Associate

Albert J. Brattesani, Research Associate

Mary A. Powers, Research Assistant

Paul J. Warwich, Jr. (partial support)

Bibliography

Two manuscripts have been submitted for publication:

Bergstrom, D.E. and Brattesani, A.J., "Halogenation of Tubercidin by N-Bromosuccinimide and N-Chlorosuccinimide. A Direct Route to 5-Bromotubercidin and 5-Chlorotubercidin", submitted to J. Org. Chem..

Bergstrom, D.E.; Brattesani, A.J.; Ogawa, M.K. and Schweichert, M.J., "Pyrrolo[2,3-d]pyrimidine Nucleoside Antibiotic Analogs. Synthesis via Organopalladium Intermediates Derived from 5-Mercuritubercidin", submitted to J. Org. Chem..

References

1. Bergstrom, D.E., and Schweichert, M.J., J. Carbohydrates-Nucleosides-Nucleotides, 5, 285-96 1978.

2. Hinshaw, B.C.; Gerster, J.F.; Robins, R.K., and Townsend, L.B., J. Heterocyclic Chem., 6, 215-21 (1969).

3. Cassar, L.; Foa', M.; Montanari, F., and Marinelli, G.P., J. Organomet. Chem., 173, 335-339 (1979).

4. Sekiya, A., and Ishikawa, N., Chem Lett., 277-278 (1975).

5. Watanabe, S., and Ueda, T., Nucleic Acids Research, Symposium Series No. 8, p. S21-26 (1980).

6. Rane, L.; Rane, D.S., and Kinnamon, K.E., "Screening Large Numbers of Compounds in a Model Based on Mortality of Trypanosoma rhodesien e Infected Mice", Amer. J. Trop. Med. Hyg., 25, 395-400 (1976).

Summary

5-Mercuritubercidin has proven to be a useful intermediate in synthesis of C-5 substituted tubercidin derivatives. Because of limitations of the coupling reaction between olefins, lithium palladium chloride, and 5-mercuritubercidin, recent efforts have concentrated on synthetic transformations of readily synthesized C-5 modified tubercidin derivatives, and on direct electrophilic substitution of tubercidin. Limitations of the coupling reaction are exemplified the finding that none of four halogenated olefins, vinyl fluoride, vinyl br de, 1,1-difluoroethene, and 3,3,3-trifluoropropene gave appreciable amounts of duct on reaction with 5-mercuritubercidin and lithium palladium chloride. 5 Iodotubercidin, which is obtained by the reaction of 5-mercuritubercidin wi iodine, did not give 5-cyanotubercidin with any of reagents commonly used t transform aryl iodides to nitriles. 5-Hydroxymethyltubercidin was studied a an intermediate for the synthesis of 5-alkoxymethyltubercidin and 5-alkylaminomethyltubercidin. Preliminary evidence suggests that the transformation to alkoxy derivations is workable, but the difficulty in obtaining sufficient 5-hydroxymethyltubercidin to produce enough of any alkoxy derivatives for biological testing has greatly limited the approach.

Finally the potential of direct electrophilic substitution of tubercidin was explored. Bromination of tubercidin by N-bromosuccinimide in N,N-dimethylformamide gave 5-bromotubercidin when unbuffered and 6-bromotubercidin when buffered with sodium acetate. 5,6-Dibromotubercidin was a minor product under both conditions. N-Chlorosuccinimide and tubercidin gave 5-chlorotubercidin and 5,6-dichlorotubercidin. Nitration of tubercidin by nitronium tetrafluoborate in sulfolane gave at least five unidentified products. When the ribosyl of tubercidin was protected by tert-butyldimethylsilyl groups a single unidentified product was obtained. The results of preliminary biological testing show that C-5 substituted derivatives of tubercidin have little activity against Leishmania donovani and Schistosoma mansoni. Activity against Trypanosoma rhodesiense was pronounced, leading in some cases to cures. The compounds are unlikely to be of therapeutic value however because of the high does levels required and the ensuing toxicity.

Table II Tubercidin Derivatives Submitted for Biological Testing

Compounds	WRIAR No.	Quantity (g)	Date Received	Test Results[a] L	T	S
5-(1-Methoxyethyl)	BH 73823	0.676	78/08/03	TX	A	NA
5-(1-Methoxyethyl)	BH 86455	1.097	78/11/01			
5-(2-Cyanoethenyl)	BH 86464	0.600	78/11/01		A	NA
5-(2-Methoxycarbonylethenyl)	BH 86473	1.013	78/11/01		NA	NA
5-(2-Carboxamidoethenyl)	BH 89536	0.650	78/12/01			NA
5-(2-Cyanoethyl)	BJ 30609	1.027	79/07/20			TX
5-(1-Methoxy-2-Methoxycarbonylethyl)	BJ 30618	0.904	79/07/20			NA
5-(2-Methoxycarbonylethyl)	BJ 36610	0.843	79/08/31		NA	
5-(2-Phenylethenyl)	BJ 45351	1.000	79/12/26		TX	
5-Hydroxymethyl	BJ 45360	1.023	79/12/26		A	
5-Methoxycarbonyl	BJ 45379	1.004	79/12/26		TX	
5-Iodo	BJ 51840	0.830	80/03/18			
5-(2-Bromoethenyl)	BJ 51859	0.900	80/03/18			
5-(2-Butenyl)	BJ 51868	1.003	80/03/18			
5-(2-Carboxyethenyl)	BJ 51877	1.001	80/03/18			
6-Bromo	BJ 51886	1.005	80/03/18			
5,6-Dichloro	BJ 51895	1.005	80/03/18			
5-Chloro	BJ 51902	1.005	80/03/18			
5-Bromo	BJ 64267	0.761	80/08/06			

[a]L= Activity in Leishmania donovani test in hamsters.

T= Antitrypanosomal Activity.

S= Primary curative test for Schistosoma mansoni.

A= Active.

NA= Not active.

TX= Toxic.

Table III Activity of 5-Hydroxymethyltubercidin Against T. Rhodesiense in ICR/NA Swiss Mice[a]

Dose (g/kg)	Cure	Mean Survival Time[b] (dose)	Toxicity[c]
(1) 0.0265	0	5.2	0
0.053	0	7.4	0
0.106	60%	6.5	0
0.212	80%	12	0
0.424	40%	10	2
(2) 0.106	60%	7.5	0
0.424	80%	-	1

[a]The compound was administered subcutaneously following the procedure of Rane et al[6]. Five female mice were tested at each dose level.

[b]Mean survival time of uncured mice.

[c]Number of mice dying prior to day 3 because of drug toxicity.

Appendix A

Halogenation of Tubercidin by N-Bromosuccinimide and N-Chlorosuccinimide. A Direct Route to 5-Bromotubercidin and 5-Chlorotubercidin.

D. E. Bergstrom* and A. J. Brattesani

Department of Chemistry
University of California
Davis, California 95616

ABSTRACT

Tubercidin may be directly brominated by reaction with N-bromosuccinimide in DMF to give 5-bromotubercidin, a reversible inhibitor of RNA synthesis. When buffered with sodium acetate the major product is 6-bromotubercidin. 5,6-Dibromotubercidin is formed in minor amounts under both conditions. N-Chlorosuccinimide and tubercidin give 5-chlorotubercidin and 5,6-dichlorotubercidin.

INTRODUCTION

5-Bromotubercidin (2) has been suggested as a tool for the study of polynucleotide metabolism in eukaryotic cells. Reich and coworkers showed it to be a reversible inhibitor of heterogeneous nuclear RNA and ribosomal RNA synthesis in cultures of chick embryo fibroblasts,[1] in addition to blocking viral RNA synthesis.[1-3] The unavailability of 2 has been an important factor in its infrequent use. In contrast C-5 brominated pyrimidine nucleosides and C-8 brominated purine nucleosides have found extensive use in nucleic acid research. These are readily available by direct bromination of commonly available nucleosides.[4] The only reported synthesis of 2 invoked bromination of 4-chloro-7-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine by N-bromoacetamide in CH_2Cl_2, followed by treatment with methanolic NH_3.[5,6] 5-Chlorotubercidin and 5-iodotubercidin were synthesized via the same intermediate.

N-Bromosuccinimide (NBS) in DMF has been demonstrated to be a particularly mild combination of reagent and solvent for electrophilic bromination.[7] The combination of NBS in DMF has been used successfully for the synthesis of 8-bromoguanosine.[8] On the basis of these results the reaction of NBS with tubercidin was carefully examined.

RESULTS AND DISCUSSION

We have found that tubercidin (1) can be directly brominated or chlorinated under carefully controlled conditions. Treatment of tubercidin in N,N-dimethylformamide with N-bromosuccinimide (NBS) at room temperature leads to two products, 5-bromotubercidin (2) and 5,6-dibromotubercidin (3) (Scheme I). These are readily separated by silica gel chromatography and purified by recrystallization from methanol. Chlorination of 1 by N-chlorosuccinimide gives 5-chlorotubercidin (5) and 5,6-dichlorotubercidin (6) under similar conditions.

When the reaction mixture was buffered with potassium acetate the outcome was entirely different. The major product was now 6-bromotubercidin (4) which was isolated in 52% yield. By HPLC analysis the yield of 5-bromotubercidin in this reaction mixture was less than 2%. 5,6-Dibromotubercidin was again a minor product (3% yield).

In the absence of potassium acetate the amount of 6-bromotubercidin formed in the reaction was less than 1%. Thus the reaction is exceptionally selective for either 2 or 4 depending on the absence or presence of an acetate buffer. We have also tried N-iodosuccinimide in DMF but got no reaction. However, 5-iodotubercidin can be synthesized in two steps from tubercidin via 5-mercuritubercidin.[9,10]

Structure proofs were based on ^{1}H NMR, ^{13}C NMR, UV and elemental analysis. NMR assignments were based on data obtained for a large number of C-5 substituted tubercidin analogs.[10] The effect of the halogen at C-6 on the ^{1}H NMR signal of H-2' is noteworthy. In one study the signal for H-2' for adenosine was observed at δ 4.15 in contrast to 8-bromoadenosine where the signal fell at δ 4.77.[11] Other sugar protons were not significantly shifted. We have observed a shift of similar magnitudes for the H-2' resonance between 5-bromotubercidin and 5,6-dibromotubercidin and between 5-chlorotubercidin and 5,6-dichlorotubercidin (Table I).

SCHEME I

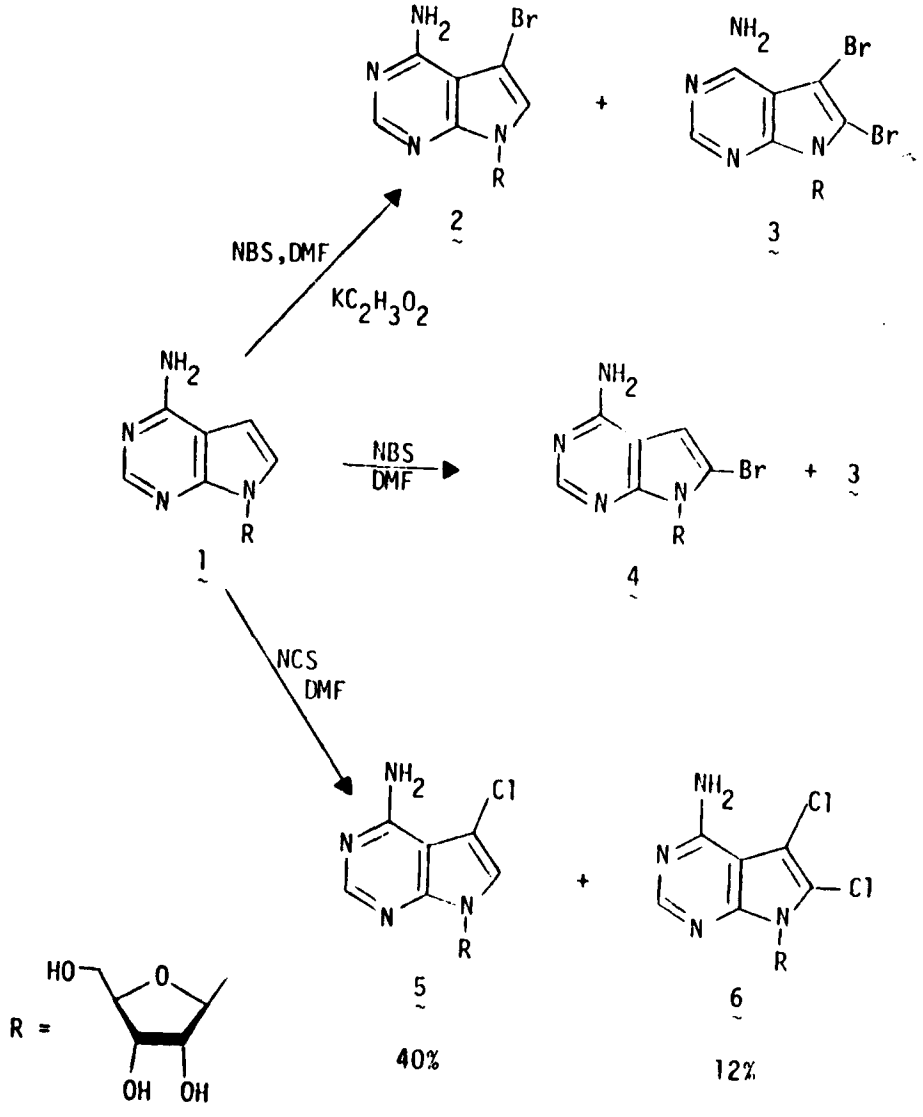
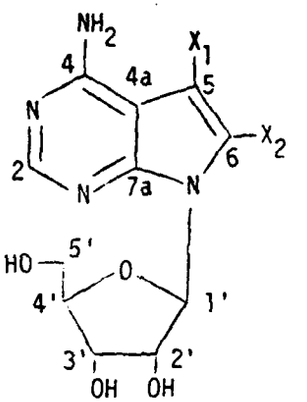


Table I. NMR Studies on 5-Halogenated and 5,6-Dihalogenated Tubercidin[a]

^{1}H NMR (δ values)

Compound	H-2	H-5	H-6	H-1'	H-2'	H-3'	H-4'	H-5'
5-Bromotubercidin	8.15 s,1H		7.64 s,1H	6.10 d,1H,J=6Hz	4.38 m,1H	4.67 m,2H		3.66 m,2H
6-Bromotubercidin	8.09 s,1H	6.87 s,1H	—	5.94 d,1H,J=6Hz	5.10 m,1H	4.20 m,1H	4.01 m,1H	3.67 m,2H
5,6-Dibromotubercidin	8.16 s,1H	—	—	6.00 d,1H,J=6Hz	5.10 m,1H	4.22 m,1H	3.94 m,1H	3.65 m,2H
5-Chlorotubercidin	8.14 s,1H	—	7.60 s,1H	6.10 d,1H,J=6Hz	4.38 m,1H	4.02 m,2H		3.62 m,2H
5,6-Dichlorotubercidin	8.16 s,1H	—	—	5.95 d,1H,J=6Hz	5.02 m,1H	4.20 m,1H	3.92 m,1H	3.60 m,2H



^{13}C NMR (ppm)

Compound	C-2	C-4	C-4a	C-5	C-6	C-7a	C-1'	C-2'	C-3'	C-4'	C-5'
5-Bromotubercidin	152.34	156.79	—[b]	—[b]	121.94	149.48	87.08	73.98	70.56	85.25	61.49
6-Bromotubercidin	151.32	156.33	102.74	103.72	109.10	149.36	89.82	70.99	70.72	85.90	62.17
5,6-Dibromotubercidin	152.22	156.12	102.42[c]	—[b]	112.00	149.24	91.16	71.26	70.74	86.17	62.28
5-Chlorotubercidin	152.59	156.68	100.00[c]	102.62[c]	119.19	149.19	86.71	73.80	70.38	85.07	61.42
5,6-Dichlorotubercidin	152.40	155.87	99.71[c]	100.89[c]	118.57	147.41	88.72	70.99	70.28	85.80	61.85

[a] Spectra were run in DMSO-d^6 and are referenced to internal TMS. ^{1}H NMR spectra are at 60 mHz and ^{13}C NMR spectra at 25.2 mHz.

[b] Signal too weak to be observed.

[c] Assignment uncertain - may be either C-4a or C-5.

The C-6 position of tubercidin is located in the same relative position as C-8 in adenosine. The large shift in the H-2' signal reflects the conformational change about the glycosidic bond induced by addition of a bulky substituent.[12]

The UV maxima of 2 and 4 were in agreement with the values reported previously by Townsend and coworkers.

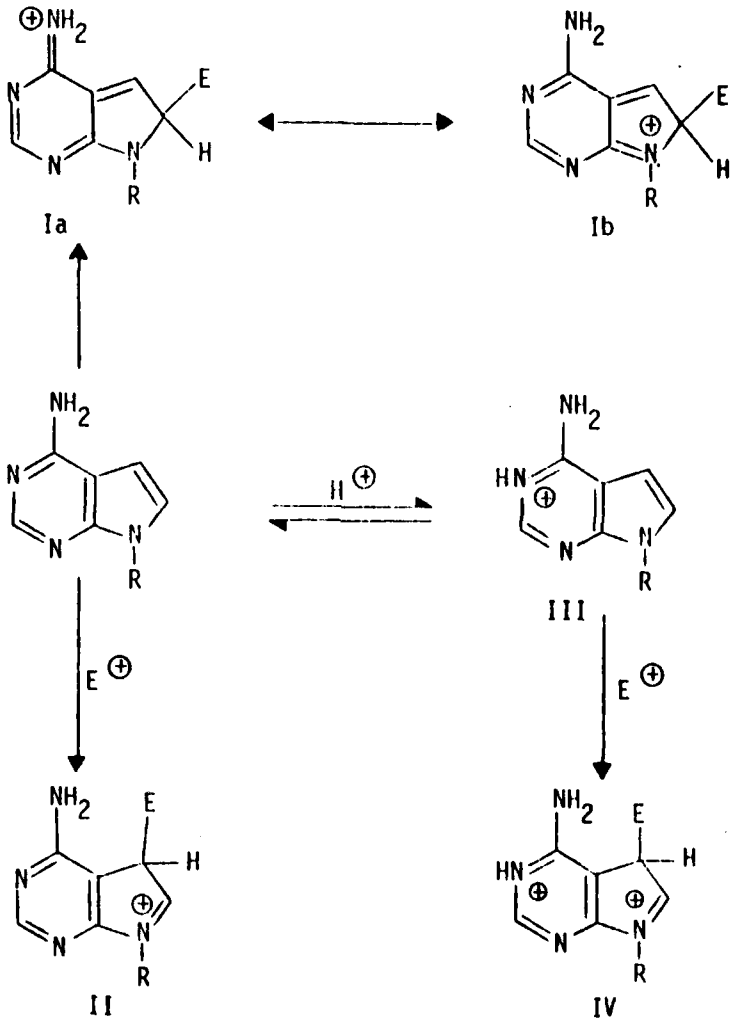
The switch from preference for electrophilic substitution at C-6 in sodium acetate buffered DMF to preference for C-5 substitution in unbuffered DMF was unexpected. There is however a reasonable explanation for these results. Simple resonance structures provide the best means for visualizing relative reactivity at C-5 and C-6. The site of substitution depends on the relative energies of the transition states leading to sigma complexes, Ia and II (Scheme II).

On addition of an electrophile to the neutral reactant both N-4 and N-7 can contribute electron density to the developing σ complex. This contribution is depicted by resonance structures Ia and Ib. In contrast, only N-7 can contribute electron density by resonance when addition occurs at C-5. IIa would be the major contributor to the resonance hybrid.

It is difficult a priori to predict which site would be most favored since C-6 but not C-5 substitution requires the participation of electrons from the adjacent pyrimidine ring. By way of comparison the preferential site of electrophilic substitution on indole is at C-3 (equivalent to C-5) and not C-2. Consequently the deciding factor must be the activation of C-6 by the amino group at C-4.

Finally, protonation of N-3 or N-4 would deactivate C-6 to a greater extent than C-5. Attack of III by an electrophile at C-5 would lead to IV in which N-7 continues to play a significant role in stabilization of the σ complex. In contrast, resonance structures like Ia and Ib could not make as significant a contribution to the σ complex resonance hybrid structure because of the positive

SCHEME II Resonance Contribution to Stabilization of Intermediate σ-Complexes in Electrophilic Substitution of Tubercidin



charge on the pyrimidine.

In the absence of the sodium acetate buffer, HBr produced as a side product of the bromination reaction may protonate tubercidin to give III, and hence under these conditions C-5 substitution predominates. On the basis of these results, C-5 substitution by other electrophiles under conditions where N-3 is either protonated or otherwise complexed with a Lewis acid should predominate. Probably very few examples of C-6 substitution will be observed since most electrophilic substitutions require conditions and reagents that would either protonate or form a complex at N-3, N-4. Oddly enough the chlorination with N-chlorosuccinimide in DMF buffered with sodium acetate gave very little product and no 6-chlorotubercidin was isolated.

EXPERIMENTAL

Nuclear magnetic resonance spectra were taken on Joel, Model PS 100 Fourier Transform NMR and a Varian EM 360 NMR. Ultraviolet spectra were measured on a Cary 17 spectrometer in methanol. Column chromatography was done on E. Merck silica gel (70-230 mesh). Thin layer chromatography (TLC) was performed on Merck silica gel 60 F-254 0.20 mm plastic sheets (solvent system: chloroform; methanol; ammonia (75;25;0.5)). HPLC was done on a Waters μ-C-18 Bondapak column. (Solvent system: aqueous 0.01 M $NH_4H_2PO_4$; acetonitrile.) All reported products were homogeneous under these conditions on TLC and HPLC. Tubercidin was purchased from Upjohn, Fine Chemicals Division, Kalamazoo, Michigan. Elemental analyses were performed by the microanalytical laboratory at the University of California, Berkeley. Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected.

5-Bromotubercidin (2). A solution of 178 mg (1.0 mmol) N-bromosuccinimide in 5 mL dry DMF was added dropwise to a stirred solution of 266 mg (1.0 mmol) tubercidin in 5 mL dry tubercidin at room temperature. The red mixture was stirred an additional 30 minutes, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 3 g silica gel. Chromatography on 150 g silica gel eluting with a methanol-chloroform gradient gave, after crystallization from methanol, 83 mg (0.24 mmol, 24%) 5-bromotubercidin. 5-Bromotubercidin softens at 191° and melts after much darkening and decomposition at 232-235°.

6-Bromotubercidin (4) and 5,6-Dibromotubercidin (3). A solution of 3.56 g (20.0 mmol) N-bromosuccinimide in 20 mL dry DMF was added dropwise to a stirred solution of 2.66 g (10.0 mmol) tubercidin and 1.96 g (20.0 mmol) potassium acetate in 40 mL of dry DMF at 20°C. The red reaction mixture was stirred an additional 10 minutes, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 10 g of silica gel. Chromatography on 300 g silica gel eluting with a methanol-chloroform gradient gave, after crystallization from methanol, 1.784 g (5.17 mmol, 51.7 %) 6-bromotubercidin, λ_{max}^{MeOH} m.p. 197-200° d, and 123 mg (0.029 mmol, 2.9%) 5,6-dibromotubercidin, λ_{max}^{MeOH} 284 nm, m.p. 225-226°.

5-Chlorotubercidin (5) and 5,6-Dichlorotubercidin (6). A solution of 266 mg (2.0 mmol) N-chlorosuccinimide in 5 mL dry DMF was added dropwise to a stirred solution of 266 mg (1.0 mmol) tubercidin in 5 mL dry DMF at room temperature. The light orange reaction mixture was stirred an additional 2 hours, the DMF evaporated at reduced pressure, and the residue dissolved in methanol and evaporated with 3 g silica gel. Chromatography on 150 g silica gel eluting with a methanol-chloroform gradient gave, after crystallization from methanol, 120 mg (0.40 mmol, 40%) 5-chlorotubercidin, λ_{max}^{MeOH} 275 nm, and 40 mg (0.12 mmol, 12%) 5,6-dichlorotubercidin, λ_{max}^{MeOH} 281 nm, m.p. 124-125°. 4 softens at 191-192°C and melts with decomposition at 226-228°.

Table II. Elemental Analyses

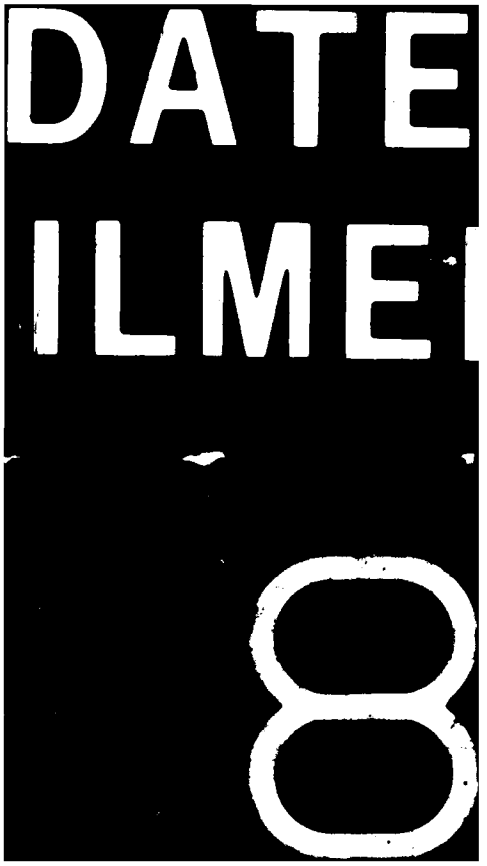
Compound	Formula	Calculated					Found				
		C	H	N	Br	Cl	C	H	N	Br	Cl
5-Bromotubercidin	$C_{11}H_{13}BrN_4O_4$	38.28	3.80	16.23	23.15	—	38.42	4.00	16.27	22.9	—
6-Bromotubercidin	$C_{11}H_{13}BrN_4O_4$	38.28	3.80	16.23	23.15	—	37.99	4.05	15.98	23.0	—
5,6-Dibromotubercidin	$C_{11}H_{12}Br_2N_4O_4$	31.16	2.85	13.21	37.69	—	31.45	3.08	12.86	37.5	—
5-Chlorotubercidin	$C_{11}H_{13}ClN_4O_4$	44.23	4.39	18.09	—	11.87	43.97	4.46	18.37	—	12.1
5,6-Dichlorotubercidin	$C_{11}H_{12}Cl_2N_4O_4$	39.42	3.61	16.72	—	21.16	39.49	3.76	16.58	—	21.2

REFERENCES

1. Brdar, B; Rifkin, D.B.; Reich, E. *J. Biol. Chem.* 1973, *248*, 2397-2408.
2. Reich, E.; Ossowski, L. *Virology* 1972, *50*(3), 630-39.
3. Easton, T.G.; Reich, E. *J. Biol. Chem.* 1972, *247*, 6420-31.
4. Goodman, L. In "Basic Principles in Nucleic Acid Chemistry"; Ts'o, Paul O.P., Ed.; Academic Press: New York, 1974; Vol. I, p. 93-208.
5. Hinshaw, B.C.; Gerster, J.F.; Robins, R.K.; Townsend, L.B. *J. Heterocyclic Chem.* 1969, *6*, 215-21.
6. 4-Chloro-7-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine is prepared in two steps from 7-deazainosine. Gerster, J.F.; Carpenter, B.; Robins, R.K.; Townsend, L.B. *J. Med. Chem.*, 1967, *10*, 326-31.
7. Mitchell, R.H.; Lai, Y-H.; Williams, R.V. *J. Org. Chem.* 1979, *44*, 4733-35.
8. Srivastava, P.C.; Nagpal, K.L. *Experientia* 1970, *26*, 220.
9. Bergstrom, D.E.; Schweickert, M.J. *J. Carbo·Nucleosides·Nucleotides* 1978, *5*, 285-96.
10. Bergstrom, D.E.; Schweickert, M.J.; Ogawa, M.K.; Brattesani, A.J. in preparation.
11. Westhof, E.; Röder, O.; Croneiss, I.; Lüdemann, H-D. *Z. Naturforsch* 1975, *30c*, 131-40.
12. Bugg, C.E.; Thewalt, U. *Biochem. Biophys. Res. Commun.* 1969, *38*, 496.

DISTRIBUTION LIST

5 copies	Director Walter Reed Army Institute of Research ATTN: SGRD-UWZ-C Walter Reed Army Medical Center Washington, DC 20012
4 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-RMS Fort Detrick, Frederick, MD 21701
12 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDA Cameron Station Alexandria, VA 22314
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20014
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234


DATE
ILME
8