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THE SYNTHESIS OF NEW ANTIMALARIAL DRUGS

Edward R. Atkinson, et al

Arthur D. Little, Incorporated

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THE SYNTHESIS OF NEW ANTIMALARIAL DRUGS

FINAL REPORT
combined with
ANNUAL REPORT NO. 2

by
Edward R. Atkinson and Felix E. Granchelli

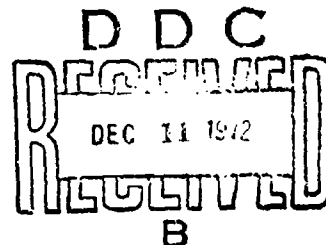
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ARTHUR D. LITTLE, INC.
Cambridge, Massachusetts 02140

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The purpose of the work performed on the subject contract was to prepare compounds belonging to four chemical classes for evaluation as antimalarial drugs. In two formal reports, of which this is the last, we have described 34 compounds submitted for appropriate pharmacological evaluation. Of these, 16 were target compounds and 18 were intermediates. α-(Di-n-butylaminomethyl)-2-phenyl-3',4',6,8-tetrachloro-1,2,3,4-tetrahydro-4-quinolinemethanol and its N-acetyl and N-ethyl derivatives were prepared to test the hypothesis that reduction of the pyridine ring in WR 30090 would eliminate an undesirable phototoxic side-effect. Phototoxicity and antimalarial activity were decreased in an equivalent fashion. Fluorenamethanols having aminoalcohol sidechains at the 1- and 4-position, and related compounds having 9-hydroxy or 9-keto groups were prepared. None had antimalarial activity. However, when chlorine atoms were introduced at the 2,7-positions high activity resulted and was not affected by the state of oxidation at the 9-position. Efforts to enhance the activity by replacing the chlorine atoms by trifluoromethyl groups failed when we were unable to carry out the necessary syntheses. The effects of replacing the chlorine atoms by other groups, and of moving the chlorine atoms to positions other than 2- and 7- were not studied.			

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13. ABSTRACT (Continued)

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A synthesis of a pyrrocolinemethanol, α -(di-n-butylaminomethyl)-7,9-dichloro-5-pyrrolo[1,2-a]quinolinemethanol, was undertaken in order to determine the effect of replacing the 2-aryl group of a conventional 2-aryl-4-quinolinemethanol antimalarial by a cyclic structure. The synthesis proceeded to 7,9-dichloro-5-methylpyrrolo[1,2-a]quinoline but then was abandoned when we were unable to convert the 5-methyl group to an appropriate aminoalcohol sidechain.

In addition to the above contract work we prepared, at no expense to the government, 2 amic acids and 2 related imides by condensing 5,5'-bis(trifluoromethyl)diphenic anhydride with 2-aminofluorene and with 2-aminofluorenone. The compounds were submitted as a gift. None was active in the mouse antimalarial screen.

14	KEY WORDS	LINK A		LINK B		LINK C	
		ROLE	WT	ROLE	WT	ROLE	WT
	Malaria Organic Chemistry Synthesis Pharmacology Antimalarial Drugs Tetrahydroquinolinemethanols Fluorenemethanols Aminobenzothiazoles Pyrrocolinemethanols Diphenimides Phototoxicity						

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FOREWORD

As indicated in its title, this report is designed to serve both as a Final Report for the subject contract and as an Annual Report for the second contract year.

As a Final Report it lists in its Table of Contents the location of all experimental data obtained during the two contract years; this material has been arranged so that this single Table of Contents can also serve as an index to both Annual Reports. As a Final Report it also contains all pharmacological data obtained from compounds prepared by us.

As Annual Report No. 2 it contains the details of experimental work carried out during the second contract year (August 1, 1971 - August 1, 1972).

ACKNOWLEDGMENT

The authors wish to thank Dr. Richard E. Strube, Walter Reed Army Institute of Research, for his advice and encouragement given throughout the past two years during which he served as our Project Officer.

Spectroscopic data were collected by the staff of the Arthur D. Little, Inc., Analytical Laboratory. Elemental analytical services were provided by the Galbraith Laboratories (Knoxville, Tenn.), the Spang Analytical Laboratory (Ann Arbor, Mich.), and by the late Dr. Stephen M. Nagy (Belmont, Mass.) who, until the time of his death on May 28, 1972, had served this laboratory and laboratories throughout the world for 25 years.

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THE SYNTHESIS OF NEW ANTIMALARIAL DRUGS

I. INTRODUCTION

The general background of our work under the present contract was described in Annual Report No. 1, p. 9. During the second contract year our work was limited to those classes described in Sections III - VI below. Of these, the class of diphenimides (Section VI) had not been included in our proposal. For this reason our preparation of these compounds was not carried out at government expense and the 4 compounds prepared were submitted as a gift to WRAIR.

Experimental details for the synthesis of 6 target compounds and 4 intermediates submitted for pharmacological evaluation during the second contract year are presented in Section VII; incompleted syntheses are described in Section VIII. Antimalarial activities for most of the compounds submitted during the entire contract are recorded in Section IX, whose Tables include structural formulas and WRAIR bottle numbers for each compound submitted.

The authors participated in a conference on antimalarials held at WRAIR on March 28-29 (1972) where our work performed during the first 18 months of the contract was described.

II. TETRAHYDROQUINOLINEMETHANOLS

During the second contract year no additional work with this class was carried out. See Annual Report No. 1, p. 11, for a formal report on earlier work.

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III. FLUORENEMETHANOLS

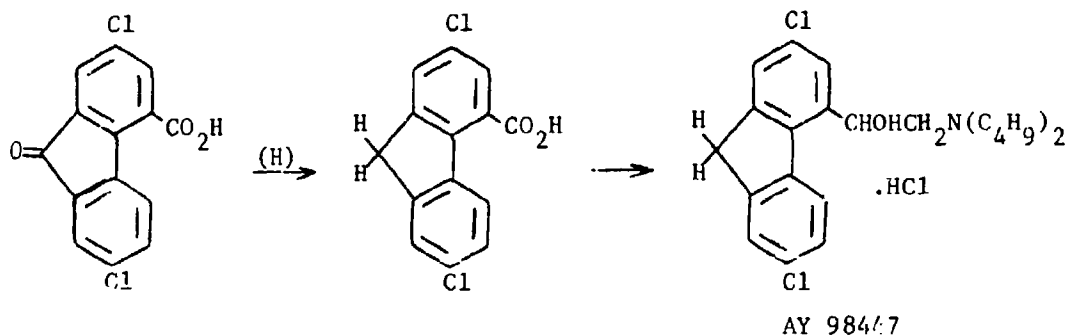
The origin of our interest in this class was described in Annual Report No. 1. p. 19. During the second contract year we have continued our work in this area, but at a much lower level of effort because of a change in emphasis in the WRAIR program from suppressive drugs to prophylactic drugs.

Because of the significant antimalarial activity of α -(di-n-butylaminomethyl)-2,7-dichloro-9-keto-4-fluorenemethanol (AX 68051) and of the 9-hydroxy analog (AY 60658), it was obvious that a variety of structural analogs should be prepared in an effort to optimize antimalarial activity. The following structural modifications were proposed:

- Additional variations at the 9-position.
- Variations in the amino sidechain, to include the 2-piperidyl group.
- The replacement of chlorine atoms by other substituents such as methyl, methoxy, and trifluoromethyl groups.
- Dichloro compounds in which the chlorine atoms were at positions other than 2 and 7.

The following specific syntheses were undertaken with the advice and encouragement of our Project Officer.

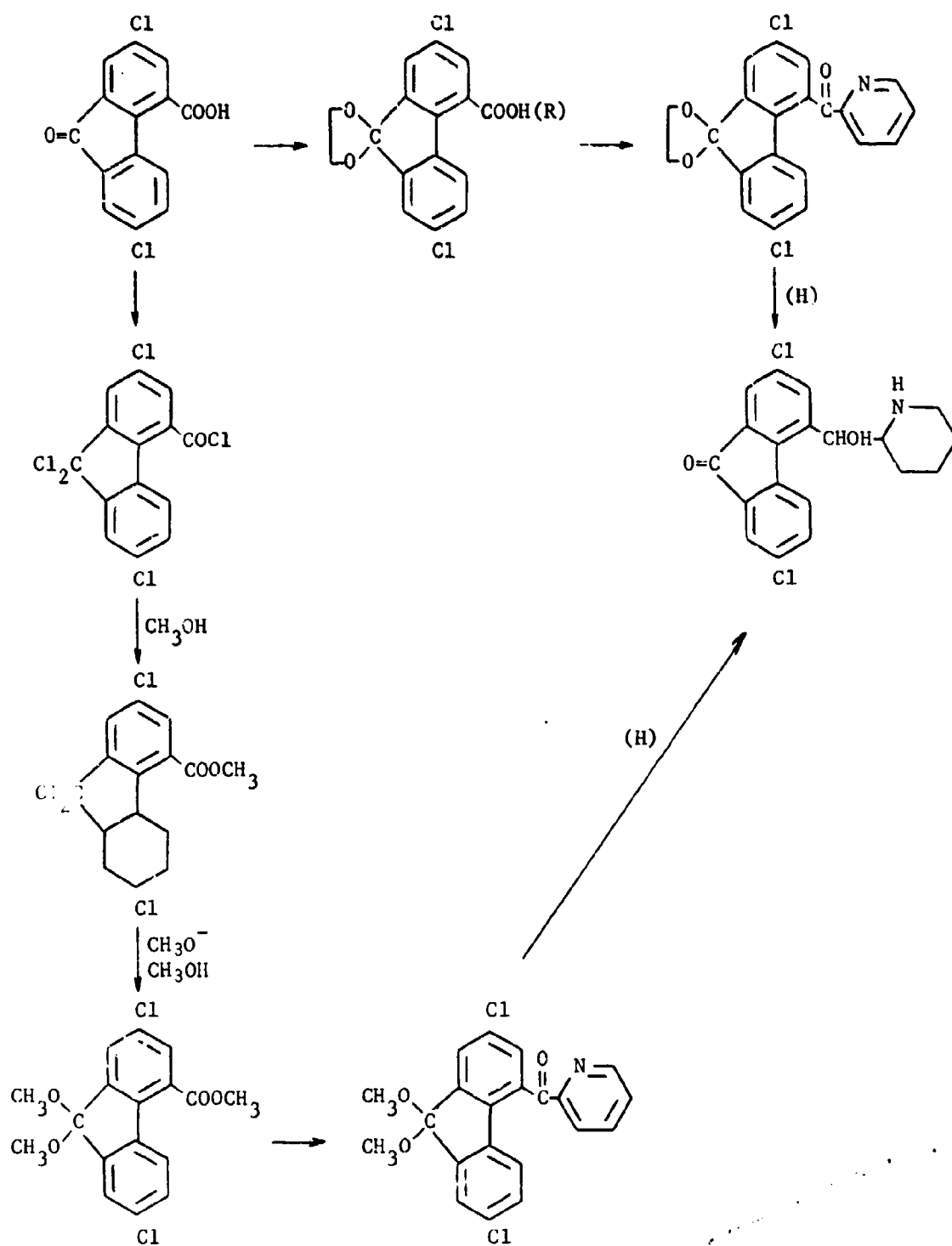
(1) α -(Di-n-butylaminomethyl)-2,7-dichloro-4-fluorenemethanol hydrochloride (AY 98447) was prepared by a conventional synthesis from 2,7-dichlorofluorenene-4-carboxylic acid. The replacement of the carboxyl group by the amine sidechain was so efficient that 5 g of acid gave 1.9 g of the target compound.



The antimalarial activity of AY 98447 was found to be very similar to that previously observed for the 9-keto (AX 68051) and 9-hydroxy (AY 60658) analogs; the compound was active at 20 mg/kg and gave 5/5 cures at 80 mg/kg. In the bird screen for prophylactic activity the compound was also quite active. We concluded that within the variations studied the exact nature of the 9-position was not critical.

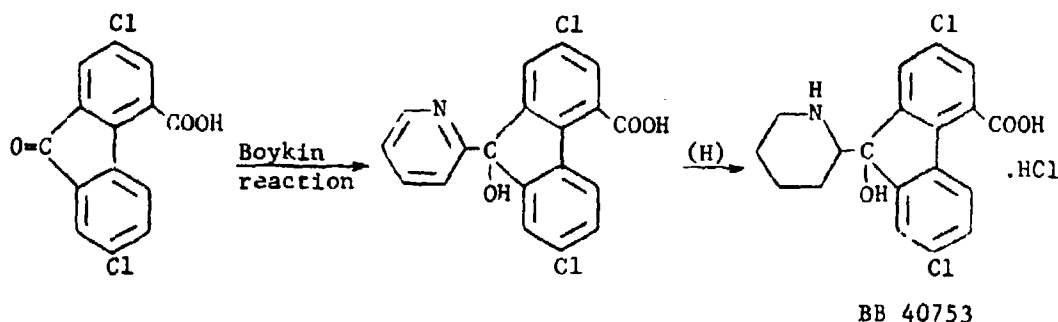
(2) We undertook the synthesis of an analog of AX 68051 (or AY 60658) in which the di-n-butylaminomethyl sidechain was to be replaced by a 2-piperidyl group. In the 9-phenanthrenemethanol series there are several cases where such a replacement leads to significantly increased antimalarial activity.

We set out to prepare the desired target compound by one of the following modifications of the Boykin reaction.



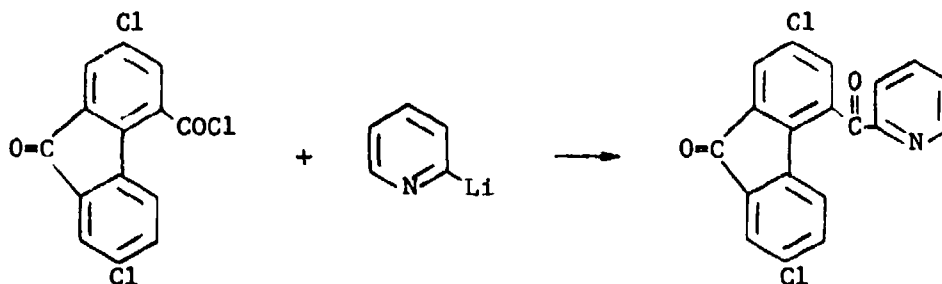
The conversion of fluorenone to either the dimethyl ketal or the cyclic ethylene ketal is well known¹ and the stability of such ketals to the conditions of the Boykin reaction are firmly established.² We recognized the likelihood of our obtaining esters during the ketal synthesis. If such were the case, we intended to carry out the reaction with 2-pyridyllithium, for it is known that such a reaction, with limited amounts of reagent, can give the desired 2-pyridyl ketone intermediate.³

We were unable to prepare a ketal of 2,7-dichlorofluorenone-4-carboxylic acid, either directly or by way of the reaction of methoxide ion with methyl 2,7,9,9-tetrachlorofluorene-4-carboxylate. When the Boykin reaction was carried out with the unprotected keto acid a reaction occurred at the 9-keto group to give a 9-pyridylmethanol which was then reduced to a 9-piperidylmethanol (BB 40753). The latter compound was inactive in both the conventional mouse screen and in the bird prophylactic screen.



The Boykin reaction was also carried out with 2,7-dichlorofluorene-4-carboxylic acid but only unreacted acid was recovered. It was likely that the 2-pyridyllithium reagent was destroyed by the reactive 9-methylene group; a similar interference with the Boykin reaction has been reported by Verbiscar.⁴

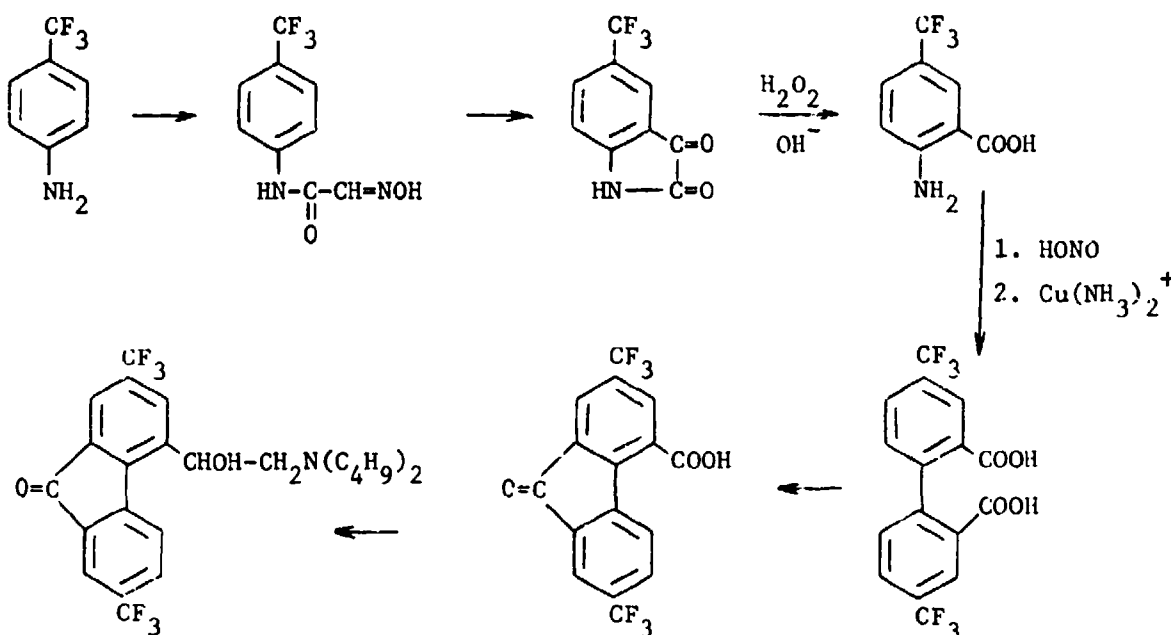
The desired pyridyl ketone was finally obtained by a conventional ketone synthesis involving the acid chloride of 2,7-dichlorofluorenone-4-carboxylic acid and 2-pyridyllithium.



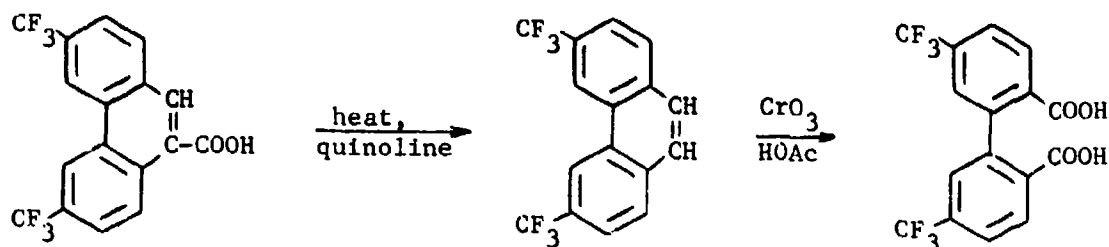
At the conclusion of the contract we were engaged in studying the reduction of the ketone to the desired target compound.

(3) We undertook the synthesis of a bis(trifluoromethyl)fluorene-methanol analogous to AX 68051. The superior contribution of trifluoromethyl groups to antimalarial activity is well known.

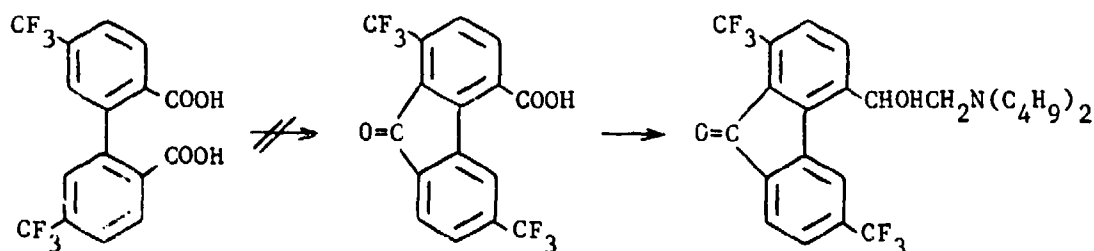
Ideally, we should have prepared the direct analog of AX 68051, 2,7-bis(trifluoromethyl)- α -(di-n-butylaminomethyl)-9-keto-4-fluorene-methanol. The intermediate isatin is known⁵ and should be easily converted to the



anthranilic acid. The remainder of the synthesis would involve the same steps used in the synthesis of the model dichloro compound. The first steps in the synthesis were in progress when we learned that the novel 5,5'-bis(trifluoromethyl)diphenic acid had been prepared by J. L. Neumeyer (under the auspices of WRAIR) from an intermediate available in adequate quantities.



We simplified Neumeyer's synthesis by oxidizing the phenanthroic acid to the diphenic acid in a single high-yield step. The balance of the synthesis was then undertaken but we were unable to cyclize the diphenic



acid to the desired fluorenonecarboxylic acid by a wide variety of agents. Fluorenone formation occurred under several conditions, but was accompanied by excessive loss of fluorine as hydrogen fluoride.

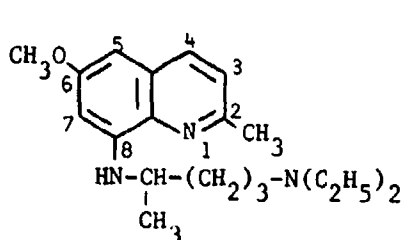
At the time the contract expired we were preparing a small quantity of one other bis(trifluoromethyl)diphenic acid to determine whether failure to cyclize under tolerable conditions was characteristic of the series.

Because of the commercial availability of a number of substituted anthranilic acids, it is now apparent that the expense involved in the trifluoromethyl work might easily have supported the preparation of several of the desired fluorenonemethanols.

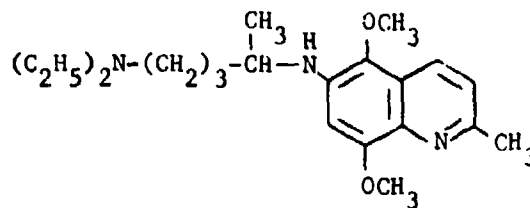
IV. AMINOBENZOTHAZOLES

Because of the growing interest in prophylactic antimalarials, we proposed to start work in this area during the second contract year with the preparation of benzothiazole analogs of 6- and 8-aminoquinoline prophylactic antimalarials.

The history of the development of compounds I and II is well known; convenient summaries have been presented.^{6,7}

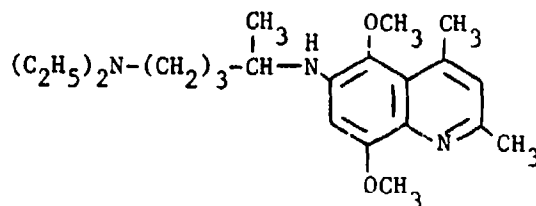


I



II

More recently the compound III has been found to be a potent causal prophylactic antimalarial.⁸



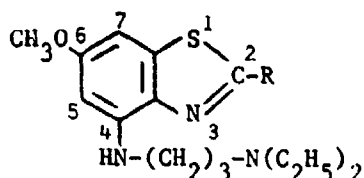
III

In the 6-aminoquinoline series compounds related to II having a variety of sidechains have been prepared in order to study the effect on antimalarial activity and toxicity⁷ but pharmacological data are not yet available.

The pharmacological analogy between quinolines and benzothiazoles was pointed out clearly in 1922 by Marston Taylor Bogert⁹ who prepared 2-phenylbenzothiazole-6-carboxylic acid as an analog of cinchophen (atophan). The analogy was later expressed by Elderfield.¹⁰ During the present WRAIR

malaria program several benzothiazole methanols derived from the 6-carboxylic acid were prepared and were found to be inactive. A few other inactive benzothiazoles derived from 2-amino- or 2-mercaptobenzothiazole are listed in Coatney.¹¹

Of particular interest to us was an early report by Knunyants¹² that compound IV was inactive (test system unknown). At about the same



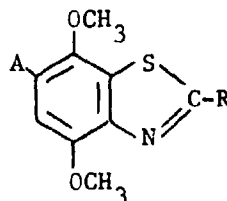
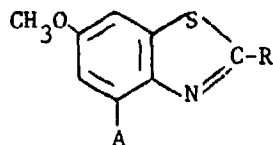
IV R = CH₃

V R = H; sidechain = NH(CH₂)₂N(C₂H₅)₂

VI R = CH₃; sidechain = NH(CH₂)₆N(C₂H₅)₂

time Bogert¹³ prepared V but no test data were recorded since Bogert was discouraged by Knunyants' negative results. During the World War II malaria program Mary Sherrill¹⁴ prepared VI, which was found to be inactive in the test system then in vogue.¹¹

We were not prejudiced by the earlier results cited above because the test systems used at the time were not standardized, nor were they designed to detect prophylactic activity. We therefore proposed to prepare a variety of aminobenzothiazole compounds, some containing chlorine substituents, and also to prepare a closely related series of benzothiazole-methanols (as potential curative drugs). From among the many compounds proposed we agreed to limit our work to the preparation of the following analogs of I and II, some of which were closely related to IV and VI:



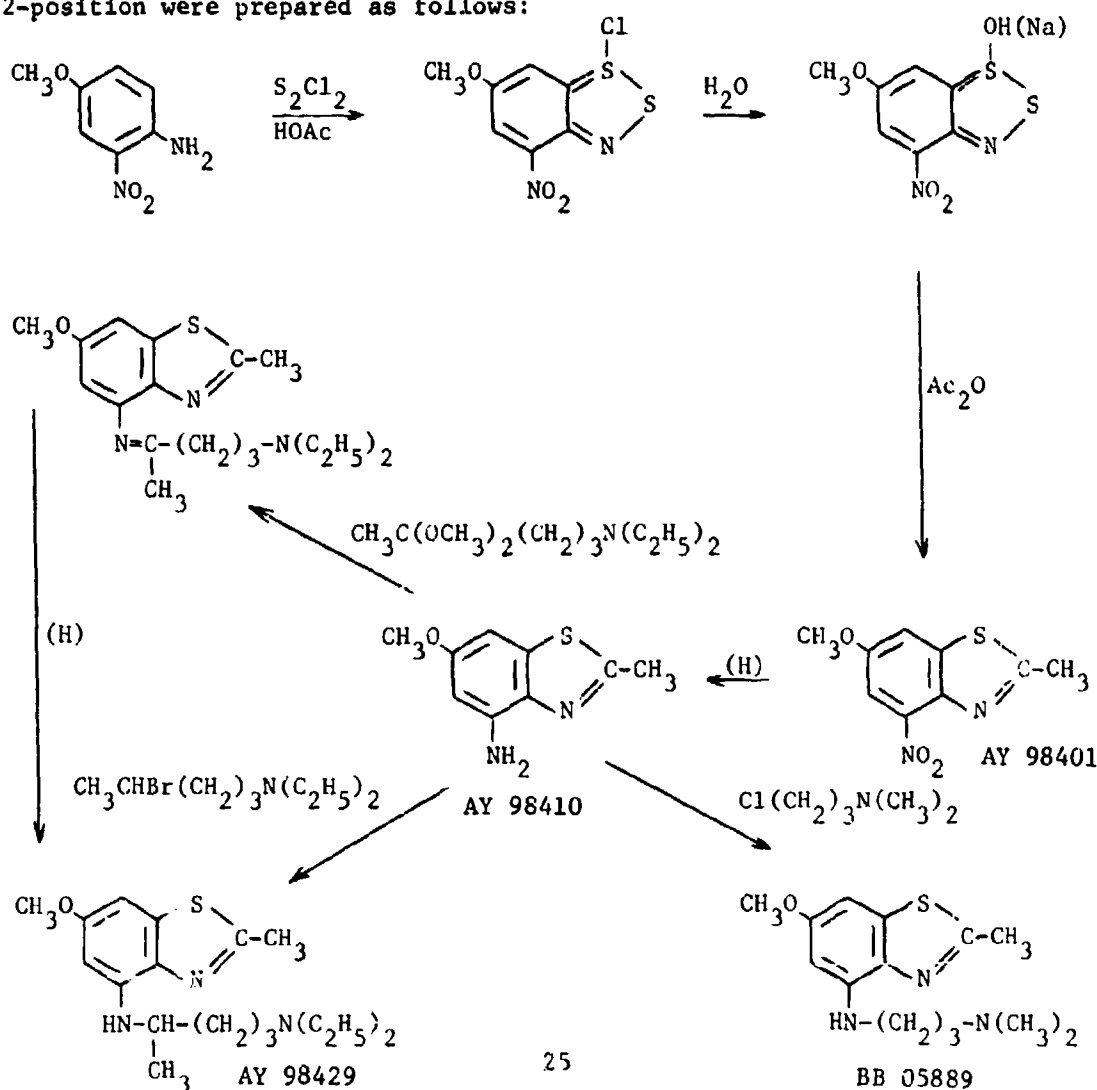
R = CH₃ and 4-chlorophenyl

A = NHCH(CH₃)-(CH₂)₃-N(C₂H₅)₂ and NH(CH₂)₃N(CH₃)₂

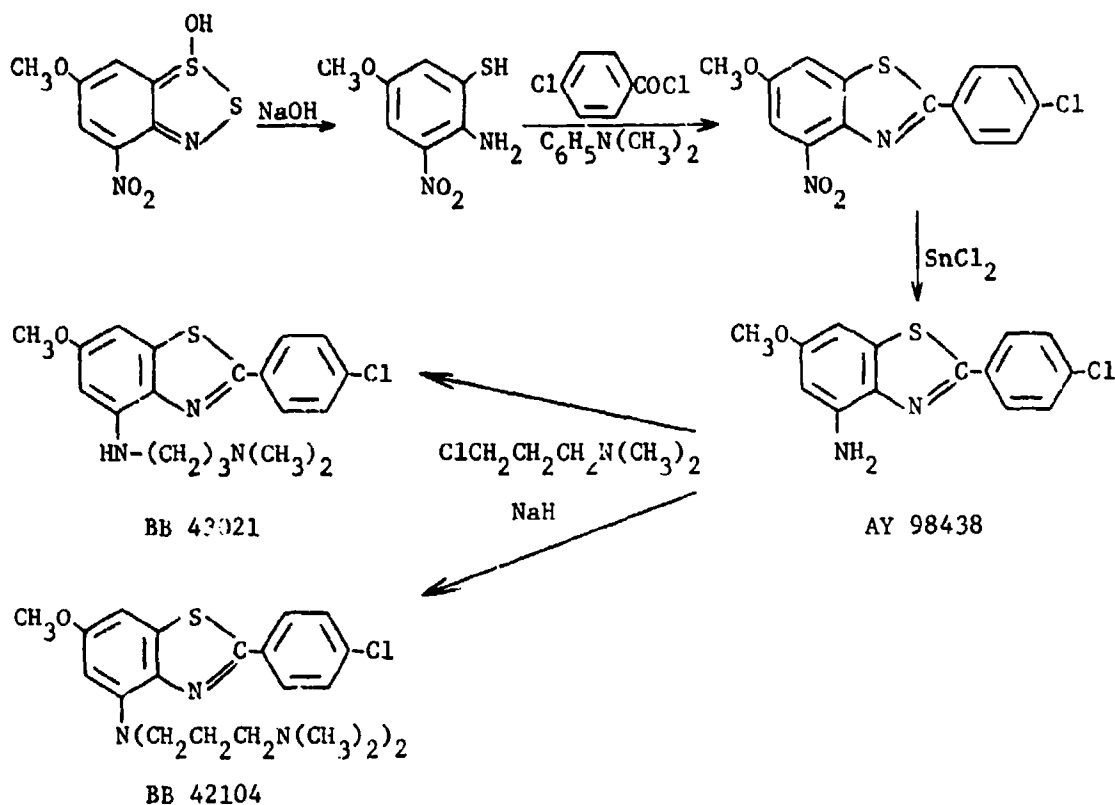
At the outset we did not plan to use the $\text{NH}(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$ sidechain but later found it desirable to use it in pilot alkylation studies with commercially available 3-dimethylaminopropyl chloride.

The selection of the 4-chlorophenyl group for the 2-position involved some risk because it is known that the antimicrobial 2-phenylbenzothiazoles are phototoxic.¹⁵ If this side effect were found in our compounds it would be necessary to replace the 4-chlorophenyl group by 4-chlorobenzyl, which does not cause phototoxicity in this series.

Four target compounds were prepared in which the amine sidechain is at the 4-position of the benzothiazole system. No work has yet been carried out in the 6-amino series. Compounds having a methyl group at the 2-position were prepared as follows:



Compounds having a (4-chlorophenyl) group at the 2-position were prepared as follows:



The bis-alkylated BB 42104 was obtained during an early preparative run for BB 43021.

The alkylation of AY 98438 to give a branched sidechain on nitrogen was not carried out because of the expiration of the contract.

Very few pharmacological test data for these compounds are yet available. AY 98429 appears to be too toxic in the preliminary bird screen for prophylactic activity.

V. PYRROCOLINEMETHANOLS

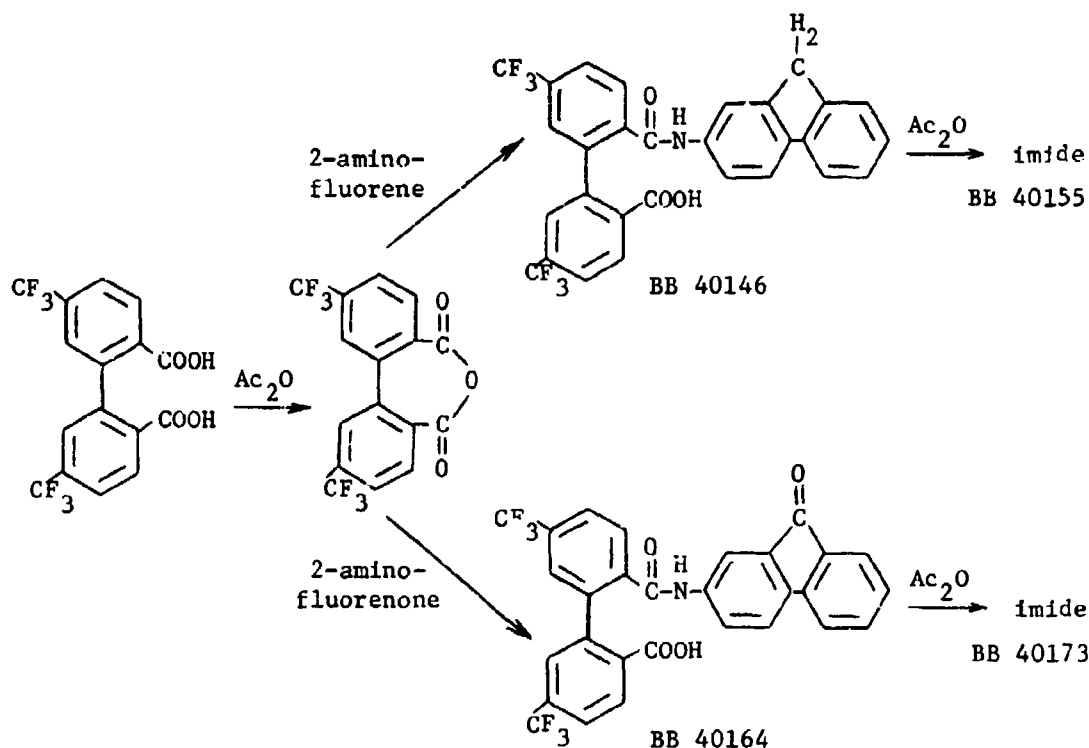
The background for the work and a summary of our progress during the first contract year was given in Annual Report No. 1. p. 15.

During the present year we continued briefly our study of the oxidation of 7,9-dichloro-5-methylpyrrolo[1,2-a]quinoline to the aldehyde or carboxylic acid. We concluded that the pyrrocoline ring was being destroyed, and the synthesis was abandoned.

VI. DIPHENIMIDES

Our preparation of 4 members of this class was the result of Fletcher's observation^{16,17} that, among 32 new diphenamic acids and related diphenimides, N-2-fluorenyldiphenimide had shown low antimalarial activity. It occurred to us that, from a 5-g supply of 5,5'-bis(trifluoromethyl)diphenic acid, which we had on hand from other work, we could prepare analogs of Fletcher's compound in which trifluoromethyl groups might enhance anti-malarial activity. These preparations were also of interest to us because of our involvement in fluorene chemistry.

The compounds were prepared as follows in high yield. Because the preparations were not part of our proposal, they were carried out at our expense and the products were submitted to WRAIR as gifts.



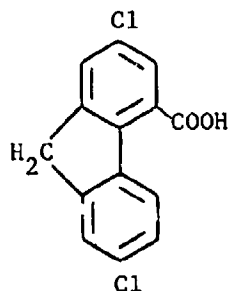
None of the compounds were active in the mouse screen.

VII. COMPOUNDS SUBMITTED

Experimental procedures used for our syntheses of compounds submitted during the second contract year are presented below. Technics used for melting points, recording of spectra, and the like are described in Annual Report No. 1, p. 25. The first 24 compounds submitted under this contract were described in Annual Report No. 1.

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25. 2,7-Dichlorofluorene-4-carboxylic Acid (AY 98456)



$C_{14}H_8Cl_2O_2$

M.W. 279.13

m.p. 257-263°

ADL 17311-2

0.5 g, January 11, 1972

The acid had been prepared previously by the chlorination of methyl fluorene-4-carboxylate, followed by saponification.¹⁸ We elected to prepare it by the Wolff-Kishner reduction of 2,7-dichlorofluorenone-4-carboxylic acid using a procedure similar to that we had used for the reduction of fluorenone-4-carboxylic acid to fluorene-4-carboxylic acid (Annual Report No. 1, p. 57).

4,4'-Dichlorodiphenic Acid

During the period of the present report 2 additional runs were carried out at 1.5 times the scale described in Annual Report No. 1, p. 60, to give 50 g (74%), m.p. 260-270°, and 57 g (84%), m.p. 252-264°. We found that the separation of the red crystalline material during diazotization of 5-chloro-2-aminobenzoic acid could be avoided by simply adding the sodium nitrite solution more rapidly (during about 10 min.).

2,7-Dichlorofluorenone-4-carboxylic Acid

The procedure described in Annual Report No. 1, p. 61, was used in 3 runs to give a total of 100 g (88-91%), m.p. 249-251°; 256-260°, whose purity was adequate for use in the next step. Because we have not stressed the point adequately in our previous reports, it is important in this preparation to use an adequate volume of dilute aqueous bicarbonate in separating the nonacidic materials, the sodium salt of the desired product is not highly soluble in water. In the present run the volume of solution from which the nonacidics were filtered by gravity was 4500 ml. When the precipitated and dried product was found to form a cloudy solution in acetone it was leached with 1500 ml of hot water (acidified to Congo Red) to remove occluded salt.

2,7-Dichlorofluorene-4-carboxylic Acid

After two small-scale preliminary runs 28 g (0.0955 mole) of 2,7-dichlorofluorenone-4-carboxylic acid was dissolved in 300 ml of warm diethylene glycol. To the warm solution there was added with stirring 11.2 g of solid potassium hydroxide followed by 12.5 g (0.37 mole) of "95% hydrazine" (Eastman 902). In the smaller runs equivalent amounts of "85% hydrazine hydrate" were also adequate.

The blue-green solution was refluxed for 90 min. at which time the temperature of the solution was 165°. The condenser was removed and volatile material was allowed to escape until the mixture reached 200°. Refluxing was then continued for an additional 5 hr. Excess hydrazine was still present.

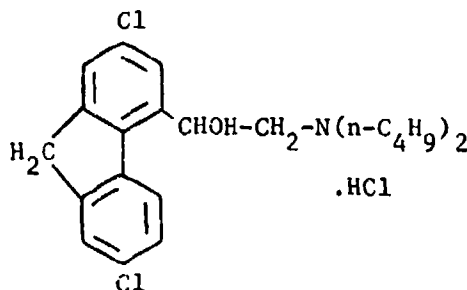
The cooled mixture was stirred into 2 liters of cold water, the brown solution that formed was warmed to 50°, and then was acidified carefully with 6N hydrochloric acid. The cream-colored solid that separated was washed once by stirring with water in a beaker and then was suspended in 1700 ml of water at 50°. Solid sodium bicarbonate was stirred in until gas evolution stopped and the mixture had pH 9. The mixture was filtered by gravity to remove about 1 g of nonacidics, then was acidified carefully at 50° with 6N hydrochloric acid to give 24 g (90%) of a cream-colored solid, m.p. 225-255°.

A 10-g portion was recrystallized from glacial acetic acid to give 5.7 g of light tan needles, m.p. 257-263°; lit.¹⁸ 262-263°. An infrared spectrum showed no maximum corresponding to a 9-keto group. A 0.5-g sample of ADL 17311-2 was submitted to WRAIR on January 11, 1972.

The balance of crude product from this and previous runs was recrystallized from glacial acetic acid (20 ml/g, with use of decolorizing carbon) to give a succession of crops, the least pure of which had m.p. 246-258°. The total yield of recrystallized material, whose purity was adequate for use in the next step, was 18.4 g (68%). An additional 4 g of inferior material was obtained by diluting the final filtrate with water.

The acid is very soluble in methyl alcohol, ethyl alcohol and acetone. It appears to be stable under ordinary laboratory conditions.

26. α -(Di-n-butylaminomethyl)-2,7-dichloro-4-fluorenemethanol Hydrochloride (AY 98447)



$\text{C}_{23}\text{H}_{29}\text{Cl}_2\text{NO}\cdot\text{HCl}$

M.W. 442.86

m.p. 99-103°

ADL 17254-41

1.8 g, January 11, 1972

2,7-Dichlorofluorene-4-carbonyl Chloride

A mixture of 5 g (0.018 mole) of 2,7-dichlorofluorene-4-carboxylic acid and 10 ml of thionyl chloride (Fisher T-104) was refluxed for 1 hr., then evaporated to dryness. Residual thionyl chloride was removed from the residue by co-distillation with benzene under vacuum. We obtained 5 g (93%), m.p. 145-150°, not additionally characterized.

Diazomethyl 2,7-Dichloro-4-fluorenyl Ketone

5 g (0.016 mole) of the acid chloride was added rapidly to a stirred solution of approximately 4.5 g (0.1 mole) of diazomethane¹⁹ in 500 ml of ether at 0°. The mixture was stirred at 0° for 40 min. and then was stored overnight at 0°. It was filtered to remove 150 mg of unreacted starting material and then was evaporated under vacuum to give 4.4 g (86%) of the diazomethyl ketone, m.p. 136° dec., whose infrared spectrum showed the expected strong absorption at 2100 cm^{-1} . The product was not additionally characterized.

Bromomethyl 2,7-Dichloro-4-fluorenyl Ketone

A suspension of 4.3 g (0.0142 mole) of the diazomethyl ketone in 100 ml of benzene was stirred at 5° and cold saturated ethereal hydrogen bromide was added dropwise. Nitrogen was evolved and the suspended solid had dissolved by the time 11-12 ml of the ethereal hydrogen bromide had been added. An additional 3 ml was added and gas evolution stopped. The mixture was stirred at 5° for 50 min. and then was taken to dryness under

vacuum. Ether was added to the residue and then evaporated so as to remove remaining traces of hydrogen bromide. The oily residue crystallized when scratched to give 4.8 g (95%) of a solid which was then recrystallized from 40 ml of n-hexane/chloroform (1:1) to give 3.05 g, m.p. 124-128°.

Anal. Calcd for $C_{15}H_9BrCl_2O$: C, 50.60; H, 2.55; Br, 22.44; Cl, 19.92.
Found: C, 50.41; H, 2.55; Br, 22.03; Cl, 19.62.

The infrared spectrum showed strong absorption at 1680 cm^{-1} that is characteristic of the bromomethyl aryl ketone function. The nuclear magnetic resonance spectrum showed δ 4.4 (s, 2H); 3.75 (s, 2H); 7.0-7.75 (m, 5H).

2,7-Dichloro-4-fluorenylethylene Oxide

1.18 g (0.0314 mole) of sodium borohydride (Alfa 14121) was added in portions to a stirred suspension of 2.8 g (0.0078 mole) of the bromomethyl ketone in 25 ml of methanol at 30-40°. The mixture was stirred for 2 hr. and then an additional 1 g of sodium borohydride was added. The clear mixture was diluted with 70 ml of methanol and allowed to stand at room temperature overnight during which time a colorless solid separated. This apparently was the desired oxide for when it and its supernatant solution were stirred after adding a solution of 1.3 g of sodium hydroxide in 10 ml of water, no observable change occurred. The solid was washed with water and dried to give 1.8 g (82%), m.p. 128-132°. A sample for analysis was prepared by recrystallization from n-hexane and then had m.p. 132-134°.

Anal. Calcd for $C_{15}H_{10}Cl_2O$: C, 65.01; H, 3.64; Cl, 25.58. Found: C, 65.30; H, 3.51; Cl, 25.59.

The nuclear magnetic resonance spectrum showed δ 2.74 (dd, 1H); 3.25 (dd, 1H); 3.75 (s, 2H); 4.25 (br, 1H); 7.1-7.7 (m, 5H).

α -(Di-n-butylaminomethyl)-2,7-dichloro-4-fluorenemethanol

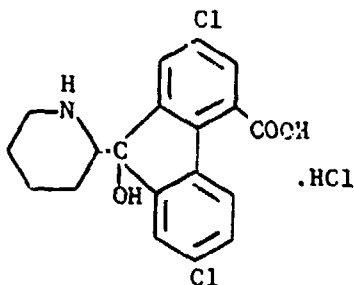
A mixture of 1.5 g (0.0054 mole) of 2,7-dichloro-4-fluorenylethylene oxide and 6.95 g (0.054 mole) of di-n-butylamine (Eastman 1260) was heated at 110-115° for 17 hr. in a stoppered flask. The mixture was steam distilled and the non-volatile residue was dissolved in ether. The

ether solution was washed with water, dried over magnesium sulfate, and evaporated to give a red oil. The oil was heated at 55°/1 mm to remove traces of di-n-butylamine, then was converted to the hydrochloride by the addition of ethereal hydrogen chloride to an ether solution of the base. An infrared spectrum was recorded and a 1.8-g sample of ADL 17254-41 was submitted to WRAIR on January 11, 1972.

Anal. Calcd for $C_{23}H_{29}Cl_2NO.HCl$: C, 62.38; H, 6.83; Cl, 24.02; N, 3.16.
Found: C, 62.30; H, 6.91; Cl, 23.85; N, 3.22.

The compound is moderately soluble in methyl and ethyl alcohols and in chloroform; insoluble in water, dilute acid and ether. Its nuclear magnetic resonance spectrum showed: δ 1.0 (t, 6H); 1.6 (m, 8H); 3.0 (br, 4H); 3.4 (br, 2H); 3.6 (s, 2H); 6.1 (br, 2H); 6.8-7.8 (m, 5H). The compound appeared to be stable under ordinary laboratory conditions.

27. 2,7-Dichloro-9-hydroxy-9-(2-piperidyl)-4-fluorencarboxylic Acid
Hydrochloride (BB 40753)



$C_{19}H_{17}Cl_2NO_3 \cdot HCl$

M.W. 414.71

m.p. 244-245° dec.

ADL 17520-17-2

0.5 g, March 22, 1972

While unsuccessful attempts were being made to prepare a ketal of 2,7-dichlorofluorenone-4-carboxylic acid (see VIII.2. below), we elected to apply the Boykin reaction^{20,21,22} directly to the keto acid. The only product isolated was that involving reaction at the 9-keto group rather than at the carboxylic acid group. The pyridyl adduct so obtained was then reduced to the piperidyl compound.

2,7-Dichloro-9-hydroxy-9-(2-pyridyl)-4-fluorencarboxylic Acid

A solution of 1.42 g (0.009 mole) of freshly distilled 2-bromopyridine in 15 ml of dry tetrahydrofuran was added dropwise at -60° to -70° to a mixture of 15 ml of tetrahydrofuran and 4 ml of 2.25M butyllithium in hexane. The mixture was stirred at -70° for 1 hr. There was then added dropwise a solution of 0.87 g (0.003 mole) of 2,7-dichlorofluorenone-4-carboxylic acid (Annual Report No. 1, p. 61) in 20 ml of tetrahydrofuran. The resulting cloudy viscous mixture was stirred at -70° for 3 hr. The mixture was then warmed to -10° and treated with 10 ml of wet ether, then with 8 ml of water. The mixture was allowed to stand overnight at room temperature. It was evaporated to dryness and the dark oily residue was extracted with several portions of boiling n-hexane, leaving a dark brown solid residue. The residue was also triturated carefully with water. When dried the light brown solid appeared to be a salt. It was suspended in water and acidified with hydrochloric acid to convert it to 0.35 g (31%) of an almost colorless solid, m.p. 213-220° dec. A portion was recrystallized from acetonitrile and then had m.p. 250-252° dec. The infrared

spectrum showed strong absorption at 3350 (OH), 1670 (COOH) and 1420 cm^{-1} (pyridyl).

Anal. Calcd for $\text{C}_{19}\text{H}_{11}\text{Cl}_2\text{NO}_3$: C, 61.31; H, 2.98; Cl, 19.05; N, 3.76.
Found: C, 61.35; H, 3.03; Cl, 19.02; N, 3.98.

The reaction was repeated at ten times the scale described above to give 2.5 g (22%), m.p. 224–232°. The purity of this material was adequate for use in the next step.

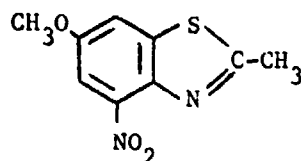
Reduction of the Pyridyl Intermediate

A mixture of 0.35 g (0.00094 mole) of the pyridyl intermediate, 70 ml of ethyl alcohol and 0.5 ml of concentrated hydrochloric acid was hydrogenated over 70 mg of Adams' platinum oxide at 25 psig and room temperature. Absorption of hydrogen was complete after about 17 hr. The filtered solution was evaporated to dryness and the yellow residue was triturated with acetonitrile to give 0.37 g (95%) of a colorless solid, m.p. 244–245° dec. An additional 0.37 g was obtained in a second run. The infrared spectrum showed strong broad absorption at 1435, 1700, and 3000–3400 cm^{-1} . A blend of the products of the two runs was submitted to WRAIR on March 22, 1972.

Anal. Calcd for $\text{C}_{19}\text{H}_{17}\text{Cl}_2\text{NO}_3 \cdot \text{HCl}$: C, 55.03; H, 4.37; Cl, 25.65; N, 3.38.
Found: C, 54.84; H, 4.56; Cl, 25.51; N, 3.39.

The compound is insoluble in dilute acid, acetone, chloroform, ether and benzene; moderately soluble in water, dilute base, methyl alcohol and ethyl alcohol. It appears to be stable under ordinary laboratory conditions.

28. 6-Methoxy-2-methyl-4-nitrobenzothiazole (AY 98401)



$C_9H_8N_2O_3S$

M.W. 224.22

m.p. 140-146°

ADL 16851-99-1

0.5 g, January 11, 1972

1-Chloro-6-methoxy-4-nitrobenzodithiazole

Four preparations were carried out using variations of a literature procedure.¹³ The largest run, in which we used variations examined in earlier runs, is described here. A total of 376 g was prepared in yields of about 55%.

To a suspension of 225 g (1.34 moles) of 4-methoxy-2-nitroaniline (Eastman 2094) in 1350 ml of glacial acetic acid there was added 660 ml (1110 g; 8.24 moles) of sulfur monochloride (Eastman P716; cooled to 0°). The mixture was stirred at room temperature for 2 hr., at 70-75° for 4 hr., at 80° for 1.5 hr., and at 90° for 1.5 hr., then was allowed to stand overnight at room temperature. During the reaction there was a copious evolution of hydrogen chloride. The mixture was filtered and the pasty solid was washed with benzene and dried at room temperature. We obtained 204 g (57%), m.p. 175° dec. As noted previously¹³ the melting point varied considerably with the rate of heating, values as high as 220° having been observed when heating rates were high.

1-Hydroxy-6-methoxy-4-nitrobenzodithiazole

The hydrolysis of the 1-chloro precursor was carried out by modification of literature procedures.^{13,14,23} The product was isolated and purified in the following single run.

30 g (0.113 mole) of the chloro compound was stirred in 4 liters of water at room temperature for 1 hr. The fluffy solid was filtered off and stirred with another 4 liters of water for 1 hr. The solid was sucked as dry as possible on the filter and then was recrystallized from 2 liters

of 95% ethanol to give 6 g (21%) of a brown crystalline material, m.p. 161° dec.; lit.¹³ m.p. 162.5° dec.

The dissolution of the chloro compound, followed by separation of the hydrolysis product¹⁴ was not observed by us.

6-Methoxy-2-methyl-4-nitrobenzothiazole

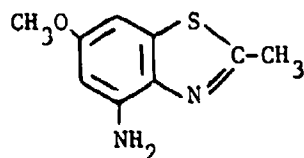
Following the procedure of Fox and Bogert¹³ the material was prepared directly from the chloro compound described above, without purification of the hydroxy compound described above.

In our best run 79 g (0.3 mole) of 1-chloro-6-methoxy-4-nitrobenzodithiazole was stirred in 9 liters of water for 40 min. The hydroxy compound that formed was filtered off and stirred for another 30 min. with 7 liters of water. The material was isolated by filtration and was then suspended in 880 ml of water. Addition of 112 ml of 20% aqueous sodium hydroxide converted the hydroxy compound to the soluble sodium salt. The dark solution was filtered to remove a small amount of insoluble material and then 135 ml of acetic anhydride was added to the stirred filtrate, with a little external cooling. The volume of acetic anhydride was sufficient to complete precipitation of the orange product. The resulting suspension was warmed briefly on a steam bath, was stirred at room temperature for 1 hr., and then was filtered. The crude was recrystallized from 95% ethanol to give 29 g (43%), m.p. 142-145°; lit.¹³ m.p. 147°. The infrared and nuclear magnetic resonance spectra were in accord with the structure. A 0.5-g sample of ADL 16851-99-1 was submitted to WKAIR on January 11, 1972.

Two earlier runs at one-quarter the scale described above gave yields of 35-40%. In our original run we did not isolate the hydroxy compound by filtration, but added sodium hydroxide directly to the suspension. In this case insoluble gums formed and the yield of desired benzothiazole was just 11%.

The compound is moderately soluble in methyl and ethyl alcohols, chloroform, and ether; insoluble in water, dilute acids, and dilute base. It appears to be stable under ordinary laboratory conditions.

29. 4-Amino-6-methoxy-2-methylbenzothiazole (AY 98410)



$C_9H_{10}N_2OS$

M.W. 194.24

m.p. 94-96°

ADL 16851-103B-1

0.5 g, January 11, 1972.

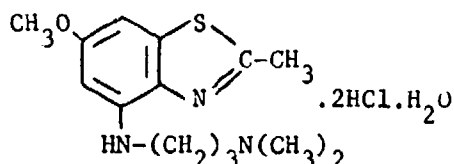
Reduction of the nitro group by iron and acid was carried out in 4 runs based on a literature procedure.¹³ The same reduction had also been carried out using stannous chloride.¹² We have not yet identified the product obtained by reduction in ethanol solution over palladium-on-carbon at 40 psi in a single run.

In our best run 29 g (1.29 mole) of 6-methoxy-2-methyl-4-nitrobenzothiazole was stirred in a mixture of 30 ml of concentrated hydrochloric acid and 300 ml of water while 26.4 g of iron powder (Alfa, 170-325 mesh) was added in portions. When the initial reaction subsided the mixture was refluxed for 3 hr., then was cooled and extracted thoroughly with boiling benzene. The latter procedure involved both leaching of insoluble material with hot solvent and also boiling a mixture of the aqueous phase and benzene.

The benzene extract was dried over magnesium sulfate and evaporated to dryness under vacuum to give an oil. The oil was dissolved in the minimum volume of a mixture of equal volumes of benzene and chloroform and the solution was placed on a 3" x 12" Florisil (60-100 mesh) chromatography column. The column was eluted with the same solvent mixture. Using experience with an earlier small-scale run as a guide, the eluate was collected in 300-ml portions, evaporation of which led to the accumulation of 10.4 g, m.p. 91-94°, and 4 g, m.p. 88-91°; lit. m.p. 95-96°. The combined yield of material suitable for the subsequent alkylation step was 14.4 g (57%). An infrared spectrum (mineral oil mull) showed absorption at 3420 and 3280 cm^{-1} (free NH_2) and at 3180 cm^{-1} (bonded NH_2). In earlier small-scale runs we obtained 6.5 g of product in yields of 58-62%. A 0.5-g sample of ADL 16851-103B-1 was submitted to WRAIR on January 11, 1972.

The compound is moderately soluble in methyl and ethyl alcohols, chloroform, ether, and dilute acids; insoluble in water and dilute base. It appears to be stable under ordinary laboratory conditions.

30. 4-Dimethylaminopropylamino-6-methoxy-2-methylbenzothiazole Dihydrochloride Monohydrate (BB 05889)



$C_{14}H_{21}N_3OS \cdot 2HCl \cdot H_2O$

M.W. 370.33

m.p. 188-195° dec.

ADL 16851-134-3

2 g, November 19, 1971

Alkylations of analogous aminoquinolines have been described by several authors.^{6,7,24} One gets the impression in studying these descriptions that the process is not simple, even when short-chain alkylaminoalkyl halides are used as alkylating agents. Incomplete alkylation seems common and the separation of unreacted aromatic amine is difficult. Elslager⁶ used sodamide to promote alkylation and isolated the alkylated products as β -resorcylic acid salts. Hydrochloride salts were eschewed by most investigators and analytical data are strangely missing in one recent case.⁷

In the benzothiazole series alkylations of 4-amino-6-methoxybenzothiazoles with dialkylaminoalkyl halides by conventional techniques have given acceptable yields, the unalkylated material being separated from the desired product by vacuum distillation.^{12,13,14}

We first attempted to alkylate 4-amino-6-methoxy-2-methylbenzothiazole with 3-dimethylaminopropyl chloride hydrochloride (Aldrich D14,520) in aqueous suspension at temperatures up to 100°. The technic has been used previously with success for aminoquinoline alkylations by 2-bromo-5-diethylaminopentane.^{24,25} In our hands only unreacted starting materials were recovered.

An inefficient but successful alkylation occurred when a mixture of 2.5 g (0.0129 mole) of 4-amino-6-methoxy-2-methylbenzothiazole, 2.6 g (0.0155 mole) of 3-dimethylaminopropyl chloride hydrochloride and 10 ml of absolute ethanol was refluxed for 24 hr. The procedure was a conventional one and was recently used with success in the quinoline series.⁷ The dark reaction mixture was allowed to stand at room temperature during which time

the bulk of the unreacted aminobenzothiazole separated as its hydrochloride salt, m.p. 186-190°, identified by direct comparison with an authentic sample and by conversion to the free base, m.p. 92-95°.

The filtrate was diluted with 100 ml of water and extracted with ether; the extract gave only an additional 0.2 g of impure aminobenzothiazole.

The aqueous phase was then made basic by adding solid potassium carbonate and then was extracted with ether. The extract was washed with saturated aqueous sodium chloride until neutral, then was dried over sodium sulfate and evaporated to give 1 g of a green oil. Bulb-to-bulb distillation at 175-180°/0.01 mm gave 0.65 g of an almost colorless oil. A small additional quantity of unreacted aminobenzothiazole distilled at a lower temperature.

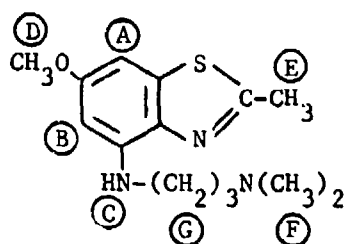
In a second run a mixture of 5.6 g (0.0288 mole) of 4-amino-6-methoxy-2-methylbenzothiazole, 5.5 g (0.0346 mole) of 3-dimethylaminopropyl chloride hydrochloride and 30 ml of absolute ethanol was refluxed for 3 days, then was worked up as described above. We obtained 3.5 g of unreacted amine (hydrochloride) and 2.2 g of crude alkylated product. The latter on distillation gave 1.9 g of an almost colorless oil.

The oily base was converted to the hydrochloride in the usual way and a 2-g sample of ADL 16851-134-3 was submitted to WRAIR on November 19, 1971. The m.p. 188-195° dec. was observed in a sealed capillary. The hydrate character was assigned on the basis of the analysis.

Anal. Calcd for $C_{14}H_{21}N_3OS \cdot 2HCl \cdot H_2O$: C, 45.41; H, 6.80; Cl, 19.15; N, 11.35. Found: C, 45.64; H, 6.29; Cl, 19.43; N, 11.33.

The compound is moderately soluble in water, dilute acid, and the lower alcohols; it is insoluble in ether, chloroform, and petroleum ether. It appears to be stable under ordinary laboratory conditions.

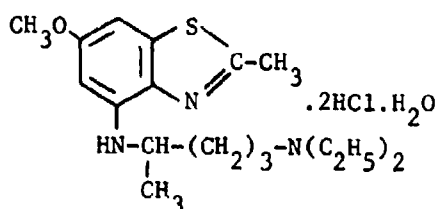
The infrared spectrum of the free base showed NH absorption at 3400 cm^{-1} and broad aromatic absorption at 1600 cm^{-1} . The nuclear magnetic resonance spectrum was recorded in deuteriochloroform solution.



- (A) 6.75 (d); 1H
- (B) 6.35 (d); 1H
- (C) 5.5 (br); 1H
- (D) 3.92 (s); 3H
- (E) 2.8 (s); 3H
- (F) 2.27 (s); 6H
- (G) 3.37 (m)
1.8-2.5 (m)

The spectra of the dihydrochloride monohydrate described above in water and in deuterium oxide presented an anomaly. The hydrate character was clearly shown in deuterium oxide solution but the aromatic absorptions were absent. When a portion of the sample was reconverted to the base, the two aromatic protons re-appeared; they also were observed when the hydrochloride salt was examined in water. The presence of exchangeable aromatic protons is unexpected. The same phenomenon was observed in an analogous case described in 31 below.

31. 4-(4-Diethylamino-1-methylbutylamino)-6-methoxy-2-methylbenzothiazole
Dihydrochloride Monohydrate (AY 98429)



C₁₈H₂₉N₃OS.2HCl.H₂O

M.W. 426.44

m.p. 110° dec. (shrinks at 84°)

ADL 17254-30-5

1 g, January 11, 1972

It was our original intention to prepare the compound by the alkylation of 4-amino-6-methoxy-2-methylbenzothiazole with 2-bromo-5-diethylaminopentane hydrobromide. One such alkylation was carried out in which we used Elderfield's aqueous procedure.²⁵ 5-Diethylamino-2-pentanone (Aldrich D8820-8) was reduced by sodium borohydride to 5-diethylamino-2-pentanol²⁶ and the latter was converted to the alkylating agent by thionyl bromide.²⁶ While the workup of the alkylation reaction mixture was in progress an alternative and obviously superior alkylation procedure, which had been developed by another contractor (for the alkylation of 1,4-dimethoxy-2-methylamine) and provided to us by WRAIR, was used successfully.

5-Diethylamino-2,2-dimethoxypentane

Dry hydrogen chloride in excess was bubbled through a solution of 15.7 g (0.1 mole) of 5-diethylamino-2-pentanone in 100 ml of methanol at 0°. Excess hydrogen chloride was removed by passing nitrogen through the solution, and the solution was allowed to stand overnight at room temperature. The solution was evaporated to dryness under vacuum and the residue was dissolved in 100 ml of methanol. To the solution there was added 12.7 g (0.12 mole) of freshly distilled methyl orthoformate (MC & B 6860), the dark solution was refluxed for 30 min., and then was allowed to stand overnight at room temperature. The solution was poured into 200 ml of saturated aqueous sodium carbonate. The resulting mixture was extracted with ether and the extract was washed with saturated aqueous sodium chloride. The washed extract was dried over anhydrous potassium carbonate and then was distilled to give 14 g (72%), b.p. 108-112°/23 mm, n_D^{28} 1.4284; lit.²⁷

b.p. 106°/8 mm, n_D^{20} 1.4346. The infrared spectrum showed no significant carbonyl absorption at 1720 cm^{-1} and strong carbon-oxygen absorption at 1050-1120 cm^{-1} .

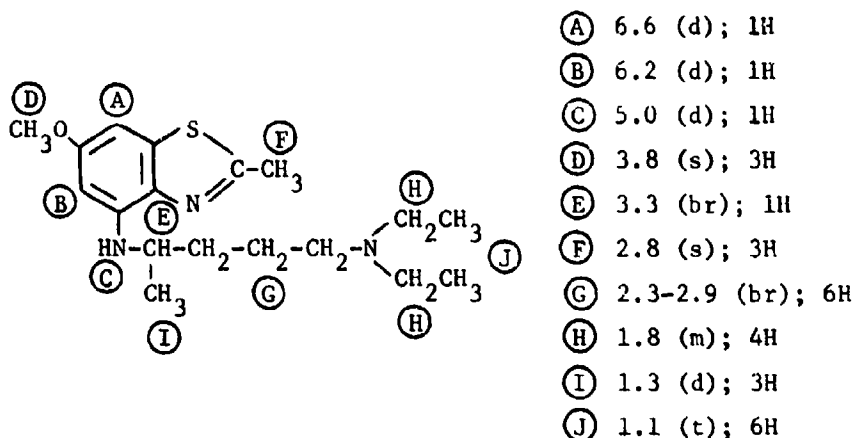
In a second run 60 g of the ketone gave 45 g of the ketal, b.p. 94-95°/10 mm.

Reductive Alkylation

A mixture of 1.94 g (0.01 mole) of 4-amino-6-methoxy-2-methylbenzothiazole, 2 g (0.011 mole) of 5-diethylamino-2,2-dimethoxypentane and 10 mg of p-toluenesulfonic acid was heated to 140° during 10 min. and was held at 140-144° for 20 min. The mixture was cooled, diluted with ether, and washed with saturated aqueous sodium carbonate. The ether solution was dried over solid potassium carbonate and evaporated to dryness to give 3.6 g of an oil whose infrared spectrum showed little absorption at 1670 cm^{-1} for the azomethine linkage. The entire 3.6 g, which obviously contained both reactants, was mixed with 0.5 g additional 5-diethylamino-2,2-dimethoxypentane and with 100 mg of p-toluenesulfonic acid and the mixture was heated at 140° for 17 hr. The mixture was then worked up as before to give 3.27 g of a dark oil with significant absorption at 1665 cm^{-1} .

In a second run carried out at about the same scale we observed that the prolonged reaction time was not necessary when refluxing of evolved methanol was avoided.

The 3.27 g of oil was dissolved in 50 ml of absolute ethanol and a solution of 1 g of sodium borohydride in 25 ml of absolute ethanol was stirred in rapidly. The mixture was stirred at room temperature for 3 days, then was diluted with 300 ml of water and extracted with ether. The extract was washed with saturated aqueous sodium chloride, dried over magnesium sulfate, and evaporated to give an oil. The oil was distilled in a bulb-to-bulb apparatus to give 0.7 g of a pale yellow oil, b.p. 165-175°/0.1 mm; unreacted aminobenzothiazole distilled at a lower temperature. The infrared spectrum showed no absorption at 1665 cm^{-1} (azomethine) and weak absorption at 3400 cm^{-1} (NH). The nuclear magnetic resonance spectrum was recorded in deuteriochloroform.

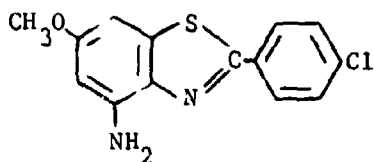


In a second run where 1.1 g of oil was obtained it was purified additionally by dissolving it in a mixture of equal volumes of chloroform and benzene and passing the solution through a Florisil column (2 x 25 cm), which effectively removed traces of unalkylated 4-amino-6-methoxy-2-methylbenzothiazole, which was not adsorbed. The desired product was leached from the column by the use of the same solvent mixture to which had been added 5% (v/v) of methanol. The 0.5 g of oil obtained by evaporation of the effluent solution was converted to the hydrochloride salt by ethereal hydrogen chloride. The salt was blended with salt prepared from the product of the first run and a 1-g sample of ADL 17254-30-5 was submitted to WRAIR on January 11, 1972. All preparations of this salt have been very hygroscopic. The sample submitted shrank at 84° and melted at 110° dec. in a capillary.

Anal. Calcd for $C_{18}H_{29}N_3OS \cdot 2HCl \cdot H_2O$: C, 50.69; H, 7.79; Cl, 16.63; N, 9.85; S, 7.52. Found: C, 50.64; H, 7.73; Cl, 16.53; N, 9.79; S, 7.67.

The compound is very soluble in water, moderately soluble in methyl and ethyl alcohols, and insoluble in chloroform, ether, and petroleum ether. Aside from its marked hygroscopicity, it appears to be stable under ordinary laboratory conditions. The free base develops a color when warm. The nuclear magnetic resonance spectrum of the salt in deuterium oxide clearly showed the hydrate character, but no aromatic protons were detected. Reconversion of the salt to the free base gave a substance whose spectrum was identical with that recorded above. A similar anomaly was described in 30 above.

32. 4-Amino-2-(4-chlorophenyl)-6-methoxybenzothiazole (AY 98438)



$C_{14}H_{11}ClN_2OS$

M.W. 290.76

m.p. 169-174°

ADL 17254-32-1

0.5 g, January 11, 1972

2-Mercapto-4-methoxy-6-nitroaniline

55 g (0.22 mole) of 1-hydroxy-6-methoxy-4-nitrobenzodithiazole (see 28 above) was added with stirring to 90 ml of 20% aqueous sodium hydroxide that had been purged with nitrogen. The dark mixture was stirred for 30 min. and then filtered. The insoluble material was then stirred into 50 ml of 20% aqueous sodium hydroxide and stirred for 30 min. before filtering. The combined basic filtrates were purged with nitrogen and acidified by dropwise addition of concentrated hydrochloric acid. The red solid that separated was filtered by suction under nitrogen, washed with water until neutral and then dried at 40° in a vacuum oven to give 27 g (61%), m.p. 110-115°. Fox and Bogert¹³ had isolated this material but reported that it oxidized rapidly in air to the related disulfide.

In a second (smaller) run we obtained 22 g (65%).

2-(4-Chlorophenyl)-6-methoxy-4-nitrobenzothiazole

The procedure used for this preparation was based on earlier preparations of 2-phenylbenzothiazoles.^{15,28} Although a total of 35 g of the compound was prepared, the procedure was not entirely satisfactory; several small-scale preliminary runs failed entirely.

A mixture of 21 g (0.12 mole) of p-chlorobenzoyl chloride (Aldrich 11,190-2) and 70 ml of N,N-dimethylaniline (MC&B, 5053) was purged with nitrogen and then 14 g (0.07 mole) of 2-mercapto-4-methoxy-6-nitroaniline was stirred in rapidly. The mixture warmed spontaneously to about 50-60° and became very stiff. The mixture was warmed during 1.5-2 hr. to 190° and held at that temperature for 1 hr.; the condensation of water was observed. When the mixture was heated to reflux, or maintained at 190° for longer periods, yields were decreased. The dark mixture was cooled

and stirred for 30 min. with a mixture of 525 ml of 6N hydrochloric acid and 525 ml of chloroform. The mixture was then shaken in a separatory funnel. The aqueous phase was extracted with chloroform and the combined chloroform solutions were extracted with 6N hydrochloric acid, water, aqueous sodium bicarbonate, saturated brine, water, then dried over magnesium sulfate, and evaporated under vacuum. The semisolid residue was triturated with alcohol, then filtered and dried to give 19.1 g (85%), m.p. 167-171°. A sample for analysis was prepared by recrystallization from a mixture of alcohol/dimethoxyethane (2/1) and had m.p. 170-171°.

Anal. Calcd for $C_{14}H_9ClN_2O_3S$: C, 52.43; H, 2.83; Cl, 11.05; N, 8.73.
Found: C, 52.52; H, 2.88; Cl, 10.93; N, 8.68.

4-Amino-2-(4-chlorophenyl)-6-methoxybenzothiazole

A mixture of 20 g (0.061 mole) of 2-(4-chlorophenyl)-6-methoxy-4-nitrobenzothiazole, 70 g (0.31 mole) of stannous chloride dihydrate (Fisher T-142), 350 ml of concentrated hydrochloric acid and 5 g of tin (Mallinckrodt 8580, 30 mesh) was heated at 70-90°. Because the nitro compound remained insoluble it was necessary to add 850 ml of glacial acetic acid to make the mixture homogeneous. The mixture was held at 90-95° for 4 hr. and then was allowed to stand at room temperature overnight. The mixture deposited a solid, which was filtered off, after chilling, and dried. The 18 g, m.p. 195°, was obviously a tin complex, for it left much ash on ignition. It was stirred with 100 ml of 20% aqueous sodium hydroxide for several hours, then washed with water until neutral and dried to give 12.5 g (71%), m.p. 160-164°. A portion was recrystallized from glacial acetic acid and then had m.p. 169-174°. A 0.5-g sample of ADL 17254-32-1 was submitted to WRAIR on January 11, 1972.

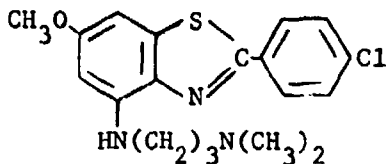
Anal. Calcd for $C_{14}H_{11}ClN_2OS$: C, 57.83; H, 3.81; Cl, 12.19; N, 9.63; S, 11.03. Found: C, 57.69; H, 3.69; Cl, 12.26; N, 9.60; S, 11.10.

In 3 previous small-scale runs an additional 8 g had been prepared.

The infrared spectrum showed absorption at 3490 cm^{-1} and 3370 cm^{-1} (NH_2 stretch) and strong absorption at $1600\text{--}1610\text{ cm}^{-1}$ (NH_2 bend). The nuclear magnetic resonance spectrum in DMSO-d_6 showed: δ 7.9 (d, 2H); 7.45 (d, 2H); 6.65 (d, 1H); 6.25 (d, 1H); 5.75 (d, 2H); and 3.75 (s, 3H).

The compound is moderately soluble in methyl and ethyl alcohols, chloroform, ether, and dilute acid; insoluble in water and dilute base. It appears to be stable under ordinary laboratory conditions.

33. 2-(4-Chlorophenyl)-4-(3-dimethylaminopropyl)amino-6-methoxybenzo-
thiazole (BB 43021)



$C_{19}H_{22}ClN_3OS$

M.W. 375.9

m.p. 97-100°

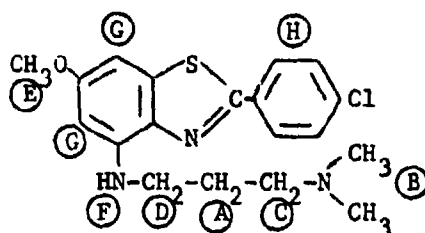
ADL 17520-52

2.0 g, May 25, 1972

A mixture of 2.32 g (0.008 mole) of 4-amino-2-(4-chlorophenyl)-6-methoxybenzothiazole, 0.34 g (0.008 mole) of sodium hydride (57% suspension in mineral oil; Alfa), 1.0 g (0.008 mole) of 3-dimethylaminopropyl chloride and 100 ml of dry toluene was stirred under nitrogen at 110-120° for 64 hr. The mixture was cooled in ice and 5 ml of water was added dropwise. The small aqueous layer was separated and extracted with toluene and the extract was combined with the principal toluene solution. The toluene solution was dried over magnesium sulfate and taken to dryness under vacuum. The brown semisolid residue (2.9 g) was dissolved in a 50/30 mixture of benzene and hexane and the solution was passed through a 3 x 30 cm column of Florisil. The first 1600 ml of eluate collected contained only a small amount of unalkylated starting material. Elution by 1 liter of benzene/hexane/methanol, 250/150/5, gave 0.2 g of a mixture of unalkylated amine and the desired product. From the following 2 liters of eluate we obtained 1.28 g of monoalkylated product, m.p. 97-100°. An analytical sample (360 mg) was obtained by recrystallizing 500 mg from 20 ml of hexane and had m.p. 98-101°.

Anal. Calcd for $C_{19}H_{22}ClN_3OS$: C, 60.71; H, 5.89; Cl, 9.43; N, 11.18; S, 8.53. Found: C, 60.68; H, 6.17; Cl, 9.16; N, 11.05; S, 8.28.

The infrared spectrum showed N-H absorption at 3240 cm^{-1} . The nuclear magnetic resonance spectrum was recorded in deuteriochloroform.



- (A) 1.84 t; 2H
- (B) 2.25 s; 6H
- (C) 2.41 t; 2H
- (D) 3.3 q (t after D₂O exchange); 2H
- (E) 3.81 s; 3H
- (F) 5.75 t (D₂O exchange); 1 H
- (G) 6.18, 6.54 d; J = 2; 2H
- (H) 7.35 d; J = 8.5; 4H
7.86 d; J = 8.5; 4H

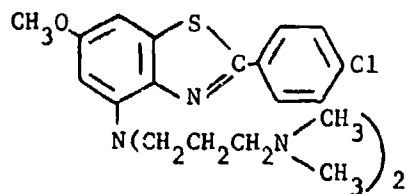
The preparation was repeated on the same scale, but the crude product was not chromatographed but instead was extracted with 60 ml of boiling hexane. The extract was cooled to room temperature and decanted from a small amount of gum that separated. The solution was then cooled to 0° and 1.5 g of yellow crystals, m.p. 93-96°, separated.

The remaining products of the two runs were combined and recrystallized from hexane to give 2.0 g of yellow needles, m.p. 97-100°, which was submitted to WRAIR as ADL 17520-52 on May 25, 1972.

The compound is insoluble in water and the lower alcohols; moderately soluble in ether, benzene, and dilute acid; very soluble in chloroform and acetone. In the solid state it appears to be stable under ordinary laboratory conditions but solutions discolor when exposed to light.

Before the above procedure was developed several unsuccessful attempts were made to carry out the alkylation in refluxing ethyl alcohol, dry dimethylformamide at 60-70°, and diethylene glycol at 175°. In all cases reaction times were 3-4 days and unreacted starting materials were recovered. We also failed to obtain azomethine formation during a reaction of the amine with 5-diethylamino-2,2-dimethoxypentane.

34. 4-Bis(3-dimethylaminopropyl)amino-2-(4-chlorophenyl)-6-methoxybenzo-
thiazole (BB 42104)



C₂₄H₃₃ClN₄OS

M.W. 461.06

m.p. 89-90°

ADL 17520-40A

1.9 g, April 28, 1972

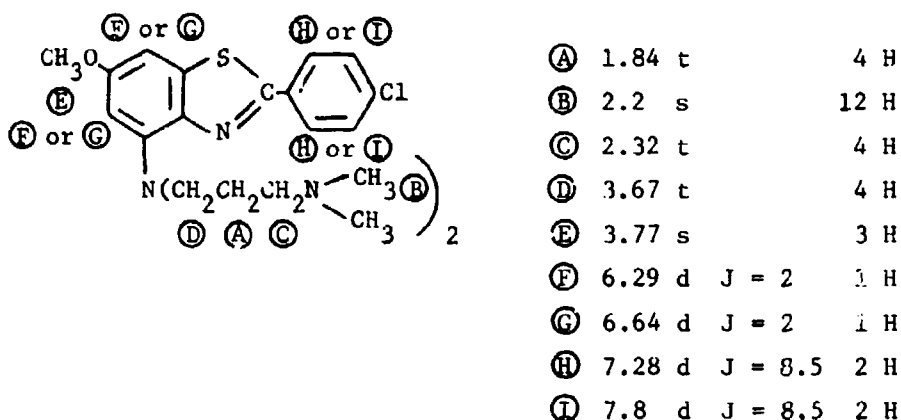
During the development of the sodium hydride alkylation procedure described in 33 above we carried out one run in which reagents were used in excess and obtained the bis-alkylated product described here.

3-Dimethylaminopropyl chloride was prepared by treating its hydrochloride salt (Aldrich D14,520) with 5% aqueous sodium hydroxide, extracting the organic base into ether, drying the extract over anhydrous magnesium sulfate, and evaporating to dryness.

A solution of 4.35 g (0.036 mole) of 3-dimethylaminopropyl chloride in 20 ml of dry toluene (distilled from sodium hydride) was added dropwise to a stirred mixture of 2.63 g (0.009 mole) of 4-amino-2-(4-chlorophenyl)-6-methoxybenzothiazole, 1.65 g (0.039 mole) of sodium hydride (as 57% suspension in mineral oil; Alfa) and 150 ml of dry toluene. Additional dry toluene was added to bring the volume of the mixture to 250 ml and the mixture was then refluxed for 54 hr. During this time no change was observed in thin layer chromatography (silica; benzene/hexane/methanol, 5/3/2; detection by fluorescence under 2537 nm light) and we concluded that no reaction had occurred. An additional 1.6 g of sodium hydride and 4.3 g of 3-dimethylaminopropyl chloride were added dissolved in 20 ml of toluene. Refluxing was then continued for another 24 hr at which time thin layer chromatography indicated the disappearance of the starting amine and the appearance of a new component. After standing at room temperature for 30 hr the mixture was treated dropwise with 40 ml of water (to destroy excess hydride). The aqueous phase was extracted once with toluene. The combined toluene phases were dried over magnesium sulfate and evaporated

to dryness under vacuum. The semisolid residue was triturated with cold petroleum ether and the insoluble yellow solid was dried to give 3 g (73%), m.p. 85-89°, which was recrystallized from 15 ml of boiling n-hexane to give 2.1 g of yellow needles, m.p. 89-90°.

An infrared spectrum was recorded; it showed no N-H absorption. The nuclear magnetic resonance spectrum was recorded in deuteriochloroform solution and showed no protons exchangeable with D₂O.



A 1.9-g sample of ANL 17520-40A was submitted to WRAIR on April 28, 1972.

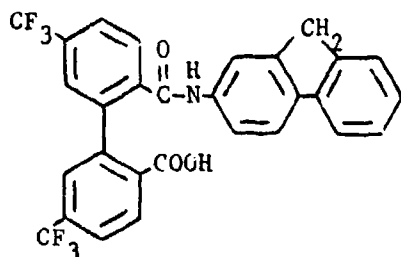
Anal. Calcd for C₂₄H₃₃ClN₄OS: C, 62.52; H, 7.21; Cl, 7.69; N, 12.15; S, 6.95. Found: C, 62.29; H, 7.27; Cl, 7.51; N, 11.88; S, 7.10.

The compound is insoluble in water and in dilute base, very soluble in dilute acid, and moderately soluble in methyl alcohol, ethyl alcohol, ether, and petroleum ether. It appears to be stable under ordinary laboratory conditions.

When an ether solution of β-resorcylic acid was added to an ether solution of the compound a yellow salt separated; it was not characterized because we found the free base to be adequate.

In thin layer chromatography the solvent system cited above does not move the compound from the origin. When a solvent mixture containing benzene (50 parts), dioxane (40 parts), diethylamine (5 parts) and ethyl alcohol (5 parts) is used, the compound has R_f 0.54; the starting amine has R_f 0.73.

35. N-(2-Fluorenyl)-5,5'-bis(trifluoromethyl)diphenamic Acid (BB 40146)



$C_{29}H_{17}F_6NO_3$
 M.W. 541.46
 m.p. 240-242°
 ADL 17311-6
 0.5 g, March 6, 1972

For the preparation of this and similar compounds reported in 36-38 below, it was necessary to prepare the novel 5,5'-bis(trifluoromethyl)diphenic anhydride. A solution of 5 g (0.013 mole) of 5,5'-bis(trifluoromethyl)diphenic acid (see VIII-3 below) in 30 ml of acetic anhydride was heated at reflux for 4 hr and then was evaporated to dryness under vacuum to give 4.6 g (97%) of a colorless solid, m.p. 139-141°, whose melting point was unchanged when a portion was recrystallized from anhydrous benzene.

Anal. Calcd for $C_{16}H_6F_6O_3$: C, 53.35; H, 1.68. Found: C, 53.23; H, 1.80.

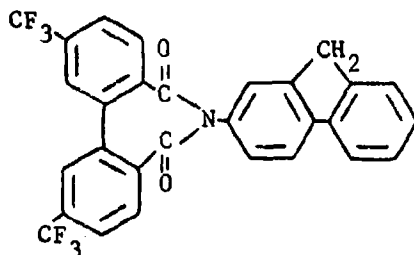
A general literature procedure^{16,17} was used.

A solution of 1 g (0.0055 mole) of 2-aminofluorene (Aldrich 5550-0) in 20 ml of methylene chloride was added with stirring to a solution of 2 g (0.0055 mole) of 5,5'-bis(trifluoromethyl)diphenic anhydride in 20 ml of methylene chloride. After 10 min. at room temperature a heavy crystalline solid separated. After washing on the filter with methylene chloride and drying at 65° there remained 2.2 g (74%), m.p. 240-242°. An infrared spectrum was recorded and a 0.5-g sample of ADL 17311-6 was submitted as a gift to WRAIR on March 6, 1972.

Anal. Calcd for $C_{29}H_{17}F_6NO_3$: C, 64.32; H, 3.16; N, 2.59. Found: C, 63.72; H, 3.42; N, 2.45.

The compound is insoluble in water and dilute acid, very soluble in base and in acetone, and moderately soluble in ethyl alcohol. It appears to be stable under ordinary laboratory conditions.

36. N-(2-Fluorenyl)-5,5'-bis(trifluoromethyl)diphenimide (BB 40155)



$C_{29}H_{15}F_6NO_2$

M.W. 523.44

m.p. 310-313°

ADL 17311-6A

1.4 g, March 6, 1972

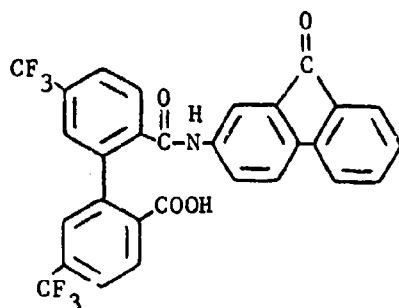
A general literature procedure^{16,17} was used.

1.6 g (0.003 mole) of the amic acid described in 35 above was dissolved in a mixture of 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate. Within a few minutes a heavy precipitation occurred, so 10 ml additional acetic anhydride was added and the mixture was stirred on a steam bath for 10 min. Water was then added and the mixture was warmed until all acetic anhydride had been destroyed. The solid product was washed well with water and dried at 65° to give 1.5 g (98%), m.p. 310-313°. An infrared spectrum showed a small residual carboxylic acid absorption. A 1.4-g sample of ADL 17311-6A was submitted as a gift to WRAIR on March 6, 1972.

Anal. Calcd for $C_{29}H_{15}F_6NO_2$: C, 66.54; H, 2.89; N, 2.68. Found: C, 65.96; H, 2.94; N, 2.63.

The compound is insoluble in water, dilute acid, and dilute base; very soluble in acetone; and moderately soluble in ethyl alcohol. It appears to be stable under ordinary laboratory conditions.

37. N-(9-Keto-2-fluorenyl)-5,5'-bis(trifluoromethyl)diphenamic Acid
(BB 40164)



$C_{29}H_{15}F_6NO_4$

M.W. 555.14

m.p. 276-279°

ADL 17311-7A

0.5 g, March 6, 1972

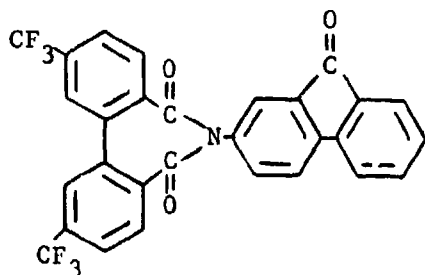
A general literature procedure^{16,17} was used.

A solution of 1.1 g (0.0055 mole) of 2-aminofluorenone (Aldrich 5580-2) in 30 ml of methylene chloride was added with vigorous stirring to a solution of 2 g (0.0055 mole) of 5,5'-bis(trifluoromethyl)diphenic anhydride in 20 ml of methylene chloride. After 10 min. at room temperature there was a heavy precipitation of an orange solid; stirring was continued for a total of 45 min. The solid was washed on the filter with methylene chloride and dried at 65° to give 2.65 g (85%), m.p. 276-279°. Evaporation of the filtrate and washings gave an additional 0.4 g of the same melting point; the yield was therefore quantitative. An infrared spectrum was recorded and a 0.5-g sample was submitted as a gift to WRAIR on March 6, 1972.

Anal. Calcd for $C_{29}H_{15}F_6NO_4$: C, 62.71; H, 2.72; N, 2.52. Found: C, 62.06; H, 2.85; N, 2.61.

The compound is insoluble in water and dilute acid, very soluble in dilute base and acetone, and moderately soluble in ethyl alcohol. It appears to be stable under ordinary laboratory conditions.

38. N-(9-Keto-2-fluorenyl)-5,5'-bis(trifluoromethyl)diphenimide (BB 40173)



$C_{29}H_{13}F_6NO_3$

M.W. 537.42

m.p. 322-325°

ADL 17311-8

1.1 g, March 6, 1972

A general literature procedure^{16,17} was used.

2.1 g (0.0038 mole) of the amic acid was dissolved in a mixture of 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate. The mixture was stirred at room temperature and a bright yellow solid soon separated. The mixture was heated on a steam bath for 10 min. Acetic anhydride was destroyed by addition of water and warming. The yellow solid was washed well with water and dried at 65° to give 2.0 g, m.p. 276-303°. The product was boiled with 40 ml of ethyl alcohol, in which uncyclized amic acid was more soluble. The suspension was filtered at room temperature. The insoluble portion was bright yellow needles, 1.2 g, m.p. 322-325°. An infrared spectrum showed the presence of a small residual carboxylic acid absorption. A 1.1-g sample of ADL 17311-8 was submitted as a gift to WRAIR on March 6, 1972.

Anal. Calcd for $C_{29}H_{13}F_6NO_3$: C, 64.81; H, 2.44; N, 2.61. Found: C, 64.78; H, 2.43; N, 2.51.

The compound is insoluble in water, dilute acid, and dilute base; very soluble in acetone; moderately soluble in ethyl alcohol. It appears to be stable under ordinary laboratory conditions.

By evaporation of the alcohol leaching above there was obtained 0.75 g of a mixture of unreacted amic acid and imide. The acetic anhydride treatment was repeated to give an additional 0.7 g of imide, m.p. 322-324°. The combined yield was thus 1.9 g (94%).

VIII. UNCOMPLETED SYNTHESSES

The following descriptions are arranged according to the target compound. A discussion of each has been given in Sections III and V of this report.

1. Pyrrocolinemethanol

In Annual Report No. 1, p. 74, we described 3 procedures for the oxidation of 7,9-dichloro-5-methylpyrrolo[1,2-a]quinoline by selenium dioxide in which we had isolated none of the desired aldehyde or carboxylic acid, either of which would have been useful for conversion to the target pyrrocolinemethanol.

A review of selenium dioxide oxidation processes²⁹ called our attention to still other alternative procedures; one of these has now been examined briefly.

6-Methoxylepidine has been oxidized in xylene solution to 6-methoxyquinoline-4-aldehyde and 6-methoxycinchoninic acid,³⁰ but no success was achieved in a repetition of the procedure³¹ unless freshly prepared selenium dioxide was used.³² On the other hand, by use of a mixture of acetic acid and acetic anhydride as a solvent, lepidine was oxidized to the aldehyde in a process where the history of the selenium dioxide was unimportant.³³ We have examined the latter procedure.

0.5 g (0.002 mole) of 7,9-dichloro-5-methylpyrrolo[1,2-a]quinoline (Annual Report No. 1, p. 41) was suspended in a mixture of 1 ml of acetic anhydride and 5 ml of glacial acetic acid. The initially yellow solution became green on standing overnight. The suspension was stirred at 85-90° and became homogeneous. 0.44 g (0.004 mole) of selenium dioxide (Alfa, 99.9%) was added during 30 min. in small portions. An immediate darkening of the mixture occurred. It was necessary to add an additional 25 ml of acetic acid to prevent the separation of a gummy solid. Stirring at 85-90° was continued for 7 hr. during which time red-brown selenium separated from the mixture. The mixture was cooled and filtered. The filtrate was stirred

with "Celite" filter aid and again filtered. About 10 ml of concentrated hydrochloric acid was added and the mixture was allowed to stand at room temperature for 2 days in order to hydrolyse any remaining acetic anhydride. The solution was then taken to dryness in a rotary evaporator; additional red selenium escaped into the condenser during this process.

The dark residue was agitated with dilute aqueous sodium bicarbonate, but very little dissolved. Acidification of the bicarbonate solution with dilute hydrochloric acid gave only a few mg of a brown solid, m.p. 170-200° slow dec.

The non-acidic product weighed 0.16 g. An infrared spectrum showed some carbonyl absorption at 1700 cm^{-1} .

2. 2,7-Dichloro-9-keto- α -(2-piperidyl)-4-fluoromethanol

When the synthesis of this target compound was undertaken it was planned to prepare the intermediate 2,7-dichloro-9-keto-4-fluorenyl 2-pyridyl ketone by a Boykin reaction between 2-pyridyllithium and a ketal of 2,7-dichlorofluorenone-4-carboxylic acid. The need to protect the 9-keto group seemed reasonable and our belief was supported by our later observation that in the Boykin reaction of the free keto acid, reaction occurred primarily at the keto group (see VII, 27 above).

After many unsuccessful attempts to prepare the desired ketal we carried out a conventional ketone synthesis between 2,7-dichlorofluorenone-4-carbonyl chloride and 2-pyridyllithium and obtained the desired ketone. As the contract expired we were attempting to reduce it to the target compound.

Four conventional procedures for ketal formation were examined. Some of them were based on procedures described for the preparation of ketals from fluorenone itself. We realized that the presence of a carboxyl group in our case might interfere with the reaction, but since the most likely side-reaction was ester formation and since esters of the ketal products would be useful in subsequent steps, we elected to examine the following procedures.

Method 1. This was based on a literature procedure¹ for the formation of the cyclic ethylene ketal of fluorenone.

A mixture of 0.6 g of 2,7-dichlorofluorenone-4-carboxylic acid, 5 ml of ethylene glycol, 25 ml of chloroform, and 30 mg of p-toluenesulfonic acid was refluxed through a Soxhlet thimble containing anhydrous magnesium sulfate for 64 hr. A conventional workup gave only unreacted starting material.

No reaction occurred in a similar experiment when benzene was used in place of chloroform and oxalic acid in place of p-toluenesulfonic acid. No evolution of water was detected and the keto acid remained insoluble.

A mixture of 1.5 g of the keto acid, 130 ml of xylene, 26 ml of ethylene glycol and 100 mg of p-toluenesulfonic acid was stirred and refluxed for 17 hr. under a Dean-Stark trap. The keto acid dissolved but the mixture had two liquid phases. On cooling there separated 0.8 g of a yellow crystalline solid which after recrystallization from absolute alcohol gave 0.66 g, m.p. 162-163°. The infrared spectrum could not distinguish between unreacted ketone carbonyl and ester carbonyl (both at 1730 cm^{-1}) but the nuclear magnetic resonance spectrum clearly excluded the cyclic ethylene ketal since only two protons appeared at 4.0 ppm (multiplet) instead of the required four protons (singlet). The material was insoluble in dilute aqueous sodium bicarbonate. We concluded that esterification had occurred, unaccompanied by ketal formation.

Method 2. This involved *trans*-ketalization with 2,2-dimethoxypropane (acetone dimethylketal).

A homogeneous mixture of 1 g of 2,7-dichlorofluorenone-4-carboxylic acid, 20 ml of 2,2-dimethoxypropane, 50 ml of benzene, and 40 mg of p-toluenesulfonic acid was stirred and refluxed for 2 hr with a slow takeoff of distillate (reflux ratio = 20/1). The still head temperature rose from 59° to 74°. On cooling the reaction mixture gave only a high recovery of unreacted keto acid.

In a second run a mixture of methanol, benzene and dimethoxypropane was used as a solvent and was allowed to distil slowly during 8 hr, while maintaining the volume of the reaction mixture constant by periodic replacement with the solvent mixture. A conventional workup gave only unreacted keto acid.

In these experiments we have no explanation for the failure of the keto acid to be converted to its methyl ester.

Method 3. This involved the use of methyl orthoformate.³⁴

A mixture of 1 g of 2,7-dichlorofluorenone-4-carboxylic acid, 1.82 g of methyl orthoformate (MC&B, 6860, redistilled), 10 ml of methanol and 30 mg of p-toluenesulfonic acid was refluxed for 5 hr and then was allowed to stand for several days at room temperature. A conventional workup gave only unreacted keto acid.

In a second run 1.3 g (0.0042 mole) of methyl 2,7-dichlorofluorenone-4-carboxylate dissolved in 5 ml of benzene was added in one portion to a solution prepared by mixing 0.68 g (0.0064 mole) of freshly distilled methyl orthoformate, 8 ml of dry benzene, 0.02 g of methanol and 1 drop of concentrated sulfuric acid and warming on a steam bath for 2-3 min.

The mixture was stirred at 75-80° for 22 hr; it did not become homogeneous. It was cooled, 1 ml of pyridine was added, followed by 50 ml of ether. A conventional workup gave only unreacted keto acid methyl ester. The keto acid methyl ester used in the latter run was prepared by the reaction of diazomethane and the keto acid. The ester was a yellow solid, m.p. 172-174°, with carbonyl absorption only at 1730 cm⁻¹.

Method 4. This involved the use of an acid ion exchange resin as a catalyst and methyl alcohol as a reagent.^{35,36}

A mixture of 1 g of 2,7-dichlorofluorenone-4-carboxylic acid, 50 ml of methanol, 3 g of an acid ion exchange resin (Rexyn 101(H), R-204; dried at 105° for 36 hr, weighed and stored under dry nitrogen), and 7 g of calcium sulfate (heated at 200° for 36 hr, weighed and stored under dry nitrogen) was stirred in the absence of moisture for 11 days at room temperature. A conventional workup gave only unreacted keto acid.

We then turned to the preparation of the dimethyl ketal.

2,7,9,9-Tetrachlorofluorene-4-carbonyl chloride was prepared by a procedure used previously^{37,38} for the conversion of fluorenone-4-carboxylic acid to 9,9-dichlorofluorene-9-carbonyl chloride.

A mixture of 1.5 g (0.0048 mole) of 2,7-dichlorofluorenone-4-carbonyl chloride (Annual Report No. 1. p. 62) and 3.4 g (0.0164 mole) of phosphorus pentachloride (Eastman P470) was heated at 160° for 4 hr; as the reaction proceeded, phosphorus oxychloride was observed to reflux. The mixture was then heated at about 130-140° under vacuum to remove excess phosphorus halides and then was cooled to give 1.6 g (91%) of product, m.p. 123-128°. In a second run 6.9 g of acid chloride gave 7.64 g (94%) of product, m.p. 129-131°. A sample for analysis was prepared by recrystallization from n-hexane and was obtained as yellow crystals, m.p. 128-131°.

Anal. Calcd for $C_{14}H_5Cl_5O$: C, 45.89; H, 1.37; Cl, 48.37. Found: C, 45.71; H, 1.35; Cl, 48.58.

The infrared spectrum showed no fluorenone carbonyl absorption at 1730 cm^{-1} but did show strong acid chloride carbonyl absorption at 1760 cm^{-1} . Strong absorption at 810 cm^{-1} is believed to be associated with the *gem*-dichloro group.

Methyl 2,7,9,9-Tetrachlorofluorene-4-carboxylate was prepared by shaking a suspension of 0.5 g (0.00136 mole) of the acid chloride in 2.5 ml of ice-cold methanol for 30 min. An infrared spectrum of an aliquot of the suspended solid showed strong residual acid chloride carbonyl absorption at 1760 cm^{-1} . After 4 hr at 0-5° absorption at 1760 cm^{-1} was still significant. After another 17 hr absorption at 1760 cm^{-1} was very weak. The entire suspended solid was collected, washed with cold methanol, with n-hexane, and dried to give 0.42 g (86%), m.p. 145-149°. In a second run 5 g of the tetrachlorocarbonyl chloride gave 4.5 g (91%), m.p. 139-143°. A sample for analysis was prepared by recrystallization from n-hexane.

Anal. Calcd for $C_{15}H_8Cl_4O_2$: C, 49.77; H, 2.23; Cl, 39.17. Found: C, 49.62; H, 2.11; Cl, 39.06.

The reaction between methyl 2,7,9,9-tetrachlorofluorene-9-carboxylate and sodium methoxide to give the desired dimethyl ketal was first studied using a procedure described earlier by Schlenk and Bergmann for the formation of 9,9-dimethoxyfluorene from 9,9-dichlorofluorene.³⁹

A mixture of 0.87 g (0.0024 mole) of methyl 2,7,9,9-tetrachlorofluorene-4-carboxylate, 0.27 g (0.005 mole) of sodium methoxide and 5 ml of anhydrous methanol was stirred at 0° for 1 hr and then was heated at reflux for 3 hr. No precipitation of sodium chloride was observed. When the mixture cooled some of the original tetrachloro ester crystallized out and was filtered off. Evaporation of the filtrate gave 0.6 g of a high-melting yellow solid that dissolved in water readily. From the solution there separated 0.26 g of an impure solid, m.p. 220°. The infrared spectra of both the high-melting solid and its hydrolysis product could not be correlated with any of the expected products of the reaction, and gave evidence that the ester function had been lost. The hydrolysis product was not an acid.

The reaction was repeated. The sodium methoxide solution was freshly prepared by the use of metallic sodium. In this run about half the starting tetrachloro ester was recovered unchanged. The workup procedure for the balance was varied somewhat but we were unable to identify it.

When dimethylformamide was used as a solvent in place of methanol, in a reaction at room temperature, color changes were observed. When the mixture was diluted with water the resulting homogeneous solution (pH 8-9) contained nothing that could be extracted into methylene chloride. We concluded that loss of the ester function had occurred, particularly when acidification of the solution caused precipitation of a solid to occur.

Carefully dried dimethyl sulfoxide was then used as a solvent. A slurry of the tetrachloro ester in DMSO was added to a solution of sodium methoxide in DMSO and the mixture was stirred at room temperature for 3.5 hr. A red color formed and the mixture became homogeneous. Thin layer chromatography showed that the tetrachloro ester had reacted completely. The mixture was diluted with ice but again we were unable to extract anything from the resulting basic solution.

We concluded that the simple metathetical reaction of Schlenk and Bergmann cannot be applied in the case of methyl 2,7,9,9-tetrachloro-fluorene-4-carboxylate.

In a closely related experiment a mixture of 0.366 g (0.001 mole) of 2,7,9,9-tetrachlorofluorene-4-carbonyl chloride was heated with 35 ml of absolute ethyl alcohol. As soon as the ester started to separate from the mixture there was injected 1.3 ml (0.0028 mole) of a standardized sodium ethoxide solution.⁴⁰ The mixture was refluxed for 3 hr during which it became homogeneous; thin layer chromatography showed the absence of starting material at the end of 2 hr. The mixture was cooled, filtered free from a small amount of solid, and evaporated to dryness. The solid residue was dissolved in 5 ml of water (pH 9-10). The solution then began to deposit a yellow precipitate and the pH of the mixture fell to about 6. The solid was washed with water and dried. There was obtained 0.17 g, m.p. 150-175°. The compound showed carbonyl absorption at both 1750 and 1720 cm^{-1} and no diethyl ketal absorption. It was not examined further.

2,7-Dichloro-9-keto-4-fluorenyl 2-pyridyl ketone was at last obtained by the following procedure, which followed 2 small-scale preliminary runs.

A solution of 2-pyridyllithium was prepared by adding at -50 to -70° a solution of 3.8 g (0.024 mole) of distilled 2-bromopyridine in 20 ml of anhydrous tetrahydrofuran to a mixture of 24 ml of tetrahydrofuran and 10.6 ml of 2.25M butyllithium in hexane. The reaction mixture was then allowed to stand at -50 to -55° for 1.5 hr.

The pyridyllithium solution was then added dropwise at -70° to a stirred suspension of 7.5 g (0.024 mole) of 2,7-dichlorofluorenone-4-carbonyl chloride (Annual Report No. 1, p. 62) in 140 ml of tetrahydrofuran. The now homogeneous reaction mixture was stirred overnight at about -60 to -70° and then was allowed to stand at room temperature for 2 days. The mixture was treated with 20 ml of water at about 0°, then an upper layer was separated and evaporated to dryness to give 8.9 g of crude product. The crude was extracted by boiling with 3 x 40 ml of methanol, then was dried under vacuum at 40° to give 6.87 g (81%), m.p. 185-190°. The infrared spectrum showed strong absorption at 1730 cm^{-1} , a weak absorption at

1670 cm^{-1} and broad absorption at 3300-3500 cm^{-1} . A product having an identical infrared spectrum, as obtained in an earlier run, had an analysis corresponding to a monohydrate.

Anal. Calcd for $\text{C}_{19}\text{H}_9\text{Cl}_2\text{NO}_2 \cdot \text{H}_2\text{O}$: C, 61.31; H, 2.98; Cl, 19.05; N, 3.76.
Found: C, 61.49; H, 3.39; Cl, 19.42; N, 3.73.

We were unable to hydrogenate the ketone over platinum oxide at 30-36 psi in either tetrahydrofuran (acidified with a little hydrogen chloride) or in glacial acetic acid.

A reaction with lithium aluminum hydride in refluxing tetrahydrofuran was carried out and the mixture was worked up in the usual way. The crude product showed hydroxyl absorption in the infrared, but strong carbonyl absorption was still present at 1720 cm^{-1} and 1590 cm^{-1} .

Because of the termination of the contract we were unable to continue our attempts to prepare the desired 2-piperidylmethanol (with or without concurrent reduction of the 9-keto group).

3. 2,7-Dichloro- α -(2-piperidyl)-4-fluorenemethanol

While engaged in the syntheses described in 2 above it occurred to us that the Boykin reaction^{20,21,22} might be applied directly to 2,7-dichlorofluorene-4-carboxylic acid, a supply of which had been acquired in other work (see VII, 25).

In the first run 0.835 g (3 millimoles) of 2,7-dichlorofluorene-4-carboxylic acid reacted with 7.5 millimoles of 2-pyridyllithium under the conventional conditions of the Boykin reaction. Insoluble material was present during the reaction and 0.5 g of unreacted acid was recovered, along with 0.33 g of 2-n-butylpyridine. In a second run the 2-pyridyllithium was increased to 15 millimoles, but most of the acid was recovered unchanged.

On the assumption that ether was not an appropriate solvent for this case we examined the use of tetrahydrofuran, which had been used in the phenanthrene series.²² The reaction mixture was homogeneous. The only material identified was a 75% recovery of unreacted acid.

4. 2,7-Bis(trifluoromethyl)- α -(di-n-butylaminomethyl)-9-keto-4-fluorene-methanol

5,5'-Bis(trifluoromethyl)diphenic acid was first prepared by J. L. Neumeyer (report to WRAIR) who sought to prepare the related dialdehyde. 3,6-Bis(trifluoromethyl)-9-phenanthroic acid was decarboxylated to 3,6-bis(trifluoromethyl)phenanthrene and the latter was oxidized by chromic anhydride in glacial acetic acid.

It occurred to us that the oxidation procedures used in the 1920's for the conversion of phenanthrene to phenanthrenequinone and thence to diphenic acid should be capable of converting 3,6-bis(trifluoromethyl)-9-phenanthroic acid directly to 5,5'-bis(trifluoromethyl)diphenic acid. The earlier procedures all involved isolation of the intermediate quinone because a major purification procedure was required to remove impurities derived from the 80% phenanthrene available at that time.

We chose to use a procedure based on a literature procedure for the conversion of phenanthrenequinone to diphenic acid.⁴¹ A generous supply of 3,6-bis(trifluoromethyl)-9-phenanthroic acid was obtained from WRAIR (sample AY 68207), m.p. 268-270°; lit.⁴² m.p. 272-273°.

The oxidant was a stock solution containing 147 g of potassium dichromate, 560 ml of water, and 110 ml of concentrated sulfuric acid; it contained about 0.03 g (2 milliequivalents) of available oxygen per milliliter.

30 g (0.084 mole) of 3,6-bis(trifluoromethyl)-9-phenanthroic acid was dissolved in 450 ml of glacial acetic acid and the solution was heated to reflux. The oxidant was added through the condenser as fast as the exothermic reaction permitted. By the time the theoretical volume (168 ml) had been added no continuing exotherm was detected. A total of 375 ml of oxidant was used and the mixture was allowed to reflux for a total of 5 hr. So rapid was the oxidation that it is likely that the overall reaction time can be shortened to one hour or less. An intermediate separation of reduced chromium salts was ignored.

The cooled mixture was poured onto ice. Crude product was separated and was redissolved in dilute aqueous sodium bicarbonate at room temperature. The solution was filtered to remove insoluble chromium salts. The filtrate was warmed to about 50° and was acidified to Congo Red by 6N hydrochloric acid. The colorless crystalline precipitate was washed with water and dried at 65°. We obtained 30 g (94%), m.p. 233-235°; lit. (Neumeyer) m.p. 232-234°.

Anal. Calcd for $C_{16}H_8F_6O_4$: C, 50.81; H, 2.13; Neut. Equiv., 189.1.
Found: C, 50.87; H, 2.83; Neut. Equiv., 190.

We were unable to cyclize the bis(trifluoromethyl)diphenic acid to the desired 1,6-bis(trifluoromethyl)fluorenone-4-carboxylic acid. The conventional synthesis of fluorenone-4-carboxylic acids involves heating the appropriate diphenic acid in concentrated sulfuric acid solution. Ring closure occurs at a measurable rate at 100° except in the case where strong electron attracting groups are present. Thus 5,5'-dinitrodiphenic acid does not cyclize at this temperature.⁴³ The effect of substituent groups on the ease of cyclization is of course a function of their position in the molecule; 5-nitrobiphenyl-2-carboxylic acid cyclized to 1-nitrofluorenone readily even at 100°.⁴⁴

Practical cyclization procedures for dichlorodiphenic acids involve heating the solution at 150-170° for 30 min. (Annual Report No. 1, p 61). In considering the application of such conditions to the case of 5,5'-bis(trifluoromethyl)diphenic acid we were concerned with the potential instability of the trifluoromethyl groups. It was known that trifluoromethyl groups do survive treatment with 55% sulfuric acid at 165° for 30 min., but do not survive treatment with 70% sulfuric acid at 190°.⁴⁵ Trifluoromethylisatins had been prepared in concentrated sulfuric acid solution at 90°;⁵ however, some evolution of hydrogen fluoride had been observed.

None of the following procedures studied by us to date has given the desired cyclization without significant concurrent hydrolysis of a trifluoromethyl group.

Concentrated Sulfuric Acid

A solution of 1 g of 5,5'-bis(trifluoromethyl)diphenic acid in 10 ml of concentrated sulfuric acid was warmed slowly. The first detectable brown color appeared at about 100°. At 140° bubbles were forming in the gradually darkening solution and acid fumes were forming at the neck of the flask. The solution was heated to 180°, held at that temperature for 30 min., cooled, and poured onto ice. The yellow solid that precipitated was washed with water and then was dissolved in dilute aqueous sodium bicarbonate, in which it was entirely soluble. Acidification of the solution by dilute hydrochloric acid gave 0.8 g of a pale yellow solid which melted at about 360-370° dec. The material was only slightly soluble in boiling glacial acetic acid; the portion that did dissolve separated on cooling and had the same melting point.

The high melting point and low solubility of the product was reminiscent of the behavior of terephthalic acids. This, along with our observation of acid fumes and a severe etching of the reaction flask, led to the conclusion that hydrolysis of at least one trifluoromethyl group had occurred. An infrared spectrum showed not only carboxylic acid carbonyl absorption at 1690 cm^{-1} , but also fluorenone carbonyl absorption at 1740 cm^{-1} .

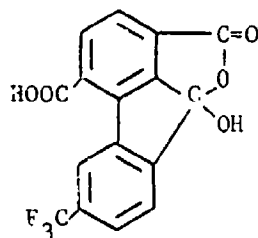
In a second run a reaction mixture was heated at just 90-100° for 4 hrs. The color deepened slowly, but once again fuming occurred and the reaction flask was etched.

In a third run the reaction flask was plunged into an oil bath preheated to 190° and held there for 5 min., then cooled rapidly. Copious fuming occurred and only high-melting material was obtained.

Methanesulfonic Acid

0.3 g of 5,5'-bis(trifluoromethyl)diphenic acid was dissolved in 5 ml of methanesulfonic acid (Eastman 6320) and the solution was heated at 155° for 3 hr. Color was first noticed at about 130°. There was pronounced fuming and the flask was later observed to be etched. The reaction mixture was poured on ice and the solid that separated was dissolved in dilute

aqueous sodium bicarbonate at room temperature. Acidification with hydrochloric acid gave 0.3 g of a pale yellow solid, m.p. 270-355° dec. Recrystallization of the material from hot glacial acetic acid gave three fractions, all of which were high melting; one fraction was colorless. Elemental analyses clearly showed loss of fluorine. The infrared spectrum of the colorless fraction (m.p. 370°) showed free hydroxyl absorption at 355 cm^{-1} and lactol carbonyl absorption at 1750 cm^{-1} . These absorptions suggested that the substance in hand had the following structure in which one trifluoromethyl



group had been hydrolysed to carboxyl.

Polyphosphoric Acid

A review of the use of polyphosphoric acid as a condensing agent⁴⁶ and an extensive description of the cyclization of substituted diphenyl ether carboxylic acids at 100° to xanthenes⁴⁷ cited no example in which trifluoromethyl groups were involved. The mechanism of the xanthone synthesis reaction is known to be identical with that of the fluorenone synthesis from diphenic acid.

1 g of 5,5'-bis(trifluoromethyl)diphenic acid proved to be so insoluble in polyphosphoric acid (M, C and B, P8096) that it was necessary to stir it with 30 g at 180° for 7 hr before all dissolved. During this time the color of the solution became a dark red-brown, indicative of fluorenone formation. Fuming was observed by the time the mixture had reached 180°.

The mixture was worked up as described above and gave 0.6 g of a yellow solid, m.p. >360°, which was essentially insoluble in boiling glacial acetic acid. The portion remaining insoluble in 100 ml of boiling glacial acetic acid weighed 0.3 g and had m.p. 365-375° dec. The only material obtained by boiling down the filtrate was also high melting.

We concluded that polyphosphoric acid was not suitable as a cyclizing agent.

Phosphorous Oxychloride

This reagent had been used in the xanthone syntheses mentioned above.⁴⁷ When a solution of 5,5'-bis(trifluoromethyl)diphenic acid was refluxed it clouded rapidly and a crystalline solid, m.p. 238-242° (sublimed), separated. No color developed. We assumed that anhydride formation had occurred.

Fluosulfonic Acid

1 g of 5,5'-bis(trifluoromethyl)diphenic acid was dissolved in 20 ml of fluosulfonic acid (B & A 1071) and the colorless solution was allowed to stand in a sealed flask at room temperature; no color developed during 24 hr. The solution was then heated gradually to 135° and held there for 90 min.; color developed at 90-100° and gradually deepened. The reaction mixture was carefully poured onto ice and the bright yellow solid that separated was then reprecipitated from aqueous bicarbonate solution to give 0.75 g, m.p. 355-370° dec. The solid seemed to become much lighter in color above 250° and a similar phenomenon was observed when the solid was heated on a spatula.

The entire solid was boiled with 80 ml of glacial acetic acid, in which it was partly soluble. That portion that separated from the solution on cooling had the same melting point and left a colorless filtrate. The infrared spectrum showed carboxylic acid carbonyl absorption at 1695 cm^{-1} and fluorenone carbonyl absorption at 1740 cm^{-1} . An elemental analysis showed no fluorine and carbon-hydrogen values corresponding to $\text{C}_{16}\text{H}_8\text{O}_7$. The material may well be fluorenone-2,6-tricarboxylic acid.

Anhydrous Oxalic Acid

The reaction of this agent with the acid chlorides of diphenyl ether dicarboxylic acid⁴⁷ give xanthone carboxylic acids is known.⁴⁷ 1 g of 5,5'-bis(trifluoromethyl)diphenic acid was converted to the acid chloride by means of thionyl chloride. The crude chloride was dissolved in 20 ml of tetrachloroethane, 0.5 g of anhydrous oxalic acid was added,

and the mixture was refluxed for 3 hr. No significant color developed. The mixture was allowed to stand overnight at room temperature and deposited crystals of 5,5'-bis(trifluoromethyl)diphenic acid.

Hexamethylphosphoramide (HMPT)

Among the various reactions of this reagent⁴⁸ are dehydration reactions that bear an admittedly distant relation to the present one. When a solution of 5,5'-bis(trifluoromethyl)diphenic acid in HMPT was heated no color was observed below 140°. The solution was held at 175° for 1 hr, then cooled and diluted with water. A viscous oil was formed, but only a portion dissolved in dilute aqueous bicarbonate solution. We were not encouraged to continue this work because of the lack of color so characteristic of fluorenones.

Aluminum Chloride

5,5'-Bis(trifluoromethyl)diphenic acid was not very soluble in either carbon disulfide or methylene chloride. When aluminum chloride was stirred into a suspension of the acid in carbon disulfide, an orange color developed and hydrogen chloride was evolved. After 24 hr. stirring at room temperature the reddish crystalline solid present was filtered off and worked up in the usual way to give a quantitative recovery of starting material containing a small amount of higher-melting solid.

The Friedel-Crafts reaction was also carried out in nitrobenzene solution for 2 hr. at room temperature. No ring closure occurred; the colorless acid was recovered.

5. Other Bis(trifluoromethyl)fluorenemethanols

Because of our continuing inability to cyclize 5,5'-bis(trifluoromethyl)diphenic acid to a fluorenone (see 4 above) we began the syntheses of small amounts of other bis(trifluoromethyl)diphenic acids in order to determine whether failure to cyclize to a fluorenone was typical of the entire class.

3-(Trifluoromethyl)isonitrosoacetanilide was prepared by a literature procedure.^{5,49}

A mixture of 80.5 g (0.5 mole) of 3-aminobenzotrifluoride (Marshallton Res. Lab. 30132), 300 ml of water and 51.2 g (43 ml; 0.52 mole) of concentrated hydrochloric acid was added with stirring to a mixture of 90 g (0.54 mole) of chloral hydrate (MC&B, 5172), 1200 ml of water and 1300 g of sodium sulfate decahydrate. To this mixture was then added a solution of 110 g (1.58 mole) of hydroxylamine hydrochloride in 500 ml of water. The mixture was then heated at reflux for 30 min., while an insoluble oil separated. The mixture was cooled and filtered to give a brown solid, m.p. 115-135°, which, after crystallization from benzene, gave 78 g (67%), m.p. 139-140°; lit.⁴⁹ 83%, m.p. 140°.

In a second run, where each and every quantity of water was doubled⁴⁹ we obtained 88 g (75%), m.p. 139-140°.

4-(Trifluoromethyl)isatin

78 g (0.336 mole) of 3-(trifluoromethyl)isonitrosoacetanilide was stirred into 240 ml of concentrated sulfuric acid at 50-60°. The solution was heated to 90-95°, held at that temperature for 30 min., cooled, and poured onto ice. The crude product was washed with water and dried at 70° to give 32 g, m.p. 160-180°. Recrystallization from 60 ml of glacial acetic acid gave only 6.4 g (9%) of dark crystals, m.p. 221-227° dec.; lit.⁵ 224° dec. Another 3 g, m.p. 210-234°, was obtained by letting the filtrate stand. Total yield was 9.4 g (13%). By dilution of the acetic acid mother liquors with water material, m.p. 128-185°, was recovered. The run was repeated on a 10-g scale and gave a similar low yield; vapors of hydrogen fluoride were observed during the heating period, as noted previously.⁵

Two 5-g runs were carried out in which heating was at just 80° for just 10 min. or 30 min., respectively. An 8% yield of product, m.p. 229° dec., was obtained in both cases. The material that precipitated when water was added to the acetic acid filtrate had m.p. 129-132° and an infrared spectrum identical with that of uncyclized starting material.

4-(Trifluoromethyl)isonitrosoacetanilide was prepared by a literature procedure⁵ when it was obvious that the conversion of the 3-isomer to the derived isatin was unusually poor. 19 g of anhydrous sodium

sulfate was added to a solution of 9 g (0.054 mole) of chloral hydrate in 125 ml of water. A solution of 8.05 g (0.05 mole) of p-aminobenzotrifluoride (Marshallton) in 110 ml of water and 6.3 ml of concentrated hydrochloric acid was then added. A thick precipitate formed. A solution of 11 g (0.158 mole) of hydroxylamine hydrochloride in 50 ml was added and the mixture was boiled for 10 min. The mixture was cooled in ice and a lower orange-colored layer solidified. The solid was separated and dissolved in a small volume of hot methyl alcohol. The solution was poured into 250 ml of cold water to give a solid which, after washing on the filter with water and drying, was 6.6 g, m.p. 102-142°. The crude was recrystallized from 35 ml of chloroform to give 3.8 g, m.p. 140-145°; lit.⁵ 148.5°.

IX. PHARMACOLOGY

This section includes all data reported to us by WRAIR prior to October 1, 1972. The data for some of the compounds in Table I were published previously in our Annual Report No. 1.

Evaluation of compounds as antimalarials in mice and chicks (Table I) was carried out in screens operated for WRAIR at the University of Miami.⁵⁰

During the second contract year 4 aminobenzothiazoles were submitted for evaluation as causal prophylactic drugs. Test data for these and for 2 fluorenemethanols are reported in Table II. The data were obtained by L. Rane at the University of Miami (S-Bird Test) and were provided by WRAIR. The test was designed to detect true causal prophylactic drugs, that is, those that affect primary exoerythrocytic parasites; most, but not all, suppressive drugs are inactive.

White Leghorn cockerels (53-57 g) are parasitized by intrajugular injection of 0.5 ml of a suspension of *Plasmodium gallinaceum* sporozoites. The sporozoite preparation is a filtered suspension of *Aedes aegypti* mosquitoes infected 9-11 days earlier by feeding on infected donor chicks. Test drugs are suspended in peanut oil and administered subcutaneously on the day of infection in a single dose. Each drug is routinely tested in 5 chicks at each of 3 dose levels.

Control chicks die between 6-11 days, with a mean survival time of 8.5 days. A drug is considered active if the mean survival time is twice as long as that of the control group, or if any chicks survive to 30 days. Deaths before day 6 are considered to be the result of drug toxicity. Sulfadiazine, a positive control drug included in each experiment, produces almost 100% survivals.

Drug activity detected in the S-Bird screen must be confirmed by subsequent testing in other species.

TABLE I. ANTIMALARIAL ACTIVITY OF COMPOUNDS SUBMITTED

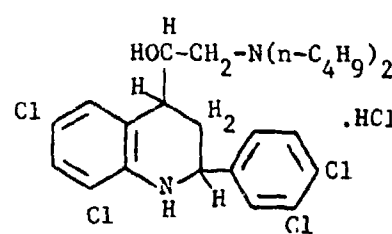
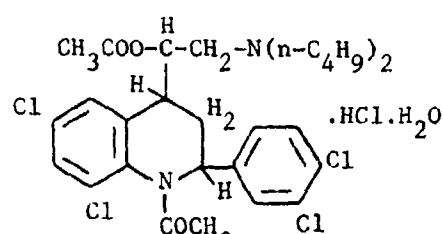
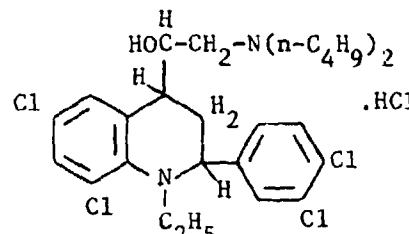
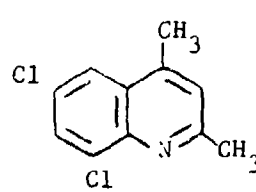
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AV 40722 ADL 15878-29A	1, p 26	10 20 40 80 160 320 640	1.8 3.8 8.8 Active 1 Cure 3 Cures 5 Cures 5 Cures		
 AX 46108 ADL 15878-46	1, p 28	640	0.3	160	0.0
 AX 46091 ADL 15878-47	1, p 30	10 20 40 80 160 320 640	0.3 0.5 2.9 8.9 Active 15.9 Active 3 Cures 5 Cures	120	0.0
 AX 63163 ADL 16771-2	1, p 33	640	0.9	120	0.0

TABLE I (CONT.)

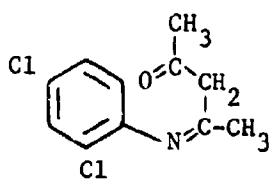
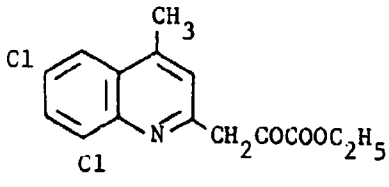
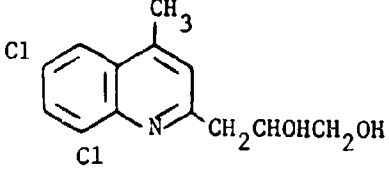
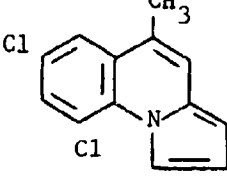
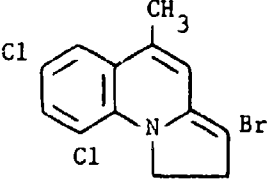
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AX 66324 ADL 16771-5	1, p 35				
 AX 66315 ADL 16851-8A	1, p 37	640	0.5	160	0.0
 AY 60667 ADL 16851-39-2	1, p 39	640	0.1		
 AY 60676 ADL 16851-40-3	1, p. 41	640	0.3		
 AY 67906 ADL 16851-79	1, p 43	640	0.3	120	0.0

TABLE I (CONT.)

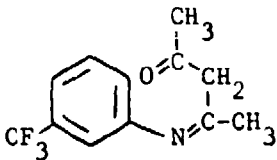
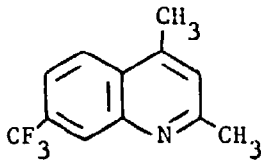
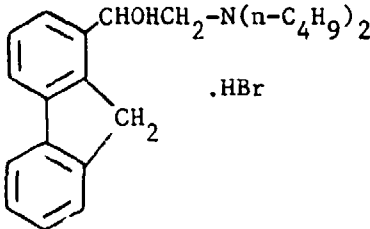
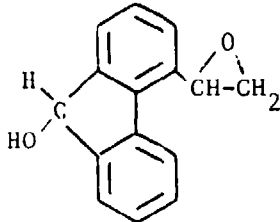
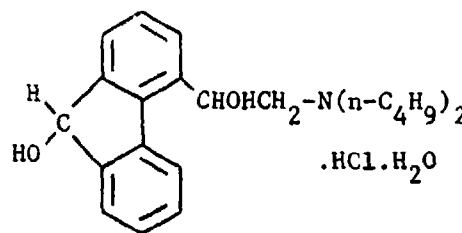
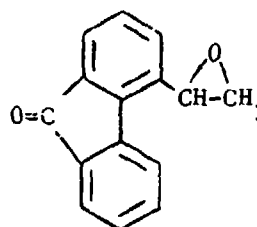
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AX 66333 ADL 16771-7	1, p 45	20	0.3		
		40	0.3		
		80	0.3		
		160	0.5		
		320	4.9		
		640	12.1	Active	
 AX 66342 ADL 16771-23	1, p 47	640	1.1	120	0.0
 AX 63136 ADL 16764-28A	1, p 49	640	0.5	320	0.0
 AX 63145 ADL 16764-6	1, p 52	640	0.1	100	0.0

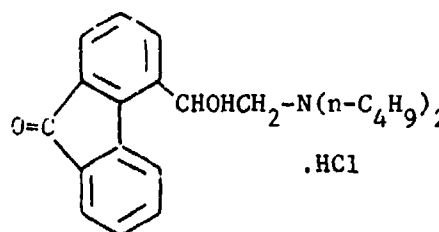
TABLE I (CONT.)

Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 <chem>CCN(CC)CC(O)c1ccccc1-c2ccccc2C1=CC=CC=C1</chem> .HCl.H ₂ O	1, p 54	640	0.3	160	1.4
				320	1.8

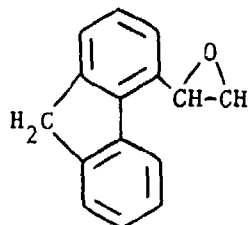
AX 63118
ADL 16764-11C

 <chem>O=C1C(=Cc2ccccc2-c3ccccc3C1=CC=CC=C1)CCOC</chem>	1, p 55	640	0.5	120	0.0

AX 63154
ADL 16764-26

 <chem>CCN(CC)CC(O)c1ccccc1-c2ccccc2C1=CC=CC=C1C(=O)c3ccccc3</chem> .HCl	1, p 56	640	0.7	320	0.0

AX 63127
ADL 16764-26B

 <chem>O=C1C(=Cc2ccccc2-c3ccccc3C1=CC=CC=C1)CCOC</chem>	1, p 57				

AX 64688
ADL 16764-40A

TABLE I (CONT.)

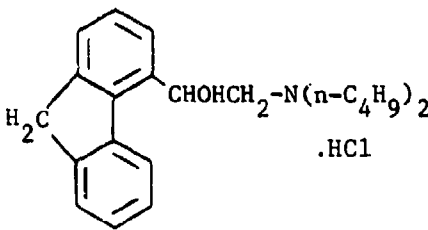
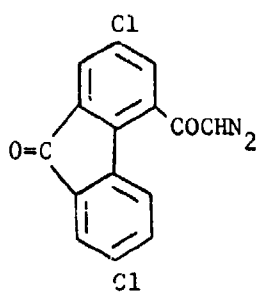
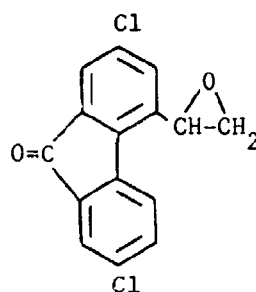
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AX 64679 ADL 16764-42A	1, p 59				
 AX 68079 ADL 16851-16-2A	1, p 60	640	0.1	120	0.0
 AX 68060 ADL 16851-28-1	1, p 64	640	0.3	160	0.0

TABLE I (CONT.)

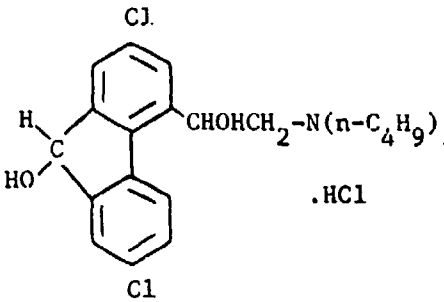
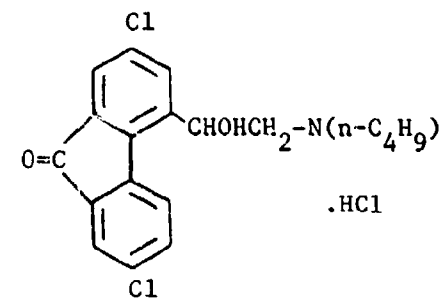
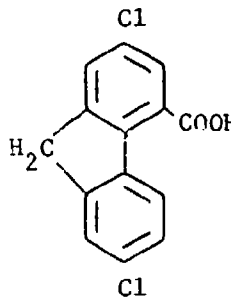
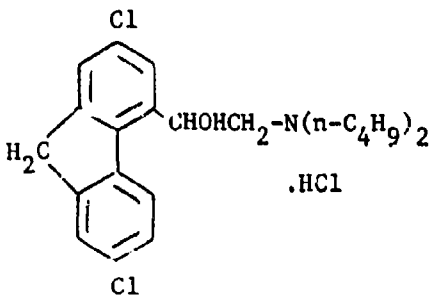
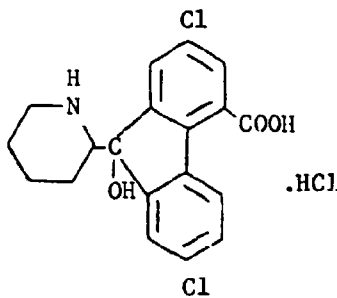
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AY 60658 ADL 16851-45-1	1, p 67	160 320 640	7.3 Active 11.3 Active 15.1 Active	320	0.0
 AX 68051 ADL 16851-30	1, p 69	10 20 40 80 160 320	5.1 8.1 Active 10.5 Active 12.9 Active 2 Cures 5 Cures	160	0.0
 AY 98456 ADL 17311-2	2, p 32	640	0.3		

TABLE I (CONT.)

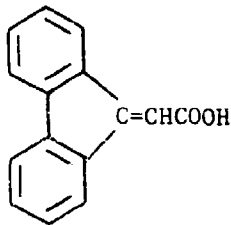
Synthesis in Annual Report No.	Mouse		Chick	
	Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time



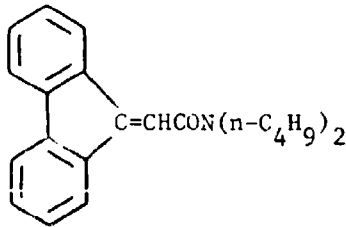
AY 98447
ADL 17254-41



BB 40753
ADL 17520-17-2



AY 67915
ADL 16851-60



AY 67899
ADL 16851-67-2

TABLE I (CONT.)

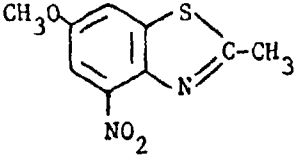
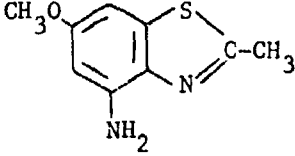
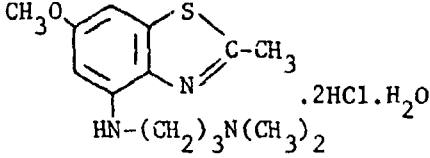
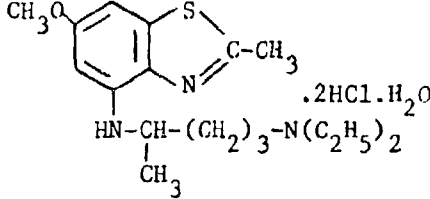
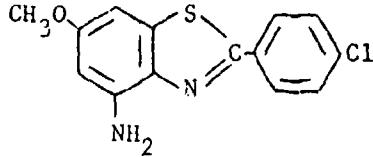
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 AY 98401 ADL 16851-99-1	2, p 39	640	0.3	100	0.0
 AY 98410 ADL 16851-103B-1	2, p 41	640	0.5	100	0.0
 BB 05889 ADL 16851-134-3	2, p 43	640	0.3	160	0.0
 AY 98429 ADL 17254-30-5	2, p 46				
 AY 98438 ADL 17254-52-1	2, p 49	640	0.1		

TABLE I (CONT.)

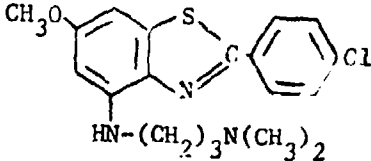
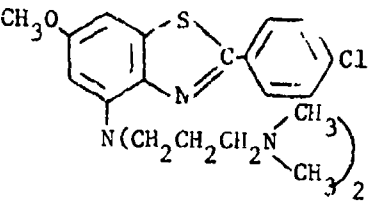
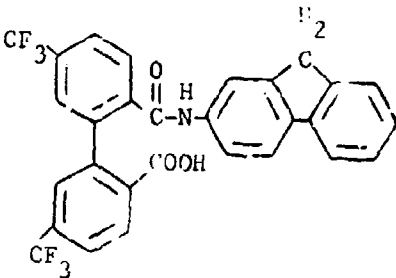
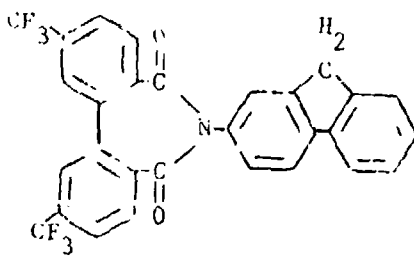
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 CH ₃ O- HN-(CH ₂) ₃ N(CH ₃) ₂	2, p 52	640	0.1		
BB 43021 ADL 17520-52					
 CH ₃ O- N(CH ₂ CH ₂ CH ₂ N(CH ₃) ₂)	2, p 54				
BB 42104 ADL 17520-40A					
 CF ₃ H ₂ COOH	2, p 56	640	0.1		
BB 40146 ADL 17311-6					
 CF ₃ H ₂	2, p 57	640	0.3		
BB 40155 ADL 17311-6A					

TABLE I (CONT.)

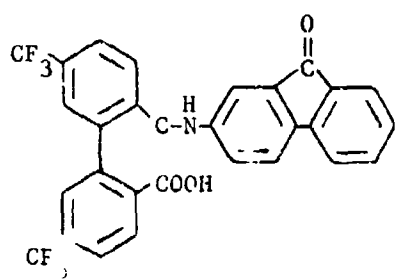
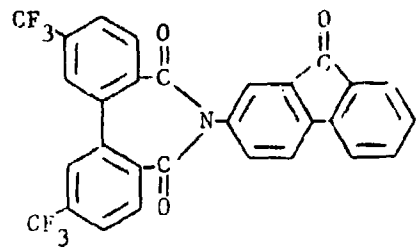
Compound	Synthesis in Annual Report No.	Mouse		Chick	
		Dose mg/kg	Increase in Mean Survival Time	Dose mg/kg	Increase in Mean Survival Time
 BB 40164 ADL 17311-7A	2, p 58	640	0.3		
 BB 40173 ADL 17311-8	2, p 59	640	0.3		

TABLE II. CAUSAL PROPHYLACTIC ACTIVITY (S-BIRD)

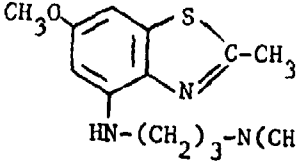
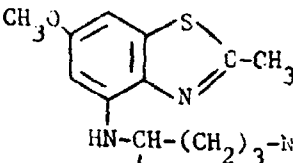
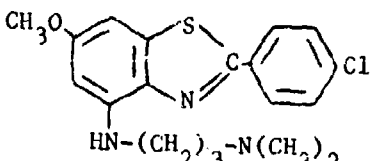
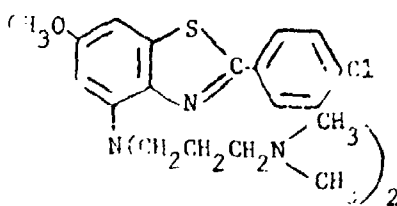
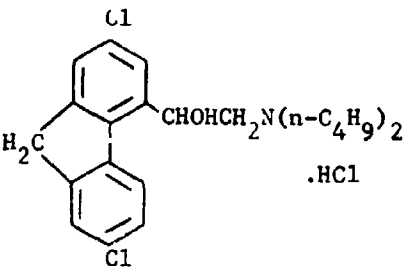
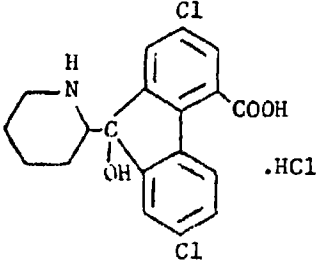
Compound	Synthesis in Annual Report No.	Dose mg/kg	Increase in Mean Survival Time
 <chem>COc1ccc2c(c1)nc(C)c(s2)NCCCN(C)C</chem> .2HCl.H ₂ O	2, p 43		
BB 05889 ADL 16851-134-3			
 <chem>COc1ccc2c(c1)nc(C)c(s2)NCCCN(C)C</chem> .2HCl.H ₂ O	2, p 46	10 20 40 80 160 320	5.9 3.5 6.3 4.4 Toxic Toxic Toxic
AY 98429 ADL 17254-30-5			
 <chem>COc1ccc2c(c1)nc(Cl)c(s2)NCCCN(C)C</chem>	2, p 52		
BB 43021 ADL 17520-52			
 <chem>COc1ccc2c(c1)nc(Cl)c(s2)NCCCN(C)C</chem>	2, p 54		
BB 4. ADL 1752 10A			

TABLE II (CONT.)

<u>Compound</u>	<u>Synthesis in Annual Report No.</u>	<u>Dose mg/kg</u>	<u>Increase in Mean Survival Time</u>
 AY 98447 ADL 17254-41	2, p 34	10 40 160 40	4 Cures 3 Cures 4 Cures 4.3 (repeat)
 BB 40753 ADL 17520-17-2	2, p 37	120	0.3

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