THE TOTAL SYNTHESIS OF (±)-ASPIROCHLORINE

Submitted by

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WE HEREBY RECOMMEND THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY GREGORY F. MIKNIS ENTITLED "THE TOTAL SYNTHESIS OF (±)- ASPIROCHLORINE" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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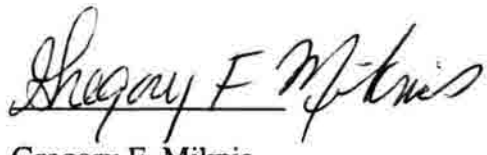
ABSTRACT OF DISSERTATION

THE TOTAL SYNTHESIS OF (±)-ASPIROCHLORINE

Aspirochlorine is a unique epidithiodioxopiperazine isolated from *Aspergillus oryzae*, *Asp. tamarii* and *Asp. flavus*. The molecule contains a highly unusual bicyclo [3.2.2]disulfide ring system which has previously never been prepared.

The first total synthesis of (±)-Aspirochlorine was achieved from commercially available 5-chlororesorcinol **324** in 16 steps. The key step in the synthesis was an efficient intramolecular cycloaddition reaction of hydroxamic ester **344** to form the parent spiro [benzofuran-2(3H),2'-piperazine] ring system **345** as a single stereoisomer. In addition the synthesis employed a 2-nitrobenzyl moiety as a novel amide protecting group. The 2-nitrobenzyl group could be removed in 72% yield under photolytic conditions. Synthetic aspirochlorine was identical to natural material in comparison by ^{1}H NMR, IR and HPLC.

Comparison of the biological activity of aspirochlorine versus other epidithiodioxopiperazines was investigated as a function of superoxide production. Although aspirochlorine was shown to be capable of producing superoxide as evidenced in DNA plasmid nicking and NBT reduction assays, the observed activity was less than the 6-membered epidithiodioxopiperazines.



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The author would like to extend a special token of gratitude to present and past members of the R.M. Williams' research group. This group of highly motivated individuals provided many hours of stimulating conversation (not necessarily about chemistry). Members of the group provided friendship, and encouragement in times of distress. Special thanks to Rena Gallegos for her unremitting patience and assistance in tutoring the author through a "crash course" in microbiology. In addition, the author would like to extend personal gratitude to Mark Flannigan and Tom Gant for the much needed excursions up the Poudre Canyon during frustrating times.

The author would like to thank Dr. Williams for providing laboratory space, chemicals, equipment and financial support. More importantly, Dr. Williams provided the author freedom to pursue his own intellectual interests. The hands-off approach taken by Dr. Williams provided an invaluable opportunity for professional growth.

Lastly, the author would like to express his appreciation for the support of his parents and family throughout life. Their never-ending support, encouragement and confidence is gratefully and lovingly appreciated.

Autobiographical Sketch

Gregory Francis Miknis was born in Laramie, Wyoming on November 2, 1963, and is one of four children. Being the son of a chemist and a nurse promoted the author's interest in the biological sciences from an early age. Greg was raised in Laramie and graduated from Laramie Senior High School in 1982. A native Wyoming resident, he enjoys outdoor activities and is an avid flyfishing enthusiast.

The author attended the University of Wyoming from 1982-1987, obtaining a B.S. in chemistry in the spring of 1987. During his undergraduate tenure, he married his wife Lisa on August 16, 1986.

At the University of Wyoming Greg was actively involved in undergraduate research programs with Dr. David A. Nelson and Dr. Bruce Barner. Greg also enjoyed working as a teaching assistant for the undergraduate chemistry labs. As a result of his efforts, Greg was the recipient of the Rebecca Raulins Research Award and the Senior Award for Academic Excellence from the University of Wyoming Department of Chemistry.

Greg attended graduate school at Colorado State University specializing in organic synthesis under the guidance of Dr. Robert M. Williams. During his graduate career, Greg was the recipient of both a teaching award and the Syntex Fellowship Award for excellence in organic chemistry. Greg completed the first total synthesis of (±)-Aspirochlorine in 1992. More significantly during his graduate studies, his daughter Ashley Nicole was born March 21, 1991.

Greg was offered both the National Institute of Health (NIH) and American Cancer Society (ACS) postdoctoral fellowships. He accepted the ACS fellowship to be conducted at the University of Texas at Austin with Professor Philip Magnus.

Dedication

This dissertation is dedicated to my wife Lisa. Her companionship, patience and encouraging words were, and continue to be, an invaluable asset. Without her love and support, this thesis may not have been possible.

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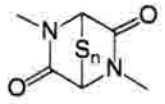
Chapter 1
Epipolythiodioxopiperazines

The epipolythiodioxopiperazines (ETP) are a unique class of fungal metabolites characterized by a sulfur-bridged diketopiperazine moiety **1**. Since the discovery in 1932 of gliotoxin,[1] almost twenty distinct families of metabolites have been characterized.



1, n=1,2,3,4

2

The production of epipolythiodioxopiperazines is widespread among fungi and it is common for a particular compound to be isolated from a variety of different sources. There is strong evidence suggesting production of these metabolites occurs in response to natural stimuli. The disulfide form **1**(n=2) is the most prevalent, although it is often accompanied by epitrisulfides **1**(n=3), epitetrasulfides **1**(n=4), and the corresponding reduced bis(methylthio) analogs **2.** The presence of the derivatives suggests a possible biosynthetic relationship between the various congeners.

Various aspects of epipolythiodioxopiperazines including production/isolation, biosynthesis, chemical reactivity, and biological properties have been extensively reviewed.[2] Epipolythiodioxopiperazines in general possess a variety of physiological properties and several compounds have been implicated in several animal and/or plant diseases. In most cases the disulfide bridge is required for the biological activity; reduction to the dithiol or conversion to the bis(methylthio) derivative usually eliminates or diminishes activity.[3,36,34b] Epipolythiodioxopiperazines are also characterized by high mammalian toxicity which has precluded their use in clinical or commercial applications.

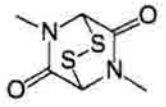
Recent evidence suggesting epipolythiodioxopiperazines exhibit immunomodulating properties has renewed interest in this class of fungal metabolites.

The material in this chapter is divided into three sections. The first section is intended to provide a general overview of the physical/chemical properties common to all epidithiodioxopiperazines. The second section introduces the structures of naturally occurring epipolythiodioxopiperazines and related metabolites which have been characterized. Lastly, the third section is devoted to a discussion of the more interesting biosyntheses of several of the metabolites.

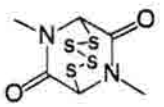
Physical/chemical Properties of epipolythiodioxopiperazines

The epipolythiodioxopiperazine ring system possesses a variety of physical properties which ultimately control the chemical reactivity of this unusual ring system. The disulfide bridge is highly strained and is easily reduced with mild reducing agents such as sodium borohydride. The strain inherent in the disulfides is a direct consequence of the small CSSC dihedral angles found in the epidithiodioxopiperazines compared to CSSC dihedral angles found in acyclic disulfides. Normal dihedral angles of 74-105° are often observed in acyclic disulfides,[4] whereas the CSSC dihedral angles present in the epidithiodioxopiperazines range from 8-18°.[2d] Analysis of X-ray structures indicate the S-S bonds in the epidithiodioxopiperazines are longer (2.059 vs 2.03-2.05 Å) than in acyclic disulfides.[2d]

Analysis of the X-ray structures of the epidithiodioxopiperazines also indicates the



1, n=2



1, n=4

disulfide bridge adopts a helical twist. Instead of lying directly above the diketopiperazine ring, the sulfur atoms are spatially closer to adjacent carbonyl carbon atoms of the adjacent amides than to the nitrogen atoms. This geometry is attributed to non-bonded interactions between sulfur and the amide nitrogens.[5] In the related tetrasulfide case, the inner sulfur atoms lie in closer proximity to nitrogen, while the outer sulfur atoms bonded to the diketopiperazine ring are positioned closer to the carbon of the carbonyl.

Analysis of epipolythiodioxoiperazines using circular dichroism revealed an important discovery which has become an invaluable tool in the identification of unknown compounds.[6] The C.D. spectra of epidithiodioxopiperazines are quite complex although bands at 340, 310, 270 and 235 nm are generally present. The sign of the Cotton effect at 270 nm has been shown to correlate with the absolute configuration about the epidithiodioxopiperazine ring. Compounds which exhibit a negative Cotton effect are assumed to contain the (3S,6S) absolute stereochemistry about the bridgehead positions and compounds with a positive Cotton effect are assigned the (3R,6R) configuration about the bridgehead carbons.

The epidithiodioxopiperazine ring undergoes a variety of general reactions and numerous examples of these reactions are presented in the following section describing the natural products. As mentioned earlier, the disulfide group is easily reduced and the intermediate dithiols readily alkylated with methyl iodide. The sulfur bridge can be removed completely upon reaction with aluminum amalgam or Raney nickel, and depending on the substrate, hydrogenation may proceed with retention of configuration.

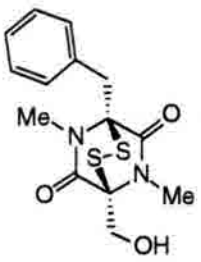
An unusual reaction characteristic of the epidithiodioxopiperazines is the interconversion of various sulfur homologs. Treatment of disulfides with polysulfur reagents such as H_2S_2 or elemental sulfur often leads to formation of tetrasulfides and is postulated to occur via branched sulfur chains. Treatment of tetrasulfides or trisulfides with triphenyl phosphine results in desulfurization to give disulfides. Reaction of

disulfides with triphenylphosphine leads to the formation of the monosulfides with inversion of configuration.

Naturally Occurring Epipolythiodioxopiperazine and Derivatives

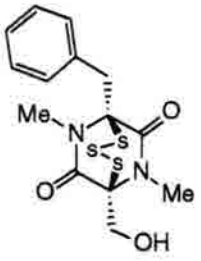
The simplest epipolythiodioxopiperazines characterized are the Hyalodendrins (**3-6**). The structures of the hyalodendrins and the related compounds is shown in Figure 1.

Figure 1. Structure of the Hyalodendrins



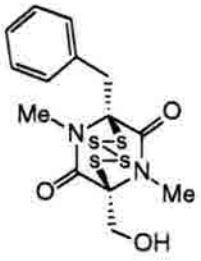
Hyalodendrin

3



Hyalodendrin-S_3

4



Hyalodendrin-S_4

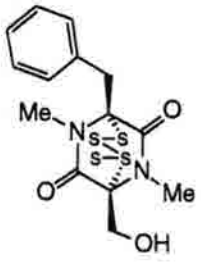
5

bisdethiodi(methylthio)-hyalodendrin

6

A26771A

7



A26771C

8

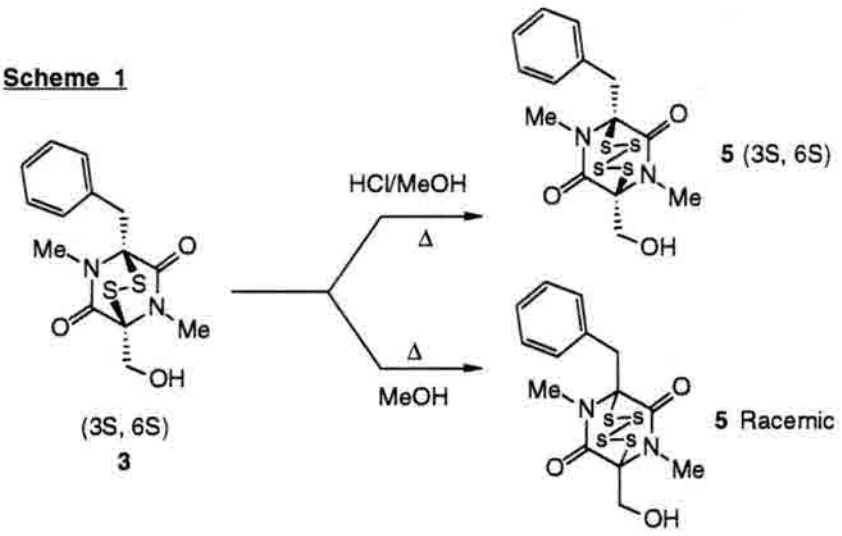
Gliovictin (A26771E)

9

Hyalodendrin (**3**) was originally isolated from a species of imperfect fungus *Hyalodendron sp.* by Strunz in 1973 and contains the (3S,6S) stereochemistry about the bridgehead positions.[7] The bis(methylthio) derivative **6** and epitetrasulfide **5** were also subsequently isolated from *Hyalodendron sp.*[8] Hyalodendrin, bis(methylthio)-hyalodendrin and epitrithiohyalodendrin (**4**) were also reported from an unidentified fungus (NRRL 3888) by DeVault and Rosenbrook.[9]

During chemical manipulations, hyalodendrin underwent an unusual dis-proportionation reaction upon heating in methanol and the results are shown in Scheme 1.

Scheme 1



In the presence of HCl, **3** generated the epitetrasulfide **5** in modest yield with retention of configuration and the tetrasulfide was identical to the naturally occurring material. In the absence of the acid catalyst, **3** generated the same tetrasulfide **5** in racemic form.

The epimer of hyalodendrin (A26771A) (**7**) was isolated from *P. turbatum* by researchers at Lilly contains the (3R,6R) absolute stereochemistry and the structure was elucidated by x-ray analysis.[10] The bis(methylthio)dioxopiperazine, gliovictin (A26771E) (**9**), and tetrasulfide (A26771C) (**8**) were also identified from the same source. Gliovictin has also been isolated from *Helminthosporium victoriae* and is identical to A26771E.[11]

Gliovictin has also been identified from the marine deuteromycete *Asteromyces cruciatus* by Fenical in 1987.[12]

Hyalodendrin displays antimicrobial activity and was superior to cryptosporiospin, nystatin, and scytalidin in inhibiting fungi which cause plant/tree diseases. In addition, hyalodendrin demonstrated favorable activity against *C. ulmi*, the causative agent of Dutch Elm Disease.[13]

Figure 2.

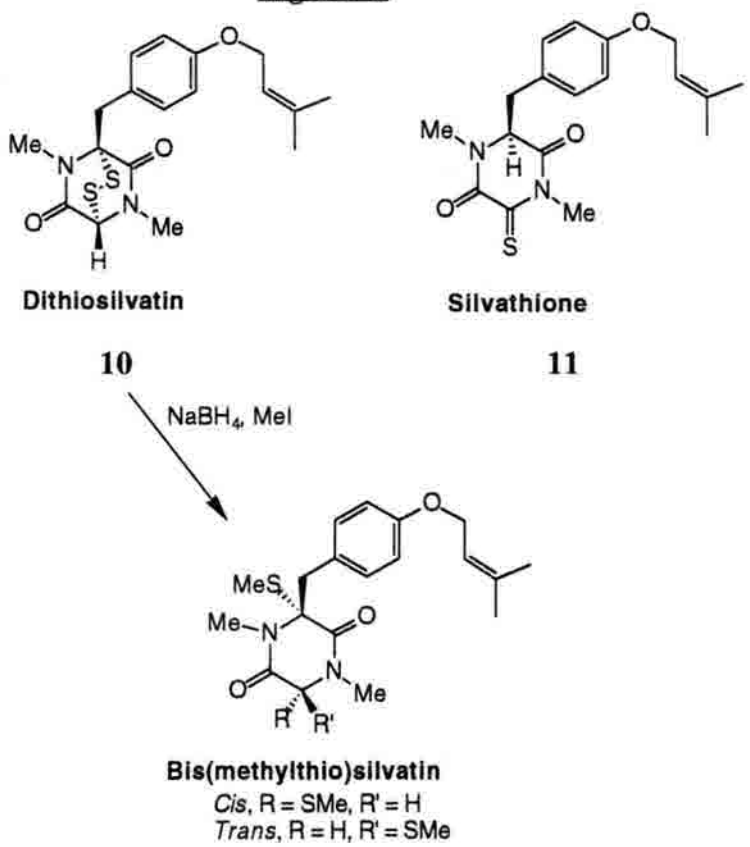
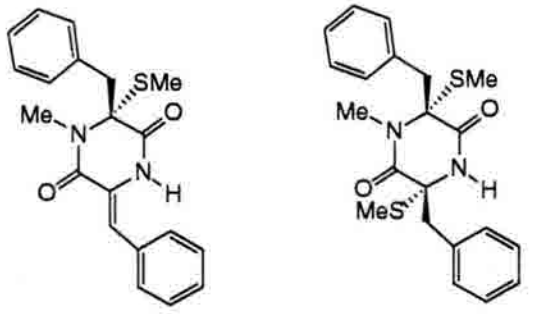


Figure 2 shows the structures of dithiosilvatin (**10**) and silvathione (**11**) which were isolated from *Asp. silvaticus* in 1987 by Kawai and contain isoprenylated tryrosine residues.[14] Silvathione is a rare example of a dioxopiperazinethione and is considered to be a precursor in the formation of trioxopiperazines from epidithiodioxopiperazines. The structures of both **10** and **11** are based on spectroscopic and chemical evidence. Reductive

methylation of **10** afforded a mixture of *cis* and *trans* bis(methylthio)silvatin **12**. *Cis*-bis(methylthio)silvatin was shown to be identical to a metabolite isolated from *G. deliquescens* by Hanson and O'Leary.[15] No data is available on biological activity.

Figure 3. Structure of Emethacins



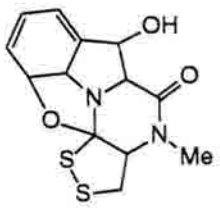
Emethacin A — **13**

Emethacin B — **14**

The most recent examples of the simple sulfur-containing dioxopiperazines are Emethacins A,B isolated from *Emericella heterothallica* (ATCC 16824) and are shown in figure 3.[16] Emethacin A (**13**) is the first example of a naturally occurring mono(methylthio)dioxopiperazine to be isolated from a fungal source. Emethacin B (**14**) was found to be identical to (3R,6R) bis(methylthio)dioxopiperazine **32** isolated from *Asp. terrus* by Kirby in 1983.[17] The structure of emethacin A was confirmed through synthesis from **14** by refluxing in a solution of aqueous acetone and sodium carbonate.

Gliotoxin and related metabolites are shown in Figure 4. Gliotoxin (**16**) was first isolated by Weidling in 1932 from *Trichoderma lignosum* and continues to generate the most interest. The structure was originally believed to involve the pentacyclic ring system shown as **15**, but revised to an epidithiodiketopiperazine moiety in 1966 when an x-ray structure confirmed the structure proposed by Woodward a few years earlier. [18,19]

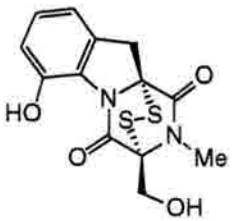
Figure 4. Structures of Gliotoxins



15

R= H, **Gliotoxin**
R= Ac, **Gliotoxin acetate**

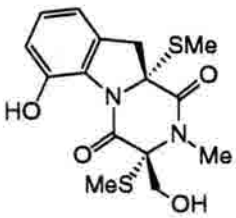
16, 17



Dehydrogliotoxin

Bisdethiodi(methylthio)gliotoxin

18



Bisdethiodi(methylthio)-dehydrogliotoxin

19

Gliotoxin E

20

Gliotoxin G

21

22

Gliotoxin has been identified in cultures of *Gliocladium fimbriatum*,[20] *Tricoderma verde*,[21] *Gliocladium deliquescens*,[22] *Aspergillus fumigatus*,[23] *Aspergillus terrus*,[24] *Penicillium obscurum*,[25] *Penicillium cinerascens*,[26] *Penicillium terlikowskii.*,[27] and

recently from *Thermoascus crustaceus*.[28] The related metabolite, gliotoxin acetate (**17**) was isolated from *P. obscurum* [29]and *P. terlikowskii*.[27]

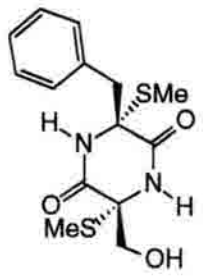
Gliotoxin displays a broad spectrum of biological activities which have been recently reviewed.[3g] To summarize, gliotoxin possesses potent antiviral/antifungal and antimicrobial properties,[30] inhibits protein/DNA synthesis[31] as well as viral RNA-dependent RNA polymerase.[32] Identification of gliotoxin and related metabolites from the pathogenic fungus *A. fumigatus* and the thermophilic fungus *T. crustaceus* has lead to inferences regarding the role of epipolythiodioxopiperazines in diseases (including an interesting speculative role in AIDS).[33]

Recent reports demonstrated gliotoxin displays immunomodulating properties *in vitro*,[34] oxidatively damages cellular, plasmid and genomic DNA,[35] inhibits platelet aggregation,[36] induces apoptosis in macrophages,[37] exhibits antiphagocytic activity *in vitro*,[38] and exhibited promising activity in the treatment of Graft-versus-host disease.[39]

Dehydrogliotoxin (**18**) was isolated from *P. terlikowskii*[40] and recently from *Gliocladium virens* .[41] In an extensive examination of the metabolites of *G. virens* using [^{35}S]sulphate as a tracer, Kirby et al. identified gliotoxin (**16**) along with trace amounts of bisdethiobis(methylthio)gliotoxin (**19**), bisdethiobis(methylthio)dehydrogliotoxin (**20**), and the novel trisulfide gliotoxin E (**21**).

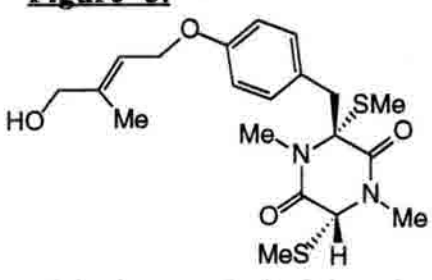
The novel sulfur containing diketopiperazines shown in figure 5 were also detected

Figure 5.



Bis-N-norgliovictin

23



3-hydroxymethylbut-2-enyl ether

24

from the [^{35}S] study carried out by Kirby. Norgliovictin is unusual and contains unsubstituted amides. These diketopiperazines may provide key evidence for the sequence of steps involved in the biosynthesis of gliotoxin and related epipolythiodioxopiperazines.

The reduced derivatives bisdethiobis(methylthio)gliotoxin (**19**) and bisdethiobis-(methylthio)dehydrogliotoxin (**20**) were originally detected in culture extracts of *G. deliquescens*. The structures were confirmed by synthesis from gliotoxin and dehydrogliotoxin.[42] Bisdethiobis(methylthio)gliotoxin (**19**) has recently been identified as a co-metabolite of gliotoxin in extracts of *T. crustaceus*, and **20** has been identified as a minor metabolite of *P. terlikowskii*.[43]

Compound **19** has been shown to be a potent inhibitor of a platelet activating factor (PAF) and displayed an IC_{50} of 8.4 μM in tests measuring inhibitory activity *in vitro*.[44] Gliotoxin in similar experiments exhibited weak antagonistic activity with a reported IC_{50} of 93.0 μM. Investigation of a variety of synthetic derivatives **25a-i** shown in Figure 6

Figure 6 Synthetic derivatives 25a-i

a R_1 = Ac, R_2 = Me
b R_1 = H, R_2 = Et
c R_1 = H, R_2 = n-butyl

d R_1 = OH, R_2 = H
e R_1 = OAc, R_2 = OAc
f R_1 = H, R_2 = H

g R= H
h R= OH
i R= OAc

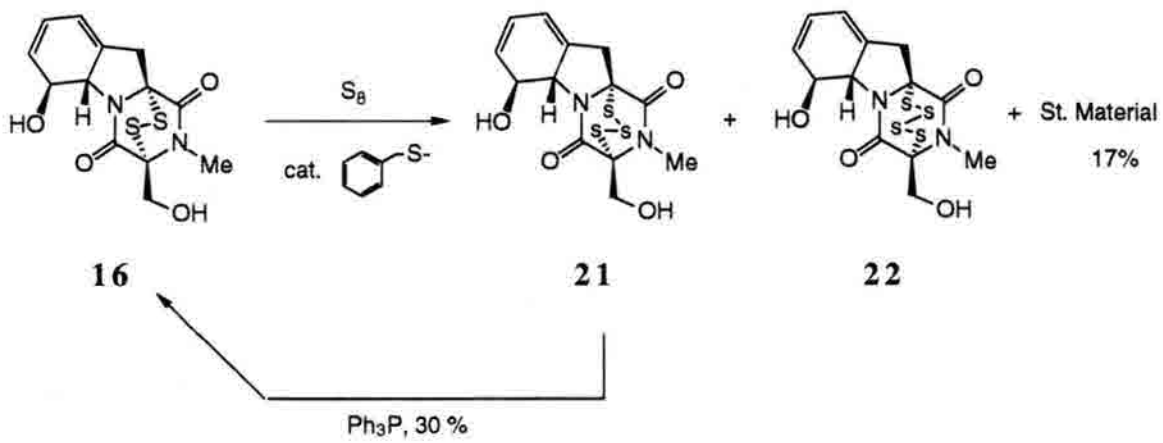
lead to the following structure-activity relationships. Long chain alkyl groups on sulfur and removal of the C3-hydroxymethyl group diminished inhibitory activity of analogs compared to the normal substrates. Compounds containing the dihydrobenzene ring system were as active as the phenol substituted compounds. Of all the analogs tested, **25g** displayed the most acute PAF inhibitory activity with an IC_{50} of 4.4 μM. Further *in vivo* studies demonstrated bisdethiobis(methylthio)gliotoxin (**20**) was an effective inhibitor of

PAF-induced bronchoconstriction in guinea pigs at 0.1 mg/kg , but did not prevent PAF-induced hypotension in mice.[45]

The epitrisulfide and epitetrasulfide gliotoxin E (**21**) and G (**22**) have been recently identified from a variety of fungal extracts. Gliotoxin E was originally identified in extracts of *P. terlikowskii* by Waring and coworkers in 1987.[43] Subsequent work identified gliotoxin E as a trace metabolite in *A. fumigatus* and *T. crustaceus* and most recently in *G. virens*.[41] Production of gliotoxin E from *T. crustaceus* is accompanied by the production of bisdethiobis(methylthio)gliotoxin (**19**). Gliotoxin G has only been identified in *A. fumigatus* and *T. crustaceus*..[46]

The absolute stereochemistry of **21** and **22** was determined via chemical methods since comparison of the CD curves could not provide definitive results. Initial evidence for the proposed structures and absolute stereochemistries was obtained by conversion of gliotoxin E into gliotoxin (30%) via desulfurization with triphenyl phosphine and occurred with retention of configuration about the bridgehead positions (Scheme 2).[43]

Scheme 2. Interconversion of Gliotoxin derivatives



Subsequent work by Kirby confirmed the structures of gliotoxin E and G. Treatment of gliotoxin with excess rhombic sulfur in the presence of a catalytic amount of

phenylmethyl thiolate resulted in formation of a mixture of gliotoxin (17%), gliotoxin E (26%), and gliotoxin G (60%).[41]

Gliotoxin E and G display the same activity as gliotoxin in macrophage adherence assays commonly used as an indication of antiphagocytic activity. All three compounds exhibited ED_{50} values in the range of 20±10 ng/mL.[34d] The S,S'-methyl derivatives of gliotoxin were devoid (< 2%) of biological activity suggesting the disulfide bridge is essential for activity.

The aranotins (**26-32**) are a group of epipolythiodioxopiperazines structurally related to gliotoxin and characterized by the presence of at least one oxepine ring. The various derivatives are shown in figure 7. Aranotin (**26**), acetylaranotin (LL-S88α) (**27**), bisdethio-bis(methylthio)acetylaranotin (LL-S88β) (**28**), apoaranotin (**29**), and bisdethiobis(methylthio)acetylapoaranotin (**30**) were all isolated from *Arachniotus aureus* (Eidam) Schroeter (NRRL 3205) in 1968.[47] The structures and absolute stereochemistry of **27** and **28** were deduced from crystallographic analysis,[48] and have been isolated from *Asp. terrus*.[49] Structural similarities between gliotoxin and the aranotins are most evident in apoaranotin (**29**), which can be considered as a hybrid of gliotoxin and aranotin.

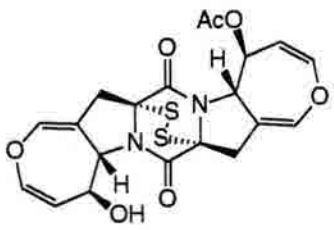
New additions to the aranotin family isolated from *Asp. terrus* include the simple diketopiperazine **32** which contains free amides. The first mono-oxepine derivative asteroxepin (**31**) was isolated in 1986.[50]

The aranotins are noted for their potent antiviral activity and appear to be specific for RNA viruses such as the influenza-, rhino-, or polio-viruses. The compounds appear to exert their antiviral effects by interfering with RNA-dependent RNA synthesis.

Closely related to the aranotins are a novel group of oxepin-substituted epipolythiodioxopiperazines known as the emethallicins (**33-38**) and shown in figure 8.

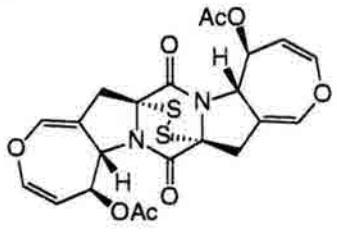
Emethallicin A (**33**) was originally isolated form mycelial extracts of *Emericella heterothallica* (mating type A) in 1989 by Kawai.[51] The fungus *E. heterothallica* is the

Figure 7. Aranotins



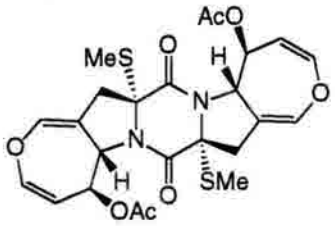
Aranotin

26



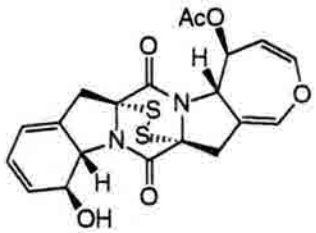
Acetylaranotin (LL-S88α)

27



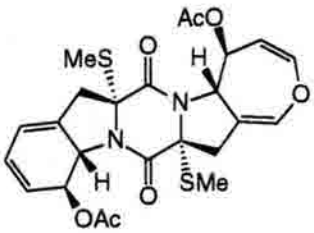
Bisdethio-(dimethylthio)-acetylaranotin (LL-S88β)

28



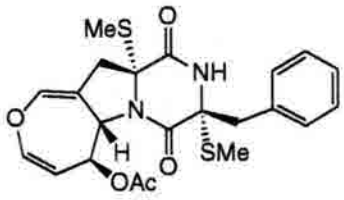
Apoaranotin

29



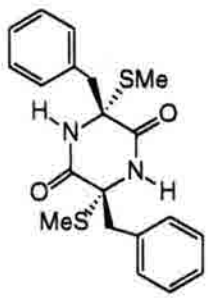
Bisdethiodi(methythio)-acetylapoaranotin

30



Asteroxepin

31



cis-3,6-dibenzyl-3,6-bis(methylthio)-dioxopiperazine

32

same fungus responsible for production of the related simplified bis(methylthio)-dioxopiperazines emethacin A (**13**) and B (**14**).

Emethallicin A has the same skeleton as apoaranotin (**29**) and differs only in the ester moieties at C2 and C6. The structural similarities (including absolute stereochemistry) were demonstrated by conversion of emethallicin A into apoaranotin via basic hydrolysis. Emethallicin A undergoes facile reductive methylation to generate epidithiobis-(methylthio)emethallicin in the presence of sodium borohydride and methyl iodide.

Isolation and identification of the tetrasulfides emethallicin B (**34**) and C (**35**) from *E. heterothallica* was reported in 1989.[52] Compound **34** undergoes facile desulfurization upon treatment with triphenyl phosphine to give the disulfide emethallicin A and subsequently converted into apoaranotin (**29**) confirming the proposed structure.

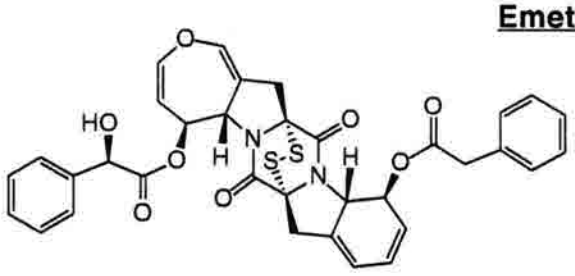
Analysis of emethallicin C (**35**) by proton and/or carbon NMR revealed only half the number of resonances as Emethallicin B indicating a symmetrical structure. The structure of emethallicin C resembles acetylaranotin (**27**) and contains two oxepine rings.

The trisulfide derivative emethallicin D (**36**) was recently isolated as the acetate from mycelial extracts of *E. heterothallica* in 1990 by Kawai and coworkers.[53] Attempts to obtain the natural product emethallicin D (**36,** R=H) from emethallicin D-acetate (**36**, R=Ac) have been unsuccessful. Treatment of emethallicin D-acetate with sodium bicarbonate in aqueous acetone resulted in disproportionation forming the disulfide emethallicin A-acetate and tetrasulfide emethallicin B-acetate. The structures of the acetates were identical to products obtained from acetylation of natural emethallicin A and B. This interesting disproportionation reaction of the trisulfide is similar to the reaction observed by Waring et al. involving gliotoxin E.[43]

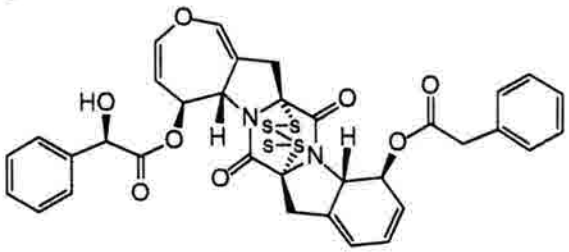
The disulfides emethallicin E (**37**) and F (**38**) are the most recent additions.[54] Emethallicin E is a symmetrical compound and does not contain oxepin rings. Emethallicin F is identical to emethallicin A with the exception of the side chain residues. Emethallicin F was obtained from the basic hydrolysis and phenylacetylation of emthallicin A.

Figure 8

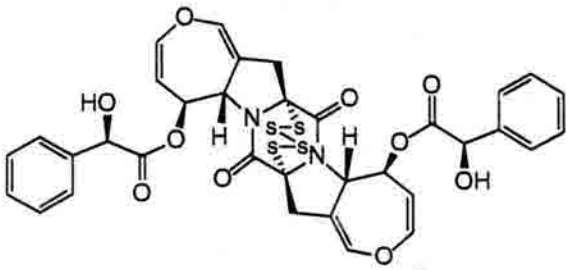
Emethallicins



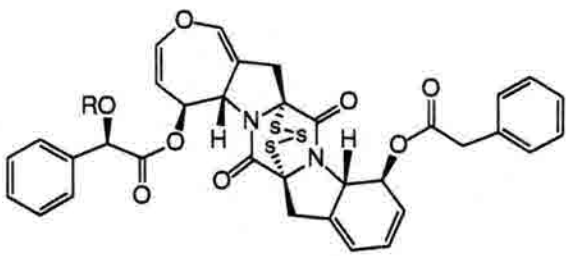
33 Emethallicin A



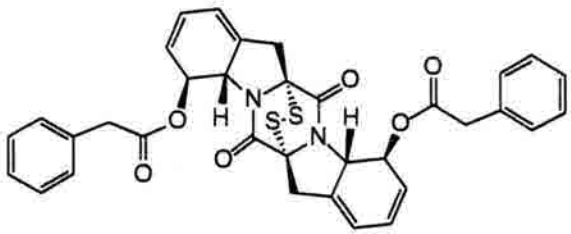
34 Emethallicin B



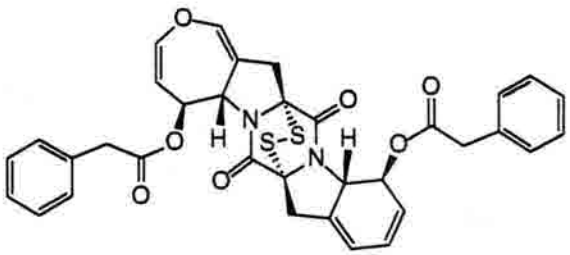
35 Emethallicin C



36 Emethallicin D
R= H, Ac



37 Emethallicin E

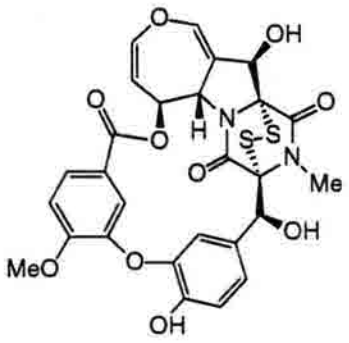


38 Emethallicin F

The emethallicins are extremely potent inhibitors of histamine release from mast cells and IC_{50} values ranging from 1×10^{-6} to 8×10^{-8} M have been reported. In addition the emethallicins have been shown to be effective inhibitors of 5-lipoxygenase with reported activities in the micromolar range.

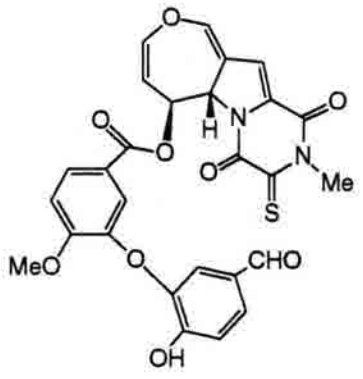
Another class of metabolites characterized by the presence of an oxepine ring system are the macrocyclic emestrins (**39-42**) shown in figure 9. Emestrin (**39**) was first isolated in 1985 from mycelial extracts of *Emericella striata* collected in Nepal by Kawai and coworkers.[55] Further investigations have identified **39** as the mycotoxin produced

Figure 9 Emestrin and related metabolites



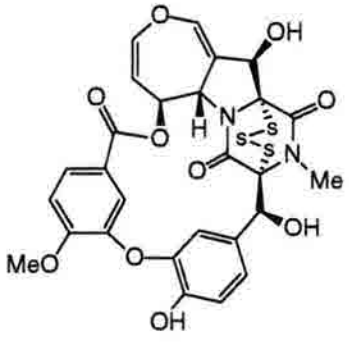
Emestrin

39



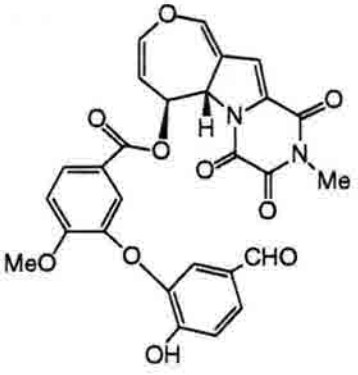
Aurantioemestrin

40



Emestrin B

41

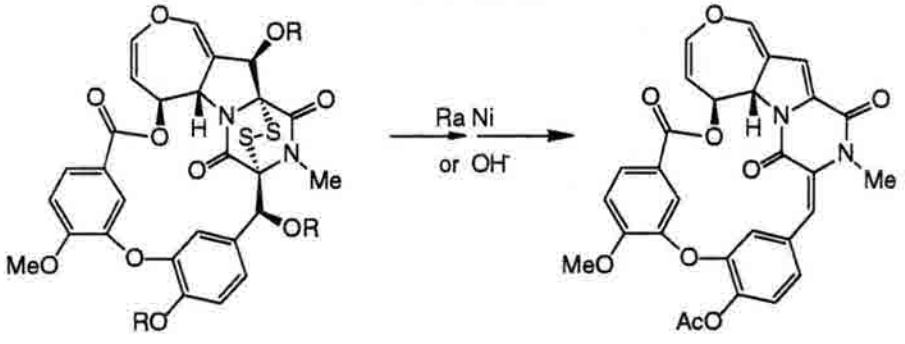


Desthiosecoemestrin

42

by *E. quadrilineata*, *E. foveolata*, *E. acristata*, and *E. parvathecia*.[56] The structure of emestrin was determined from x-ray crystallography and the absolute configuration inferred from comparison of the CD spectra with the CD spectra of gliotoxin and acetylaranotin.[57] Emestrin is the first example of a epidithiodioxopiperazine possessing a macrocyclic ring system and believed to be derived from two molecules of phenylalanine and benzoate. Emestrin (**39**) was converted to **43** by treatment with Raney nickel or under basic hydrolysis resulted in deacetylation and simultaneous desulfurization giving rise to O-acetyldianhydrodidethiosecoemestrin (**43**) as depicted in Scheme 3.

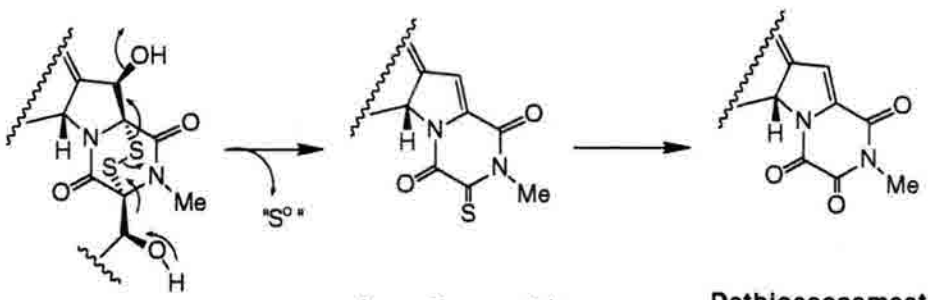
Scheme 3



39 **43**

The piperazinethione aurantioemestrin (**40**), and trisulfide emestrin B (**41**) were subsequently isolated from *E. striata* by Kawai.[58] The trioxopiperazine **42** dethio-secoemestrin, was also identified as a metabolite of *E. striata*.. The similarity in structures between these metabolites suggests a possible biosynthetic relationship. Dethiosecoemestrin is postulated to be biogenetically derived from emestrin via aurantioemestrin as depicted in Scheme 4. Compound **42** is proposed to be a possible key intermediate in the biosynthetic conversion of epidithiodioxopiperazines into trioxopiperazines.

Scheme 4.



Emestrin **Aurantioemestrin** **Dethiosecoemestrin**

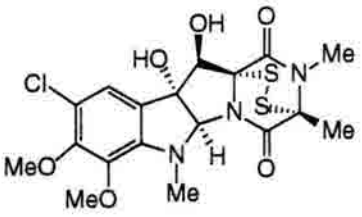
39 **40** **42**

Emestrin displays strong antifungal/antibacterial activity *in vitro* (0.78- 1.56 μg/mL against *Tricophyton spp.*, 3.1- 6.3 μg/mL against *Microsporum spp.* and 25μg/disc against *B.Subtilus* and *E. Coli.*). Unfortunately, **39** also exhibits strong toxicity in mammals (eg. LD_{50} = 13 mg/kg in mice) characteristic of this family of metabolites.

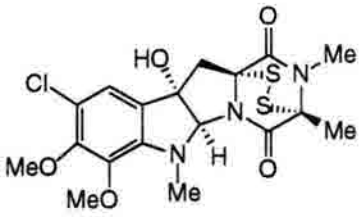
The sporidesmins (**44-52**) are indole-containing epipolythiodioxopiperazines produced by the fungus *Pithomyces chartarum* and are shown in Figure 10.[59] This fungus is the causative agent of the disease facial eczema which afflicts grazing animals in New Zealand and Australia. Because of their role in disease, the sporidesmins have been the focus of a considerable amount in research over the last three decades.[2b] All the derivatives **44-52** have been completely characterized. The trisulfide sporidesmin C (**46**) is one of the most unusual of the sporidesmins containing the novel [4.3.3] ring system.

As a result of the extensive investigations into the sporidesmins, a number of reactions were discovered and have since been shown to be common to many epipolythiodioxopiperazines. For example, desulfurization of the trisulfide sporidesmin E (**48**) to generate disulfide **44** can be efficiently carried out using triphenyl phosphine. Both the di and trisulfides can be converted to a higher homolog by insertion of sulfur using hydrogen polysulfide or dihydrogen disulfide and this was demonstrated by the interconversion of sporidesmin A (**44**) and E (**48**) into sporidesmin G (**50**).

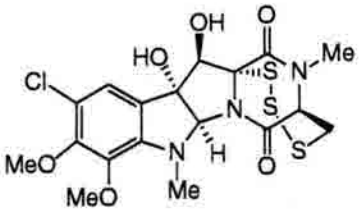
Figure 10. Structures of the Sporidesmins



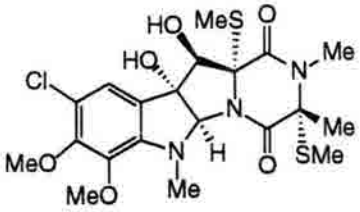
44 A



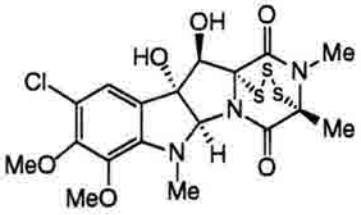
45 B



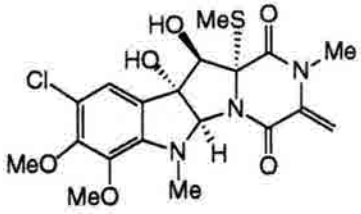
46 C



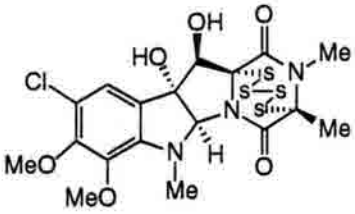
47 D



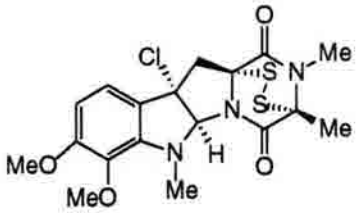
48 E



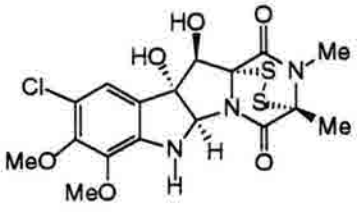
49 F



50 G



51 H

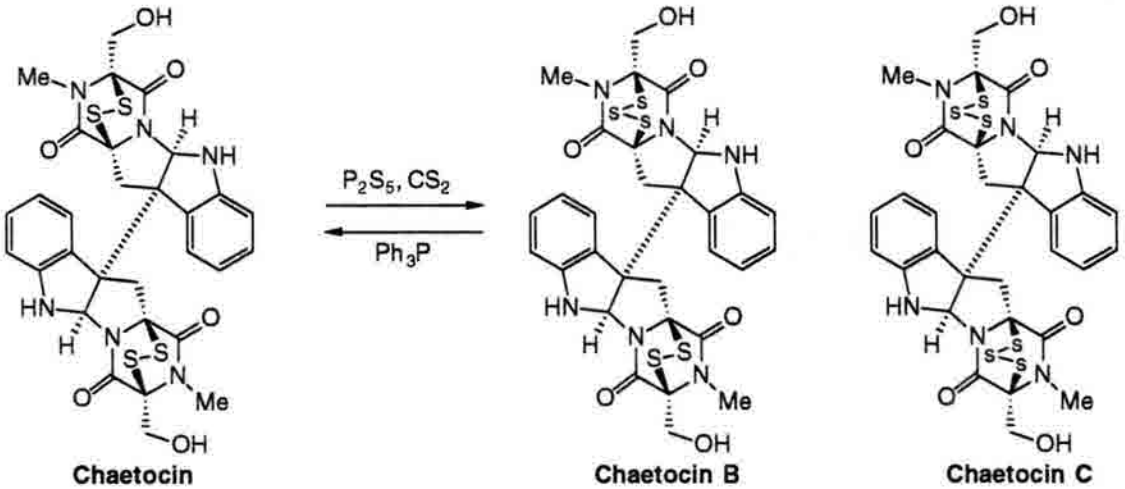


52 J

A distinctive group of dimeric epipolythiodioxopiperazines include the chaetocins (**53-54**),[60] verticillins (**58-60**),[61] melinacidins (**61-63**),[62] and chetomins (**64,65**).[63] All of these compounds contain the indolopyrrolopyrazine skeleton found in sporidesmin. In general the dimeric alkaloids are ineffective against Gram-(-) organisms, but display remarkable activity against Gram-(+) species. In addition, IC_{50} values against HeLa cells in the range of 0.02-0.07 μg/mL have been observed, although acute toxicity precludes their use.

The structures of chaetocin (**53**), chaetocin B (**54**) and chaetocin C (**55**) are shown in Scheme 5. Circular dichroism experiments and x-ray analysis demonstrated the configuration about the diketopiperazine moiety of chaetocin (**53**) is antipodal with respect to gliotoxin, aranotin and sporidesmin.

Scheme 5. Interconversion of Chaetocin derivatives



Chaetocin **53**

Chaetocin B **54**

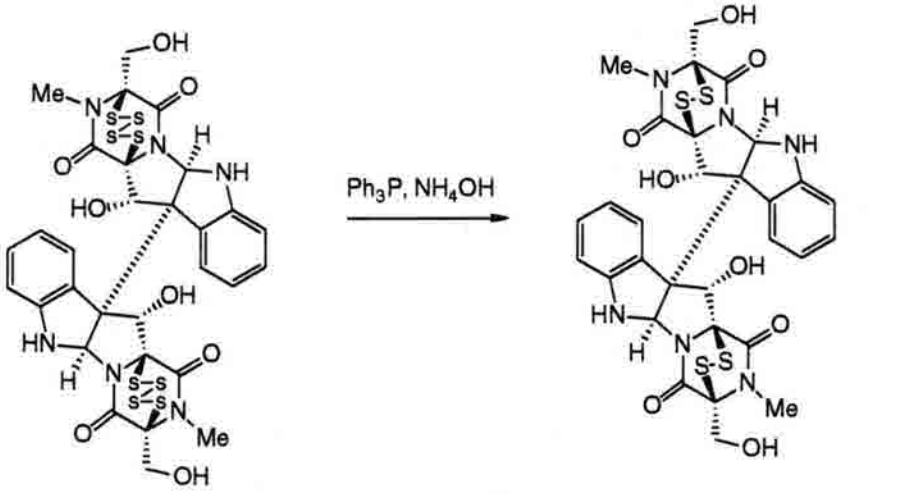
Chaetocin C **55**

Recently, the penta and hexasulfide homologs **54, 55** of chaetocin were isolated from *Chaetomium spp* in 1988.[64] The structures of chaetocin B and C were inferred from NMR and fast atom bombardment mass spectrometry. Chaetocin B is a pentasulfide

containing both di and tri-sulfide bridges, while chaetocin C is symmetrical possessing two trisulfide bridges. Affirmation of the proposed structures was obtained by interconversion of chaetocin (**53**) with B (**54**) and C (**55**) as shown in Scheme 5. Desulfurization of **54** and **55** with triphenyl phosphine afforded chaetocin, while sulfurization of chaetocin using phosphorus pentasulfide in carbon disulfide generated chaetocin B and C.

Also isolated from *Chaetomium spp.* was the novel dimeric alkaloid chetracin A (**57**).[65] Chetracin A is the first example of a dimeric tetrasulfide isolated. The structure was determined from conversion to 11α,11'α-dihydroxychaetocin (**56**) upon desulfurization with triphenyl phosphine is depicted in Scheme 6 and has been confirmed from x-ray analysis.

Scheme 6

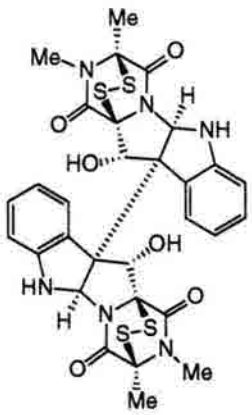


Chetracin A **11α-11'α-dihydroxychaetocin**

57 **56**

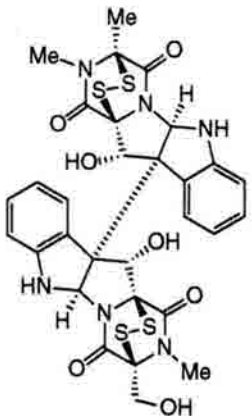
The remaining indole dimers are shown in figure 11. Verticillins A, B and C (**58-60**) were isolated from *Verticillium* (TM-759).[58b] Verticillin B differs from A by the presence of a single hydroxymethyl group attached to a diketopiperazine moiety. Verticillin C is believed to be a S_5-homolog on the basis of mass spectral analysis and attempts to convert verticillin C into B have been unsuccessful.

Figure 11.Structures of Indole Dimers



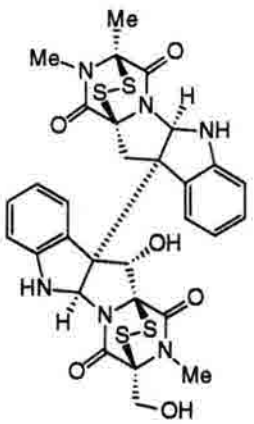
Verticillin A

58



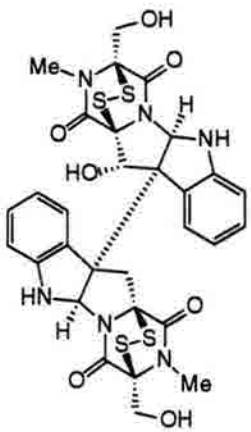
Verticillin B
Verticillin C (S_5 homolog)

59, 60



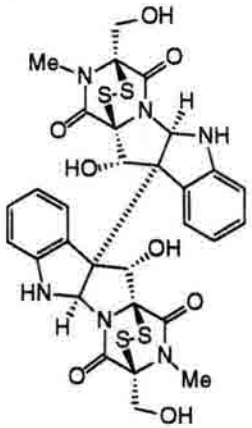
Melinacidin II

61



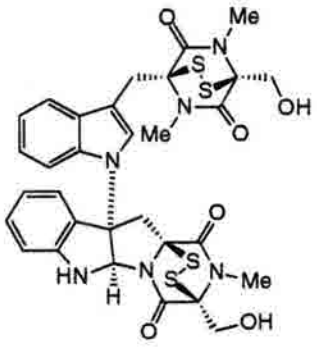
Melinacidin III

62



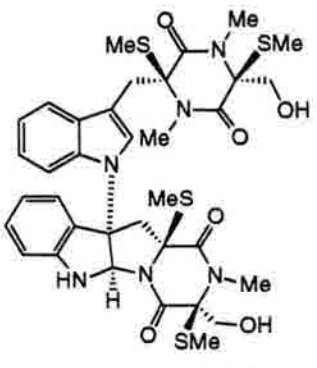
Melinacidin IV
(11a-11a'-dihydroxychaetocin)

63



Chetomin

64



Dethio-tetra(methylthio)Chetomin

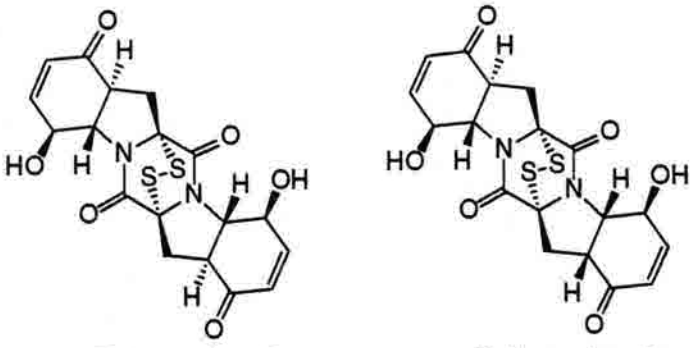
65

The melinacidins (**61-63**) are produced from *Aerostalagmus cinnabarinus var. melinaccidinus*. Melinacidin IV is identical to 11α,11'α-dihydroxy chaetocin (**56**) produced by *Verticillium tenerum*..

Chetomin (**64**) was isolated from *Chaetomium cochliodes* , a fungus associated with poor growth in grazing animals. Chetomin differs from the other indole dimers in the position of the bond connecting the two indole groups together. In chetomin the linkage occurs at the β-position of one indole with the nitrogen of the other indole. Dethiotetra-(methylthio)chetomin (**65**) has been recently characterized from extracts of *Chaetoium globosum kinze ex fr.*[66]

The epicorazines consisting of epicorazine A (**66**) and B (**67**) were isolated from the airborne fungi *Epicoccum nigrum*,[67] and more recently in extracts from *Epicoccum purpurascens*.[68] Both the structure and absolute conformation of **66** and **67** have been

Figure 12. Epicorazines



Epicorazine A **Epicorazine B**

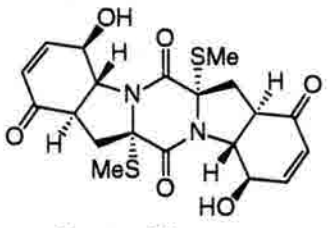
66 **67**

confirmed by X-ray analysis and contain the same configuration about the diketopiperazine moiety as gliotoxin.[69] Epicorazine A and B differ only in the absolute stereochemistry of the proton at C6. Both **66** and **67** display marginal activity against *Staphylococcus aureus* and *Streptococcus*, but are inactive against Gram-(-) organisms.

Remarkably similar to the epicorazines are a group of natural products called the exserhilones and are shown in Figure 13.[70] Exserhilone (**68**) and 9,10-dihydroexserhilone (**69**) were isolated from *Exserohilum holmi*, a pathogenic fungus. The structures of **68** and **69** were determined by X-ray, and contain the 3S,6S stereochemistry about the diketopiperazine moiety.

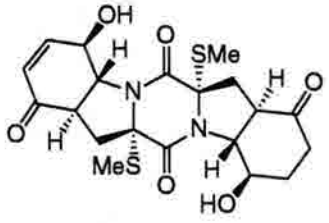
Epoxyexserhilone (**70**) was isolated from *Nigrospora sphaerica* and the structure also confirmed by X-ray. This unique metabolite contains the antipodal R,R configuration about the diketopiperazine moiety. Epoxyexserhilone was devoid of activity against Gram-(+) and Gram-(-) bacteria. No mention was made regarding a possible biosynthetic relationship between these unique metabolites and the epicorazines, although the similarities in structure are remarkable.

Figure 13 Structure of the Exserhilones.



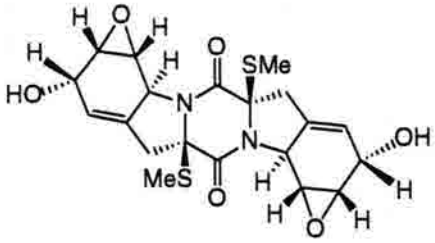
Exserohilone

68



9,10-dihydroexserhilone

69

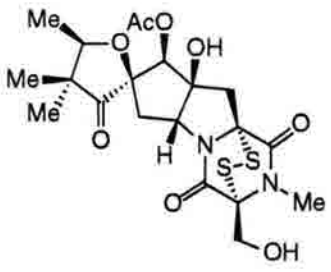


Epoxyexserohilone

70

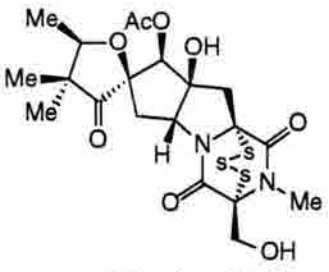
The sirodesmins (**71-76**) shown in Figure 14 are an emerging group of epipolythiodioxopiperazines characterized by a spirofused tetrahydrofuran-cyclopentyl-pyrrolidine skeleton.[71] Isolation of these metabolites has been confined to the fungi

Figure 14 Sirodesmins



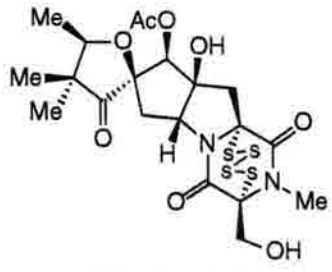
Sirodesmin A

71



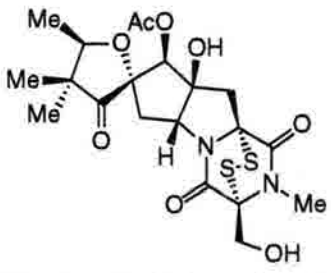
Sirodesmin C

72



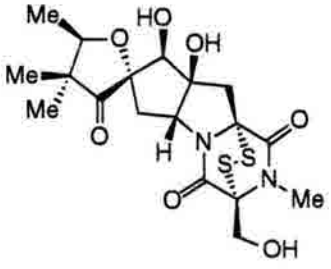
Sirodesmin B

73



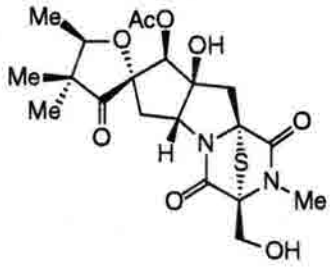
Sirodesmin G / Sirodesmin PL

74



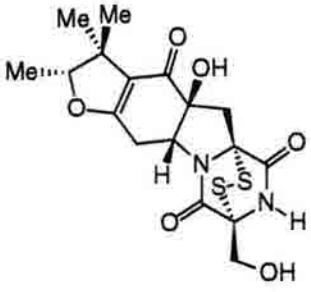
Desacetyl-sirodesmin PL

75



Sirodesmin H

76



Phomalirazine

77

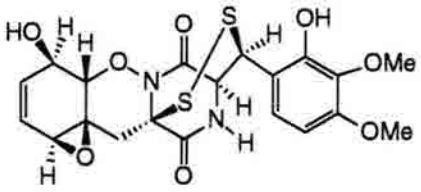
Sirodesmium diversum and *Phoma lingam*. The structures and absolute configuration of sirodesmin A (**71**) and sirodesmin PL (**74**) were determined from X-ray and C.D. analyses. Sirodesmins A (**71**), B (**72**), and C (**73**) contain the R configuration at the spirocenter while sirodesmins G (**74**), PL (**75**), and H (**76**) are antipodal with respect to

the configuration at this center. Sirodesmin H (**76**) was isolated from *P. lignam* and is the first example of a naturally occurring monosulfide.[72]

Conversion of the disulfide Sirodesmin A into a mixture of the tetrasulfide sirodesmin B and trisulfide sirodesmin C occurred upon treatment with sulfur in pyridine. Sirodesmins B and C are unstable in ethanol or pyridine; upon standing a mixture of sirodesmins A, B, and C was obtained. The sirodesmins are potent antiviral agents, and particularly active against the rhinovirus.

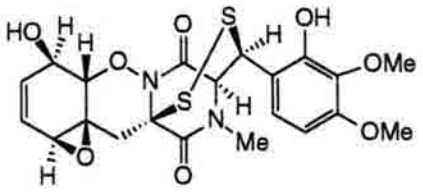
Phomalirazine (**77**) was recently characterized from *P. lingam*, believed to be the causative agent of "blackleg disease".[73] The structure of phomalirazine was deduced by X-ray analysis. Compound **77** is proposed to be a key intermediate in the biosynthesis of sirodesmin PL.

Figure 15. N-alkoxy Epidithiodioxopiperazines



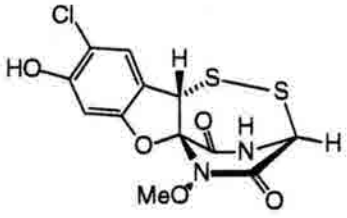
Gliovirin

78



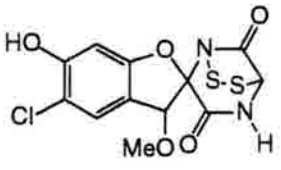
FA-2097

79

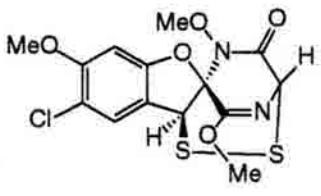


Aspirochlorine (A30641)

80



81



82

A number of epidithiodioxopiperazines containing an N-alkoxy substituted diketopiperazine ring have been identified and the structures shown in Figure 15. This group includes gliovirin (**78**), FA-2097 (**79**) and aspirochlorine (**80**).

Gliovirin was isolated in 1982 by Stipanovic from cultures of *Gliocladium virens* and the structure deduced from X-ray crystallographic analysis.[74] Gliovirin is believed to be derived from two equivalents of phenylalanine. N-methyl gliovirin (FA-2097) was recently described and the structure determined by comparison of spectral data with **78**.[75]

Aspirochlorine was isolated from *Aspergillus tamari* in 1976 and subsequently identified as the active constituent in cultural extracts from *Asp. flavus* and *Asp. oryzae*.[76] The original structure proposed contained a novel bicyclo [3.2.1] N-1,3 disulfide bridge depicted in **81**.[77] The correct structure of aspirochlorine was deduced in 1987 from a crystal structure of the semi synthetic derivative **82**.[78] Aspirochlorine is proposed to be the only known epidithiodioxopiperazine to be derived from glycine and contains a rare example of a secondary amide adjacent to the sulfur bridge. Aspirochlorine displays marginal biological activity.

Biosynthesis of epipolythiodioxopiperazines

All of the epipolythiodioxopiperazines are believed to be derived biosynthetically from the aromatic amino acids phenylalanine, tyrosine, or tryptophan which allows for a convenient method to classify each family.[2e] Gliotoxins, hyalodendrins, gliovictins, aranotins, epicorazines and emethacins are believed to be derived from phenylalanine. The emestrins, emethallicins, dithiosilvatin, gliovirin or dithiosilvatin families can be derived from either phenylalanine or tyrosine. Sirodesmins, phomalirazine and aspirochlorine are believed to be derived from tyrosine. Tryptophan-derived metabolites include the sporidesmins, chaetocin, verticillin, melinacidins, chetracins and chetomins.

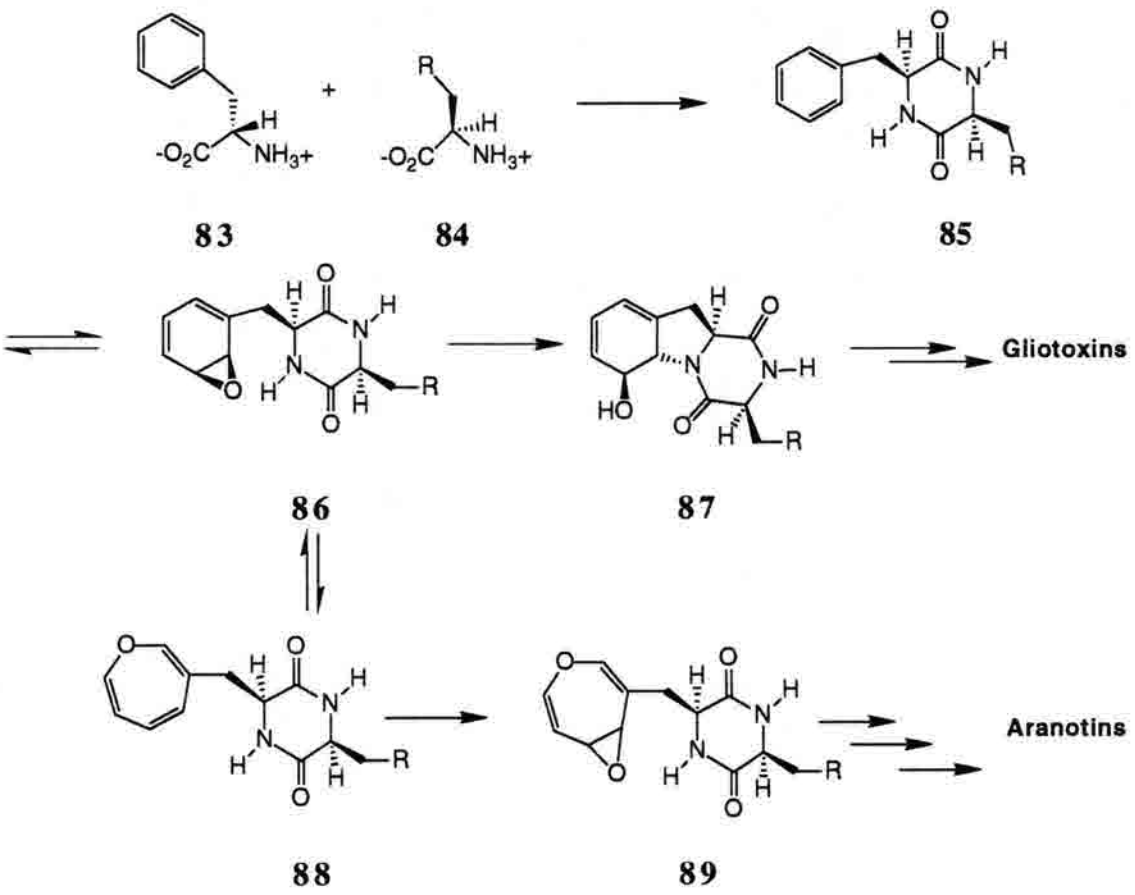
The biosynthesis of gliotoxin has been the focus of considerable investigation and is the most well understood of all epidithiodioxopiperazines.[2e] Because of the similarity in

structure between gliotoxin , aranotin and the simple epipolythiodioxopiperazines, all three classes are believed to be biosynthesized via a similar chain of events.

Suhadolnik and Chenoweth in 1968 demonstrated gliotoxin is derived from phenylalanine and serine.[79] Phenylalanine is believed to be incorporated completely, although radiolabeling studies by Johns indicates possible stereospecific removal of the pro-R hydrogen may occur .[80]

Neuss in 1968 proposed the biosynthesis of gliotoxin proceeds via the arene oxide intermediate **86** shown in Scheme 7.[81] Furthermore the elegant proposed biosynthesis

Scheme 7. Proposed biosynthesis via arene oxide



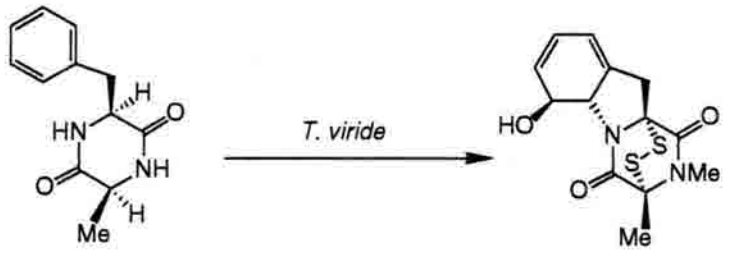
83 **84** **85**

86 **87**

88 **89**

provides a convenient entry into the aranotins by ring enlarging tautomerism of the arene oxide to an oxepine (ie. **86→88**). Support for the proposal by Neuss was obtained from feeding experiments from several groups in which cyclo (L-Phe-L-Ser) (**85**) was found to be the most efficiently incorporated.[82]

One of the more convincing feeding studies was carried out by Kirby who demonstrated the biosynthesis of unnatural 3α-deoxygliotoxin (**91**) in *T. viride* from U-^{14}C-labeled cyclo-(L-alanyl-L-phenylalanyl) (**90**).[83]

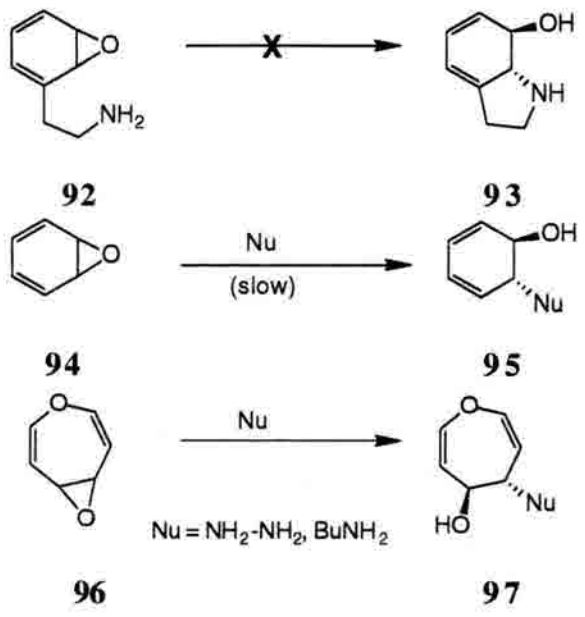


90 **91**

Interestingly, confirmation of the proposed biosynthesis using model arene oxides shown in Scheme 8 could not be carried out efficiently in the lab. Attempted cyclization of the model 3-(β-aminoethyl)benzene oxide **92** failed to cyclize to **93** under a variety of conditions.[84]

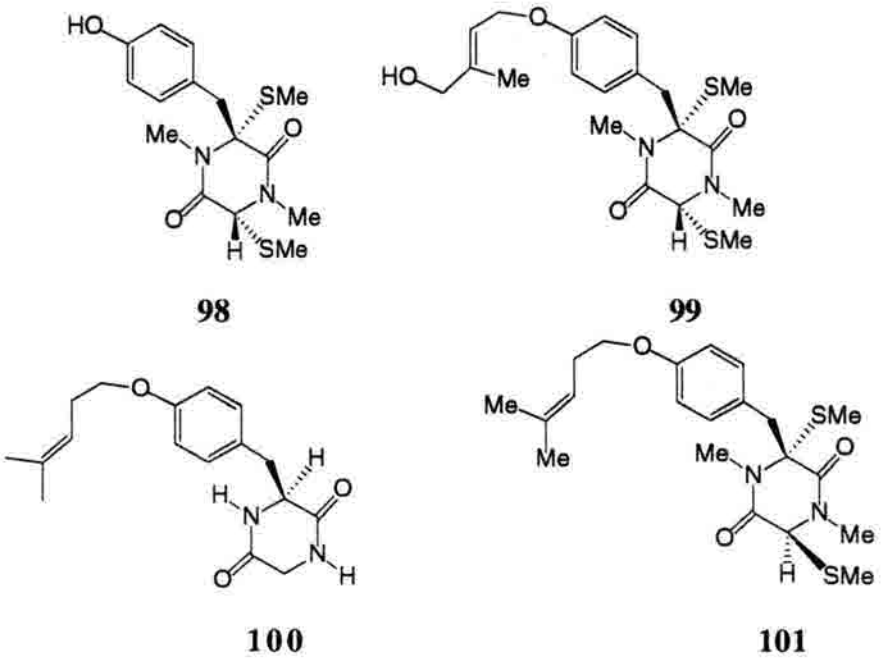
Treatment of benzene oxide **94** with several amine nucleophiles resulted in formation of the desired ring opened products **95**, although the reactions were prohibitively slow.[85] 11% of **95** was obtained from reaction of **94** with butyl amine after 7 weeks. Ironically a similar cyclization of *sym*-oxepin oxide **96** which is related to the aranotins, proceeded readily and additions were completed within 72 hours. The results lead Rastetter to conclude the ring closure proposed by Neuss was feasible, although the proposed cyclization of the arene oxide to generate the indole nucleus may occur under enzymatic control.

Scheme 8. Attempted arene oxide cyclizations

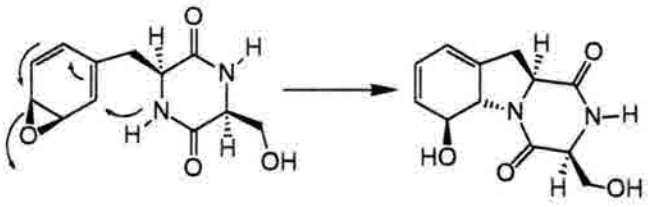


The failure of model arene oxides to undergo cyclization, and the recent identification of hydroxylated co-metabolites such as the simple diketopiperazines **98-101** shown in Figure 16 has lead to an alternative mechanism for the biosynthesis.

Figure 16 Hydroxylated diketopiperazines



Hanson and O'Leary suggested a modification to the Neuss proposal which invokes the tautomeric arene oxide **102** as the active intermediate.[86]

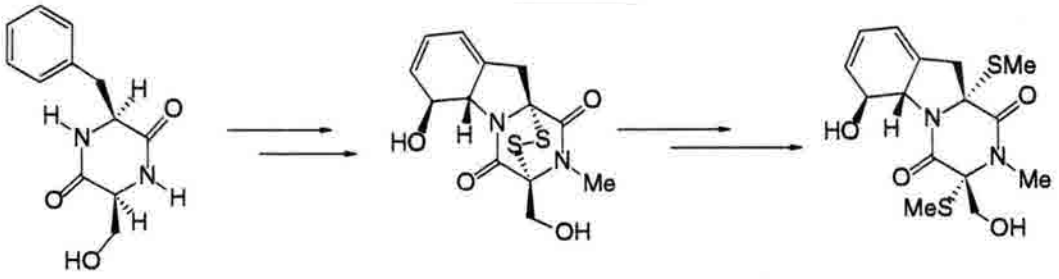


102 **87**

This proposal has been disproved in recent feeding experiments by Johns and Kirby using [phenyl-^{3}H]-phenylalanine and [^{3}H]-labeled m-tyrosine. Incorporation of tritium into gliotoxin was only observed with phenylalanine and demonstrates the biosynthesis of gliotoxin proceeds from phenylalanine and not a hydroxylated derivative such as m-tyrosine or other hydroxy benzene intermediate.[87]

Although feeding experiments provided evidence for the proposed biosynthesis via the arene oxide, key mechanistic information for the sequence of steps along the biosynthetic pathway (such as N-methylation, oxidative cyclization, incorporation of the sulfur bridge and formation of the reduced bis(methylthio) derivatives) has been more difficult to obtain. A series of reports by Kirby et. al., have provided insights into these fundamental questions.

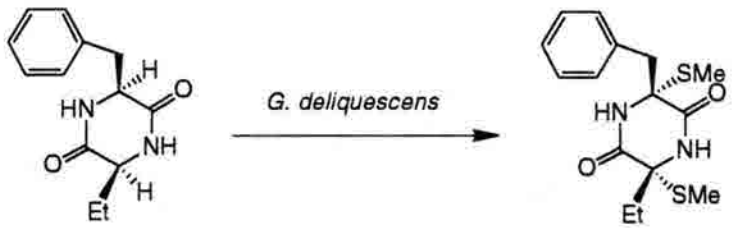
Scheme 9



^{14}C-103 **^{14}C-16** **^{14}C-20**

As depicted in Scheme 9, using radiolabeled gliotoxin (**16**) prepared from L-[U-^{14}C]-Phe (**103**), Kirby isolated ^{14}C-labeled bisdethiobis(methylthio)gliotoxin (**20**). This results suggest the bis(thiomethyl)derivatives are produced from the disulfides *in vivo* .[88]

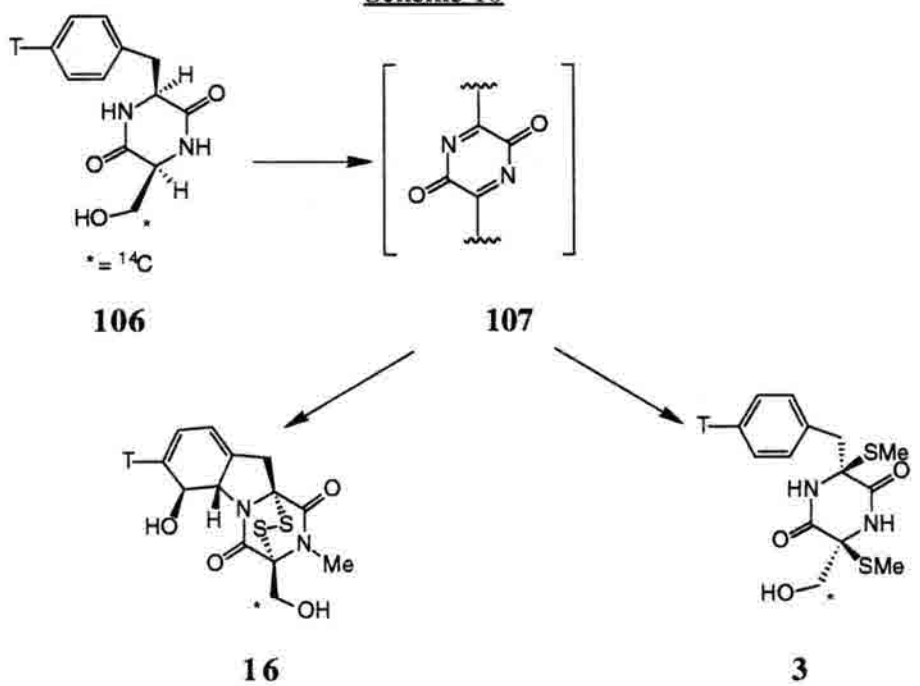
A few years later during feeding experiments using cyclo-(L-2-aminobutanoyl-L-phenyl-alanyl) (**104**), the "unnatural" metabolite **105** containing a pendant ethyl group and unsubstituted amide nitrogens was positively identified.[89]



104 **105**

The existence of **105** provides evidence introduction of sulfur can occur prior to N-methylation or oxidative cyclization. A similar result was obtained in feeding experiments investigating the biosynthesis of aranotins using *Asp. terreus*.[90]

Scheme 10



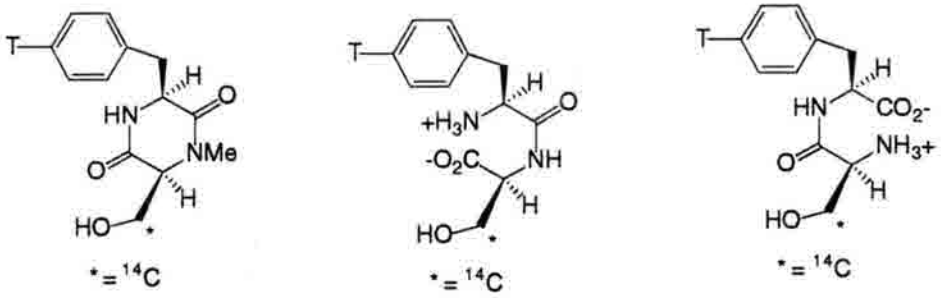
106 **107**

16 **3**

The similarity in structure between gliotoxin and the hyalodendrins has led to some interesting inferences regarding a possible common biosynthetic pathway depicted in Scheme 10. Using the double labeled cyclic dipeptide cyclo-(L-[4'-^{3}H]Phe-L-[3-^{14}C]Ser (**106**), Kirby and Pita Boenta established that **106** is efficiently incorporated into both gliotoxin and hyalodendrin presumably via an acyl imine intermediate **107**.[91]

Further investigations using cyclo (L-[U-^{14}C]-phe-N-methyl-L-Ser) (**108**) (Figure 17) resulted in little incorporation into either gliotoxin or hyalodendrin. Based on these findings, Kirby et al., concluded N-methyl diketopiperazines are not biosynthetic intermediates, indicating incorporation of sulfur precedes N-methylation. Additional feeding experiments with the acyclic dipeptides **109, 110** shown in Figure 17 exhibited moderated incorporation. In addition, feeding experiments with **109,110** resulted in a change in the ratio of ^{3}H:^{14}C incorporated, indicating possible hydrolysis of the dipeptides occurred prior to incorporation.

Figure 17. Potential biosynthetic precursors



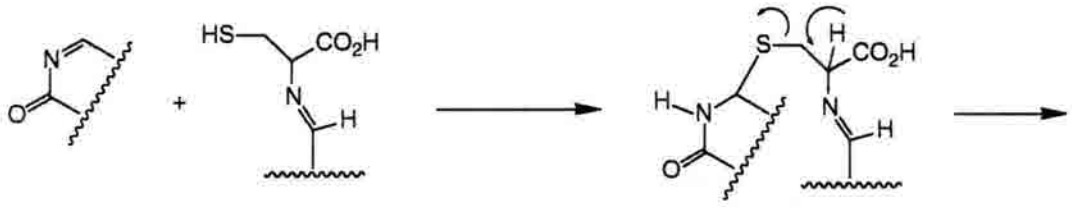
108 **109** **110**

Incorporation of sulfur into epidithiodioxopiperazines is not well understood and a variety of mechanisms have been proposed.[2e, 92] Interpreting results from feeding experiments carried out with [^{35}S] is usually difficult due to transfer of sulfur among various potential donors. The ultimate source of sulfur is usually an ion such as SO_4^{2-}, $S_2O_3^{2-}$, or SO_3^{2-} although the immediate donor is not as well characterized. Both

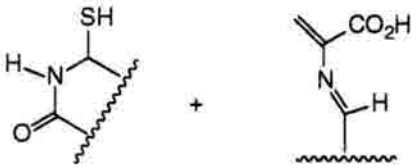
methionine and cysteine have been shown to be donors, although cysteine is more efficient.[2e]

A commonly proposed method is presented in Scheme 11 between cysteine or a cysteine/pyrodoxal complex eg. **112** and a dehydrodioxopiperazine moiety **111**.

Scheme 11. Proposed incorporation of Sulfur via cysteine



111 **112** **113**

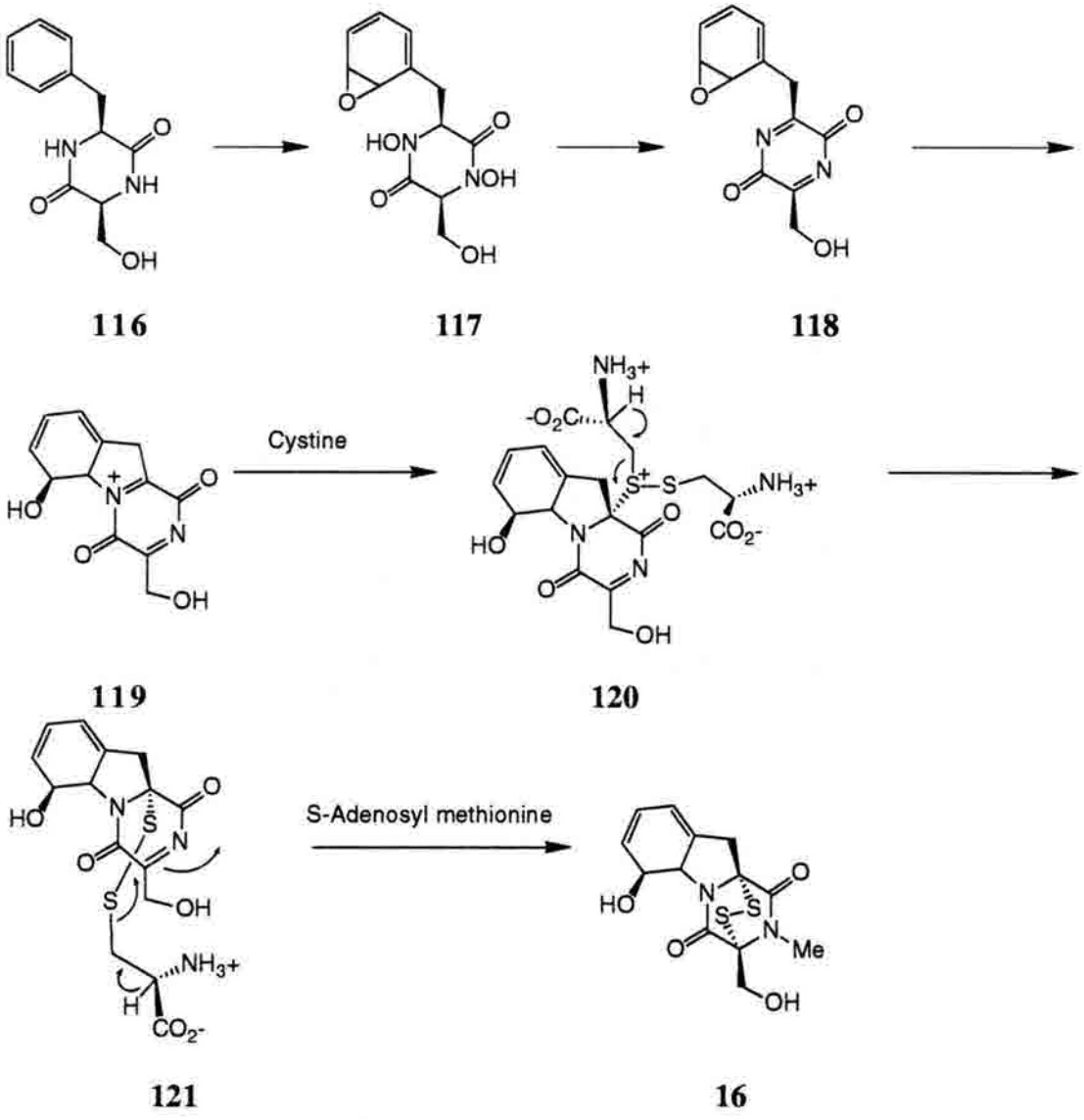


114 **115**

The dehydrodioxopiperazine **111** may arise from the decomposition of N-alkoxy amides and Ottenheijm has proposed a biosynthesis of gliotoxin based on the intermediacy of N-alkoxy amino acids which is shown in Scheme 12.[93] Furthermore, the order of events proposed by Ottenheijm are consistent with results from the recent labeling experiments carried out by Kirby.

The proposed biosynthesis from cyclo(L-Phe-L-Ser) (**116**) involves initial oxidation to give di-N-hydroxybenzene oxide **117** which decomposes to the N-acylimine species **118**. Ring closure of **118** generates the highly electrophilic N-acylimminium ion **119** which undergoes nucleophilic addition of sulfur supplied by cystine, generating the episulfonium ion **120**. β-elimination of the cystine moiety followed by nucleophilic

Scheme 12 Proposed biosynthesis of gliotoxin from N-alkoxy amino acids

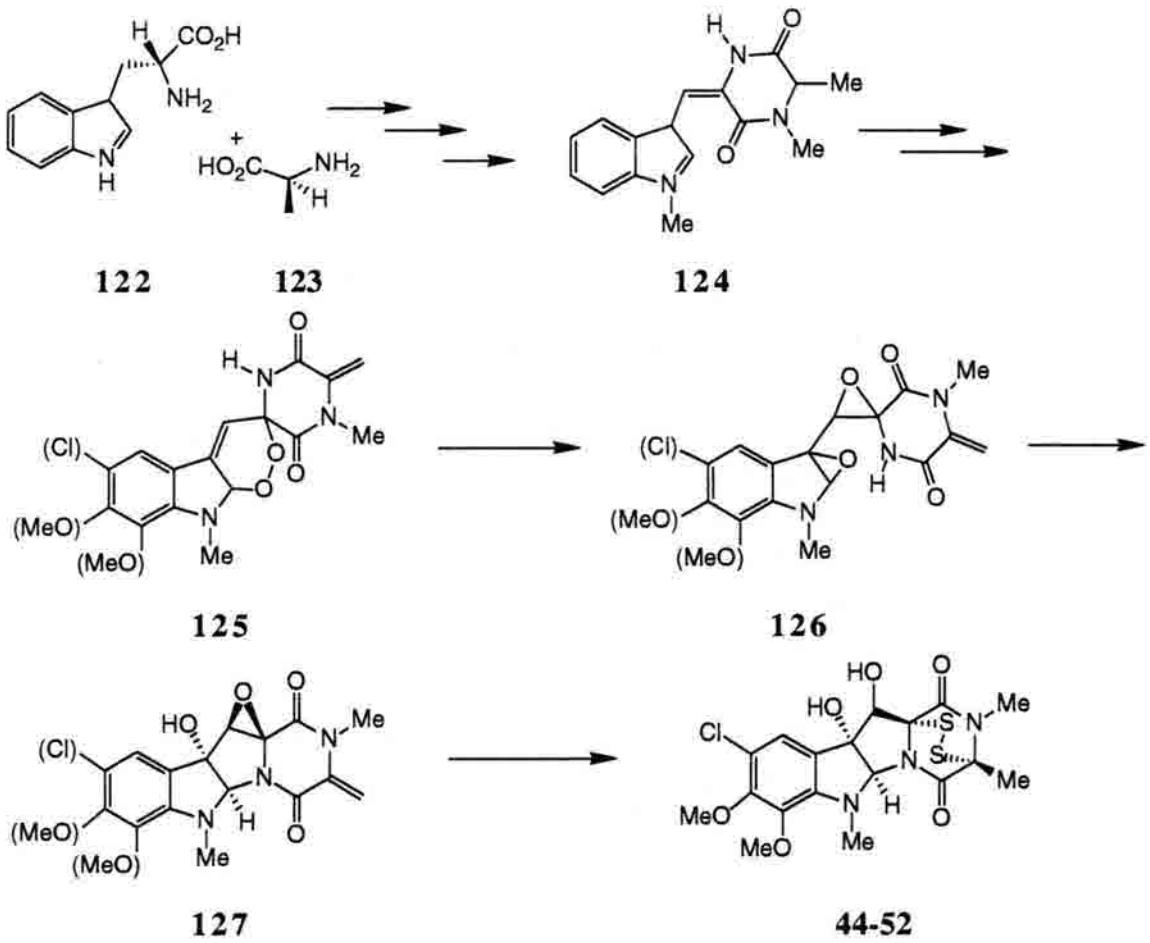


addition of the disulfide and N-methylation (**120→121→16**) affords gliotoxin. The proposed biosynthesis by Ottenheijm is unique, suggesting the disulfide bridge is incorporated in a single step.

The biosynthesis of the sporidesmins from tryptophan (**122**) and alanine (**123**) is presented in Scheme 13. The process involves an extensive series of transformations and is still not well understood. The general steps were first proposed by Sammes and are

considered to be similar to the sequence proposed for gliotoxin involving an oxidative ring closure of the intermediate arene oxide **126** to give the eserin ring system **127**.[94]

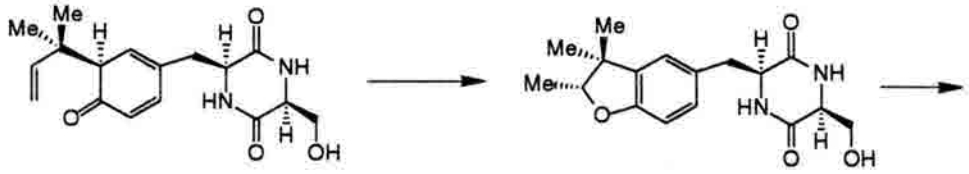
Scheme 13 Proposed biosynthesis of sporidesmins



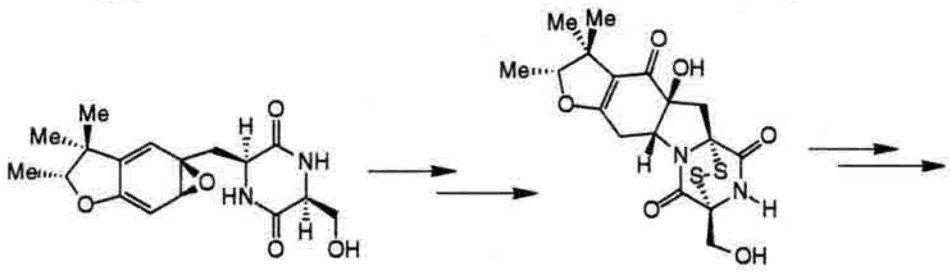
The biosynthesis of the sirodesmins is fairly well understood and the pathway is consistent with the mechanism proposed for gliotoxin. The original pathway from tyrosine (**128**) and serine (**84**) shown in Scheme 14 was proposed by Curtis et al. in 1977.[77] The mechanism has been supported by several feeding experiments.[95] Isolation of the proposed intermediate phomamide (**129**),[96] and the related sulfur-functionalized phomalirazine (**77**) from *P. lingam tode* provides additional evidence for the pathway.

Scheme 14 Proposed biosynthesis of Sirodesmins

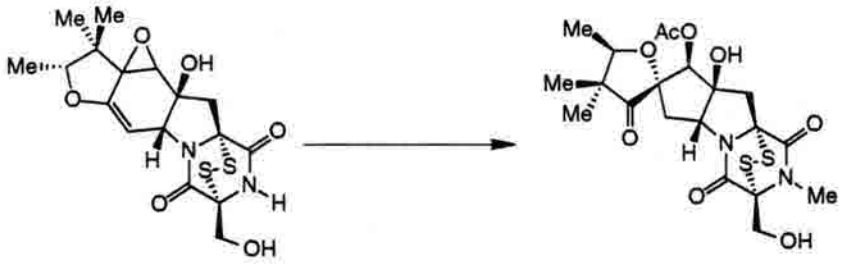
128 **84** **129, phomamide**



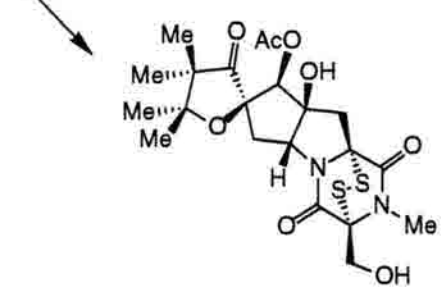
130 **131**



132 **77, phomalirazine**



133 **74, Sirodesmin PL**



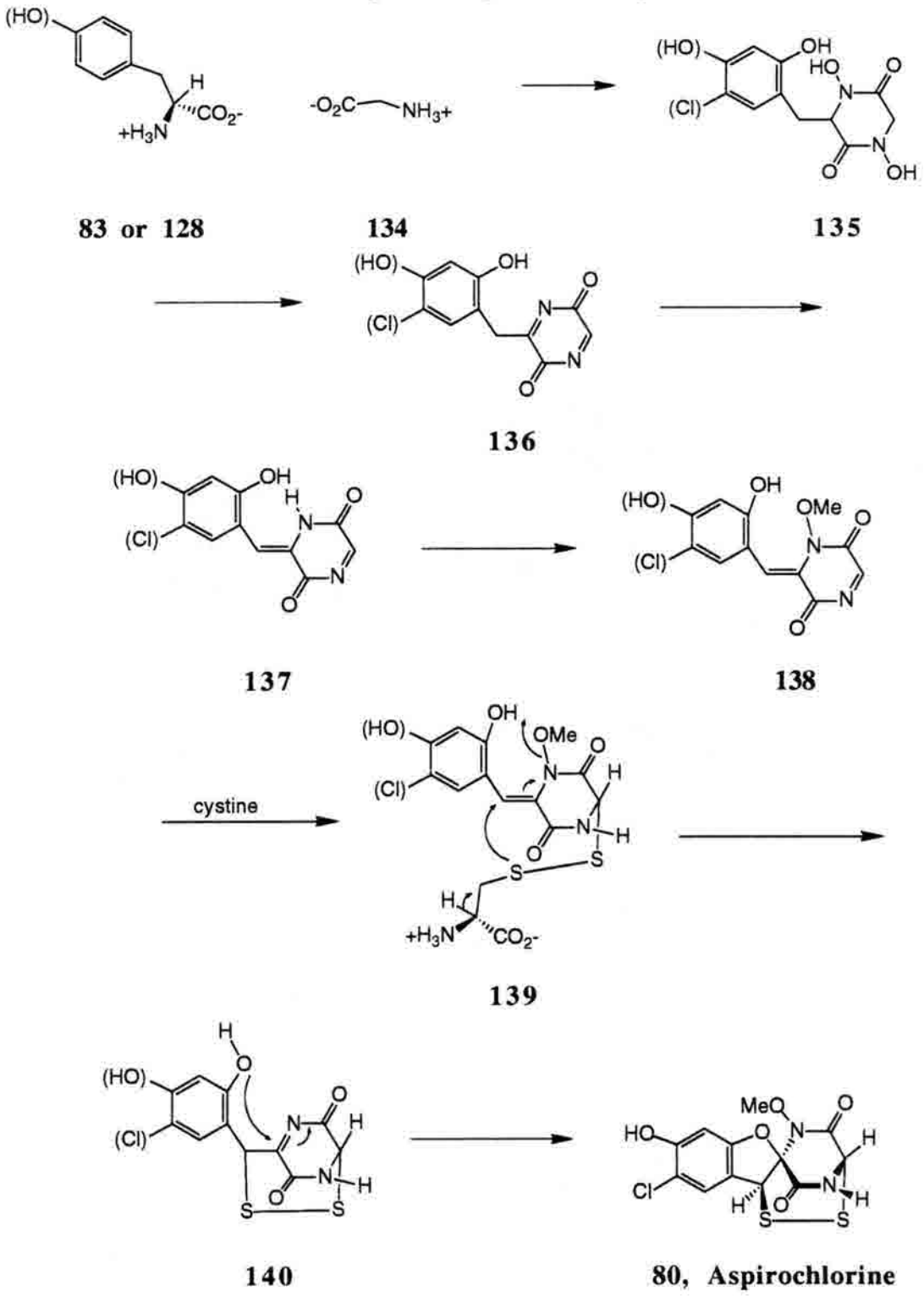
71-73, Sirodesmins A, B, C

The biosynthesis involves a Claisen rearrangement of the O-dimethylallyl tyrosine derivative **129** to afford cyclohexadienone **130** and subsequent cyclization to the benzofuran **131**. Formation of the pyrrolidinone ring is believed to occur via oxidative ring closure of arene oxide **132** to give ultimately **77** or **133.** Subsequent biosynthetic transformations including a ring contraction which converts **133** into both sets of sirodesmins.

No formal proposal for the biosynthesis of N-alkoxy containing epidithiodioxopiperazines has been developed. Scheme 15 presents a possible biosynthesis of aspirochlorine based on the proposed pathways presented for the other classes of epidithiodioxopiperazines. The presence of N-methoxyl groups in aspirochlorine indicates the biosynthesis most likely involves N-alkoxy amino acids as intermediates. With this assumption in mind, the pathway presented in Scheme 15 resembles the biosynthesis proposed by Ottenheijm for gliotoxin.

Formation of the diketopiperazine from either tyrosine **128** or phenylalanine **83** with glycine **134** is followed by oxidation to give the intermediate N-hydroxy diketopiperazine **135**. Elimination of two equivalents of water generates the intermediate bis N-acylimine **136** which rearranges to the more stable exocyclic olefinic N-acylimine **137**. Initial oxidation of the free amide followed by methylation generates the highly electrophilic N-methoxyl acylimine **138.** In the presence of cystine, incorporation of sulfur occurs initially at the α-position of the diketopiperazine moiety to afford the disulfide **139.** β-elimination of the cystine residue followed by S_N2' addition of the sulfur gives the disulfide **140.** Furan ring closure and subsequent oxidation/methylation leads to aspirochlorine.

Scheme 15 Proposed biosynthesis of Aspirochlorine



Chapter 2
Synthetic Studies of Epipolythiodioxopiperazines

The structural complexity and potent biological activity of the epipolythiodioxopiperazines has generated a tremendous amount of research. Over the past 20 years, the epidithiodioxopiperazine family has continued to steadily grow. Unfortunately, advances in new synthetic methodologies have not kept pace and as a result, only a handful of epidithiodioxopiperazines have been synthesized in either racemic or optically active form.

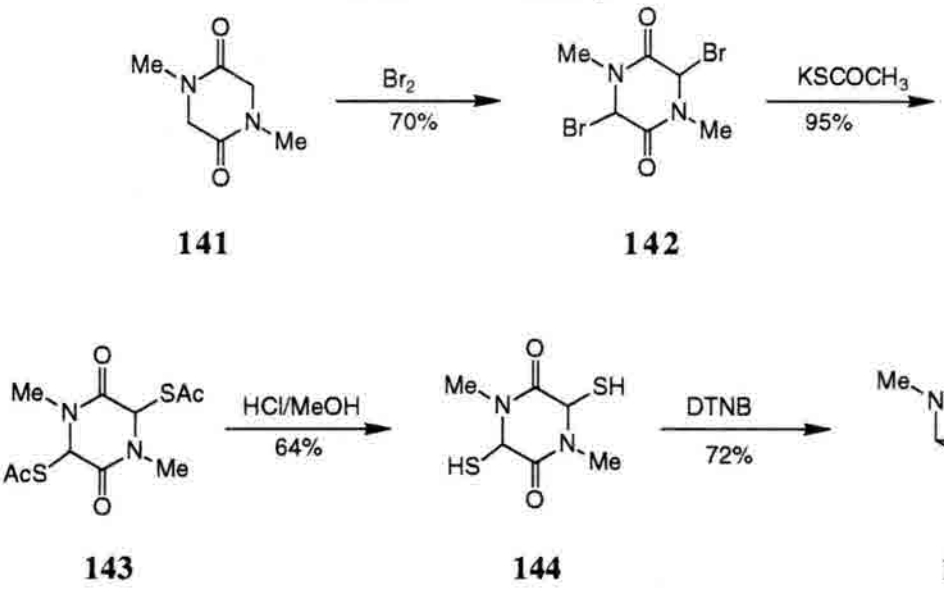
A major concern in the synthesis of these unique metabolites is the stereoselective introduction of sulfur, a problem which still persists. The most successful approaches developed thus far incorporate sulfur into a preformed diketopiperazine ring.

The synthesis of epidithiodioxopiperazines is further complicated by the sensitivity of the polysulfide bridge to a variety of conditions commonly encountered during a total synthesis. The disulfide bridge is easily oxidized, reactive towards reducing agents and readily decomposes under basic conditions. Only under acidic conditions is the disulfide bridge relatively stable. The sensitivity to base is accentuated by hydroxymethyl substituents at the α-position which are present in gliotoxin and related metabolites. In light of the sensitivity to most conditions, incorporation of the disulfide bridge is usually reserved for the end of a synthesis.

The early literature prior to 1980 has been extensively reviewed.[2] For the sake of brevity, only the most useful methods that have been instrumental in the synthesis of natural products will be discussed in this chapter.

Scheme 1 represents the first synthesis of the epidithia-2,5-piperazinedione ring **145** by Trown in 1968, and this approach is still one of the most useful methods.[97] Bromination of sarcosine anhydride (**141**) with bromine afforded the intermediate

Scheme 1 Trown synthesis

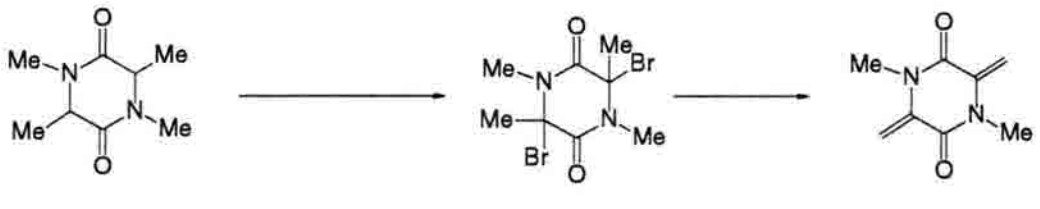


141 **142** **143** **144** **145**

dibromide **142** as a mixture of *cis* and *trans* isomers. Conversion to a mixture of thioacetates **143** was effected upon workup with potassium thioacetate. Acidic hydrolysis to the intermediate dithiol **144** followed by oxidation with 5,5'-dithiobis(2-nitrobenzoic acid, DTNB) completed the synthesis.

A drawback to methodology developed by Trown is the lack of stereochemical control resulting from non-selective bromination. Furthermore reports have indicated the oxidation using DTNB is difficult to reproduce leading to a mixture of products.[98]

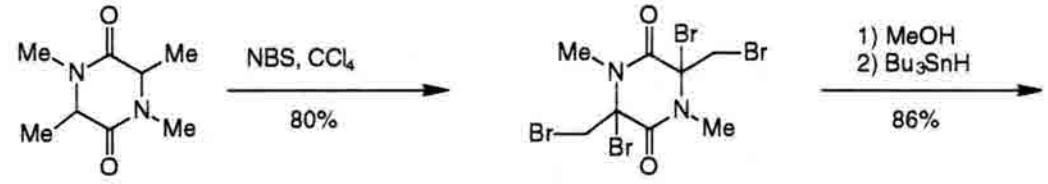
The methodology has also been reported to be unsuited for substituted diketopiperazines such as **146** resulting in formation of dehydro diketopiperazines such as **148** from facile elimination of HBr from the intermediate dibromide **147**.



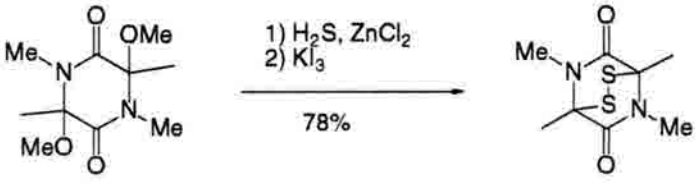
146 **147** **148**

Yoshimura attempted to avoid formation of dehydro derivatives such as **148** and the approach is presented in Scheme 2.[99] Exhaustive bromination of dimethyl sarcosine anhydride (**146**) afforded the tetrabromide **149** in 80% yield. Substitution of the more labile bromides with methoxide followed by hydrogenation of the primary bromides with tributyltin hydride generated the α-methoxy diketopiperazine **150.** Sulfenylation was carried out using H_2S/ $ZnCl_2$ followed by oxidation with KI_3 to give disulfide **151** in 78% yield.

Scheme 2 Yoshimura et al.

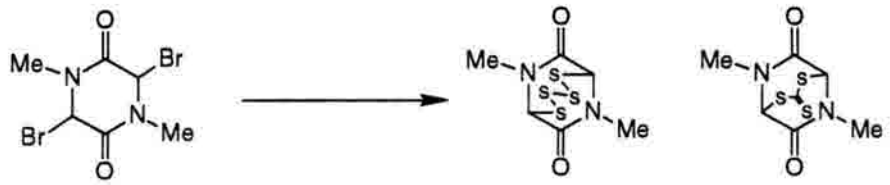


146 **149**



150 **151**

There have been a variety of methods designed to introduce the disulfide bridge intact thus (in theory) avoiding the formation of mixtures of *cis* and *trans* dithiols. Schmidt demonstrated dibromide **142** could be converted into tetrasulfides **152** or trithiocarbonates **153** by reaction with nucleophilic difunctional sulfur reagents such as sodium tetrasulfide

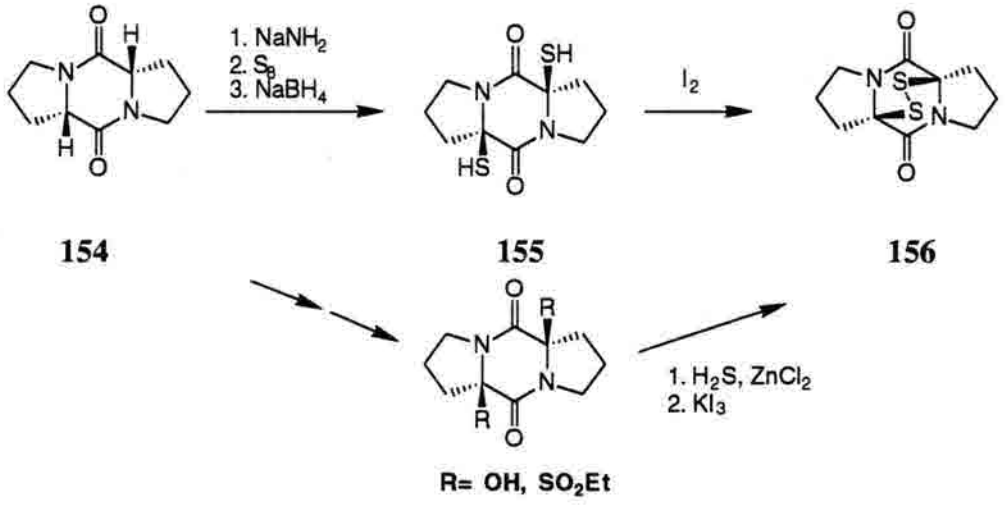


142 **152** **153**

(Na_2S_4 or sodium sulfide in the presence of sulfur) or sodium trithiocarbonate.[98, 100] Reduction of the tetrasulfide **152** leads to stereochemically pure *cis* dithiols.[59f]

Attempts to incorporate the disulfide bridge using electrophilic forms of sulfur have also been investigated by a number of groups. Schmidt et al., in a series of papers investigated the reaction of elemental sulfur with the dianion generated from metallation of cyclo-L-prolyl-L-proline (Scheme 3 **154→155**).[101] Reductive workup of the intermediate oligosulfides followed by oxidation with iodine or KI_3 lead to disulfide **156** in good yields. In addition, **156** retained the absolute configuration of the starting diketopiperazine and therefore was optically active.

Scheme 3. Summary of Schmidt et al.



154 **155** **156**

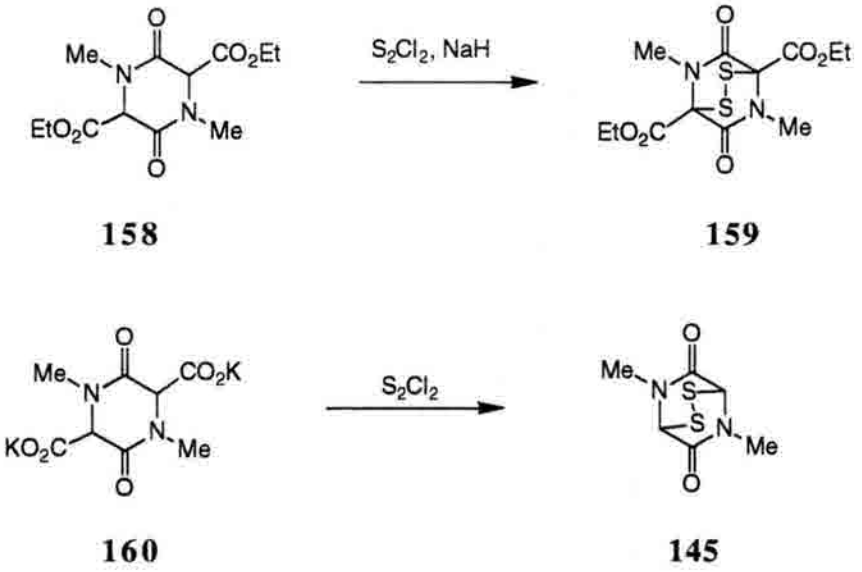
157

Further studies by Schmidt, demonstrated the facile nucleophilic displacement of hydroxyl or sulfone derivatives **157** by thiolates in the presence of zinc chloride could be efficiently carried out (ie Scheme 3 **154→155→156**).[102]

Hino and Sato devised a protocol to introduce the disulfide bridge in a single transformation as shown in Scheme 4.[103] Heating 3,6-diethoxycarbonyl-1,4-

dimethyldiketopiperazines **158** with sulfuryl dichloride in the presence of sodium hydride resulted in formation of the desired disulfide **159** in 17% yield. In addition varying

Scheme 4. Hino and Sato

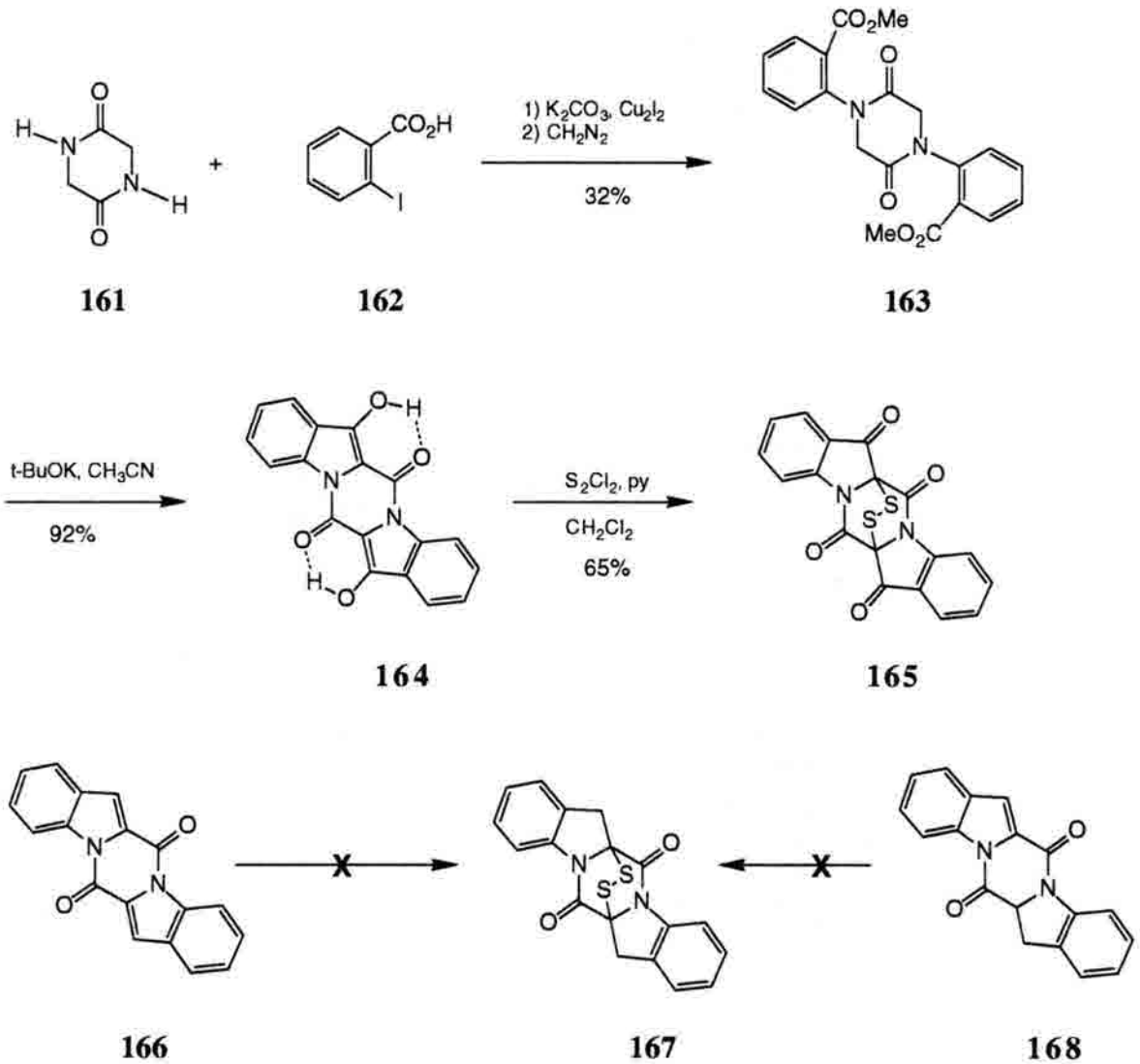


amounts of mono,di,tri and tetrasulfides were observed. Attempted reduction of **159 to**to the corresponding dithiol with sodium borohydride regenerated the starting diester **158** via elimination of sulfur.

The methodology was improved slightly by heating the dipotassium dicarboxylate salts **160** with sulfuryl dichloride in dioxane affording disulfide **145**. Sulfenylation occurs with concurrent dicarboxylation to give the epidithiodioxopiperazines in 33% yields.[104]

A similar approach was successfully used by Coffen in the synthesis of a variety of aromatic analogues of aranotin displayed in Scheme 5.[105] Reaction of the symmetric indole **163** with sulfuryl chloride in pyridine resulted in 65% conversion to the disulfide **165**. Ironically, Coffen was unable to introduce sulfur into the indole derivatives **166** or **168** which were considered to be possible biosynthetic intermediates.

Scheme 5



161 **162** **163**

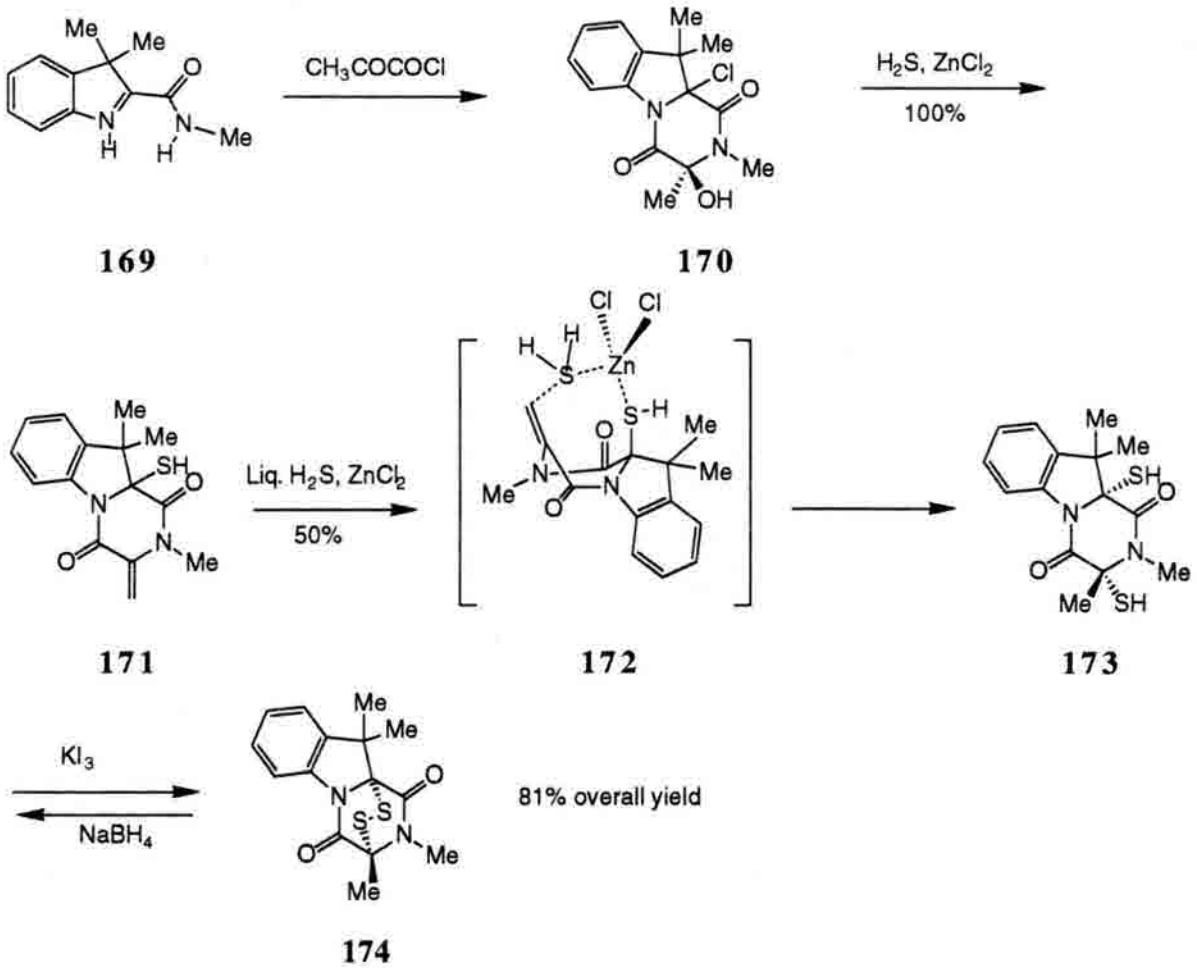
164 **165**

166 **167** **168**

Of the various methods developed to incorporate sulfur in simple models, the Lewis acid-assisted nucleophilic substitution reaction by thiols has been the most useful. This is exemplified in Ottenheijm's 3-step synthesis of gliotoxin analogues shown Scheme 6.[106]

Treatment of chloride **170** with a saturated solution of H_2S in methylene chloride resulted in clean conversion to the mono thiol alkene **171**. Under more pressing conditions (liquid H_2S in the presence of $ZnCl_2$) the cis dithiol **173** was obtained as a single isomer.

Scheme 6

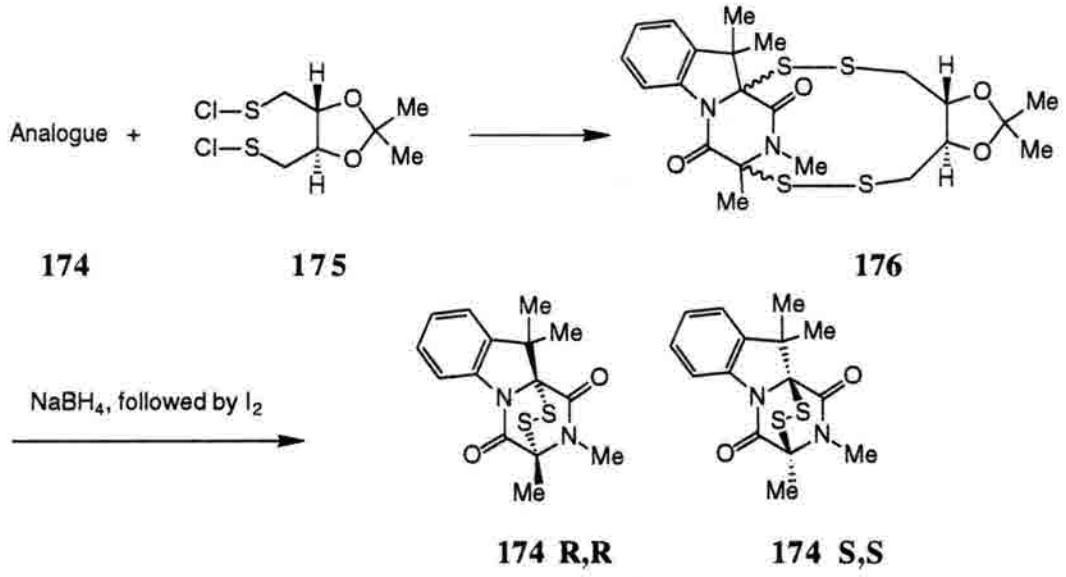


169 **170** **171** **172** **173** **174**

Formation of the cis dithiol **173** was proposed to occur via the chelation-controlled transition state **172** in which zinc directs the approach of H_2S to the olefin face.

During subsequent investigations into the synthesis of gliotoxin and analogues such as **174**, Ottenheijm developed a unique method for the resolution of epidithiodioxopiperazines depicted in Scheme 7.[107] The method outlined in Scheme 7 involves sulfenylation of dithiols **173** with the optically active sulfenyl chloride **175** obtained from optically active Cleland's reagent (DTT). The resulting diastereomeric mixture of mixed disulfides **176** could be easily separated by chromatography. Reduction of the individual disulfide diastereomers **176** using sodium borohydride followed by

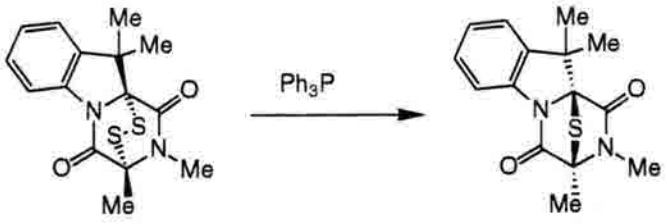
Scheme 7 Optical resolution



oxidation afforded the enantiomerically pure epidithiodioxopiperazines **174.** The absolute configuration of each enantiomers was verified by X-ray analysis.

An unusual reaction characteristic of epipolythiodioxopiperazines is the desulfurization with triphenyl phosphine. Early reports by Taylor involving reactions with dehydrogliotoxin suggested the desulfurization proceeded with inversion of configuration at the bridgehead positions of the diketopiperazine ring.[59g]

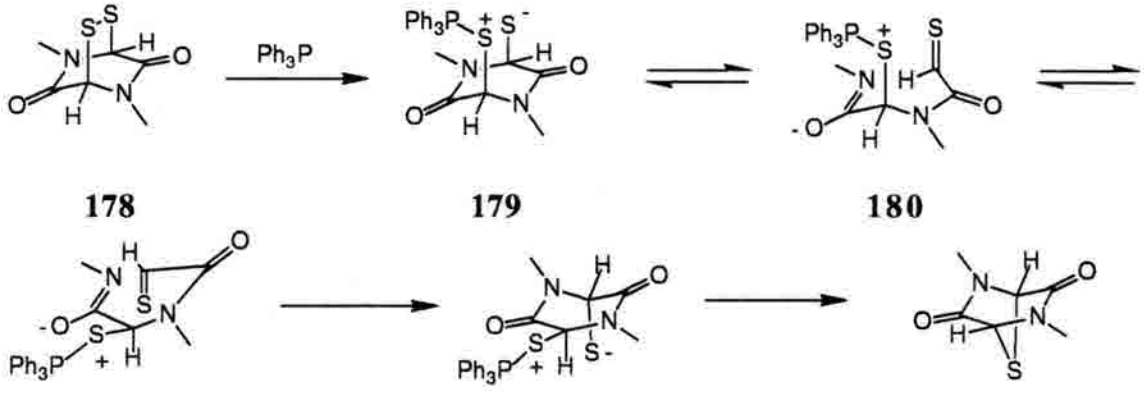
Using the enantiomerically pure analogs **174**, Ottenheijm investigated the desulfurization reaction using triphenyl phosphine and established that the reaction does proceed with inversion of configuration.[108] Reaction of (R,R)-**174** with triphenyl phosphine generated the (S,S)-monosulfide **177.** The structure of the monosulfide was confirmed by NMR experiments using chiral shift reagents and verified by X-ray analysis.



174 R,R **177 S,S**

The generally accepted mechanism for inversion is illustrated in Scheme 8. Nucleophilic attack on sulfur by triphenyl phosphine generates a thiophosphonium ion **179**. Decomposition of the sulfonium ion to thione **180** results in cleavage of the diketopiperazine ring. **180** undergoes bond rotation to **181** followed by ring closure resulting in net inversion at one bridgehead position to give **182**. The nucleophilic substitution results in formation of the monosulfide **183** with net inversion of configuration.

Scheme 8. Proposed Mechanism of Inversion



178 **179** **180**

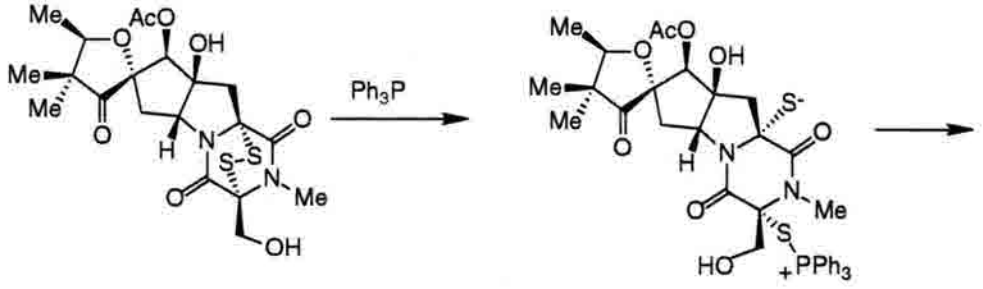
181 **182** **183**

The proposed mechanism is similar to mechanisms proposed for the conversion of *trans* dithiols to *cis* disulfides.[109]

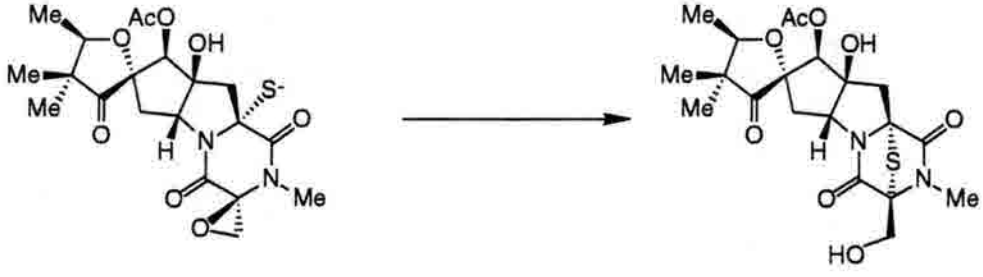
In an unusual turn of events, Férézou and coworkers observed desulfurization of sirodesmin PL **74** with triphenyl phosphine occurred with apparent net retention of

configuration about the diketopiperazine moiety.[110] To account for this result the following mechanism was proposed and is illustrated in Scheme 9. The mechanism is consistent with the mechanism by Ottenheijm/Taylor.

Scheme 9. Desulfurization of Sirodesmin PL with triphenyl phosphine



74, sirodesmin PL **184**

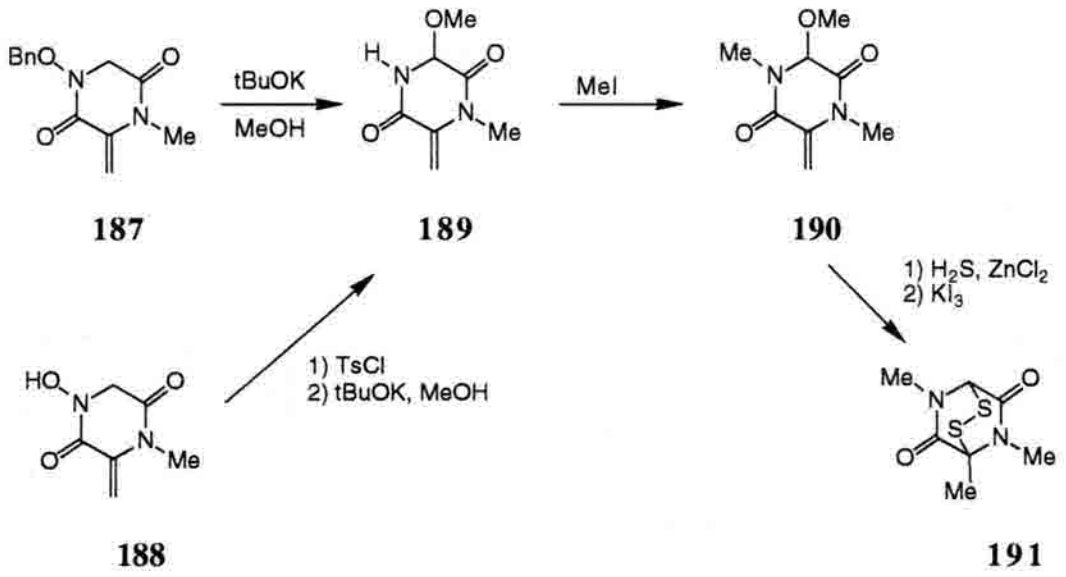


185 **186**

Nucleophilic addition of triphenylphosphine to the least hindered sulfur results in ring opening generating the thiophosphonium ion **184** which rapidly undergoes nucleophilic substitution by the β-hydroxy group generating the intermediate epoxide **185**. Ring opening of the epoxide by thiolate anion results in formation of the monosulfide **186** with net retention of configuration about the diketopiperazine group.

A recent approach to incorporate sulfur into diketopiperazines in a biosynthetic fashion has been developed by Herschied and Ottenheijm and the general approach shown in Scheme 10.[93a,b]

Scheme 10.



187 **189** **190**

188 **191**

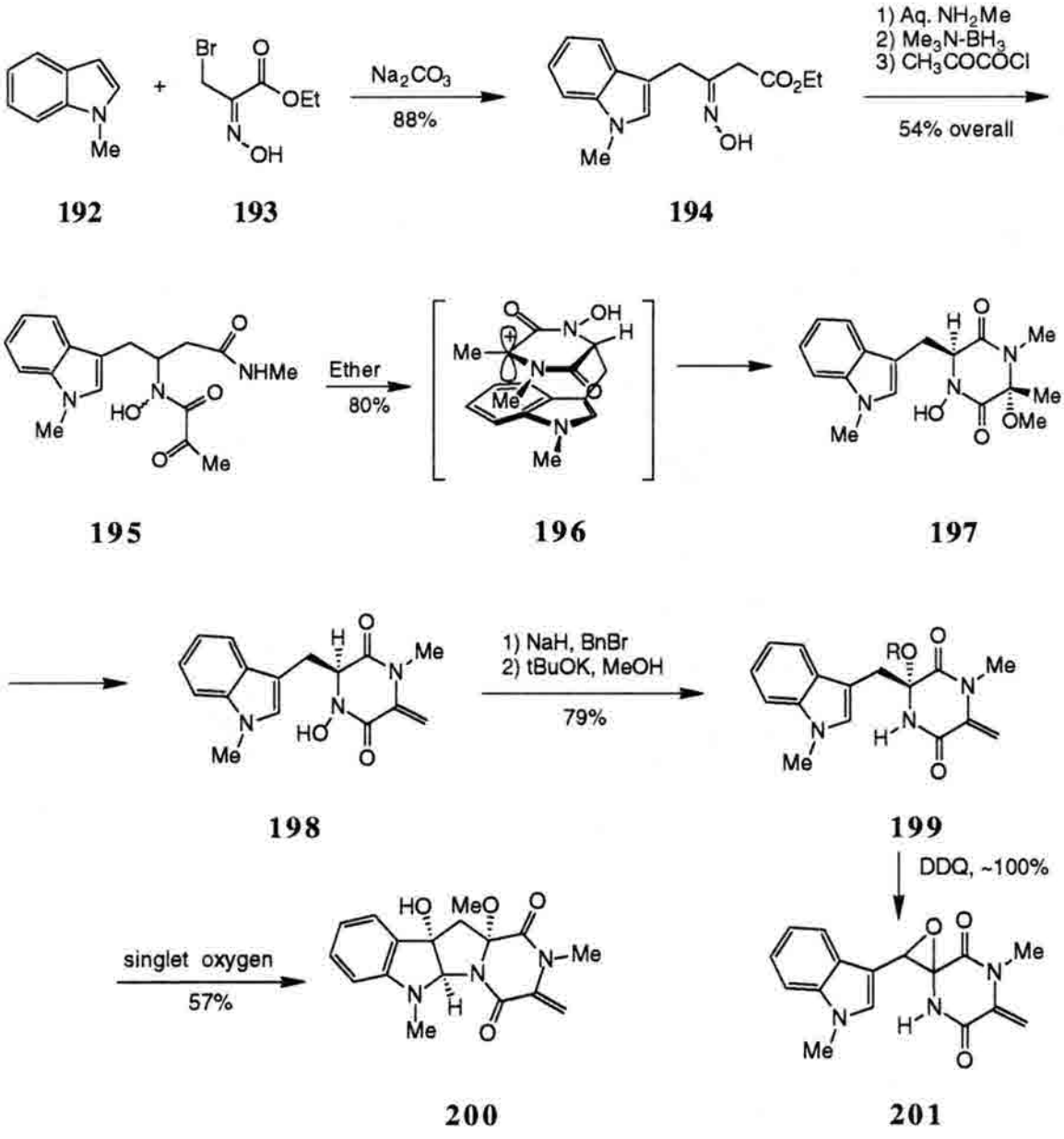
The method involves treating N-alkoxy diketopiperazines **187,** or N-hydroxy diketopiperazine **188** with potassium tert-butoxide in the presence of methanol, generating an intermediate N-acyl imine which undergoes rapid nucleophilic addition by solvent to give **189**. Following N-alkylation, the α-methoxy diketopiperazines **190** are efficiently converted into the epidithiodioxopiperazine **191** using $H_2S/ZnCl_2$ followed by oxidation.

This methodology was exploited in the synthesis of N-hydroxytryptophans which were purported to be possible sporidesmin biosynthetic intermediates. The synthetic approach is shown in Scheme 11.[111]

Coupling of Gilchrist's reagent **193** and readily available N-methyl indole **192** afforded the intermediate oxime **194**. Aminolysis using methylamine, reduction of the oxime with trimethylamine borane complex followed by N-acylation with pyruvoyl chloride generated the N-pyruvoyl-N-hydroxtryptophan **195.** Compound **195** underwent spontaneous cyclization to afford the dehydro diketopiperazine **198**.

The cyclization is believed to occur via the cationic transition state **196** in which the diketopiperazine is folded over the indole ring. Evidence for the proposed transition

Scheme 11 Synthesis of Sporidesmin Biosynthetic Intermediates



192 **193** **194** **195** **196** **197** **198** **199** **200** **201**

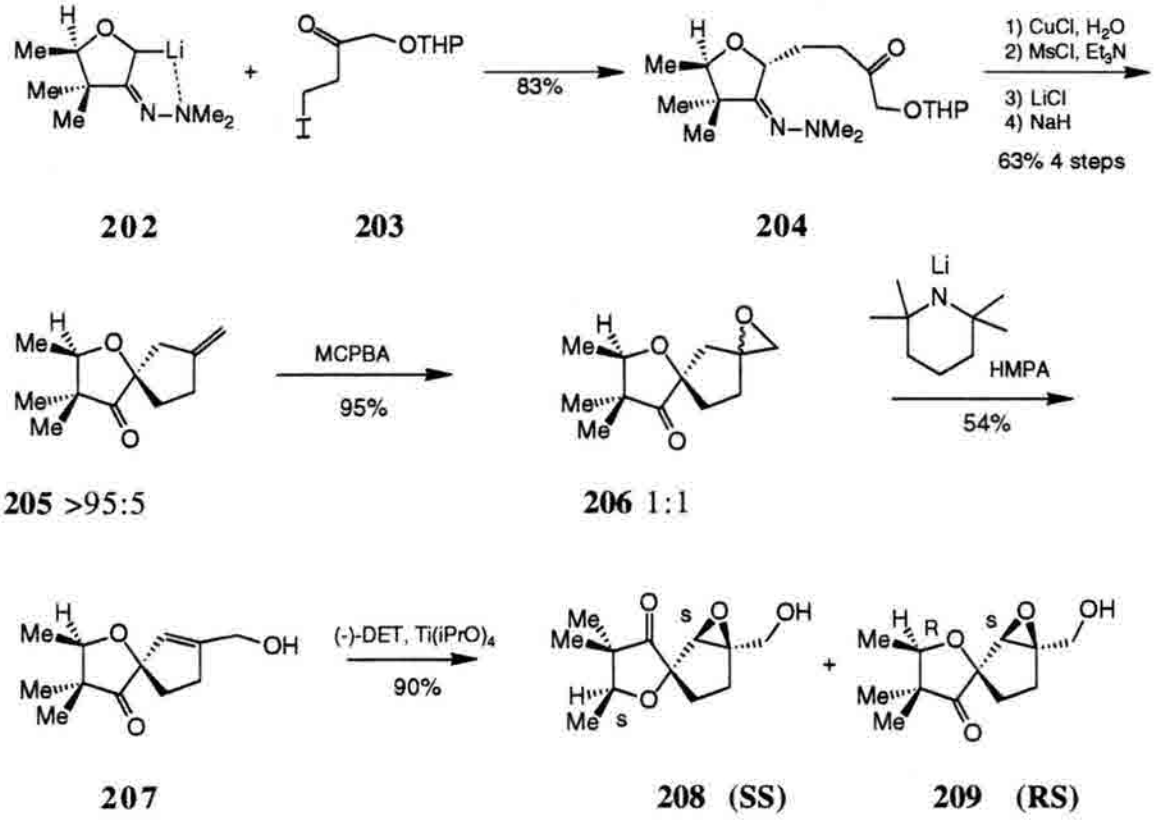
state was obtained from quenching experiments with methanol which generated the α-methoxy substituted **197** as a single stereoisomer. Compound **197** slowly underwent elimination of methanol to **198**. N-Benzylation with sodium hydride/benzyl bromide followed by treatment with t-butoxide in methanol produced the desired rearranged indole derivative **199.**

Oxidation of **199** with DDQ results in the synthesis of α,β-epoxy tryptophan **201** which serves as a model for the proposed biosynthesis of sporidesmins (see chapter 1, Scheme 12, **126→127**). Unfortunately all attempts to convert **201** into other proposed biosynthetic intermediates have failed.

Treatment of **199** with singlet oxygen generated the tetracyclic eserine derivative **200** containing the correct relative stereochemistry present in the sporidesmins. The conversion of **200** into the sporidesmins has been prohibited by the inability to substitute the methoxy group with sulfur.

The sirodesmins have also been the focus of an attempted total synthesis. Rastetter and Adams synthesized the spirofuran portion of sirodesmin A in a stereoselective fashion

Scheme 12. Synthesis of the spirofuran moiety of Sirodesmin A

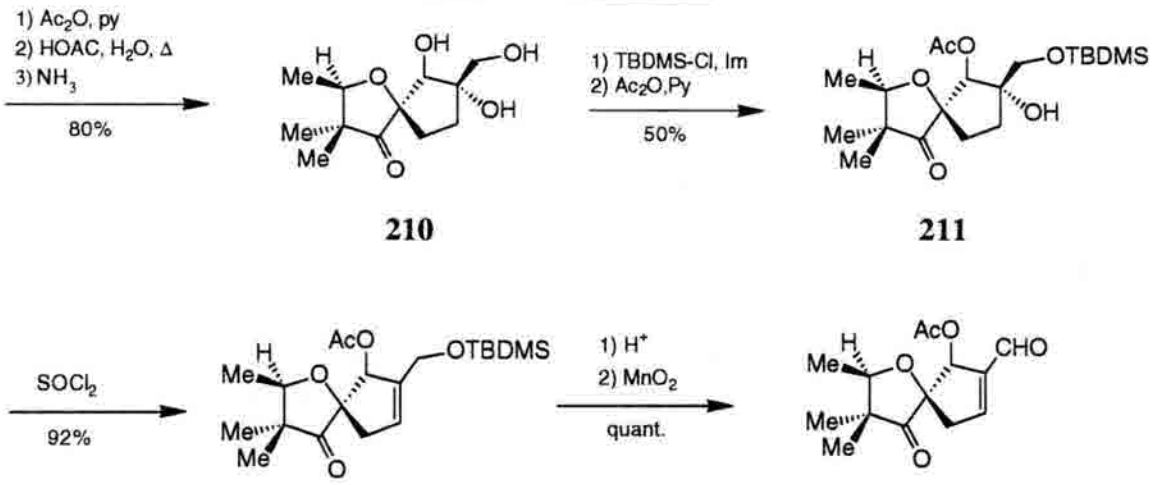


202 **203** **204**

205 >95:5 **206** 1:1

207 **208 (SS)** **209 (RS)**

Scheme 12 continued

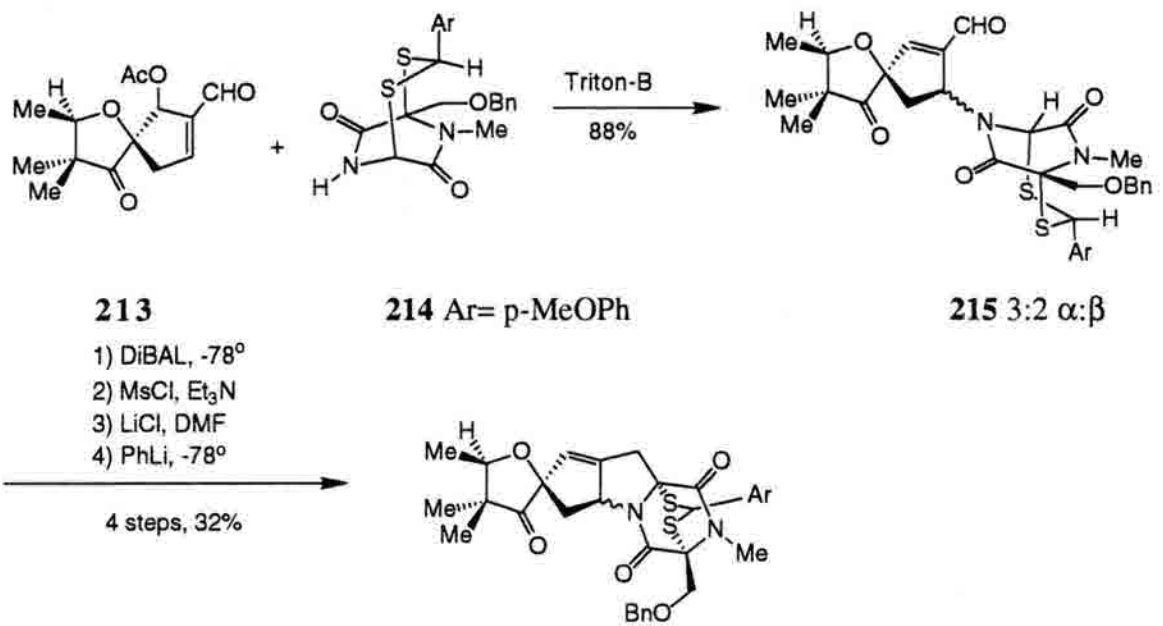


210 **211**

212 **213**

depicted in Scheme 12. [112] The correct relative and absolute configuration was established in a highly enantioselective Sharpless epoxidation of **207** to give the epoxy ketones **208** and **209**. Expoxy ketone **209** contains the correct absolute configuration for sirodesmin A and was converted to the protected spirofuranone **213** is a series of protection steps.

Scheme 13. Synthesis of skeleton of Sirodesmin A



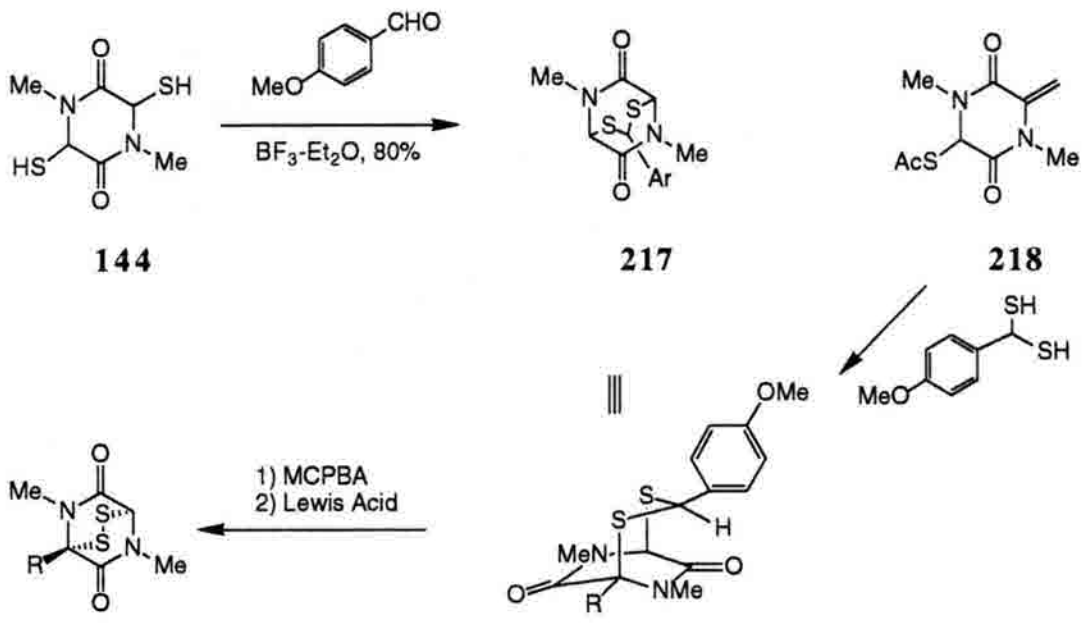
213 **214** Ar= p-MeOPh **215** 3:2 α:β

216

Basak utilized the work of Rastetter and reported the first total synthesis of the skeleton of sirodesmin in 1985 shown in Scheme 13.[113] Coupling of **213** with the dithioacetal protected diketopiperazine **214** in the presence of Triton B resulted a 3:2 mixture of diastereomers **215.** The diastereomers could be separated by column chromatography. Conversion of the aldehyde in **215** into an allylic chloride was achieved in several steps. Ring closure to the protected form of sirodesmin A **216** was obtained in 32% overall yield from the adduct **215**. No mention was made regarding the conversion or attempted conversion of **216** into sirodesmin PL.

In 1973 Kishi described a novel procedure to synthesize epidithiodioxopiperazines using a dithioacetal moiety as a protecting group for the disulfide bridge. The methodology is presented in Scheme 14.[114] Treating a mixture of dithiols **144** with *p*-anisaldehyde

Scheme 14. Kishi's dithioacetal Protection

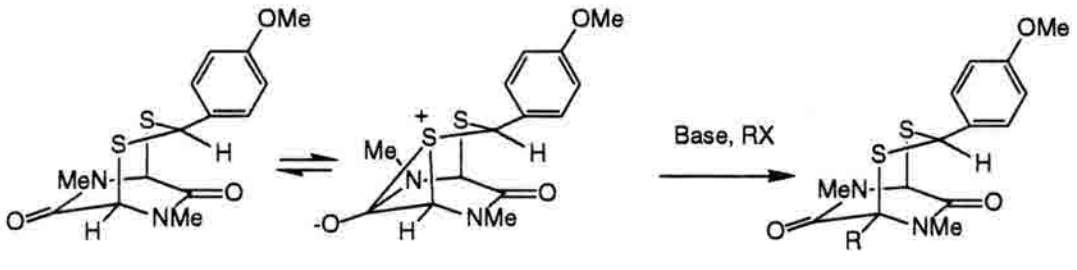


in the presence of strong Lewis acids such as boron trifluoride etherate afforded the dithioacetal **217** in 80% yields. Deprotection to the disulfide **219** was achieved in a high-yield two-step oxidative process. Initial treatment of dithioacetal **217** with m-CPBA (*m*-chloroperbenzoic acid) affords an intermediate sulfoxide, which when treated with strong Lewis acids decomposes to the disulfide directly. Interestingly, deprotection fails using dithioacetals derived from formaldehyde, acetaldehyde or benzaldehyde, indicating electron-rich aldehydes are required.

A variation of the protocol utilized the dithiane derivative of anisaldehyde. Reaction of the diketopiperazine **218** with thioacetal of p-anisaldehyde extended the methodology to the synthesis of methyl-functionalized diketopiperazines **219** (R= Me) commonly found in the sporidesmins.

The dithioacetal protected diketopiperazines **217** are stable to acidic, basic and reducing conditions. The stability of the dithioacetal moiety makes it highly attractive in a total synthesis endeavor allowing introduction of the thiol groups at any stage.

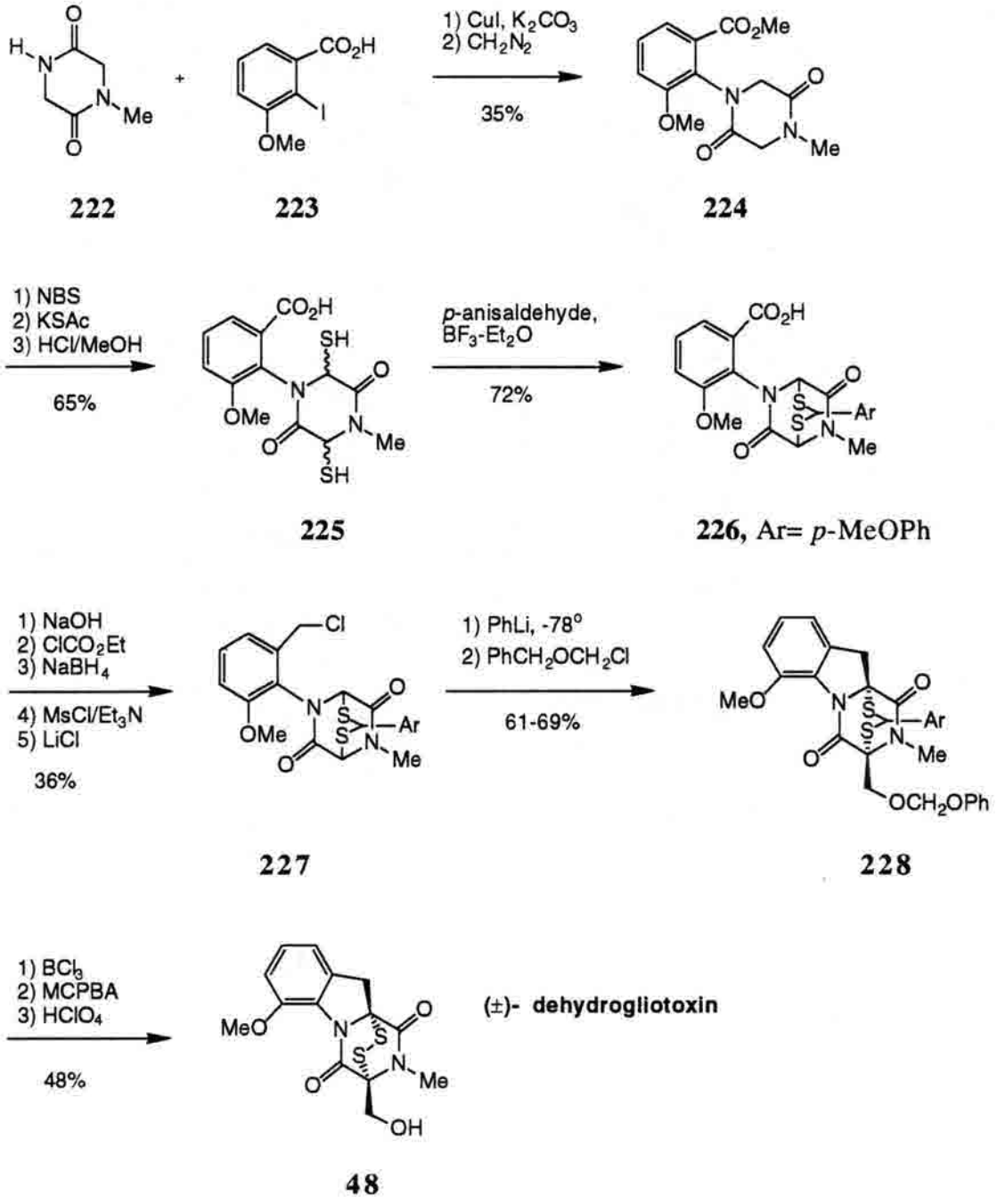
Extensive investigations of the chemical reactivity of the dithioacetal **217** indicated that the compounds can be regioselectively alkylated or acylated under kinetic conditions. Furthermore, under carefully controlled conditions, both bridgehead carbons can be functionalized in a predictable manner. The observed diastereoselectivity in the alkylation reactions is believed to be controlled by the orientation of the anisaldehyde group as depicted below. The anisaldehyde group is believed to force one of the sulfur atoms to be



217 **220** **221**

closer to the carbonyl carbon. Non-bonded interactions between the sulfur lone pairs and the carbonyl carbon (eg. **220**) causing a decrease in the pKa of the bridgehead proton resulting in regiospecific carbanion formation and upon alkylation generates **221.**

Scheme 15. Total Synthesis of (±)-dehydrogliotoxin



222 **223** **224**

225 **226,** Ar= *p*-MeOPh

227 **228**

48 (±)- **dehydrogliotoxin**

The importance of Kishi's dithioacetal protection strategy is apparent in the numerous total syntheses carried out using this methodology and include (±)-dehydro gliotoxin, (±)-gliotoxin, (+)-gliotoxin , (±)- sporidesmin A and B and (±)- hyalodendrin.

The synthesis of (±)-dehydrogliotoxin is shown in Scheme 15.[115] Coupling of N-methyl glycine anhydride **222** with 2-iodo-3-methoxy benzoic acid **223** in the presence of copper iodide affords the N-phenylated diketopiperazine. Esterification with diazomethane afforded the functionalized diketopiperazine **224**.

Bromination and sulfenylation using conditions reported by Trown followed by acidic hydrolysis generated a mixture of *cis* and *trans* mercaptans **225**. The dithiols were converted into a 1:1 mixture of *p*-anisaldehyde protected dithioacetals **226** in 72% yield.

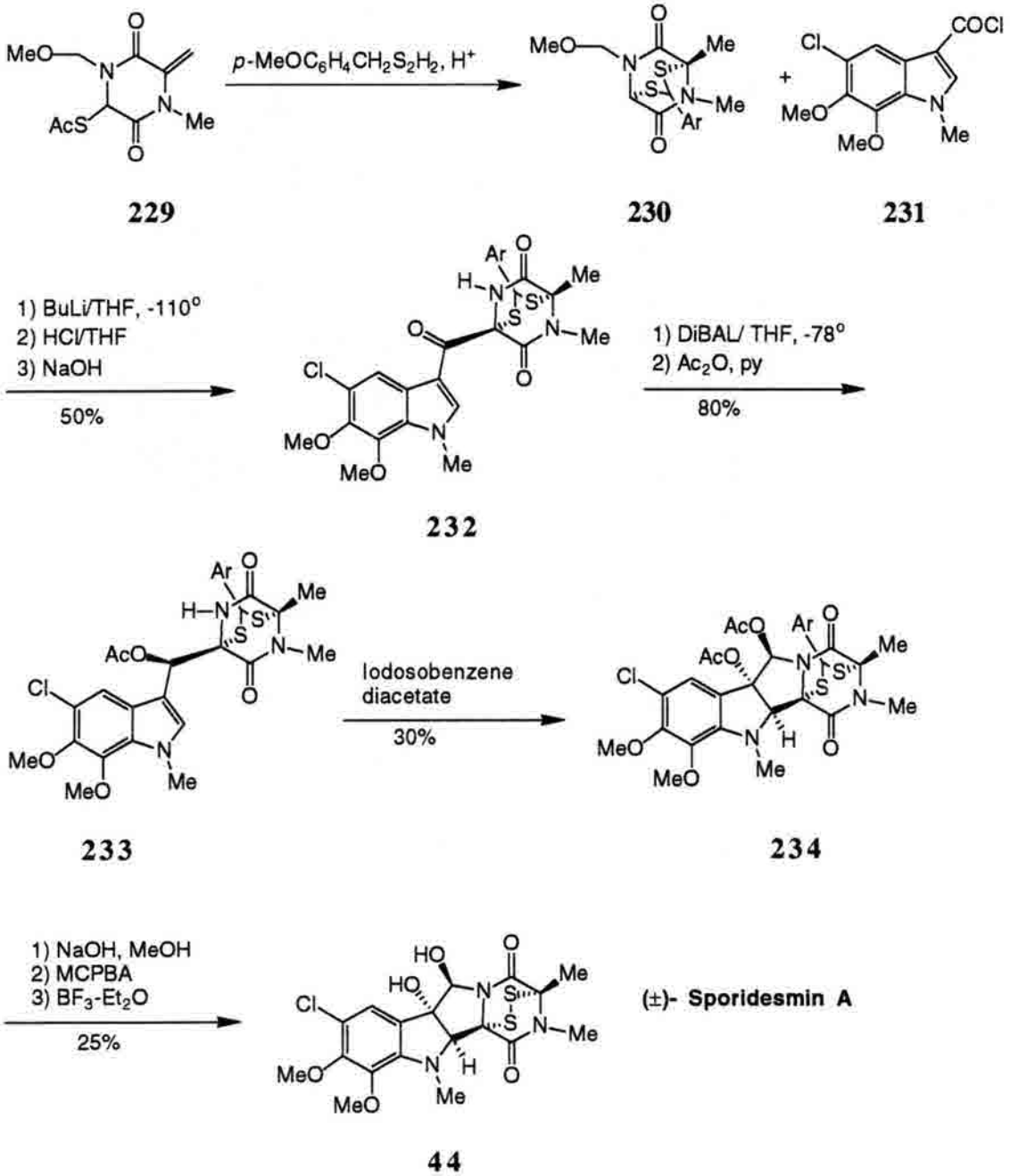
Following a series of transformations, the mixture of thioacetals **226** was converted into the benzylic chlorides **227**. Stereoselective ring closure to the indole nucleus **228** and the incorporation of the benzyloxymethyl moiety was achieved by lithiation with phenyl lithium followed by treatment with phenoxymethyl chloride. Deprotection with boron trichloride followed by oxidative deprotection afforded (±)-dehydrogliotoxin **48**.

The total synthesis of sporidesmin A was carried out in a similar fashion and is shown in Scheme 16.[116] The key dithioacetal-protected diketopiperazine **230** was obtained using a slight modification of Kishi's original protocol. Conversion of the thioacetate **229** into **230** was performed by treatment with the dithiane derivative of *p*-anisaldehyde in the presence of acid.

Condensation of dithioacetal-protected **230** with acid chloride **231** followed by removal of the nitrogen methoxymethyl protecting group afforded **232**. Chelation controlled stereoselective reduction of the ketone was carried out using diisobutyl aluminum hydride at low temperature, and the secondary alcohol protected as an acetate to give **233** in 80% yield. Oxidative cyclization was performed using iodosobenzene diacetate and the tetracyclic derivative **234** was obtained in 30% yield.

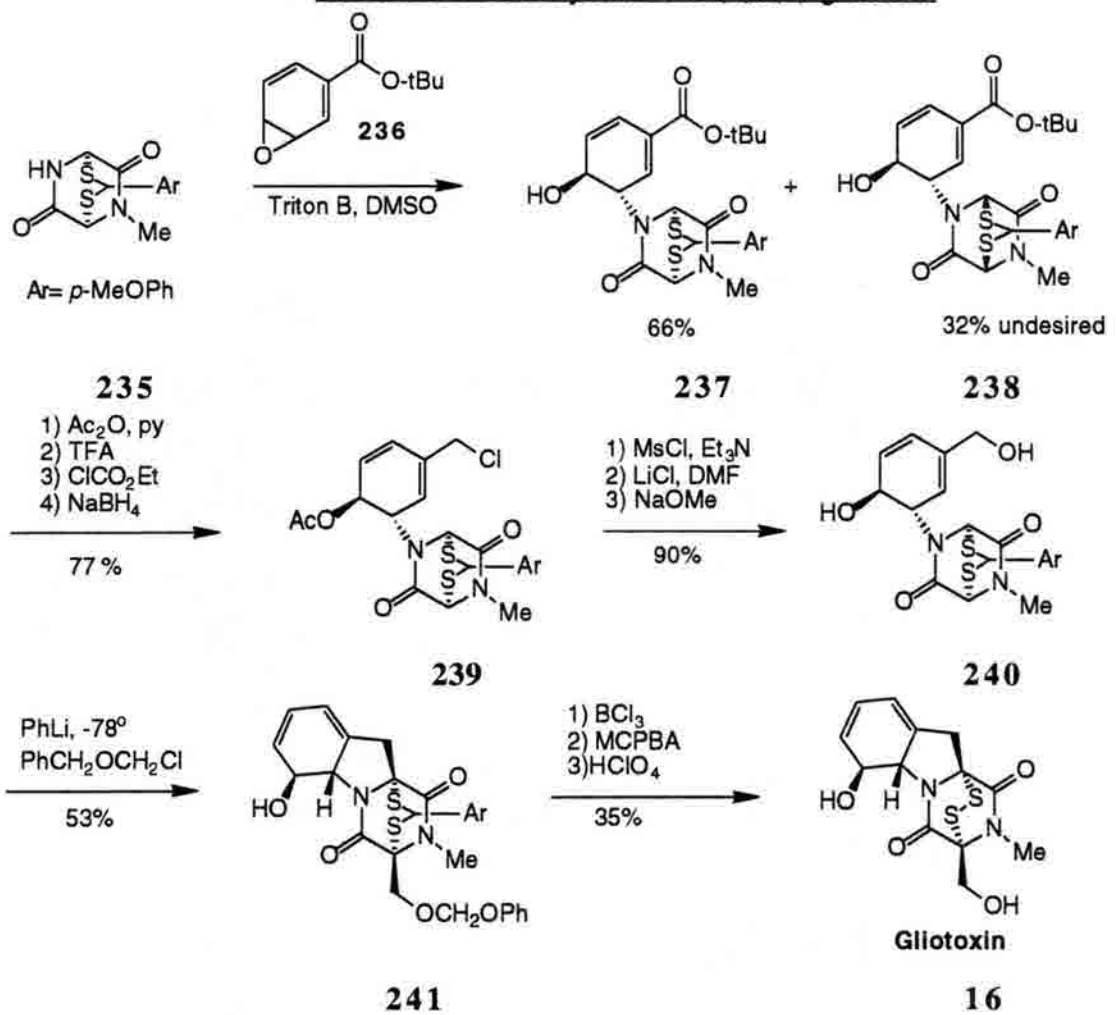
Hydrolysis of the acetate followed by deprotection of the dithioacetal lead to the total synthesis of (±)-sporidesmin A. The same series of reactions was utilized in the synthesis of the isomeric (±)-sporidesmin B.[117]

Scheme 16. Total synthesis of (±)-Sporidesmin A



The synthesis of (±)-gliotoxin was completed in 1976 and is presented in Scheme 17.[118] The synthesis of (+)-gliotoxin was achieved in 1981 using the same route starting from optically active dithioacetal **235** obtained from resolution.[119] The key step in the gliotoxin synthesis is the stereoselective formation of the dihydrobenzene moiety.

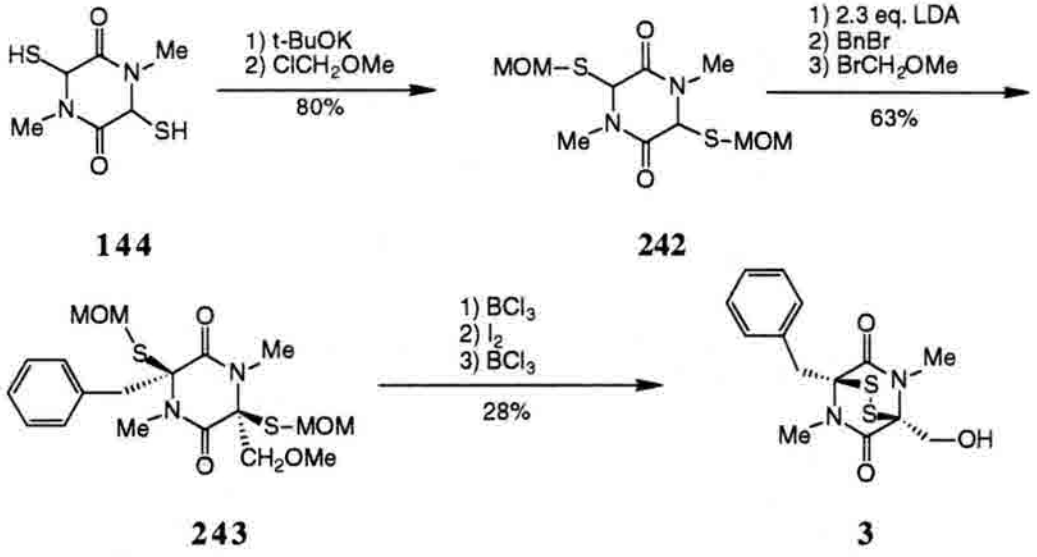
Scheme 17. Total synthesis of (±), (+)-gliotoxin



Coupling of the dithioacetal protected diketopiperazine **235** with t-butoxy arene oxide **236** in the presence of triton B resulted in Michael-type addition and afforded the dihydrobenzene adducts **237** and **238** in a 3:1 ratio (desired:undesired). Conversion of the major adduct **237** into gliotoxin was achieved in similar fashion as described for the synthesis of (±)-dehydrogliotoxin.

An alternative dithioacetal protection using methoxymethyl thioethers was developed by Kishi et al. in the total synthesis of (±)- hyalodendrin shown in Scheme 18.[120] Formation of the bisthioethers **242** was achieved by alkylation of the thiolates with

Scheme 18. Total synthesis of (±)-hyalodendrin

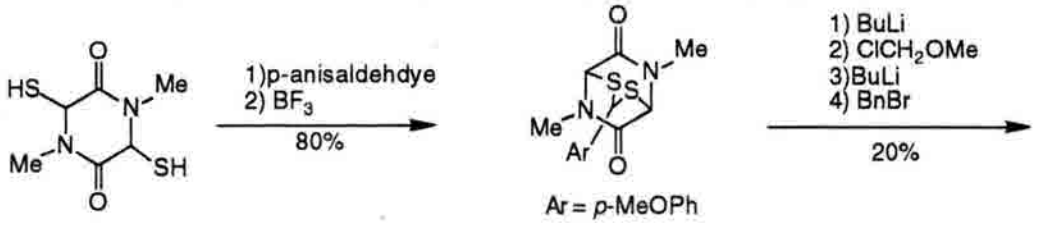


144 **242** **243** **3**

methoxymethyl chloride. Selective dialkylation with benzyl bromide and methoxymethyl bromide generated the cis diketopiperazine derivative **243** with net cis addition.

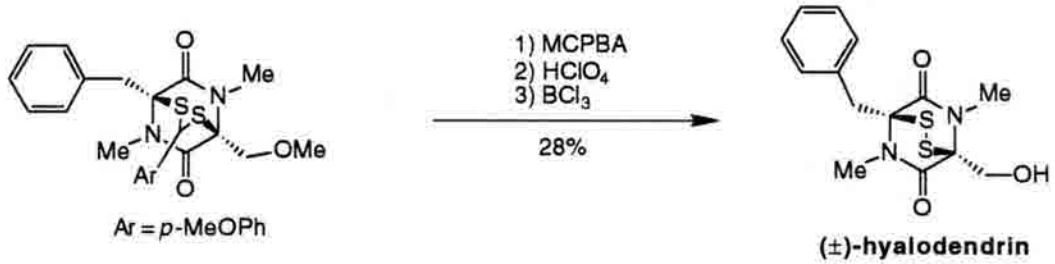
Deprotection of the methoxymethyl groups with boron trichloride, oxidation and removal of the hydroxyl protecting group completed the total synthesis. Interestingly, Strunz and Kakushima reported a synthesis of (±)-hyalodendrin based on Kishi's original *p*-anisaldehyde dithioacetal and is presented in Scheme 19.[121]

Scheme 19 Total synthesis of (±)-hyalodendrin by Strunz et. al



144 **244**

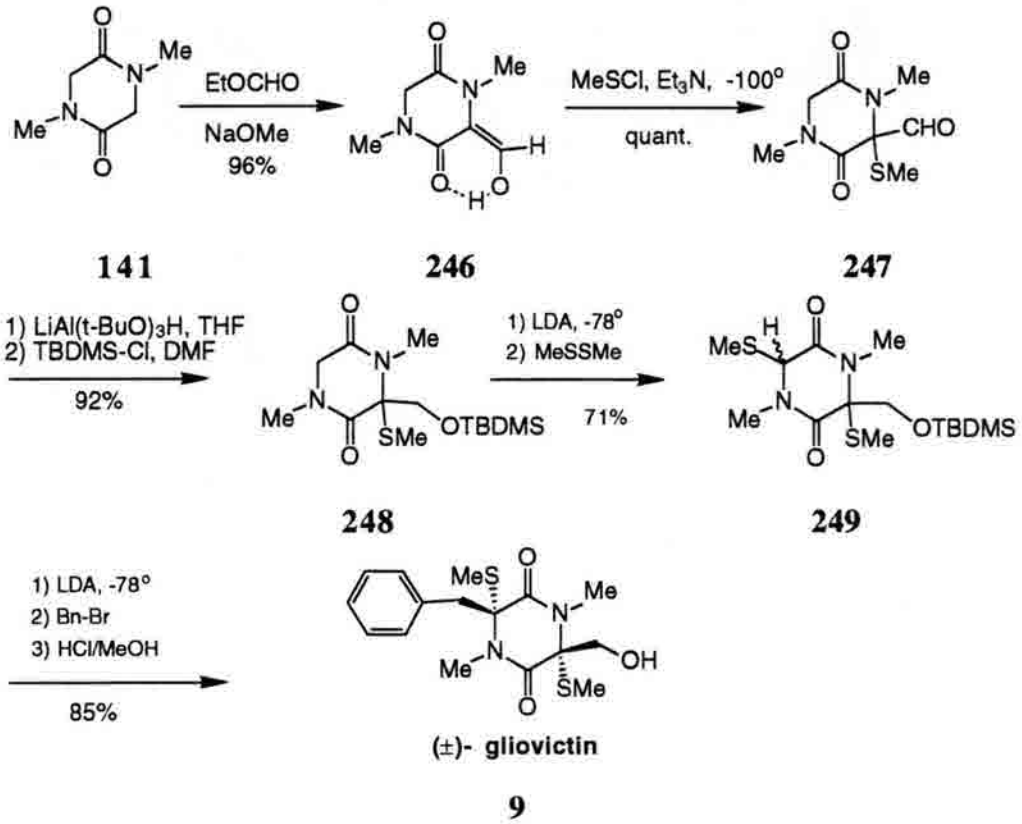
Scheme 19 continued



245 **3**

Williams and Rastetter reported a general method applicable to the synthesis of both (±)-gliovictin and (±)-hyalodendrin and is presented in Schemes 20 and 21.[122]

Scheme 20 Total synthesis of (±)-gliovictin by Williams et. al.



141 **246** **247**

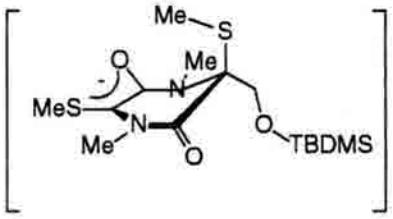
248 **249**

9

The synthesis of gliovictin commences with aldol condensation between sarcosine anhydride (**141**) and ethyl formate to generate after acidic workup enol **246** in 95% yield. Sulfenylation with methyl sulfenyl chloride at -100° afforded the thiocarboxyaldehyde **247** in quantitative yield. Reduction of the aldehyde and protection of the alcohol as the t-butyldimethyl silyl ether gave **248**.

Sulfenylation of **248** with dimethyldisulfide gave a mixture of diastereomeric bismethyl sulfides **249**. The benzylic residue was incorporated in a stereoselective fashion from alkylation of the preformed lithium enolate and, following acidic deprotection afforded (±)- gliovictin.

The observed stereoselective introduction of the benzyl group was rationalized via transition state **250**. In this transition state, the bulky siloxy group adopts a pseudo-equatorial position forcing the thiomethyl residue to reside in an axial orientation.



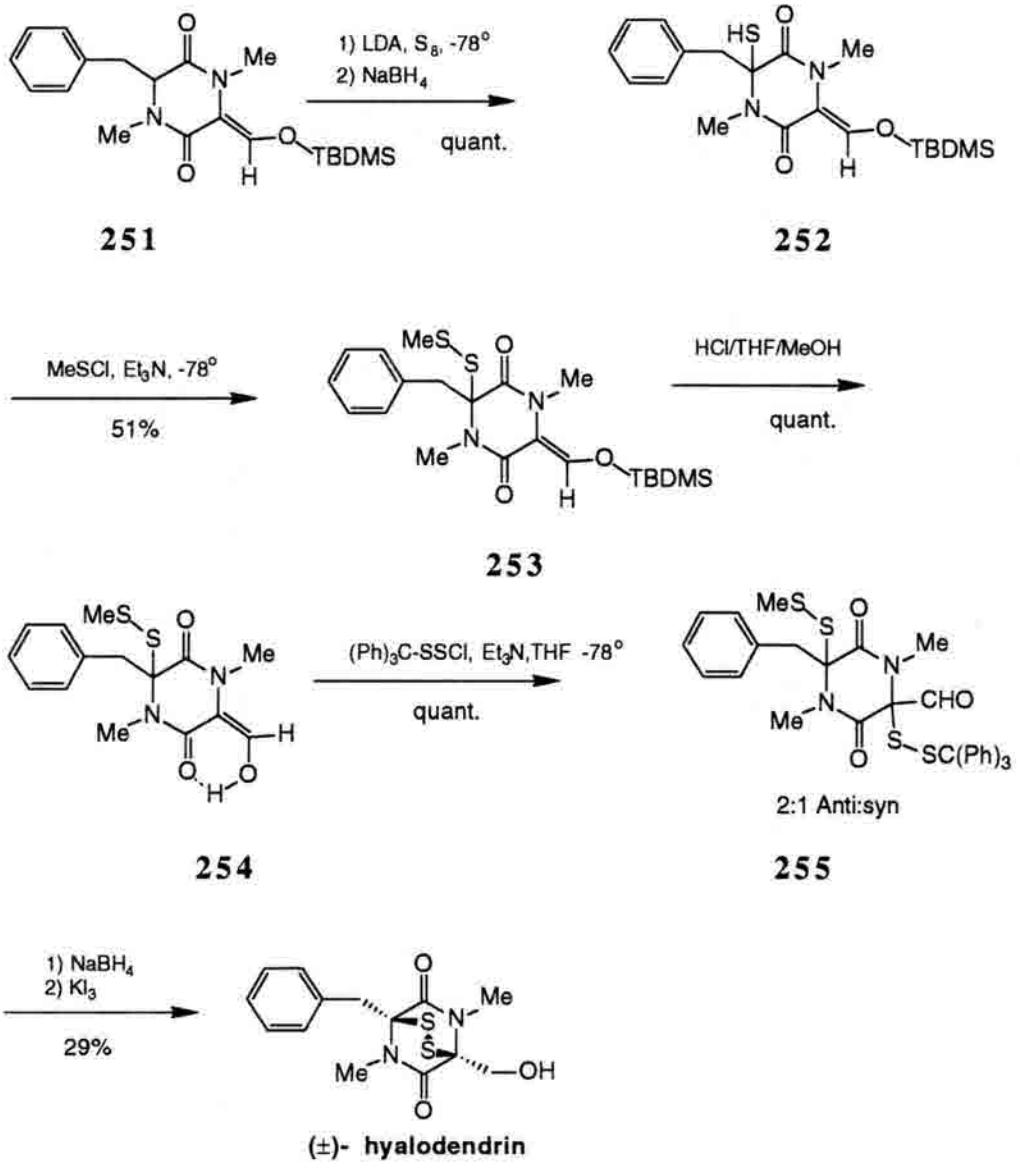
250

This conformation is stabilized by anomeric interactions with the nitrogen lone pair. The axial orientation of the thiomethyl group blocks the top face of the enolate resulting in alkylation occurring from the opposite side.

Application of an analogous methodology resulted in the synthesis of (±)-hyalodendrin shown in Scheme 21. Sulfenylation of the substituted silyl enol ether **251** with monoclinic sulfur (obtained from heating rhombic sulfur under vacuum, ~100°) followed by reductive workup afforded thiol **252** in essentially quantitative yield. Protection of the thiol group as a mixed disulfide was achieved by reaction with methylsulfenyl chloride to give **253**.

Acid hydrolysis of the silyl group produced the protected enol ether **254**. Sulfenylation with triphenylmethyl chlorodisulfide afforded the mixed disulfide **255** in a 2:1 ratio of anti:syn favoring the undesired anti isomer. Reduction of the mixed disulfides with sodium borohydride followed by oxidation gave (±)-hyalodendrin in 29% yield.

Scheme 21. Total synthesis of (±)-hyalodendrin by Williams et. al.



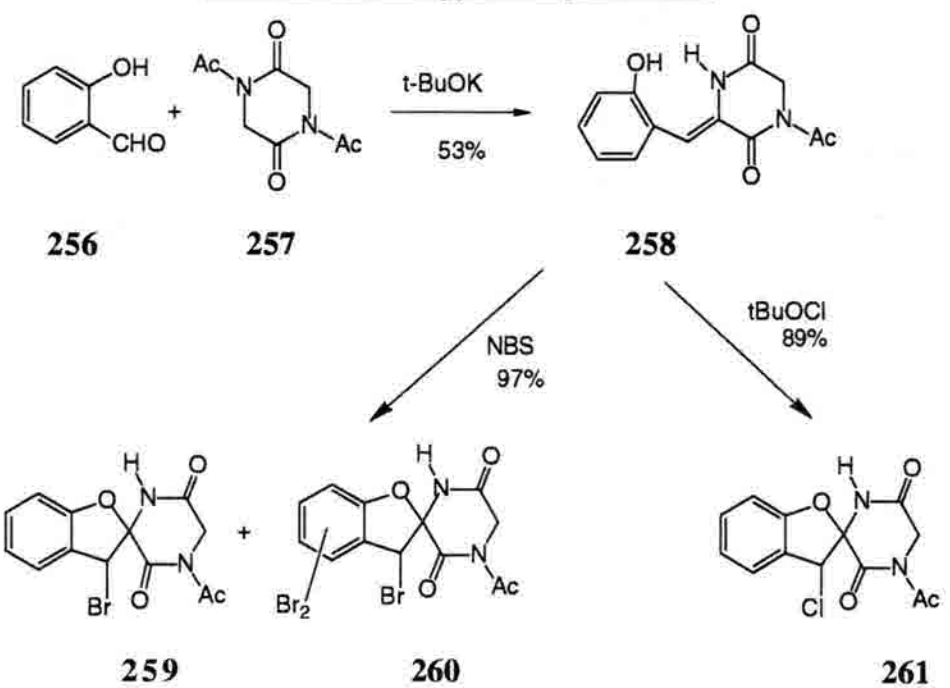
251 **252** **253** **254** **255** **3**

Chapter 3
Total synthesis of (±)-Aspirochlorine

Aspirochlorine has several features which render the molecule a synthetic challenge. Foremost among the synthetic challenges is establishing control of both the relative and absolute stereochemistries about the spiro center. Other synthetic challenges to address include formation of the N-methoxyl substituted diketopiperazine ring, and synthesis of the bicyclo[3.2.2] disulfide ring system which had not been prepared previous to this study. Aspirochlorine also contains a free NH adjacent to the disulfide bridge and the chemical reactivity of this arrangement of atoms was also of concern. The first section of this chapter describes the model studies directed at preparing the tricyclic ring system of aspirochlorine. The second section of this chapter is devoted to the first total synthesis of (±)-aspirochlorine.

Model Studies

Scheme 1. Initial approach by Shin et al.



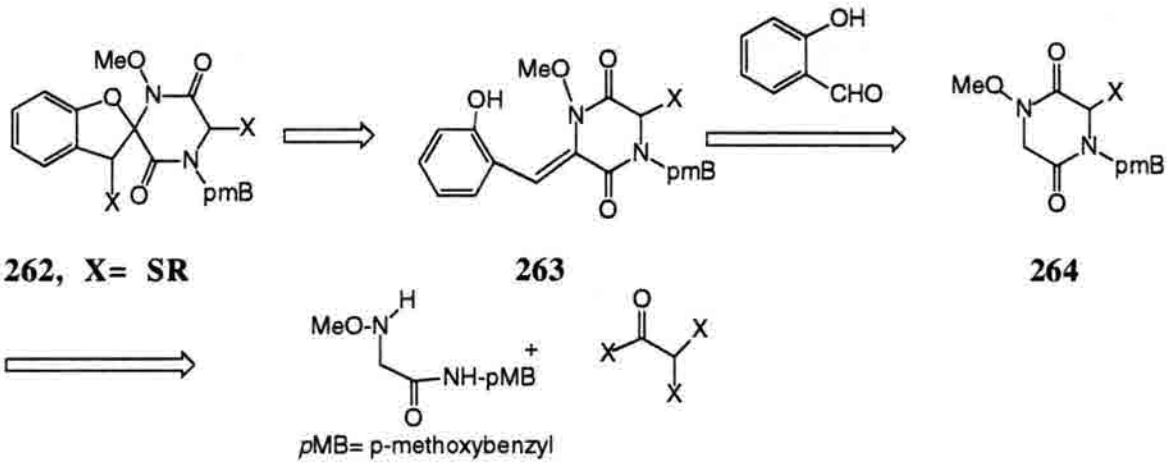
259 260 261

In 1984 before the correct structure of aspirochlorine was elucidated, Shin and co-workers developed a facile entry to the spiro[benzofuran-2(3H),2'-piperazine-3',6'-dione] ring system shown in Scheme 1.[123] Condensation of salicylaldehyde **256** with N,N-1,4-diacetyl-2,6-piperazinedione **257** occurred via a nitrogen to oxygen acyl transfer to generate **258** with the Z-geometry about the double bond.

Treatment of **258** with N-bromosuccinimide gave a 1:3 mixture of the desired bromide **259** and tribromide **260** in 97% overall yield. Treating **258** with t-butyl hypochlorite gave the chloride **261** of unknown stereochemistry in 89% yield.

The simplicity of Shin's methodology formed the basis of the initial model study. The retrosynthetic plan which was adopted is shown below in Scheme 2. Regioselective coupling of salicylaldehyde **256** with the unsymmetrical N-methoxyl diketopiperazine **264**

Scheme 2 Initial Retrosynthetic Plan.



262, X= SR **263** **264**

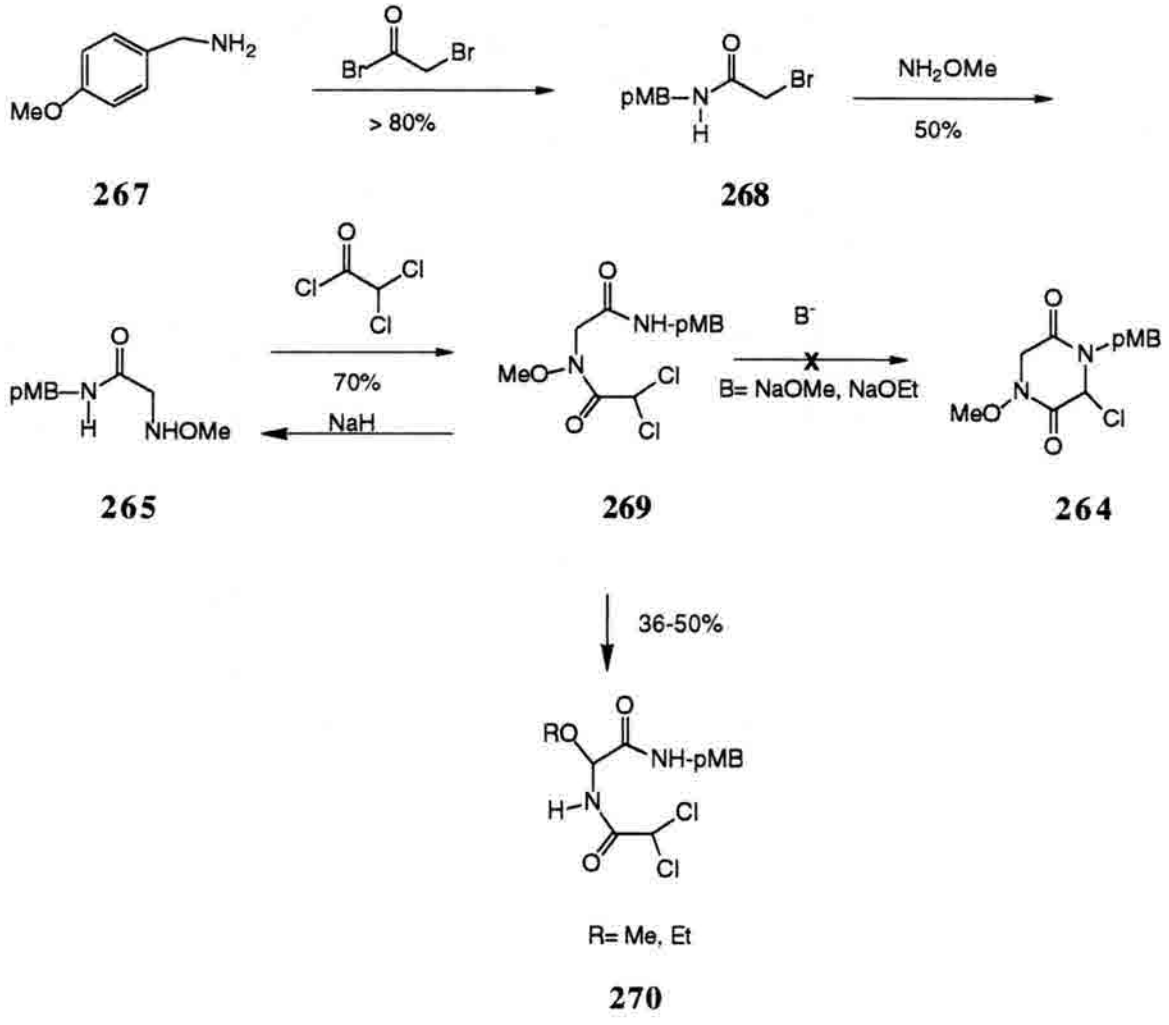
265 **266, X= Br, Cl**

should afford the desired condensation product **263** analogous to the method by Shin. Oxidative ring closure of **263** to the tricyclic core **262** followed by incorporation of the mercaptan groups was anticipated to provide a facile route to the correct structure of aspirochlorine depicted as **262** .

Preparation of diketopiperazine **264** was envisioned to be accessible from 2-(N-methoxy)-acetamide **265** from condensation with dichloroacetyl chloride or similar reagents. Subsequent ring closure was anticipated to give the desired functionalized diketopiperazine **264** with the correct regiochemistry.

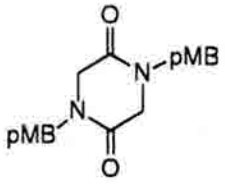
The attempted synthesis of **264** is shown in Scheme 3. Acylation of p-methoxybenzyl amine **267** with bromoacetyl bromide afforded 2-bromo acetamide **268**

Scheme 3. Attempted synthesis of N-methoxyl diketopiperazine **264**



in quantitative yield (5g scale or less), but upon scale-up the yield dropped to 80%. The acylation reaction is highly exothermic, and care must be observed when adding the acetyl bromide. If the addition is too fast none of the desired product was isolated.

Conversion of **268** into the N-methoxyl acetamide **265** was experimentally more difficult than anticipated. Treating **268** with equimolar amounts of methoxylamine hydrochloride salt in the presence of triethyl amine, sodium carbonate, or pyridine rendered only starting material. In the presence of stronger bases, the symmetrical diketopiperazine **271** was isolated in up to 60% yield (eg. 1.1 eq, NH_2OMe, 2.1 eq. NaH, DMF).

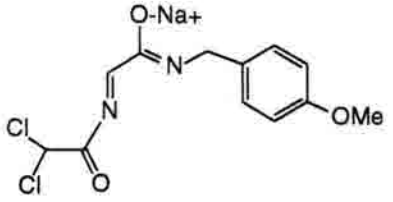


271

Conversion of **268** to **265** was achieved in 10-50% yield by using 4-10 equivalents of methoxyl amine (obtained from free-basing the hydrochloride salt with 50% KOH).[124] N-Acylation of **265** with dichloroacetyl chloride afforded the requisite dichloride **269** in 70% yield.

All attempts to cyclize **269** under basic conditions using sodium methoxide, sodium ethoxide, and potassium t-butoxide in methanol or ethanol failed to produce diketopiperazine **264**. Instead of the desired diketopiperazine, a series of α-alkoxy peptides **270** were isolated as the major products in 36-50% yields.

Formation of the α-alkoxy dipeptides results from facile elimination of the N-methoxyl group generating the possible intermediate N-acylimine species **272** which undergoes nucleophilic addition by the solvent. This type of rearrangement/addition reaction of N-alkoxy amino acids is well documented by Ottenheijm and α-alkoxy amino acids are efficiently prepared by this route.[214]

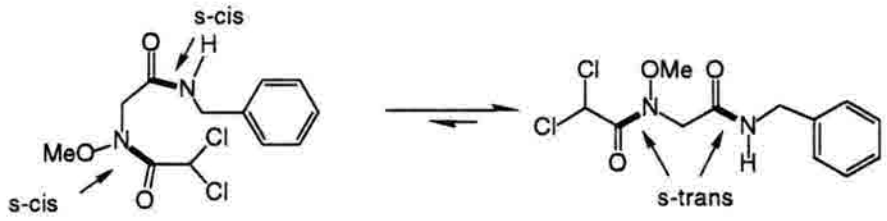


272

Treatment of dichloride **269** with one equivalent of sodium hydride in THF at -10° gave a mixture of products, and acetamide **265** was identified as a side product. Compound **265** can arise from loss of dichloroketene which is easily hydrolyzed to dichloroacetic acid upon workup. Attempts to generate **264** under acidic conditions using silver salts (silver triflate, silver nitrate) as catalysts were also unsuccessful and lead to mixtures of unidentifiable products.

The inability to generate any of the desired diketopiperazine is the result of a variety of factors. Although amide nitrogens are considered basic (pKa ~15-17) and can be alkylated with a variety of reagents, the corresponding anion in **269** is probably not sufficiently nucleophilic to favor cyclization.

A more likely explanation for the lack of cyclization is related to the conformation of dipeptide **269**. Amides in general exist as a mixture of cis and trans rotamers that can be interconverted by rotation around the central C-N bond. In the s-cis conformation, groups attached to nitrogen and the carbonyl adopt a cis relationship to each other as depicted in **269-cis.**



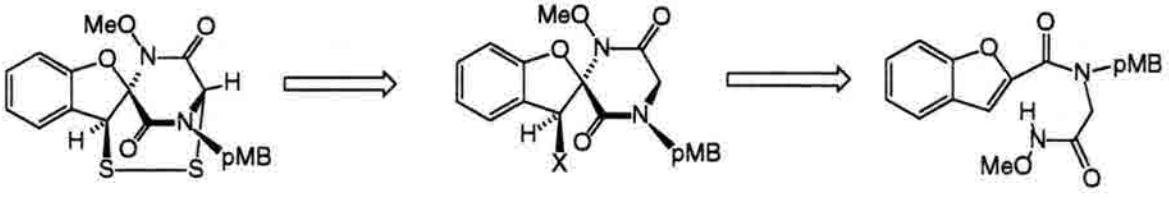
269-cis **269-trans**

In the s-trans conformation depicted as **269-trans**, groups attached to nitrogen and the carbonyl are anti to each other with respect to the central C-N bond. The barrier for rotation about the C-N bond is generally accepted to be ~16 kcal/mole.[125] Furthermore, the energy difference between the two conformations is substrate dependent and is related to the size of the substituents attached. For amides with sterically large groups, the trans geometry can be the exclusive conformation.

Based on this analysis, **269** exists predominantly in the extended trans conformation about both amide residues (ie. **269-trans**) minimizing steric interactions among the various groups. Cyclization to the diketopiperazine requires the interconversion of both amides into the cis geometry depicted as **269-cis** and is highly disfavored. The net result is the two reactive ends of **269** cannot become proximal prohibiting cyclization.

Formation of rearranged products such as **270**, and the documented base-catalyzed rearrangement of N-alkoxy amino acids, exposes a serious flaw in the original retrosynthetic design. The original method proposed coupling of the N-methoxyl diketopiperazine **264** with an aldehyde under basic conditions. Based on the present experimental evidence, the desired coupling was unlikely to occur and this approach to aspirochlorine was abandoned.

Scheme 4 Intramolecular cyclization



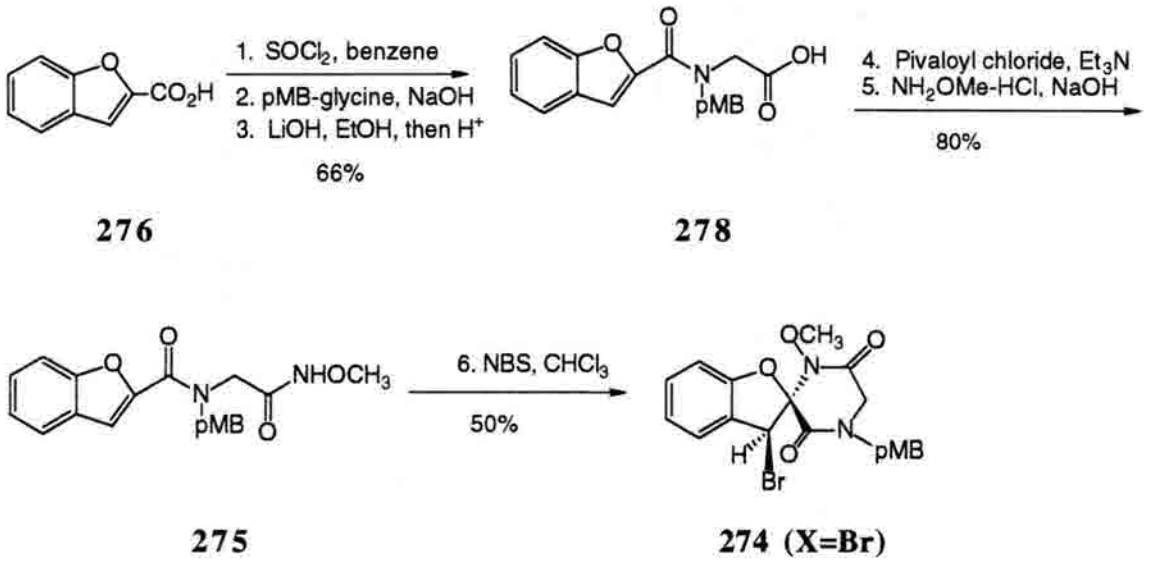
273 **274** **275**

276 **277**

During this time an alternative approach was conceived and the retrosynthetic plan shown in Scheme 4. The idea was to synthesize the tricyclic core **274** by treatment of hydroxamic ester **275** with electrophiles resulting in intramolecular cyclization. Compound **275** should be readily obtained from coupling of commercially available coumarilic acid **276** and N-alkylated glycine ethyl ester **277** followed by aminolysis with methoxyl amine.

The successful approach to the spiro[benzofuran-2(3H),2'piperazine] ring system based on the proposed intramolecular cyclization reaction is shown in Scheme 5.

Scheme 5



276 **278**

275 **274 (X=Br)**

Commercially available coumarilic acid (**276**) was converted into the acid chloride by reaction with thionyl chloride and coupled with N-(*p*-methoxybenzyl)glycine ester (**277**) affording the desired benzofuranyl-glycine ethyl ester. Saponification with lithium hydroxide gave coumarilic glycinate (**278**) in 66% overall yield from **276**. O-methyl hydroxamic ester (**275**) was most efficiently prepared from **278** via mixed anhydride (pivaloyl chloride, triethyl amine) followed by aminolysis with methoxylamine to give **275** in 80-95% yields.

Treatment of **275** with 1.2 eq. N-bromosuccinimide in ethanol-free chloroform resulted in formation of the desired tricyclic bromide **274** in 50% isolated yield as a single diastereomer with the desired *trans* relative stereochemistry. The structure of bromide **274** was confirmed by X-ray crystal analysis and the structure is shown in Figure 1.

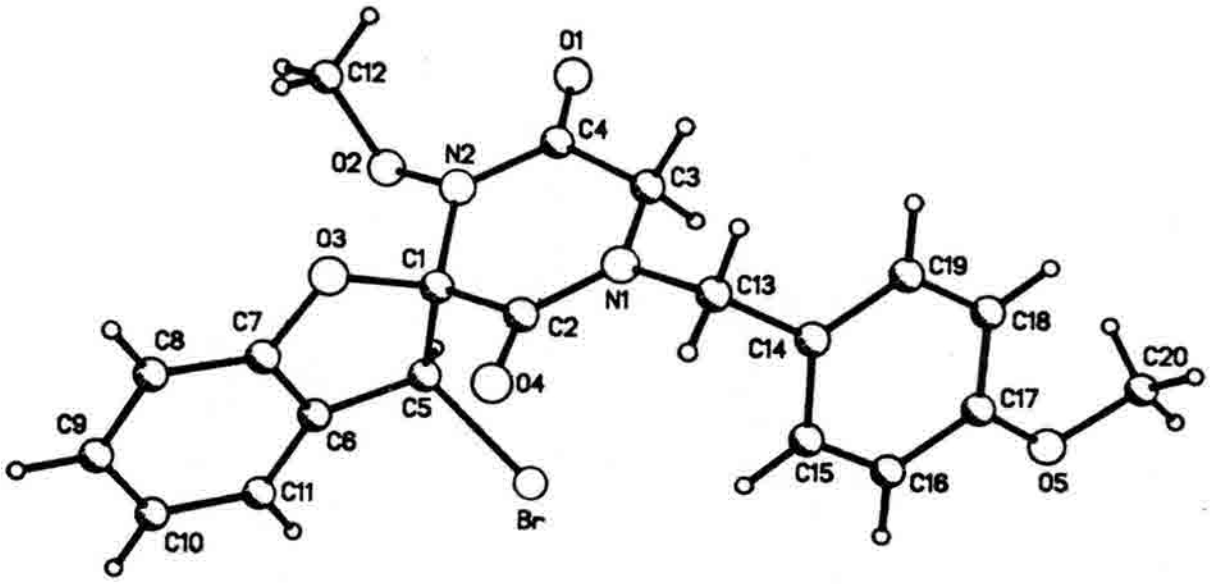
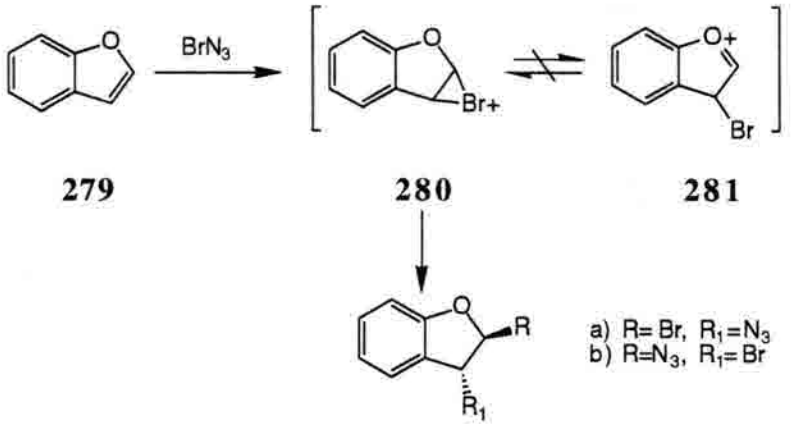


Figure 1. X-ray crystal structure of bromide **274**.

Investigation of other electrophilic agents such as Br_2, IBr, pyridinium bromide perbromide, or t-butyl hypochlorite often gave mixtures of products, none of which appeared to have undergone cyclization. Increasing the concentration of NBS in an attempt to improve the yield lead to decreases in the purity of product and lower overall yield.

The *trans* relative stereochemistry in **274** and the absence of other stereoisomers suggests cycloaddition may occur via a bromonium ion rather than an oxonium ion intermediate. Evidence supporting the intermediacy of a bromonium ion was obtained by Okuyama and the results shown in Scheme 6.[126]

Scheme 6 Electrophilic bromination of benzofuran



279 **280** **281**

282a,b

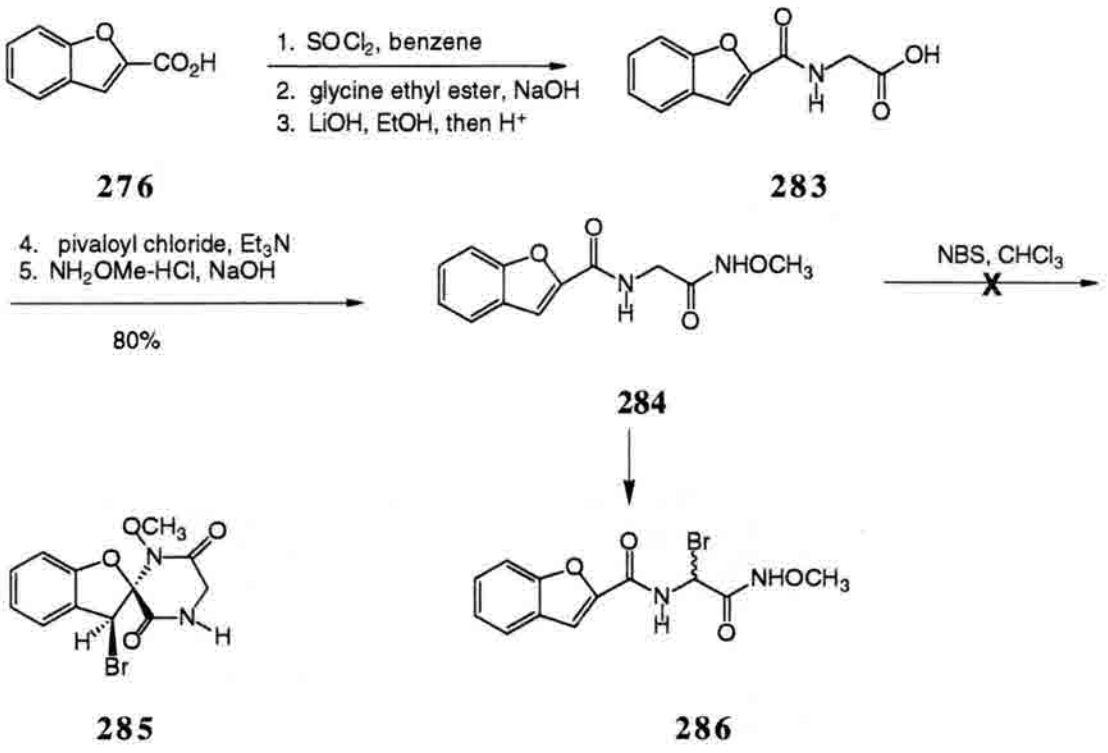
Electrophilic bromination of unsubstituted benzofurans **279** with BrN_3 resulted in exclusive formation of 3-azido benzofuran **282a**. To rationalize this result, Okuyama proposed the electronic structure of benzofuran is similar to styrene in reactivity rather than phenyl vinyl ether.

The absence of the isomeric 2-azido benzofuran (**282b**) was taken as evidence the oxonium ion **281** does not contribute significantly to the reaction. Based on the above arguments, generation of the desired 6-membered diketopiperazine **274** versus a 7-membered derivative can be attributed to differences in the rates of formation for 6 and 7-membered rings.

Further investigations into the scope of the spirocyclization reaction were carried out involving the unprotected hydroxamic ester **284** shown in Scheme 7. Cyclization of **284** would provide a distinct improvement over the current approach by avoiding subsequent deprotection of the amide nitrogen.

Compound **284** was readily obtained from peptide coupling of **276** with glycine ethyl ester, followed by hydrolysis to give the carboxylic acid **283.** Mixed anhydride formation and aminolysis with methoxylamine afforded **284** in 80% yield.

Scheme 7 Preparation of unprotected hydroxamic ester **284**



Treatment of **284** with NBS under the same conditions used previously ($CHCl_3$, rt, 12 hrs) did not afford any of the desired tricyclic bromide **285** and starting material was recovered. The same result was observed in reactions conducted in refluxing carbon tetrachloride. The only discernable products obtained under these conditions were small amounts of α-brominated acyclic compounds **286** identified from ^1H NMR.

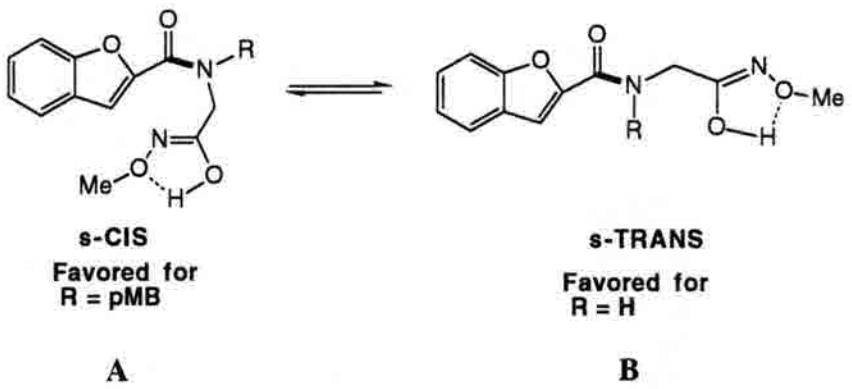
The formation of cyclized products is easily discerned by ^1H NMR. The line shapes of the acyclic precursors are extremely broad due to the interconversion of rotamers. In the cyclized product, all the resonances are sharpened due to restricted degrees of freedom.

The facile cyclization of hydroxamic ester **275** compared to the inability of **284** to undergo the same reaction is mechanistically interesting. Analysis of the conformational

preferences for the central amide bond in hydroxamic esters **275** and **284** suggests a reason for the differences in reactivity.

Formation of the diketopiperazine ring is proposed to occur via transition state **A** shown in Figure 2. In this geometry, the central amide adopts the s-cis geometry minimizing steric interactions between the bulky p-methoxybenzyl group and the benzofuran moiety. As a result, the hydroxamic ester side chain is able to reside in

Figure 2. Proposed transition state geometries for the cycloaddition



A **B**

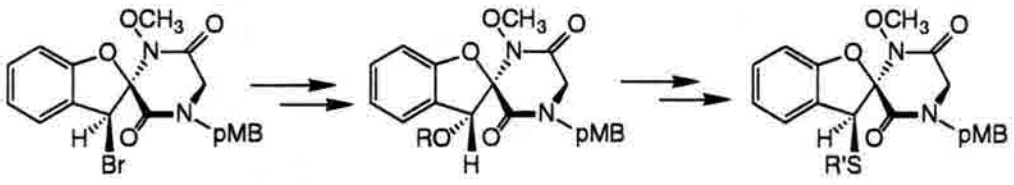
proximity of the benzofuran ring much of the time. Furthermore, the hydroxamic ester moiety presumably exists in a 5-membered hydrogen bonded conformation also generating the s-cis geometry required for cyclization.

The inability of **284** to undergo the desired cycloaddition can be rationalized using the same arguments. Compounds such as hydroxamic ester **284** containing a secondary amide will adopt the lower energy extended s-trans geometry **B** in which the benzofuran and N-methoxyl group are distal. Due to the high energy barrier required to generate the s-cis geometry needed for cyclization, unprotected hydroxamic esters are unlikely to undergo diketopiperazine formation. Based on these conformational arguments, application of the cycloaddition reaction towards the synthesis of aspirochlorine requires a bulky tertiary amide group to be present at the central nitrogen.

Having established a stereoselective route to the correct structure of aspirochlorine, efforts were directed at the stereoselective functionalization of the benzylic bromide. Based on the established reactivity of benzylic halides as excellent leaving groups in nucleophilic substitution, it was anticipated that the relative stereochemistry obtained in the cycloaddition could be preserved. The general idea is illustrated in Scheme 8.

This process was envisioned to occur by initial conversion of bromide **274** into the epimeric alcohol or ether. Displacement of the alcohol or ether by thiolates should give the desired product in a stereoselective fashion with overall net retention of configuration.[127]

Scheme 8 Proposed double inversion.



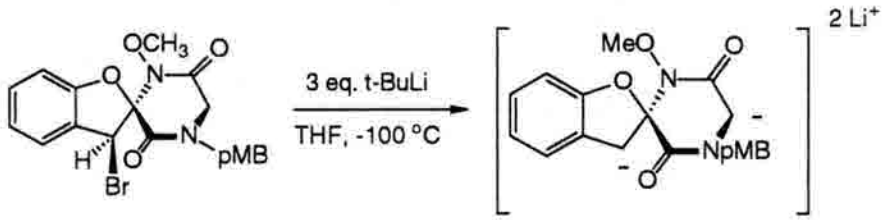
274 **287** **288**

All attempts to convert bromide **274** into **287** or **288** with nucleophiles such as sodium methoxide, sodium methylthiolate, or sodium hydrogen sulfide were unsuccessful and resulted in a multitude of products by TLC. The lack of substitution was attributed to the N-methoxyl group partially blocking the back face of the furan ring preventing substitution from readily occurring.

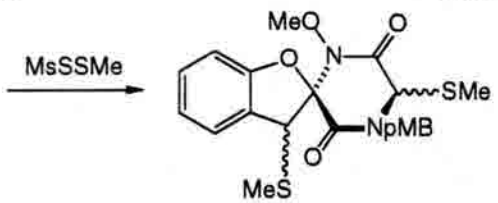
Attempted conversion of **274** into an epidithio derivative such as **290** depicted in Scheme 9 using halogen-metal exchange were equally unsuccessful. Treating **274** in THF with three equivalents of t-butyl lithium at low temperature (-100º) resulted in formation of a deep red solution assumed to be the dianion **289.** Subsequent treatment with elemental sulfur according to the improved method developed by Williams,[122] followed by reductive

workup and oxidation with KI_3 did not produce any of the desired disulfide and only resulted in decomposition of the starting bromide.

Scheme 9. Attempted metallation



274 **289**



290

Treatment of dianion **289** with electrophilic sources of sulfur such as mesylmethylsulfide gave a complex mixture of products, though a small amount (<20%) of the possible bis(thiomethyl) derivative of unknown stereochemistry **290** was obtained. The NMR of **290** is shown in Figure 3.

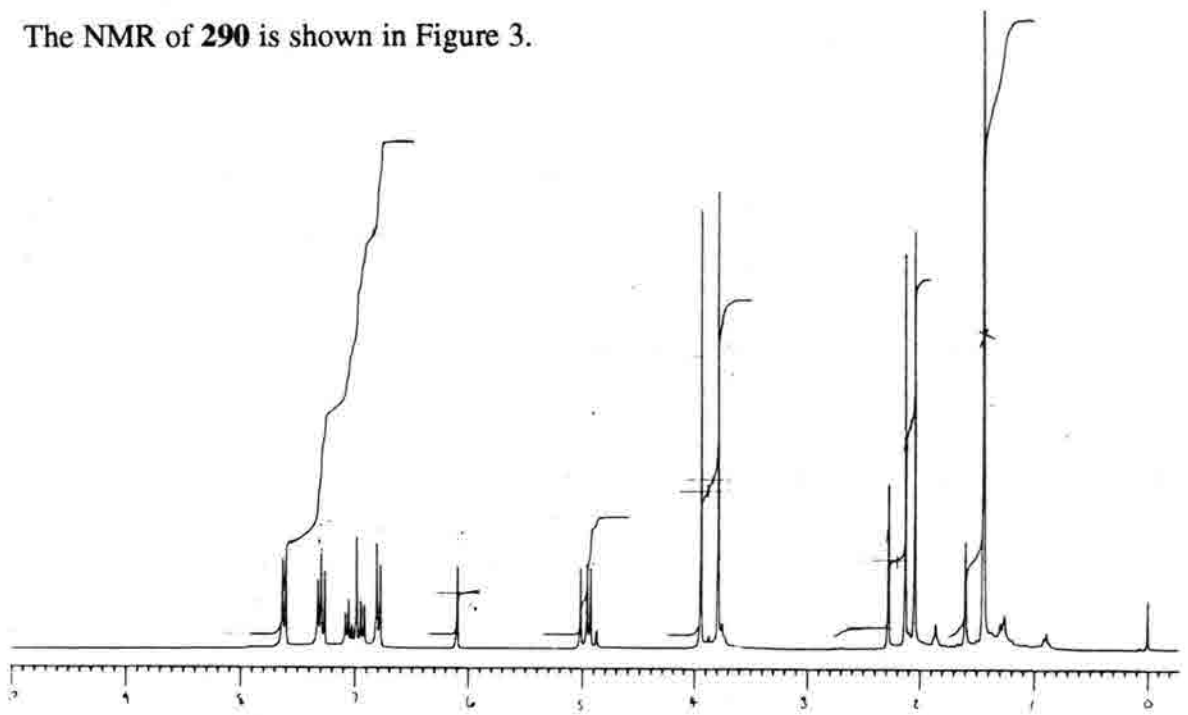
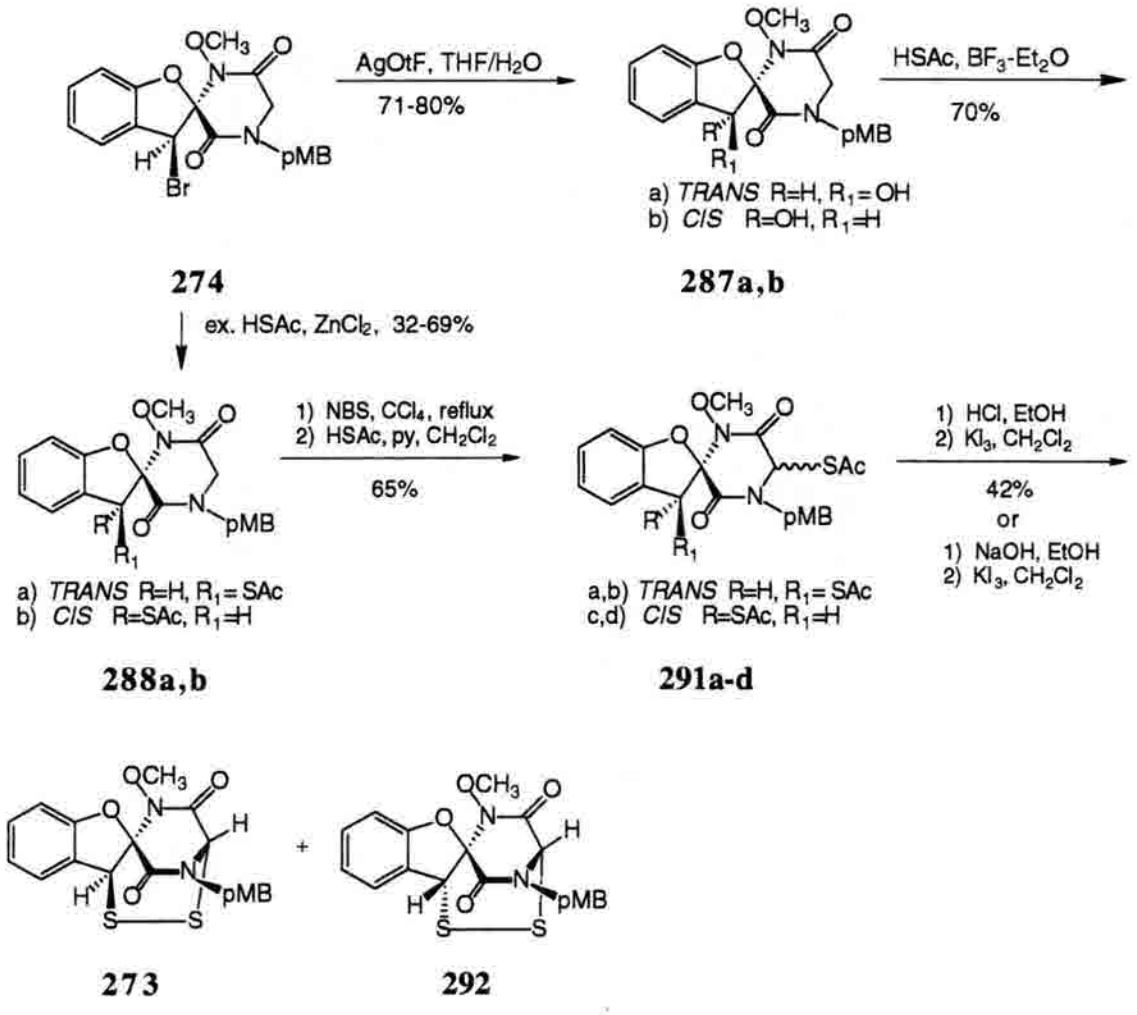


Figure 3. $CDCl_3$ 270 mHZ NMR of proposed bis thiomethyl ether **290**.

Functionalization of **274** was ultimately achieved using Lewis-acid (S_N1-type) conditions and ultimately led to the completion of the model study shown in Scheme 10. Treatment of **274** with a slight excess of silver triflate in aqueous THF led to a 1.6:1.0 (trans:cis) mixture of alcohols **287a,b** in 75% combined yield. The alcohols were easily separated by column chromatography and carried on individually.

Scheme 10. Completion of *p*-methoxybenzyl model study



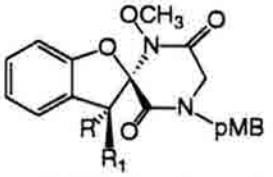
The relative configuration about the benzylic position for alcohols **287a,b** and for subsequent related compounds, was inferred from ^1H NMR / NOE experiments.

In the case of the *trans* -diastereomer **287a**, irradiation of the benzylic methine proton at 5.80 ppm resulted in a slight positive NOE enhancement of the N-methoxyl signal at 3.88 ppm. Likewise, irradiation of the N-methoxyl group exhibited a weak positive NOE enhancement of the signal assigned to the benzylic proton indicating the two groups are positioned near each other. Analogous NOE experiments involving the *cis* -diastereomer **287b** did not reveal NOE enhancements upon irradiation of either signal (benzylic methine 5.59 ppm, N-methoxyl 3.81 ppm). The NOE experimental results are included in the appendix.

Several attempts were made to achieve optimal conditions for preparation of the desired thioacetate **288a,b** and the results are listed in Table 1. The ratio of thioacetate diastereomers **288a,b** from the reactions was readily determined by integration of the benzylic methine signals. In the desired *trans* stereoisomer **288a,** the proton appeared at 5.74 ppm. In the *cis* stereoisomer **288b** the proton is slightly shifted downfield at 5.81 ppm.

Treatment of alcohols **287a,b** under a variety of conditions (entries 2-9) routinely gave mixtures of oxygen and sulfur acetylated compounds. Reactions using an excess of both thiolacetic acid and boron trifluoride etherate favored formation of thioacetates **288a,b**.

The identity of the O-acetylated compounds **293a,b** was confirmed by acetylation of **287a** and **287b** with acetyl chloride. Comparison of the ^{1}H NMR spectra indicated the compounds were identical.



a) *TRANS* R=H, R_1 = OAc
b) *CIS* R=OAc, R_1 =H

293a,b

Table 1. Conversion of 274, 287a,b to thioacetates 288a,b.

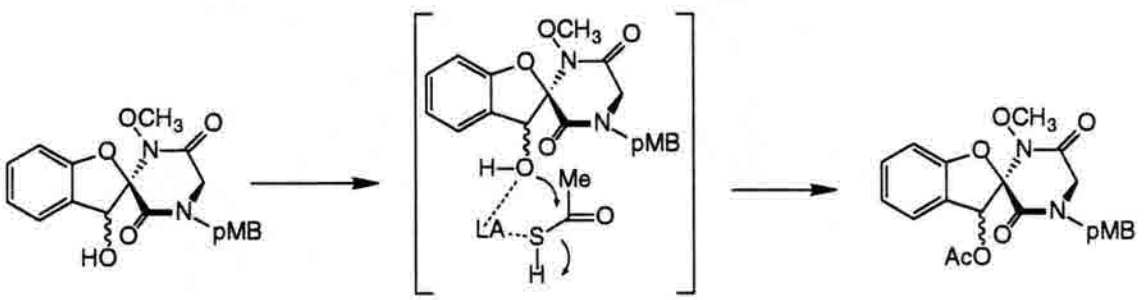
Entry	Substrate	Conditions[a]	Ratio O vs. S Acetylation[b]	Ratio of Trans:cis[c]	Combined Yield (%)
1	**287a**	3 eq. HSAc, $ZnCl_2$, benzene, reflux 6 hrs	1 : trace	---	50
2	**287a**	ex. HSAc, $ZnCl_2$, benzene, rt, 24 hrs	9 : 1	2.5 : 1	77
3	**287a**	ex. HSAc, $ZnCl_2$, benzene, rt, 6 days	4 : 1	2 : 1	75
4	**287a**	12 eq. HSAc, 6 eq BF_3-Et_2O, CH_2Cl_2	1 : 3	1.5 : 1	70
5	**287b**	3 eq. HSAc, $ZnCl_2$, benzene, reflux 6 hrs	1 : trace	---	54
6	**287b**	ex. HSAc, $ZnCl_2$, benzene, rt, 24 hrs	9 : 1	3 : 1	63
7	**287b**	ex. HSAc, $ZnCl_2$, benzene, rt, 6 days	1 : 1	2 : 1	80
8	**287b**	3 eq. HSAc, 0.5 eq BF_3-Et_2O, CH_2Cl_2	1 : trace	---	50
9	**287b**	12 eq. HSAc, 6 eq BF_3-Et_2O, CH_2Cl_2	1 : 3	3 : 1	64
10	**274**	ex. HSAc, $ZnCl_2$, $CHCl_3$, rt, 24 hrs	trace : 1	3 : 1	52
11	**274**	ex. HSAc, $ZnCl_2$, acetone, rt, 24 hrs	trace : 1	5 : 1	32
12	**274**	ex. HSAc, $ZnCl_2$, benzene, rt, 24 hrs	trace : 1	3.6 : 1	62
13	**274**	ex. HSAc, $ZnCl_2$, benzene, rt, 36 hrs	trace : 1	3 : 1	83

[a]HSAc= thiolacetic acid. All reactions were carried out in flame-dried flasks under nitrogen in anhydrous solvents. The zinc chloride was anhydrous and a large excess of thiolacetic acid used. Reactions carried out in chloroform used ethanol-free chloroform. [b]Ratios determined by NMR analysis of crude reaction. [c]Ratio of trans/cis thioacetates **288a,b**.

Treatment of alcohol **287a** or **287b** in refluxing benzene with 3 equivalents of thiolacetic acid in the presence of excess anhydrous zinc chloride resulted in exclusive formation of the O-acetyl compounds **293a,b** (entries 1, 5) and could be isolated in 50% yield.

Formation of acetates **293a,b** may occur via a Fischer type esterification shown in Scheme 11. The reversal in reactivity is the result of complexation of the thiol group of thiolacetic acid with the Lewis acids boron trifluoride etherate or zinc chloride causing the carbonyl to become more electrophilic. Nucleophilic addition by the hydroxyl results in a net S→O acyl transfer.

Scheme 11. Proposed formation of O-acetates **293a,b**

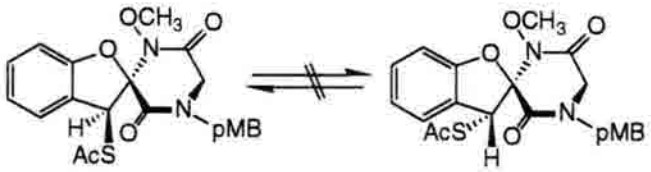


287a,b **293a,b**

Formation of the O-acetylated intermediates could be avoided by starting from bromide **274**. Treatment of **274** with excess of thiolacetic acid (>30 eq, essentially solvolysis conditions) in the presence of zinc chloride at room temperature resulted in exclusive formation of **288a,b** as a mixture of stereoisomers (entries 10-13). Surprisingly, the reactions involving **274** routinely gave higher ratios of **288a:288b** (eg. compare entry 2 and 12)

The major diastereomer obtained in all the thioacetylation reactions possessed the desired *trans*- relative stereochemistry. To determine if the observed product ratio reflected an intrinsic thermodynamic stability for the trans configuration, the individual

diastereomers **288a** and **288b** were re-subjected to the reaction (excess thiolacetic acid/$ZnCl_2$ benzene) and the ratio of products determined from NMR.



288a **288b**

Analysis of the crude NMR's revealed epimerization did not take place under the reaction conditions. The lack of interconversion between **288a** and **288b** indicated the observed product ratios in the thioacetylation reactions reflected kinetic, not thermodynamic factors.

Introduction of the thiol group at the α-position of the diketopiperazine moiety in **288a,b** was achieved using the two-step process developed by Trown.[96] Bromination of *trans* **288a** with NBS followed by reaction of the unstable bromide intermediates with a mixture of thiolacetic acid/pyridine afforded a 1:1 mixture of bis-thioacetates **291a,b** in 65% combined yield epimeric at the α-position. Treatment of the *cis* thioacetate **288b** under the same conditions gave *cis* **291c,d** in 50% isolated yield also as a 1:1 mixture of epimers at the α-position.

Attempts to firmly establish the stereochemistry at the α-position for each of the individual diastereomers **291a-d** by NOE experiments were unsuccessful. Furthermore, since conversion of *trans*-dithiols to disulfides has been experimentally observed,[128,218] determination of the stereochemistry at the α-position in bisthioacetates **291a-d** was not critical for subsequent transformations. Initially, the four diastereomers **291a-d** were separated and carried on individually. Subsequent experiments revealed it was more convenient to carry each pair **291a,b** or **291c,d** on as a mixture of epimers.

Deprotection of *trans*- **291a** with acid (HCl saturated ethanol, rt 6 hrs) followed by oxidation with aqueous KI_3 generated a surprising 1.0:1.4 ratio of the natural and unnatural

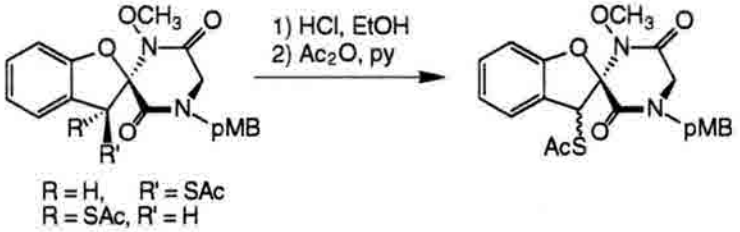
disulfides **273** and **292** in modest 32-44% combined yield. Attempts to improve the yield by using milder conditions (10% HCl/MeOH, catalytic acid) failed to give any of the deprotected material and recovery of starting material resulted.

A similar ratio of disulfides **273:292** was obtained starting from the epimer *trans* **291b**. The relative configurations of disulfides **273** and **292** were deduced from single X-ray crystal structural analyses and are shown in Figure 4.

The crystal structure of the natural disulfide **273** displayed a CSSC dihedral angle of 56.7° and the S-S bond length of 2.04 Å. The cis disulfide **292** contained a dihedral angle of 56.3° and the S-S bond length was 2.056 Å.

Deprotection of the *cis* diastereomers **291c,d** under the same acid conditions afforded a surprisingly low 1:5 to 1:11 ratio of the natural:unnatural disulfides in 30-49% combined yield.

In an attempt to gain insight into when epimerization occured, a series of experiments were carried out using the diastereomeric mono thioacetates **288a,b**.

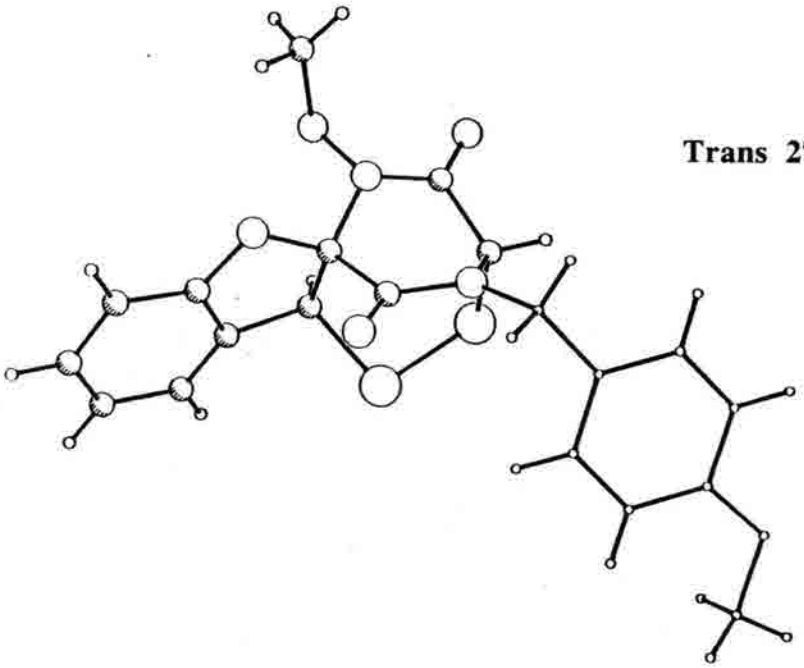


288a or b **288a,b**

Acid hydrolysis of **288a** (saturated HCl, EtOH) followed by acetylation with acetic anhydride afforded a 3:1 *trans:cis* **288a,b** in 40% combined yield. Analogous reaction of *cis* thioacetate **288 b** gave a 1:5.6 *trans:cis* ratio in 60% combined yield.

These experiments indicated epimerization occurs during acid hydrolysis of the thioacetate moiety. Furthermore, the cis diastereomer **288b** appears to be more stable to acidic conditions and undergoes epimerization to a smaller degree as evidenced by the

lower *trans:cis* ratio of products formed in both disulfide and thioacetate epimerization reactions.



Trans 273

Cis 292

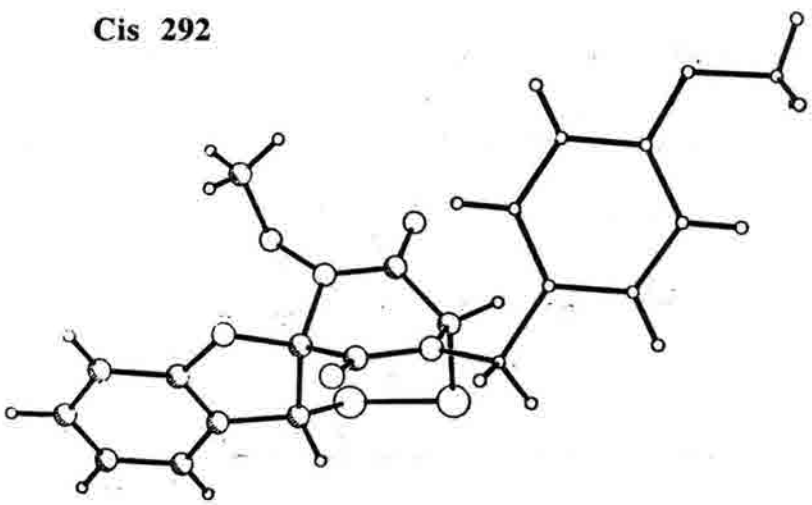
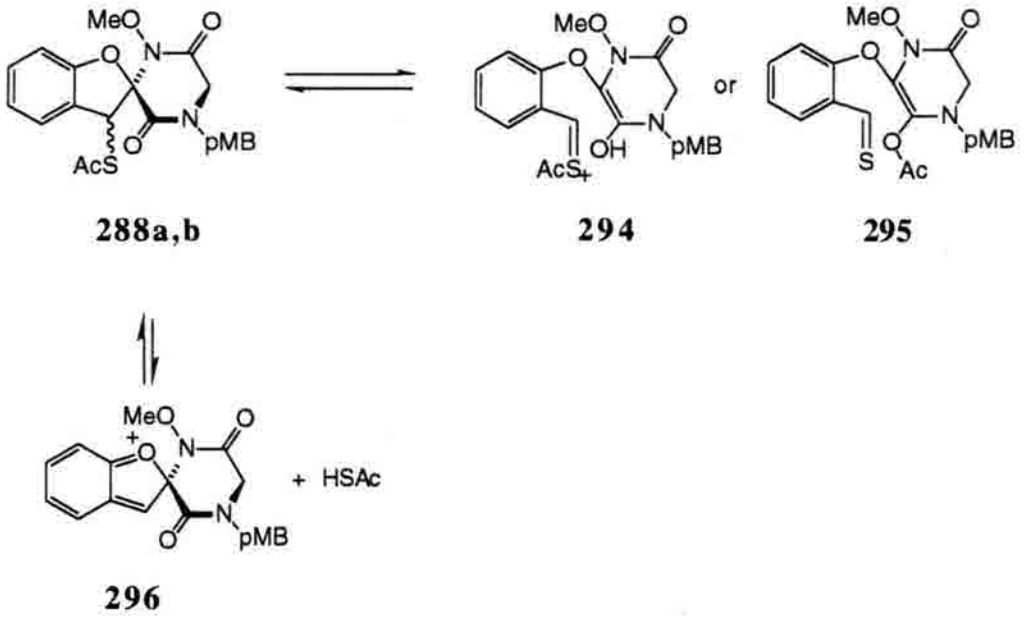


Figure 4. X-ray crystal structures of disulfides **273** and **292**

Epimerization may involve formation of the incipient thionoacetate ions **294** or **295** as intermediates shown in Scheme 12. This mechanism is similar to the mechanism proposed for the interconversion of trans dithiols to cis disulfides.[108] The alternative route involving oxonium ion **296** is not likely since no products were detected resulting from trapping of **296** with solvent (eg ethanol).

Scheme 12 Proposed mechanism of acid-catalyzed epimerization



288a,b **294** **295**

296

The acid-catalyzed epimerization was avoided by conducting the final deprotection/oxidation sequence under basic conditions. Deprotection of either *trans* **291a** or **291b** under basic conditions (0.2 M sodium hydroxide in aqueous ethanol) followed by oxidation with KI_3 resulted in formation of the desired disulfide **273** in 32% yield. Similar results were obtained using NH_4OH/methanol, but the overall yield dropped to 18%. Comparable results were obtained using the mixture of diastereomeric *cis* **291c,d** giving rise to the *cis* disulfide **292** in 20-34% yield.

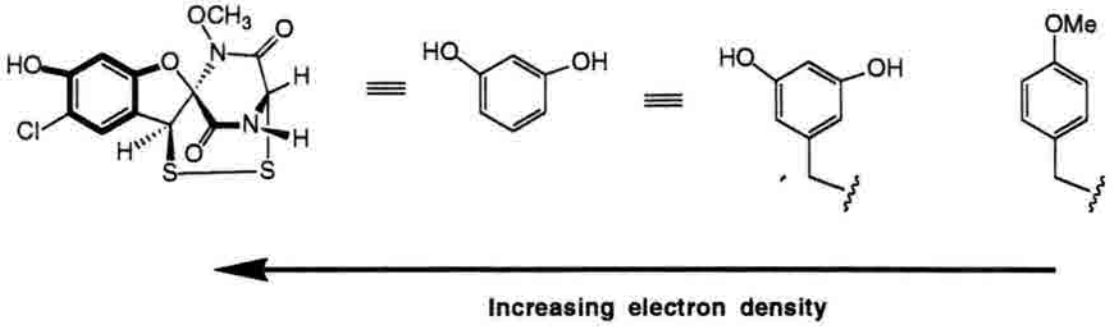
A major drawback of the model system was the inability to remove the *p*-methoxybenzyl amide protecting group. As previously discussed, a bulky tertiary amide was required for the spirocyclization reaction to proceed efficiently. The initial choice of the *p*-methoxybenzyl protecting group was predicated on the assumption that strongly acidic conditions would be capable of removing this group and literature precedent indicated epipolythiapiperazinediones were reasonably stable to strong acid.

Unfortunately, removal of the N-*p*-methoxybenzyl group from disulfides **273, 292** (or related spirocyclic compounds such as **274**) failed. Attempted removal using ceric ammonium nitrate[129] resulted in decomposition and no discernable products obtained.

Attempts to remove the *p*-methoxybenzyl group using concentrated acids such as H_2SO_4 or TFA were unsuccessful. In fact **274** displayed remarkable stability to concentrated sulfuric acid. After 30 minutes at 0°C followed by warming to room temperature in neat H_2SO_4 the majority of **274** was recovered unchanged.

The inability to remove the *p*-methoxybenzyl group in the model study precluded its use for the total synthesis. A closer inspection of the structure of aspirochlorine led to additional restrictions depicted in Figure 5 for any potential protecting group.

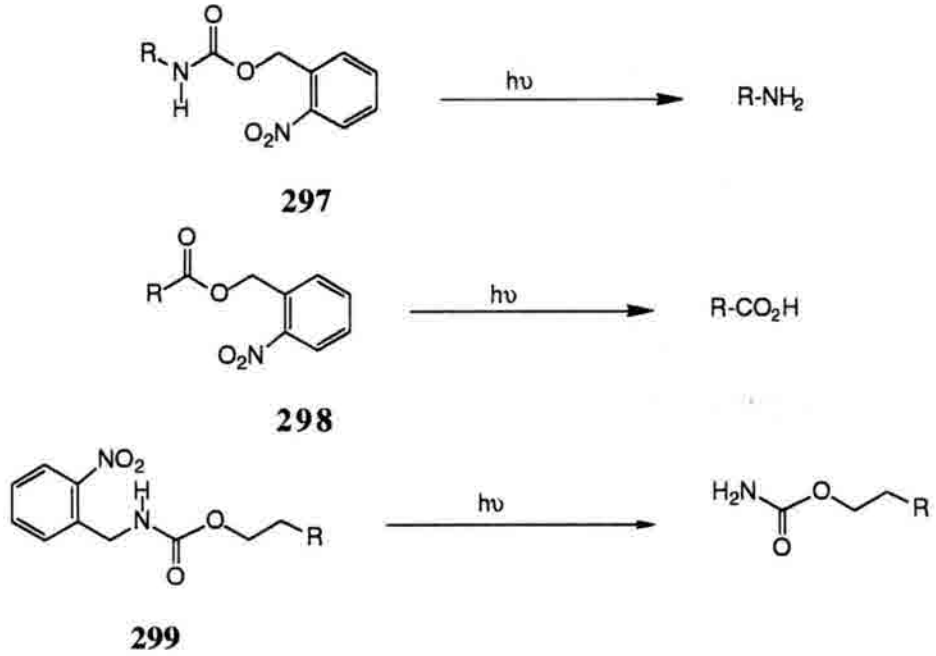
Figure 5. Comparison of electron density of various aromatic groups



The aromatic portion of aspirochlorine can be considered to be a catechol derivative and therefore electron rich compared to the *p*-methoxybenzyl group used in the model study. The increase in electron density about the aromatic ring in aspirochlorine necessarily precludes any type of oxidative deprotection strategies since this moiety would be equally reactive under such conditions. Therefore a nitrogen protecting group which could be removed under mild, non-oxidative conditions was sought.

A variety of groups have been used to protect amides including methoxymethyl (MOM), methoxythiomethyl (MTM), acetyl, and benzyl derivatives.[130] An attractive alternative not reported in the literature as an *amide* protecting group was the *o*-nitrobenzyl group. Previous uses of the o-nitrobenzyl group are shown in Scheme 13.[131]

Scheme 13. *o*-nitrobenzyl protections



297

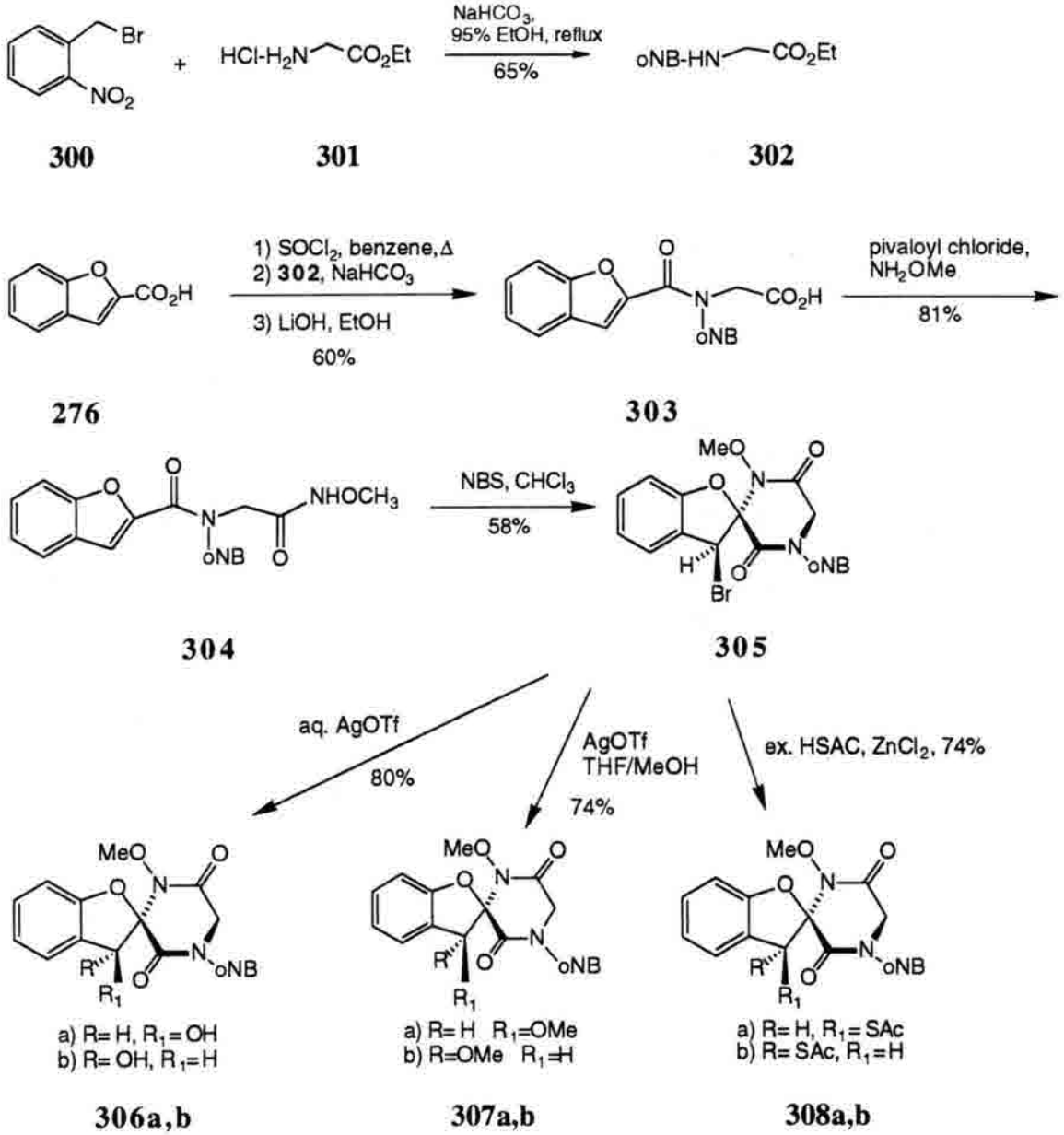
298

299

The *o*-nitrobenzyl group had been widely used as a protecting group for amino and carboxyl groups by way of the corresponding urethane **297** or ester **298**. Photolysis using

a mercury vapor lamp results in clean conversion of **297** or **298** generating the amine or carboxylic acid. During the course of these studies, a report appeared in which the *o*-nitrobenzyl was used as a carbamoyl protecting group shown as **299** and photolyzed using a laser.[132]

Scheme 14. Preparation of *o*-nitrobenzyl protected compounds



300 **301** **302** **276** **303** **304** **305** **306a,b** **307a,b** **308a,b**

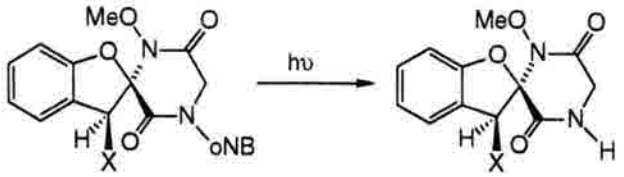
The *o*-nitrobenzyl protected compounds were synthesized in analogous fashion following the methodology developed for the p-methoxybenzyl series. The synthesis is presented in Scheme 14.

Incorporation of the *o*-nitrobenzyl group was easily achieved by alkylation of glycine ethyl ester (**301**) with o-nitrobenzyl bromide (**300**) to afford *o*-nitrobenzyl glycine ethyl ester (**302**) in 65% yield. Coupling of **302** with coumarilic acid chloride under Schotten-Baumann conditions followed by saponification afforded carboxylic acid **303.** Acid **303** was converted into the hydroxamic ester **304** in 81% yield (48% overall yield from coumarilic acid **276**). Cyclization of **304** generated the desired bromide **305** in 58% yield without incident.

Bromide **305** was converted into a mixture of alcohols **306a,b** in 80% yield using aqueous silver triflate in THF. Alternatively, **304** was converted into a 3:1 *trans:cis* mixture of methyl ethers **307a,b** in 74% yield from reaction of silver triflate in methanol/THF. A 1.5:1.0 *trans:cis* mixture of thioacetates **308a,b** was obtained in 74% by treatment of **305** with excess thiolacetic acid in benzene with zinc chloride.

The photolytic deprotection of compounds **305-308** was investigated and results from initial studies are summarized in Table 2. As expected, photolysis of bromide **305** resulted in decomposition due to the lability of the carbon-bromide bond.

Photolysis of alcohol **306** under a variety of conditions resulted in conversion to the unprotected derivatives **310** in 28-49% isolated yields. Furthermore, deprotection of **308** occurred by simply exposing a solution of the compound to direct sunlight resulting in formation of thioacetate **311** in 52% yield. Although moderate yields were obtained from the initial studies, the reactions successfully demonstrate the viability of the *o*-nitrobenzyl group as an amide protecting group.



305-308 **309 X=Br, 310 X=OH, 311 X=SAc**

Table 2. Photolytic Deprotections

Entry	Substrate X=	Conditions[a]	% Yield
1	Br	quartz, EtOH, 3 hrs	decomp.
2	OH	quartz, THF, 3 hrs.	38
3	OH	quartz, dioxane, 3 hrs.	28
4	OH	quartz, THF, 2 hrs.	33
5	OH	quartz, EtOH, 2 hrs.	49
6	SAC	sunlight, 30% aq. THF, 2 hrs	17
7	SAC	sunlight, 30% aq. THF, 4 hrs	46
8	SAC	sunlight, 30% aq. THF, 5 hrs	52

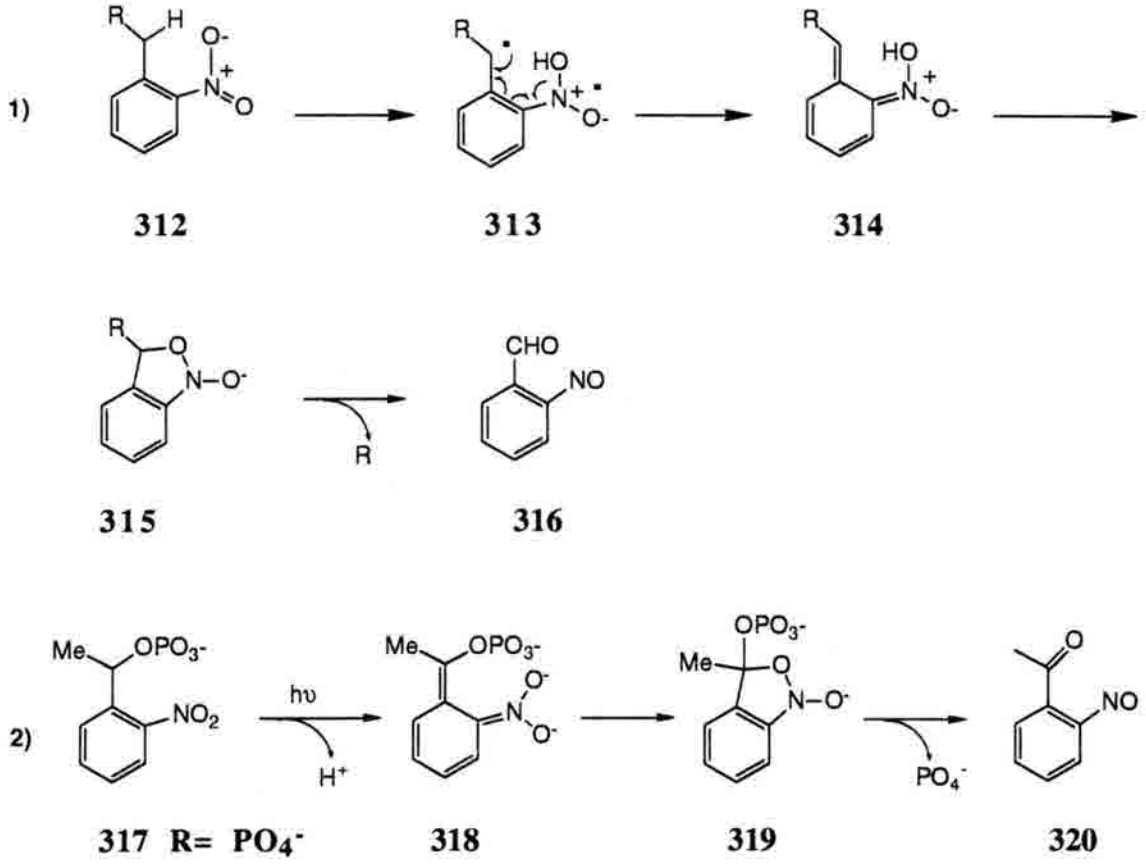
[a]Photolysis carried out using a mercury vapor lamp.

The mechanism for the photolytic deprotection is not clearly defined and is the topic of some debate. The two commonly proposed mechanisms are shown in Scheme 15. The reaction depicted in equation 1 originally proposed hydrogen atom abstraction from the *o*-alkyl side chain in **312** generating the radical cation **313.** Cation **313** undergoes rearrangement to the *o*-quinoid *aci*-nitro intermediate **314** which rearomatizes to give **315.** Decomposition of **315** results in loss of the R group generating the nitro aldehyde **316** as a by product.[133]

More recent mechanistic work carried out by Trentham using o-nitrobenzyl protected phosphates has lead to a variation of the proposed mechanism and is shown in

eq. 2.[134] The deprotections were carried out in aqueous media using pulsed laser photolysis. Formation of the aci-nitro intermediate **318** was followed spectroscopically, as well as the pH of the solution, during the photolysis reactions.

Scheme 15 Proposed mechanisms for photolytic deprotection of o-nitrobenzyl residue



312 **313** **314** **315** **316**

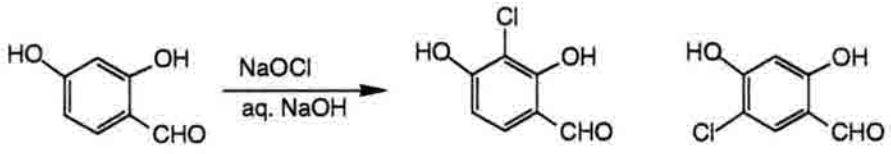
317 R= PO_4^- **318** **319** **320**

The formation of **318** from photolysis of **317** was observed to be preceded by an increase in pH. The increase in pH suggests the initial step in generation of **318** involves rapid loss of a proton from the *o*-alkylphosphate side chain, not a hydrogen atom abstraction. The authors also commented the mechanism presented in eq. 2 may not be applicable to the photolytic deprotection of o-nitrobenzyl alcohols, aldehydes or carboxylic acids.

In summary, the various model studies described demonstrated the tricyclic skeleton of aspirochlorine could be synthesized efficiently and stereoselectively from an acyclic hydroxamic ester. The cyclization reaction proceeds with the desired anti relative stereochemistry and requires the presence of a tertiary amide at the central position. In addition, model studies demonstrated the utility of the *o*-nitrobenzyl group as an amide protecting group. Application of the model studies towards the total synthesis of (±)-aspirochlorine is the focus of the following section.

The Total Synthesis of (±)-Aspirochlorine

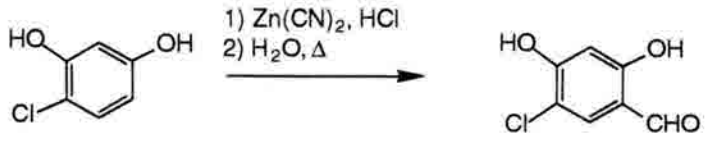
The total synthesis of aspirochlorine commenced with the synthesis of 2,4-dihydroxy-5-chlorobenzaldehyde (**323**) and a literature search revealed two methods. The simpler of the two methods reported by Hopkins and Chisholm involved electrophilic chlorination of 2,4-dihydroxybenzaldehyde (**321**) using sodium hypochlorite (bleach).[135]



321 **322** (50%) **323** (0%)

Attempted chlorination as described by Hopkins and Chisholm lead to exclusive formation of the undesired 3-chlorobenzaldehyde regioisomer **322**. The regiochemical assignment was based on ^{1}H NMR coupling constants for the 3 and 5-chloro regio isomers. In the 200 MHz ^{1}H NMR, **322** exhibits two doublets at 7.57 and 6.68 ppm (J= 8.69 Hz) consistent with *ortho*- protons indicating chlorination occurred at the 3-position.

The desired regioisomer **323** could be obtained from Gatterman formylation of chlororesorcinol (**324**) using a mixture of zinc cyanide/HCl.[136] Formylation consistently

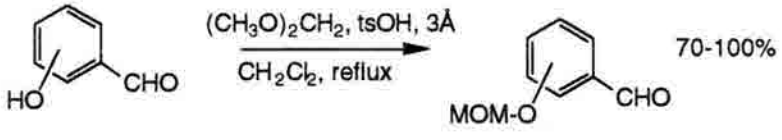


324 **323**

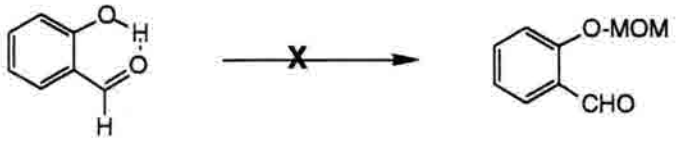
produced the desired benzaldehyde **323** in 40-50% yields and could be performed on 500 gram scale. The 200 MHz ^{1}H NMR of **323** exhibits two singlets at 7.61 and 6.59 ppm consistent for the *para*- substitution pattern present in **323**.

Yardley and Fletcher reported selective protection of hydroxy benzaldehydes **325** could be achieved under acidic conditions as depicted in Scheme 16.[137] Reaction of hydroxy benzaldehydes with dimethoxymethane in the presence of acid produced methoxymethyl protected phenols **326** in 70-100% yields. Furthermore, Yardley et al. reported 2-hydroxybenzaldehydes **327** were unreactive under the conditions due to internal hydrogen bonding between the hydroxyl group and carbonyl.

Scheme 16. Selective protection of hydroxy benzaldehydes



325 **326**



327 **328**

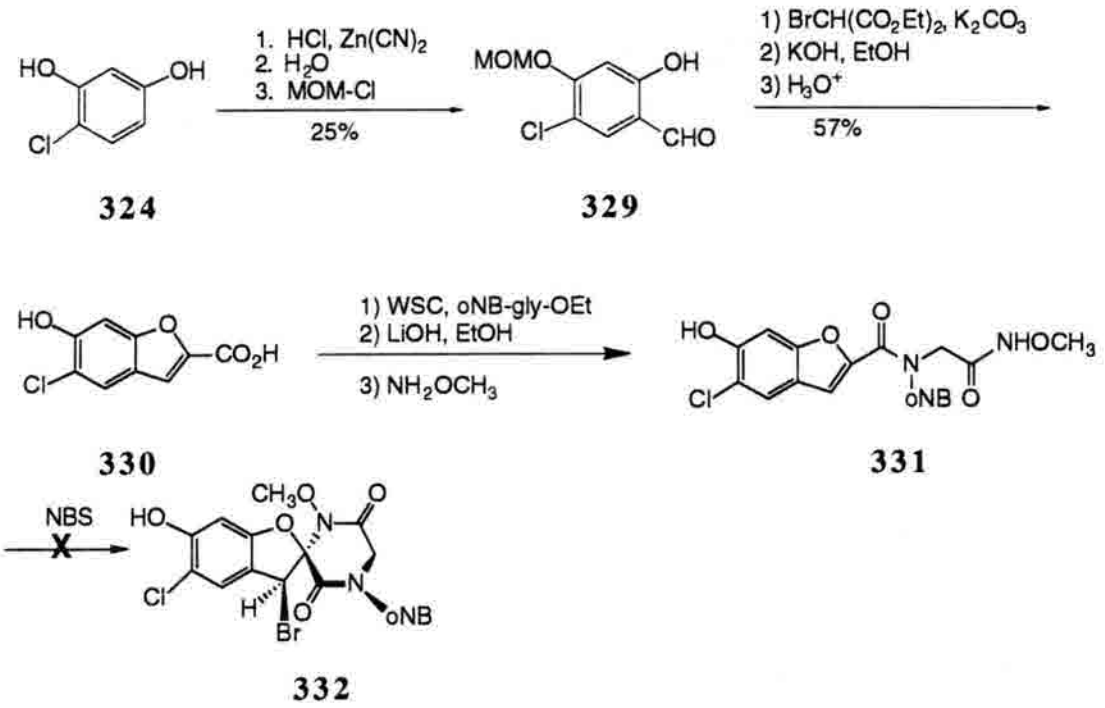
Attempts to protect **323** using Yardley's conditions resulted in mixtures of mono- and dialkylated products. Selective protection of the 4-hydroxyl group in **323** was

achieved with moderate success (49%) by reaction with chloromethyl methyl ether in the presence of triethyl amine. The desired methoxymethyl (MOM) protected aldehyde **329** was accompanied by the expected over-protected compound in 10-25% yield.

The initial approach to aspirochlorine is shown in Scheme 17. Benzaldehyde **329** was converted into the coumarilic acid derivative **330** in 50-77% yield using a procedure developed by Tanaka.[138] Acidic workup removed the methoxymethyl protecting group.

Conversion of **330** into hydroxamic ester **331** was straight forward and achieved in several steps. Coupling of **330** with o-nitrobenzyl glycine ethyl ester was performed using a water-soluble carbodiimide to generate the intermediate ester which was hydrolyzed

Scheme 17 Initial approach to aspirochlorine



324 **329** **330** **331** **332**

to the corresponding carboxylic acid without isolation. Conversion to O-methyl hydroxamate **331** was achieved by using the water-soluble carbodiimide in the presence of free methoxylamine. The overall yield of **331** from **330** for the 3 step process was 40%.

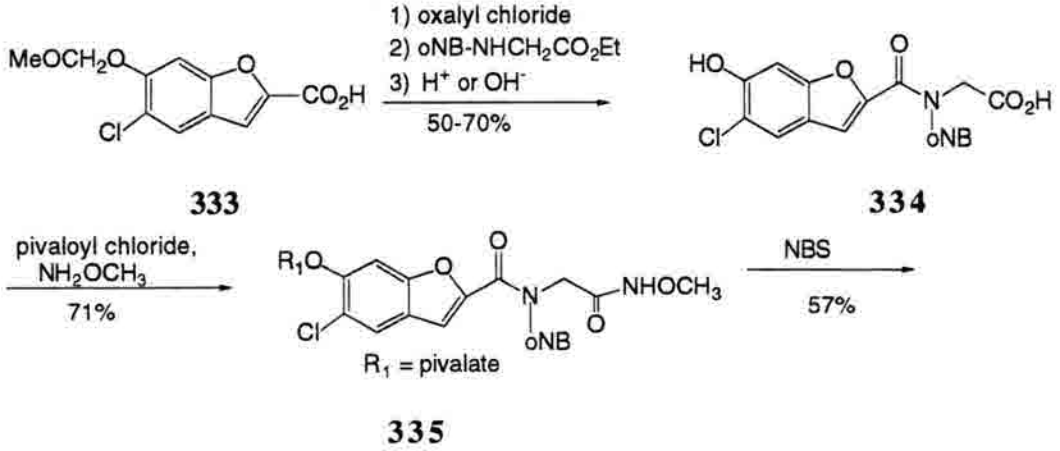
In a surprising result, **331** did not undergo the desired cyclization to **332** and the majority of starting material was isolated unchanged. The lack of reactivity of **331** strongly suggests the free C6-hydroxyl group has a pronounced influence of the electronic nature of the C2-C3 double bond presumably via resonance effects. To test this proposal, the hydroxyl group was protected as an electron-withdrawing ester group to minimize any donation of electron density from the hydroxyl group into the benzofuran ring system. The synthesis of the pivalate (t-butyl ester) protected compounds is shown in Scheme 18.

Reaction of carboxylic acid **334** with an excess of pivaloyl chloride followed by reaction with methoxylamine afforded the protected hydroxamic ester **335** in 71% yield. Cyclization of **335** afforded the desired bromide **336** in 57% yield.

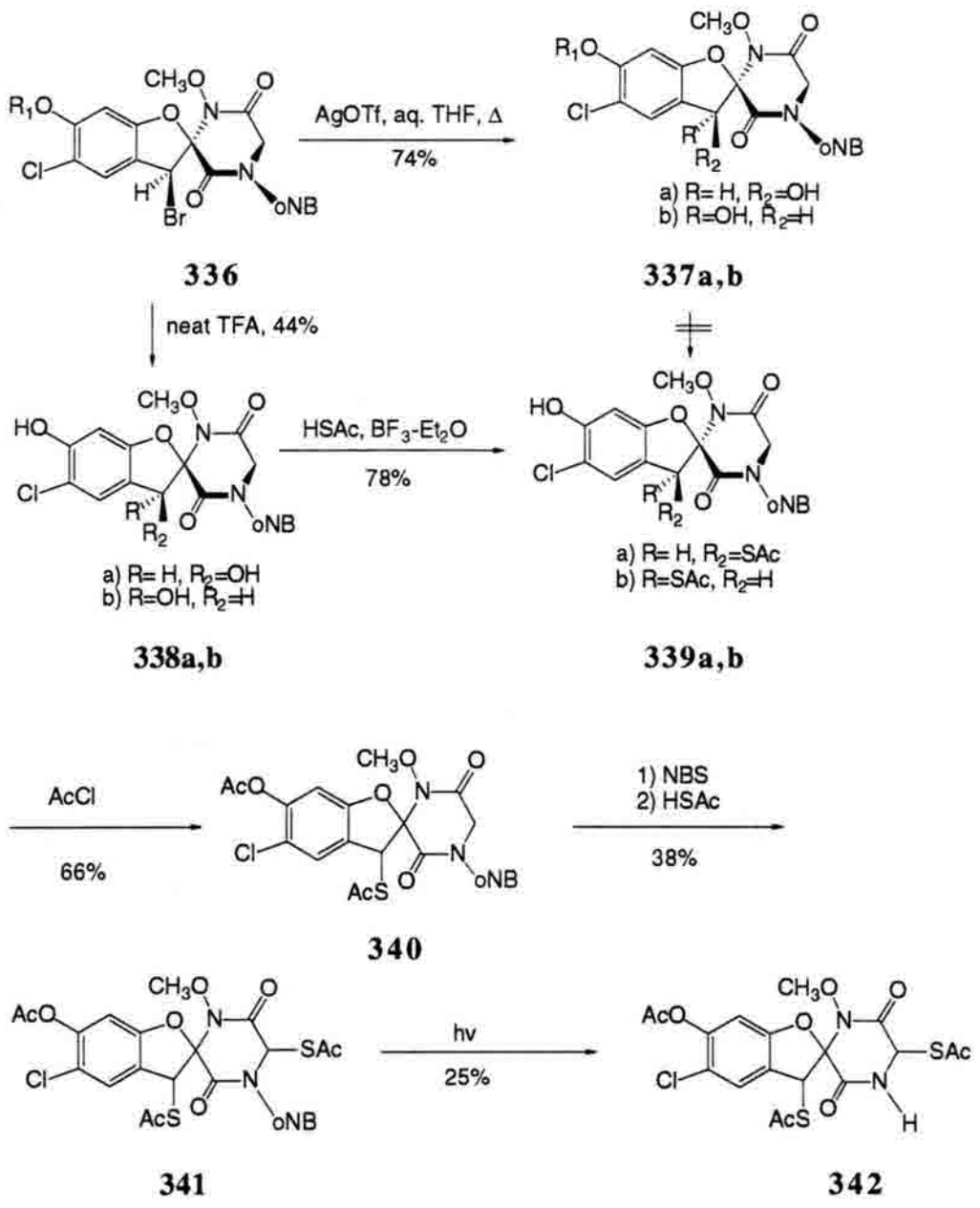
Bromide **336** was converted into the corresponding pivalate-protected alcohols **337a,b** in 74% yield without incident. In addition, **336** was converted into a mixture of deprotected alcohols **338a,b** containing the free phenol moiety in 44% by acid hydrolysis in neat TFA followed by aqueous workup.

Alcohols **338a,b** ultimately lead to the synthesis of the bisthioacetates **342** although the steps involved proved problematic. Irreproducible results and low overall

Scheme 18 Pivalate protected synthetic approach.



Scheme 18 continued



336 **337a,b** **338a,b** **339a,b** **340** **341** **342**

yields in several key transformations hampered the successful completion of this approach. For example, all attempts to convert alcohols **337a,b** into thioacetates **339a,b** failed.

Synthesis of **339a,b** was achieved by initial removal of the pivalate protecting group (neat trifluoroacetic acid) to give alcohols **338a,b**. Thioacetylation of alcohols

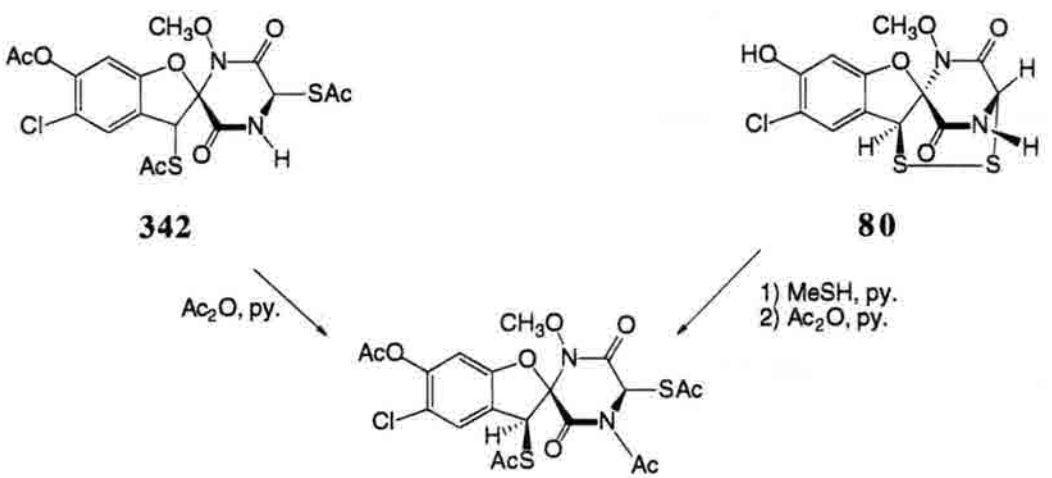
338a,b using boron trifluoride etherate lead to inseparable mixtures of S-acetylated compounds **339a,b.**

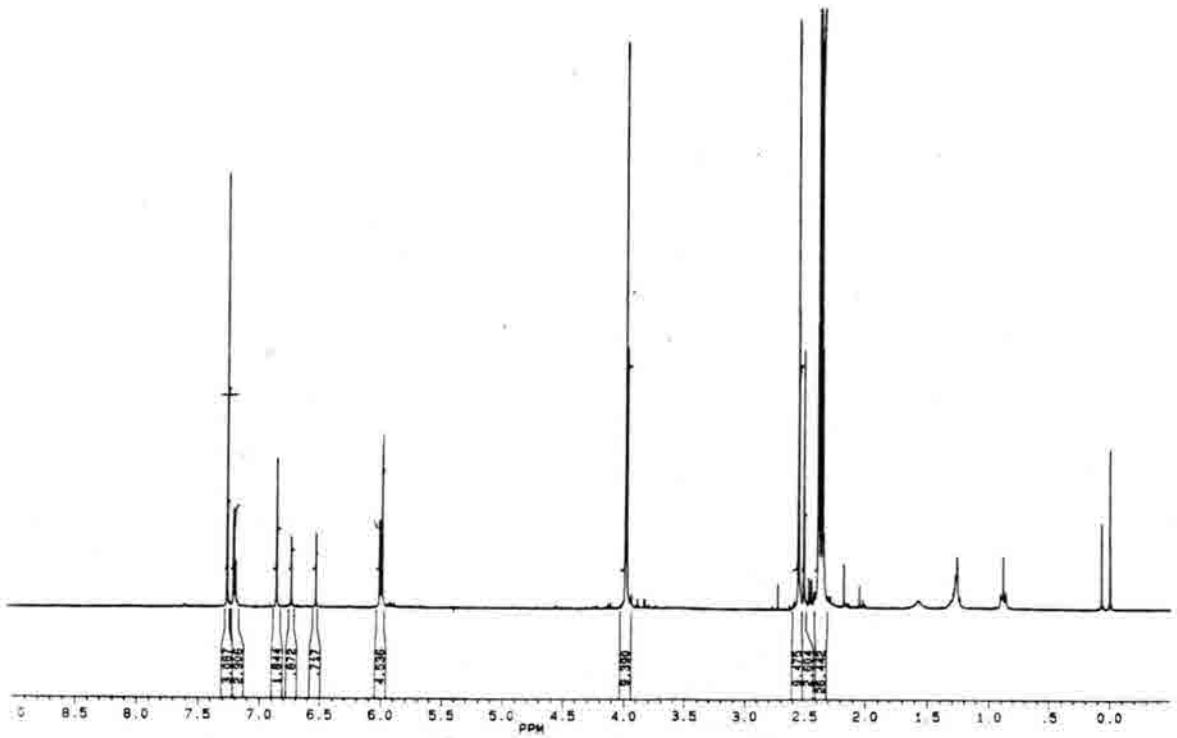
Bromination and thioacetylation of **340** resulted in a completely random mixture and **341** was isolated and tentatively identified. Because of the presence of diastereomers, the stereochemistry as well as the purity of **341** could not be accurately determined.

The mixture of bis-thioacetates **341** of unknown stereochemistry was photolyzed and a small amount (~25% yield) of material tentatively identified as **342** was isolated. The structure of **342** was confirmed following the sequences depicted in Scheme 19.

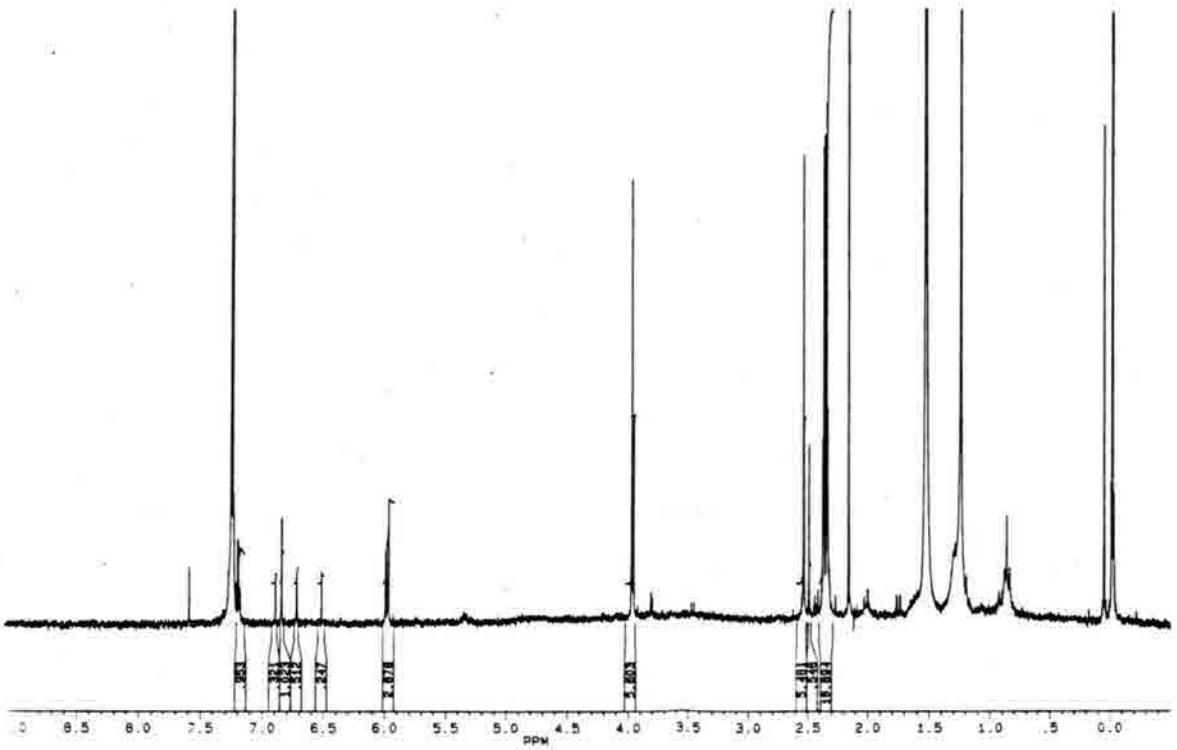
N-acetylation of **342** with acetic anhydride afforded **343** in moderate yield. Reduction of (+)-aspirochlorine (**80**) with sodium borohydride followed by acetylation with acetic anhydride also generated **343** confirming the structure. Figure 6 displays the NMR data obtained from the individual reactions. All attempts to convert either **342** or **339** into aspirochlorine failed.

Scheme 19. Confirmation of the structure of bis thioacetate **342**





^{1}H NMR of **343** from reduction and acetylation of aspirochlorine



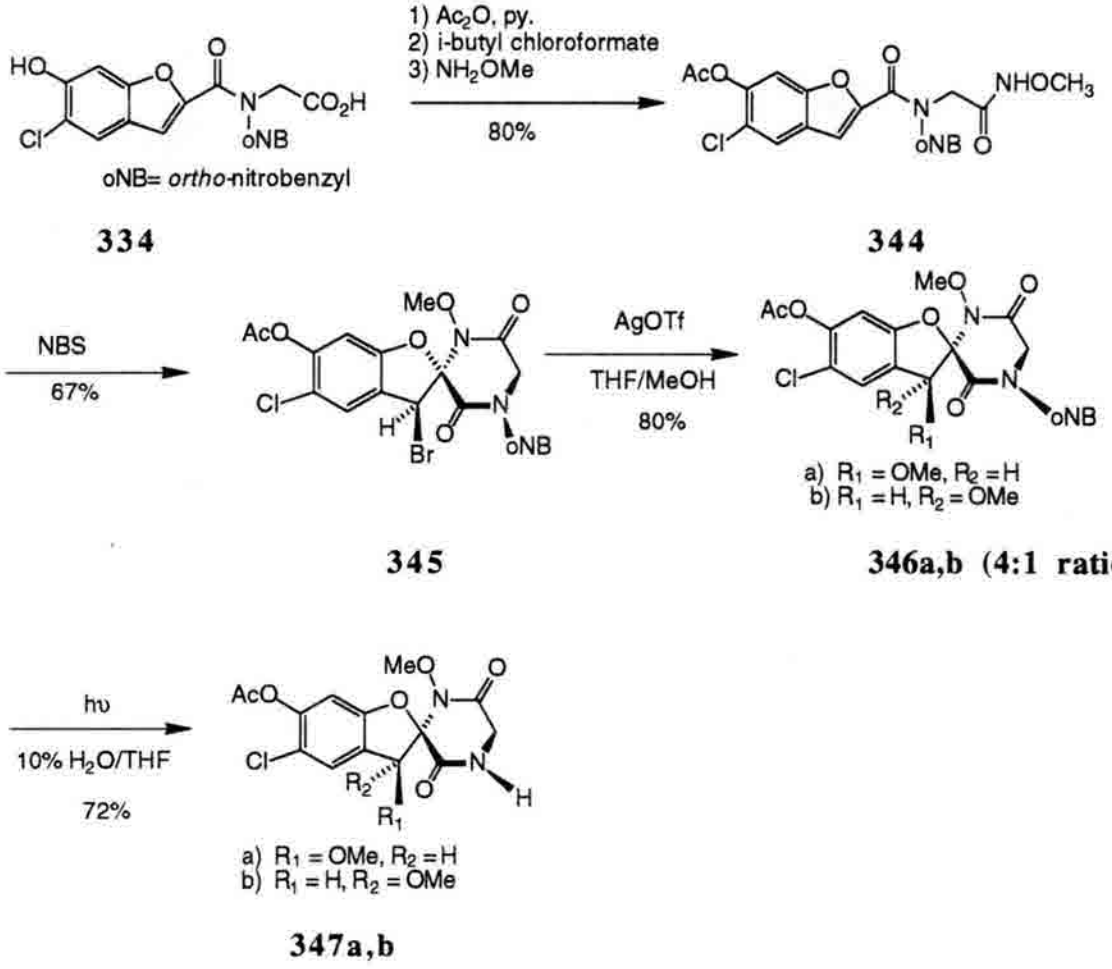
^{1}H NMR of synthetic **343**

Figure 6. Comparison of 300 mHz NMR's of bis thioacetate **343** in $CDCl_3$

Although the pivaloyl protected series of compounds appeared to be headed in the right direction, the approach had several drawbacks. A great concern was the disappointingly low yields and complex mixtures of inseparable products obtained in the final steps. In addition the overall synthetic design was inefficient, requiring a number of protecting group interchanges. At this time the decision was made to abandon the pivalate protecting group and incorporate an acetate as the phenol protecting group throughout the synthesis. This new series of compounds ultimately lead to the first successful total synthesis of (±)-aspirochlorine.

Incorporation of the acetate protecting group is shown in Scheme 20. The acetate

Scheme 20. Acetate protected compounds



334 **344** **345** **346a,b (4:1 ratio)** **347a,b**

moiety was efficiently introduced by treating **334** with excess acetic anhydride. Hydroxamic ester **344** was obtained in 70% yield via mixed anhydride formation using isobutyl chloroformate. Treatment of **344** with NBS gave the desired tricyclic compound **345** in 57-63% isolated yield after workup.

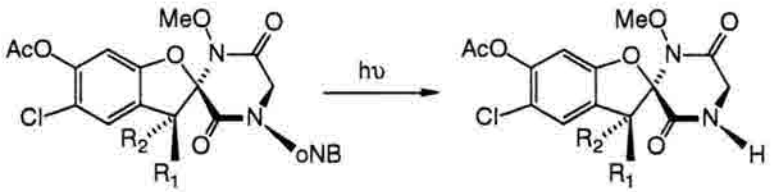
Although conversion of **345** into a mixture of alcohols proceeded as expected (silver triflate, aqueous THF, 73%, ~1:1 ratio), bromide **345** was converted into a mixture of methyl ethers **346a,b** in 73% yield instead. The change in strategy was designed to avoid formation of O-acetate side products which plagued previous model studies.

The conversion of **345** to **346a,b** proceeded in a stereoselective fashion resulting in a 4:1 ratio of **346a**:**346b**. More importantly, the methyl ethers **346a,b** could be separated by careful column chromatography and carried on independently simplifying the approach. Determination of the stereochemistry for the methyl ethers was carried out by NOE experiments previously described.

Photolytic deprotection of **346a,b** was examined under a variety of conditions and **347a,b** could be obtained in 40-50% yields. Table 3 summarizes several attempts to improve the yield of the photodeprotection.

During the course of the photolytic deprotection the solution darkens and this change in color was perceived to be contributing to quenching of the reaction. Addition of pyrex beads to the solution (entry 2) to increase the transmittance through the solution resulted in improving the yield to 58%.

Further improvements in the yield were obtained by decreasing the amount of water in the reaction from 30% to 10% resulting in 66% yield. A final change in the duration of the photolysis from 18 hrs to 5 hours increased the yield to an optimal 68-72%. Addition of an aldehyde trapping agent (10 eq. semicarbazide hydrochloride, entry 5) did not affect the overall yield for the photolysis.



TRANS R_1 = OMe, R_2 = H
CIS R_1 = H, R_2 = OMe

346a,b

TRANS R_1 = OMe, R_2 = H
CIS R_1 = H, R_2 = OMe

347a,b

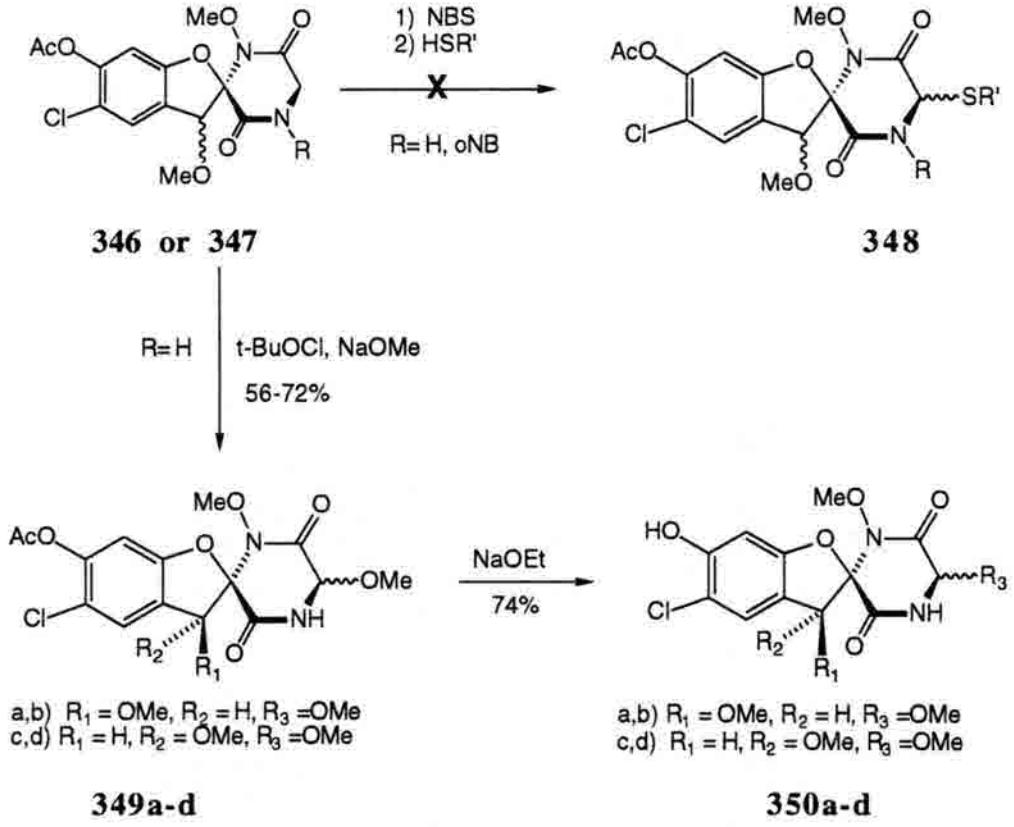
Table 3. Photolysis conditions for the conversion of 346a,b to 347a,b.[a]

Entry	Solvent,conditions	Time (hrs)	% Yield **343a,b**[b]
1	30% H_2O/THF	18	40-50
2	30% H_2O/THF, beads	18	58
3	10% H_2O/THF, beads	18	66
4	10% H_2O/THF, beads	5	72
5	10% H_20/THF, beads, 10 eq. semicarbazide-HCl	5	68

[a]All reactions were carried out at 10 mmol concentrations in a quartz tube. Photolysis was carried out using a 450-watt Conrad-Hanovia medium pressure mercury vapor lamp at 37°C. [b]Reported yields are isolated yields.

As in the pivalate series, attempted functionalization of the α-position of the diketopiperazine ring of methyl ethers **346a,b** under a variety of conditions did not give satisfactory results as depicted in Scheme 21. Attempted bromination of **346a,b** (NBS, benzoyl peroxide carbon tetrachloride, reflux) followed by trapping of the unstable intermediate bromide with thiolacetic acid resulted in low yields of the desired thioacetates **348.** The unprotected methyl ethers **347a,b** were also resistant to bromination under a variety of conditions which have been reported to be effective for bromination α- to NH groups in dipeptides (eg. NBS/CCl_4, or NBS/$CHCl_3$).[139]

Scheme 21.



Oxidation of the diketopiperazine ring was achieved via N-chlorination using t-butyl hypochlorite.[140] Treatment of **347a,**with t-butyl hypochlorite in the presence of sodium methoxide generated the bis methyl ethers *trans*-**349a,b** in 50-58% yields as an inseparable mixture of epimers. Analogous reaction was observed starting from **347b** to give *cis*-**349c,d** and the individual diastereomers separated by column chromatography.

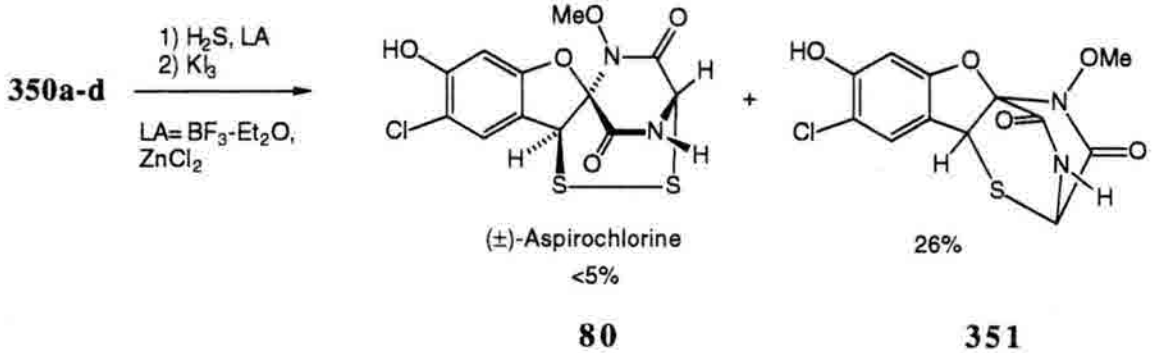
The N-chlorination/rearrangement reaction displays a pronounced solvent dependency and results from several experiments are shown in Table 4. The reaction appears to generally give higher yields when carried out in chlorinated solvents such as chloroform or methylene chloride.

Table 4. Attempted N-chlorination/rearrangement Reactions

Entry	alcohol	Reaction conditions	%Yield **345**
1	**347a,b**	1.1 eq. t-BuOCl, 1.9 eq, NaOMe Dioxane, 0°C	16
2	**347a,b**	1.1 eq. t-BuOCl, 0.9 eq, NaOMe Et_2O, 0°C	28-38
3	**347a,b**	1.1 eq. t-BuOCl, 0.9 eq, NaOMe THF, 0°C	46
4	**347a,b**	1.1 eq. t-BuOCl, 1.0 eq, NaOMe MeOH, 0°C	32-52
5	**347a,b**	1.2 eq. t-BuOCl, 1.0 eq, NaOMe, CH_2Cl_2, 0°C	53
6	**347a**	1.3 eq. t-BuOCl, 1.1 eq, NaOMe CH_2Cl_2, 0°C	58
7	**347b**	1.3 eq. t-BuOCl, 1.1 eq, NaOMe $CHCl_3$, 0°C	55

The acetate protecting group in **349a-d** was efficiently removed by treatment of the individual diastereomers with sodium ethoxide in absolute ethanol at 0°C to give the free phenols **350a-d** in 72-74% yields. In addition, all four diastereomers of **350a-d** were easily separated by chromatography. The direct conversion of **347** to **350** could be carried out using excess base, but better overall yields were obtained if the reactions were carried out separately.

Incorporation of the sulfur moieties into **350a-d** using $H_2S/ZnCl_2$ was anticipated to lead directly to aspirochlorine based on numerous examples in the literature of similar transformations.[99] Treating **350a,b** with H_2S in the presence of $ZnCl_2$ or BF_3-Et_2O

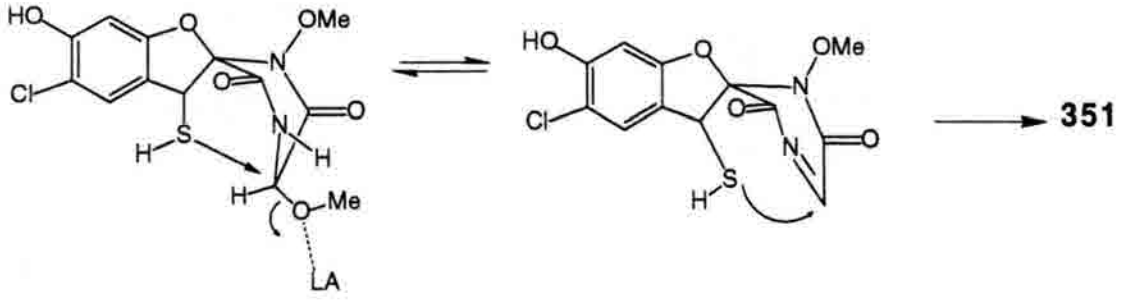


80 **351**

under numerous conditions followed by KI_3 oxidation resulted in complex mixtures and only trace amounts of the natural product. Treatment of **350c,d** with $H_2S/ZnCl_2$ followed by oxidation gave aspirochlorine in a disappointing 5-10% yield.

A possible explanation for the lack of natural product in reactions using H_2S was provided by the isolation and tentative identification of the 6-membered monosulfide **351**. The structural assignment was based on mass spectral analysis evidence (M^+=328 corresponding to $C_{12}H_8N_2O_5SCl$), although rigorous structural elucidation was not carried out. The ^1H NMR and mass spectral data of **351** are included in the appendix.

Formation of **351** can be rationalized to occur via intermediate **352**. Generation of the N-acylimine **353** as the result of loss of methanol is followed by a rapid intramolecular substitution leading to the monosulfide **351**.

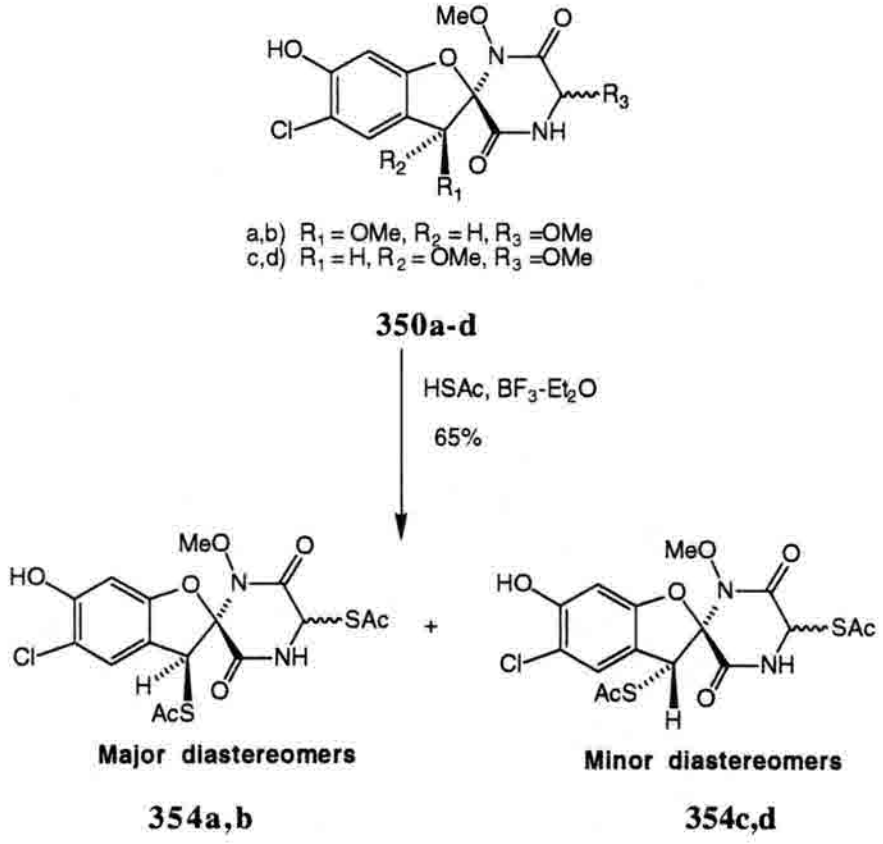


352 **353**

Conversion of methyl ethers **350a-d** to the corresponding bis thioacetates **354a-d** is shown in Scheme 22. The reaction was carried out using 12 eq. thiolacetic acid, 6 eq. BF_3-Et_2O (reflux, 8 hours) and unfortunately resulted in a complex mixture of diastereomers **354** in approximately 65% combined yield.

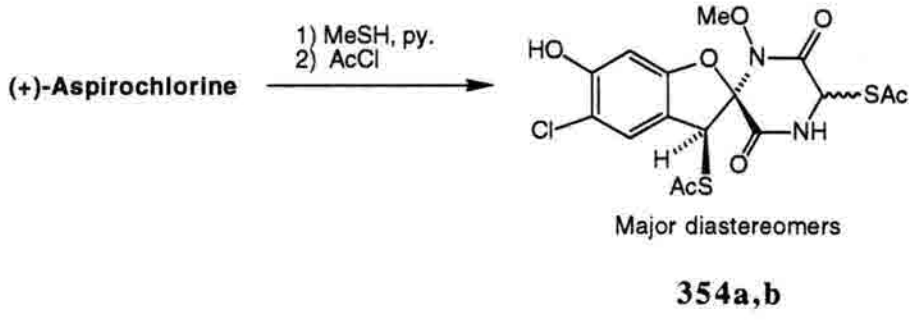
The mixture of thioacetates could be separated into two sets by chromatography. The non-polar minor mixture **354c,d** (ca. < 20%) was assumed to contain the unnatural configuration about the benzylic position while the more polar major set of diastereomers

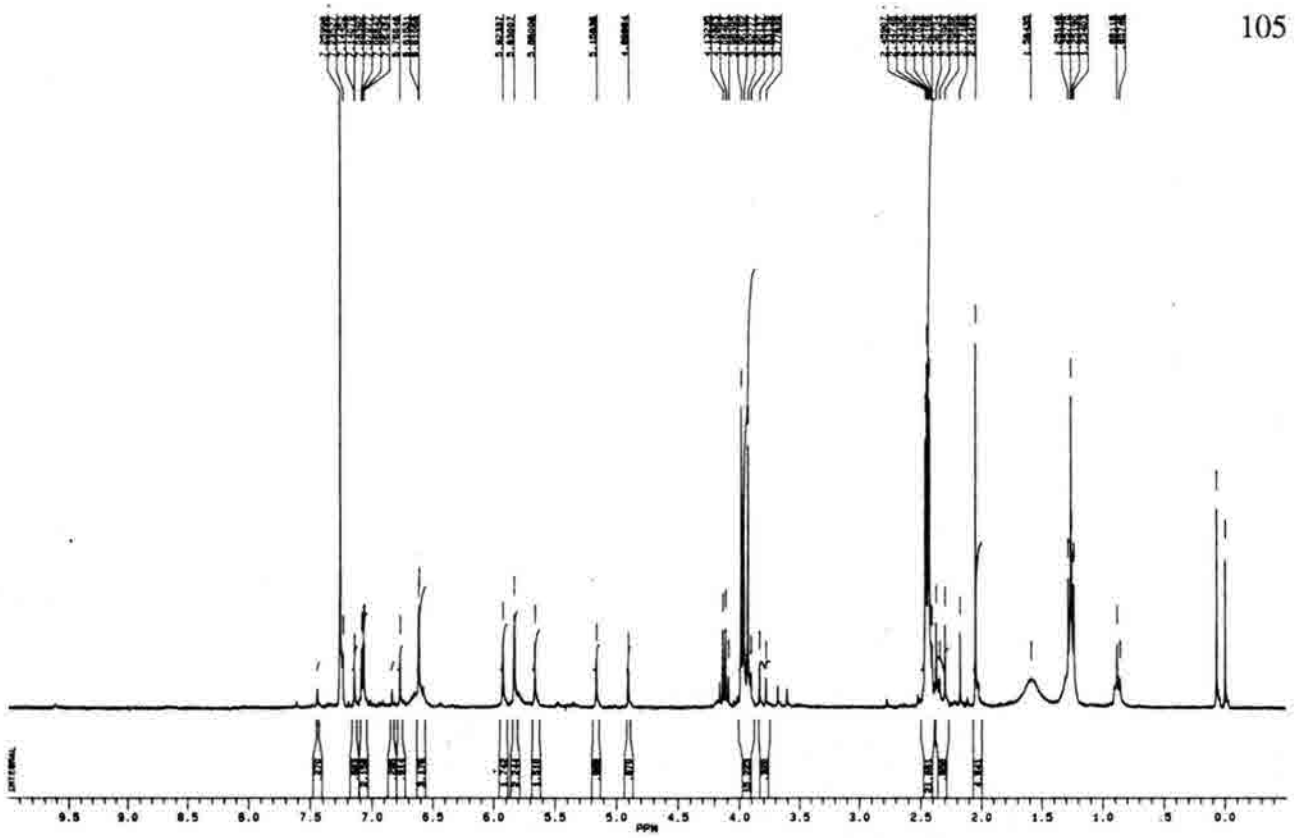
Scheme 22 Conversion of **350** to bisthioacetates **354**



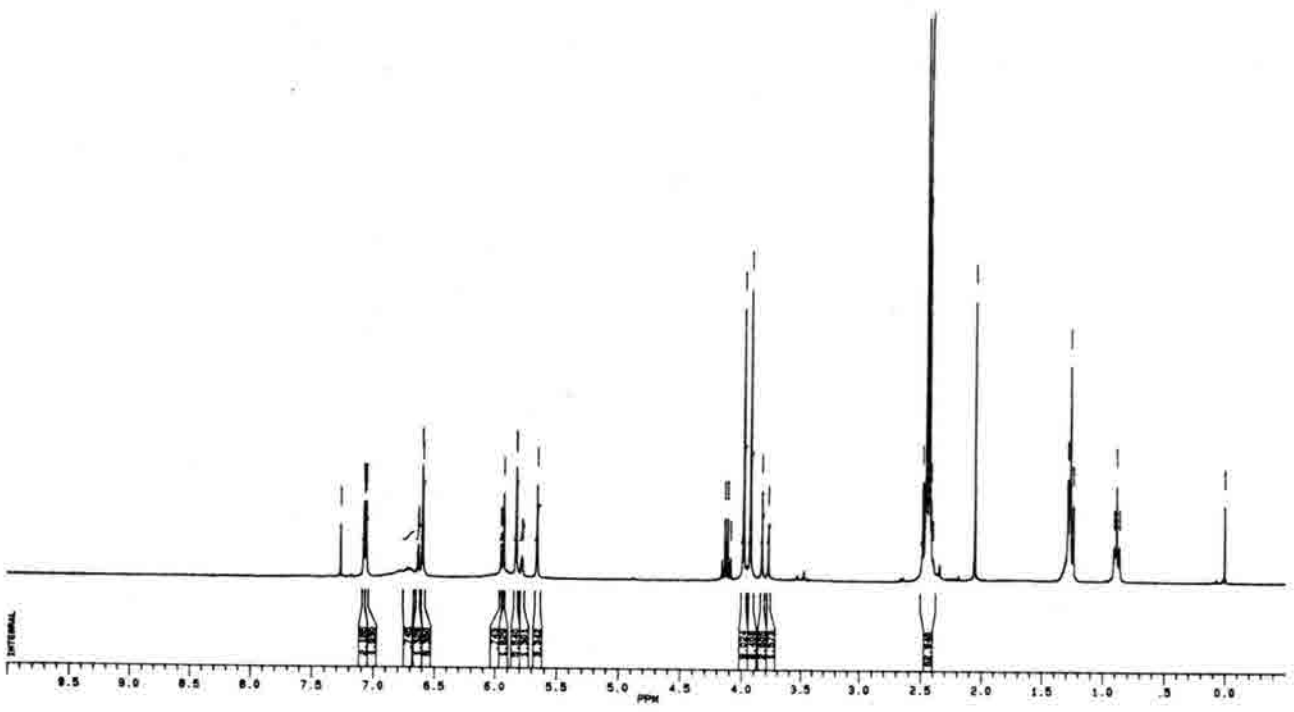
354a,b was considered to contain the desired natural stereochemistry. All attempts to further separate the individual diastereomers were unsuccessful.

The structural assignment was based on the precedents observed in the previous model studies. In all cases investigated, the major product contained the *trans* relative stereochemistry. The stereochemical assignment was verified by reduction of an authentic





^{1}H NMR of **354a,b** from reduction and acetylation of aspirochlorine

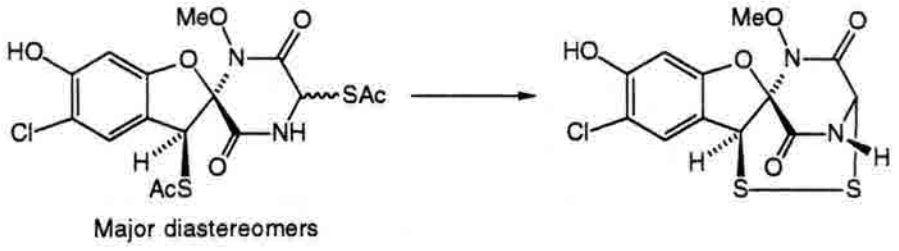


^{1}H NMR of synthetic **354a,b**

Figure 7. 300 mHz ^{1}H NMR comparison of bisthioacetates **354a,b** in $CDCl_3$.

sample of aspirochlorine using excess methyl mercaptan in pyridine followed by trapping of the dithiol intermediate with acetyl chloride to generate **354a,b**. Comparison of the ^{1}H NMR spectra from the reaction with the ^{1}H NMR of thioacetates **354a,b** obtained from thioacetylation established the thioacetates were identical. Comparison of the ^{1}H NMR spectra are shown in Figure 7.

The conversion of thioacetates **354a-d** into aspirochlorine also proved problematic and Table 5 summarizes the various attempts to afford the final transformation.



354a,b **(±)-Aspirochlorine, 80**

Basic conditions such as sodium methoxide, aqueous sodium bicarbonate, sodium hydrogen sulfide, or ammonium hydroxide under either anaerobic or aerobic conditions resulted in disappearance of starting material **354a,b** but did not produce any aspirochlorine upon oxidative workup.

Deprotections using a non-basic nucleophile such as cyanide anion[141] (0.1 eq aqueous sodium cyanide, methanol, reflux) gave only traces of the natural product by ^{1}H NMR. Reactions of **354a,b** with chloroaniline, reported to cleave base-sensitive thioacetates, gave no reaction.[142]

Removal of the acetate protecting groups in **354a,b** under acidic conditions was achieved but with limited success. Treatment of thioacetates **354a,b** with saturated ethanolic HCl followed by oxidation resulted in complex mixtures from which aspirochlorine could be isolated in 5-15% yield. Numerous attempts at varying the

Table 5. Attempted deprotections of 354a,b

Entry	Conditions	Result
1	1) aq. $NaHCO_3$ (anaerobic): 2) KI_3	decomposition
2	1) 3.1 eq NaOEt (anaerobic), 2) KI_3	decomposition
3	1) 2.0 eq NaOEt (anaerobic), 2) KI_3	decomposition
4	1) NH_4OH/MeOH 0°C, 2) KI_3	decomposition
5	0.2 eq NaCN/EtOH reflux	decomposition
6	1) 0.1 eq NaCN/EtOH rt , 2) KI_3	trace
7	0.2 eq KCN/EtOH	decomposition
8	1) HCl/EtOH, 2) KI_3	10-15%
9	TFA/thioanisole	No reaction
10	1) cat. H_2SO_4, TFA/H_2S, 2) KI_3	9%
11	1) NaSH, HCl/EtOH, 2) KI_3	15-20%
12	1) H_2S, CSA benzene, 2) KI_3	28%
13	1) EtSH, CSA (neat), 2) KI_3	No reaction
14	1) DMAP, CH_2Cl_2	decomposition
15	chloroaniline, benzene reflux, 4 days	No reaction
16	1) NH_2OMe (neat), CSA, benzene, 2) KI_3	20-38%

conditions (aq. HCl/EtOH, cat. HCl, aqueous acid, neat TFA/thioanisole, etc.) did not substantially improve the yield.

The use of sulfur nucleophiles to effect the deprotection gave mixed results. Saturating a solution of **354a,b** with hydrogen sulfide afforded upon workup aspirochlorine in 28% yield. Unfortunately, the reaction was difficult to reproduce with any consistency. Attempted deprotection with ethanethiol did not give any reaction and starting material was recovered.

The low yield and substantial amount of decomposition observed, indicated the possibility that **354a,b** was unstable to the reaction conditions. Furthermore, the oxidation of the intermediate dithiol to the 7-membered disulfide may not readily proceed.

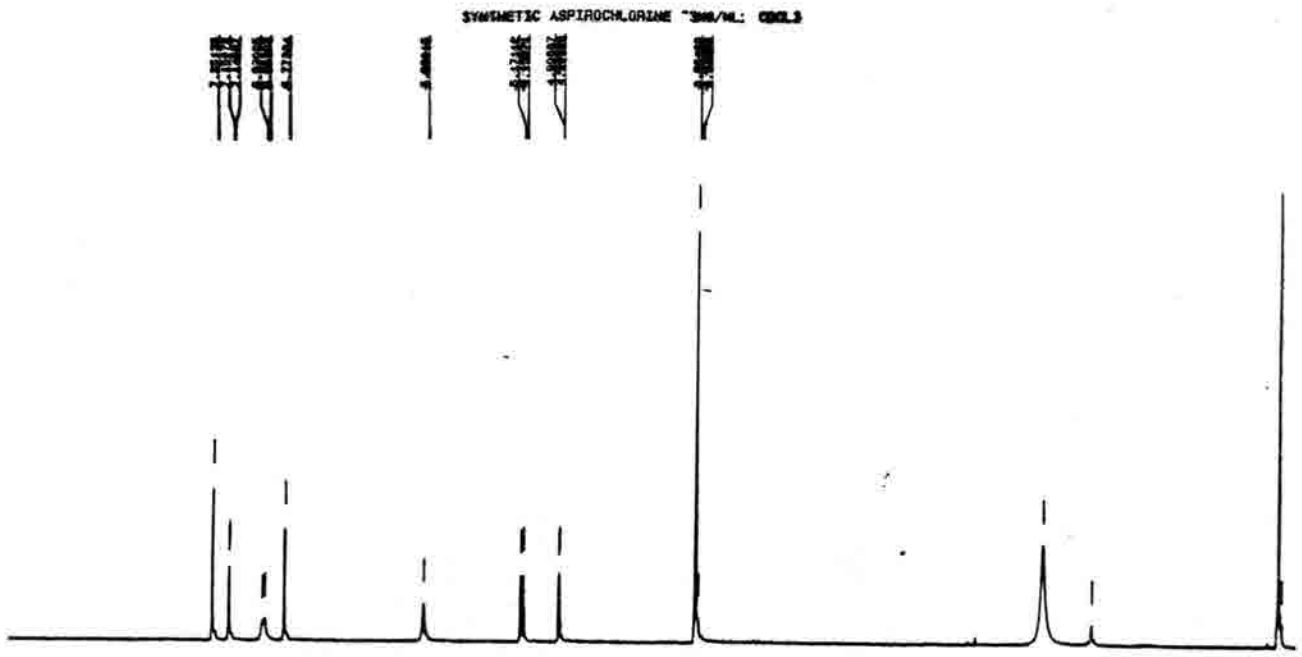
To test whether oxidation of the intermediate dithiol was contributing to the low yield, a sample of natural aspirochlorine was reduced (sodium borohydride, ethanol, 0°C) and immediately oxidized with aqueous KI_3. Although the reaction appeared to be clean by TLC, aspirochlorine was only isolated in 38% from the reaction, indicating the oxidation does not take place as readily as anticipated. Attempt to improve the oxidation step by using alternative agents such as O_2, $FeCl_3$, and Ellman's reagent were unsuccessful.

After several additional attempts, the thioacetates were cleaved under relatively mild conditions using aminolysis. Treating a solution of **354a-d** with excess methoxylamine in the presence of camphor sulphonic acid (CSA) routinely gave (±)-aspirochlorine in 20-38% isolated yield. The synthetic aspirochlorine possessed identical ^1H NMR, IR and HPLC retention time when compared with the natural substrate.

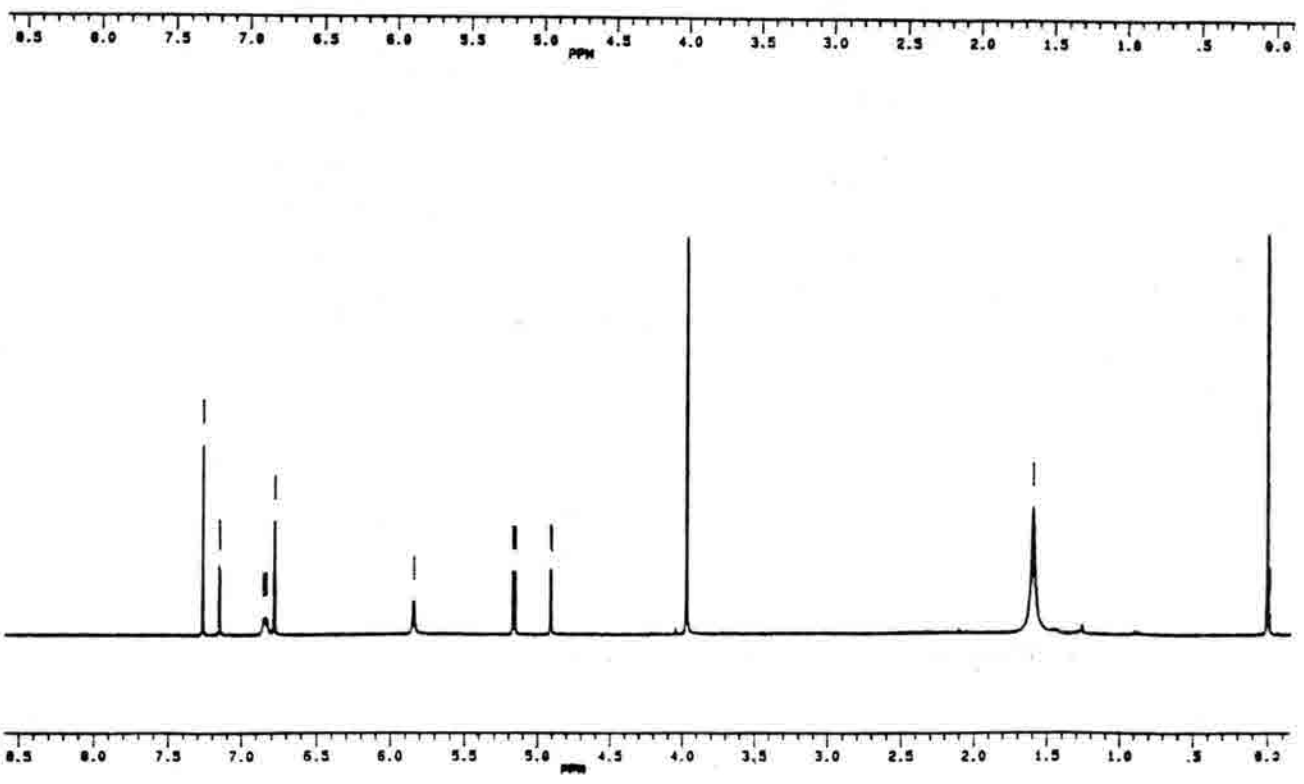
Figure 8 presents the comparison of the ^1H NMR's of synthetic and natural aspirochlorine and the IR comparison is shown in Figure 9. Comparison of the HPLC retention times is included in the appendix.

At the present time, epi-aspirochlorine **355** has not been positively identified from reactions involving methyl ethers **350a-d** or from thioacetates **354a-d**. Several possible compounds were isolated, although a consistent NMR was never obtained from the various reactions. The lack of evidence for **355** does not rule out formation of epi-aspirochlorine and may reflect problems associated with stability.

The first total synthesis of (±)-aspirochlorine has been achieved in 16 steps from 4-chlororesorcinol and is summarized in Scheme 23. The synthesis proceeds with moderate stereoselectivity, and demonstrates the use of the *o*-nitrobenzyl group as a photolabile amide protecting group.



a) Synthetic (±)-aspirochlorine



b) Authentic (+)-aspirochlorine

Figure 8. Comparison of ^{1}H NMR of a) synthetic and b) natural aspirochlorine.

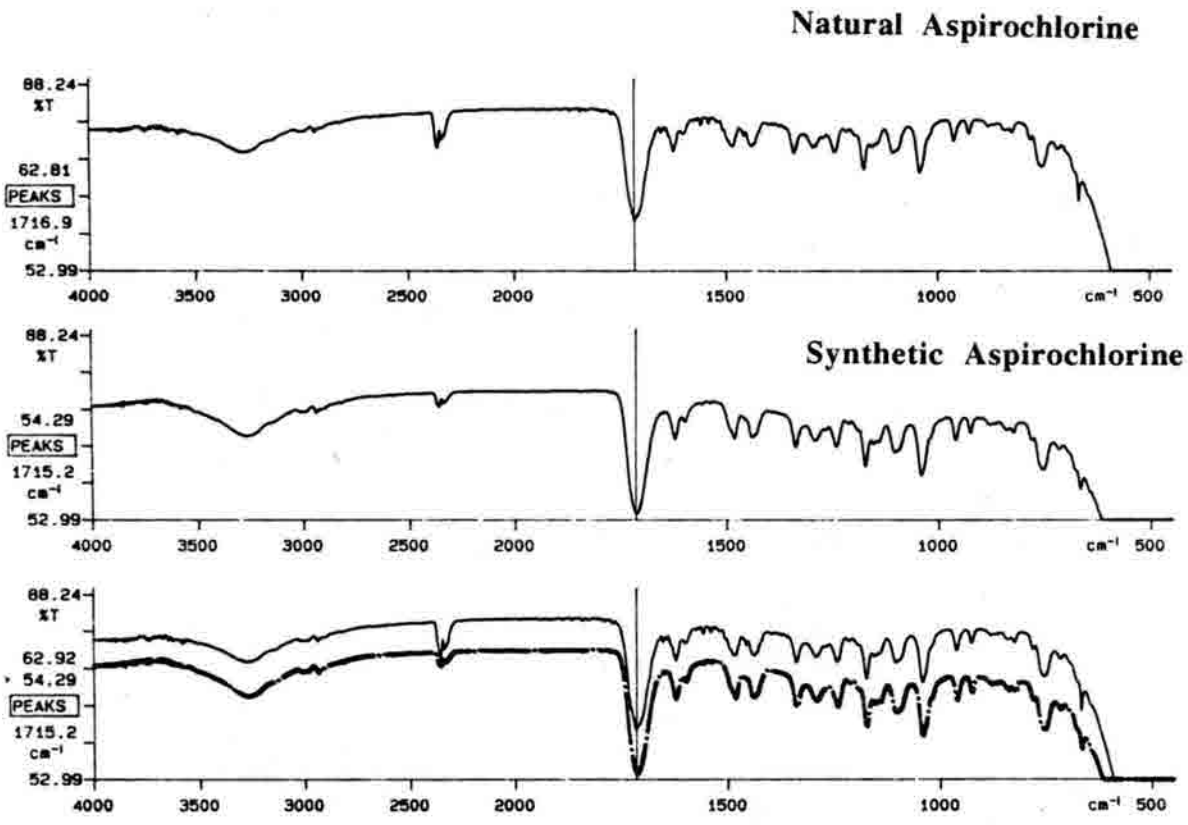
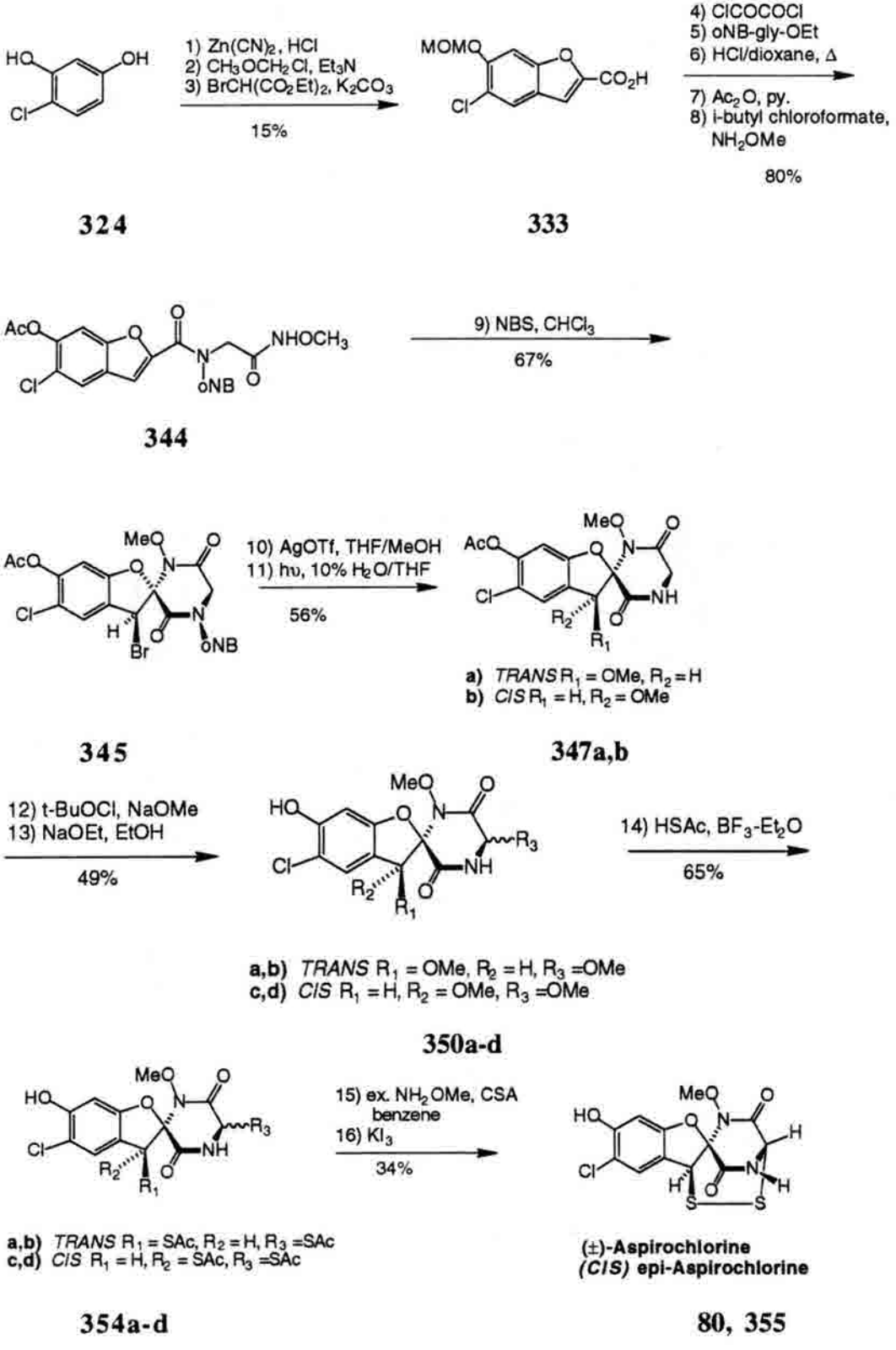


Figure 9. Comparison of IR spectra of synthetic and natural aspirochlorine

Scheme 23. Total synthesis of (±)-Aspirochlorine



324 **333** **344** **345** **347a,b** **350a-d** **354a-d** **80, 355**

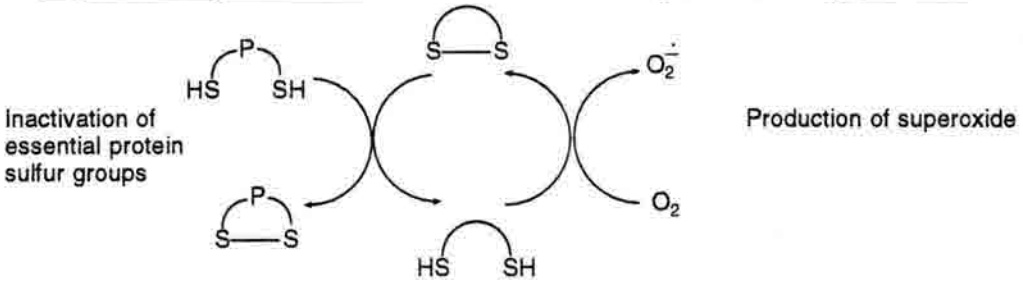
Chapter 4
Mechanism of Action

The epipolythiodioxopiperazines possess a wide range of biological properties. Early evidence indicating the epidithiodioxpiperazine ring was responsible for biological activity was obtained by Trown who observed that the simple N,N-dimethyl epidithiodioxopiperazine **145** displayed biological activity.[96] Further evidence supporting this proposal includes a number of studies demonstrating reduction to the dithiol or conversion to the bis(methylthio) derivatives diminishes or abrogates biological activity.[143]

The marginal antiviral properties exhibited by aspirochlorine are unusual compared to the potent activity displayed by the majority of epipolythiodioxopiperazines. The lack of activity exhibited may be due to subtle differences in redox properties inherent in the 7-membered disulfide ring versus the more commonly found 6-membered ring. The occurrence of aspirochlorine provides a unique opportunity to study the differences in redox properties of epidisulfides as a function of ring size.

Although the antimicrobial effects of epidithiodioxopiperazines have been studied for decades, only recently has a molecular basis been presented to account for the biological activity. With the exception of the bis(methylthio)gliotoxins (**19, 20**) the basis for antimicrobial activity is believed to involve facile dithiol/disulfide exchange of the epidithiodioxopiperazine moiety. This group can react in two intimately related processes depicted in Figure 1.

Figure 1. Proposed Mechanisms of Action for Epidithiodioxopiperazines



The first pathway involves thiol/disulfide exchange reaction between the epidithiodioxopiperazine moiety and protein-bound thiols resulting in inactivation of the proteins. A more intriguing mechanism is the second pathway involving generation of superoxide during oxidation of dithiols. Since thiol/disulfide exchange and oxidation of thiols readily occur, the epidithiodioxopiperazines are believed to exert their influence in a catalytic fashion. Moreover, the rates of both thiol/disulfide exchange and oxidation may be affected by the size of the disulfide ring.

The first part of this chapter describes attempts to measure thiol/disulfide exchange for several disulfides. The remaining section is devoted to several studies that attempted to measure the relative reactivity of various disulfides and dithiols as a function of superoxide production.

Attempted thiol/disulfide exchange.

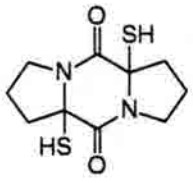
Thiol/disulfide exchange occurs via S_N2 displacement of a thiolate anion on the disulfide bridge. The reaction follows a Brönsted relationship and the optimum rate occurs when the pH of the solution is equal to the pKa of the thiol.[144]

In a series of extensive investigations designed to produce strongly reducing dithiols, Whitesides has studied the factors influencing thiol/disulfide exchange.[145] From these studies Whitesides concluded the major factor in the preparation of strongly reducing dithiols is the strain energy involved in oxidation of the dithiol to the disulfide bridge. The strain energy correlates with the observed CSSC dihedral angle found in the native disulfides. The torsional strain in the CSSC angle is due to steric repulsion between the lone pairs on sulfur and is known as the *gauche effect.*.[146]

Whitesides et al. demonstrated dithiols which generate strain free 6-membered dithianes upon oxidation are most strongly reducing.[145h] Dithiols that generate 5 and 7-membered rings upon oxidation were shown to be approximately one order of magnitude less strongly reducing in dithiol/disulfide exchange reactions.

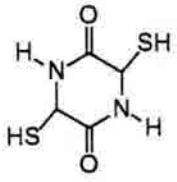
The epidithiodioxopiperazines may constitute an exception to this rule. Determination of the dithiol/disulfide equilibrium constant and corresponding reduction potential ($E^{o'}$) for 3,6-dimercaptoprolylproline anhydride **356** indicated this compound to be slightly more reducing towards disulfides than dithiothreitol **358** shown in Figure 2. The increase in reducing ability of **356** versus **358** may be due to the small amount of reorganization required for **356** upon oxidation.

Figure 2. Comparison of reduction potential ($E^{o'}$) for various dithiols

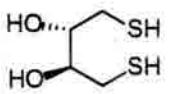


356

$E^{o'}$= -0.344 v



357



358

$E^{o'}$=-0.323 v

Interestingly, a recent molecular modeling study comparing the equilibrium constants for reaction between dithiols and oxidized butanedithiol (**359**) led to a different conclusion.[145e] The equilibrium constant (Keq) for the reaction of **357** with **359** was

Scheme 1. Molecular modeling study

BDT^{ox} BDT

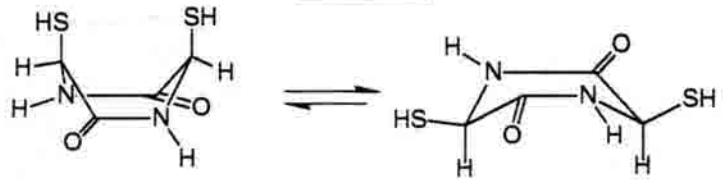
359 **360**

$$K_{eq} = \frac{[RS_2][BDT]}{[HS\text{-}R\text{-}SH][BDT^{ox}]}$$

predicted to be $2.0x10^{-3}$ M, and for the reaction with DTT (**358**), Keq=6.0 M. These two studies suggest the possibility that epidithiodioxopiperazines such as **356** or **357** are thermodynamically more reducing, although kinetically the epidithiodioxopiperazines are slower.

Another possible explanation for the discrepancy in reducing abilities maybe due to the conformation of the individual dithiols demonstrated in Scheme 2. In the proline derivative **356**, the mercaptan groups are held in position by the rigid nature of the proline ring. In the diketopiperazine **357,** the dithiols can be either pseudo axial or pseudo equatorial and the rate of interconversion may affect the rate of oxidation to the disulfide.

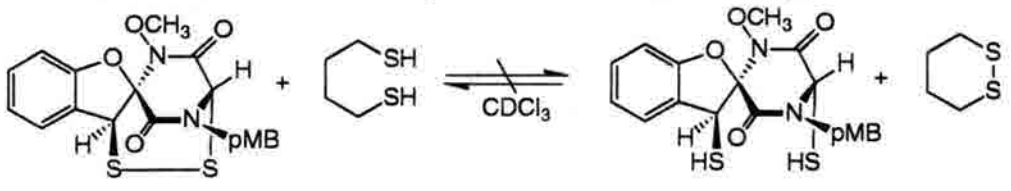
Scheme 2.



357-axial **357-equatorial**

Investigation of thiol/disulfide interchange is often carried out by following the reaction of interest by NMR.[147] Several attempts to follow dithiol/disulfide exchange between the model disulfide **273** and butanedithiol (**360**) (Scheme 3) according to the method used by Whitesides were unsuccessful.[145j] Even after a couple of weeks, NMR

Scheme 3. Attempted dithiol/disulfide exchange



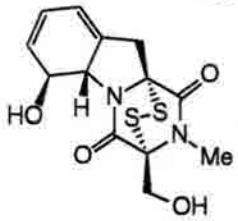
273 **360** **361** **359**

of the reaction mixture did not show any exchange had taken place. Changing the dithiol to DTT was unsuccessful due to the insolubility of DTT in chloroform as well as attempts to change solvents.

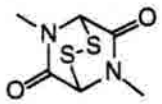
Similar results were observed in attempts to monitor dithiol/disulfide exchange between dithiol **360** and the simple epidisulfide **145**. In hindsight, the lack of reactivity is attributed to the absence of any thiolate anion in the reactions which is necessary to initiate the exchange process. Limited by the availability of the various disulfides, more sensitive methods to measure thiol/disulfide exchange were sought.

Attempts to measure the equilibrium constant for thiol/disulfide exchange for the various disulfides shown in Figure 3 using cyclic voltametry lead to an interesting series of

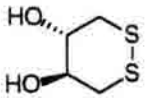
Figure 3. Disulfides used in Cyclic Voltametry experiments.



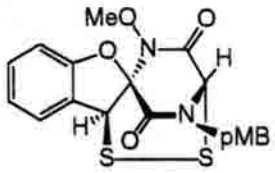
16, gliotoxin



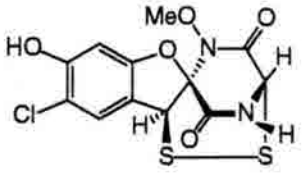
145



362



273



80, aspirochlorine

results. In all cases, reduction of the disulfides to corresponding dithiols occurred. Unfortunately the reverse oxidation to disulfide was characterized by surface waves.[148] Figure 4 presents a typical cyclic voltamogram from the reactions.

The occurrence of the surface waves indicates irreversible reaction between the intermediate dithiol and electrode surface occurred during oxidation. Despite the overall

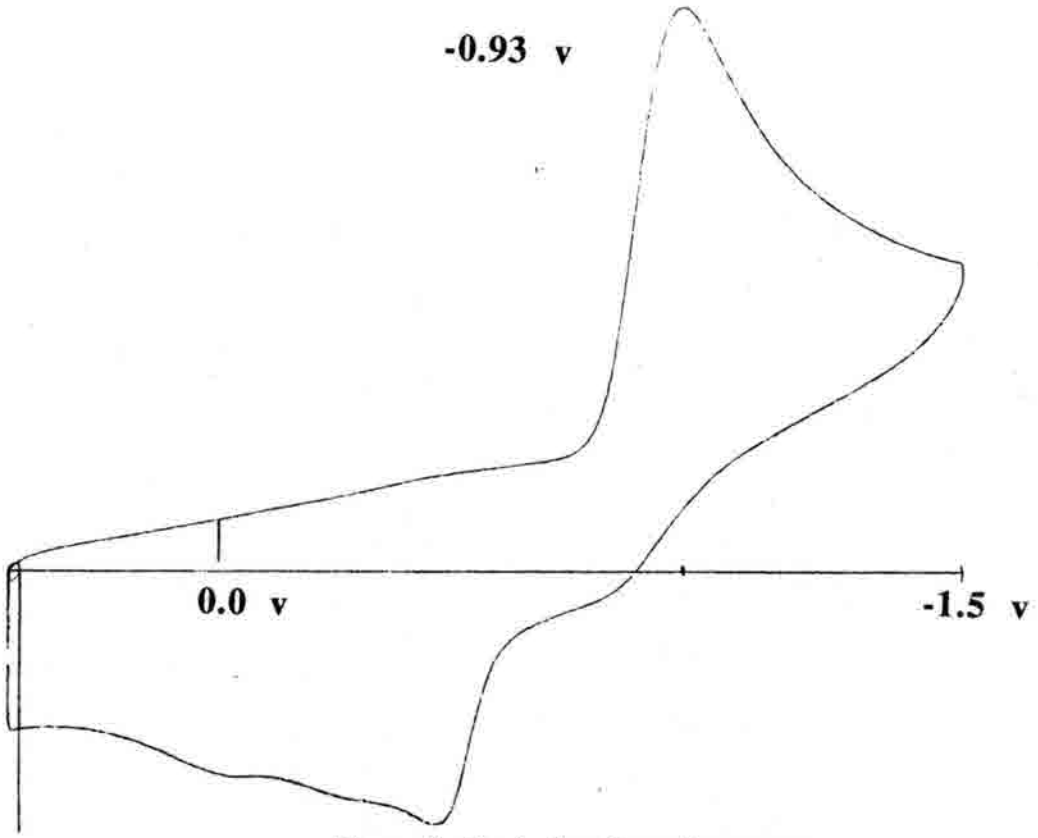


Figure 4. Typical cyclic voltamogram

Table 1. Observed reduction potentials for various disulfides

Entry	Disulfide	CSSC dihedral angle	Observed Potential [a]
1.	**145**	8-18 ^{o}	-0.93
2.	**16**	8-18^{o}	-1.16
3.	**80**	~56^{o}	-1.6
4.	**273**	~56^{o}	-1.35
5.	**362**	58-66^{o}	-2.14

[a]Potential measured versus the sodium chloride saturated calomel electrode (SCCE)

---

process being irreversible, a general trend in the observed reduction potentials was noted. Values for the experimentally measured reduction potential of the disulfides and CSSC dihedral angle present in the disulfide are presented in Table 1.

The relative ordering of the observed potentials appears to directly correlate with the dihedral angle present in each disulfide and therefore may reflect the relative stabilities of the various disulfides in the ground state. The ground state energy of the disulfides is dominated by strain energy which is directly proportional to the CSSC dihedral angles. Minimal strain occurs when the dihedral angle is near 90°, and deviations from this optimal value results in increasing strain leading to higher ground state energies.[4,146]

Based on the small dihedral angles present in both 6-membered epidithiodioxopiperazines **16** and **145** (dihedral angles range from 8-18° in naturally occurring epidithiodioxopiperazines),[2d] and disulfides **16** and **145** can be considered highly strained and therefore possess the highest ground state energies. As a consequence of the high ground state energies, reduction should require the least amount of energy and is reflected in the less negative half-cell potentials observed.

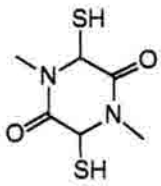
On the other hand, the oxidized form of DTT (**362**) exists in a chair conformation with dihedral angles ranging from 58-66° and considered to be strain free.[145e] The stability of the disulfide is reflected in the observed more negative potential required for reduction.

The seven-membered aspirochlorine (**80**) and analogue **273** represent an intermediate situation. Normal 1,2-dithiepanes (7-membered disulfides) exist in a twist chair conformation and have been calculated to contain dihedral angles close to ideal (81-89°) indicating the disulfide form is relatively strain free.[145e] The rigid nature of the diketopiperazine ring present in **80** and **273** prohibits formation of the desired twist chair conformation. This results in a decrease in the CSSC dihedral angle causing elevation of the ground state energy. Analysis of the crystal structure of disulfide **273** indeed revealed that the dihedral angle has been compressed to 56°. The net effect is the ground states of aspirochlorine and **273** lie somewhere between **145** and **362**; therefore reduction of the seven-membered disulfides requires an intermediate potential which is experimentally observed.

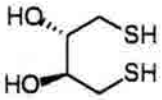
Superoxide production

The inability to measure either directly or indirectly the equilibrium constant for thiol/disulfide exchange led to a series of experiments in which the relative reactivity of the disulfides could be qualitatively compared. The goal of these studies was to determine the reason for the difference in biological activity displayed by aspirochlorine compared to other epidithiodioxopiperazines. The ability of the disulfides in Figure 3 and the dithiols shown in Figure 5 to generate superoxide was followed as a function of DNA plasmid nicking and in reduction of nitroblue tetrazolium (NBT) assays.

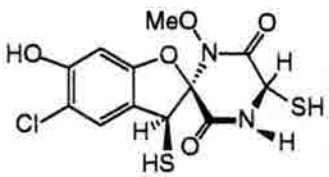
Figure 5. Structure of various Dithiols



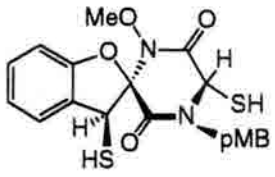
363



358



364



361

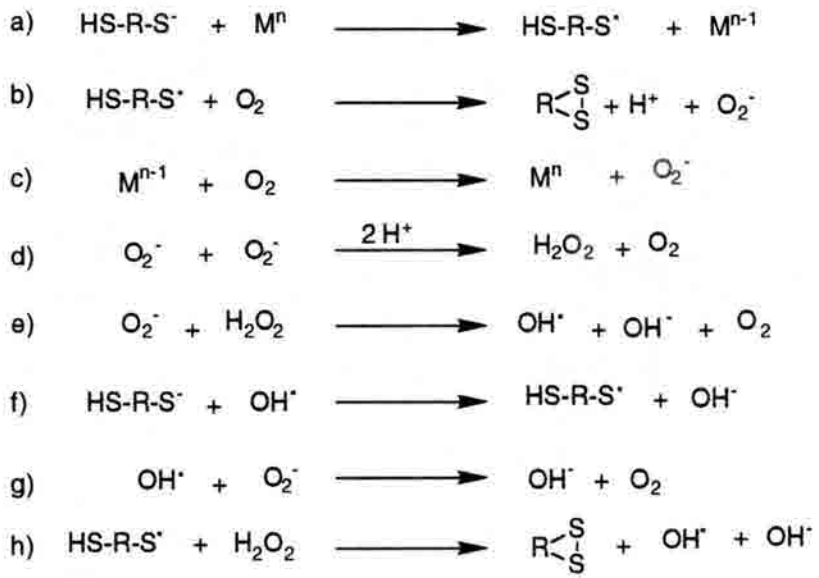
As previously mentioned, oxidation of dithiols can occur with concomitant reduction of molecular oxygen to generate superoxide. The oxidation of thiols by oxygen is well known, and believed to proceed via a sulfur-metal complex. The reaction is complex and the precise steps involved in the reaction are unknown.[148,149]

Production of superoxide during the oxidation of thiols was observed *in vivo* by Misra in studies with dithiothreitol (DTT) and was detected by the reduction of NBT.[150] The rate of NBT reduction increased as the pH of the solution increased suggesting the thiolate anion is required. The rate of NBT reduction was decreased by addition of EDTA indicating formation of superoxide also requires a metal ion. Inhibition of NBT reduction

by addition of superoxide dismutase is evidence that superoxide was being produced during the course of the reaction.

Additional reactions carried out by Misra under anaerobic conditions resulted in NBT reduction suggesting thiols are capable of directly reducing NBT via a non-oxygen dependent pathway. Based on the experimental data, Misra proposed the following series of reactions illustrated in Scheme 4.

Scheme 4 Proposed mechanism for the reduction of oxygen during oxidation of thiols



Transition metals such as Fe^{+3} or Cu^{+2} are proposed to initiate thiol oxidation through electron transfer between thiolate and the metal ion generating a thiyl radical and the reduced metal ion. The intermediate thiyl radical can react with oxygen resulting in formation of the disulfide and superoxide. In addition, the metal catalyst can undergo oxidation producing superoxide in the process shown in equation c. Equations d-h are possible chain propagation steps involved in the conversion of superoxide into hydroxyl radicals.

Evidence for the proposed catalytic activity of epidithiodioxopiperazines was obtained several years later by Munday.[151] The observed rate of NBT reduction was greater for the reaction of sporidesmin disulfide/glutathione than during autoxidation of sporidesmin dithiol. In subsequent studies, Munday demonstrated oxidation of sporidesmin dithiol is accompanied by the production of superoxide, hydrogen peroxide and hydroxyl radical.[152] Furthermore, the reactive oxygen radicals were also detected in reactions involving the epidisulfide in the presence of reducing agents. The mechanism proposed by Munday to account for the production of the oxygen radicals by sporidesmin dithiol is essentially the same mechanism by Misra (HS-R-S$^{\cdot}$ = sporidesmin dithiol, M= Cu^{+2})

Recently Eichner et al. demonstrated the DNA cleaving abilities of gliotoxin **16** and **145** occurs via an oxygen-dependent pathway involving superoxide.[35a,b] The nicking activity of disulfides requires the presence of a suitable reducing agent. Additional evidence for the intermediacy of superoxide was demonstrated by enhanced nicking in the presence of Fe^{+3}/EDTA and inhibition of nicking by the strong iron chelating agent desferral or oxygen radical scavengers.

Addition of superoxide dismutase to the reactions was reported to actually increase the amount of nicking. The increase in nicking is due to the normal function of the enzyme which catalyzes the formation of hydrogen peroxide from superoxide. The increase in hydrogen peroxide concentration in the presence of iron leads to an increase in formation of hydroxyl radical which is the ultimate source of the DNA nicking.

The autoxidation of **363** was investigated by Eichner et al., and shown to produce hydrogen peroxide during the course of the reaction. Evidence was also obtained suggesting that the autoxidation of **363** occurs via a UV distinguishable intermediate of unknown identity.

The recent reports by Eichner demonstrating DNA-nicking activity of gliotoxin proceeds via superoxide provided a convenient starting point for the comparison of the

reactivity of the various disulfides and dithiols. Incubation of supercoiled plasmid DNA with the various disulfides in the presence of glutathione under a variety of conditions resulted in formation of open circular DNA and the results are shown in Figure 6 and summarized in Table 2.

As reported by Eichner, the disulfides were inactive except in the presence of reducing agents such as DTT or in this case reduced glutathione. The observed DNA nicking activity was also enhanced by the presence of Fe^{+3}/EDTA.

In all experiments conducted, the simple disulfide **145** consistently produced more (up to 70% conversion) of the open circular form of DNA compared to other disulfides at equal molar concentrations. The nicking activities of the natural products aspirochlorine (**80**) and gliotoxin (**16**) appeared to be similar at 250 μM concentration resulting in 41 and 32% conversion to open circular DNA respectively. Oxidized DTT (**362**) and the synthetic analogue **273** gave < 10% conversion by scanning densitometry measurements. The lack of nicking activity of **273** is ascribed to the extremely low water solubility of this compound.

At 500 μM concentrations, **145** gave almost complete conversion to the open circular form (97%) while the percent conversion for aspirochlorine **80** and gliotoxin **16** increased to 70% and 52%. Incorporation of superoxide dismutase (10 μg/mL) did not inhibit nicking activity of the disulfides, although incorporation of 10 μg/mL catalase inhibited nicking in all cases.

Analysis of the DNA nicking abilities of the dithiols **358**, **364**, **361** and **363** revealed similar trends and is shown in Figure 7 and also summarized in Table 3. The conversion of supercoiled plasmid to open circular DNA was observed in reactions of dithiols and plasmid DNA.

Incorporation of Fe^{+3}/EDTA accentuated conversion to open circular DNA in all cases. The simple dithiol **363** consistently produced more of the open circular form (53%) of DNA compared to other dithiols. Aspirochlorine dithiol (**364**) caused 40% conversion

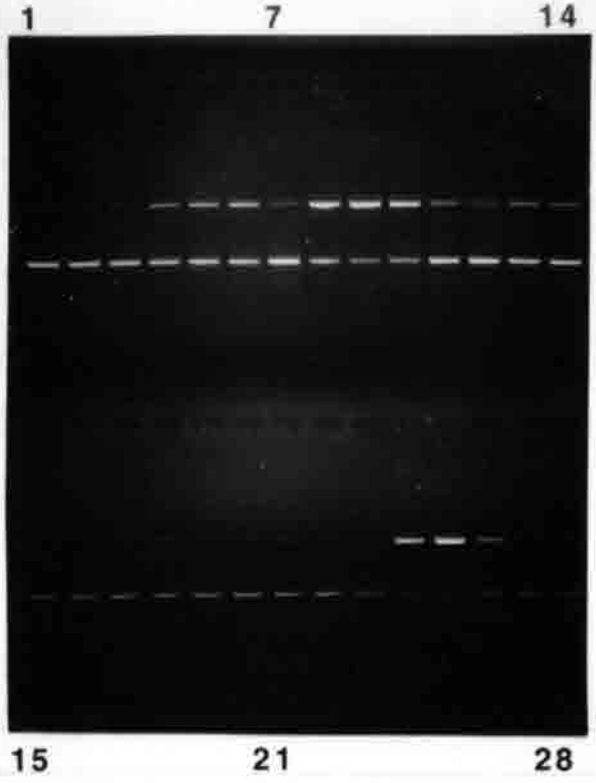


Figure 6. DNA plasmid nicking by disulfides. All reactions carried out in 20 mM phosphate buffer, pH 7.6. The reactions were incubated for one hour at 37°C prior to electrophoresis. All lanes contained DNA, and Lanes 1-3 were control lanes. Lane 1) no addition; Lane 2) 20 μM Fe^{+3}/EDTA; Lane 3) 20 μM Fe^{+3}/EDTA plus 40 μM glutathione. Lane 4) 250 μM aspirochlorine (**80**) plus 20 μM Fe^{+3}/EDTA; Lane 5) 250 μM aspirochlorine (**80**), 20 μM Fe^{+3}/EDTA plus 40 μM glutathione; Lane 6) 250 μM aspirochlorine (**80**), 20 μM Fe^{+3}/EDTA , 40 μM glutathione plus 10 μg/mL SOD; Lane 7) 250 μM aspirochlorine (**80**), 20 μM Fe^{+3}/EDTA, 40 μM glutathione plus 10 μg/mL catalase. Lanes 8-11 same as 4-7 and contained 250 μM disulfide **145**. Lanes 12-15 were the same as 4-7 and contained 250 mM gliotoxin (**16**). Lanes 16-19 were the same as 4-7 and contained 250 μM disulfide **273**. Lanes 20-23 were the same as 4-7 and contained 250 μM disulfide **362**. Lanes 24-28 contained 20 μM Fe^{+3}/EDTA, 40 μM glutathione and 500 μM of the individual disulfides **80**, **145**, **16**, **273** and **362** respectively.

Table 2. Summary of DNA results from various disulfides

Lane	Reagents and conditions[a]	%OC[b]
1	DNA, 10 μM Fe^{+3}/EDTA, 40 μM glutathione---CONTROL	0
2	250 μM aspirochlorine **80**	41
3	250 μM aspirochlorine **80,** 10 μg/mL SOD	46
4	250 μM aspirochlorine **80,** 10 μg/mL catalase	10
5	250 μM **145**	70
6	250 μM **145** 10 μg/mL SOD	62
7	250 μM **145,** 10 μg/mL catalase	25
8	250 μM gliotoxin **16**	32
9	250 μM gliotoxin **16,** 10 μg/mL SOD	32
10	250 μM gliotoxin **16,** 10 μg/mL catalase	6
11	250 μM **273**	6
12	250 μM **273,** 10 μg/mL SOD	24
13	250 μM **273,** 10 μg/mL catalase	13
14	250 μM DTT_{ox} **362**	13
15	250 μM DTT_{ox} **362,** 10 μg/mL SOD	17
16	250 μM DTT_{ox} **362,** 10 μg/mL catalase	9
17	500 μM aspirochlorine **80**	70
18	500 μM **145**	97
19	500 μM gliotoxin **16**	52
20	500 μM **273**	17
21	500 μM DTT_{ox} **362**	9

[a]All reactions carried out in 20 mM phosphate buffer, pH 7.6 containing 10 μM Fe^{+3}/EDTA and 40 μM glutathione. Incubated for one hour at 37°C [b]Percent open circular form of DNA determined from scanning densitometry measurements.

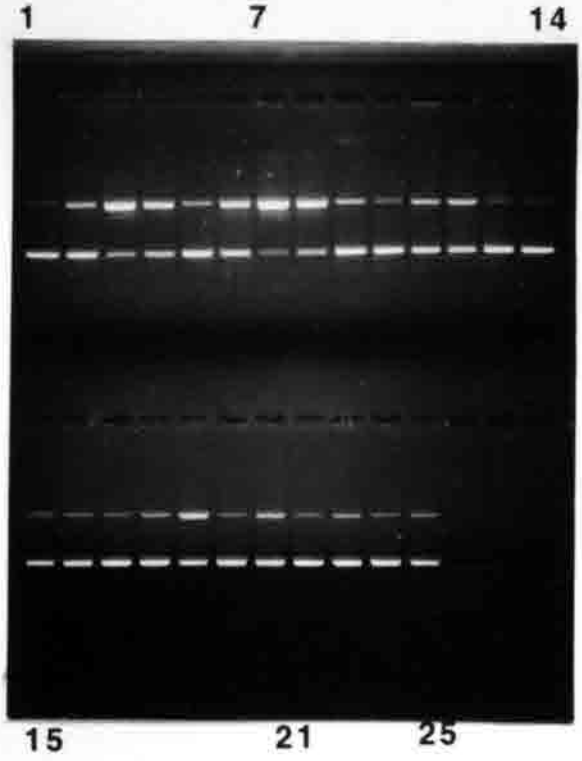


Figure 7. DNA plasmid nicking by dithiols. All reactions carried out in 20 mM phosphate buffer, pH 7.6. The reactions were incubated for one hour at 37°C prior to electrophoresis. All lanes contained DNA. Lane 1) 10 μM Fe^{+3}/EDTA-control; Lane 2) 200 μM aspirochlorine dithiol (**364**); Lane 3) 10 μM Fe^{+3}/EDTA plus 200 mM aspirochlorine dithiol (**364**); Lane 4) 10 μM Fe^{+3}/EDTA, 200 μM aspirochlorine dithiol (**364**) plus 10 μg/mL SOD; Lane 5) 10 μM Fe^{+3}/EDTA, 200 μM aspirochlorine dithiol (**364**) plus 10 μg/mL catalase. Lanes 6-9 were the same as 2-5 and contained 200 μM of dithiol **363**. Lanes 10-13 were the same as 2-5 and contained 200 μM dithiol **361**. Lanes 14-17 were the same as 2-5 and contained 200 μM dithiol **358**. Lane 18) 50 μM aspirochlorine dithiol (**364**); Lane 19) 50 μM aspirochlorine dithiol (**364**) plus 10 μM Fe^{+3}/EDTA. Lanes 20 and 21 were the same as 18 and 19 and contained 50 μM dithiol **363**. Lanes 22,23 were the same as 18 and 19 containing 50 μM dithiol **361**. Lanes 24,25 were identical to 18,19 and contained 50 μM dithiol **358**.

Table 3. Summary of DNA results from various dithiols

entry	Reagents and conditions[a]	%OC[b]
1	DNA, 10 μM Fe^{+3}/EDTA---CONTROL	0
2	DNA, 200 μM aspirochlorine dithiol **364**	5
3	DNA, 200 μM **364,** 10 μM Fe^{+3}/EDTA	40
4	DNA, 200 μM **364,** 10 μM Fe^{+3}/EDTA, 10 μg/mL SOD	33
5	DNA, 200 μM **364,** 10 μM Fe^{+3}/EDTA, 10 μg/mL catalase	6
6	DNA, 200 μM **363**	15
7	DNA, 200 μM **363,** 10 μM Fe^{+3}/EDTA	53
8	DNA, 200 μM **363,** 10 μM Fe^{+3}/EDTA, 10 μg/mL SOD	82
9	DNA, 200 μM **363,** 10 μM Fe^{+3}/EDTA, 10 μg/mL catalase	48
10	DNA, 200 μM **361**	15
11	DNA, 200 μM **361,** 10 μM Fe^{+3}/EDTA	50
12	DNA, 200 μM **361,** 10 μM Fe^{+3}/EDTA, 10 μg/mL SOD	65
13	DNA, 200 μM **361,** 10 μM Fe^{+3}/EDTA, 10 μg/mL catalase	32
14	DNA, 200 μM **358**	2
15	DNA, 200 μM **358,** 10 μM Fe^{+3}/EDTA	11
16	DNA, 200 μM **358,** 10 μM Fe^{+3}/EDTA, 10 μg/mL SOD	4
17	DNA, 200 μM **358,** 10 μM Fe^{+3}/EDTA, 10 μg/mL catalase	3

[a]All reactions carried out in 20 mM phosphate buffer, pH 7.6. Incubated at 37°C for 1 hour. [b]Percent open circular form of DNA determined from scanning densitometry.

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to open circular DNA and the synthetic analogue **361** resulted in 50% conversion to the open circular form. DTT (**358**) displayed only minimal DNA nicking (11%) at 200 μM concentration.

In agreement with previous experiments involving thiol/disulfide exchange, enhanced DNA nicking was observed in the presence of superoxide dismutase (10 μg/mL) and inhibition of nicking activity was evident in reactions including 10 μg/mL catalase. The results obtained from the DNA nicking studies of both disulfides and dithiols are consistent with the results obtained by Eichner.

At the onset of the NBT reduction experiments, the goal was to apply the guidelines proposed for thiol/disulfide exchange to provide a rational basis for the differing rates of NBT reduction which were observed. Such an approach is difficult since thiol/disulfide exchange occurs in a nucleophilic manner, and reduction of oxygen by thiols involves a metal catalyzed electron transfer. Furthermore, the rate of superoxide production does not necessarily have to correlate with dithiol oxidation since superoxide can be produced from a variety of sources (eg. equation b,c in Scheme 4).

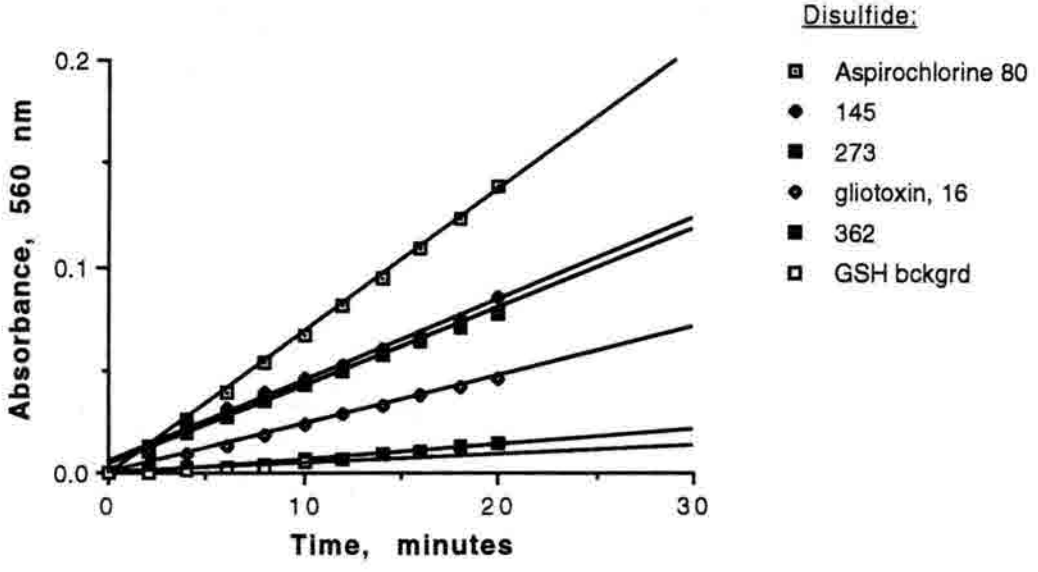

Figure 8. Average rate of NBT reduction as a function of disulfide structure. Change in absorbance at 560 nm was recorded as a function of time using the following conditions: 25 °C, 50 mM pH 7.6 phosphate buffer, 250 μM NBT, 100 μM EDTA, 1 mM reduced glutathione and 40 μM disulfide (final concentration of acetonitrile = 2% v/v).

The reduction of NBT in reactions between glutathione and the various disulfides was investigated using conditions similar to those used by Munday.[151] The results from several experiments are presented in Figure 8 and summarized in Table 4.

The rate of NBT reduction was followed at 560 nm for reaction of the various disulfides in the presence of excess glutathione and measured as the change in absorbance versus time. Reduction of NBT by glutathione alone at this concentration (1 mM) was determined to be negligible compared to the rate of reduction observed in the presence of a

Table 4. Rate of NBT reductions involving Glutathione/disulfide exchange

Entry	Disulfide	Total Δ OD/min[a]	SOD Δ OD/min[a,b]	% inhibition	Net ΔOD/min[a,c]
1.	**80**	6.87 ± 0.05	3.59 ± 0.02	47	3.28
2	**273**	3.81 ± 0.18	1.99 ± 0.02	47	1.81
3.	**145**	3.93 ± 0.15	1.14 ± 0.06	71	2.79
4.	**16**	2.35 ± 0.23	0.49 ± 0.04	79	1.86
5.	**362**	0.73 ± 0.23	0.25 ± 0.03	65	0.48

[a]Change in absorbance values x10^{-3} [b]10 μg/mL SOD added to reaction. [c]Total ΔOD/min - ΔOD/min in the presence of SOD.

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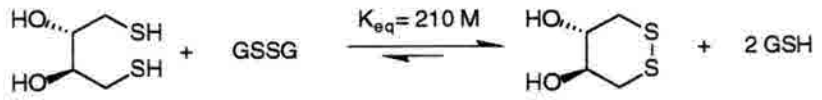
disulfide. From the rates of NBT reduction in Figure 8, the following trend was observed: aspirochlorine (**80**) > **145** ≈ analogue **273** > gliotoxin (**16**) >> DTT_{ox} (**362**).

The rate of NBT reduction for reactions using oxidized DTT did not vary significantly above the background rate produced during the autoxidation of glutathione itself. This lack of reactivity is consistent with kinetic data provided in the literature.

Scheme 5.



358 **362**

$$K_{eq} = \frac{[DTT_{ox}][GSH]^2}{[DTT_{red}][GSSG]}$$

The experimentally determined Keq between the reduced form of DTT (**358**) and oxidized glutathione has been suggested to be 210 M from the equilibrium shown in Scheme 5.[145a] The value for the equilibrium constant indicates that the reaction (eg **362→358**) used in this assay is highly unfavored, and only trace amounts of reduced DTT (**358**) is available for reaction at any given time.

The faster rate of NBT reduction displayed by aspirochlorine (**80**) compared to disulfide **145** is somewhat surprising considering **145** consistently exhibited more nicking of plasmid DNA.

Addition of 10 μg/mL of superoxide dismutase (SOD) to the reactions involving glutathione and disulfides resulted in a decrease in the rate of NBT reduction. In the presence of SOD, the rate of NBT reduction for the seven-membered disulfides aspirochlorine (**80**) and analogue **273** was reduced only 47%. Based on this result, it appears that almost half of the observed total rate of NBT reduction occurs via a non-oxygen dependent pathway. Increasing the concentration of superoxide dismutase to 20 μg/mL did not lead to a significant (<2%) change in the amount of inhibition. In the case of the six-membered disulfides **145** and gliotoxin (**16**), addition of SOD decreased the rate of NBT reduction 71-79%.

Based on results from the SOD studies, the net rate of NBT reduction (ie., total Δ OD/min -Δ OD/min in the presence of SOD) is a more realistic measure of the ability of the

various disulfides to produce superoxide. Comparison of the net Δ OD/min in Table 4 for the various disulfides indicates aspirochlorine (**80**) reduces NBT only slightly faster than the 6-membered disulfide **145.**

Furthermore, the relative amounts of inhibition suggest that 6-membered disulfides (eg. **145**) may be more efficient at producing superoxide than 7-membered disulfides such as aspirochlorine (**80**). These results are consistent with observations made in the DNA nicking experiments.

Attempts to directly measure the rate of NBT reduction during autoxidation of dithiols **361**, **363**, **364** and **358** gave mixed results. Dithiol **363** was obtained from sodium borohydride reduction of the disulfide **145** and purified by recrystallization.

Aspirochlorine dithiol (**364**) and the *p*-methoxybenzyl protected dithiol **361** were obtained from sodium borohydride reduction of the corresponding disulfides carried out under anaerobic conditions. Dithiol **364** and **361** could be obtained in >85% purity by this method based on the crude ^{1}H NMR. Attempts to further purify **364** or **361** only resulted in increasing amounts of the disulfides being formed. Therefore, dithiols **364** and **361** were used "as is" directly from the sodium borohydride reduction. Dithiols **361**, **364** were relatively stable if stored under an inert atmosphere at -80°.

Reduction of NBT during the autoxidation of dithiols **358, 361, 363,** and **364** was initially carried out using conditions similar to those reported by Misra[150] and the results are shown in Figure 9 and Table 5. Analogous reactions were conducted at pH 7.6 and results from this series of reactions are also included in Table 5.

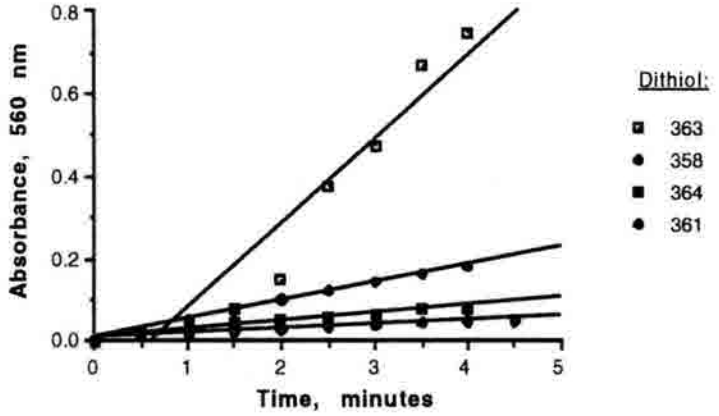


Figure 9. NBT reduction during autoxidation of selected dithiols at pH 10.2. All measurements made using 25 °C, 50 mM pH 10.2 sodium carbonate buffer, 250 μM NBT, 100 μM EDTA and 20 μM dithiol.

Table 5. NBT reduction data for selected dithiols[a]

		Δ OD/min,		Δ OD/min ,	
Entry	Dithiol	pH 10.2	% inhibition	pH 7.6	% inhibition
1.	**363**	0.204	80	0.0061	53
2.	**358**	0.045	83	0.0008	32
3.	**364**	0.018	71	0.0033	75
4.	**361**	0.011	52	0.0017	57

[a]All reactions were conducted at 25°C, in 50 mm buffer. The reactions contained 20 μM dithiol, 250 μM NBT and 100 μM EDTA.

NBT reduction in the presence of the dithiols was initially conducted at pH 10.2 to minimize any differences as a consequence of differing pKa values of the various dithiols. At pH 10.2, DTT (**358**) reduced NBT at a faster rate than the 7-membered dithiols **361** or

364. Interestingly, this is consistent with Whitesides' observations suggesting the major factor in formation of the disulfide is due to strain energy since aspirochlorine dithiol should have a distinct entropic advantage over the acyclic DTT during oxidation.[145e,h]

Dithiol **363** reduced NBT at a much higher rate overall, although the rate of reduction was not linear as observed with the other dithiols. The response observed during the autoxidation of dithiol **363** vs DTT (**358**) at pH 10.2 is shown in Figure 10 and illustrates the complex process involved in the autoxidation. The general shape of the curve for **358** is typical of the dithiols examined. The complex behavior exhibited by **363** is consistent with results obtained by Eichner who suggested autoxidation of **363** proceeds via an unknown intermediate.

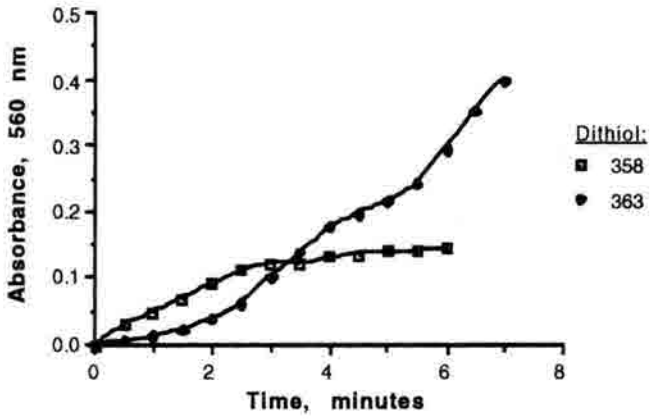


Figure 10. Comparison of NBT reduction by DTT **358** and dithiol **363** at pH 10.2. The shape of the curve for DTT was generally observed for the remaining dithiols.

The low rate of NBT reduction observed in reactions of the dithiol **361** is probably artificially low as a result of the previously mentioned inability to obtain pure samples of the dithiols.

Similar results for the autoxidation of dithiols were observed at pH 7.6 and are also summarized in Table 5. Compared to the reactions conducted at pH 10.2, the observed

change in absorbance per minute at pH 7.6 was generally slower for each dithiol, and this is consistent with the fact thiolate is required for oxidation.

The slight reduction of NBT by DTT (**358**) at pH 7.6 is a direct consequence of the pKa of the dithiol (pKa of DTT= 9.2). At pH 7.6, only a small fraction of DTT exists in the ionized thiolate form.

Dithiol **363** achieved the fastest rate of reduction, although the response still was not linear. Aspirochlorine dithiol **364** and the synthetic analogue **361** efficiently reduced NBT, although the rate of reduction was not as efficient as in the case of dithiol **363.**

Based on the conflicting results obtained in the various NBT reduction studies, the difference in reactivity between 6-membered epidisulfides versus the 7-membered aspirochlorine is not obvious. With the exception of ring size, structural differences between the various compounds do not suggest a reasonable molecular rationale to explain the different rates observed in the reduction of NBT. In addition the complex response observed during the autoxidation of dithiol **363** indicates a complex process is occurring. The possibility of NBT reduction by thiols directly further complicates the issue.

Interpretation of the results from the NBT studies strictly in terms of the rate of thiol/disulfide exchange or rate of autoxidation of dithiols is unreasonable. From the NBT reduction studies the only meaningful conclusion that can be drawn at this time is to suggest 6-membered epidithiodioxopiperazines appear to be more reactive in generating superoxide than the corresponding seven membered compounds. This difference may suggest the reason for the difference in biological activity reported for aspirochlorine.

In conclusion, attempts to quantitatively determine the basis for the observed differences in biological activity between a series of 6 and 7-membered disulfides was unsuccessful. Results from DNA plasmid nicking, and NBT reductions involving both glutathione/disulfide and dithiol indicate the production of superoxide was greatest in reactions involving the 6-membered disulfides since these compounds consistently displayed greater activity than related 7-membered compounds.

The application of the general principles developed by Whitesides for thiol/disulfide exchange to the NBT reduction experiments was not possible due to fundamental differences in reaction mechanisms. Thiol/disulfide exchange is an uncomplicated nucleophilic substitution reaction, whereas the autoxidation of dithiols occurs via electron transfer and involves transition metal catalysis.

Chapter 5
Experimental Section

^{1}H, ^{13}C NMR spectra were recorded on a Bruker 300 MHz FT NMR or on an IBM WP270 MHz FT NMR and chemical shifts are reported relative to TMS. NOE, DEPT and variable temperature NMR were conducted on the Bruker 300 mHz NMR. NMR data collected in methanol-d_4 are reported relative to the methanol peak at 3.30 ppm. IR were collected on a Perkin-Elmer 1600 FT IR either as KBr pellets or as thin films from chloroform. Melting points were obtained using a Mel Temp apparatus and are uncorrected. Elemental analysis were performed by MHW labs, Phoenix, Arizona. Analytical thin-layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} glass plates. Preparative flash column chromatography was performed using Grade 60 230-400 mesh silica gel purchased from Aldrich and radial chromatography was carried out on a Chromatotron Model 7924 using 1,2 or 4 mm silica plates as needed. HPLC separation of aspirochlorine was carried out using Waters 6000 pump equipped with a 254 nm fixed wavelength detector, utilizing an 8x10 cm silica radial compression cartridge, using 2% methanol/chloroform at a flow rate of 2 mL/min.

Unless otherwise stated, all reactions were carried out in flame-dried flasks under a nitrogen or argon atmosphere. Compounds containing the *o*-nitrobenzyl group were stored in flasks wrapped in aluminum foil to minimize photodecomposition. Tetrahydrofuran and diethyl ether were dried over sodium/benzophenone ketyl while methylene chloride was dried over calcium hydride. Transfer of solvents were carried out via flame-dried syringe. Ethanol-free chloroform was obtained by first washing with water, drying over magnesium sulfate followed by distillation from phosphorus pentoxide and storage in an amber bottle. Triethyl amine, pyridine and any acyl chlorides were first filtered through alumina prior to use.

TLC's of thiol and thioacetate-containing compounds were visualized either using ethanolic I_2/NaN_3 stain or 5% Ellmans' reagent in dimethylformamide. Photolysis reactions were conducted using a 450-watt Conrad-Hanovia 7825 medium pressure lamp in a pyrex well at 37 °C. Coumarilic acid was obtained from Lancaster Synthesis and 5-chlororesorcinol was obtained from Aldrich. Methoxylamine hydrochloride was converted into the free base and stored over potassium hydroxide at 0°C. Prior to use, the amine was filtered through a plug of alumina. Tertiary butyl hypochlorite was prepared fresh and stored in an amber bottle over calcium chloride at 0°C. All other reagents used were of commercial purity (Aldrich) unless otherwise stated.

Caution! Reactions involving either the highly toxic hydrogen sulfide or zinc cyanide/hydrogen chloride mixtures were carefully performed in a well ventilated fume hood. In addition, reactions using the potent mutagen chloromethyl methyl ether were carried out using appropriate laboratory attire and all glassware washed with concentrated base immediately after use.

Dithiothreitol (DTT), nitroblue tetrazolium (NBT), and tetrabutylammonium hexafluorophosphate ($TBAPF_6$) were obtained from Aldrich. $TBAPF_6$ was recrystallized from ethanol prior to use. Reduced glutathione (GSH) was purchased from Sigma. Fresh solutions of DTT, GSH were prepared in acetonitrile prior to analysis. Gliotoxin was kindly provided by Dr. Paul Waring, John Curtin School of Medical Research, Australian National University, and an authentic aspirochlorine was a generous gift from the Eli Lilly company. All disulfides were stored in acetonitrile at -20 °C and solutions of the dithiols in acetonitrile were prepared fresh from a stock solution kept at -80 °C. Superoxide dismutase (SOD), and catalase were obtained from Boehringer Mannheim and stored in solution at 4 °C.

DNA nicking

Plasmid DNA (pUC 19) was prepared from E. Coli MC 1061 and crude plasmid was obtained from the transformed cells using a Promega Magic Maxiprep. The plasmid

was purified by low melt agarose gel eletrophoreseis according to the method described by Maniatis and was stored in sterile water. All reactions were carried out in 20 mM pH 7.6 phosphate buffer and a final volume of 15 μL. Solutions of the disulfides and dithiols were made up in acetonitrile. An aliquot of the disulfides or dithiols was added to an eppendorf tube and the solvent removed in vacuum. The drug was resuspended by addition of the DNA buffer and the remainder of reagents (2 μL additions) added. The reactions were initiated by the addition of Fe^{+3}/EDTA. The tubes were gently vortexed to ensure mixing, and incubated at 37 °C for 1 hour. The reactions were cooled to 0 °C, 4 μL of a loading dye was added and 15 μL of the reaction mixture analyzed by 1.2 % neutral agarose gel electrophoresis (containing 1 mg/mL ethidium bromide; 0.04 M tris HCl buffer pH 8.0 containing 10 mM EDTA.; 55 volts constant voltage); visualized by ultraviolet light and quantitated by scanning densitometry.

Nitroblue tetrazolium (NBT) reductions by thiol/disulfide exchange or dithiols

The change in absorbance at 560 nm was measured using a Varian DMS 80 UV spectrometer equipped with a thermostated sample cell. Solutions of the disulfide or dithiol were prepared in acetonitrile such that the final concentration of acetonitrile in solution was 2% (v/v). In all cases, reactions were initiated by adding the disulfide/dithiol (20 μL) to a cuvette containing a solution consisting of 50 mm buffer, 100 μM EDTA, 250 μM NBT and 0.03% Triton X-100. The reactions were quickly mixed, placed in the UV spectrometer, and the change in absorbance followed as a function of time.

Attempted Cyclic voltametric determination of equilibrium constant for dithiol/disulfides

Cyclic voltametry experiments were conducted using a PAR model 173 potentiostat/galvanostat equipped with a PAR model 175 universal programmer, model 179 digital coulometer and recorded on an HP model 7045A X-Y recorder. The cell apparatus consisted of a platinum electrode and utilitzed a SCCE electrode as the reference. The

disulfides were dissolved in a 0.1 M tetrabutylammonium hexafluorophosphate/acetonitrile solution such that the final concentration of disulfide was 1 mM. The solution was placed in the cell, purged with nitrogen and the reduction followed at a scan rate of 100 mv/s.

268

2-bromo-N-(4-methoxybenzyl)-Acetamide (268)

To a tetrahydrofuran solution containing triethylamine (1.1 mL, 8.0 mmol, 1.1 eq.), and 4-methoxybenzylamine (1.0 mL, 7.0 mmol, 1.0 eq.) at 0 °C was added bromoacetyl bromide (0.6 mL, 7.0 mmol, 10 eq.) slowly dropwise. After 15 minutes the reaction mixture was poured into diethyl ether, etracted with cold brine, dried over sodium sulfate, filtered and concentrated to an off-white solid. The amide was recrystallized from ethyl acetate/hexanes to give fine white needles, mp 119-120 °C. 90% yield.

^{1}H NMR (270 mHz, $CDCL_3$) δ=7.13 (2H, d, J= 8.5 Hz); 6.80 (2H, d, J= 8.6 Hz); 6.65 (1H, bs, exchangeable); 4.32 (2H, d, J= 5.7 Hz); 3.82 (2H, s); 3.72 (3H, s).

IR (KBr), υ=3280, 1650, 1540, 1510, 1244, 1125 cm^{-1}.

Anal. Calcd. for $C_{10}H_{12}NO_2Br$: C 46.53, H 4.69, N 5.42, Br 30.96; Found C 46.60, H 4.64, N 5.44, Br 31.18.

268 **265**

2-(N'-methoxy)-N-(4-methoxybenzyl)-acetamide (265)

A solution of **268** (100 mg, 0.39 mmol, 1.0 eq.) in 25 mL tetrahydrofuran was added to a tetrahydrofuran solution of methoxylamine (73 mg, 1.54 mmol, 4.0 eq.) and triethylamine (270 μL, 1.93 mmol, 5.0 eq.). The reaction was stirred at room temperature for 24 hours. The reaction was filtered, and concentrated to a colorless oil which crystallizes upon standing. The product was purified by radial chromatography using 3:1 ethyl acetate/hexanes as the eluent. 52% yield of white needles from ethyl acetate/hexanes, mp 72- 73 °C.

^{1}H NMR (270 mHz, $CDCL_3$) δ= 7.72 (2H, d, J= 8.6 Hz); 6.86 (2H, d, J= 8.6 Hz); 6.85 (1H, bs, exchangeable); 5.95 (1H, bs, exchangeable); 4.43 (2H, d, J= 5.7 Hz); 3.79 (3H, s); 3.55 (2H, s); 3.49 (3H, s).

IR (KBr), υ= 3350, 3220, 2920, 1660, 1515, 1280, 1100 cm^{-1}.

Anal. Calcd. for $C_{11}H_{16}N_2O_3$: C 58.91, H 7.19, N 12.49; Found C 58.79, H 7.09, N 12.31.

265 **269**

2-[N-(2',2'-dichloroacetyl)-N-(methoxy)]-N'-(4-methoxybenzyl)-acetamide (269)

To a solution of **265** (20 mg, 0.09 mmol, 1.0 eq.) in 1 mL tetrahydrofuran was added triethylamine (26 μL, 0.09 mmol, 1.1 eq.). Solution stirred for five minutes before adding dichloroacetyl chloride (8 μL, 0.09 mmol, 1.1 eq). After 20 minutes the reaction was diluted with tetrahydrofuran, filtered, and concentrated to an off-white solid. The product was purified by PTLC using2:1 ethyl acetate/hexanes. Obtained 21 mg of a white solid which was recrystallized from ethyl acetate/hexanes, 70% yield, mp 116- 117 °C.

^{1}H NMR (270 mHz, $CDCl_3$) δ= 7.17 (2H, d, J= 8.6 Hz); 6.84 (2H, d, J= 8.6 Hz); 6.51 (1H, s); 6.30 (1H, bs, exchangeable); 4.38 (2H, d, J= 5.6 Hz); 4.32 (2H, s); 3.84 (3H, s); 3.78 (3H, s).

IR (KBr), υ= 3280, 1680, 1640, 1555, 1505, 1440, 1248, 1123 cm^{-1}.

Anal. Calcd. for $C_{12}H_{16}N_2O_4Cl_2$: C 46.58, H 4.81, N 8.36, Cl 21.15; Found C 46.39, H 4.66, N 8.26, Cl 21.24.

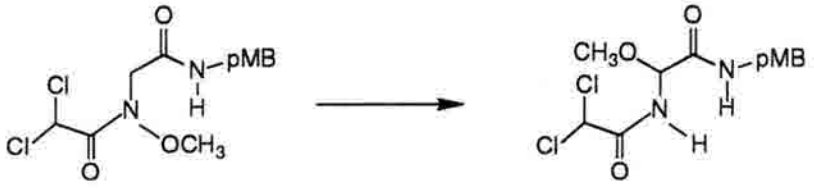
269 **270 (R= Et)**

2-[N-(2',2'-dichloroacetyl)-N-methoxyl)]-1-ethoxy-N'-(4-methoxybenzyl)-acetamide (270 R= Et)

A solution of **269** (70 mg, 0.21 mmol, 1.o eq.) in 5 mL of absolute ethanol was added to sodium ethoxide (29.8 mg, 0.44 mmol, 2.1 eq.) contained in 5 mL of ethanol. The resulting solution was refluxed for 3 hours. The reaction was poured into methylene chloride, washed with saturated ammonium chloride, water, dried over magnesium sulfate, filtered and concentrated to a yellow film. The product was purified as an off-white solid from radial chromatography (2:1 ethyl acetate/hexanes). Obtained 26 mg 37% yield, mp 144-147 °C.

^{1}H NMR (270 mHz, $CDCL_3$) δ=7.38 (1H, Bd, J= 8.0 Hz); 7.23 (2H, d, J= 8.5 Hz); 6.89 (2H, d, J= 8.6 Hz); 6.80 (1H, bs) ; 5.99 (1H, s); 5.54 (1H, d, J= 8.4 Hz); 4.45-4.40 (2H, m); 3.80 (3H, s); 3.70 (2H, q, J= 7.1 Hz); 1.22 (3H, t, J= 7.1 Hz).

IR (KBr), υ= 3290, 1680 (shoulder), 1650, 1505, 1140, 1022 cm^{-1}.



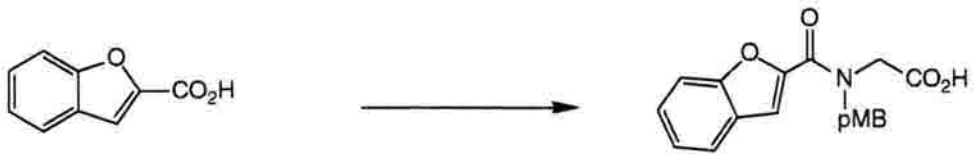
269 **270 (R=Me)**

2-[N-(2',2'-dichloroacetyl)-N-methoxyl)]-1-methoxy-N'-(4-methoxy-benzyl) -acetamide (270 R=Me)

A solution of **269** (50 mg, 0.14 mmol, 1.0 eq.) in 2.5 mL of absolute methanol was added to a solution of sodium methoxide (20.3 mg, 0.3 mmol, 2.0 eq.) in 2.5 mL methanol. The solution was stirred at ambient temperature for 12 hours. The solution was diluted with methylene chloride, washed with ammonium chloride, water, dried over magnesium sulfate, filtered and concentrated to a white solid. The solid was dissolved in chloroform and purified by PTLC. The product was recrystallized from ethyl acetate/hexanes. 50% yield, mp 161-162 °C.

^{1}H NMR (300 mHz, $CDCL_3$) δ= 7.34 (1H, s); 7.23 (2H, d, J= 8.5 Hz); 6.89 (2H, d, J= 8.6 Hz); 6.74 (1H, bs); 5.99 (1H, s); 5.47 (1H, d,J= 8.4 Hz); 4.43-4.40 (2H, m); 3.80 (3H, s); 3.45 (3H, s).

IR (KBr), υ= 3270, 1675 (shoulder), 1650, 1505, 1240 cm^{-1}.



278

N-[benzofuranyl-2-carbonyl-(3H)]-N-(4-methoxybenzyl)-glycine (278)

A solution of coumarilic acid (1.98 g, 12.2 mmol, 1.0 eq) and thionyl chloride (3.1 mL, 38.9 mmol, 3.0 eq) in 150 mL of dry benzene was refluxed for 4 hrs. The solution was concentrated under reduced pressure. The crude acid chloride was taken up in methylene chloride and added to a vigorously stirred aqueous solution containing N-(4-methoxybenzyl) glycine ethyl ester **11** (3.0 g, 13.0 mmol, 1.1 eq) and sodium bicarbonate (1.1 g, 13.0 mmol, 1.1 eq). The solution was stirred at room temperature for 1 hour. The layers were separated and the organic phase washed with water, dried over magnesium sulfate, and filtered to give a yellow oil. The crude ester was taken up in 100 mL of ethanol, cooled to 0°C and saponified using lithium hydroxide (15 mL, 15.0 mmol, 1.2 eq.) The solution was warmed to room temperature and stirred for 24 hrs. The white solid was collected by filtration and taken up in water. After acidification with 2M HCl, the acid was extracted into methylene chloride, dried over magnesium sulfate, filtered, and concentrated to a light yellow solid. The product was recrystallized from boiling ethyl acetate/hexanes. 66% yield. mp 153-154 °C.

^{1}H NMR (300 mHz, DMSO-d_6, T= 370 K) δ= 7.71 (1H, d, J= 7.7 Hz); 7.54 (1H, d, J= 8.1 Hz); 7.44- 7.37 (2H, m); 7.32- 7.24 (3H, m); 6.89 (2H, d, J= 8.6 Hz); 4.75 (2H, s); 4.17 (2H, s); 3.74 (3H, s).

IR (NaCl, film) υ= 2938, 1738, 1611, 1248, 1176 cm^{-1}.

Anal. Calcd. for $C_{19}H_{17}NO_5$: C 67.25, H 5.05, N 4.13, Found C 67.19, H 5.11, N 4.18.

283

N-[benzofuran-2-carbonyl-(3H)]-glycine (283)

Coumarilic acid (1.0 g, 6.16 mmol. 1.0eq) dissolved in 100 mL of dry benzene and thioyl chloride (1.45 mL, 18.5 mmol, 3.0 eq) were refluxed for 3 Hrs. The solvent was evaporated to give the crude acid chloride which was stored under vacuum until needed.

Glycine ethyl ester hydrochloride (0.947 g, 6.78 mmol, 1.1 eq) was dissolved in 50 mL of water. Potassium hydroxide (0.380 g, 6.78 mmol, 1.1 eq) was added and the free amine was extracted into methylene chloride.

The acid chloride was dissolved in methylene chloride and added to the solution of glycine ethyl ester. A solution of sodium bicarbonate (0.569 g, 6.78 mmol, 1.1 eq) in 100 mL of water was added to the methylene chloride solution with vigorous stirring. The layers were separated after 1 hr and the organic layer extracted with saturated ammonium chloride and water. The methylene chloride solution was dried over sodium sulfate, filtered and evaporated to give a highly crystalline solid. The ester was recrystalized from ethyl acetate-hexanes to give 530 mg of white needles as the first crop, and 300 mg as the second crop. M.P. 96-99^{o}C. 54 %Yield

The glycine ethyl ester (687.5 mg, 2.78 mmol, 1.0eq) was dissolved in 50 mL ethanol and cooled to 0^{o}C. Lithium hydroxide (2.80 mL, 2.80 mmol, 1.1 eq) was added and the solution allowed to warm to room temperature. Reaction stirred for 2 days. The ethanol was removed under reduced pressure and the residue taken up in water, which was acidified with 1 molar HCl. The white precipitate was extracted into methylene chloride, dried over sodium sulfate. After filtration and removal of solvent, the white solid was recrystalized in ethyl acetate/hexanes to give 400 mg of the acid. M.P. 189-191^{o}C. 65% Yield.

Benzofuran-2-carbonyl-(3H)-glycine ethyl ester

^{1}H NMR (DMSO-D_6, 200mHz) ∂= 9.14 (1H, t, J= 5.9 Hz) 7.8 (1H, d, J= 7.7 Hz); 7.68 (1H, d, J= 8.3 Hz); 7.6 (1H, s); 7.49 (1H, m); 7.35 (1H, m); 4.13 (2H, q, J= 7.2 Hz); 4.03 (2H, d, J= 6 Hz); 1.21 (3H, t, J= 8 Hz).

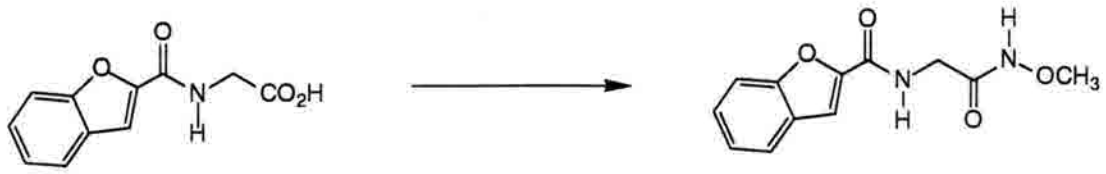
IR (KBr) υ= 3366, 1748, 1659, 1520 cm^{-1}.

Benzofuran-2-carbonyl-(3H)-glycine (283)

^{1}H NMR (DMSO-D_6, 200mHz) ∂=12.73(1H, bs); 9.02 (1H, t, J= 6Hz); 7.79 (1H, d, J= 7.7Hz); 7.68 (1H, d, J= 8.3Hz); 7.66 (1H, s); 7.49 (1H, m); 7.35 (1H, m); 3.95 (2H, d, J= 6Hz).

IR (KBr) υ= 3267, 3057, 1725, 1660 cm^{-1}.

Anal. Calcd for $C_{11}H_9NO_4$: C 60.27, H 4.14, N 6.39; Found C 60.25, H 4.25, N 6.28.



283 **284**

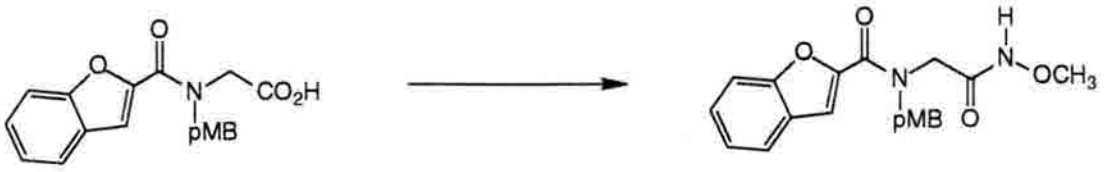
N-[2-(methoxyamino)-2-oxoethyl]-N-2-benzofurancarboxamide (284)

To a tetrahydrofuran solution containing **283** (1.6 g, 7.3 mmol, 1.0 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.79 g, 14.6 mmol, 2.0 eq) was added a tetrahydrofuran solution of methoxylamine (0.686 g, 14.6 mmol, 2.0 eq). The resulting solution was stirred for 48 Hrs, poured into water and extracted with methylene chloride. The methylene chloride was dried over magnesium sulfate, filtered and evaporated to give a white solid. The solid was recrystalized from ethyl acetate/hexanes to give 976 mg of product. M.P. 181°C. 53% Yield.

^{1}H NMR (DMSO-D_6, 200mHz) ∂=11.25 (1H, bs); 8.9 (1H, bt); 7.8 (1H, d, J= 7.7Hz); 7.68 (1H, d, J= 8.3Hz); 7.58 (1H, s); 7.49 (1H, m); 7.35 (1H, m); 3.80 (2H, d, J= 5.7Hz); 3.60 (3H, s).

IR (KBr) υ= 3275, 3196, 3001, 1684(shoulder), 1648 cm^{-1}.

Anal. Calcd. for $C_{12}H_{12}N_2O_4$: C 58.06, H 4.87, N 11.29; Found C 58.26, H 4.99, N 11.25.



278 **275**

N-[2-(methoxyamino)-2-oxoethyl]-N-(4-methoxybenzyl))-2-benzofuran carboxamide (275)

To a solution containing 1 g **278** (2.94 mmol, 1.0 eq) dissolved in 250 mL of tetrahydrofuran cooled to 0°C was added triethylamine (616 mL, 4.42 mmol, 1.5 eq). Pivaloyl chloride (400 mL, 1.1 eq) was added causing the solution to turn bright yellow. The reaction was stirred at 0°C for 90 minutes before adding methoxylamine (500 mL, 9.4 mmol, 3.2 eq). Solution stirred at 0°C until the yellow color dissipated (about 2 hours). The reaction was poured into ethyl acetate, washed with saturated Sodium bicarbonate, water, dried over magnesium sulfate, filtered, and concentrated to a thick, colorless oil which left standing crystallized. The hydroxamic ester was purified by recrystallization from boiling ethyl acetate/hexanes. Obtained 1.03 grams of a white solid. Typical yields 80-95%.

^{1}H NMR (300 mHz, DMSO-d_6, T= 370 K) δ= 10.78 (1H, bs); 7.48 (1H, d, J= 7.7 Hz); 7.55 (1H, d, J= 8.2 Hz); 7.44- 7.37 (2H, m); 7.33- 7.24 (3H, m); 6.89 (2H, d, J= 8.6 Hz); 4.74 (2H, s); 4.0 (2H, s); 3.75 (3H, s); 3.58 (3H, s).

IR (NaCl, film) υ= 3203, 2999, 2936, 1677, 1613, 1513 cm^{-1}.

Anal.Calcd. for $C_{20}H_{20}N_2O_5$: C 65.20, H 5.47, N 7.61, found C 65.25, H 5.61, N 7.69.

275 **274**

Trans-3-bromo-1'-methoxy-4'-(4-methoxybenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (274).

To a solution of glycine hydroxamate **275** (1 g, 2.71 mmol, 1eq) in 50 mL of ethanol-free chloroform was added N-bromosuccinamide (578 mg, 3.25 mmol, 1.2 eq) in one portion and the solution stirred at room temperature overnight. The reaction was diluted with additional chloroform, extracted with saturated sodium thiosulfate, followed by twice with water. The organic layer dried over sodium sulfate, filtered and evaporated to give a thick yellow residue. The residue was taken up in ethyl acetate, and the white precipitate which subsequently forms collected. The mother liquor was chromatographed using 3:2 hexane/ethyl acetate, and the product recrystallized from boiling ethyl acetate/hexanes. 50% combined yield. mp 142-143 °C.

^{1}H NMR ($CDCl_3$, 300 mHz) ∂= 7.33- 7.26 (4H, m); 7.28 (1H, d, J= 8.6 Hz, super imposed over multiplet); 7.07- 7.02 (1H, m); 6.97- 6.95 (1H, m); 6.86 (1H, d, J= 8.6 Hz), 6.01 (1H, s); 4.69 (1H 1/2 ABq, J = 14.3Hz); 4.50 (1H, 1/2 ABq, J= 14.3 Hz); 5.99 (2H, d, J= 1.4 Hz); 3.92 (3H, s); 3.80 (3H, s).

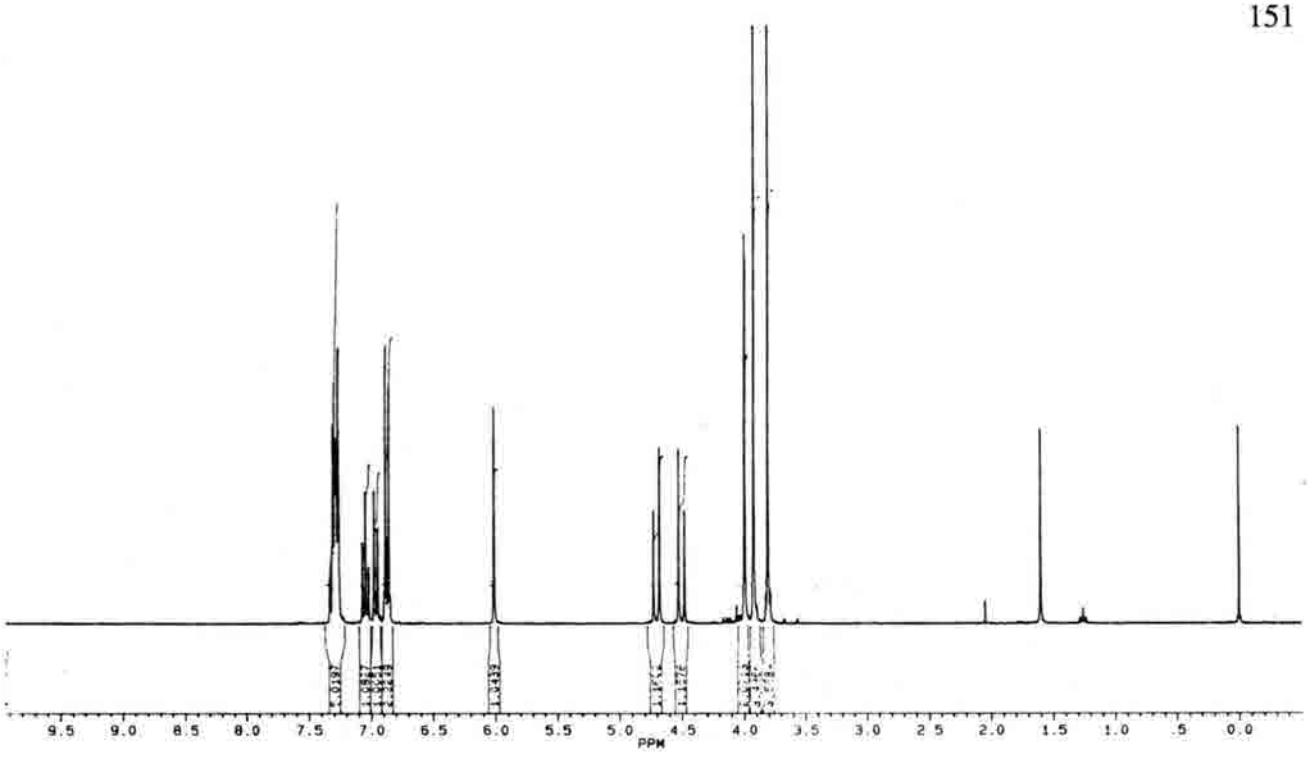
^{1}H NMR (DMSO-d_6, 270 mHz) ∂= 7.34 - 7.29 (5H, m); 7.06- 7.02 (2H, m); 6.92 (1H, d, J= 8.6 Hz); 6.24 (1H, s); 4.69 (1H, 1/2 ABq, J= 14.3 Hz); 4.38 (1H, 1/2 ABq, J= 14.3 Hz); 4.19 (1H, 1/2ABq, J= 18.3 Hz); 4.04 (1H, 1/2 ABq, J= 18.3 Hz); 3.74 (3H, s).

^{13}C NMR ($CDCl_3$, 75.47 mHz) ∂=162.1 (C); 160.93 (C); 159.8 (C); 157.9 (C); 131.18 (CH), 131.08(CH); 130.63 (CH); 125.93 (C); 125.44 (CH); 122.68 (CH); 114.28 (CH);

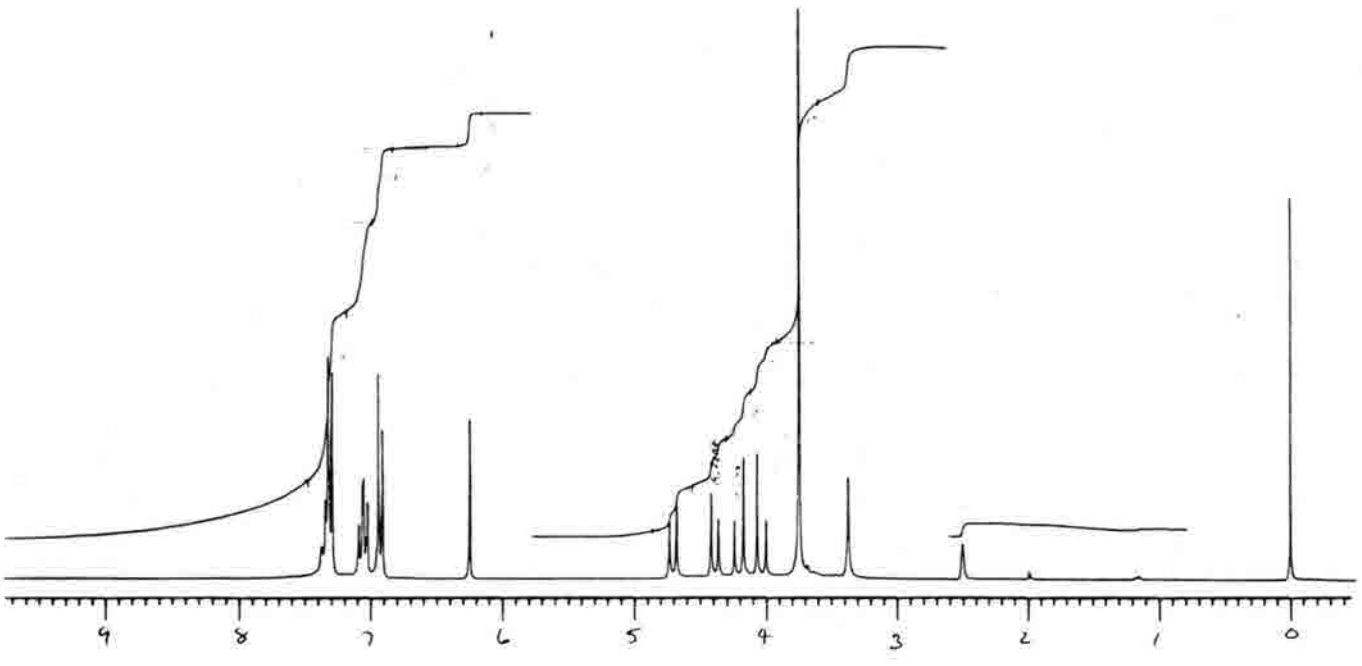
109.69 (CH); 100.68 (C); 65.71 (CH_3); 55.28 (CH_3); 50.15 (CH); 49.24 (CH_2); 47.96 (CH_2).

IR (neat, NaCl) υ= 1693, 1613, 1513, 1245 cm^{-1}.

Anal. Calcd. for $C_{20}H_{19}N_2O_5Br$: C 53.70, H 4.28, N 6.26, Br 17.87; found C 53.62, H4.22, N 6.26, Br 18.00

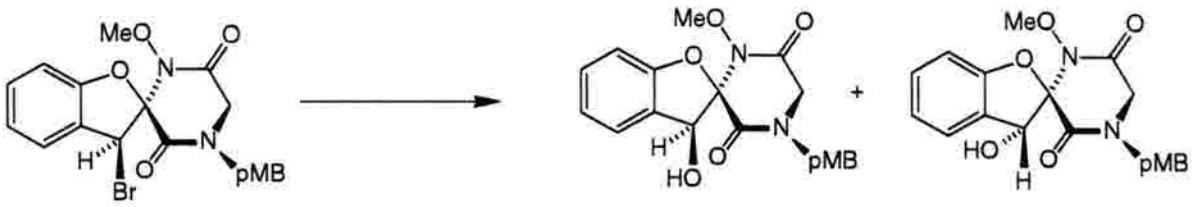


300 mHz NMR in $CDCl_3$



270 mHz NMR in DMSO-d_6

Figure 1. NMR of bromide **274** in $CDCl_3$ and DMSO-d_6.



(±)-**274** (±)-**287a** (±)-**287b**

Cis,Trans-3-hydroxy-1'-methoxy-4'-(4-methoxybenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (287a,b)

Bromide **274** was dissolved in a solution of 1:1THF/$H_2$0 at room temperature. A solution of silver triflate (1.2 eq) dissolved in tetrahydrofuran was added and the resulting solution stirred for 30 minutes. The solution was diluted with ethyl acetate and saturated sodium chloride solution added. The solution was filtered through a plug of celite to remove the silver salts. The filtrate was washed with brine, water, dried over magnesium sulfate, filtered and after evaporated to a colorless oil which upon standing solidifies. The residue was taken up in ethyl acetate and chromatographed using 1:1 ethyl acetate/hexanes. Both isomers were recrystallized from ethyl acetate/hexanes. 80% combined yield.

287a TRANS (R_f= 0.17, 1:1 ethyl acetate/hexanes)

^{1}H NMR: (270 mHz, $CDCl_3$) δ = 7.39- 7.19 (4H, m); 7.06- 7.01 (1H, m); 6.91- 6.87 (3H, m); 5.80 (1H, d, J= 12.3 Hz, singlet in D_2O); 4.60 (1H, 1/2ABq, J= 14.4 Hz); 4.49 (1H, 1/2ABq, J= 14.4 Hz); 3.98 (2H, s); 3.88 (3H, s); 3.80 (3H, s); 3.55 (1H, d, J= 12.4 Hz, exchangeable in D_2O).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 161.49 (C); 160.99 (C); 159.73 (C); 158.47 (C); 130.65 (CH); 129.97 (CH); 127.19 (C); 125.81 (C); 125.0 (CH); 122.44 (CH); 114.46 (CH); 109.86 (CH); 99.92 (C); 77.50 (CH); 65.30 (CH_3); 55.29 (CH_3); 48.95 (CH_2); 47.99 (CH_2).

IR: (NaCl, neat film) υ= 3407, 1683 cm^{-1}.

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: C 62.49, H 5.24, N 7.29, Found C 62.49, H 5.25, N 7.26. mp 135-136 °C.

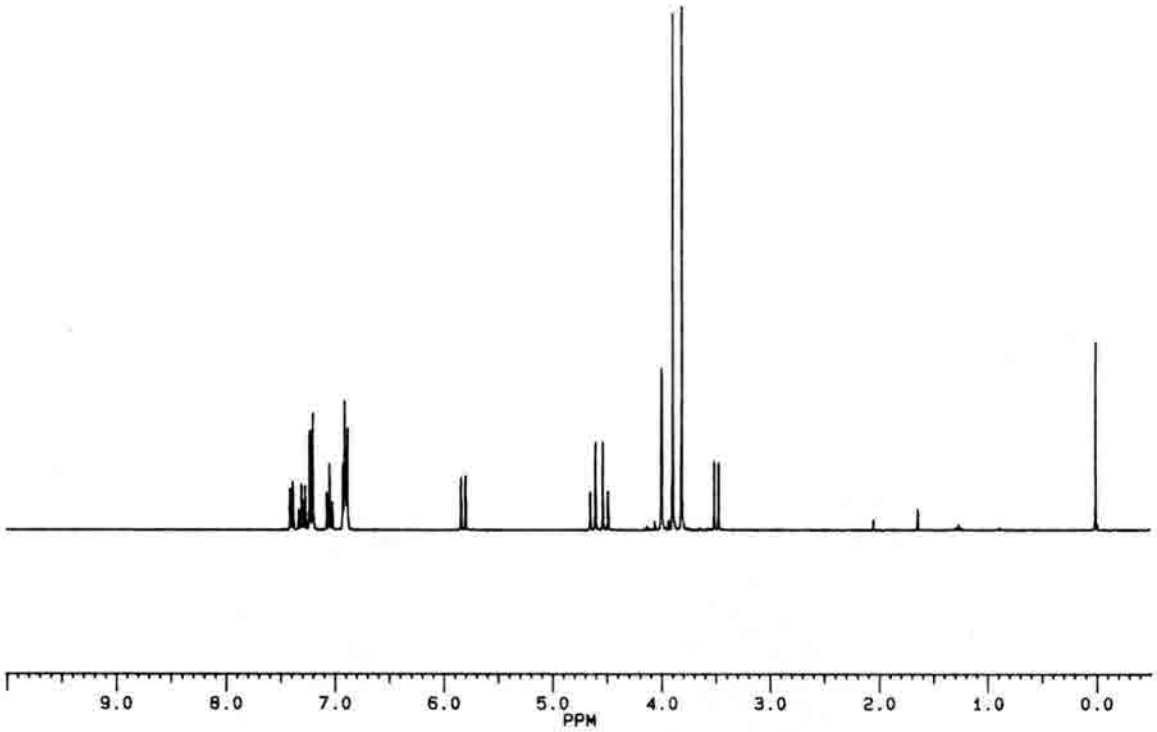
287b Cis (R_f= 0.1, 1:1 ethyl acetate/hexanes)

^{1}H NMR: (270 mHz, $CDCl_3$) δ = 7.36- 7.28 (3H, m); 7.24 (1H, 1/2ABq, J= 8.6 Hz); 7.07- 6.96 (3H, m); 6.98 (1H, 1/2ABq, J= 8.6 Hz); 5.59 (1H, d, J= 12.6 Hz, singlet in D_2O); 4.69 (1H, 1/2ABq, J= 14.3 Hz); 4.48 (1H, 1/2ABq, J= 14.3 Hz); 4.01 (2H, s); 3.81 (3H, s); 3.78 (3H, s); 3.0 (1H, d, J= 12.8 Hz, exchangeable in D_2O).

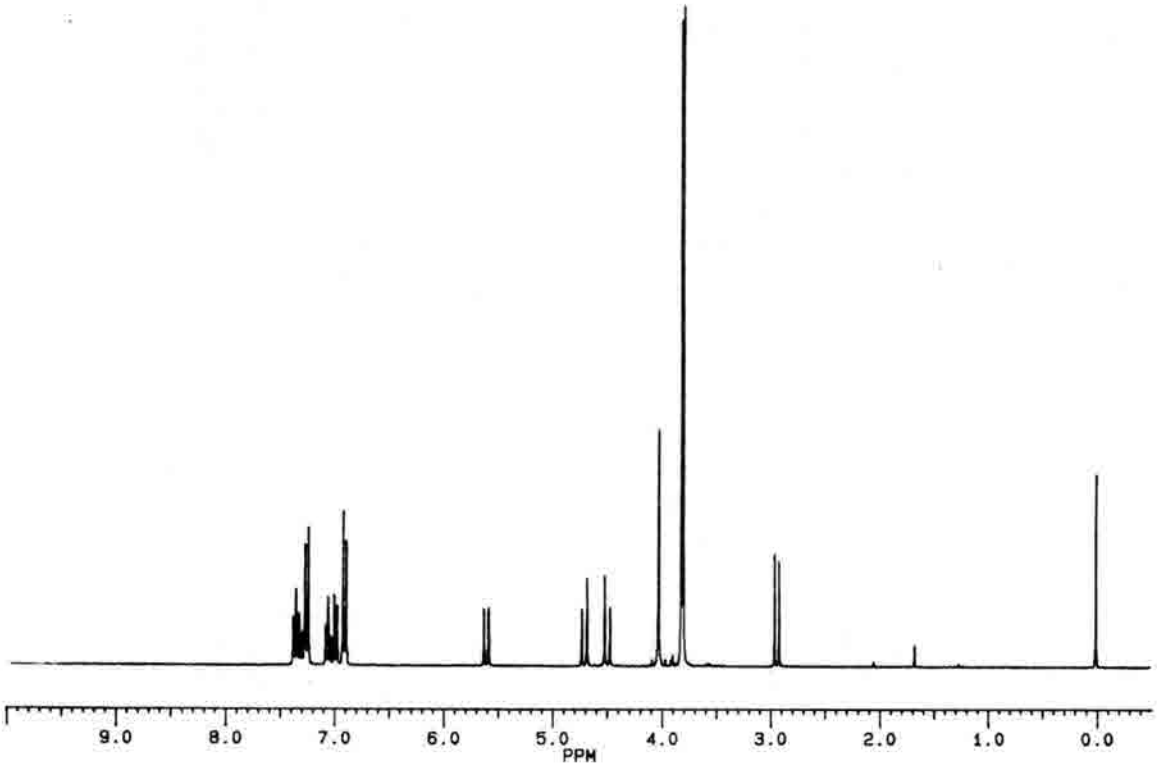
^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 162.95 (C); 161.78 (C); 159.75 (C); 157.46 (C); 130.79 (CH); 130.16 (CH); 126.27 (C); 126.20 (C); 124.57 (CH); 122.58 (CH); 114.47 (CH); 109.76 (CH); 98.28 (C); 79.08 (CH); 65.54 (CH_3); 55.33 (CH_3); 49.28 (CH_2); 48.11 (CH_2).

IR (NaCl, neat film) υ= 3392, 2940, 1683, 1513, 1248 cm^{-1}.

Anal. Calcd. for $C_{20}H_{20}N_2O_6$: C 62.49 H 5.24, N 7.29, Found C 62.44, H 5.21, N 7.20. mp 140-142 °C.

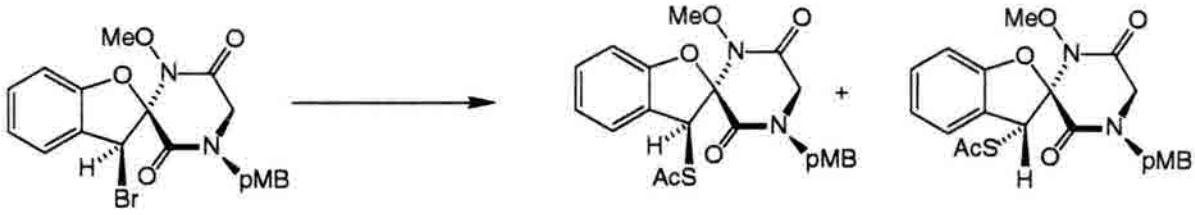


trans 287a



cis 278b

Figure 2. 300 mHz NMR of cis, trans **278a,b** in $CDCl_3$



274 **(±)-288a** **(±)-288b**

Cis,Trans-3-(S-acetyl)-1'-methoxy-4'-(4-methoxybenzyl)-spiro-[benzofuran-2(3H),2'-piperazine]-3',6'-dione (288a,b)

75 mg of **274** was dissolved in 4 mL benzene and excess thiolacetic acid (0.5 mL) was added followed by a spatula tip of anhydrous zinc chloride. The solution was stirred for 36 hours at room temperature. The reaction was filtered, diluted with ethyl acetate, washed 3 times with saturated sodium bicarbonate, water, dried over magnesium sulfate, filtered and concentrated to a foul-smelling yellow film. The diastereomers were separated via column chromatography using 10% acetone in carbon tetrachloride, and can be isolated as a mixture using 2:1 hexanes/ethyl acetate as the eluent. Yield 69-80%.

TRANS 288a (R_f= 0.3, 20% acetone/carbon tetrachloride)

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.28- 7.24 (4H,m); 7.14 (1H, d, J= 7.5 Hz); 6.97-6.85 (3H, m); 5.74 (1H, s); 4.87 (1H, 1/2ABq, J= 14.1 Hz); 4.16 (1H, 1/2ABq, J= 14.1 Hz); 3.94 (1H, 1/2ABq, J= 18 Hz); 3.91 (3H, s); 3.83 (1H, 1/2ABq, J 18.2 Hz); 3.79 (3H, s); 2.14 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 195.2 (C); 161.48 (C); 160.58 (C); 159.7 (C); 158.1 (C); 130.69 (CH); 129.91 (CH); 126.41 (C); 124.31 (CH); 123.91 (C); 122.17 (CH); 114.25 (CH); 109.36 (CH); 101.3 (C); 65.4 (CH_3); 55.31 (CH_3); 50.11 (CH); 49.24 (CH_2); 48.45 (CH_2); 29.62 (CH_3).

IR (NaCl, neat film) υ= 1694, 1513, 1462, 1244 cm^{-1}. White crystals from ethyl acetate/petroleum ether. mp 164- 165°C.

Anal. Calcd. for $C_{22}H_{22}N_2O_6S$: C 59.71, H 5.01, N 6.33, S 7.25; Found C 59.93, H 5.18, N 6.07, S 7.48.

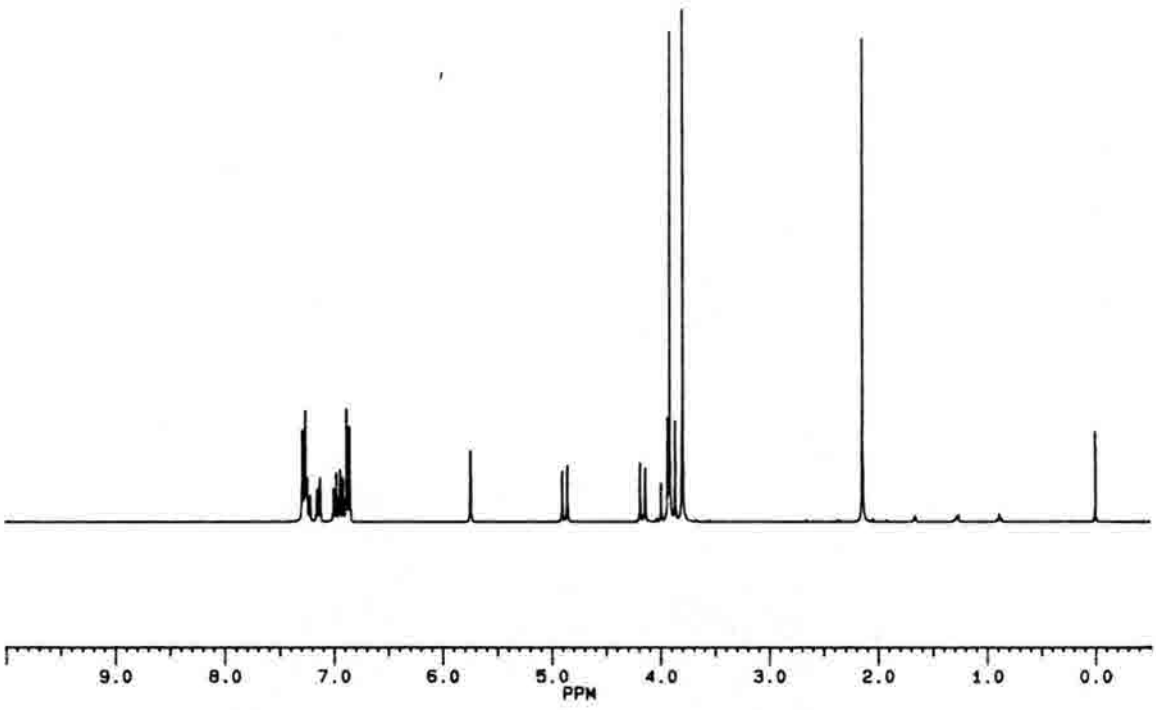
CIS 288b (R_f= 0.4, 20% acetone/carbon tetrachloride)

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.29- 7.28 (4H, m); 7.14 (1H, d, J= 7.5 Hz); 7.11-6.88 (3H, m); 5.81 (1H, s); 4.81 (1H, 1/2ABq, J= 14.3 Hz); 4.47 (1H, 1/2ABq, J= 14.3 Hz); 3.91 (1H, 1/2ABq, J= 18 Hz); 3.81 (3H, s); 3.77 (3H, s) superimposed over 3.73 (1H, 1/2ABq, J = 18 Hz); 2.36 (3H, s).

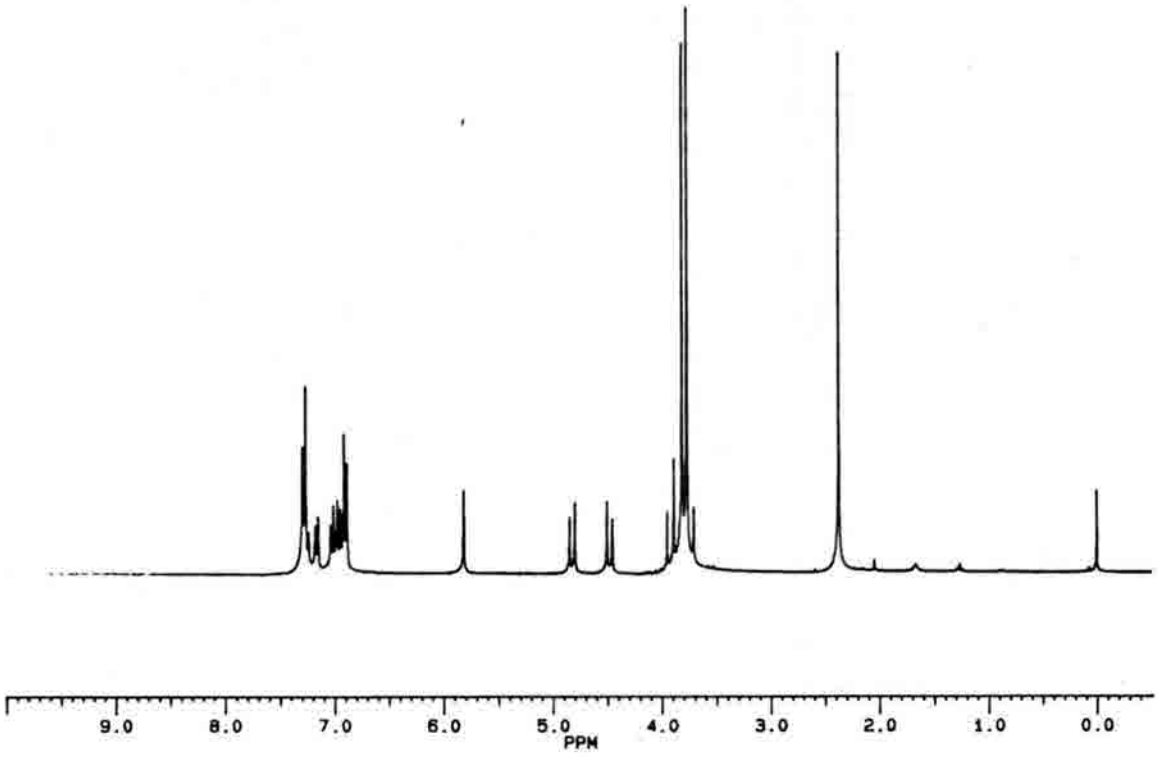
^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 195.61 (C); 162.9 (C); 160.32 (C); 159.67 (C); 157.19 (C); 130.33 (CH); 129.78 (CH); 126.14 (C); 123.97 (CH); 123.59 (C); 122.33 (CH); 114.31 (CH); 109.26 (CH); 99.50 (C); 65.04 (CH_3); 55.29 (CH_3); 52.74 (CH); 49.45 (CH_2); 48.16 (CH_2); 29.91 (CH_3).

IR (NaCl, neat film) υ= 1706, 1691, 1612, 1513, 1461, 1239 cm^{-1}. White plates from ethyl acetate/petroleum ether. mp 102- 104°C.

Anal. Calcd. for $C_{22}H_{22}N_2O_6S$: C 59.71, H 5.01, N 6.33, S 7.25; Found C 59.61, H 5.23, N 6.11, S 7.00.

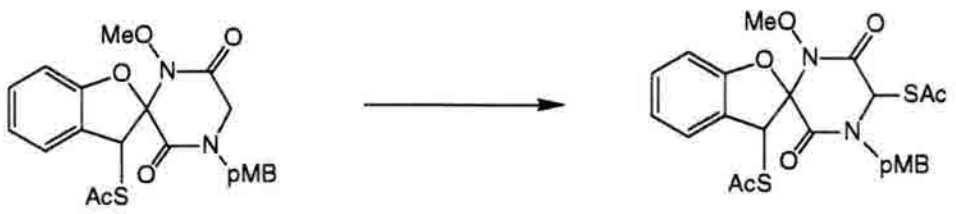


trans 288a



cis 288b

Figure 3. 300 mHz NMR of thioacetates **288a,b** in $CDCl_3$



288a,b **291a-d**

Cis,Trans-3,5'-(S-acetyl)-1'-methoxy-4'-(4-methoxybenzyl)-spiro [benzofuran-2(3H),2'-piperazine]-3',6'-dione (291a-d)

288a (600 mg, 1.35 mmol, 1.0 eq) and N-bromosuccinamide (362 mg, 2.03 mmol, 1.5 eq) were dissolved in 50 mL carbon tetrachloride under argon and the solution was heated and refluxed for 3 1/2 hrs. The solution was cooled and the solvent removed. The orange residue was taken up in methylene chloride and a methylene chloride solution of pyridine (408 µL, 5.42 mmol, 4.0 eq) and thiolacetic acid (387 µL, 5.42 mmol, 4.0 eq) was added. The combined solution was stirred at room temperature for 30 minutes. The solution was extracted with saturated sodium bicarbonate, water, dried over magnesium sulfate, filtered and concentrated to an obnoxious smelling foam. The diastereomers were separated using flash column chromatography using 5% acetone in carbon tetrachloride or using 2:1 hexane/ethyl acetate to obtain a mixture. Obtained 493 mg of both diastereomers. 65 % combined yield. Same experimental procedure starting with **288b** afforded **291c,d** in 55% yield.

291a Trans, (R_f= 0.4, 1:1 ethyl acetate/hexanes)

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.32 (2H, d, J= 8.5 Hz); 7.26- 7.21 (1H, m); 7.13-7.10 (1H, m); 7.00- 6.95 (1H, m); 6.90-6.85 (3H, m); 6.12 (1H, s); 5.74 (1H, s); 5.19 (1H, 1/2ABq, J= 14.5 Hz); 3.91 (3H, s); 3.80 (3H, s); 3.74 (1H, 1/2ABq, J= 14.5 Hz); 2.50 (3H, s); 2.18 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 195.18 (C); 192.31 (C); 161.64 (C); 161.13 (C); 159.72 (C); 157.35 (C); 130.97 (CH); 129.60 (CH); 126.52 (C); 125.14 (C); 124.05

(CH); 122.38 (CH); 114.14 (CH); 109.54 (CH); 101.52 (C); 64.85 (CH_3); 60.04 (CH); 55.29 (CH_3); 48.95 (CH); 46.73 (CH_2); 30.45 (CH_3); 29.64 (CH_3).

IR (NaCl, neat film) υ= 1695, 1612, 1513, 1459, 1243 cm^{-1}. White crystals from ethyl acetate/hexanes. mp 204- 205 °C.

Anal. Calcd. for $C_{24}H_{24}N_2O_7S_2$: C 55.80, H 4.68 N 5.42, S 12.41; Found C 55.64, H 4.76, N 5.24, S 12.51.

291b Trans, (R_f= 0.32, 1:1 ethyl acetate/hexanes)

^{1}H NMR (300 mHz, $CDCl_3$) δ=7.30- 7.21 (3H, m); 7.13- 7.11 (1H, m); 7.01- 6.87 (4H, m); 5.89 (1H, s); 5.88 (1H, s); 5.14 (1H, 1/2ABq, J= 14.4 Hz); 3.95 (3H, s); 3.88 (1H, 1/2ABq, J= 14.4 Hz); 3.81 (3H, s); 2.48 (3H, s); 2.46 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 193.41 (C); 191.96 (C); 161.9 (C); 160.01 (C); 159.74 (C); 157.85)C); 130.57 (CH); 129.79 (CH); 126.49 (C); 125.18 (C); 124.28 (CH); 122.37 (CH); 114.2 (CH); 109.35 (CH); 101.0 (C); 65.55 (CH_3); 59.83 (CH); 55.28 (CH_3); 49.28 (CH); 47.05 (CH_2); 30.27 (CH_3); 30.17 (CH_3).

IR (NaCl, neat film) υ=1706, 1691, 1612, 1513, 1461, 1239 cm^{-1}. White solid from ethyl acetate/petroleum ether. mp 168- 170 °C (decomposed).

Anal. Calcd. for $C_{24}H_{24}N_2O_7S_2$: C 55.80, H 4.68 N 5.42, S 12.41; Found C 55.68, H 4.84, N 5.58, S 12.16.

Cis thioacetate diastereomers:

291c (R_f= 0.42, 1:1 ethyl acetate/hexanes)

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.27- 7.25 (3H, m); 7.15- 7.13 (1H, m); 7.05- 6.87 (2H, m); 6.85 (2H, d, J= 8.54 Hz); 6.23 (1H, s); 5.36 (1H, s); 5.28 (1H, 1/2ABq, J= 8.54 Hz); 3.99 (1H, 1/2ABq, J= 14.5 Hz); 3.81 (3H, s); 3.68 (3H, s); 2.44 (3H, s); 2.37 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ=194.24 (C); 192.23 (C); 162.45 (C); 159.96 (C); 159.7 (C); 156.75 (C); 130.60 (CH); 129.47 (CH); 126.23 (C); 124.73 (C); 123.60 (CH); 122.56 (CH); 114.15 (CH); 109.31 (CH); 99.73 (C); 64.16 (CH_3); 60.09 (CH); 55.27 (CH_3); 52.45 (CH); 47.61 (CH_2); 30.48 (CH_3); 29.96 (CH_3).

IR (NaCl, neat film) υ= 2940, 1702, 1612, 1513, 1239 cm^{-1}.

Anal. Calcd. for $C_{24}H_{24}N_2O_7S_2$: C 55.80, H 4.68 N 5.42, S 12.41; Found C 55.67, H 4.76, N 5.23. White crystals from acetone/petroleum ether mp 192-193 °C.

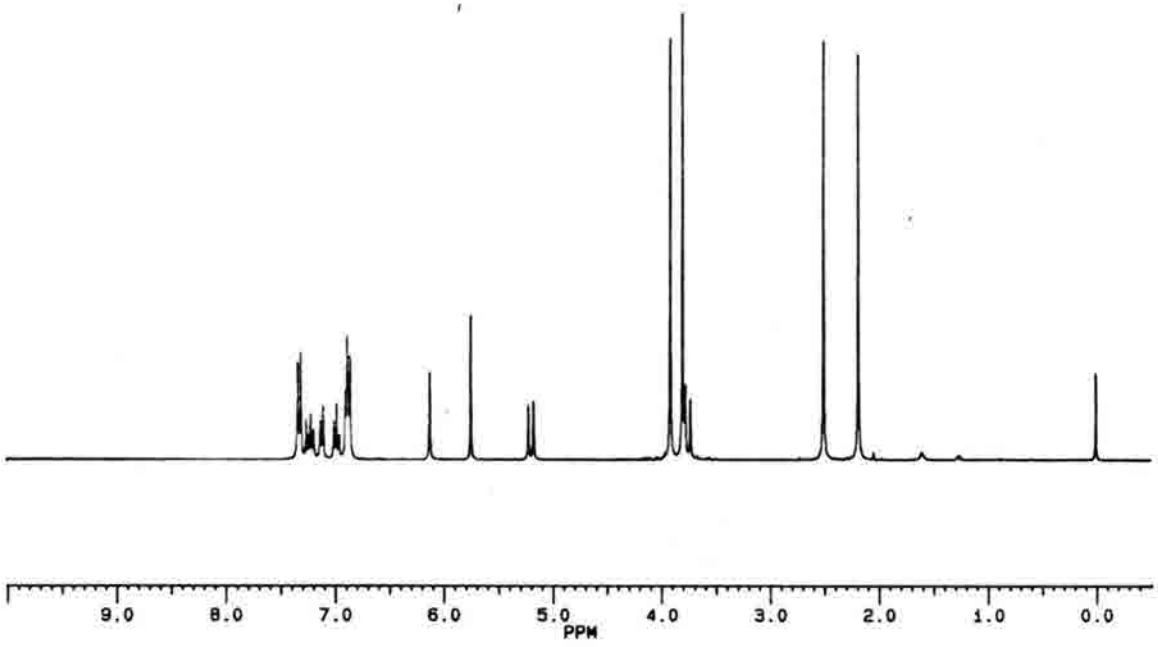
291d (R_f= 0.32, 1:1 ethyl acetate/hexanes)

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.32 (2H, d, J= 8.5 Hz); 7.26- 7.22 (1H, m); 7.13 (1H, d, J= 7.4 Hz); 7.03- 6.89 (4H, m); 5.94 (1H, s); 5.83 (1H, s); 5.27 (1H, 1/2ABq, J =14.47 Hz); 3.82 (1H, 1/2ABq, J= 14.47 Hz); 3.83 (3H, s); 3.77 (3H, s); 2.51 (3H, s);2.46 (3H, s).

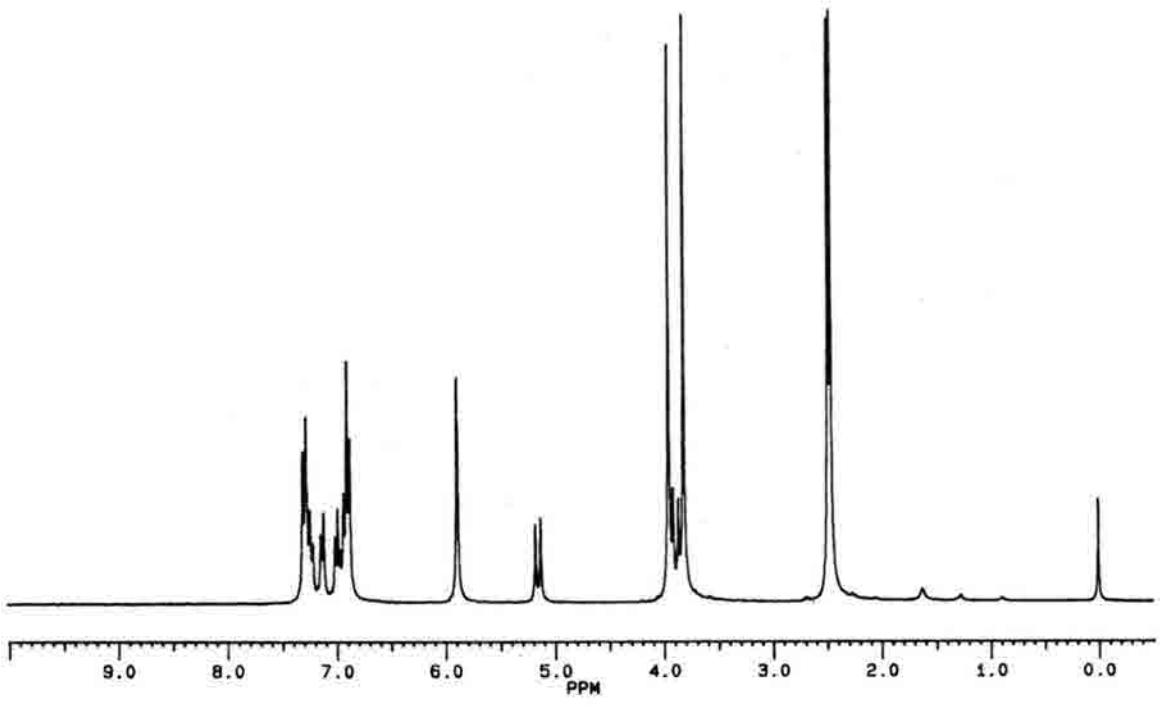
^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 194.57 (C); 192.02 (C); 163.23 (C); 159.79 (C); 159.72 (C); 156.92 (C); 130.56 (CH); 129.7 (CH); 126.58 (C); 124.19 (C); 123.92 (CH); 122.52 (CH); 114.23 (CH); 109.16 (CH); 99.0 (C); 65.34 (CH_3); 60.03 (CH); 55.27 (CH_3); 51.75 (CH); 47.24 (CH); 30.24 (CH_3).

IR (NaCl, neat film) υ= 2942, 1699, 1513, 1238 cm^{-1}. Light yellow crystals from ethyl acetate/hexanes.

Anal. Calcd. for $C_{24}H_{24}N_2O_7S_2$: C 55.80, H 4.68 N 5.42, S 12.41; Found C 55.83, H 4.73, N 5.25, S 12.14. mp 168-169 °C.

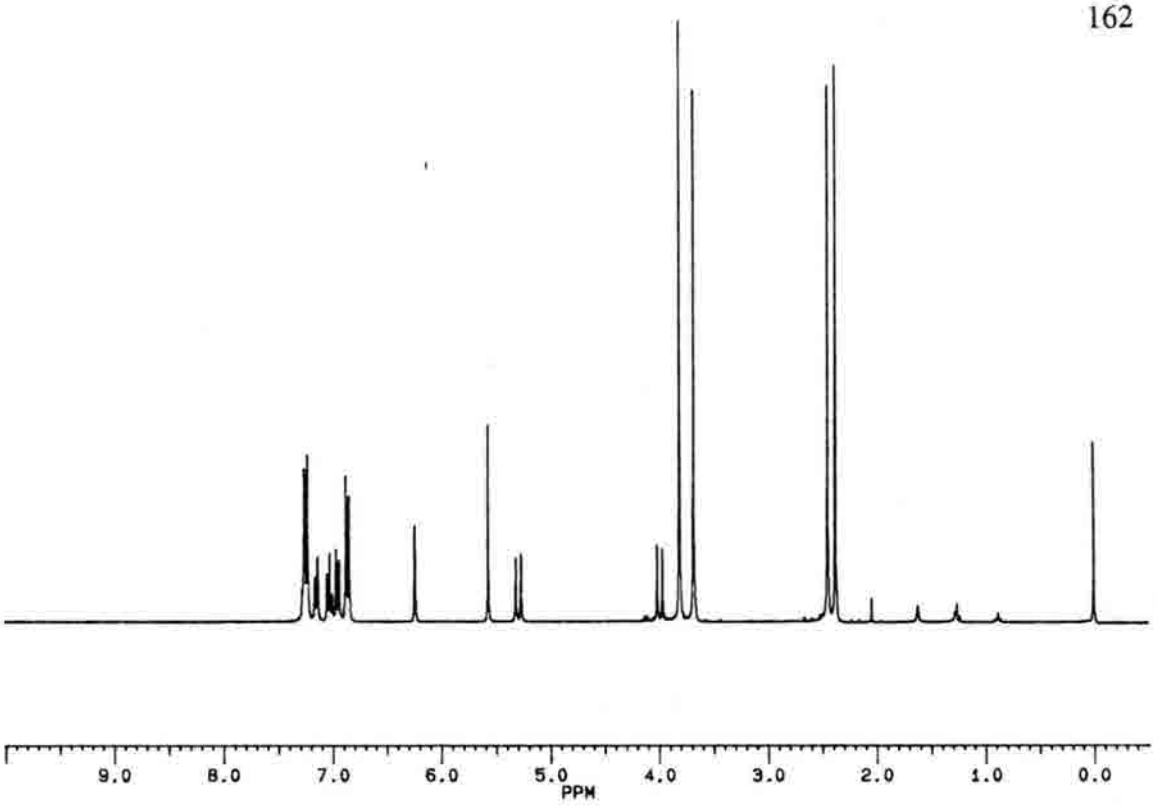


trans 291a

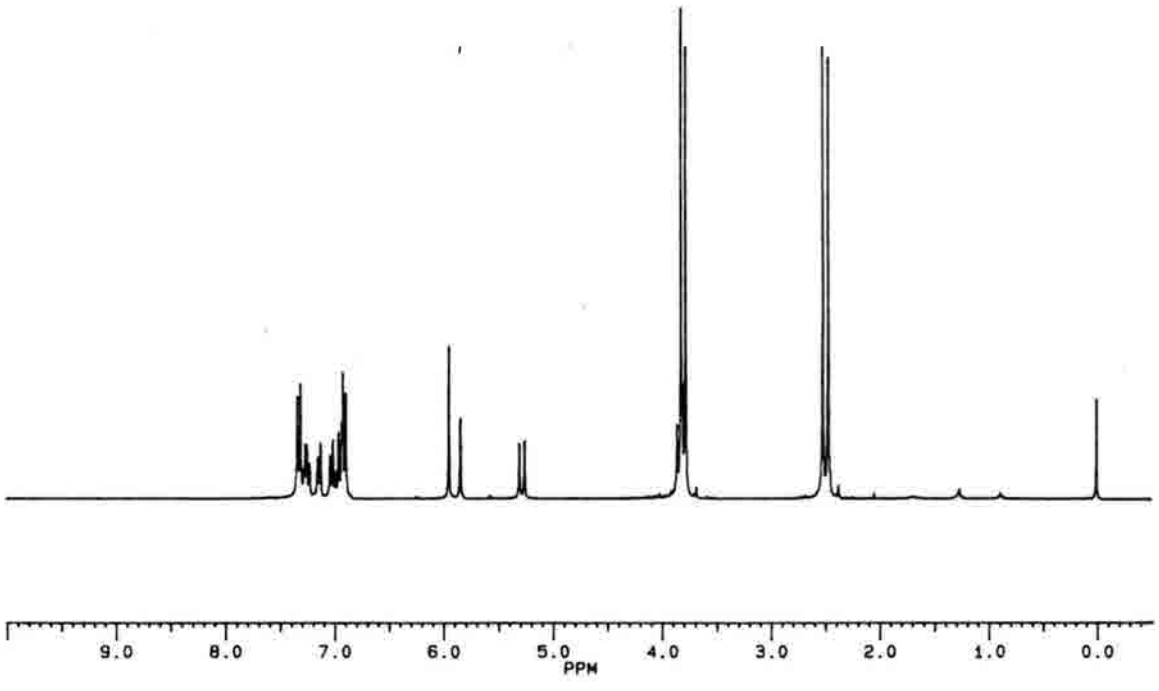


trans 291b

Figure 4. 300 mHz NMR of bisthioacetates **291a,b** in $CDCl_3$

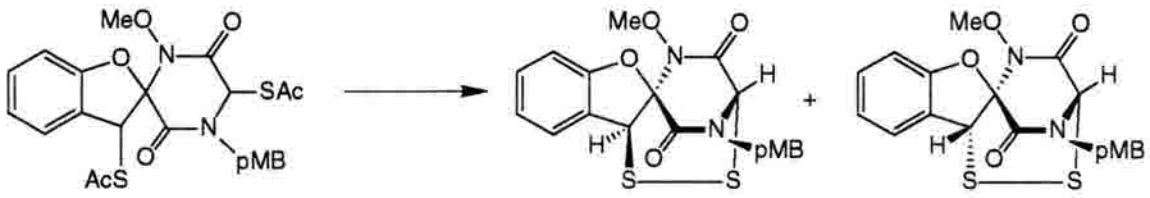


cis 291c



cis 291d

Figure 5. 300 mHz NMR of cis bisthioacetates **291c,d** in $CDCl_3$



291a-d **273** **292**

11-(N-methoxy)-4-(4-methoxybenzyl)-10bH-5a,5H-benzofuro[2,3-f]-1,2,4-dithiazepine-5,12-dione. (3α,5aα,10bα) and (3α,5aα,10bβ) (273 and 292)

Acid hydrolysis procedure:

25 mg (0.048 mmol, 1eq) of a mixture of **291a-d** was suspended in 10 mL of absolute ethanol and cooled to 0°C. Dry HCl gas was bubbled through the solution for 30 minutes, and was stirred at room temperature for an additional 5 hours. The ethanol was removed under vacuum and the yellow residue taken up in methylene chloride. The methylene chloride layer was shaken with aqueous 2% KI_3 until the organic layer was a persistent pink/purple. The organics were washed with water, dried over sodium sulfate, filtered and concentrated to a dark brown film which was purified via preparative thin layer chromatography using 3:2 hexane/ethyl acetate as the eluent. The two bands which stain white with I_2/NaN_3 were isolated. Obtained 6.7 mg of white solid. Combined yield 32%.

Basic hydrolysis procedure:

20 mg (0.041 mmol, 1eq) of a mixture of **291a-d** was suspended in 7 mL of absolute ethanol and argon was bubbled through the solution for 20 minutes. 1.5 mL of 0.2N sodium hydroxide was added with continued degassing and the solution stirred for 10 minutes. The reaction was poured into a separatory. funnel containing methylene chloride and aqueous 2% KI_3 was added until the methylene chloride layer was purple. The organic layer was washed with water, dried over sodium sulfate, filtered and concentrated to a brown film which was PTLC'd using 3:2 hexane/ ethyl acetate (or radial

chromatography using 3:1 hexane/ethyl acetate). Isolated 5.4 mg of **273** or **292** as a white solid. Yield 32%.

273 TRANS (R_f= 0.38, 2:3 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.34-7.18 (4H, m); 7.09 (1H, d, J= 8.2 Hz); 7.04-6.99 (1H, m); 6.89 (2H, 1/2 ABq, J= 8.7 Hz); 5.27 (1H, 1/2 ABq, J= 14.4 Hz); 5.00 (1H, s); 4.89 (1H, s); 3.96 (1H, 1/2 ABq, J= 14.4 Hz); 3.96 (3H, s); 3.81 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 165.53 (C); 164.24 (C); 159.97 (C); 157.98 (C); 131.23 (CH); 130.66 (CH); 125.25 (C); 124.79 (CH); 122.68 (CH); 121.19 (C); 114.56 (CH); 110.48 (CH); 101.62 (C); 66.44 (CH_3); 62.29 (CH); 55.33 (CH_3); 52.45 (CH); 47.44 (CH_2).

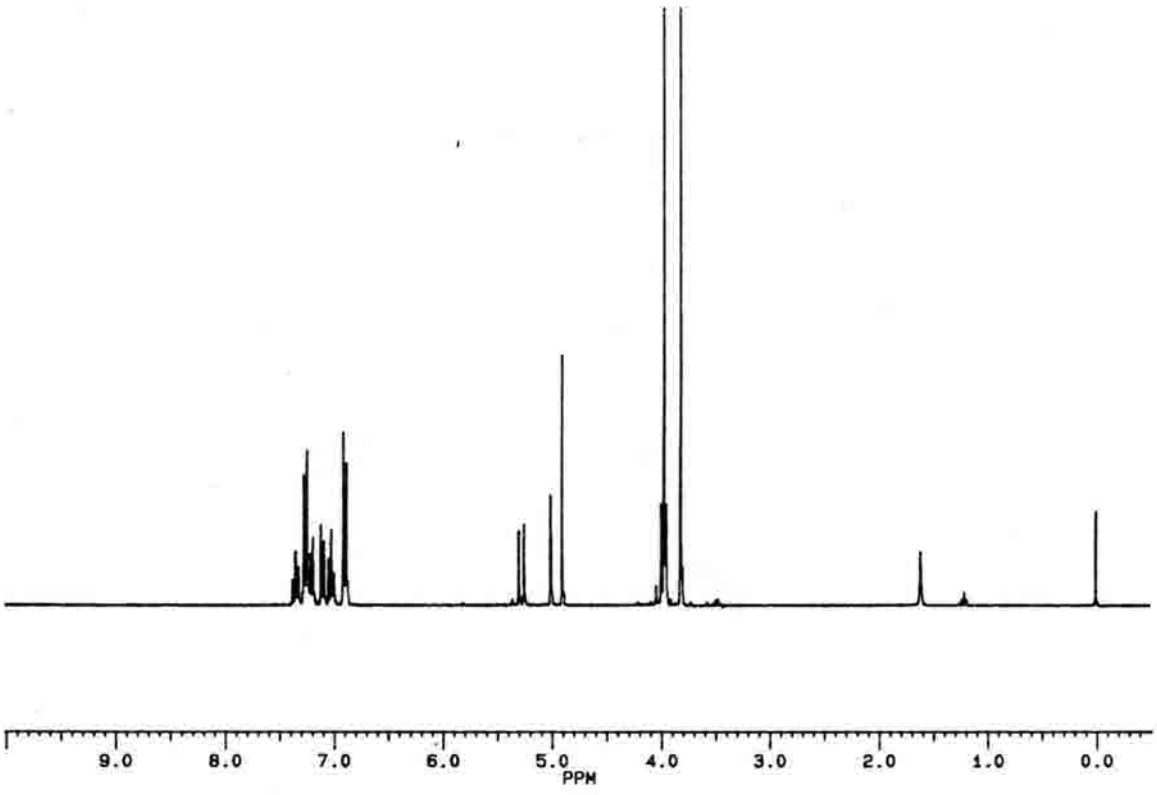
IR (NaCl, neat film) υ= 2942, 1712, 1612, 1513, 1232 cm^{-1}. Colorless crystals from diethyl ether/ pentane, mp 188-189 °C.

292 CIS (R_f= 0.29, 2:3 ethyl acetate/hexanes, recrystallized from acetone/petroleum ether)

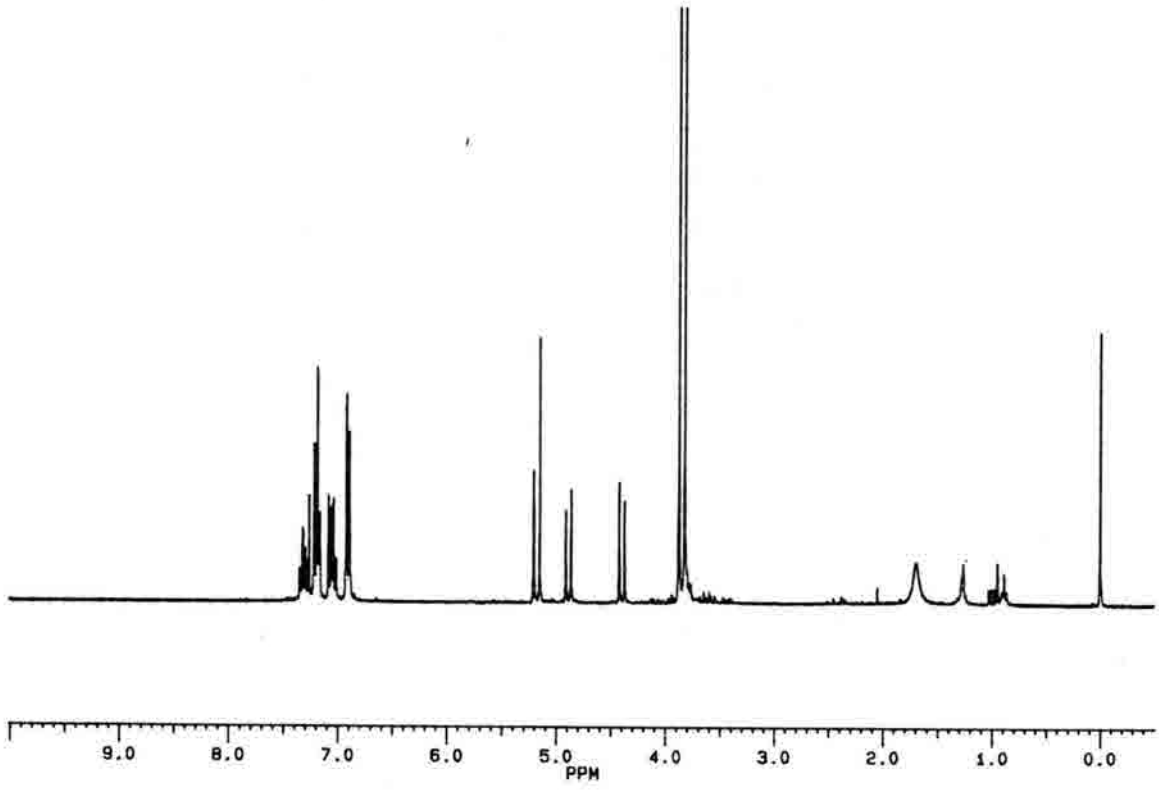
^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.26-7.16 (4H, m); 7.08-7.03 (2H, m); 6.90 (2H, d, J= 8.6 Hz); 5.20 (1H, s); 5.14 (1H, s); 4.88 (1H, 1/2 ABq, J= 14.6 Hz); 4.39 (1H, 1/2 ABq, J= 14.6 Hz); 3.87 (3H, s); 3.82 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 166.09 (C); 162.97 (C); 160.05 (C); 158.1 (C); 130.78 (CH); 130.45 (CH); 125.94 (C); 123.9 (CH); 123.07)CH); 121.48 (C); 114.76 (CH); 109.88 (CH); 100.21 (C); 66.35 (CH_3); 64.64 (CH); 56.27 (CH); 55.34 (CH_3); 48.47 (CH_2).

IR (NaCl, neat film) υ= 2943, 1708, 1513, 1458, 1249, 1030 cm^{-1}. mp 163-164 °C.



trans 273



cis 292

Figure 6. 300 mHz NMR of disulfides **273, 292** in $CDCl_3$

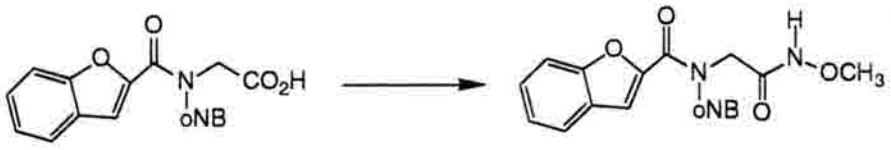
303

N-[benzofuranyl-2-carbonyl-(3H)]-N-(2-nitrobenzyl)-glycine (303)

Coumarilic acid (2.0 g, 12.3 mmol, 1.0 eq) and thionyl chloride (4.0 mL, 49.2 mmol, 4.0 eq)were dissolved in 200 mL benzene and refluxed vigorously for 6 hrs. The solution was cooled, concentrated to a white solid. The solid was taken up in 20 mL of methylene chloride and added to a methylene chloride solution containing N-(2-nitrobenzyl) glycine ethyl ester (3.0 g, 13.53 mmol, 1.1 eq). 10 mL of water containing sodium bicarbonate (1.14 g, 13.53 mmol, 1.1 eq) was added and the resulting solution vigorously stirred for 30 minutes. The layers were separated, and the organic layer collected, dried over magnesium sulfate, and after filtration concentrated to a yellow film. The film was taken up in ethanol and lithium hydroxide was added. The solution was stirred at room temperature overnight. The solid which forms during the hydrolysis was collected dissolved in water and acidified. The aqueous solution was extracted with methylene chloride, dried over magnesium sulfate, and after concentration obtained a yellow solid. The product was recrystalized from boiling ethyl acetate/hexanes. Obtained 3.67 g of light yellow crystals (mp 168- 169 ^{o}C). 88% yield.

^{1}H NMR (DMSO-D_6, 300mHz, T= 360 K) ∂=8.05 (1H, dd, J= 0.9, 8.1 Hz); 7.75-7.65 (3H, m); 7.58- 7.39 (4H, m); 7.30 (1H, ddd, J= 1.1, 7.3, 7.3 Hz); 5.16 (2H, s); 4.35 (2H, s).

IR (KBr) υ= 3045, 1740, 1602, 1556, 1520, 1192 cm^{-1}.



303 **304**

N-[2-(methoxyamino)-2-oxoethyl]-N-(2-nitrobenzyl))-2-benzofurancarboxamide (304)

303 (2.5 g, 7.38 mmol, 1.0 eq.) was dissolved in 200 mL tetrahydrofuran and cooled to 0°C. Triethyl amine (1.23 mL, 8.86 mmol, 1.2 eq) was added followed by pivaloyl chloride (1.09 mL, 8.86 mmol, 1.2 eq). The resulting solution turns yellow and a precipitate forms. The solution was stirred at 0°C for 30 minutes before methoxyl amine (2.5 mL) was added causing the yellow color to fade. The reaction was stirred and additional 15 minutes before diluting with methylene chloride, extraction with saturated sodium bicarbonate, water, and dried over sodium sulfate. After filtration the organic solution was concentrated to give an oil which when taken up in ethyl acetate begins to crystalize. Obtained 2.3 g of product (mp 123- 125 °C) from boiling ethyl acetate/hexanes. 81% yield.

^{1}H NMR (DMSO-D_6, 300mHz, T= 300 K) ∂=10.88 (1H, bs); 8.05 (1H, d, J= 8.0 Hz); 7.75- 7.63 (3H, m); 7.58- 7.40 (4H, m); 7.30 (1H, dd, J= 7.7, 7.5 Hz); 5.13 (2H, s); 4.27 (2H, s); 3.59 (3H, s).

IR: (KBr pellet) υ= 3212, 2948, 1679, 1634, 1526 cm^{-1}.

Anal. Calcd. for $C_{19}H_{17}N_3O_6$: C 59.52, H 4.47, N 10.96; Found C 59.34, H 4.60, N 10.75.

304 **305**

Trans-3-bromo-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (305)

304 (2.0 g, 5.22 mmol, 1.0 eq.) was dissolved in 200 mL of ethanol-free chloroform. N-bromosuccinamide (1.14 g, 6.26 mmol, 1.2 eq.) was added in one portion and the solution stirred at room temperature for 24 hrs. The solution was extracted with saturated sodium bicarbonate, and dried over sodium sulfate. Solution was concentrated to give a yellow foam. The residue was taken up in ethyl acetate and purified using flash chromatography with 1:1 ethyl acetate/hexane as the eluent. The white solid (mp 153- 154 ^{o}C) was recrystalized using boiling ethyl acetate/hexanes. Obtained 1.4 g of product. 58% yield.

^{1}H NMR: (300mHz, $CDCl_3$) δ= 8.05 (1H, d, J= 8.0Hz); 7.65- 7.63 (2H, m); 7.53- 7.47 (1H, m); 7.33- 7.29 (2H, m); 7.08- 7.03 (1H, m); 6.97 (1H, d, J= 8.1Hz); 6.05 (1H, s); 5.08 (1H, 1/2ABq, J= 15.8Hz); 5.0 (1H, 1/2ABq, J= 15.8Hz), 4.23 (1H, 1/2ABq, J= 18.3Hz); 4.19 (1H, 1/2ABq, J= 18.3Hz); 3.95 (3H, s).

IR: (KBr) 2943, 1699, 1519, 750 cm^{-1}.

Anal. Calcd. for $C_{19}H_{16}N_3O_6Br$: C 49.37, H 3.49, N 9.09, Br 17.29; Found C 49.56, H 3.74, H 9.16, Br 17.03.

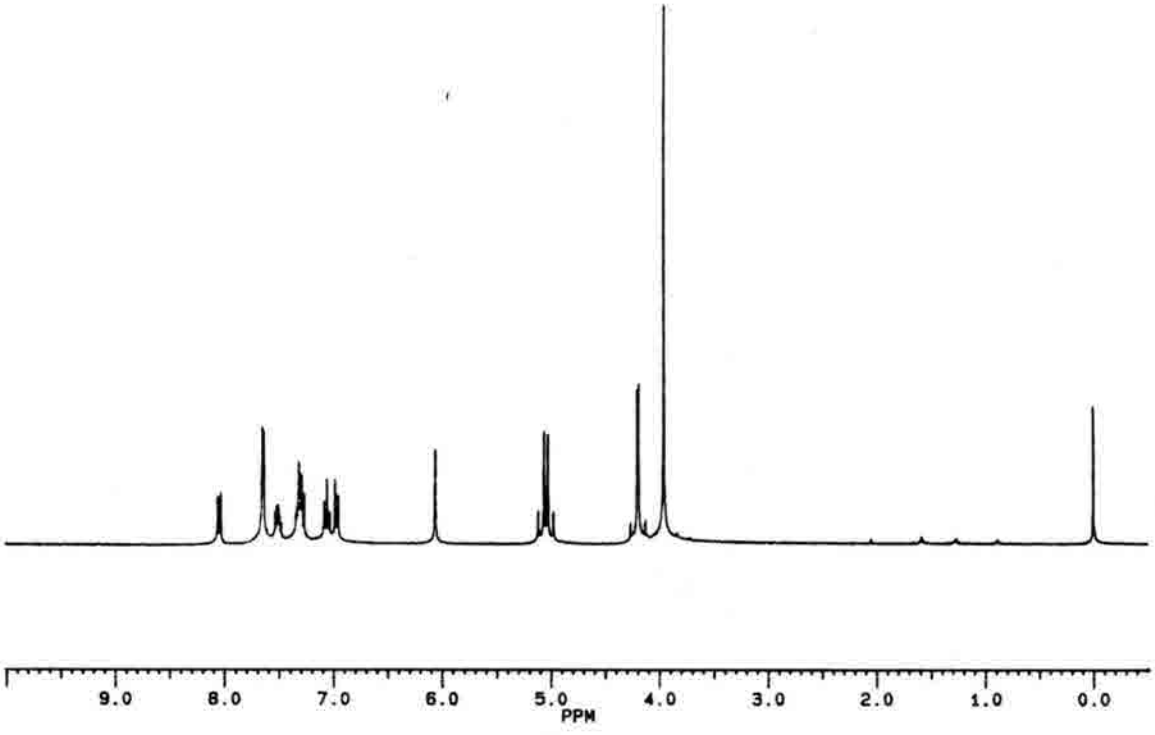
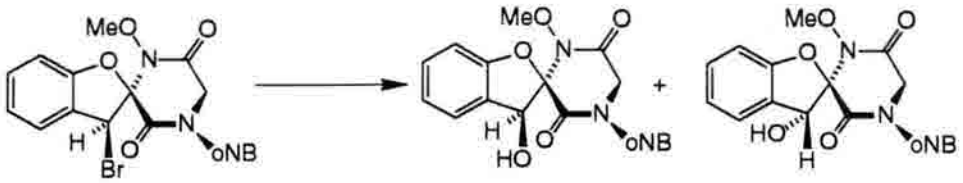


Figure 7. 300 mHz NMR of bromide **305** in $CDCl_3$



305 **306a** **306b**

Cis,Trans-3-hydroxy-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (306a,b)

Bromide **305** (888 mg, 1.92 mmol, 1.0 eq.) was dissolved in 50 mL of tetrahydrofuran followed by the addition of 30 mL water. Silver triflate (592 mg, 2.3 mmol, 1.1 eq) was added and the resulting solution was stirred for 30 minutes. The solution was filtered through a plug of celite, taken up in methylene chloride, extracted with water and dried over sodium sulfate. The solution was concentrated to a yellow film/oil which eventually solidifies. The residue was taken up in methylene chloride, adsorbed onto a small amount of silica, and chromatographed using 1:1 ethyl acetate/hexane as the eluent. Obtained 365 mg of one isomer (II-500-1) and 235 mg of the other (II-500-2). Both isomers were recyrstalized from ethyl acetate/hexanes. 78% combined yield.

306a Trans (white needles from ethyl acetate/hexanes, mp 193- 194 °C)

^{1}H NMR: (300mHz, $CDCl_3$) δ= 8.10 (1H, dd, J= 8.17Hz, J= 1.25Hz); 7.70-7.65 (1H, m); 7.54-7.28 (4H, m); 7.08-7.03 (1H, m); 6.93 (1H, d, J= 8.17Hz); 5.87 (1H, d, J= 11.97Hz); 5.19 (1H, 1/2ABq, J= 16.5Hz); 4.89 (1H, 1/2ABq, J= 16.5Hz); 4.21 (1H, 1/2ABq, J= 18.1Hz); 4.11 (1H, 1/2ABq, J= 18.1Hz); 3.94 (3H, s); 3.27 (1H, d, J=12.25Hz).

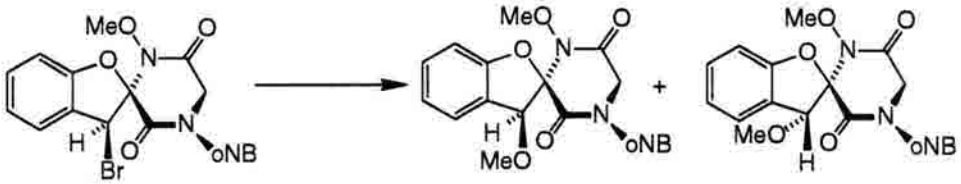
IR: (NaCl, film from $CDCl_3$) υ= 3399, 1684, 1526 cm^{-1}.

Anal Calcd. for $C_{19}H_{17}N_3O_7$: C 57.14, H 4.29, N 10.52; Found C 56.99, H 4.36, N 10.69.

306b CIS (fine white needles from ethyl acetate/hexanes, mp 143- 145 °C)

^{1}H NMR: (300mHz, $CDCl_3$) δ= 8.09 (1H, d, J= 8.13Hz); 7.71-7.66 (1H, m); 7.54-7.43 (2H, m); 7.37-7.27 (2H, m); 7.07-7.02 (1H, m); 6.98 (1H, d, J= 8.13Hz); 5.64 (1H, s); 5.29 (1H, s).

IR: (NaCl, film) υ= 3389, 2942, 1688, 1527 cm^{-1}.



305 **307a** **307b**

Cis,Trans-3-methoxy-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (307a,b)

Bromide **305** (2.0 g, 4.33 mmol, 1.0 eq) was suspended in 50 mL methanol to which 15 mL of tetrahydrofuran was added. Silver triflate (1.1 g, 6.5 mmol, 1.5 eq) was added causing the solution to instantly turn cloudy. The solution was stirred at room temperature for 30 minutes before addition of a saturated sodium chloride solution and the reaction mixture stirred an additional 15 minutes. The reaction mixture was filtered through a plug of celite, and thoroughly washed with ethyl acetate. The ethyl acetate was washed with water, dried over magnesium sulfate, filtered, and concentrated to a yellow-white solid. The reaction routinely gave a 3:1 (Trans:Cis) ratio of diastereomers by NMR. The diastereomers were separated by flash column chromatography using 2:1 hexane/ethyl acetate or 2% acetone in chloroform. Obtained 1.4 g of products (combined yield 80%).

307a TRANS (white solid from ethyl acetate/hexanes, mp 196- 197 °C).

^{1}H NMR: (300mHz, $CDCl_3$) δ= 8.05 (1H, d, J= 7.8 Hz); 7.62- 7.60 (2H, m); 7.5-7.44 (1H, m); 7.40- 7.33 (2H, m); 7.04- 6.99 (2H, m); 5.54 (1H, s); 5.27 (1H, 1/2ABq, J= 16.3 Hz); 4.81 (1H, 1/2ABq, J= 16.3 Hz); 4.12 (2H, s); 3.93 (3H, s); 3.61 (3H, s).

IR (NaCl, film) υ= 2941, 1696, 1526, 1487, 1343 cm^{-1}.

Anal. Calcd. for $C_{20}H_{19}N_3O_7$: C 58.12, H 4.63, N 10.17; Found C 58.49, H 4.57, N 10.23.

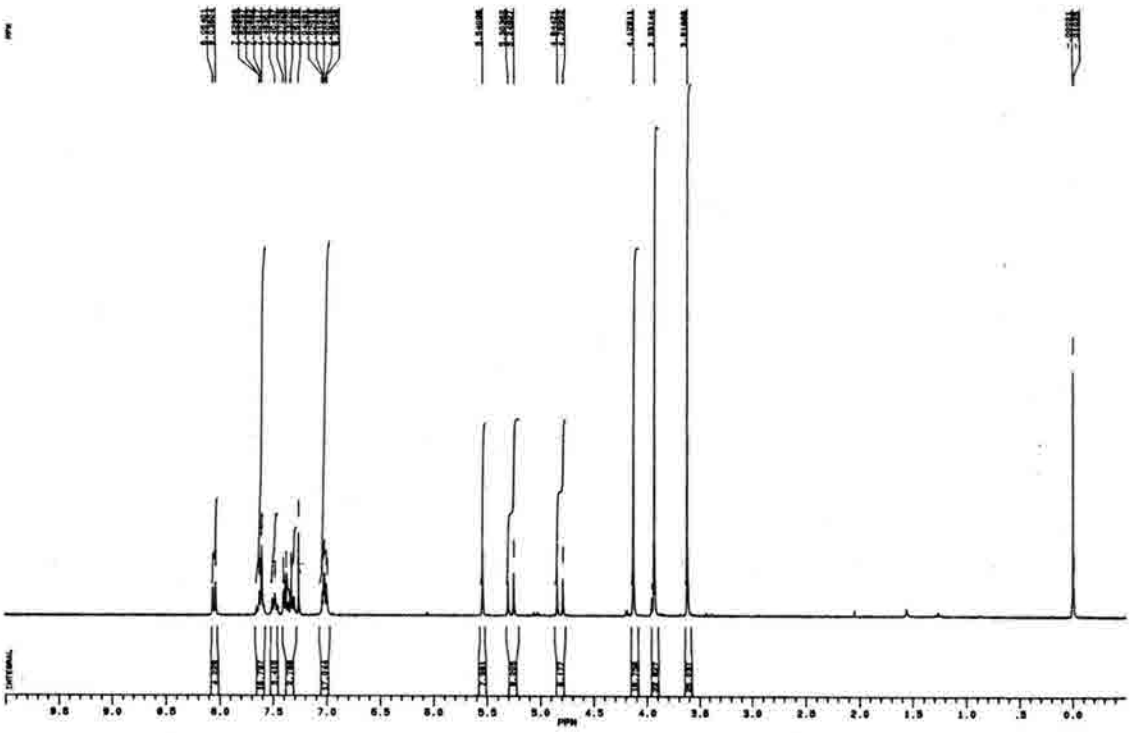
307b CIS (white solid from ethyl acetate/hexanes, mp 170- 171 °C).

^{1}H NMR: (300mHz, $CDCl_3$) δ= 8.09 (1H, d, J= 8.1 Hz); 7.69- 7.65 (1H, m); 7.56-7.52 (1H, m); 7.45 (1H, d, J= 7.7 Hz); 7.35 (1H, d, J= 7.5 Hz); 7.27- 7.25 (1H, m);

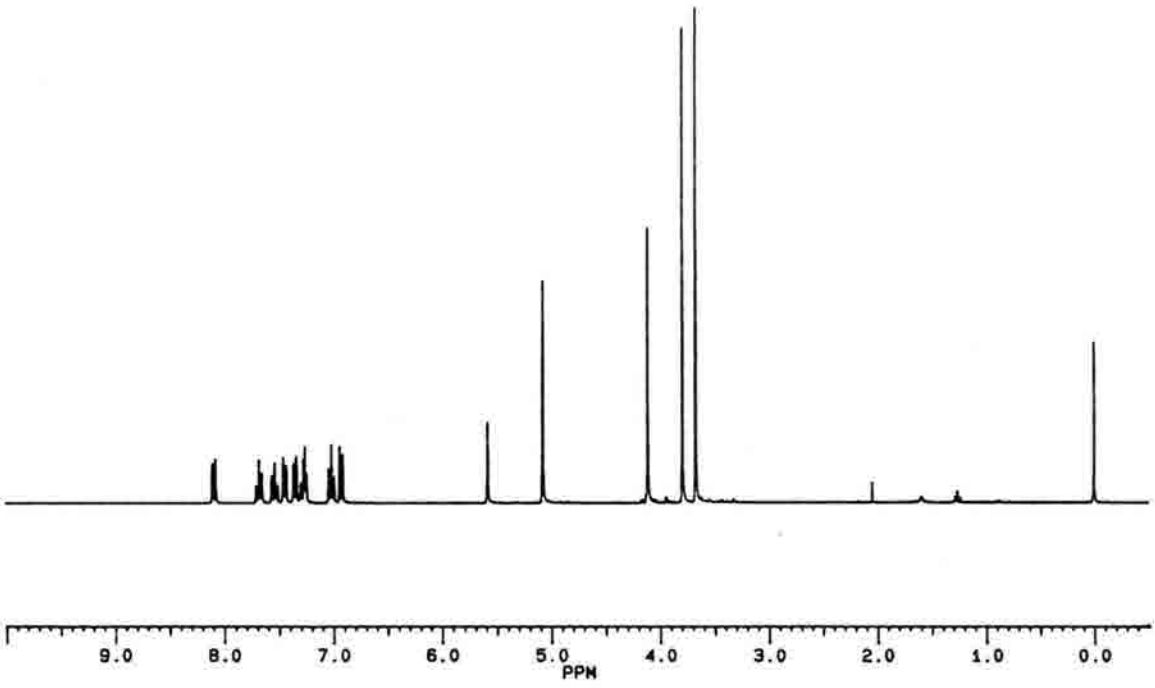
7.02 (1H, dd, J= 7.5, 7.4 Hz); 6.93 (1H, d, J= 8 Hz); 5.58 (1H, s); 5.07 (2H, s); 4.11 (2H, s); 3.79 (3H, s); 3.67 (3H, s).

IR (NaCl, film) υ= 2940, 1684, 1526, 1478, 1343 cm^{-1}.

Anal. Calcd. for $C_{20}H_{19}N_3O_7$: C 58.12, H 4.63, N 10.17; Found C 57.93, H 4.49, N 9.94.

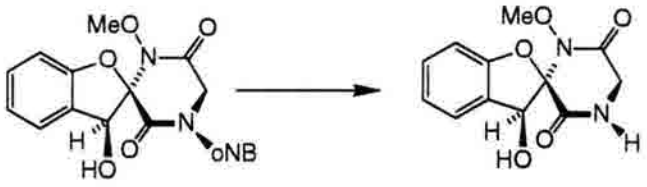


trans methyl ether **307a**



cis methyl ether **307b**

Figure 8. 300 mHz NMR of methyl ethers **307a,b** in $CDCl_3$



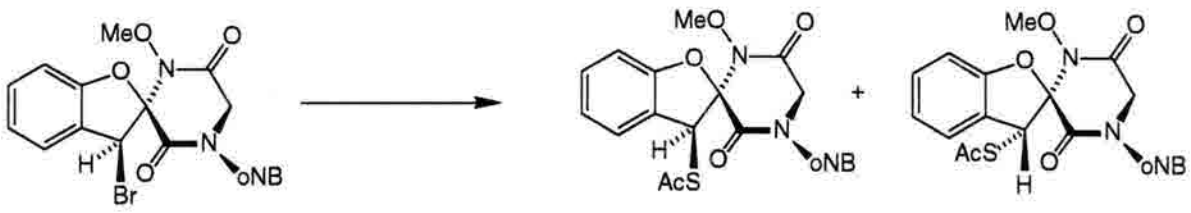
306a **310a**

Trans-3-hydroxy-1'-methoxy-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione GFM-II-501

Alcohol **306a** (20 mg, 0.05 mmol, 1.0 eq) was dissolved in 5 mL of 10% water in tetrahydrofuran in a quartz tube. The solution was irradiated with a Hanova 450-watt mercury vapor lamp for 5 hrs. The yellow solution was concentrated to a brown oil which was taken up in chloroform, extracted with saturated sodium chloride, and dried over sodium sulfate. After filtration the solution was concentrated to a film, which was purified using PTLC using 4:1 ethyl acetate/hexanes. Obtained 8.5 mg of an off-white colored solid which was recrystallized from ethyl acetate/hexanes, mp 203- 204°C. 65% yield.

^{1}H NMR: (300mHz, $CDCl_3$) δ= 7.38-7.27 (2H, m); 7.06-7.02 (1H, m); 6.90 (1H, d, J= 8.12Hz); 6.50 (1H, bs); 5.80 (1H, s); 4.16 (2H, s); 3.91 (3H, s); 3.54 (1H, bs).

IR: (KBr) υ= 3486, 3211, 2947, 1686, 1249, 1031, 745 cm^{-1}.



305 **308a** **308b**

Cis,Trans-3-(S-acetyl)-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (308a,b)

Bromide **305** (600 mg, 1.2 mmol, 1.0 eq) was dissolved in 15 mL benzene followed by the addition of thiolacetic acid (5.0 eq) and an excess of freshly fused zinc chloride. The solution was heated to 50 °C and stirred for 12 hrs. The reaction was cooled, diluted with ethyl acetate, extracted several times with water, once with saturated sodium bicarbonate, and dried over sodium sulfate. The vile smelling yellow film was purified by flash column chromatography using 2:1 hexanes/ethyl acetate to give a mixture or separately using 2% acetone in chloroform. Obtained 443 mg of a mixture of diastereomers (1.5:1.0 trans:cis) as a white solid in 74% combined yield.

308a TRANS (white needles from ethanol at 0°C, mp 138- 139 °C)

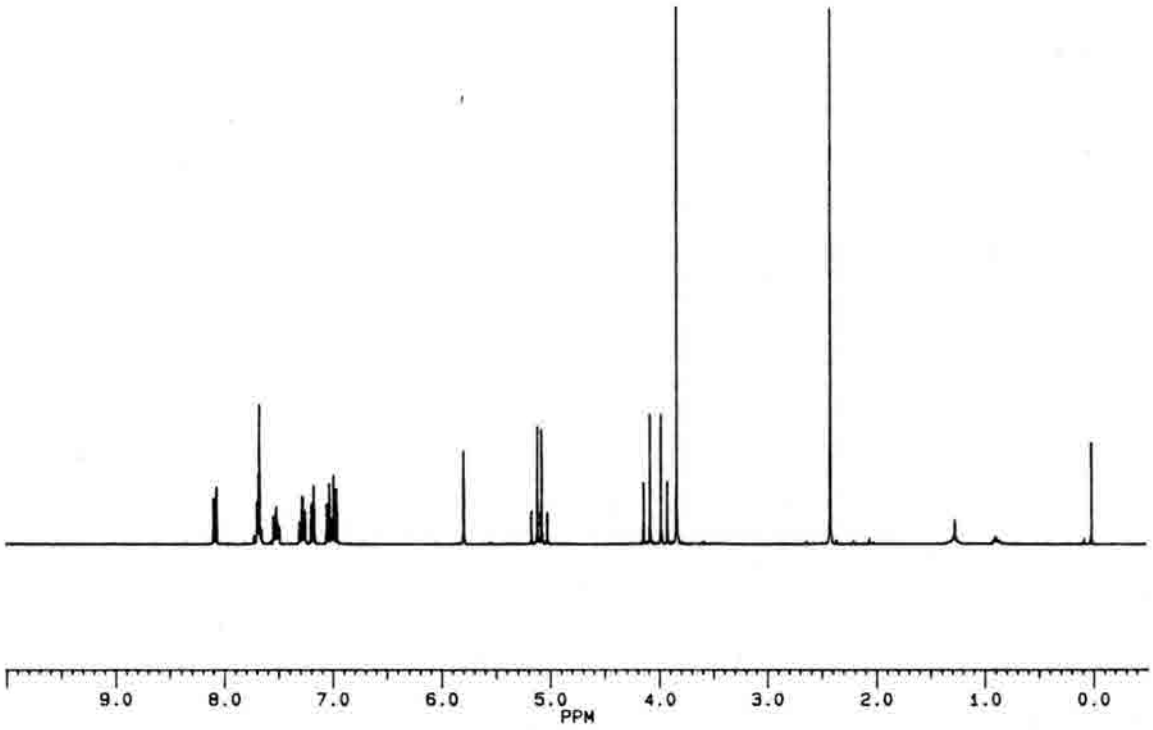
^{1}H NMR (300 mHz, $CDCl_3$) δ=8.09 (1H, d, J= 8.6 Hz);7.69- 7.66 (2H, m); 7.53- 7.48 (1H, m); 7.29- 7.16 (2H, m); 7.04- 6.95 (2H, m); 5.78 (1H, s); 5.12 (1H, 1/2ABq, J= 16.2 Hz); 5.05 (1H, 1/2ABq, J= 16.2 Hz); 4.09 (1H, 1/2ABq, J= 17.9 Hz); 3.94 (1H, 1/2ABq, J= 17.9 Hz); 3.82 (3H, s); 2.41 (3H, s).

IR (NaCl film from chloroform) υ= 2941, 1690, 1526, 1478, 1344, 1239, 728 cm^{-1}.

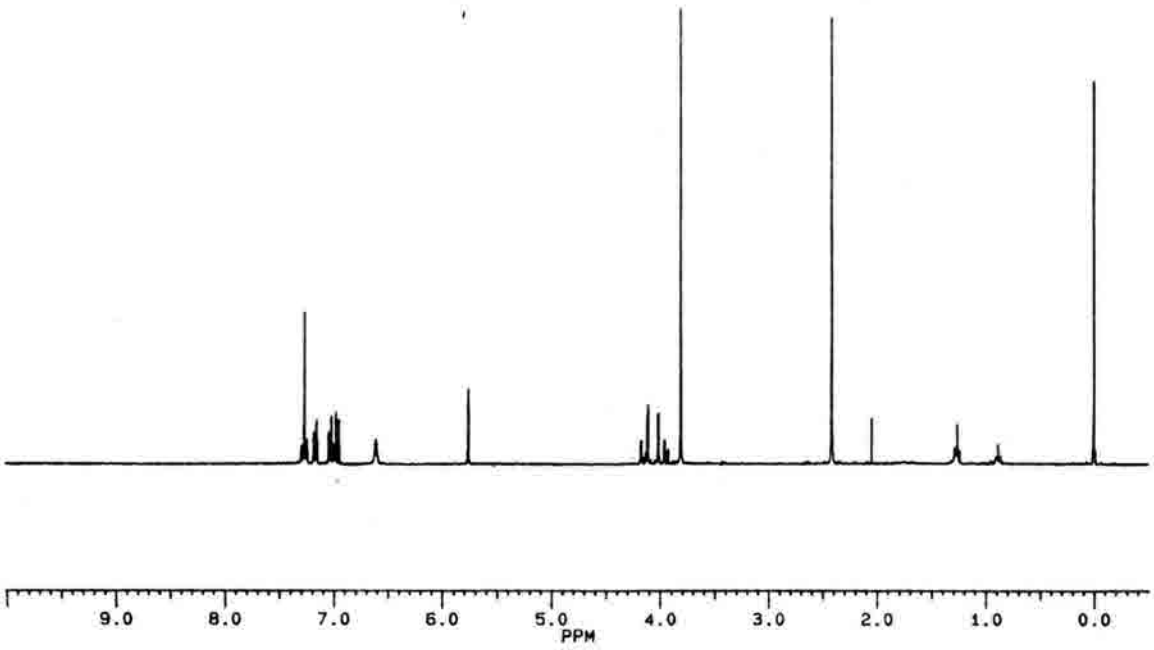
308b CIS (white solid from ethanol, 64- 65°C)

^{1}H NMR (300 mHz, $CDCl_3$) δ=8.05 (1H, dd, J= 1.1, 8.0 Hz); 7.65- 7.62 (1H, m); 7.55- 7.48 (2H, m); 7.24- 7.13 (2H, m); 7.01- 6.91 (2H, m); 5.85 (1H, s); 5.03 (1H, 1/2ABq, J= 15.8 Hz); 4.91 (1H, 1/2ABq, J= 15.8 Hz); 4.09 (1H, 1/2ABq, J= 18.0 Hz); 4.01 (1H, 1/2ABq, J= 18.0 Hz); 3.95 (3H, s); 2.34 (3H, s).

IR (NaCl film from chloroform) υ= 2925, 1696, 1526, 1478, 1346, 1241, 727 cm^{-1}.



Trans 308a



cis 308b

Figure 9. 300 mHz NMR of thioacetates **308a,b** in $CDCl_3$

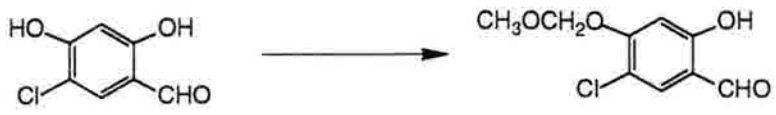
323

5-Chlororesorcylaldehyde (323)

Prepared following the procedure of Chakravarti and Ghosh. In a flame-dried 1-liter, 3-necked flask equipped with a condensor and a mercury seal stirrer under an inert atmosphere was added 4-chlororesorcinol (25 g, 0.17 mol, 1 eq.) and anhydrous zinc cyanide (40 g, 0.34 mol, 2.0 eq). Anhydrous diethyl ether (200 mL) was added and the reaction cooled to 0°C. Dry HCl gas was passed through the rapidly stirred solution for 2 hrs until a solid mass formed. The ether was decanted and 250-300 mL of water was added. The reaction heated to reflux and any residual ether distilled off. The reaction was refluxed until the solid mass dissolved entirely. Upon cooling the crude chlororesorcylaldehyde separates as a red solid and was collected. Recystallization from water gave the desire product in sufficient purity to carry on. Repeated recrystallizations gave chlororesorcylaldehyde as yellow needles in 40% yield.

^{1}H NMR (300 MHz, DMSO-d6) δ= 11.39 (1H, bs, exchangeable), 10.89 (1H, bs, exchangeable), 9.98 (1H, s), 7.60 (1H, s), 6.58 (1H, s).

IR (KBr) υ= 3378, 1630, 1495, 1266, 723 cm^{-1}.



323 **329**

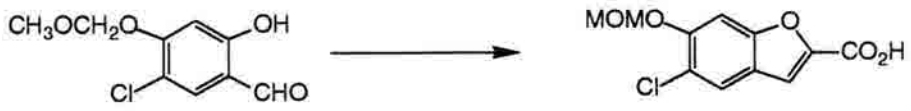
2-hydroxy-4-(methoxymethyl)-5-chlorobenzaldehyde (329)

A solution containing 20 grams (116 mmol, 1.0 eq) of chlororesorcylaldehyde **323** and triethylamine (16.2 mL, 116 mmol, 1.0 eq) in 750 mL tetrahydrofuran was cooled to 0°C. Chloromethyl methyl ether (13.2 mL, 173.8 mmol, 1.5 eq) was added subsurface and the solution warmed to room temperature and stirred for 3 hours. The solution was filtered through a celite plug diluted with diethyl ether, extracted 3 times with 0.2 M sodium hydroxide. The etheral layer (containing dialkylated material) was discarded while the aqueous layer was carefully acidified to pH 4-5 with cold, 0.1 M sulfuric acid and extracted with ethyl acetate. The ethyl acetate was washed with water, dried over magnesium sulfate, filtered, and concentrated to an orange solid which was taken up in a 3:1 hexane:ethyl acetate solution and filtered through a plug of 25% alumina in silica gel . The colorless filtrate was concentrated and the product recrystallized from hexane. Obtained 12.5 g of a white crystalline solid. 49% yield. mp 68-70 °C.

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 11.29 (1H, s, exchangeable in D_2O); 9.70 (1H, s); 7.35 (1H, s); 6.76 (1H, s); 5.31 (2H, s); 3.52 (3H, s).

IR (NaCl film) υ= 2962, 2839, 1647, 1487 cm^{-1}.

Anal. Calcd. for $C_9H_9O_4Cl$: C 49.90, H 4.19, Cl 16.36, Found C 50.15, H 4.29, Cl 16.42.



329 **333**

5-chloro-6-(methoxymethyl)-Coumarilic Acid (333)

A slurry consisting of 4 grams (18.46 mmol, 1.0 eq) **329**, diethyl bromomalonate (3.5 mL, 20.31 mmol, 1.1 eq) and potassium carbonate (5.1 g, 36.9 mmol, 2.0 eq) were heated to reflux in 15 mL of acetone. After 5 hours the solution was concentrated to a thick yellow oil taken up in water, acidified to pH 5 with cold, 0.1 M sulfuric acid, and extracted with ethyl acetate. The ethyl acetate was washed with water, concentrated and the residue taken up in 20 mL of ethanol and an alcoholic solution of potassium hydroxide (3.0 eq) was added and refluxed for 2 hours. The solution was cooled, concentrated, and the residue taken up in dilute sodium hydroxide, extracted with diethyl ether which was discarded. The aqueous layer was acidified to pH 3-4 with 0.1M sulfuric acid and the precipitate which gradually forms collected. The carboxylic acid was further purified by recrystallization from ethyl acetate/hexanes. Obtained 3.4 grams of a fine white solid, mp 193- 194 °C. 72% yield.

^{1}H NMR: (300 mHz, DMSO-d_6) δ= 13.55 (1H, bs, exchangeable in D_2O); 7.89 (1H, s); 7.59 (1H, s); 7.56 (1H, s); 5.39 (2H, s); 3.44 (3H, s).

IR (KBr) υ= 3415, 2907, 2554, 1684, 1571 cm^{-1}.

Anal. Calcd. for $C_{11}H_9O_5Cl$: C 51.48, H 3.53, Cl 13.81, Found C 51.38, H 3.54, Cl 13.64.

302

N-(2-nitrobenzyl) glycine ethyl ester (302).

Glycine ethyl ester hydrochloride (52 g, 370.3 mmol, 4.0 eq.) was dissolved in 1.5 liters of 95% ethanol. Sodium bicarbonate (38.8 g, 462.8 mmol, 5.0 eq) was added and after 5 minutes 2-nitrobenzyl bromide (20 g, 92.5 mmol, 1.0 eq) was added and the solution refluxed for 20 hrs. The solution was cooled, filtered and concentrated to a viscous yellow oil which was taken up in ethyl acetate, washed with water, dried over magnesium sulfate, filtered, and concentrated to a thick yellow/brown oil. 14.5 g of product (yellow oil) was obtained from flash column chromatography using 3:2 hexane/ethyl acetate as the eluent along with 2 g of dialkylated side product. 65% yield.

^{1}H NMR: (300 mHz), $CDCl_3$) δ= 7.97 (1H, d, J= 8.0 Hz); 7.66-7.57 (2H, m); 7.45-7.40 (1H, m); 4.18 (2H, qt, J = 7.1 Hz); 4.10 (2H, s); 3.44 (2H, s); 1.27 (3H, t, J= 7.1 Hz).

IR: (NaCl, neat film) υ= 3354, 1736, 1526 cm^{-1}.

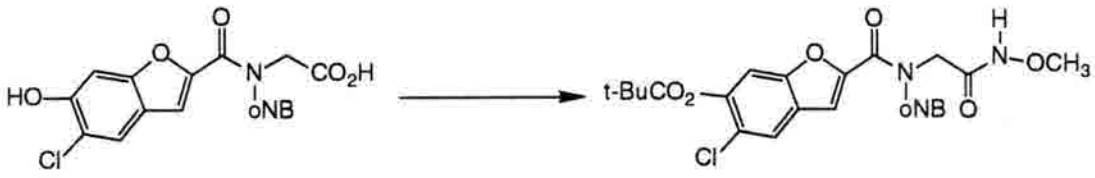
333 **334**

N-[5-chloro-6-hydroxy-benzofuranyl]-N-(2-nitrobenzyl)-glycine (334)

5-chloro-6-(methoxymethyl)-coumarilic Acid **333** (9 g, 35.1 mmol, 1.0 eq) was suspended in 500 mL methylene chloride under N_2. Tetrahydrofuran was added to the solution until all the starting material was completely dissolved. 1 mL of DMF was added followed by 3.5 mL oxalyl chloride (38.57 mmol, 1.1 eq). After approximately 5 minutes the solution turned cloudy and continued to stirr at room temperature for 1 hour. The acid chloride solution was concentrated to approximately 1/2 the original volume and was added to a vigorously stirred solution containing N-(2-nitrobenzyl) glycine ethyl ester **302** (8.35 g, 35.1 mmol, 1.0 eq) and sodium bicarbonate (4.4 g, 52.6 mmol, 1.5 eq) in 100 mL of water. The resulting solution was vigorously stirred for 45 minutes. The layers were separated and the organic layer washed with 0.1 M potassium carbonate, acidified with 10% HCl, washed with water, dried over magnesium sulfate, filtered, and concentrated to a thick yellow oil which solidifies on standing. Obtained 16.5 grams of crude ester (>95% by TLC). The crude ester was taken up in 500 mL of dioxane, 55 mL of 2M HCl was added and the solution refluxed for 36 hours. The solution was cooled, made basic with 0.2 M sodium hydroxide, and washed with diethyl ether. The aqueous solution reacidified with aqueous HCl. The crude acid was extracted into ethyl acetate, washed with water, dried over magnesium sulfate, filtered and concentrated to a light yellow solid. Obtained 12.9 grams of crude acid which was carried on without further purification.

^{1}H NMR (300 mHz, DMSO-d_6, T= 357 K) δ= 12.5 (1H, bs, exchangeable in D_2O); 10.5 (1H, bs, exchangeable in D_2O); 8.04 (1H, d, J= 8.1 Hz); 7.74- 7.54 (3H, m); 7.27 (1H, s); 7.01 (1H, s); 5.11 (2H, s); 4.31 (2H, s).

IR (KBr) υ= 3413, 3141, 1733, 1608, 1556, 1525 cm^{-1}.



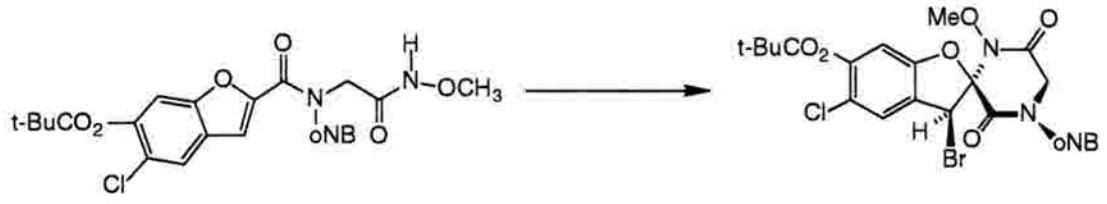
334 335

N-[2-(methoxyamino)-2-oxoethyl]-N-(2-nitrobenzyl)-2-[(5-chloro-6-(O-t-butyl carbonyl)]-benzofurancarboxamide (335)

To a tetrahydrofuran solution containing **334** (4.1 g, 10.89 mmol, 1.0 eq) and triethyl amine (5.3 mL, 38.13 mmol, 3.5 eq) was added pivaloyl chloride (4.7 mL, 38.13 mmol, 3.5 eq). The bright yellow solution was stirred at room temperature for 30-45 minutes before methoxylamine (2.3 mL, 43.5 mmol, 4.0 eq) was added and the solution stirred for an additional 30 minutes. The solution was diluted with ethyl acetate and extracted with saturated sodium bicarbonate followed by water. The organic layer was dried over magnesium sulfate. The thick orange oil/solid was chromatographed using 4:1 chloroform-acetonitrile. Obtained 3.91 g of a white solid from ethyl acetate/petroleum ether, mp 121- 122°C. 71% yield.

^{1}H NMR: (300mHz, DMSO-d_6, T= 360 K) δ=10.91 (1H, bs); 8.05 (1H, d, J= 8.2 Hz); 7.91 (1H, s); 7.75- 7.54 (4H, m); 7.41 (1H, s); 5.12 (2H, s); 4.24 (2H, s); 3.58 (3H, s); 1.37 (9H, s).

IR: (NaCl film) υ= 3210, 2977, 1759, 1675, 1636, 1526, 1459, 1340, 1140, 1098, 729 cm^{-1}.



335 336

Trans-3-bromo-5-chloro-6-(O-t-butyl carbonyl)-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (336)

To a solution of **335** (200 mg, 0.395 mmol, 1.0 eq) in 10 mL of ethanol-free chloroform was added N-bromosuccinamide (85 mg, 0.474 mmol, 1.2 eq) in one portion. The solution was stirred at room temperature for 14 hrs. The reaction was diluted with chloroform and extracted with saturated sodium bicarbonate and dried over magnesium sulfate. The residue was purified by flash column chromatography using 2:1 ethyl acetate-hexane. The oily product from the column upon standing in a small volume of ether solidifies. Obtained 96 mg of an off-white solid. Recrystallized from ethyl acetate/hexanes (mp, decomposes 183- 185°C) 41% yield.

^{1}H NMR: (300mHz, $CDCl_3$) δ=8.03 (1H, d, J= 8.3 Hz); 7.67- 7.60 (2H, m); 7.54-7.48 (1H, m); 7.34 (1H, s); 6.77 (1H, s); 5.05 (1H, 1/2ABq, J= 15.8 Hz); 5.01 (1H, 1/2ABq, J= 15.8 Hz); 4.18 (2H, s); 3.95 (3H, s); 1.38 (9H, s).

IR: (NaCl film) υ= 2977, 1759, 1698, 1527, 1476, 1145, 1098 cm^{-1}.

Anal. Calcd. for $C_{24}H_{23}N_3O_8ClBr$: C 48.30, H 3.88, N 7.04, total halogen 11.88; Found C 48.55, H 4.00, N 7.05, total halogen 11.86.

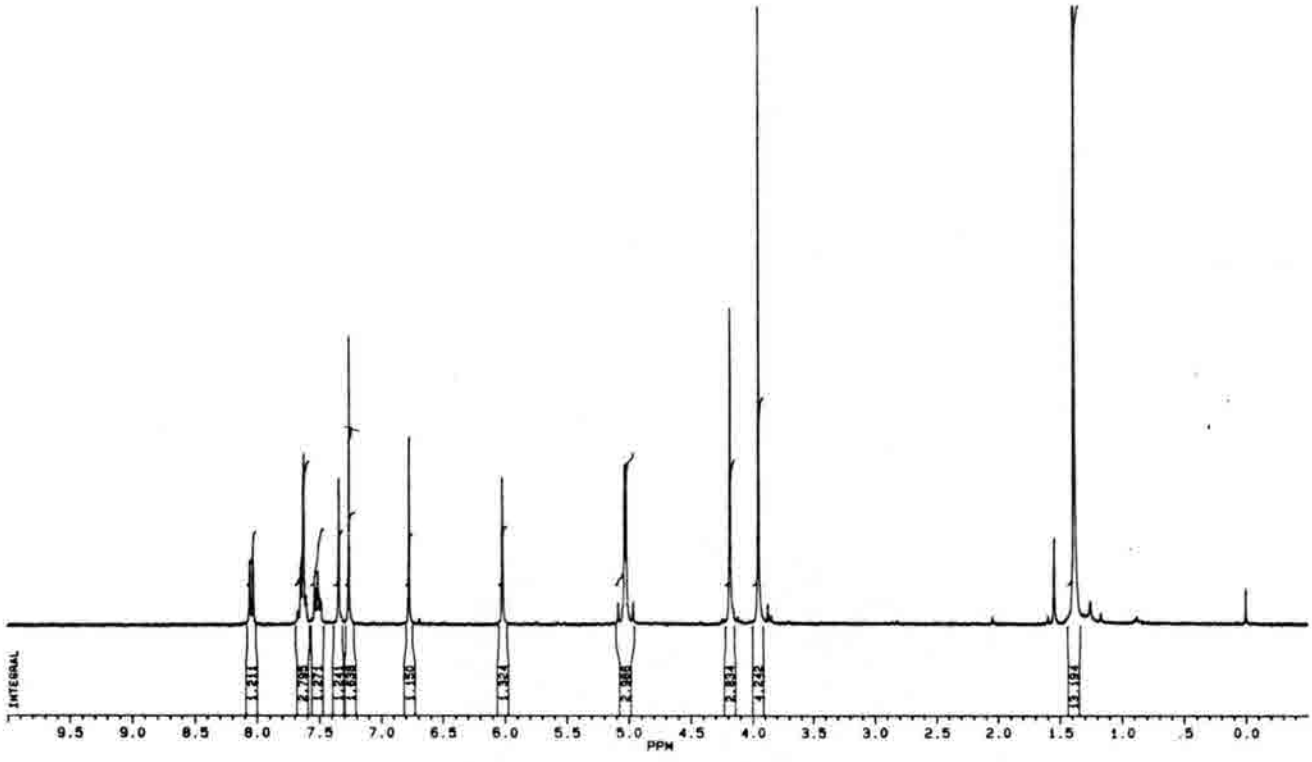
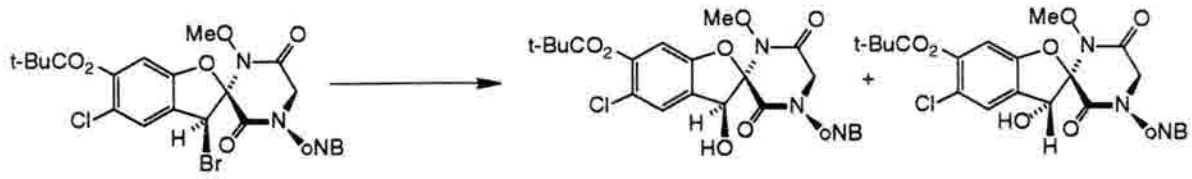


Figure 10. 300 mHz NMR of bromide **336** in $CDCl_3$



336 **337a** **337b**

Cis,Trans-3-hydroxy-5-chloro-6-(O-t-butylcarbonyl)-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (337a,b)

The bromide **336** (200 mg, 0.335 mmol, 1.0 eq) was dissolved in 10 mL of tetrahydrofuran and water was added until solution turns cloudy. The solution was vigorously stirred and heated to reflux. Silver triflate (172 mg, 0.670 mmol, 2.0 eq.) was added in one portion and the solution stirred at reflux for 30 minutes. The solution was cooled and diluted with ethyl acetate. A saturated solution of sodium chloride was added to precipitate any remaing silver salt. The mixture was filtered through celite, extracted with water and dried over magnesium sulfate. After filtration and concentration the residue was chromatographed with 1:1 ethyl acetate-hexanes as the eluent. Obtained 113 mg (combined yield) of products. 74% Yield.

337a TRANS

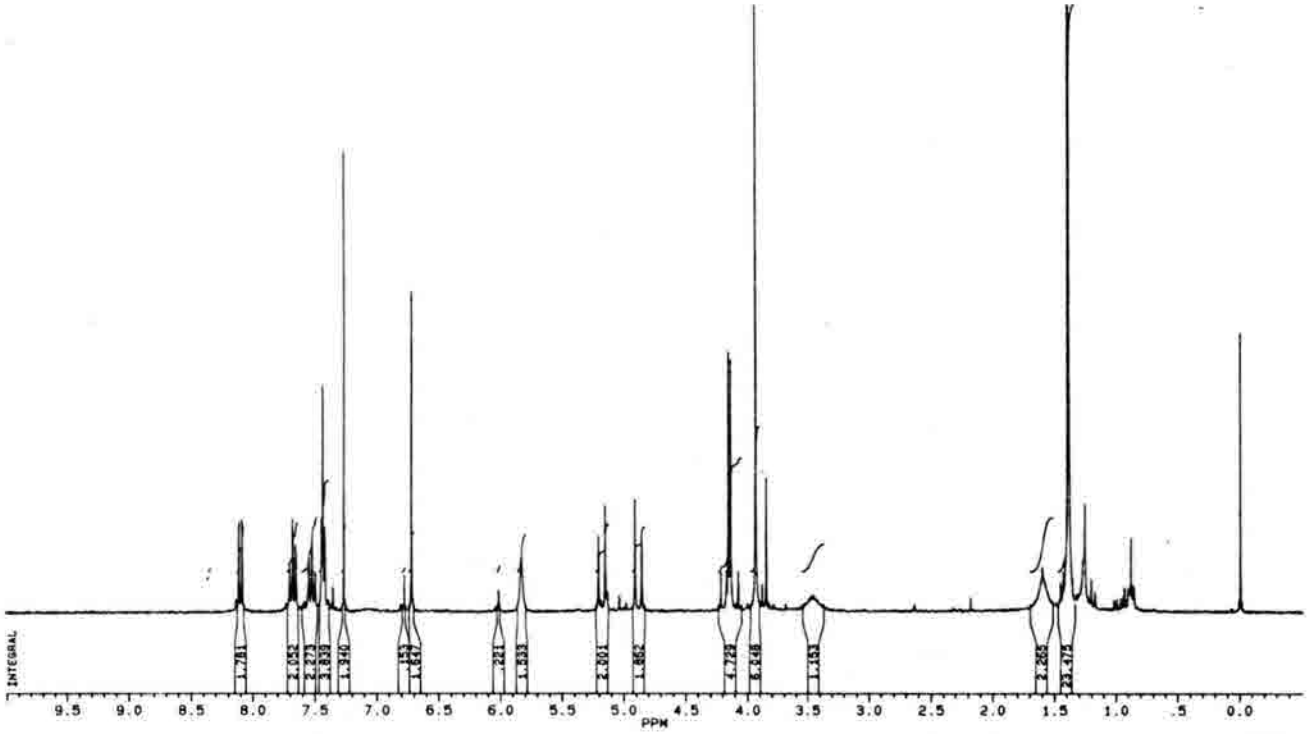
^{1}H NMR: (300 mHz, $CDCl_3$) δ= 8.09 (1H, dd, J_{ortho}= 8.17 Hz, J_{para}= 1.2 Hz); 7.7-7.64 (1H, m); 7.54- 7.52 (1H, m); 7.49- 7.41 (2H, m); 6.71 (1H, s); 5.82 (1H, bs); 5.17 (1H, 1/2ABq, J= 16.4 Hz); 4.88 (1H, 1/2ABq, J= 16.4 Hz); 4.15 (1H, 1/2ABq, J= 18.2 Hz); 4.14 (1H, 1/2ABq, J= 18.2 Hz); 3.93 (3H, s); 3.49 (1H, bs, exchangeable); 1.38 (9H, s).

IR (NaCl, film) υ= 3400, 2976, 1759, 1688, 1528, 1476, 1143, 1101 cm^{-1}.

337b CIS

^{1}H NMR: (300 mHz, $CDCl_3$) δ=8.09 (1H, dd, J_{ortho}= 8.17 Hz, J_{para}= 1.3 Hz); 7.71-7.66 (1H, m); 7.56- 7.51 (1H, m); 7.44- 7.42 (1H, m); 7.42 (1H, s); 6.78 (1H, s); 5.63 (1H, s); 5.05 (1H, s); 4.16 (2H, s); 3.93 (3H, s); 3.0 (1H, bs, exchangeable); 1.39 (9H, s).

IR (NaCl, film) υ= 3391, 2975, 1759, 1690, 1528, 1141, 1098 cm^{-1}.



trans 337a

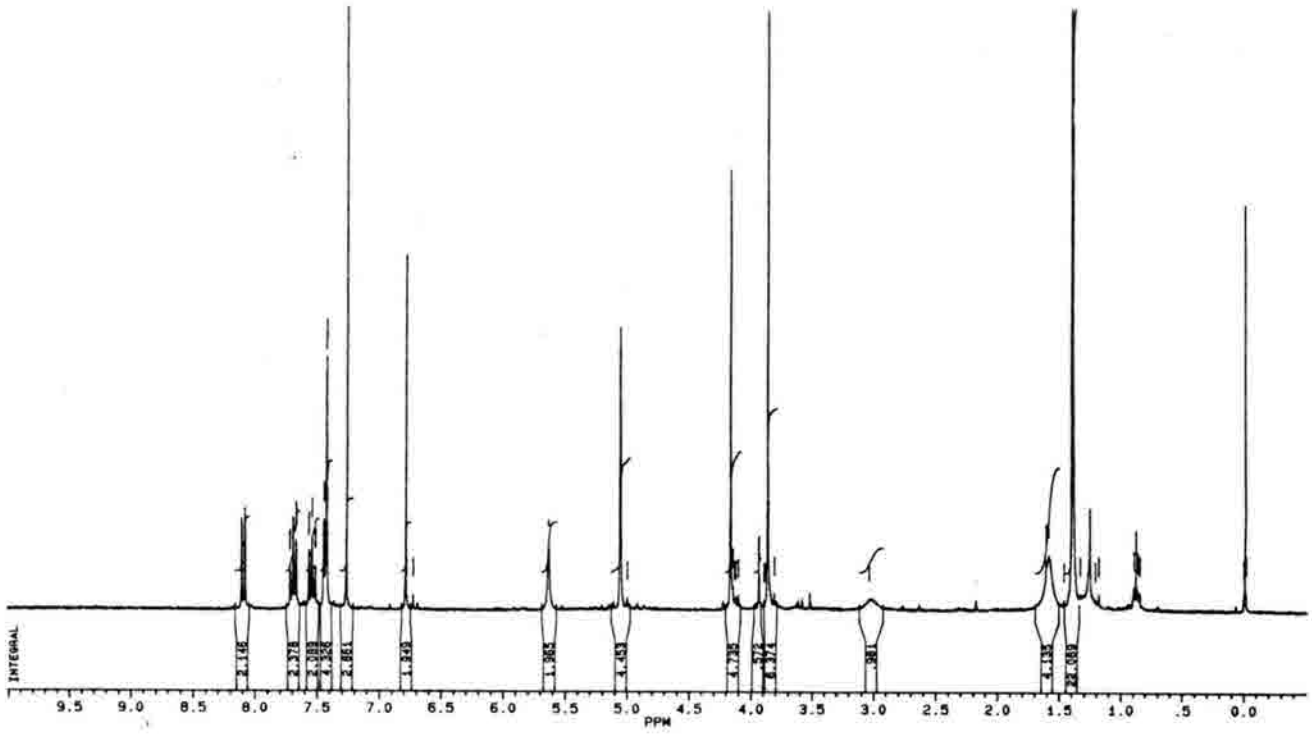
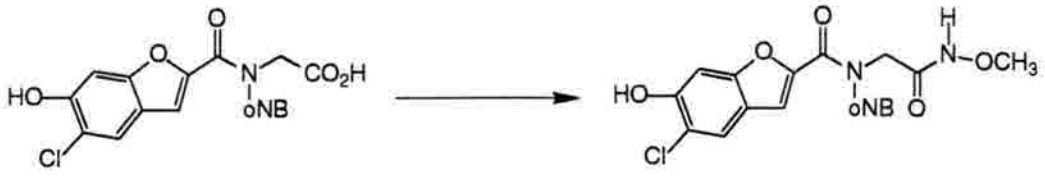


Figure 11. 300 mHz NMR of alcohols **337a,b** in $CDCl_3$



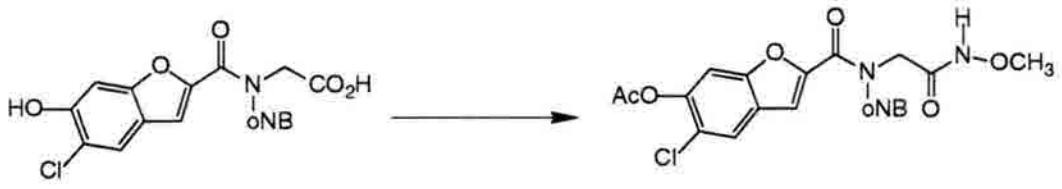
334 **331**

N-[2-(methoxyamino)-2-oxoethyl]-N-(2-nitrobenzyl)-2-[(5-chloro-6-hydroxy)]-benzofurancarboxamide (331).

To a solution of **334** (250 mg, 0.617 mmol, 1.0 eq) dissolved in 60 mL of tetrahydrofuran was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (178 mg, 0.926 mmol, 1.5 eq). (A few milliliters of water was added to help dissolve the carbodiimide). The yellow solution was stirred for ten minutes before methoxylamine (96 mL, 1.85 mmol, 3.0 eq) was added causing the color to dissipate. The solution was stirred for 16 hours before dilution with ethyl acetate, extracted with saturated sodium bicarbonate, and dried over sodium sulfate. The residue was purified using 20% methanol/chloroform. Obtained 172 mg of a white solid which was recrystallized from ethyl acetate/hexanes. 64% yield.

^{1}H NMR (300 mHz, T= 357 K, DMSO-d_6) δ= 10.81 (1H, bs); 8.21 (1H, s); 8.05 (1H, d, J= 7.9 Hz); 7.7- 7.52 (4H, m); 7.29 (1H, s); 7.06 (1H, s); 5.10 (2H, s); 4.24 (2H, s); 3.59 (3H, s).

IR (KBr) υ=3171, 2975, 1665, 1610, 1523, 1338, 1142 cm^{-1}.



334 344

N-[2-(methoxyamino)-2-oxoethyl]-N-(2-nitrobenzyl)-2-[(5-chloro-6-acetoxy)]-benzofurancarboxamide (344).

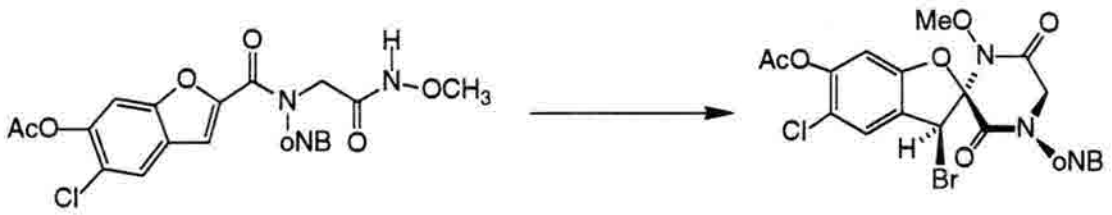
To 12.9 grams of **334** (31.97 mmol, 1.0 eq) dissolved in 30- 50 mL of pyridine was added excess acetic anhydride (30 mL at least 10 eq) and the solution stirred at room temperature for 4 hours. The majority of the solvent was removed in vacuo and the residue taken up in ethyl acetate, washed with 10% HCl followed by water, dried over magnesium sulfate, filtered and concentrated to 14 g of a yellow/orange foam (>95% by TLC) which was also carried on without further purification.

To a solution containing 2.77 g of crude carboxylic acid (6.2 mmol, 1.0 eq) dissolved in 200 mL tetrahydrofuran cooled to 0°C was added N-methylmorpholine (750 mL, 6.82 mmol, 1.1 eq). After 10 minutes isobutyl chloroformate (880 mL, 6.82 mmol, 1.1 eq) was added causing the solution to turn yellow. After 45 minutes the ice bath was removed and methoxylamine (630 mL, 12.4 mmol, 2.0 eq) was added causing the intense yellow color to dissipate, and the reaction stirred for an additional 45 minutes at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with 10% HCl followed by water, dried over magnesium sulfate, filtered and concentrated to a yellow/orange oil. The hydroxamic ester was purified from column chromatography using 2:1 ethyl acetate/hexanes. Obtained 2.1 g of a white foam which was further purified by recystallization from ethyl acetate/hexanes, mp 151-152 °C. 70% yield.

^{1}H NMR: (300 mHz, DMSO-d_6, T= 370 K)) δ= 10.81 (1H, bs); 8.02 (1H, d, J= 8 Hz); 7.91 (1H, s); 7.73- 7.27 (4H, m); 7.0 (1H, s); 5.10 (2H, s);4.23 (2H, s); 3.57 (3H, s); 2.32 (3H, s).

IR (NaCl film) υ= 3210, 2998, 1771, 1677, 1637, 1526 cm^{-1}.

Anal. Calcd. for $C_{21}H_{18}N_3O_8Cl$: C 53.01, H 3.81, N 8.83, Cl 7.45, Found C 52.92, H 3.88, N 8.68, Cl 7.25.



344 **345**

Trans-3-bromo-5-chloro-6-acetoxy-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (345).

N-bromosuccinamide (4.0 g, 22.69 mmol, 1.2 eq) was added in one portion to a solution of **344** (9.0 g, 18.9 mmol, 1.0 eq) in 600 mL of ethanol-free chloroform under N_2 at room temperature and the reaction stirred at room temperature for 20 hours. The reaction was poured into a separatory funnel containing additional chloroform, washed with saturated sodium thiosulfate followed by water, dried over magnesium sulfate, filtered and concentrated to a thick, light yellow colored oil which let standing solidifies. The oily product was immediately purified by column chromatography using 3:2 hexane/ethyl acetate as the eluent. Obtained 6.67 g of a white solid which was recrystallized from ethyl acetate/hexanes. mp 160- 162 °C. 67% yield.

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 8.04 (1H, d, J = 8.0 Hz); 7.67- 7.60 (2H, m); 7.55 - 7.50 (1H, m); 7.35 (1H, s); 6.79 (1H, s); 6.01 (1H, s); 5.05 (1H, 1/2 ABq, J= 15.7 Hz); 5.01 (1H, 1/2 ABq, J= 15.7 Hz); 4.18 (2H, s); 3.95 (3H, s); 2.35 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 168.02 (C), 161.33 (C), 161.22 (C), 156.68 (C), 148.88 (C), 148.81 (C), 133.9 (CH), 130.59 (CH), 129.42 (C), 129.30 (CH), 126.36 (CH), 125.19 (CH), 124.41 (C), 120.71 (C), 105.92 (CH), 101.75 (C), 65.81 (CH_3), 49.06 (CH_2), 48.18 (CH), 46.49 (CH_2), 20.55 (CH_3).

IR (NaCl film) υ= 2992, 1772, 1697, 1527 cm^{-1}.

Anal. Calcd. for $C_{21}H_{17}N_3O_8ClBr$: C 45.47, H 3.09, N 7.57, total halogen content (calculated as 2 Cl) 12.78, Found C 45.55, H, 3.15, N 7.58, total halogen 12.67.

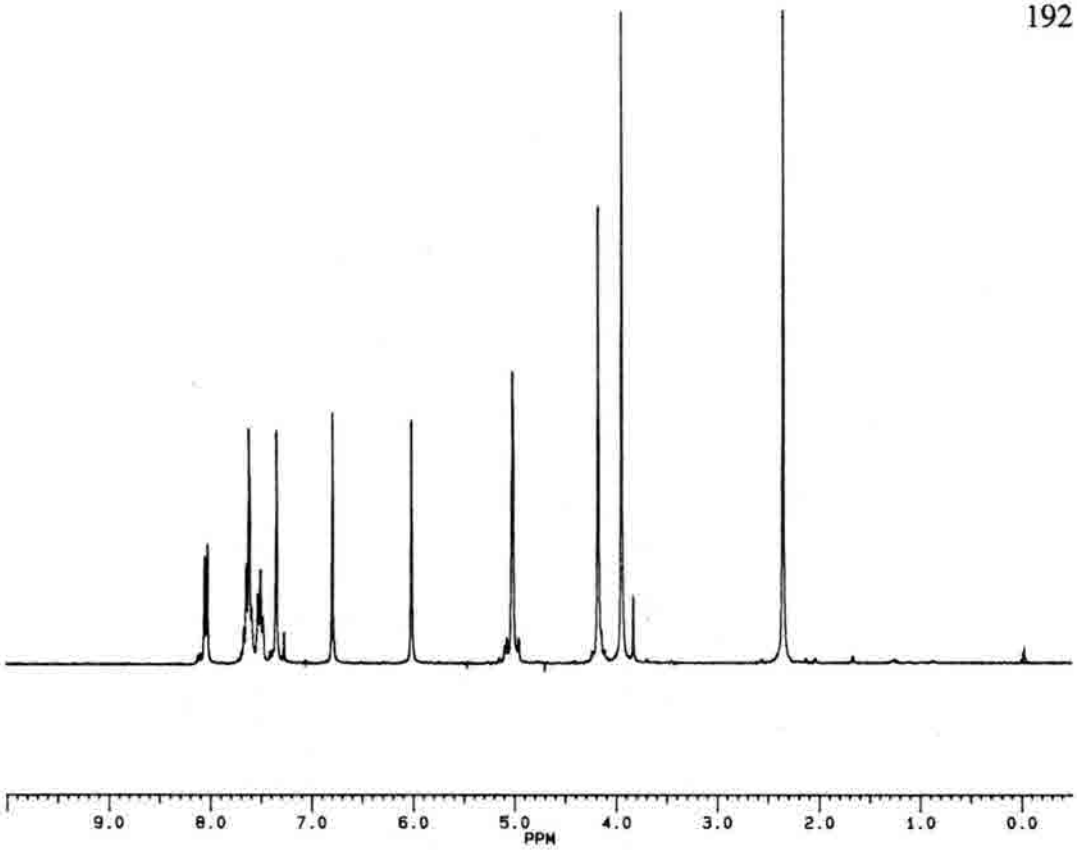
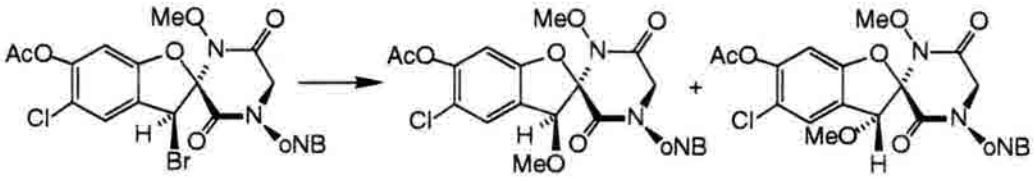


Figure 12. 300 mHz NMR of bromide **345** in $CDCl_3$



345 **346a** **346b**

Cis,Trans-3-methoxy-5-chloro-6-acetoxy-1'-methoxy-4'-(2-nitrobenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (346a,b).

A solution of silver triflate (735 mg, 4.36 mmol, 1.2 eq) in 15 mL of tetrahydrofuran was added in one portion to a rapidly stirring solution of 2.0 g **345** (3.6 mmol, 1.0 eq) dissolved in 150 mL of tetrahydrofuran in which 30 mL of methanol had been added The resulting solution was stirred at room temperature for 45 minutes. The reaction was diluted with ethyl acetate followed by the addition of saturated brine solution. The reaction was filtered through a plug of celite, and the filtrate washed with brine, water, dried over magnesium sulfate, filtered, and concentrated to a white solid. The diastereomers were purified by column chromatography using 2% acetone in chloroform. 950 mg of the trans diastereomer (non-polar) and 315 mg of the cis diastereomer (polar) were obtained in 69% combined yield (81% based on recovered starting material). Each diastereomer was recrystallized from ethyl acetate/hexanes.

346a TRANS (R_f= 0.3, 5% acetone/chloroform)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 8.05 (1H, d, J= 8.1 Hz); 7.62- 7.49 (3H, m); 7.43 (1H, s); 6.82 (1H, s); 5.52 (1H, s); 5.26 (1H, 1/2 ABq, J= 16.2 Hz); 4.88 (1H, 1/2 ABq, J= 16.2 Hz); 4.12, 2H, s); 3.93 (3H, s); 3.61 (3H, s); 2.35 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 168.07 (C), 161.97 (C), 161.15 (C), 158.03 (C), 148.67 (C), 148.65 (C), 133.85 (CH), 129.97 (C), 129.62 (CH), 128.98 (CH), 126.02 (CH), 125.19 (CH), 124.12 (C), 119.93 (C), 106.37 (CH), 101.15 (C), 84.62 (CH), 65.69 (CH_3), 59.28 (CH_3), 49.24 (CH_2), 46.56 (CH_2), 20.56 (CH_3).

IR (NaCl film) υ= 2943, 1773, 1697, 1527 cm^{-1}.

Anal. Calcd. for $C_{22}H_{20}N_3O_9Cl$: Calculated C 52.23, H 4.98, N 8.31, Cl 7.01, Found C 52.13, H 4.20, N 8.17, Cl 6.92. White crystals from ethyl acetate/hexanes mp 132- 134 ^oC.

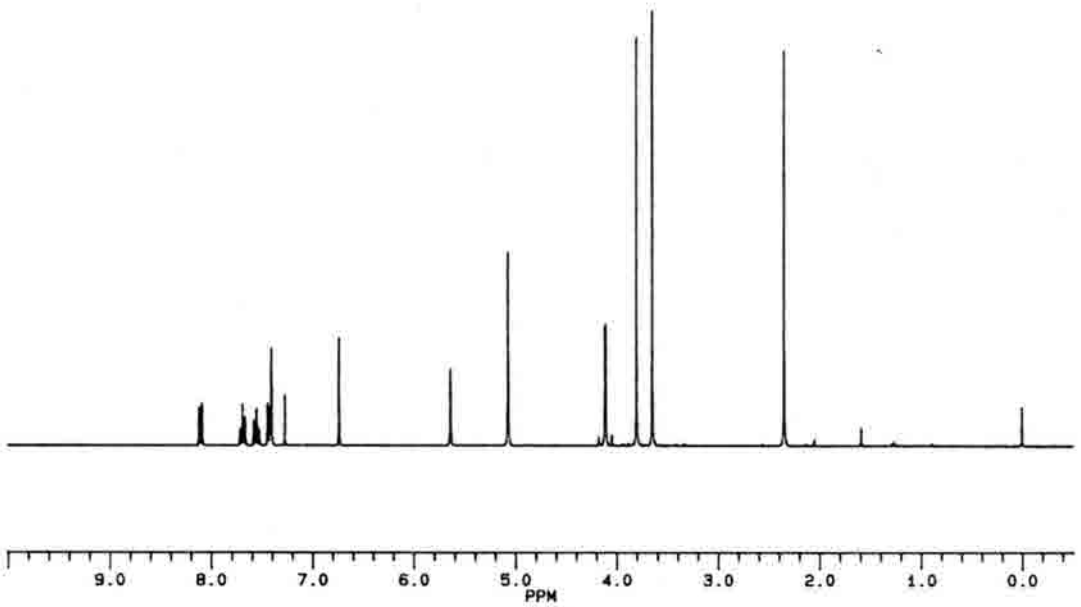
346b CIS (R_f= 0.2, 5% acetone/chloroform)

^1H NMR: (300 mHz, $CDCl_3$) δ=8.09 (1H, d, J= 8.1 Hz); 7.86 (1H, t, J = 7.5 Hz); 7.54 (1H, t, J= 8.1 Hz); 7.43- 7.39 (2H, m); 6.73 (1H, s); 5.63 (1H, s); 5.0 (2H, s); 4.13 (1H, 1/2Abq, J= 18.1 Hz); 4.09 (1H, 1/2ABq, J= 18.2 Hz); 3.80 (3H, s); 3.64 (3H, s); 2.34 (3H, s).

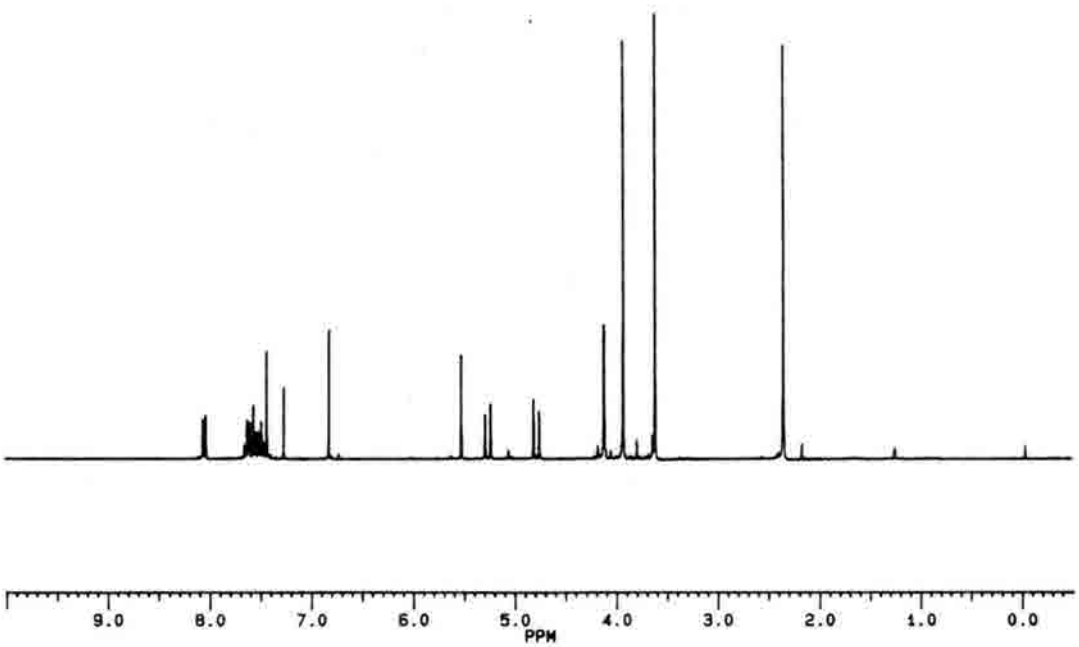
^{13}C NMR (75.47 mHz, $CDCL_3$) δ= 168.0(C), 162.89 (C), 162.37 (C), 156.05 (C), 148.74 (C), 148.07 (C), 134.1 (CH), 129.54 (CH), 129.52 (C), 129.45 (CH), 125.43 (CH), 125.12 (CH), 124.44 (C), 120.1 (C), 105.84 (CH), 98.2 (C), 84.6 (CH), 64.58 (CH_3), 60.13 (CH_3), 49.17 (CH_2), 47.04 (CH_2), 20.57 (CH_3).

IR (NaCl film) u= 2946, 1772, 1688, 1527 cm^{-1}.

Anal. Calcd. for $C_{22}H_{20}N_3O_9Cl$: C 52.23, H 3.98, N 8.31, Cl 7.01, Found C 52.49, H 4.20, N 8.39, Cl 7.09. White needles from ethyl acetate/hexanes. mp 163-164 ^oC.



trans 346a



cis 346b

Figure 13. 300 mHz NMR of alcohols **346a,b** in $CDCl_3$

346a,b **347a,b**

Cis,Trans-3-methoxy-5-chloro-6-acetoxy-1'-methoxy-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (347a,b)

Methyl ether **346a** (250 mg, 0.494 mmol) was dissolved in 50 mL of 10% water in tetrahydrofuran in a quartz tube containing pyrex beads. The solution was photolyzed at 37°C for 5 hours using a 450 watt Conrad-Hanovia medium pressure mercury vapor lamp. The reaction was filtered into a separatory funnel, containing ethyl acetate, washed with water, dried over magnesium sulfate, filtered, and concentrated to a brown film which was chromatographed using 15% acetone/chloroform. Obtained 131 mg of product **347a**. 72% yield. Analogous reaction using the cis diastereomer **346b** gave **347b** as a white solid in 71% yield.

TRANS (347a)

^{1}H NMR: (300 mHz, $CDCl_3$) δ=7.41(1H, s); 7.30 (1H, s, exchangeable in D_2O), 6.80 (1H, s); 5.44 (1H, s); 4.15 (1H, 1/2 ABq, J = 18.4 Hz, broadened by NH, d, J= 2.8 Hz); 4.07 (1H, 1/2 Abq, J = 18.4 Hz); 3.90 (3H, s); 3.60 (3H, s); 2.35 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 168.12 (C), 162.59 (C), 162.28 (C), 157.92 (C), 148.69 (C), 126.13 (CH), 124.19 (C), 120.04 (C), 106.3 (CH), 100.93 (C), 84.79 (CH), 65.71 (CH_3), 59.5 (CH_3), 44.0 (CH_2), 20.62 (CH_3).

IR (NaCl film) υ= 3273, 2942, 1772, 1708 cm^{-1}.

Anal. Calcd. for $C_{15}H_{15}N_2O_7Cl$: C 48.59, H 4.08, N 7.56, Cl 9.56, Found C 48.46, H, 4.21, N 7.35, Cl 9.46. White needles from ethyl acetate/petroleum ether. mp 181-182°C.

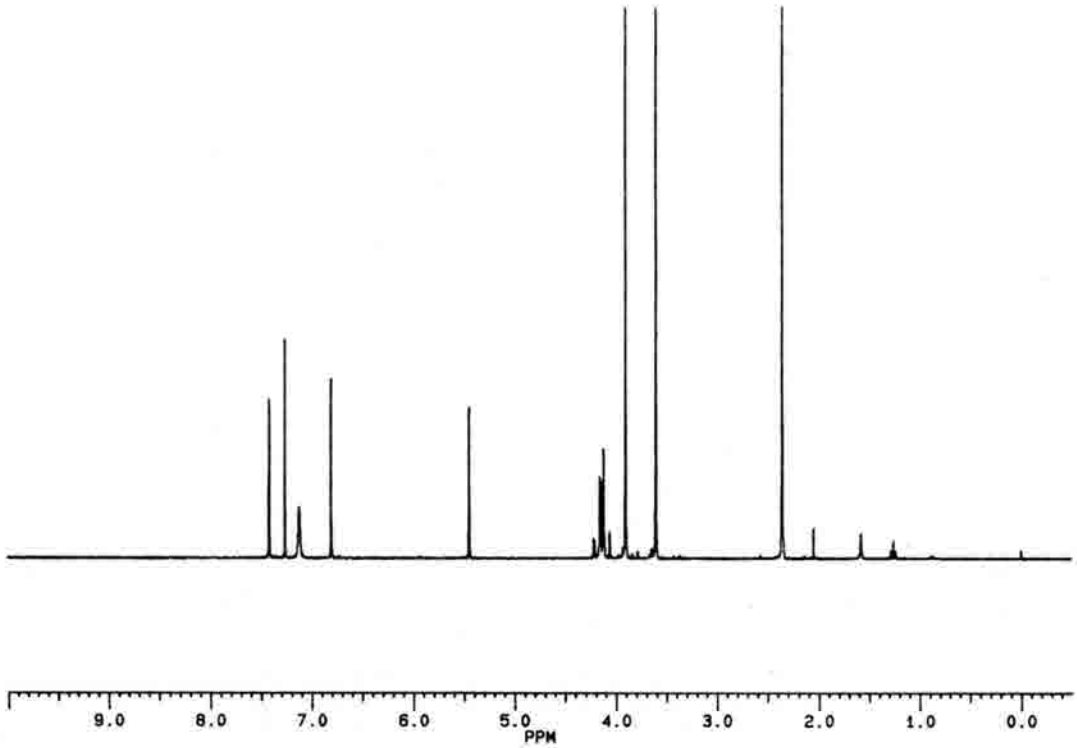
CIS (347b)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.39 (1H, s); 6.73 (1H, s); 6.57 (1H, s, exchangeable in D_2O); 5.5 (1H, s); 4.19 (1H, 1/2 ABq, J= 2.0 Hz); 4.28 (1H, 1/2 ABq, J = 1.3 Hz); 3.79 (3H, s); 3.65 (3H, s); 2.34 (3H, s).

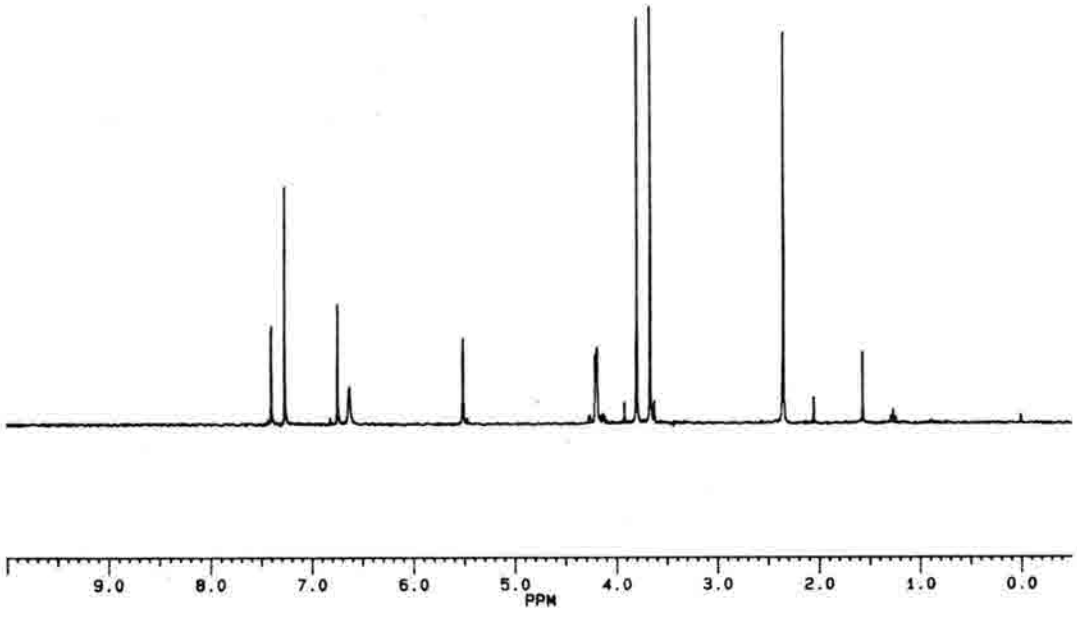
^{13}C NMR (75.47 mHz, DMSO-d_6) δ= 168.13 (C); 162.79 (C); 162.73 (C); 156.14 (C); 147.64 (C); 125.29 (CH); 125.19 (C); 118.5 (C); 105.83 (CH); 97.71 (C); 83.33 (CH); 63.70 (CH_3); 59.70 (CH_3); 43.21 (CH_2); 20.35 (CH_3).

IR (NaCl film) υ= 3274, 2942, 1770, 1703 cm^{-1}.

Anal. Calcd. for $C_{15}H_{15}N_2O_7Cl$: C 48.59, H 4.08, N 7.56, Found C 48.61, H 4.19, N 7.31. White solid from ethyl acetate/ petroleum ether, mp. decomposed >230°C.

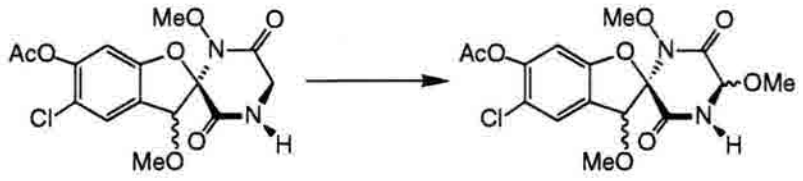


trans 347a



cis 347b

Figure 14. 300 mHz NMR of methyl ethers **347a,b** in $CDCl_3$



347a,b **349a-d**

3,5'-methoxy-5-chloro-6-acetoxy-1'-methoxy-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (349a-d)

General Procedure:

Methyl ether **347** (65 mg, 0.176 mmol, 1 eq) was dissolved in 5 mL of dry ethanol-free at room temperature. Freshly prepared t-butyl hypochlorite (25 uL, 0.193 mmol, 1.2 eq) was added followed by the dropwise addition of a freshly prepared solution of 0.1 M sodium methoxide (1.5 eq). The solution was stirred at room temperature for 1 hour. The reaction was quenched with 10% aqueous HCl, extracted with ethyl acetate, washed with water, dried over sodium sulfate, filtered and concentrated to a yellow film. In the case of **347a**, the individual diastereomers **349a,b** were purified by column chromatography using 20% acetone/chloroform as the eluent. Obtained a white solid for a combined yield of 56%. Analogous reactions using **347b** gave **349c,d** as an inseparable mixture of diastereomers which were carried on directly.

TRANS (349a) (R_f= 0.4, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.39 (1H, s); 7.32 (1H, bs, exchangeable in D_2O); 6.75 (1H, s); 5.6 (1H, s); 5.00 (1H, d, J= 2.2 Hz); 3.91 (3H, s); 3.57 (3H, s); 3.49 (3H, s); 2.35 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 168.15 (C), 161.91 (C), 160.0 (C), 157.52 (C), 148.51 (C), 126.0 (CH), 124.49 (C), 120.10 (C), 106.23 (CH), 101.16 (C), 84.7 (CH), 80.82 (CH), 65.34 (CH_3), 60.0 (CH_3), 55.56 (CH_3), 20.59 (CH_3).

IR (NaCl, film) υ= 3268, 2943, 1772, 1715, 1604, 1478, 1198, 1145 cm^{-1}.

Anal. Calcd. for $C_{16}H_{17}N_2O_8Cl$: C 47.95, H 4.28 N 6.99, Cl 8.85, found C 47.89, H 4.40, N 6.77, Cl 9.03. White needles from ethyl acetate/hexanes. mp 132- 133°C.

TRANS (349b) (R_f= 0.3, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.72 (1H, bs, exchangeable in D_20); 7.42 (1H, s); 6.83 (1H, s); 5.21 (1H, s); 4.90 (1H, d, J= 4.2 Hz); 3.86 (3H, s); 3.57 (3H, s); 3.53 (3H, s); 2.35 (3H, s).

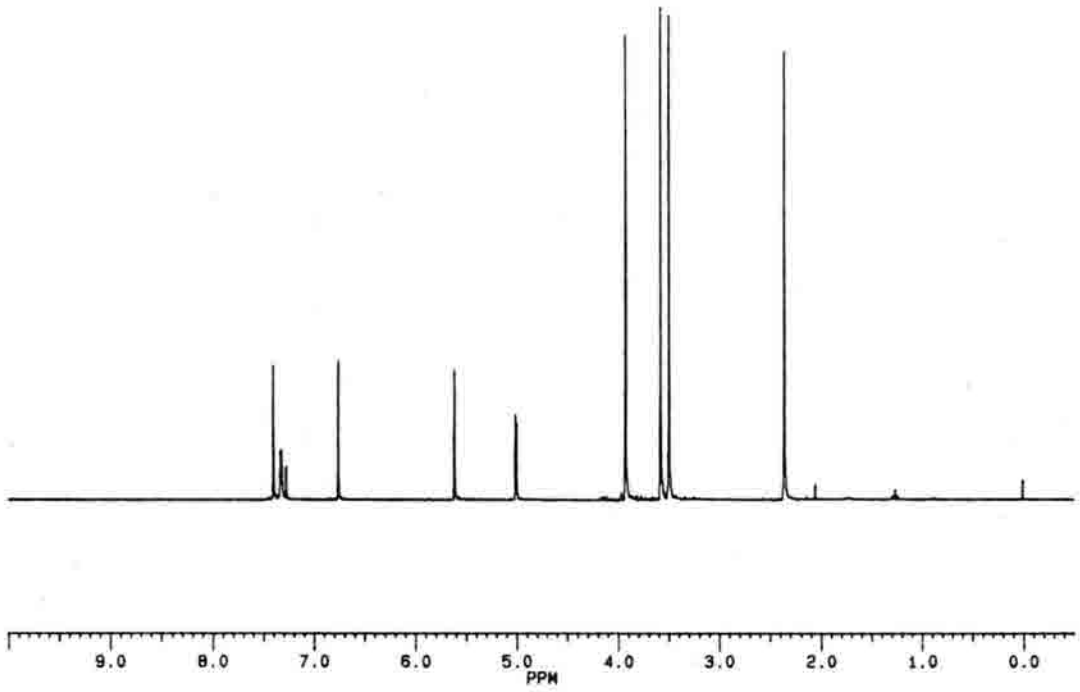
^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 168.02 (C), 163.17 (C), 160.66 (C), 158.12 (C), 148.77 (C), 126.0 (CH), 124.32 (C), 120.15 (C), 106.17 (CH), 100.33 (C), 85.9 (CH), 80.66 (CH), 65.92 (CH_3), 59.19 (CH_3), 56.35 (CH_3), 20.58 (CH_3).

IR (NaCl, film) υ= 3281, 2942, 1772, 1716, 1604, 1476, 1197, 1147 cm^{-1}.

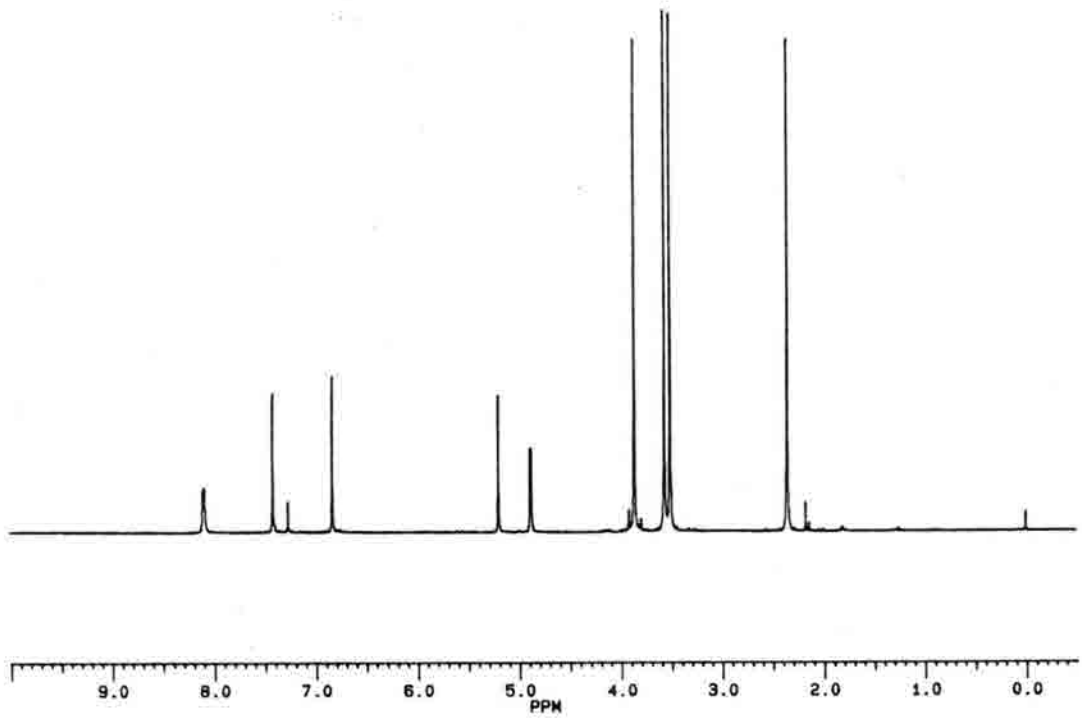
Anal. Calcd. for $C_{16}H_{17}N_2O_8Cl$: C 47.95, H 4.28 N 6.99, Cl 8.85, found C 47.88, H 4.39, N 6.71, Cl 8.90. White plates from ethyl acetate/hexanes. mp 178- 180°C.

CIS diastereomers (349c,d)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.87 (1H, d, J= 3.4 Hz); 7.60 (1H, d, J= 2.36 Hz); 7.41 (1H, s); 7.37 (1H, s); 6.77 (1H, s); 6.72 (1H, s); 5.60 (1H, s); 5.22 (1H, s); 5.08 (1H, d, J= 2.79 Hz); 4.91 (1H, d, J= 3.6 Hz); 3.82 (3H, s); 3.77 (3H, s); 3.66 (3H, s); 3.61 (3H, s); 3.57 (3H,s); 3.53 (3H, s); 2.35 (3H, s); 2.34 (3H, s).

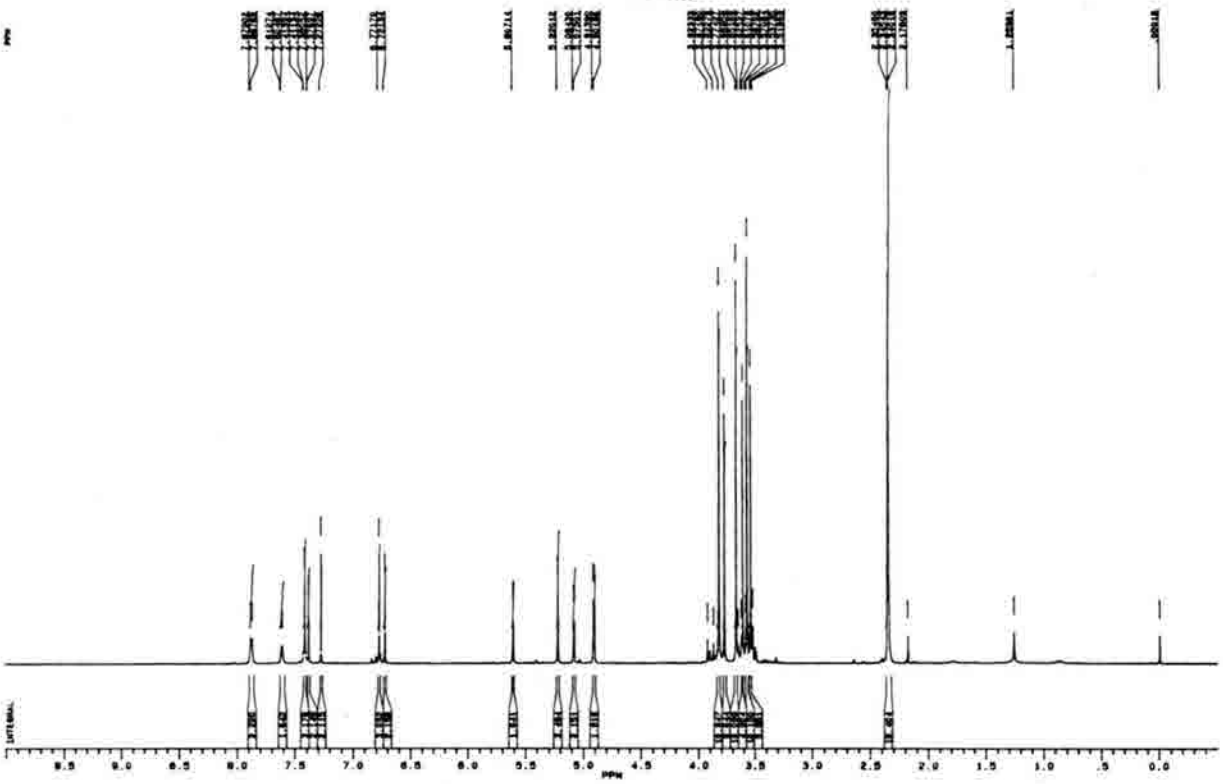


trans 349a



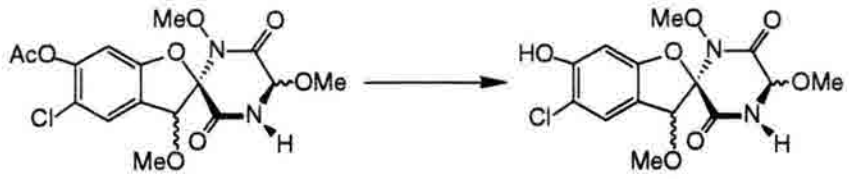
cis 349b

Figure 15. 300 mHz NMR of methyl ethers **349a,b** in $CDCl_3$



mix 349c,d

Figure 16. 300 mHz NMR of methyl ethers **349c,d**



349a-d **350a-d**

3,5'-methoxy-5-chloro-6-hydroxy-1'-methoxy-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione(350a-d)

General Procedure:

A suspension of 44 mg (0.11 mmol, 1 eq) of **349a,b** or **349c,d** in 2 mL of absolute ethanol was stirred at 0°C. Exactly 1.1 eq of 0.1 M sodium ethoxide at 0°C was added dropwise. The resulting solution was stirred at 0°C for 30 minutes before quenching the reaction by addition of 10% aqueous HCl. The reaction was poured into ethyl acetate, washed with saturated sodium chloride, dried over sodium sulfate, filtered, and concentrated to a light yellow film. Crude NMR shows >90% product. The product was purified by column chromatography using 2:1 ethyl acetate hexanes or 15% acetone/chloroform. Obtained 29 mg of a white solid. The individual diastereomers could be obtained routinely in 74% isolated yield.

TRANS 350a (R_f= 0.28, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, MeOH-d_4) δ=7.28 (1H, s); 6.49 (1H, s); 5.44 (1H, s); 5.08 (1H, s); 4.86 (2H, bs); 3.83 (3H, s); 3.51 (3H, s): 3.49 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 163.54 (C); 162.36 (C); 160.04 (C); 156.53 (C); 127.06 (CH); 118.66 (C); 115.03 (C); 102.16 (C); 99.30 (CH); 86.77 (CH); 82.12 (CH); 65.67 (CH_3); 59.65 (CH_3); 55.76 (CH_3).

IR (NaCl, film) υ= 3273 shoulder 3148, 2943, 1705, 1627.6, 1299, 1097, 1032 cm^{-1}.

Anal. Calcd. for $C_{14}H_{15}N_2O_7Cl$: C 46.87, H 4.21, N 7.81; Found C 47.00, H 4.42, N 7.69. mp decomposes-no clean melting point (180-202 °C). White crystals from diethyl ether at -11 °C.

TRANS 350b (R_f= 0.18, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, MeOH-d_4) δ= 7.35 (1H, s); 6.56 (1H, s); 5.03 (1H, s); 4.89 (1H, s); 4.83 (2H, bs); 3.79 (3H, s); 3.50 (3H, s); 3.48 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 164.78 (C); 162.99 (C); 160.65 (C); 156.78 (C); 127.12 (CH); 119.78 (C); 118.66 (C); 115.16 (C); 99.24 (CH); 87.81 (CH); 82.04 (CH); 66.35 (CH_3); 58.71 (CH_3); 56.57 (CH_3).

IR (KBr) υ= 3342 shoulder 3225, 2945, 1710, 1631, 1497, 1186, 1099, 1041 cm^{-1}.

Anal. Calcd. for $C_{14}H_{15}N_2O_7Cl$: C 46.87, H 4.21, N 7.81; Found C 46.95, H 4.32, N 7.7. Decomposes-no clean melting point. White solid from aceton/petroleum ether.

CIS 350c (R_f= 0.32, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, MeOH-d_4) δ= 7.21 (1H, d, J= 1.1 Hz); 6.46 (1H, s); 5.48 (1H, s); 5.12 (1H, s); 4.84 (2H, bs); 3.72 (3H, s); 3.55 (3H, s); 3.49 (3H, s).

^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 166.41 (C); 163.57 (C); 158.19 (C); 156.06 (C); 125.96 (CH); 118.86 (C); 115.09 (C); 99.16 (CH); 99.03 (C); 87.22 (CH); 81.59 (CH); 64.59 (CH_3); 60.40 (CH_3); 55.71 (CH_3).

IR (NaCl, film) υ= 3281, 2941, 1700, 1627, 1487, 1437, 1104 cm^{-1}.

Anal. Calcd. for $C_{14}H_{15}N_2O_7Cl$: C 46.87, H 4.21, N 7.81; Found C 46.66, H 4.37, N 7.63. White crystals from diethyl ether/petroleum ether at 0°C. mp. 158- 159°C.

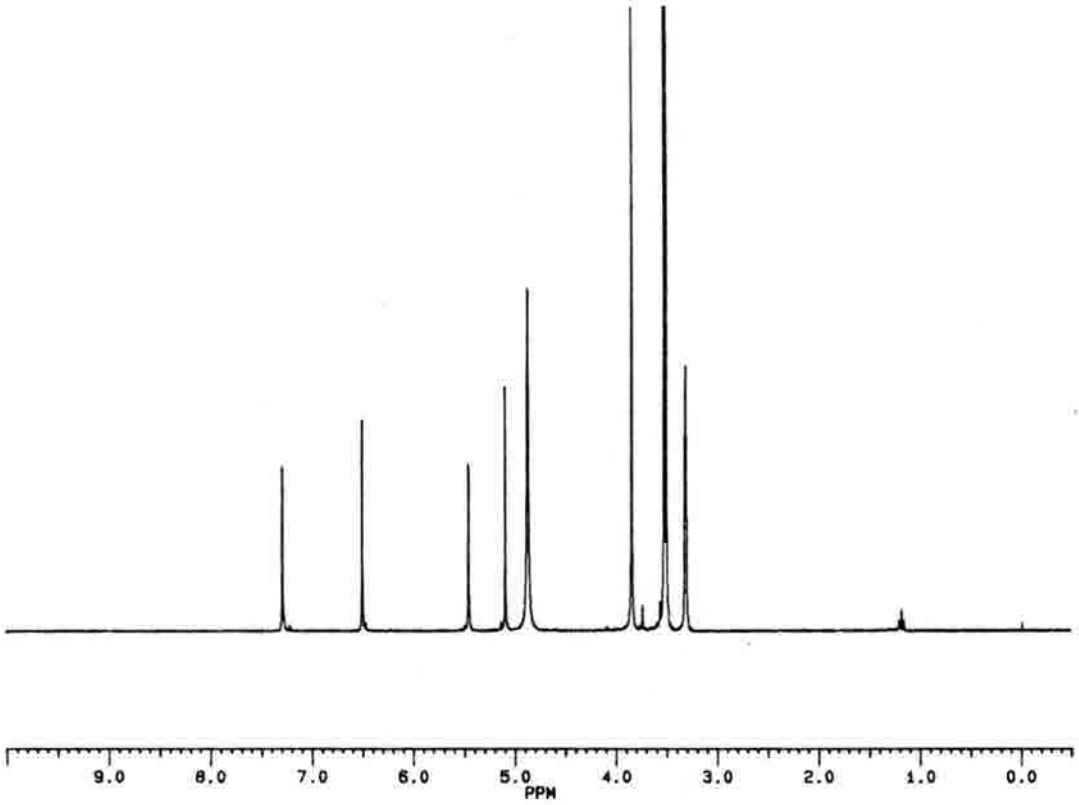
CIS 350d (R_f= 0.23, 2:1 ethyl acetate/hexanes)

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.29 (1H, s); 6.49 (1H, s); 5.10 (1H, s); 4.93 (1H, s); 4.85 (2H, bs); 3.77 (3H, s); 3.59 (3H, s); 3.53 (3H, s).

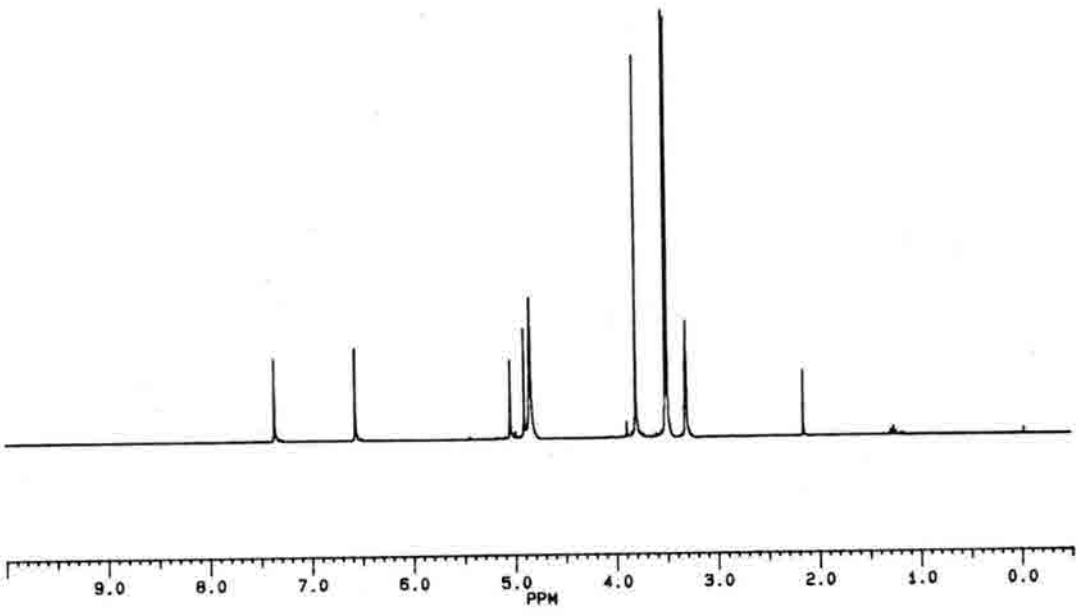
^{13}C NMR (75.47 mHz, $CDCl_3$) δ= 167.66 (C); 162.64 (C); 158.73 (C); 156.40 (C); 126.66 (CH); 118.7 (C); 115.2 (C); 99.1 (C); 99.03 (CH); 86.07 (CH); 82.48 (CH); 65.24 (CH_3); 60.57 (CH_3); 57.37 (CH_3).

IR (NaCl, film) υ= 3272, 3171 (shoulder), 2934, 1700, 1627, 1440, 1303, 1157, 1034 cm^{-1}.

Anal. Calcd. for $C_{14}H_{15}N_2O_7Cl$: C 46.87, H 4.21, N 7.81; Found C 46.63, H 4.33 N 7.57, Cl 9.84. White crystals from carbon tetrachloride/methanol 0°C. Decomposes 198-200°C.

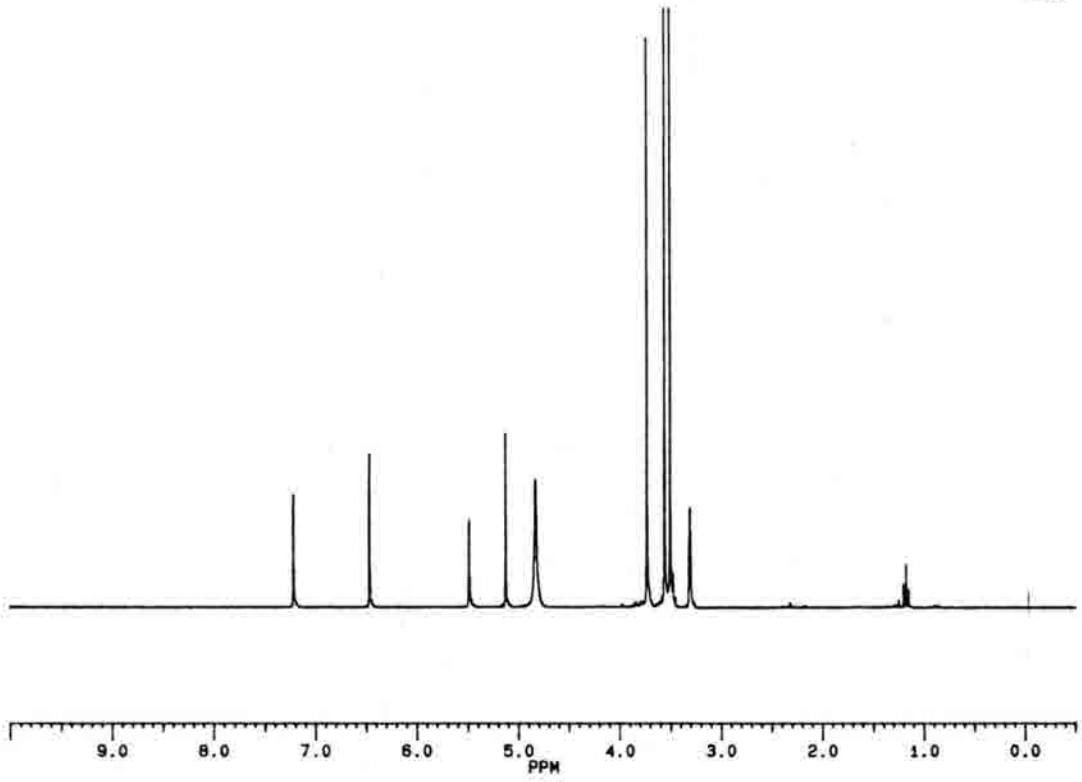


trans 350a

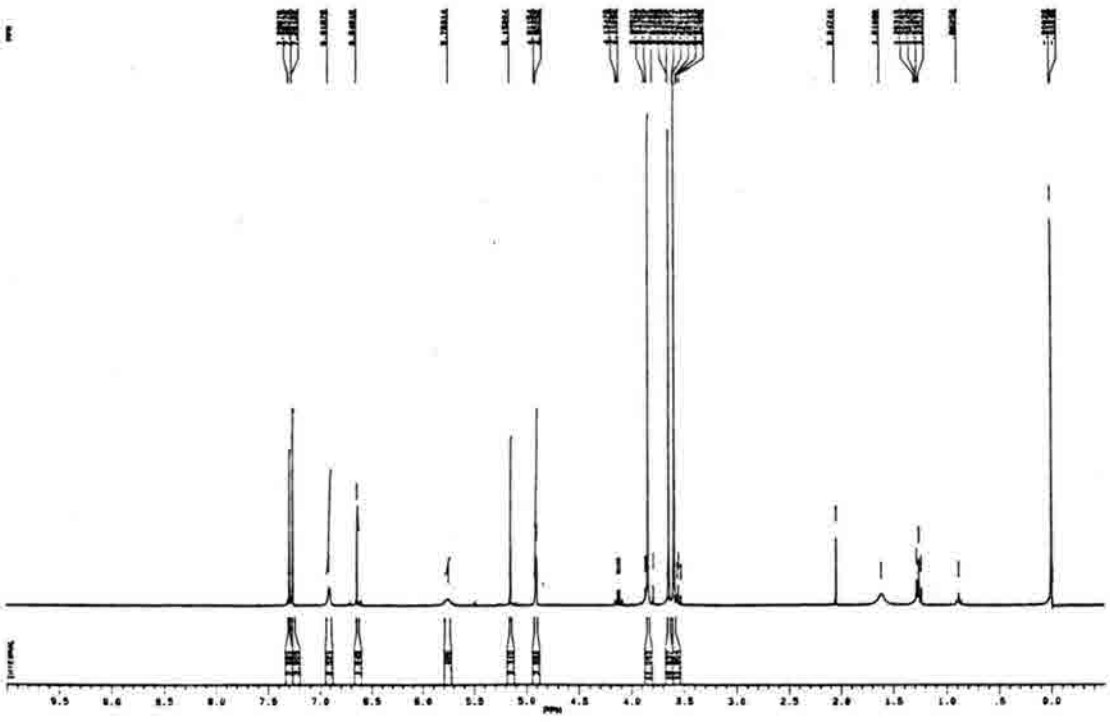


trans 350b

Figure 17. 300 mHz NMR of bismethyl ethers **350a.b** in $CDCl_3$

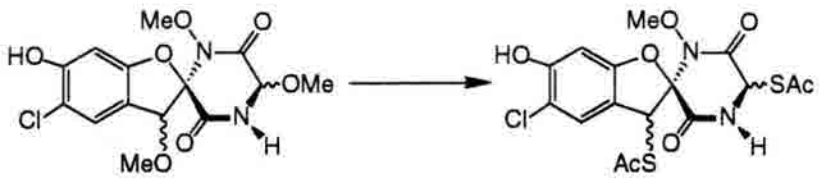


cis 350c



cis 350d

Figure 18. 300 mHz NMR of bis methyl ethers **350c,d** in $CDCl_3$



350a-d **354a-d**

Cis,Trans-3,5'-(S-acetyl)-1'-methoxy-spiro[5-chloro-6-hydroxy-benzofuran-2(3H),2'-piperazine]-3',6'-dione (354a-d)

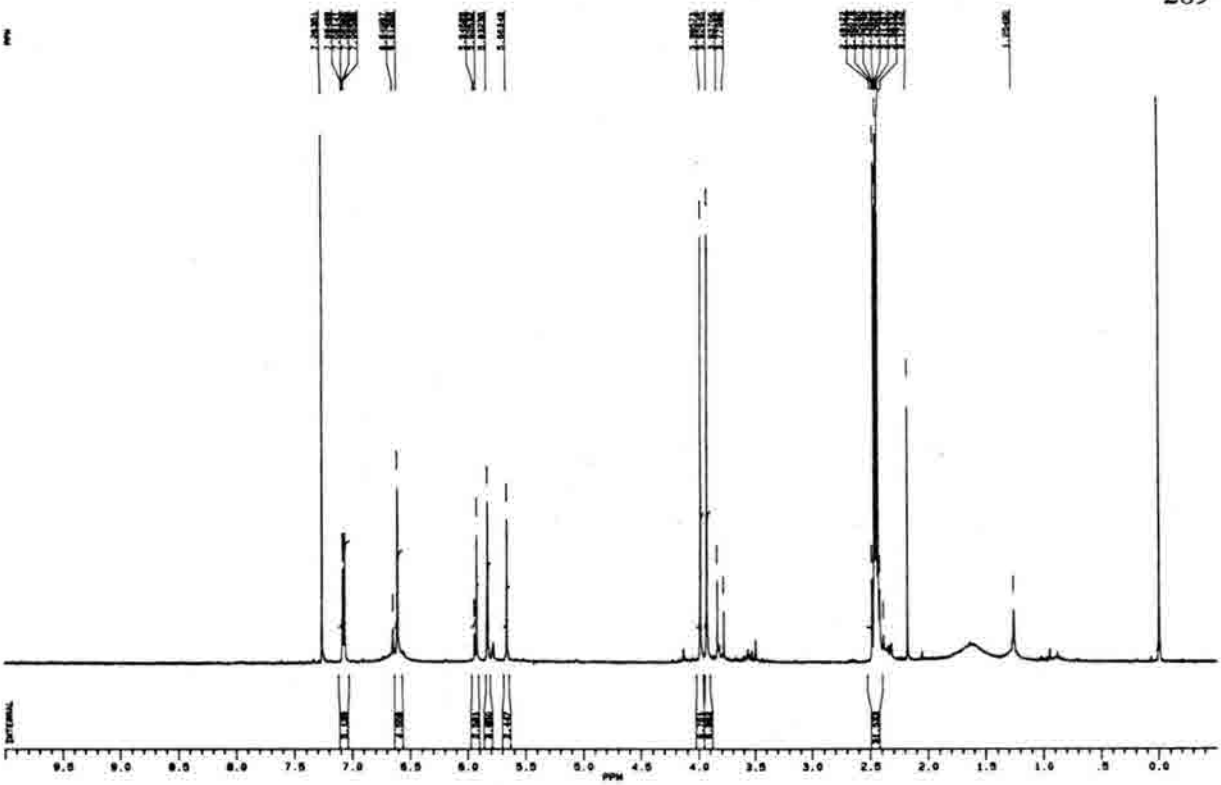
Representative procedure:

Boron trifluoride etherate (170 μL, 1.36 mmol, 8.0 eq) was added to 60 mg of bis methyl ether **350a** (1.0 eq. 0.167 mmol) in 15 mL methylene chloride. Thiolacetic acid (150 μL, 2.07 mmol, 12.0 eq) was added and the solution was heated and refluxed for 8 hrs. The solution was cooled, diluted with additional methylene chloride, washed once with saturated sodium bicarbonate, saturated ammonium chloride, water, dried over sodium sulfate, filtered and concentrated to a foul-smelling yellow film. The products were purified by column chromatography using 3% methanol/chloroform or 10% acetone/chloroform. 47 mg of a mixture of inseparable diastereomers were obtained which were carried on without further purification. 65% yield.

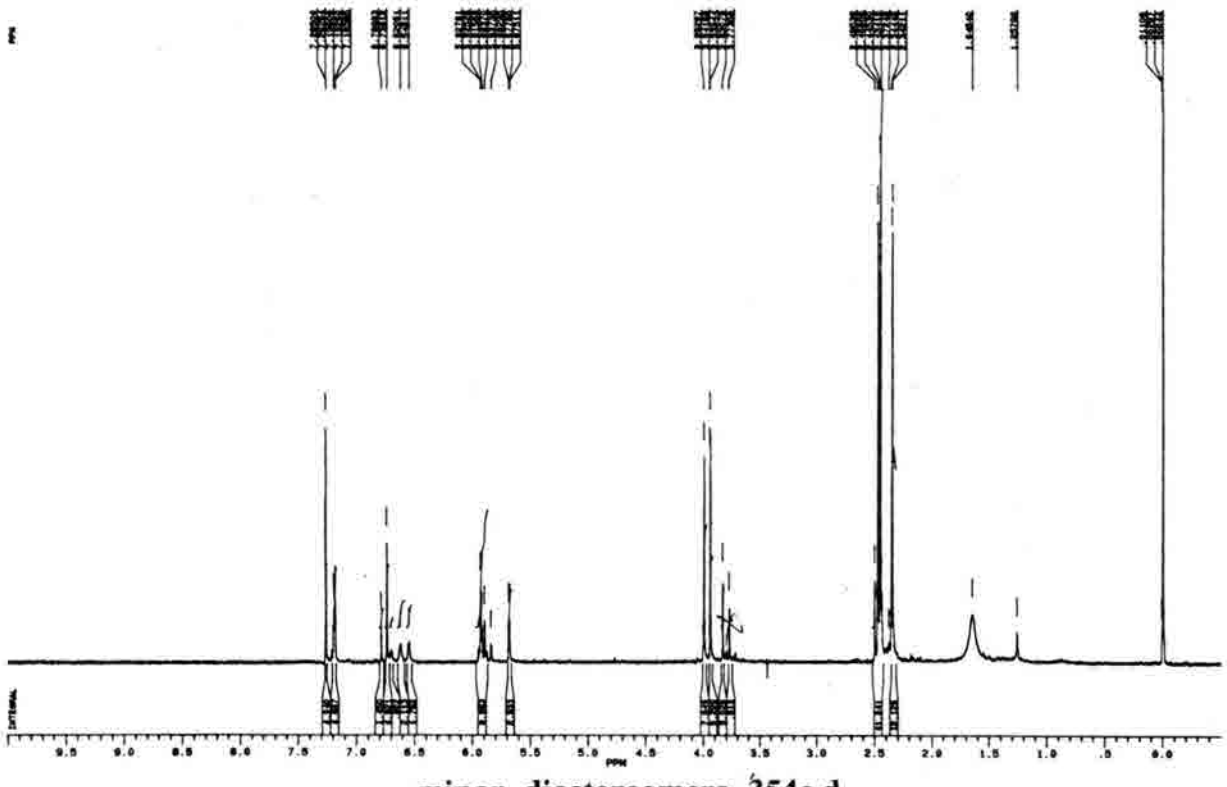
354a,b Major diastereomers:

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.08 (1H, s); 7.07 (1H, s); 6.61 (2H, s); 6.50 (1H, bs); 5.92 (1H, d, J= 2.9 Hz); 5.83 (1H, s); 5.70 (1H, bs); 5.66 (1H, d, J= 2.2 Hz); 3.98 (3H, s); 3.93 (3H, s); 2.48- 2.41 (12H, m).

IR (NaCl, film) υ= 3401, 2938, 1707, 1367, 1197, 1145 cm^{-1}.

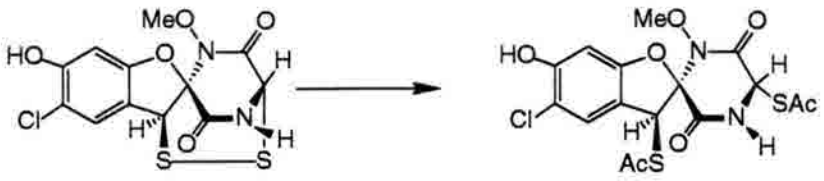


Major diastereomers 354a,b



minor diastereomers 354c,d

Figure 19. 300 mHz NMR of bisthioacetes **354a-d** in $CDCl_3$



(+)-80 **(+)-354a,b**

Methanethiol reduction and acetylation of aspirochlorine

Aspirochlorine (15 mg) was dissolved in 1 mL of dry pyridine and cooled to 0°C. Excess methanethiol was added and the solution stirred under argon for 15 minutes and at room temperature for 9 hours. The solution was concentrated under vacuum, the residue taken up in 1 mL fresh pyridine followed by the addition of 2 equivalents of acetyl chloride. The reaction was stirred for 15 minutes before diluting with ethyl acetate, washed with water, dried over magnesium sulfate, filtered and concentrated to a yellow film. ^{1}H NMR of the crude film showed the presence of thioacetates **354a,b**.

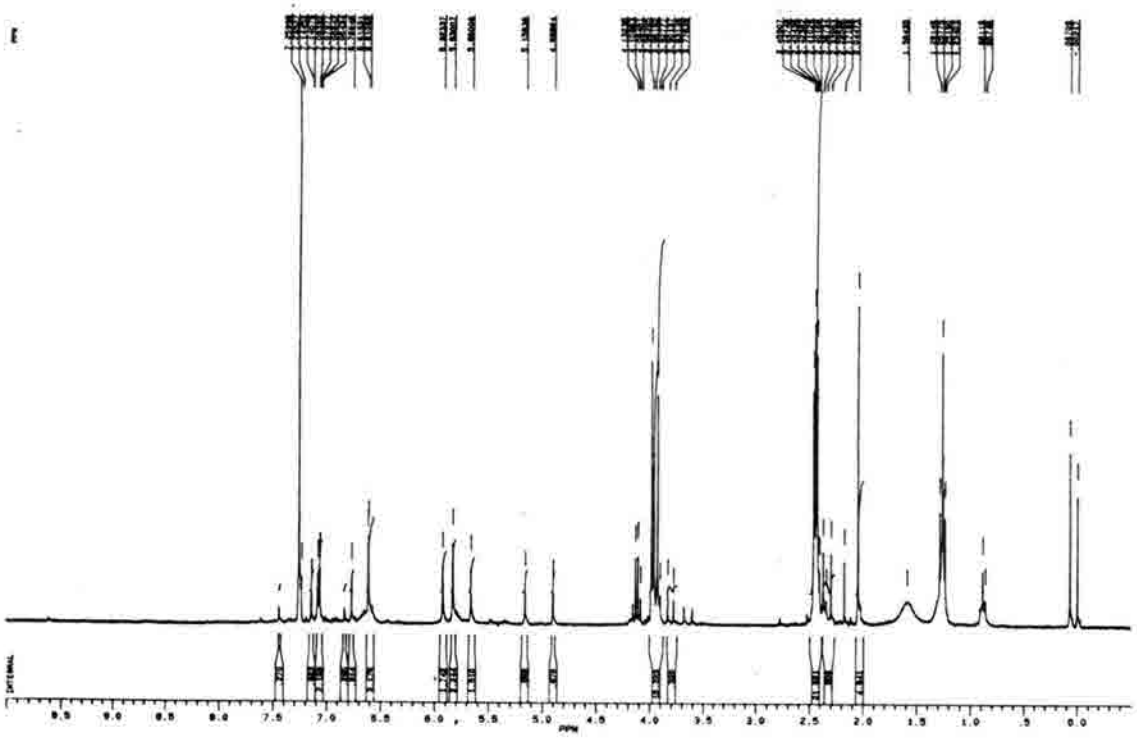


Figure 20. 300 mHz NMR of crude reaction from reduction and acetylation in $CDCl_3$

(+)-80 **(+)-80**

Sodium Borohydride reduction and KI_3 oxidation of aspirochlorine

Aspirochlorine (8 mg) was dissolved in 2 mL of absolute ethanol at 0°C and the solution degassed by passing argon through the solution for 20 minutes. A small spatula tip of sodium borohydride was quickly added and the solution stirred for 30 minutes. The solution was concentrated under reduce pressure and the residue taken up in water/chloroform and acidified to pH 5-6 with 10% sulfuric acid. Aqueous KI_3 was added dropwise until the organic phase was a light pink color. The organics were washed with water, dried over sodium sulfate, filtered, and concentrated to a brown film which was purified using PTLC. 3 mg of aspirochlorine (38% yield) was recovered.

354a,b **(±)-80**

(±)-8-hydroxy-9-chloro-11-(N-methoxy)-10bH-5a,5H-benzofuro[2,3-f]-1,2,4-dithiazepine-5,12-dione: (±)-Aspirochlorine 80

10 mg of **354a,b** and a small amount of camphor sulfonic acid was dissolved in 4 mL of tetrahydrofuran cooled to 0°C saturated with oxygen. 100 uL of methoxylamine was added and the solution warmed to room temperature and stirred for 6 1/2 hours. The reaction was concentrated and the yellow film immediately purified by PTLC using 5 % methanol/chloroform. 2 mg of a light yellow non-crystalline solid was obtained. On repeated attempts, yields of aspirochlorine routinely ranged from 20-34%. The synthetic and natural substances were identical by ^{1}H NMR, IR, TLC and HPLC. (R_f= 0.2, 5% methanol/chloroform; 0.5 in 6:4 chloroform/acetone and 0.4 in 1:1 benzene/ethyl acetate).

^{1}H NMR: (300 mHz, $CDCl_3$) δ= 7.15 (1H, s); 6.77 (1H, s); 6.73 (1H, bs, exchangeable, concentration dependent); 5.78 (1H, bs, exchangeable, concentration dependent); 5.16 (1H, d, J= 5.5 Hz); 4.90 (1H, s); 3.96 (3H, s).

IR (NaCl, film) υ= 3268, 2996, 1715, 1624, 1482, 1338, 1174, 1042, 754 cm^{-1}.

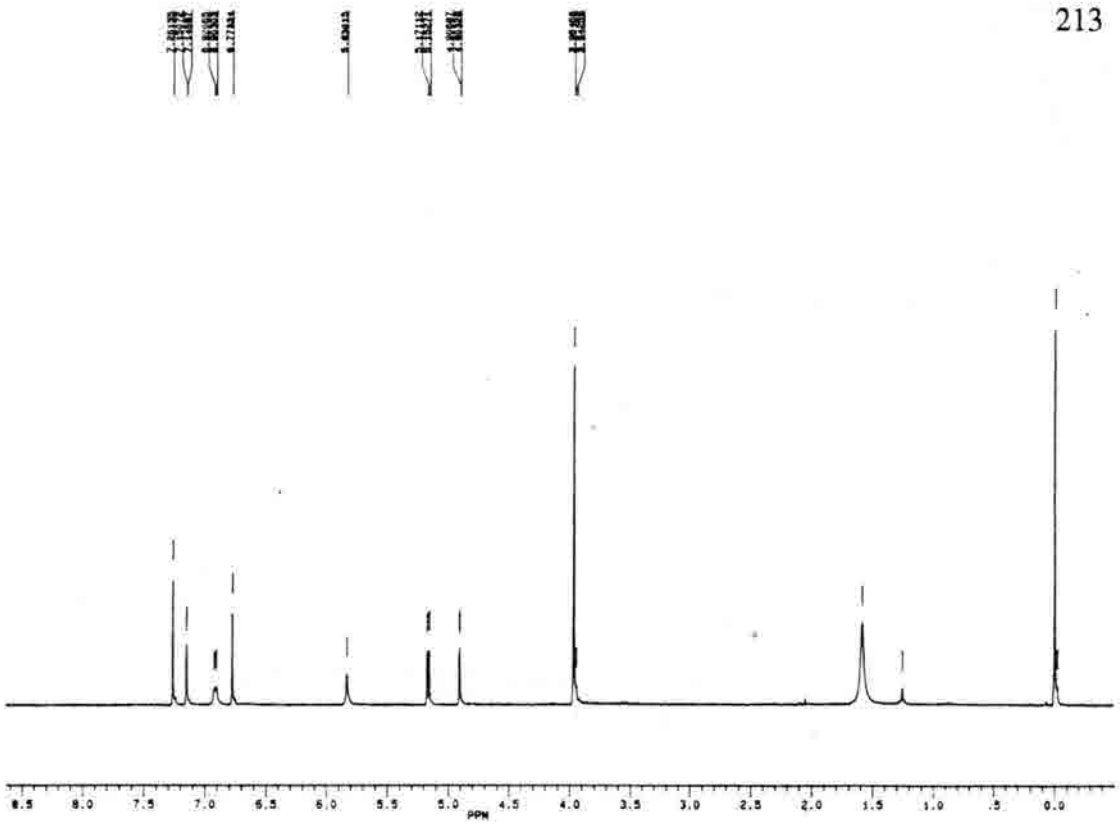
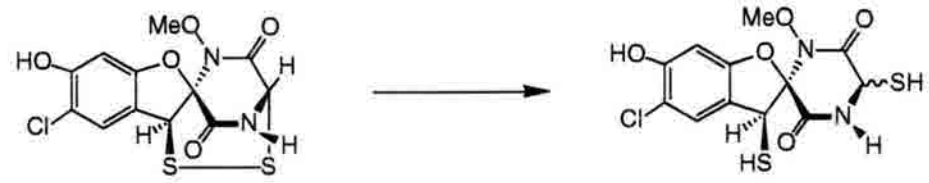


Figure 21. 300 mHz NMR of synthetic (±)-aspirochlorine in $CDCl_3$



80 **364**

Aspirochlorine dithiol (364)

9 mg (20 μmol) of aspirochlorine was dissolved in 400 μL of methanol at 0°C under argon. 2.2 mg of sodium borohydride in 200 μL methanol was added via syringe and the resulting solution stirred at 0°C for 15 minutes. 200 μL of 10% HCl was added followed by 1 mL chlorform and the resulting mixture vigorously stirred for 15 minutes. The layers were separated, and the organic phase transferred to a purged flask containing sodium sulfate. The aqueous layer was washed 3 times with chloroform (1 mL each) and the organic layers combined. After drying over sodium sulfate, the mother liquor was cannulated into a tarred flask and concentrated to a yellow film under vacuum. NMR of the crude routinely displayed >90% aspirochlorine dithiol with small amounts of the disulfide present. The sample was used without further purification, since attemtped recrystallization only resulted in increasing amounts of the disulfide. The dithiol was stored either as a solid or in acetonitrile at -80 °C under argon.

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.25 (1H, s); 6.66 (1H, bs), 6.60 (1H, s); 5.22 (1H, dd, J= 3.2, 7.5 Hz); 4.95 (1H, d, J= 12.3 Hz); 3.97 (3H, s); 3.37 (1H, d, J= 7.5 Hz); 2.31 (1H, s, J= 12.3 Hz).

IR (NaCl film) υ= 3229, 2919, 2557, 1694, 1625, 1430, 1153. 1034 cm^{-1}.

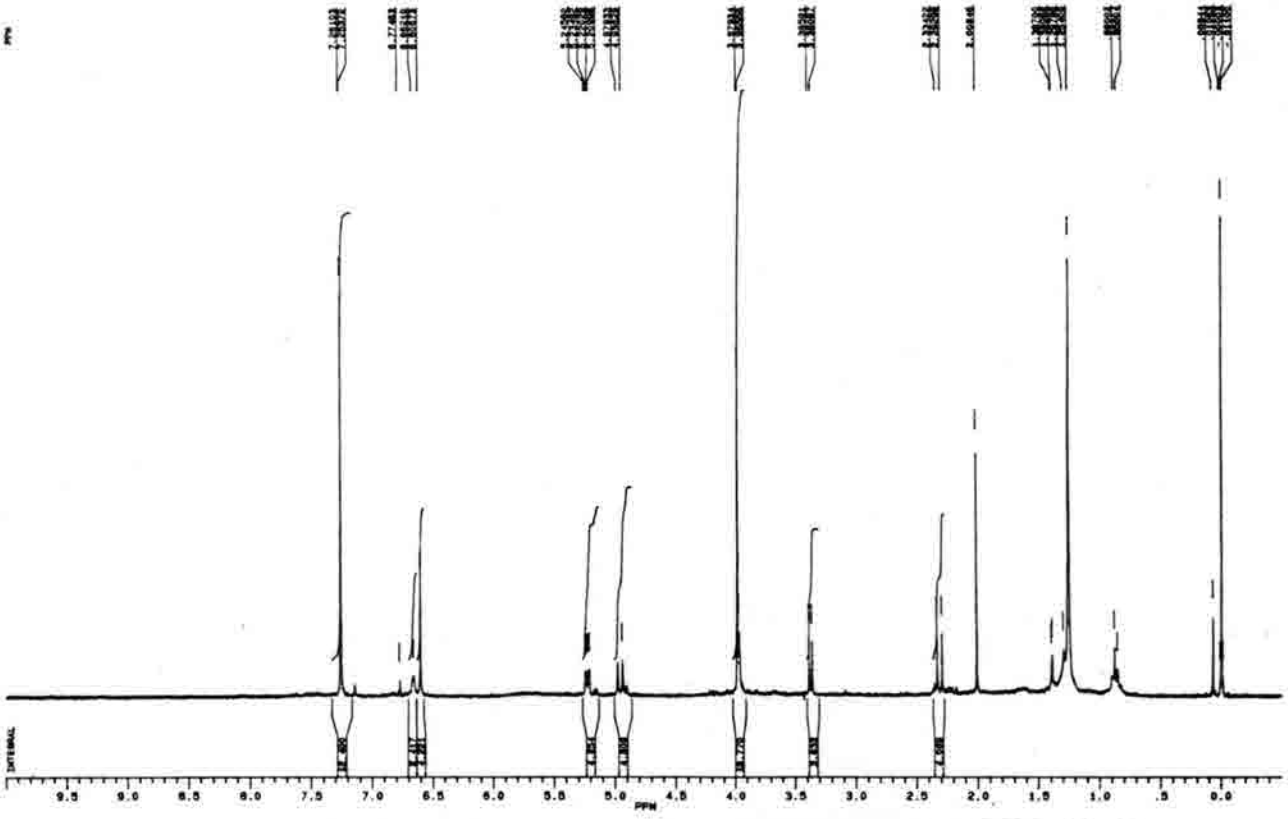
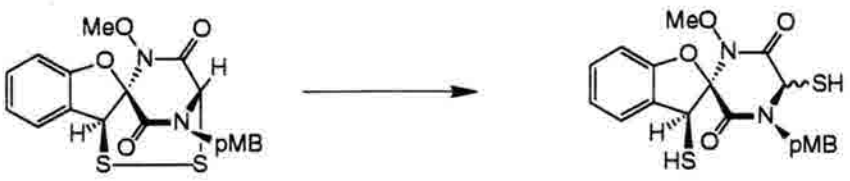


Figure 22. 300 mHz NMR of crude aspirochlorine dithiol **364** in $CDCl_3$



292a **361**

Trans-3,5'-dithio-1'-methoxy-4'-(4-methoxybenzyl)-spiro[benzofuran-2(3H),2'-piperazine]-3',6'-dione (361)

9 mg (20 μmol) of **292a** was dissolved in 400 μL of methanol at 0°C under argon. 2.2 mg of sodium borohydride in 200 μL methanol was added via syringe and the resulting solution stirred at 0°C for 15 minutes. 200 μL of 10% HCl was added followed by 1 mL chlorform and the resulting mixture vigorously stirred for 15 minutes. The layers were separated, and the organic phase transferred to a purged flask containing sodium sulfate. The aqueous layer was washed 3 times with chloroform (1 mL each) and the organic layers combined. After drying over sodium sulfate, the mother liquor was cannulated into a tarred flask and concentrated to a yellow film under vacuum. NMR of the crude routinely displayed >90% dithiol with small amounts of the disulfide present. The sample was used without further purification, since attemtped recrystallization only resulted in increasing amounts of the disulfide. The dithiol was stored either as a solid or in acetonitrile at -80 °C under argon.

^{1}H NMR (300 mHz, $CDCl_3$) δ= 7.34-7.01 (5H, m); 6.9- 6.88 (3H, m); 5.44 (1H, d, J= 14.3 Hz); 5.00(1H, d, J= 7.91 Hz); 4.18 (1H, d, J= 14.3 Hz); 3.95 (3H, s); 3.82 (3H, s); 3.18 (1H, d, J= 7.9 Hz); 2.31 (1H, d, J= 12.4 Hz).

IR (NaCl film) υ= 2981, 2557, 1680, 1511, 1238 cm^{-1}.

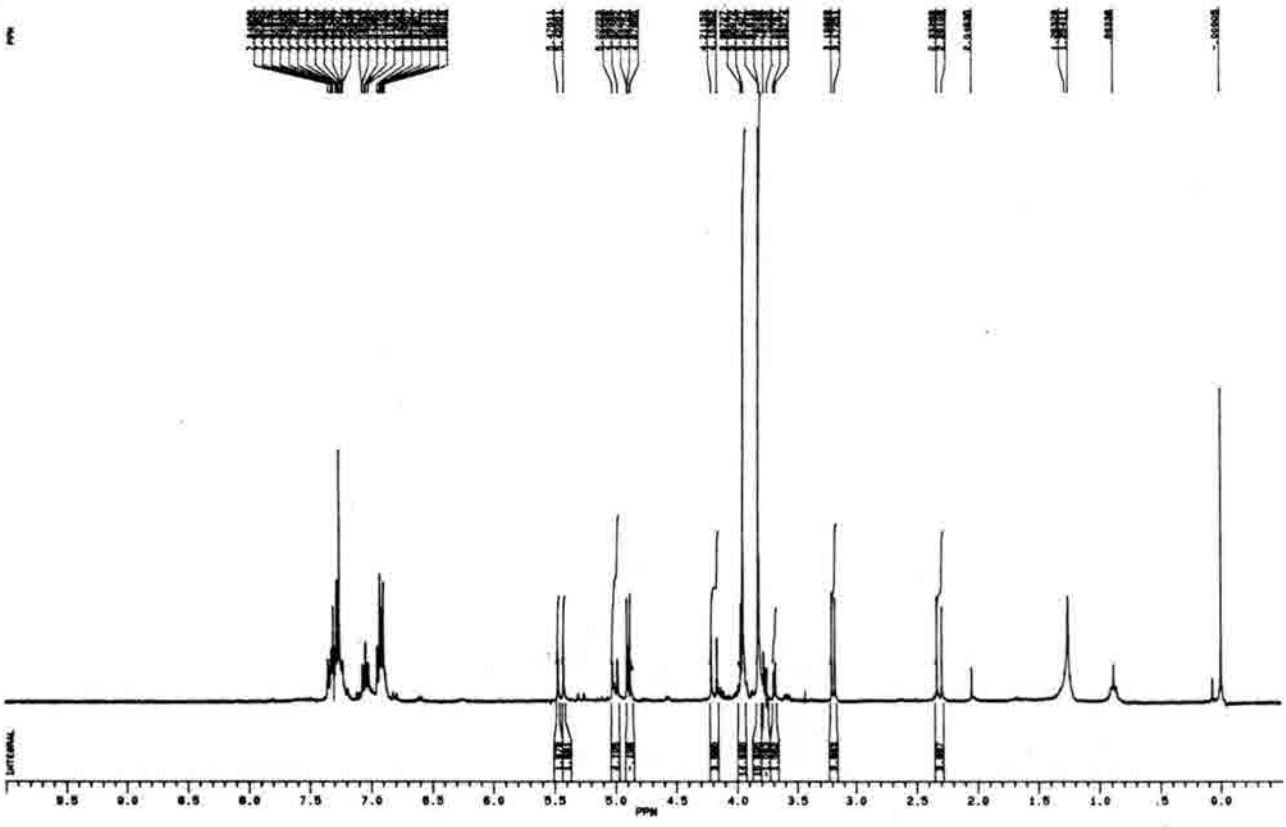


Figure 23. 300 mHz NMR of crude aspirochlorine dithiol **361** in $CDCl_3$

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Appendix 1
Additional Spectral Data

Appendix 1 contains additional spectroscopic data collected on key synthetic intermediates during the course of studies. The individual compounds are identified by the original laboratory designations. A list of the data included herein is as follows:

Figure 1. HETCOR NMR analysis of bromide **274** (GM-204).

Figure 2. X-ray crystallographic coordinates for bromide **274** (GM-204).

Figure 3. NOE ^{1}H NMR of *trans* alcohol **287a** (GFM-1-262-H).

Figure 4. NOE ^{1}H NMR of *cis* alcohol **287b** (GFM-262-L).

Figure 5. NOE ^{1}H NMR of *trans* thioacetate **288a** (GFM-1-315).

Figure 6. X-Ray crystallographic coordinates for disulfide **292a** (GFM-393-H).

Figure 7. X-ray crystallographic coordinates for disulfide **292b** (GFM-393-L).

Figure 8. NOE ^{1}H NMR of *trans* alcohol **346a** (GFM-754-1).

Figure 9. NOE ^{1}H NMR of *cis* alcohol **346b** (GFM-754-2).

Figure 10. Mass spectral analysis of monosulfide **351** (GFM-1091).

Figure 11. ^{1}H NMR of monosulfide **351** (GFM-1091).

Figure 12. HPLC analysis of synthetic and natural aspirochlorine.

Figure 1 HETCOR NMR of bromide 274

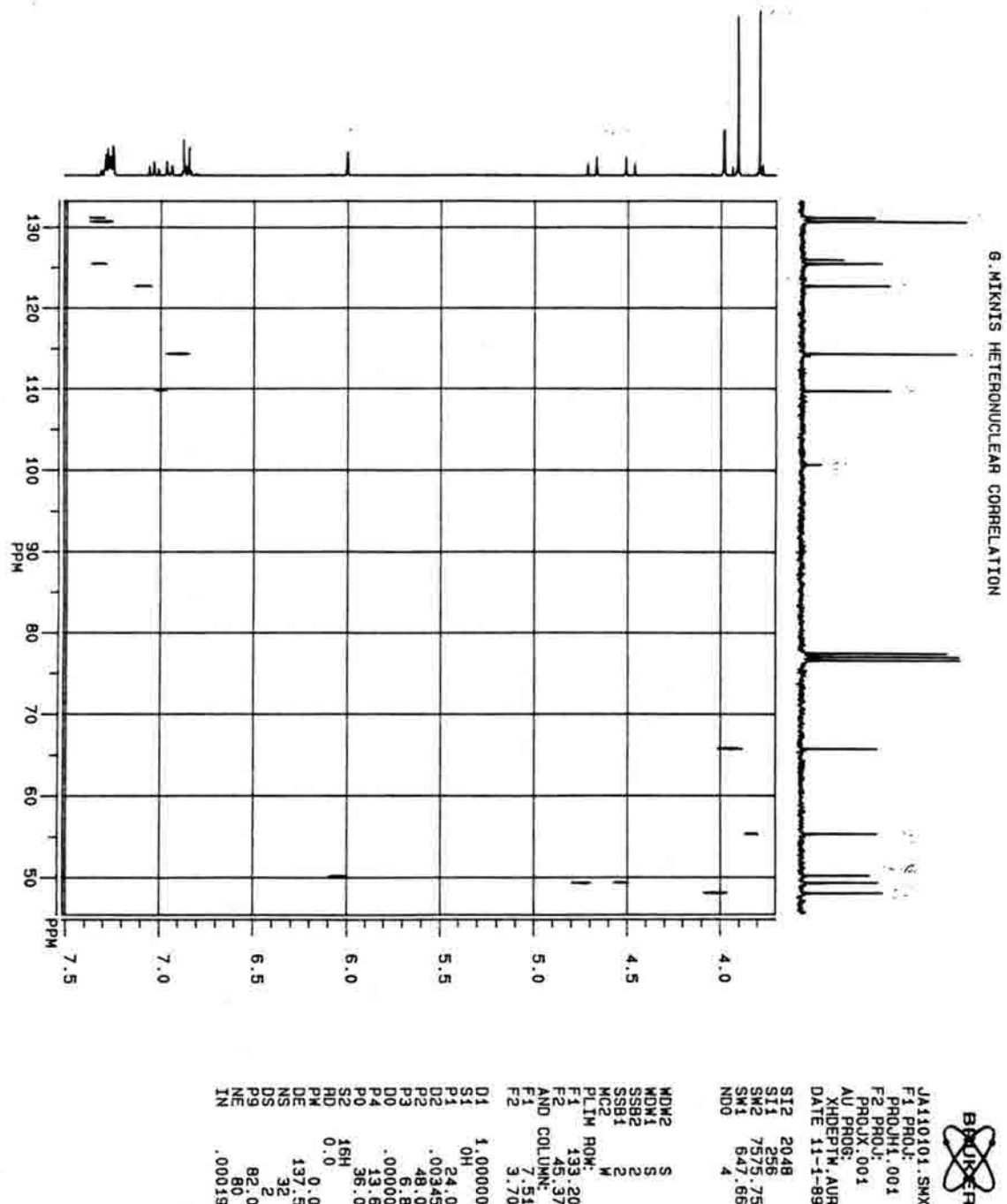


Figure 2 X-ray coordinates for bromide 274

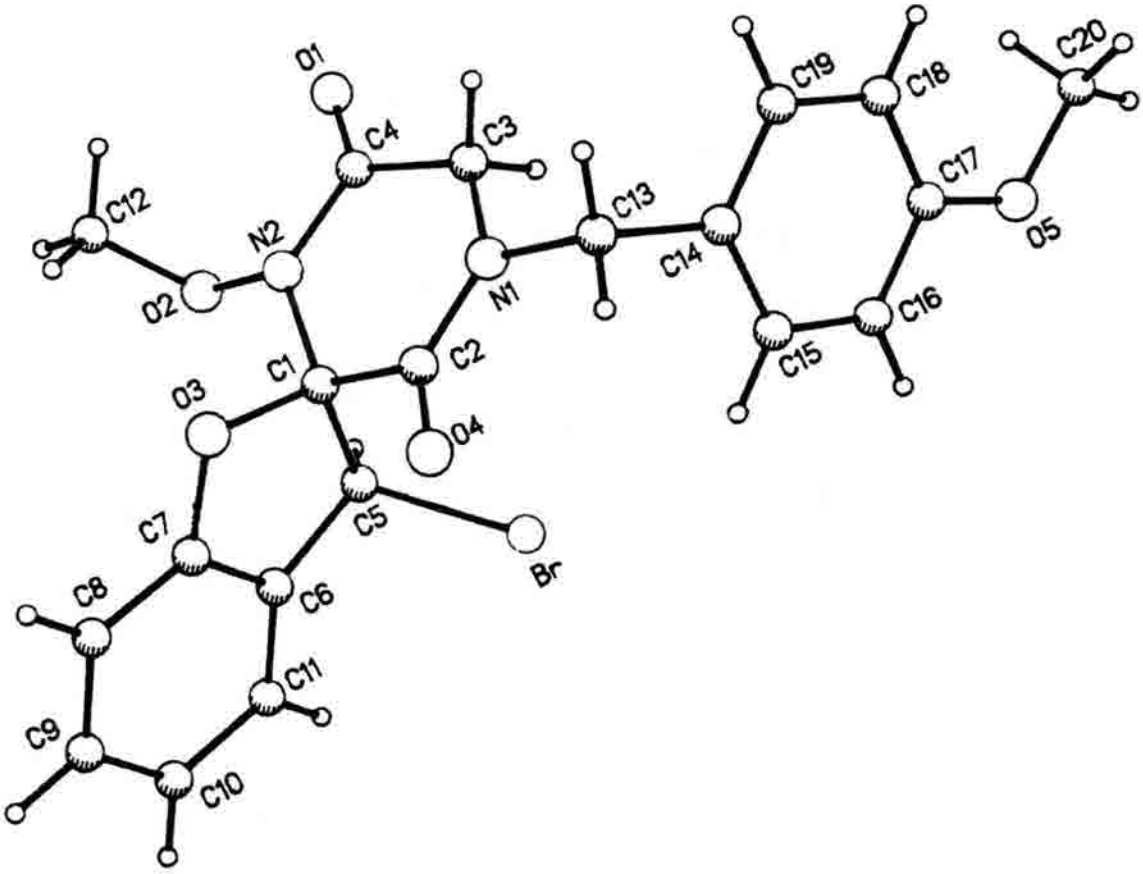


Figure 2 continued

TABLE 1 Atomic coordinates ($\times 10^4$) and isotropic thermal parameters ($Å^2 \times 10^3$)[a] for $C_{20}H_{19}BrN_2O_5$ RW19

atom	x	y	z	U^b_{iso}
Br	645(1)	9066(1)	10289(1)	36(1)*
N1	3901(7)	8112(3)	9684(3)	28(2)*
N2	5055(7)	8904(4)	10554(3)	20(2)*
O1	6579(6)	9621(3)	10008(2)	30(2)*
O2	5372(5)	9401(3)	10986(2)	26(2)*
O3	3941(6)	7998(3)	11093(2)	28(2)*
O4	2468(7)	7407(3)	10257(2)	41(2)*
O5	776(7)	9597(3)	7667(2)	38(2)*
C1	3670(9)	8487(4)	10634(3)	20(3)*
C2	3293(9)	7954(4)	10161(3)	25(3)*
C3	4928(9)	8735(4)	9581(3)	25(3)*
C4	5619(9)	9134(4)	10058(3)	27(2)*
C5	2316(9)	9023(5)	10798(3)	27(3)*
C6	1857(9)	8712(4)	11329(3)	26(3)*
C7	2833(8)	8130(4)	11467(3)	21(3)*
C8	2721(10)	7727(5)	11945(3)	26(3)*
C9	1559(10)	7936(5)	12290(3)	28(3)*
C10	544(10)	8515(5)	12157(3)	33(3)*
C11	684(10)	8910(4)	11676(3)	33(3)*
C12	6751(9)	9171(5)	11238(4)	38(3)*
C13	3496(10)	7647(5)	9215(3)	35(3)*
C14	2753(11)	8137(5)	8783(4)	31(3)*
C15	1303(11)	8416(5)	8846(4)	36(3)*
C16	680(10)	8903(5)	8468(3)	37(3)*
C17	1487(9)	9106(5)	8008(3)	26(3)*
C18	2916(10)	8823(5)	7940(3)	29(3)*
C19	3552(11)	8343(5)	8324(4)	35(3)*
C20	1535(11)	9783(6)	7170(4)	44(4)*

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) For values with asterisks, the equivalent isotropic U is defined as 1/3 of the trace of the U_{ij} tensor.

Figure 2 continued

TABLE 2 Bond lengths (Å)[a] for $C_{20}H_{19}BrN_2O_5$ RW19

Br-C5	1.946(8)	N1-C2	1.331(10)
N1-C3	1.438(10)	N1-C13	1.462(10)
N2-O2	1.407(8)	N2-C1	1.437(10)
N2-C4	1.388(10)	O1-C4	1.206(9)
O2-C12	1.427(10)	O3-C1	1.445(9)
O3-C7	1.368(9)	O4-C2	1.222(10)
O5-C17	1.358(10)	O5-C20	1.441(11)
C1-C2	1.533(11)	C1-C5	1.571(11)
C3-C4	1.505(11)	C5-C6	1.482(11)
C6-C7	1.375(11)	C6-C11	1.391(12)
C7-C8	1.384(11)	C8-C9	1.387(12)
C9-C10	1.390(12)	C10-C11	1.386(12)
C13-C14	1.521(12)	C14-C15	1.379(13)
C14-C19	1.389(13)	C15-C16	1.380(12)
C16-C17	1.391(12)	C17-C18	1.366(12)
C18-C19	1.389(12)		

(a) Estimated standard deviations in the least significant digits are given in parentheses.

TABLE 3 Bond angles (deg)[a] for $C_{20}H_{19}BrN_2O_5$ RW19

C2-N1-C3	124.7(6)	C2-N1-C13	119.8(6)
C3-N1-C13	115.5(6)	O2-N2-C1	112.1(6)
O2-N2-C4	115.4(6)	C1-N2-C4	125.0(6)
N2-O2-C12	109.4(6)	C1-O3-C7	108.6(6)
C17-O5-C20	117.5(7)	N2-C1-O3	105.4(6)
N2-C1-C2	112.7(6)	O3-C1-C2	106.5(6)
N2-C1-C5	112.5(6)	O3-C1-C5	105.7(6)
C2-C1-C5	113.2(6)	N1-C2-O4	125.1(7)
N1-C2-C1	118.0(7)	O4-C2-C1	116.9(7)
N1-C3-C4	117.6(6)	N2-C4-O1	123.1(7)
N2-C4-C3	114.9(7)	O1-C4-C3	121.9(7)
Br-C5-C1	115.5(5)	Br-C5-C6	112.7(5)
C1-C5-C6	102.9(6)	C5-C6-C7	108.6(7)
C5-C6-C11	131.6(7)	C7-C6-C11	119.7(7)
O3-C7-C6	113.8(7)	O3-C7-C8	123.3(7)
C6-C7-C8	122.9(7)	C7-C8-C9	116.9(7)
C8-C9-C10	121.3(8)	C9-C10-C11	120.6(8)
C6-C11-C10	118.5(8)	N1-C13-C14	110.9(6)
C13-C14-C15	121.3(8)	C13-C14-C19	120.4(8)
C15-C14-C19	118.3(8)	C14-C15-C16	120.7(8)
C15-C16-C17	120.7(8)	O5-C17-C16	115.9(7)
O5-C17-C18	125.2(7)	C16-C17-C18	119.0(8)
C17-C18-C19	120.3(8)	C14-C19-C18	121.0(8)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

Figure 2 continued

TABLE 4 Anisotropic thermal parameters ($Å^2 \times 10^3$)[a,b]

for $C_{20}H_{19}BrN_2O_5$ RW19 MIKNIS/WILLIAMS

atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Br	24(1)	47(1)	38(1)	20(1)	-4(1)	4(1)
N1	45(4)	11(3)	27(4)	-4(3)	-17(4)	-1(3)
N2	18(3)	19(4)	23(4)	-6(3)	-1(3)	-1(3)
O1	32(4)	20(3)	38(4)	6(3)	5(3)	-5(3)
O2	15(3)	34(3)	30(3)	-12(3)	-2(3)	3(3)
O3	32(4)	23(3)	31(3)	12(3)	4(3)	6(3)
O4	48(4)	28(3)	47(4)	5(3)	-8(4)	-15(3)
O5	33(4)	41(4)	39(4)	5(3)	-9(4)	2(4)
C1	15(4)	11(4)	33(5)	5(4)	1(4)	-1(4)
C2	25(5)	19(4)	32(6)	2(4)	-11(4)	6(4)
C3	30(5)	17(4)	28(5)	-0(4)	-7(4)	3(4)
C4	28(4)	13(4)	39(5)	-1(4)	2(5)	13(5)
C5	23(4)	26(5)	31(5)	6(5)	4(4)	-11(5)
C6	21(5)	17(4)	39(5)	-3(4)	-7(4)	-2(4)
C7	13(4)	25(5)	24(5)	1(4)	-6(4)	-5(4)
C8	23(5)	25(5)	31(5)	6(4)	-10(4)	-4(4)
C9	37(5)	31(5)	18(5)	-1(4)	-1(4)	-12(5)
C10	23(5)	43(6)	32(5)	-7(4)	4(5)	-9(5)
C11	25(4)	22(5)	53(6)	-4(4)	-6(5)	-8(5)
C12	23(5)	38(6)	52(6)	-16(5)	-14(5)	9(5)
C13	40(6)	21(5)	44(6)	-10(4)	-15(5)	-0(5)
C14	48(6)	13(5)	32(5)	-8(4)	-10(5)	-3(5)
C15	42(6)	37(6)	28(5)	1(5)	-4(5)	-3(5)
C16	20(4)	49(6)	41(5)	4(5)	-0(5)	-11(5)
C17	28(5)	19(5)	32(5)	-3(4)	0(4)	-0(5)
C18	31(5)	28(6)	29(5)	-8(4)	3(4)	1(5)
C19	32(5)	32(6)	42(6)	-12(5)	-3(5)	4(5)
C20	42(6)	58(7)	32(6)	13(5)	-4(5)	-2(6)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) The anisotropic thermal parameter exponent takes the form:

$$-2\pi^2(h^2a^{*2}U_{11}+k^2b^{*2}U_{22}+ \ldots +2hka^*b^*U_{12})$$

Figure 2 continued

TABLE 5 Hydrogen coordinates ($x10^4$) and thermal parameters ($Å^2 x10^3$) for $C_{20}H_{19}BrN_2O_5$ RW19 MIKNIS/WILLIAMS

atom	x	y	z	U_{iso}
H3A	5744	8536	9367	26
H3B	4385	9115	9378	26
H5	2623	9551	10807	30
H8	3415	7322	12033	36
H9	1454	7675	12629	31
H10	-262	8642	12400	30
H11	-13	9312	11583	45
H12A	6945	9528	11523	34
H12B	7591	9169	10993	34
H12C	6618	8666	11386	34
H13A	4395	7416	9071	38
H13B	2804	7252	9325	38
H15	720	8270	9155	38
H16	-320	9104	8522	43
H18	3484	8958	7624	27
H19	4559	8150	8271	40
H20A	942	10163	6986	43
H20B	1783	9375	6925	43
H20C	2449	10013	7302	43

Figure 3. NOE of trans alcohol 287a- CONTROL

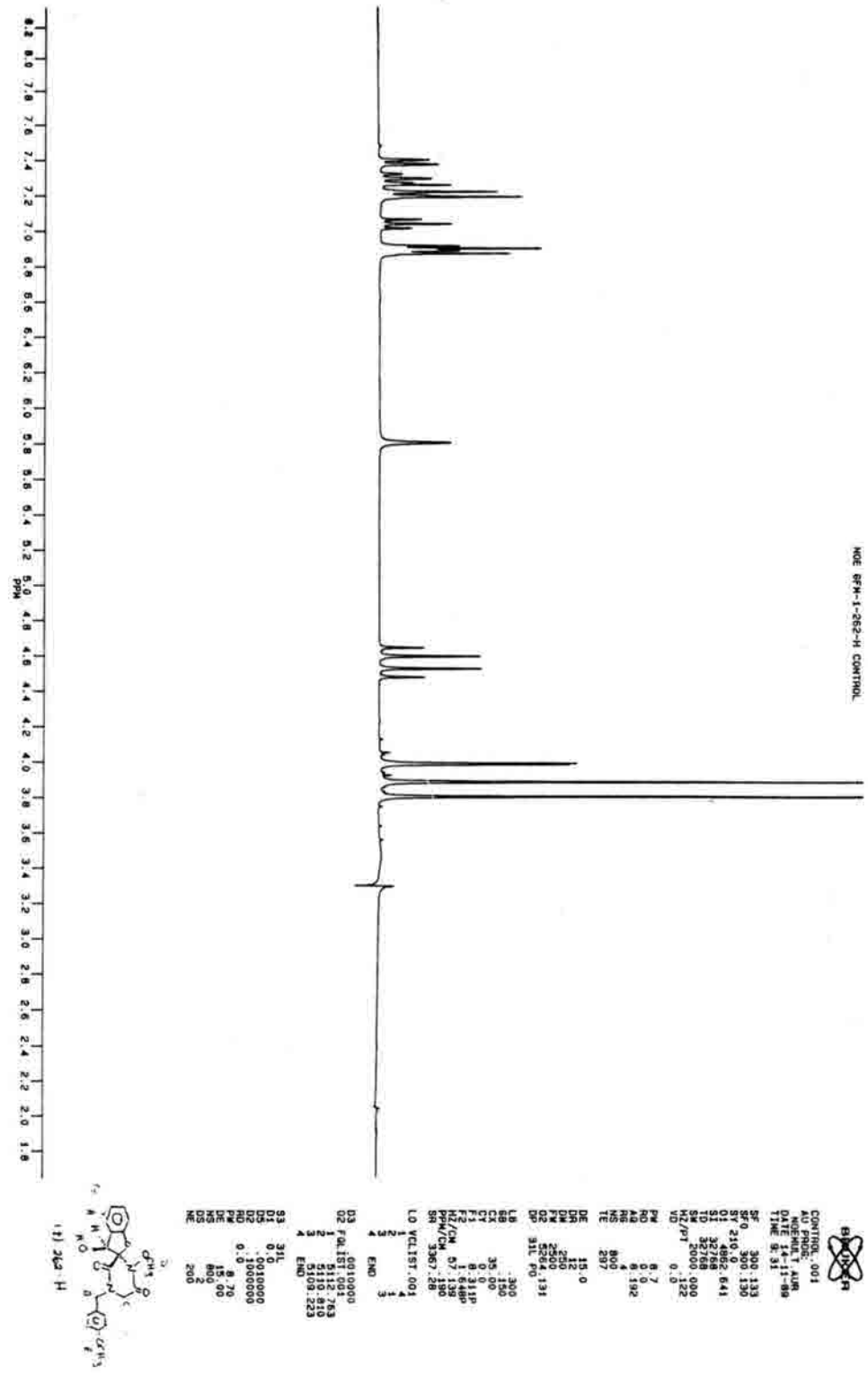


Figure 3. NOE of 287a- irradiation of Benzylic methine

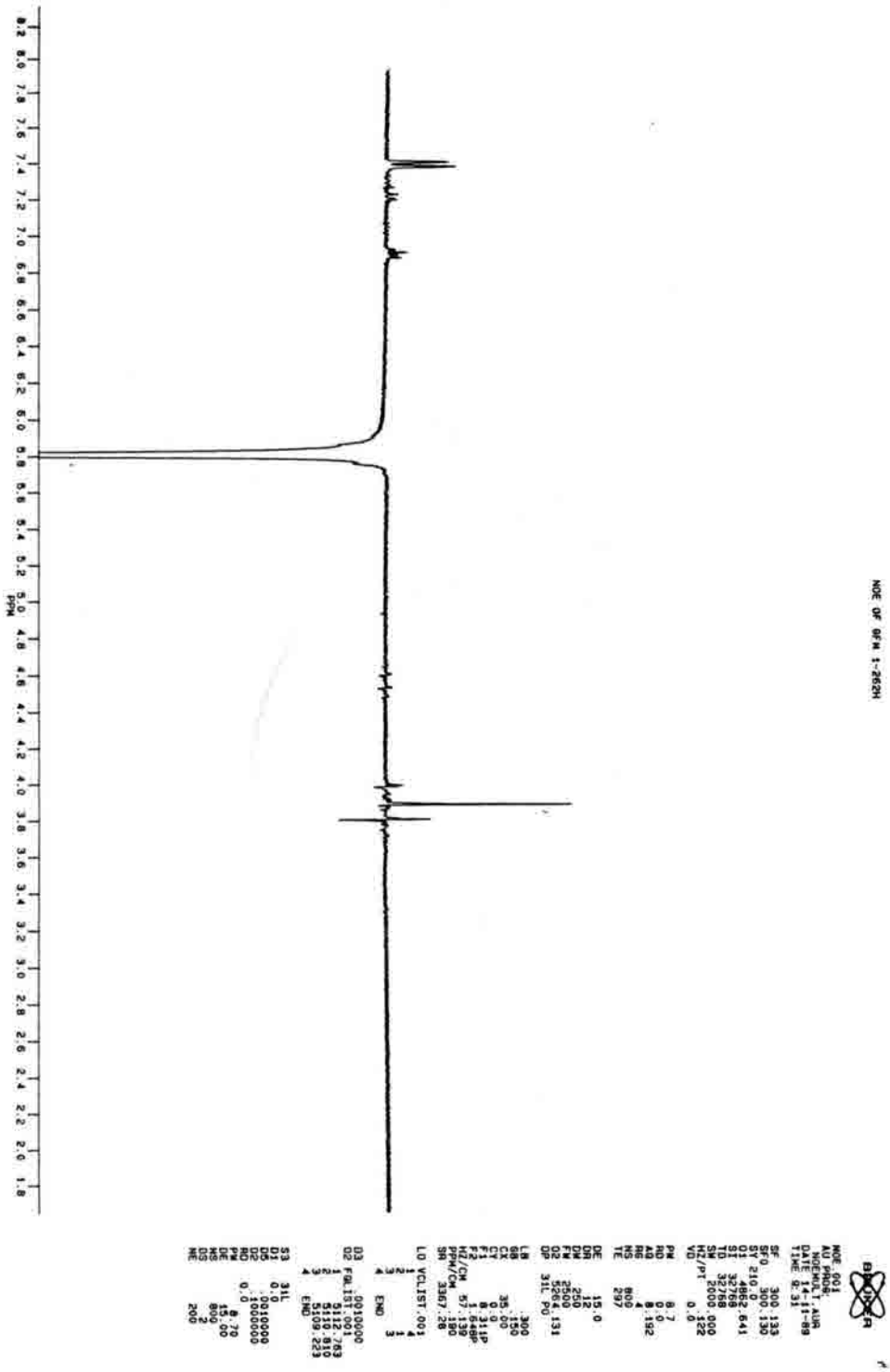


Figure 3. NOE of 287a- irradiation of N-methoxyl

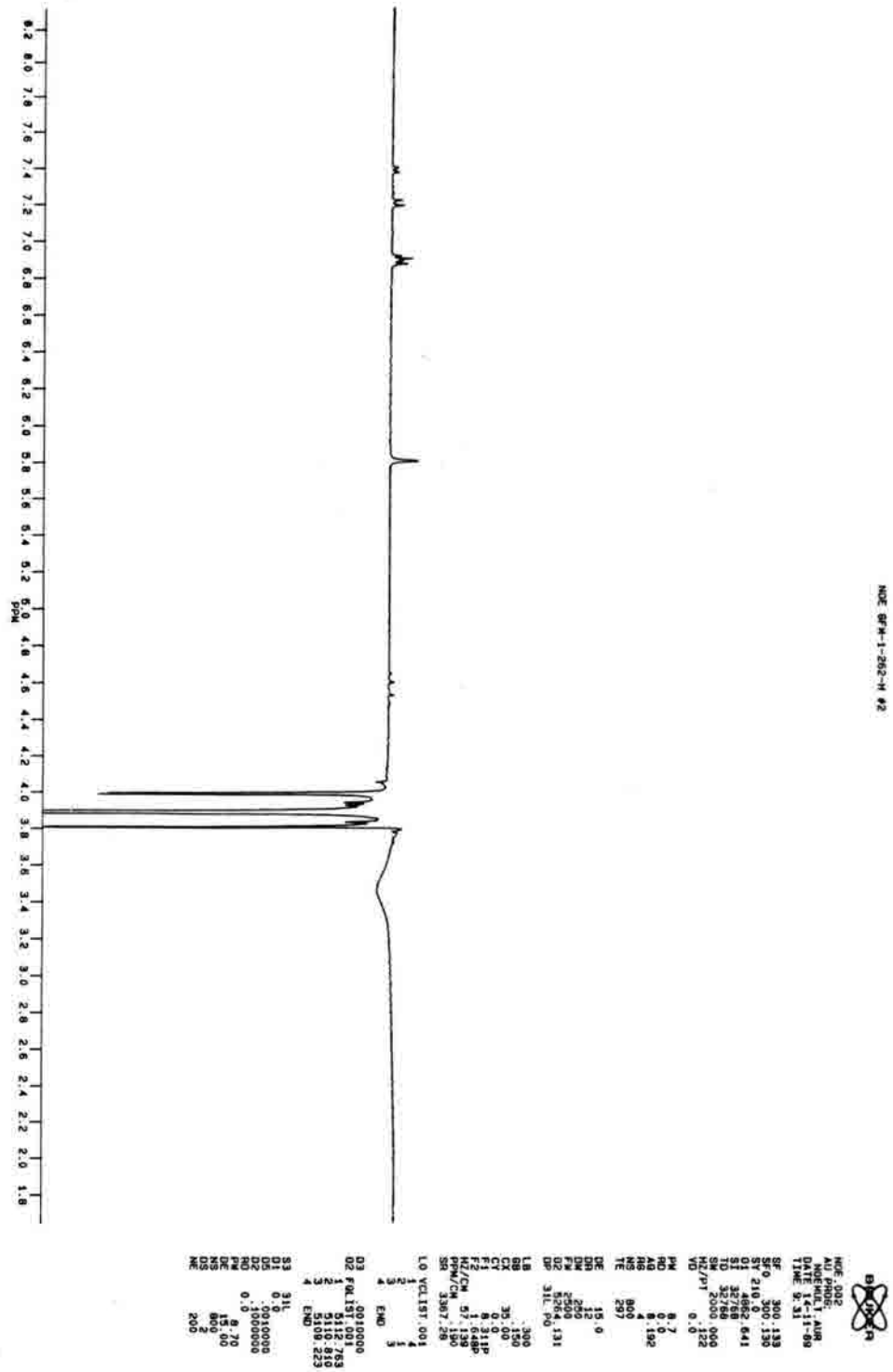


Figure 3. NOE of 287a- irradiation of benzyl O-Methyl

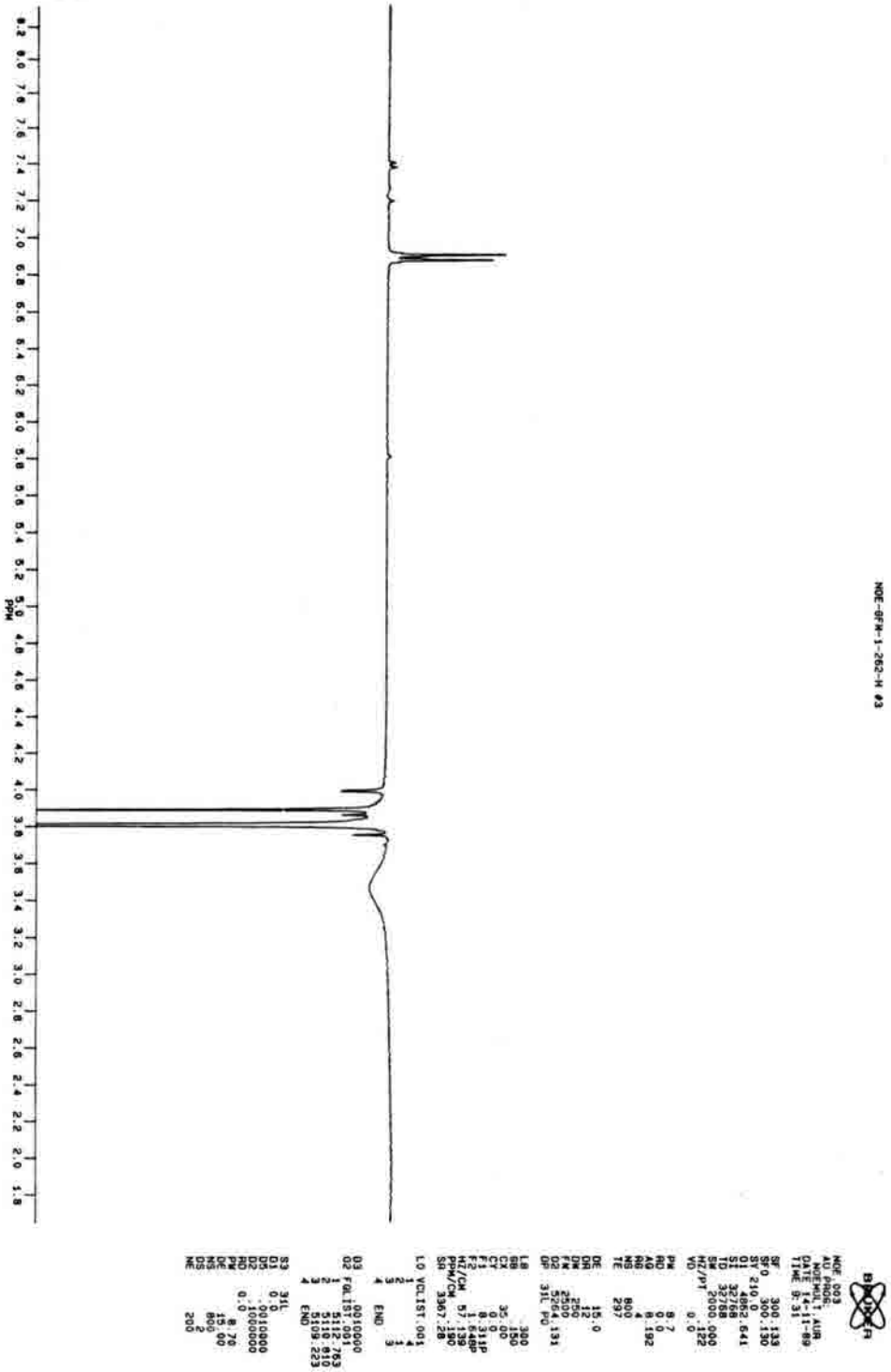


Figure 4. NOE of cis alcohol 287b- CONTROL

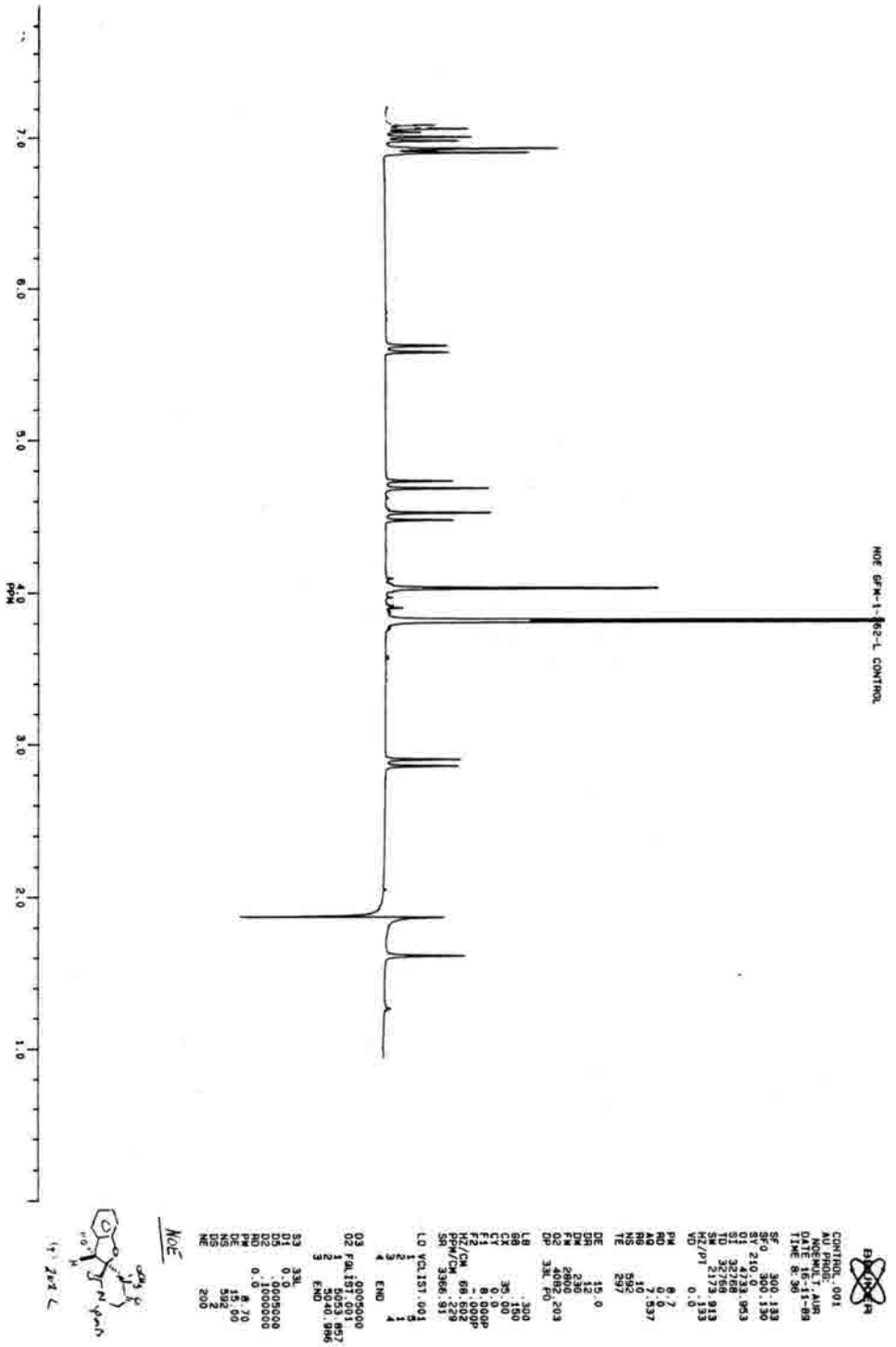


Figure 4. NOE of 287b- irradiation of Benzylic methine

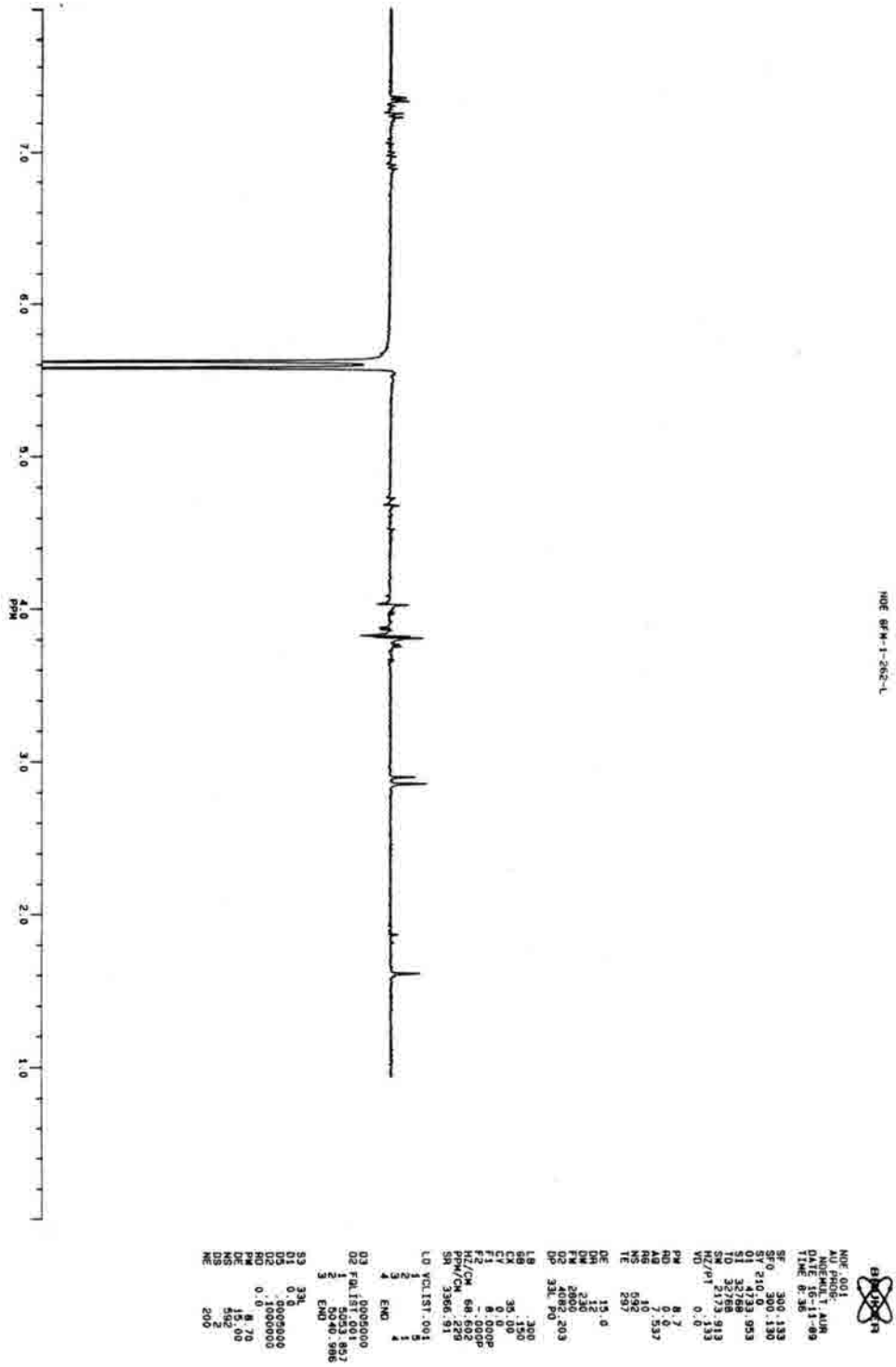


Figure 4. NOE of 287b- irradiation of Benzyl O-Methyl

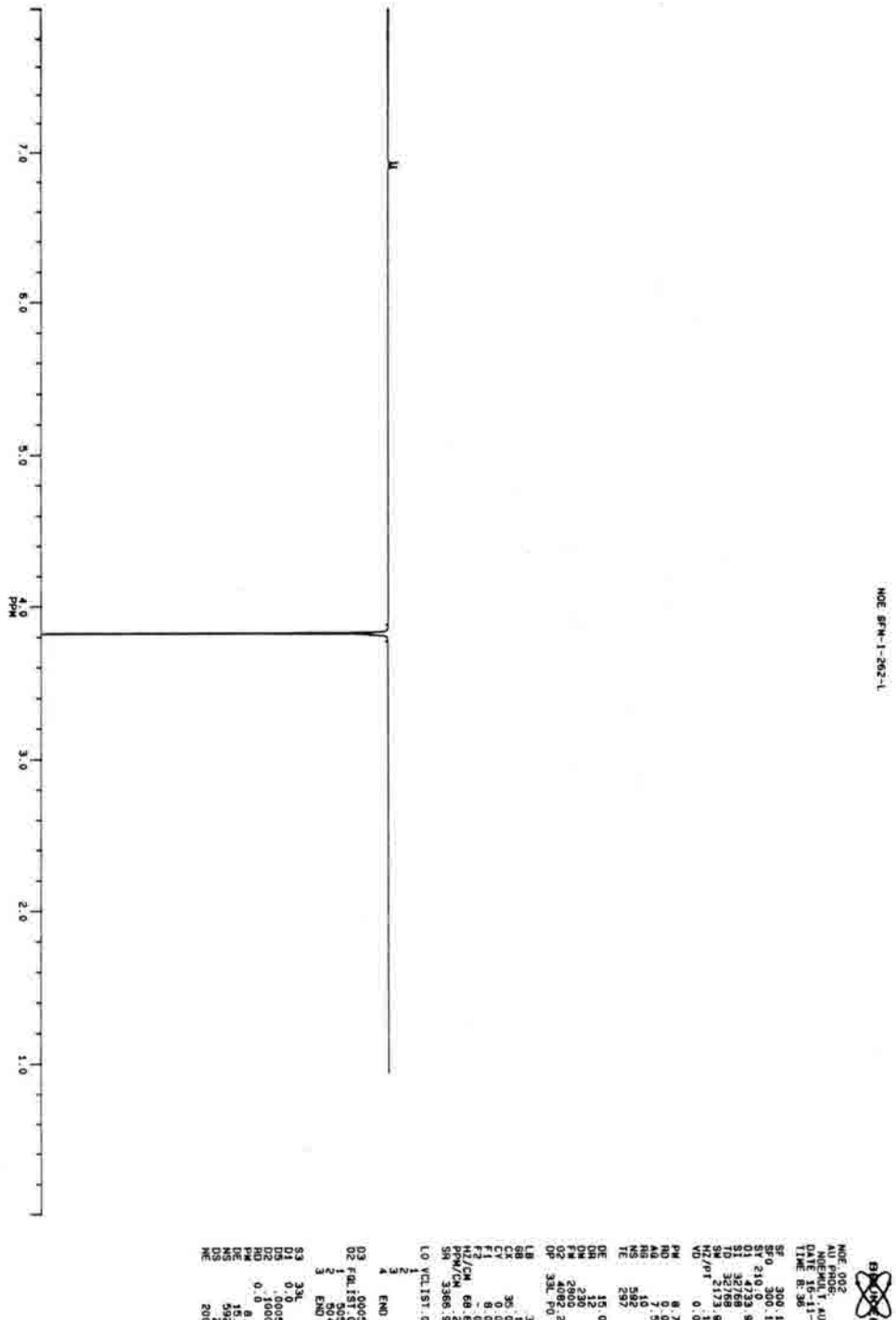


Figure 4. NOE of 287b- irradiation of N-methoxyl

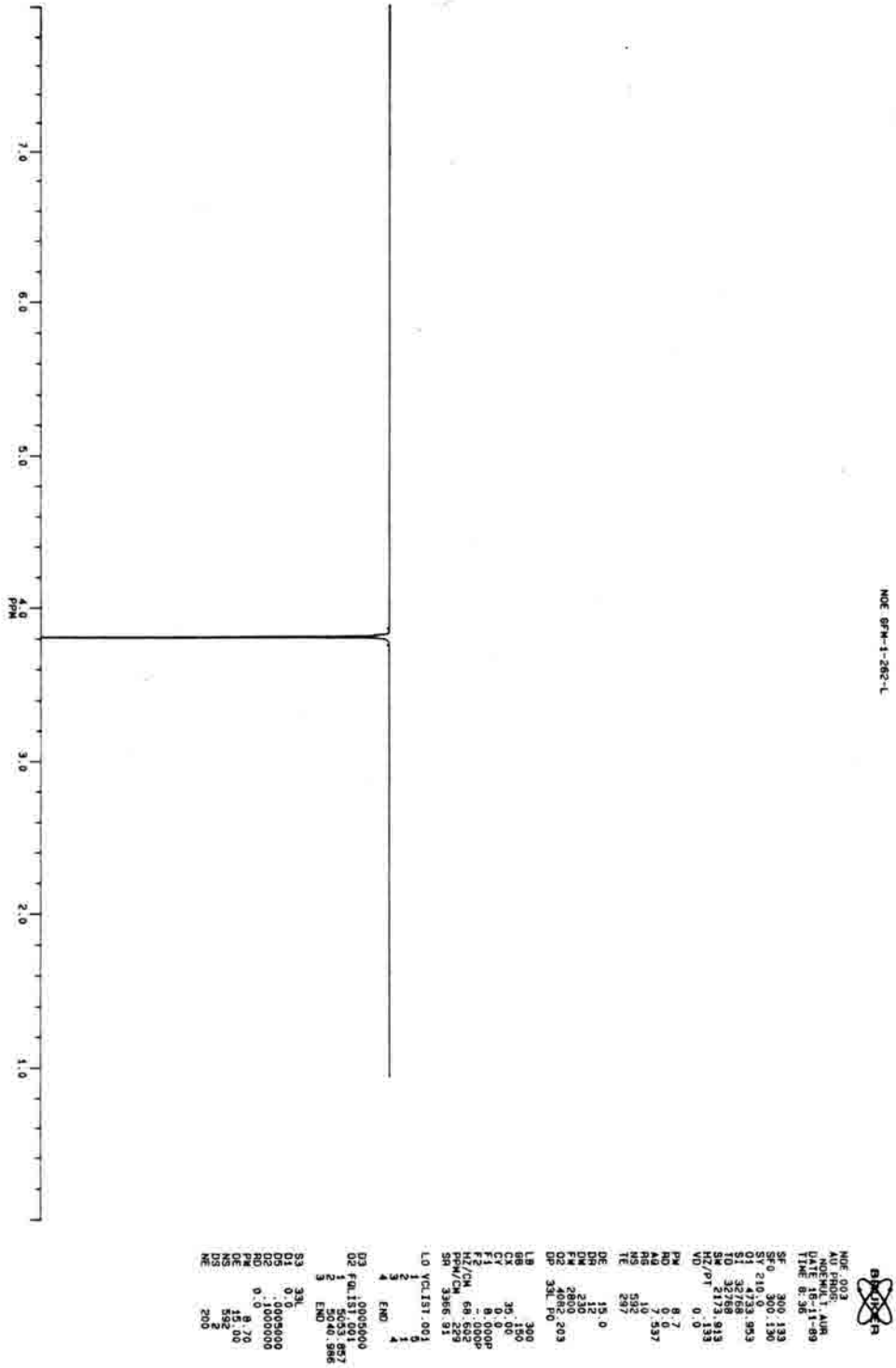


Figure 4. NOE of 287b- irradiation of Benzylic hydroxyl

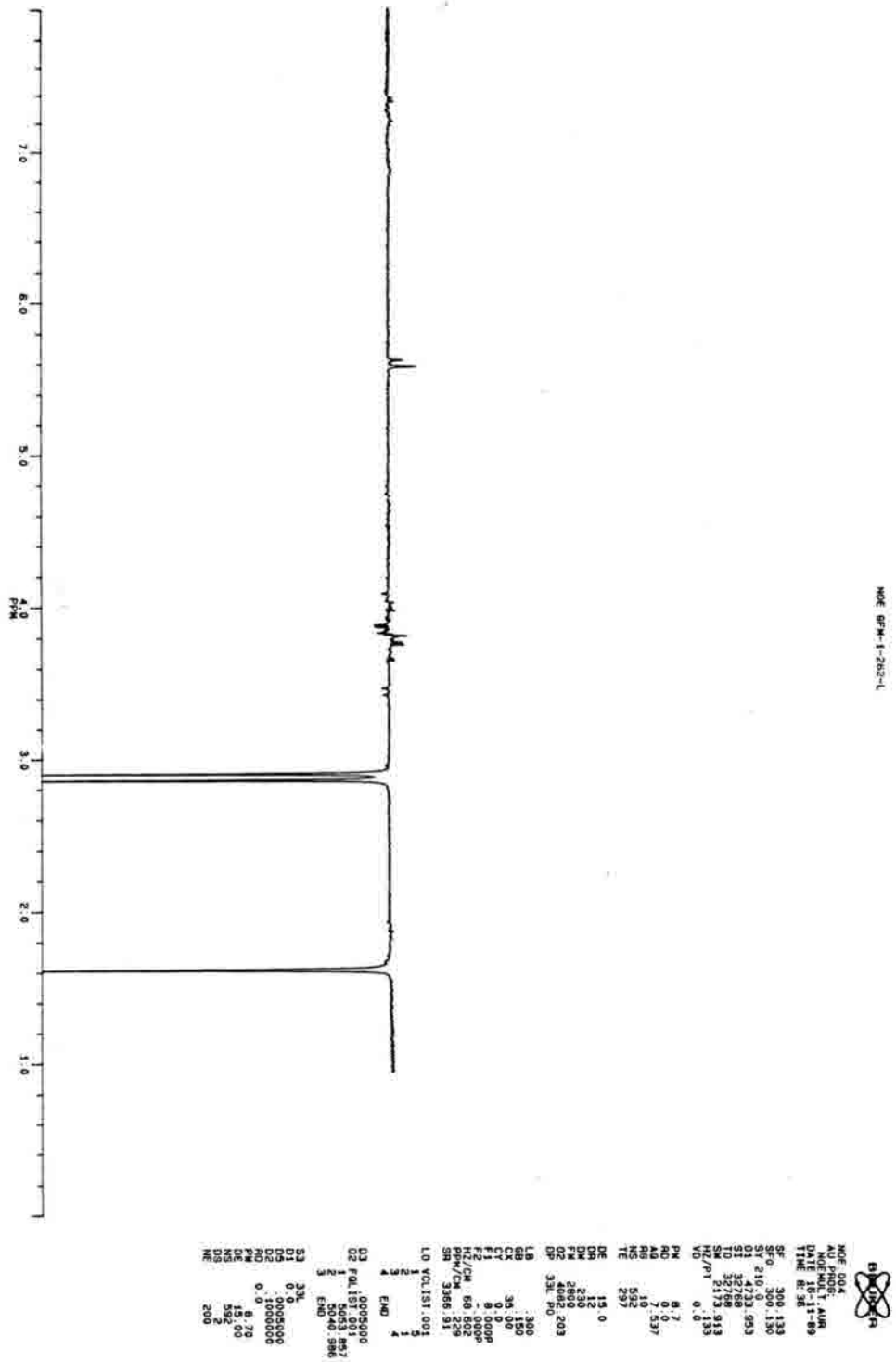


Figure 5. NOE of trans thioacetate 288a- CONTROL

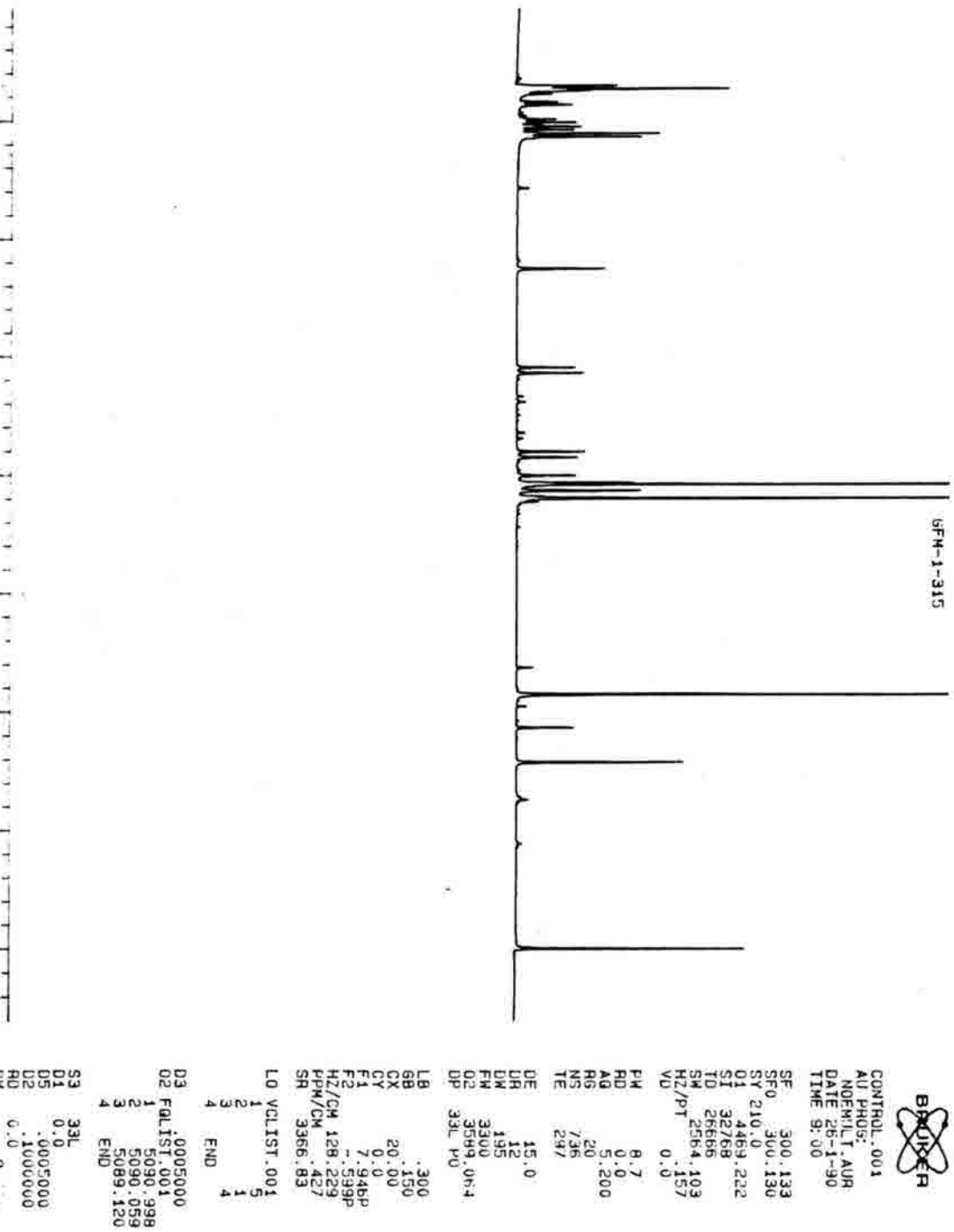


Figure 5. NOE of trans thioacetate 288a- irradiation of thioacetate

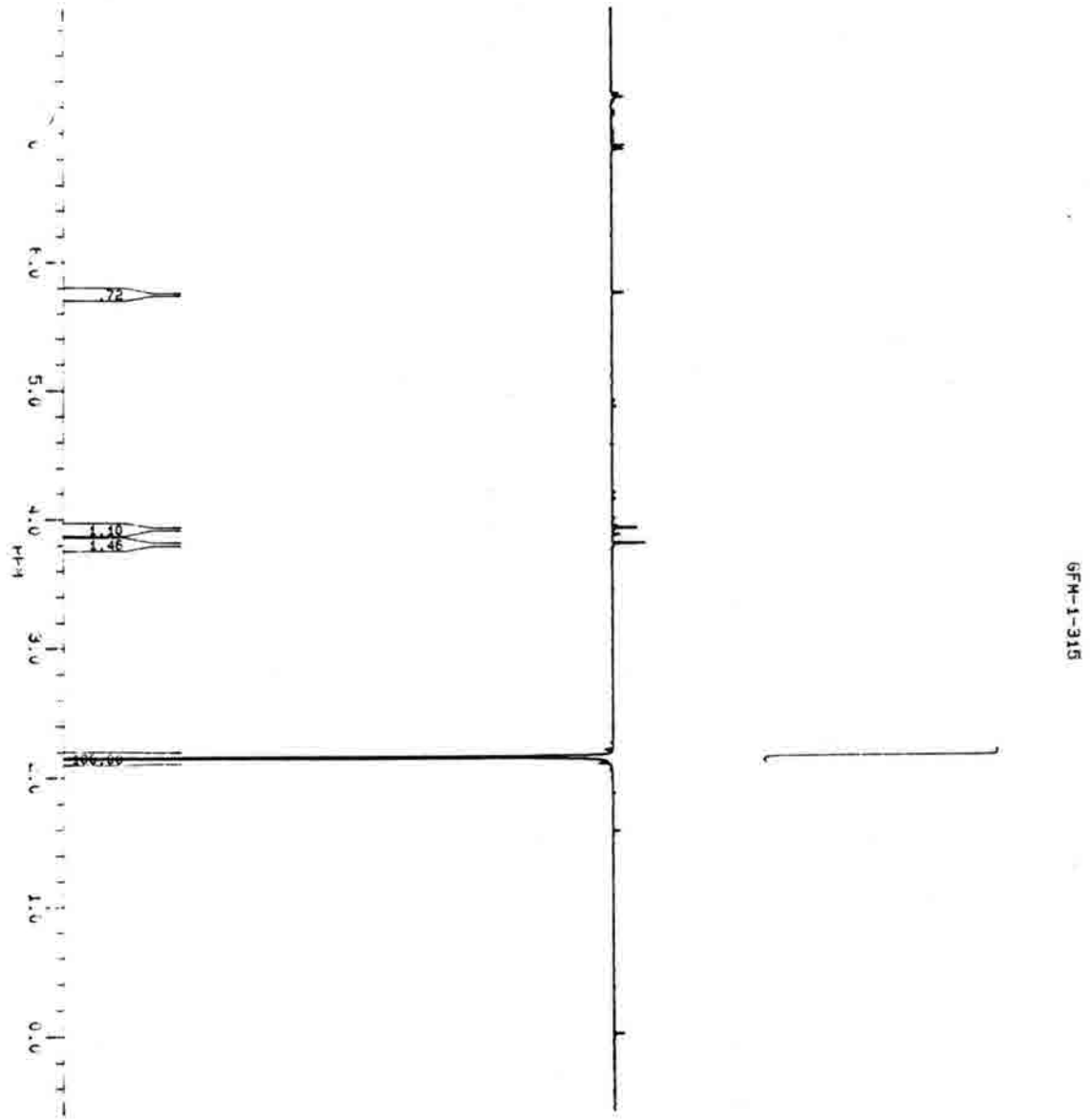


Figure 5. NOE of trans thioacetate 288a- irradiation of benzyl O-methyl

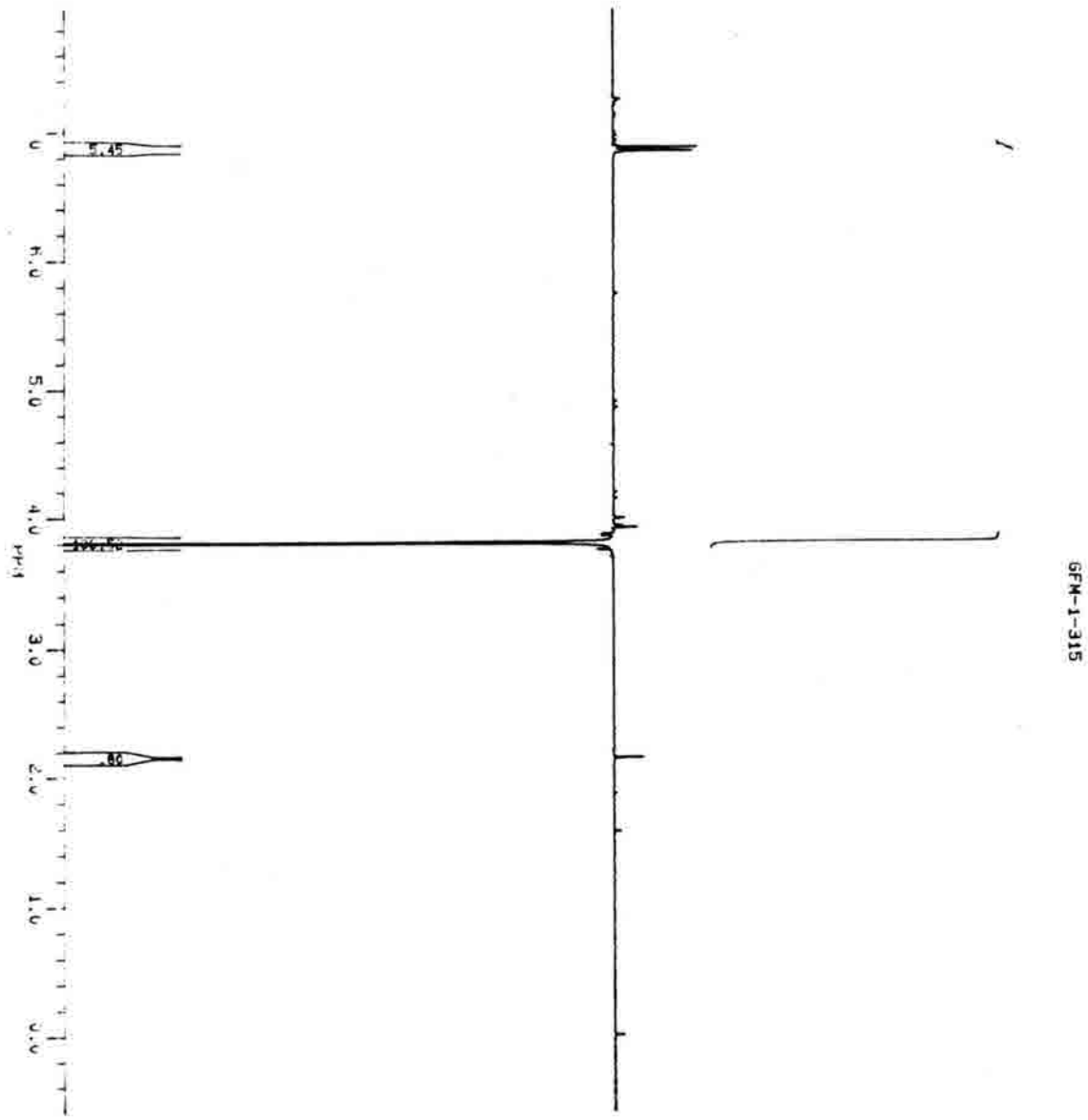


Figure 5. NOE of trans thioacetate 288a- irradiation of N-methoxyl

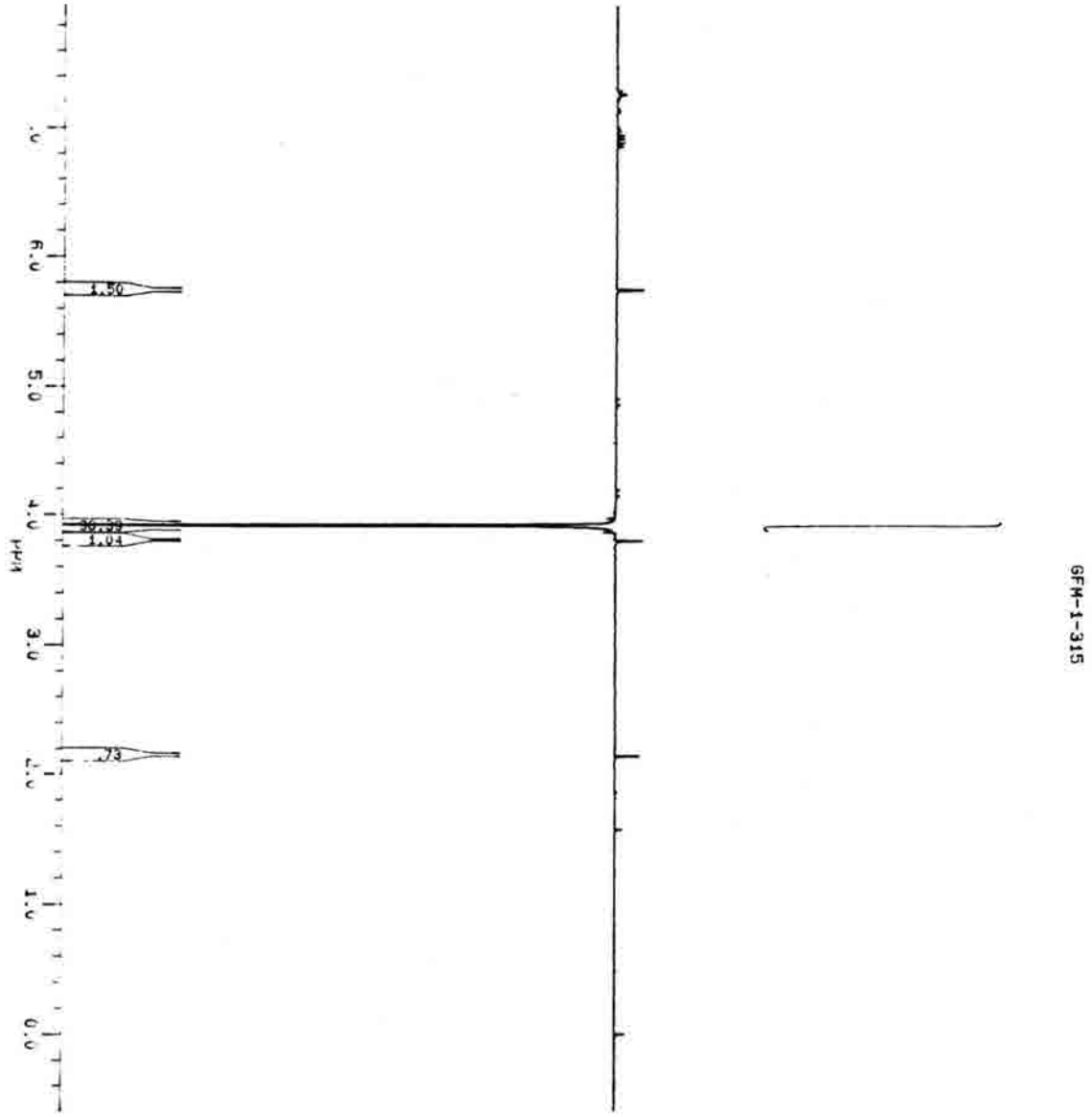


Figure 5. NOE of trans thioacetate 288a- irradiation of benzylic methine

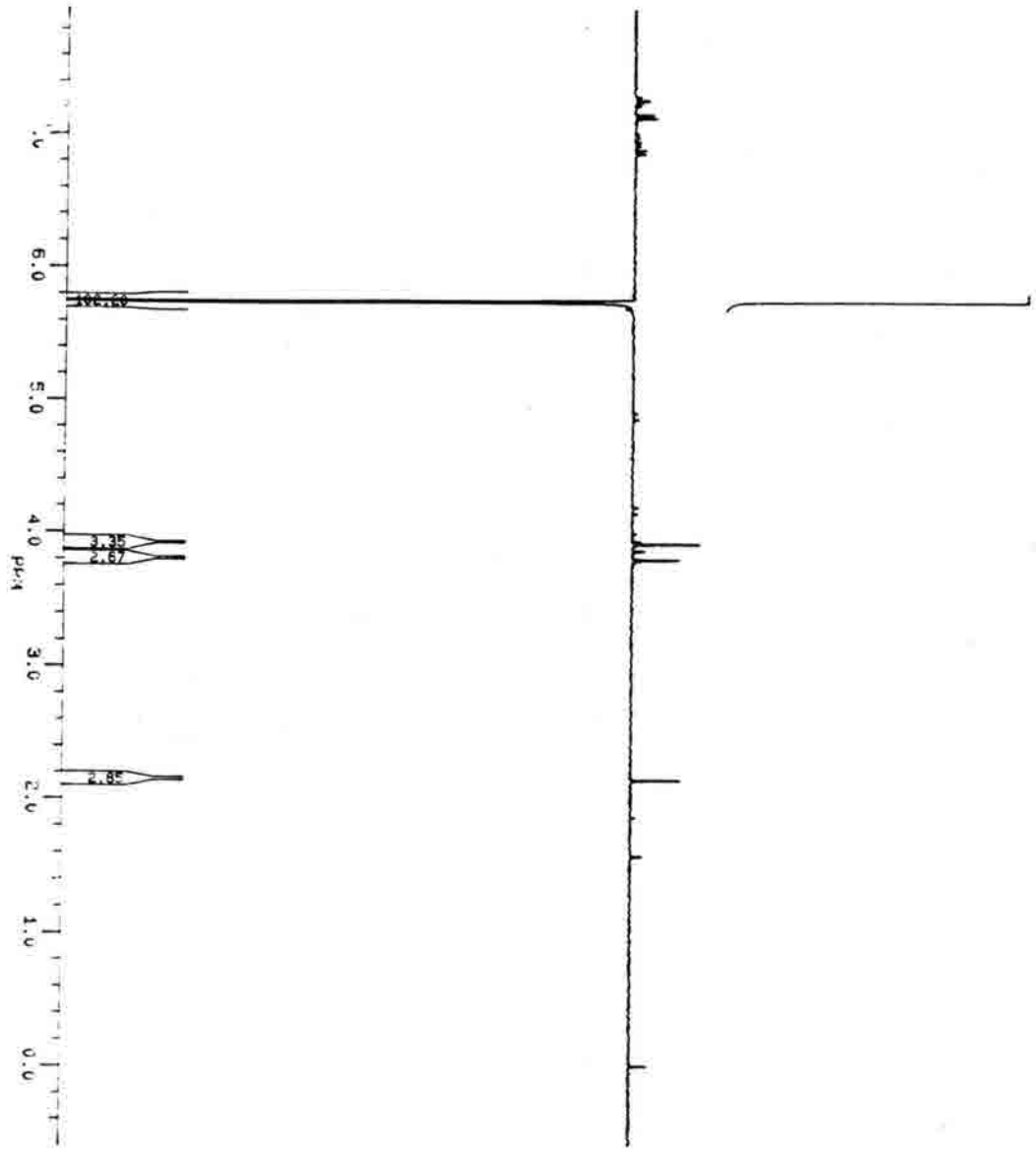


Figure 6. X-ray coordinates of trans disulfide 292a

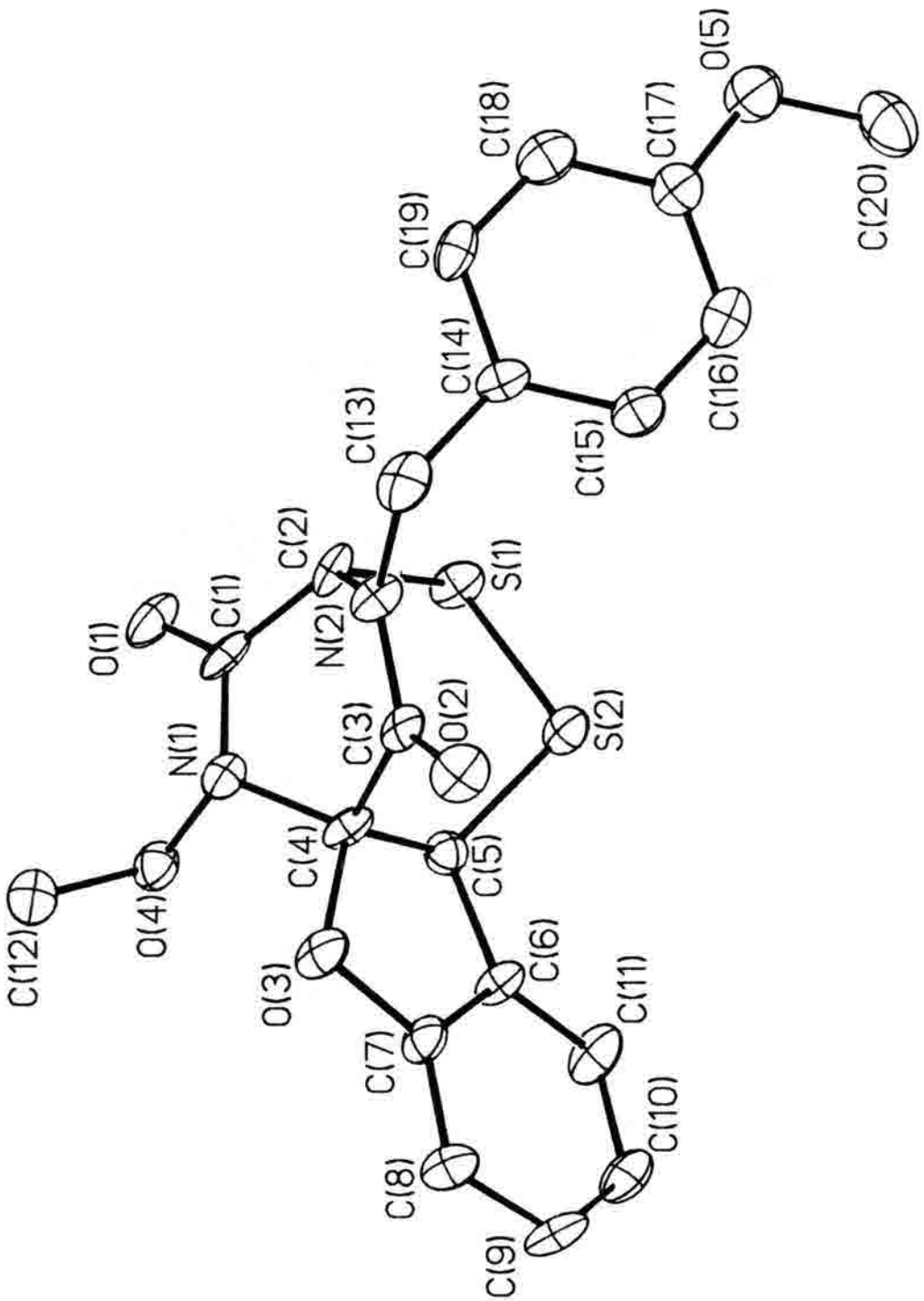


Figure 6 continued

Table 1. Atomic coordinates and isotropic thermal parameters ($A^2 x 10^3$) for 1

	x	y	z	U
S(1)	0.0244(2)	0.0800(1)	-0.2686(1)	27(1)*
S(2)	0.0661(2)	0.1775(1)	-0.3097(1)	25(1)*
C(1)	0.1310(7)	0.0801(3)	-0.0435(5)	26(2)*
C(2)	-0.0286(8)	0.0879(3)	-0.1166(5)	24(2)*
C(3)	-0.0356(7)	0.2066(3)	-0.0862(4)	20(2)*
C(4)	0.1535(7)	0.1995(3)	-0.0786(5)	20(2)*
C(5)	0.2234(7)	0.1987(3)	-0.1974(5)	22(1)
C(6)	0.2827(7)	0.2695(3)	-0.2057(5)	22(2)*
C(7)	0.2761(7)	0.2994(3)	-0.0988(5)	23(2)*
C(8)	0.3193(7)	0.3646(3)	-0.0768(5)	26(2)*
C(9)	0.3785(8)	0.3995(3)	-0.1686(6)	35(2)*
C(10)	0.3890(8)	0.3710(3)	-0.2756(5)	32(2)*
C(11)	0.3437(8)	0.3050(3)	-0.2938(5)	32(2)*
C(12)	0.3805(9)	0.1443(3)	0.1523(5)	35(2)*
C(13)	-0.2911(7)	0.1446(3)	-0.0843(5)	28(2)*
C(14)	-0.3843(7)	0.1150(3)	-0.1860(5)	22(2)*
C(15)	-0.4026(7)	0.1482(3)	-0.2894(5)	25(2)*
C(16)	-0.4974(7)	0.1224(3)	-0.3827(5)	25(2)*
C(17)	-0.5781(7)	0.0615(3)	-0.3702(5)	25(2)*
C(18)	-0.5590(8)	0.0276(3)	-0.2693(6)	32(2)*
C(19)	-0.4631(7)	0.0537(3)	-0.1762(5)	24(2)*
C(20)	-0.6979(9)	0.0665(4)	-0.5626(6)	43(3)*
N(1)	0.1968(5)	0.1409(2)	-0.0079(4)	21(2)*
N(2)	-0.1147(6)	0.1481(2)	-0.0926(4)	21(2)*
O(1)	0.1886(5)	0.0263(2)	-0.0177(4)	34(2)*
O(2)	-0.0968(5)	0.2620(2)	-0.0798(3)	27(1)*
O(3)	0.2177(5)	0.2566(2)	-0.0193(3)	26(1)*
O(4)	0.3618(5)	0.1382(2)	0.0296(3)	27(1)*
O(5)	-0.6756(5)	0.0323(2)	-0.4553(4)	35(2)*

* Equivalent isotropic U defined as one third of the trace of the orthogonalised U_{ij} tensor.

Figure 6 continued

Table 2. Bond lengths for 1 (Å)

S(1)-S(2)	2.040(2)	S(1)-C(2)	1.878(6)
S(2)-C(5)	1.822(6)	C(1)-C(2)	1.522(8)
C(1)-N(1)	1.380(7)	C(1)-O(1)	1.203(7)
C(2)-N(2)	1.438(7)	C(3)-C(4)	1.570(8)
C(3)-N(2)	1.338(7)	C(3)-O(2)	1.220(7)
C(4)-C(5)	1.553(8)	C(4)-N(1)	1.460(7)
C(4)-O(3)	1.414(7)	C(5)-C(6)	1.502(8)
C(6)-C(7)	1.392(8)	C(6)-C(11)	1.383(9)
C(7)-C(8)	1.368(8)	C(7)-O(3)	1.381(7)
C(8)-C(9)	1.404(9)	C(9)-C(10)	1.386(9)
C(10)-C(11)	1.380(9)	C(12)-O(4)	1.438(7)
C(13)-C(14)	1.487(8)	C(13)-N(2)	1.476(8)
C(14)-C(15)	1.378(8)	C(14)-C(19)	1.395(8)
C(15)-C(16)	1.391(8)	C(16)-C(17)	1.400(8)
C(17)-C(18)	1.360(9)	C(17)-O(5)	1.361(7)
C(18)-C(19)	1.396(8)	C(20)-O(5)	1.428(8)
N(1)-O(4)	1.403(6)		

Table 3. Bond angles for 1 (deg)

S(2)-S(1)-C(2)	101.7(2)	S(1)-S(2)-C(5)	100.1(2)
C(2)-C(1)-N(1)	112.5(5)	C(2)-C(1)-O(1)	122.7(5)
N(1)-C(1)-O(1)	124.8(5)	S(1)-C(2)-C(1)	105.2(4)
S(1)-C(2)-N(2)	114.5(4)	C(1)-C(2)-N(2)	113.5(5)
C(4)-C(3)-N(2)	114.0(5)	C(4)-C(3)-O(2)	119.8(5)
N(2)-C(3)-O(2)	126.1(5)	C(3)-C(4)-C(5)	113.4(4)
C(3)-C(4)-N(1)	107.5(4)	C(5)-C(4)-N(1)	114.0(5)
C(3)-C(4)-O(3)	106.7(4)	C(5)-C(4)-O(3)	107.2(4)
N(1)-C(4)-O(3)	107.6(4)	S(2)-C(5)-C(4)	110.5(4)
S(2)-C(5)-C(6)	112.9(4)	C(4)-C(5)-C(6)	101.3(5)
C(5)-C(6)-C(7)	107.9(5)	C(5)-C(6)-C(11)	132.3(5)
C(7)-C(6)-C(11)	119.8(5)	C(6)-C(7)-C(8)	123.2(6)
C(6)-C(7)-O(3)	112.6(5)	C(8)-C(7)-O(3)	124.2(5)
C(7)-C(8)-C(9)	115.5(6)	C(8)-C(9)-C(10)	122.8(6)
C(9)-C(10)-C(11)	119.6(6)	C(6)-C(11)-C(10)	119.0(6)
C(14)-C(13)-N(2)	114.6(5)	C(13)-C(14)-C(15)	121.6(5)
C(13)-C(14)-C(19)	119.8(5)	C(15)-C(14)-C(19)	118.5(5)
C(14)-C(15)-C(16)	121.7(6)	C(15)-C(16)-C(17)	118.9(5)
C(16)-C(17)-C(18)	120.1(5)	C(16)-C(17)-O(5)	123.7(5)
C(18)-C(17)-O(5)	116.2(5)	C(17)-C(18)-C(19)	120.7(6)
C(14)-C(19)-C(18)	120.2(5)	C(1)-N(1)-C(4)	117.4(4)
C(1)-N(1)-O(4)	114.3(4)	C(4)-N(1)-O(4)	113.2(4)
C(2)-N(2)-C(3)	119.4(5)	C(2)-N(2)-C(13)	119.0(5)
C(3)-N(2)-C(13)	121.4(5)	C(4)-O(3)-C(7)	107.8(4)
C(12)-O(4)-N(1)	109.2(4)	C(17)-O(5)-C(20)	117.7(5)

Figure 6 continued

Table S-1. Anisotropic thermal parameters for 1 ($Å^2 x 10^3$)

	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S(1)	35(1)	18(1)	31(1)	-5(1)	11(1)	1(1)
S(2)	34(1)	21(1)	20(1)	-2(1)	8(1)	-1(1)
C(1)	28(4)	23(3)	30(4)	10(3)	19(3)	-1(3)
C(2)	36(4)	17(3)	21(4)	8(3)	9(3)	4(3)
C(3)	29(4)	22(4)	9(3)	-1(2)	10(3)	-4(3)
C(4)	26(3)	11(3)	24(3)	-1(3)	14(3)	0(3)
C(6)	27(4)	15(3)	25(3)	2(3)	12(3)	-1(3)
C(7)	31(4)	22(3)	17(3)	2(3)	8(3)	-3(3)
C(8)	29(4)	19(3)	32(4)	-2(3)	9(3)	-2(3)
C(9)	44(4)	16(3)	47(4)	5(3)	10(3)	-9(3)
C(10)	35(4)	30(4)	33(4)	11(3)	15(3)	-4(3)
C(11)	44(4)	30(4)	25(4)	3(3)	21(3)	2(3)
C(12)	43(4)	34(4)	28(4)	4(3)	-1(3)	-4(3)
C(13)	29(4)	35(4)	23(3)	3(3)	15(3)	4(3)
C(14)	23(4)	19(3)	25(3)	-0(3)	8(3)	-4(3)
C(15)	27(4)	23(3)	25(4)	-1(3)	8(3)	-1(3)
C(16)	30(4)	21(3)	25(4)	1(3)	12(3)	11(3)
C(17)	29(4)	24(4)	21(4)	-4(3)	5(3)	0(3)
C(18)	36(4)	25(4)	36(4)	-0(3)	10(3)	-1(3)
C(19)	30(4)	24(3)	20(3)	7(3)	11(3)	9(3)
C(20)	42(5)	56(5)	30(4)	-4(4)	-6(3)	0(4)
N(1)	12(3)	24(3)	28(3)	8(2)	6(2)	4(2)
N(2)	22(3)	17(3)	24(3)	0(2)	13(2)	3(2)
O(1)	34(3)	25(3)	46(3)	19(2)	11(2)	4(2)
O(2)	35(3)	21(2)	26(2)	-6(2)	9(2)	4(2)
O(3)	40(3)	22(2)	19(2)	-2(2)	15(2)	-6(2)
O(4)	23(2)	31(3)	27(2)	7(2)	4(2)	-0(2)
O(5)	44(3)	33(3)	28(3)	-2(2)	3(2)	-7(2)

The anisotropic temperature factor exponent takes the form:
$-2\pi^2(h^2a^{*2}U_{11} + \ldots + 2hka^*b^*U_{12})$.

Figure 6 continued

Table S-2. H-Atom coordinates ($x10^4$) and isotropic thermal parameters ($Ax10^3$) for 1

	x	y	z	U
H(2)	-1048	538	-995	28
H(5)	3064	1659	-2060	31
H(8)	3097	3852	-36	41
H(9)	4131	4451	-1568	48
H(10)	4276	3969	-3367	52
H(11)	3544	2841	-3665	41
H(12A)	4947	1438	1739	56
H(12B)	3306	1066	1862	56
H(12C)	3346	1851	1788	56
H(13A)	-3102	1178	-187	35
H(13B)	-3303	1893	-739	35
H(15)	-3486	1904	-2971	45
H(16)	-5072	1458	-4545	43
H(18)	-6121	-148	-2620	46
H(19)	-4512	295	-1052	36
H(20A)	-7596	389	-6173	54
H(20B)	-5931	749	-5886	54
H(20C)	-7531	1083	-5544	54

Figure 7. X-ray coordinates of cis disulfide 292b

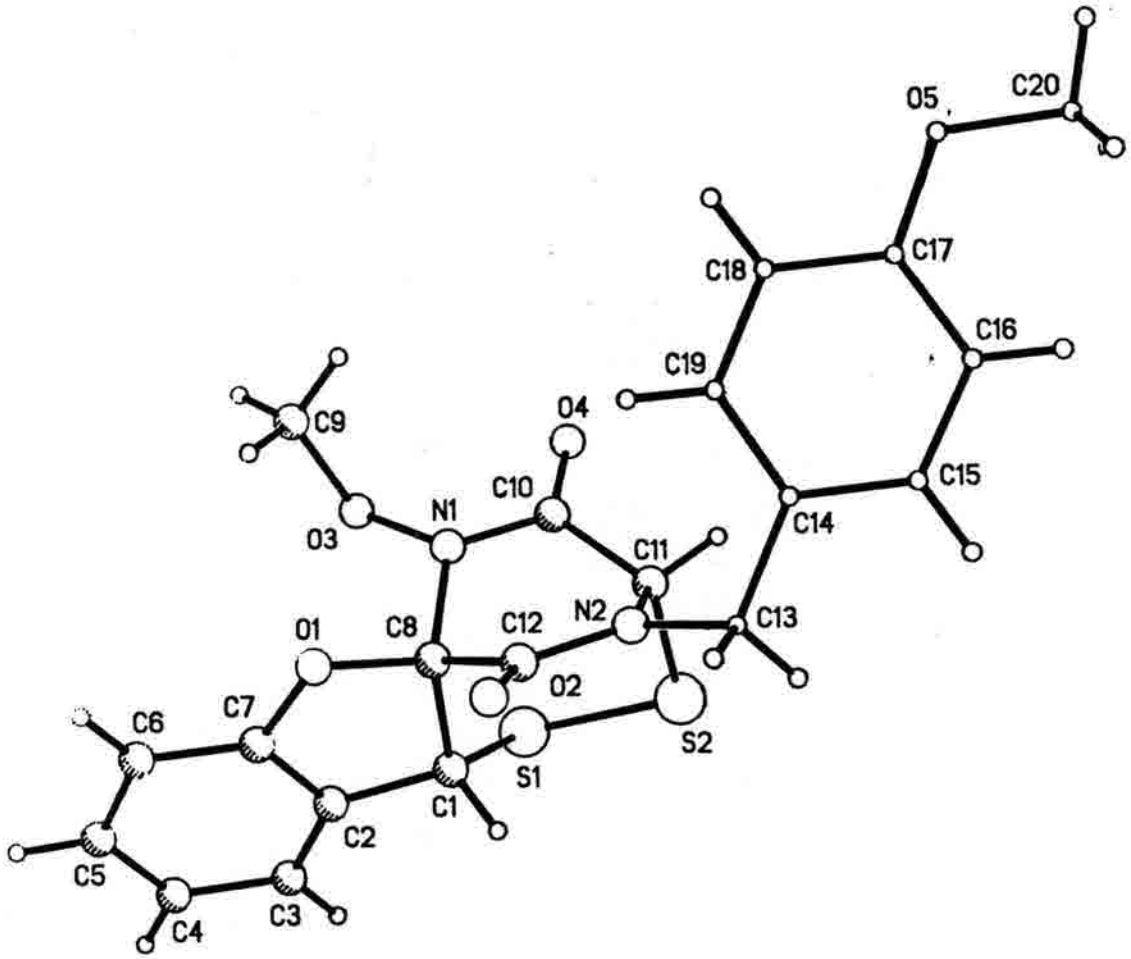


Figure 7 continued

TABLE 1. Atomic coordinates ($x10^4$) and isotropic thermal parameters ($Å^2 x10^3$)[a] for 1

atom	x	y	z	U_{iso}[b]
S1	6609(1)	10121(1)	6434(1)	25(1)*
S2	7641(1)	9897(1)	4896(1)	25(1)*
O1	7055(2)	8399(1)	8494(2)	20(1)*
O2	7561(2)	7602(1)	6473(2)	24(1)*
O3	8703(2)	9494(1)	9059(1)	24(1)*
O4	10203(2)	9998(1)	7246(2)	28(1)*
O5	14728(2)	8124(1)	4589(2)	27(1)*
N1	8682(2)	9178(1)	7854(2)	19(1)*
N2	8569(2)	8535(1)	5560(2)	18(1)*
C1	6175(3)	9223(1)	6878(2)	19(1)*
C2	5339(3)	9216(1)	7978(2)	19(1)*
C3	4175(3)	9582(1)	8192(2)	26(1)*
C4	3590(3)	9439(2)	9303(2)	31(1)*
C5	4169(3)	8936(1)	10163(3)	31(1)*
C6	5344(3)	8567(1)	9961(2)	25(1)*
C7	5897(3)	8727(1)	8856(2)	19(1)*
C8	7447(3)	8769(1)	7424(2)	17(1)*
C9	9542(3)	9076(2)	9981(2)	36(1)*
C10	9363(3)	9535(1)	6993(2)	20(1)*
C11	8959(3)	9287(1)	5640(2)	20(1)*
C12	7862(3)	8227(1)	6441(2)	19(1)*
C13	9002(3)	8125(1)	4497(2)	21(1)*
C14	10531(3)	8108(1)	4517(2)	19(1)*
C15	11108(3)	8130(1)	3384(2)	23(1)*
C16	12507(3)	8122(1)	3361(2)	22(1)*
C17	13335(3)	8107(1)	4490(2)	21(1)*
C18	12781(3)	8093(1)	5642(2)	24(1)*
C19	11388(3)	8086(1)	5642(2)	24(1)*
C20	15336(3)	8123(2)	3423(3)	32(1)*

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) For values with asterisks, the equivalent isotropic U is defined as 1/3 of the trace of the U_{ij} tensor.

Figure 7 continued

TABLE 2. Bond lengths (Å)[a] for 1

S1-S2	2.056(1)	S1-C1	1.812(2)
S2-C11	1.848(2)	O1-C7	1.393(3)
O1-C8	1.416(3)	O2-C12	1.210(3)
O3-N1	1.402(2)	O3-C9	1.442(3)
O4-C10	1.213(3)	O5-C17	1.377(3)
O5-C20	1.430(3)	N1-C8	1.475(3)
N1-C10	1.366(3)	N2-C11	1.461(3)
N2-C12	1.354(3)	N2-C13	1.463(3)
C1-C2	1.500(4)	C1-C8	1.578(3)
C2-C3	1.384(4)	C2-C7	1.377(3)
C3-C4	1.391(4)	C4-C5	1.388(4)
C5-C6	1.393(4)	C6-C7	1.375(4)
C8-C12	1.540(3)	C10-C11	1.516(3)
C13-C14	1.517(4)	C14-C15	1.383(4)
C14-C19	1.386(3)	C15-C16	1.393(4)
C16-C17	1.373(3)	C17-C18	1.390(4)
C18-C19	1.384(4)		

(a) Estimated standard deviations in the least significant digits are given in parentheses.

TABLE 3. Bond angles (deg)[a] for 1

S2-S1-C1	99.7(1)	S1-S2-C11	100.4(1)
C7-O1-C8	108.1(2)	N1-O3-C9	109.6(2)
C17-O5-C20	116.7(2)	O3-N1-C8	115.4(2)
O3-N1-C10	115.9(2)	C8-N1-C10	120.3(2)
C11-N2-C12	121.6(2)	C11-N2-C13	116.9(2)
C12-N2-C13	121.4(2)	S1-C1-C2	112.1(2)
S1-C1-C8	113.0(2)	C2-C1-C8	101.6(2)
C1-C2-C3	131.7(2)	C1-C2-C7	108.1(2)
C3-C2-C7	120.2(2)	C2-C3-C4	118.3(2)
C3-C4-C5	120.1(3)	C4-C5-C6	122.0(3)
C5-C6-C7	116.2(2)	O1-C7-C2	113.1(2)
O1-C7-C6	123.7(2)	C2-C7-C6	123.1(2)
O1-C8-N1	107.4(2)	O1-C8-C1	106.0(2)
N1-C8-C1	116.1(2)	O1-C8-C12	109.4(2)
N1-C8-C12	105.9(2)	C1-C8-C12	111.8(2)
O4-C10-N1	125.7(2)	O4-C10-C11	122.2(2)
N1-C10-C11	112.2(2)	S2-C11-N2	113.6(2)
S2-C11-C10	108.3(2)	N2-C11-C10	113.1(2)
O2-C12-N2	125.4(2)	O2-C12-C8	122.2(2)
N2-C12-C8	112.3(2)	N2-C13-C14	112.2(2)
C13-C14-C15	119.7(2)	C13-C14-C19	122.3(2)
C15-C14-C19	118.0(2)	C14-C15-C16	121.5(2)
C15-C16-C17	119.4(2)	O5-C17-C16	124.6(2)
O5-C17-C18	115.1(2)	C16-C17-C18	120.2(2)
C17-C18-C19	119.5(2)	C14-C19-C18	121.4(2)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

Figure 7 continued

TABLE S-1. Anisotropic thermal parameters ($Å^2 x10^3$)[a,b] for 1

atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
S1	30(1)	19(1)	29(1)	4(1)	11(1)	5(1)
S2	29(1)	26(1)	23(1)	6(1)	7(1)	2(1)
O1	19(1)	19(1)	22(1)	3(1)	8(1)	2(1)
O2	27(1)	18(1)	30(1)	-1(1)	7(1)	-4(1)
O3	32(1)	23(1)	17(1)	-6(1)	4(1)	-3(1)
O4	27(1)	29(1)	30(1)	-7(1)	6(1)	-13(1)
O5	16(1)	34(1)	31(1)	-2(1)	2(1)	2(1)
N1	21(1)	20(1)	17(1)	-3(1)	4(1)	-3(1)
N2	17(1)	17(1)	21(1)	-4(1)	6(1)	-3(1)
C1	19(1)	18(1)	20(1)	1(1)	4(1)	0(1)
C2	18(1)	18(1)	20(1)	-2(1)	2(1)	-1(1)
C3	21(2)	29(1)	27(1)	-0(1)	1(1)	4(1)
C4	24(2)	37(2)	35(2)	-2(1)	15(1)	5(1)
C5	30(2)	36(2)	29(1)	2(1)	15(1)	-0(1)
C6	29(2)	25(1)	24(1)	5(1)	8(1)	-0(1)
C7	16(1)	17(1)	24(1)	-3(1)	5(1)	-3(1)
C8	19(1)	16(1)	18(1)	1(1)	7(1)	-2(1)
C9	46(2)	35(2)	24(1)	0(1)	-6(1)	-8(2)
C10	16(1)	20(1)	25(1)	-1(1)	2(1)	0(1)
C11	21(1)	18(1)	22(1)	-0(1)	9(1)	-2(1)
C12	14(1)	19(1)	23(1)	-1(1)	2(1)	1(1)
C13	18(1)	25(1)	21(1)	-6(1)	4(1)	-4(1)
C14	17(1)	17(1)	22(1)	-2(1)	5(1)	-1(1)
C15	19(1)	28(1)	20(1)	-3(1)	-0(1)	1(1)
C16	16(1)	28(1)	22(1)	-4(1)	6(1)	0(1)
C17	16(1)	20(1)	27(1)	-1(1)	2(1)	2(1)
C18	24(2)	27(1)	20(1)	2(1)	-4(1)	2(1)
C19	24(2)	27(1)	20(1)	3(1)	5(1)	0(1)
C20	18(2)	40(2)	38(2)	-4(1)	8(1)	3(1)

(a) Estimated standard deviations in the least significant digits are given in parentheses.

(b) The anisotropic thermal parameter exponent takes the form:

$$-2\pi^2(h^2a^{*2}U_{11}+k^2b^{*2}U_{22}+ \ldots +2hka^*b^*U_{12})$$

Figure 7 continued

TABLE S-2. Hydrogen coordinates ($x10^4$) and thermal parameters ($Å^2 x10^3$) for 1

atom	x	y	z	U_{iso}
H1	5721	9033	6102	23
H3	3780	9926	7588	31
H4	2785	9688	9476	37
H5	3746	8840	10920	37
H6	5745	8221	10558	31
H9A	9617	9352	10751	43
H9B	10429	8991	9732	43
H9C	9114	8628	10122	43
H11	9731	9309	5168	24
H13A	8676	7644	4546	26
H13B	8620	8338	3713	26
H15	10531	8151	2595	27
H16	12889	8127	2565	27
H18	13359	8089	6431	29
H19	11007	8066	6439	28
H20A	16292	8080	3665	38
H20B	15157	8555	2944	38
H20C	15024	7721	2910	38

Figure 8. NOE of trans alcohol 346a-CONTROL

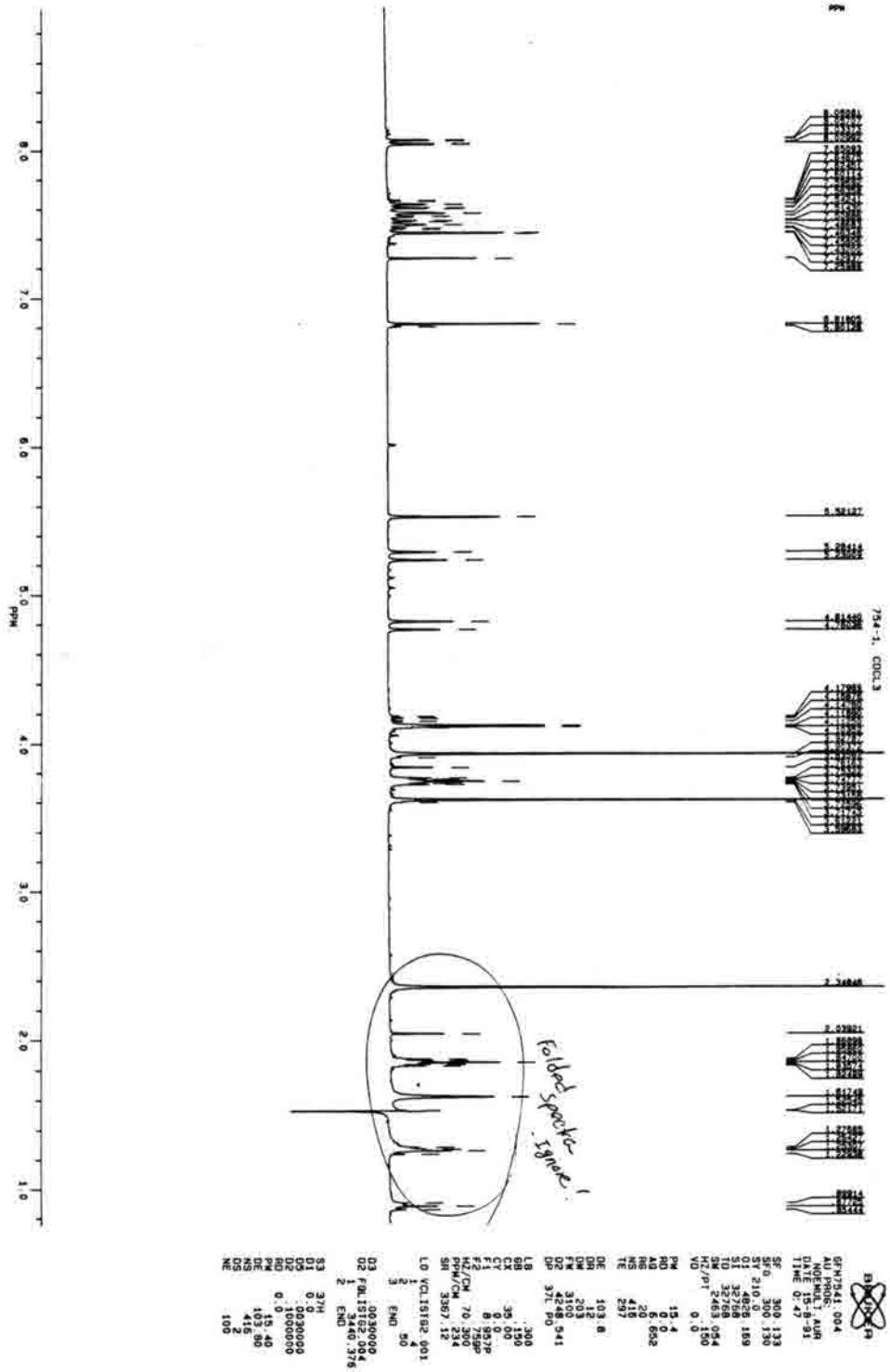


Figure 8. NOE of trans alcohol 346a-irradiation of benzylic methine

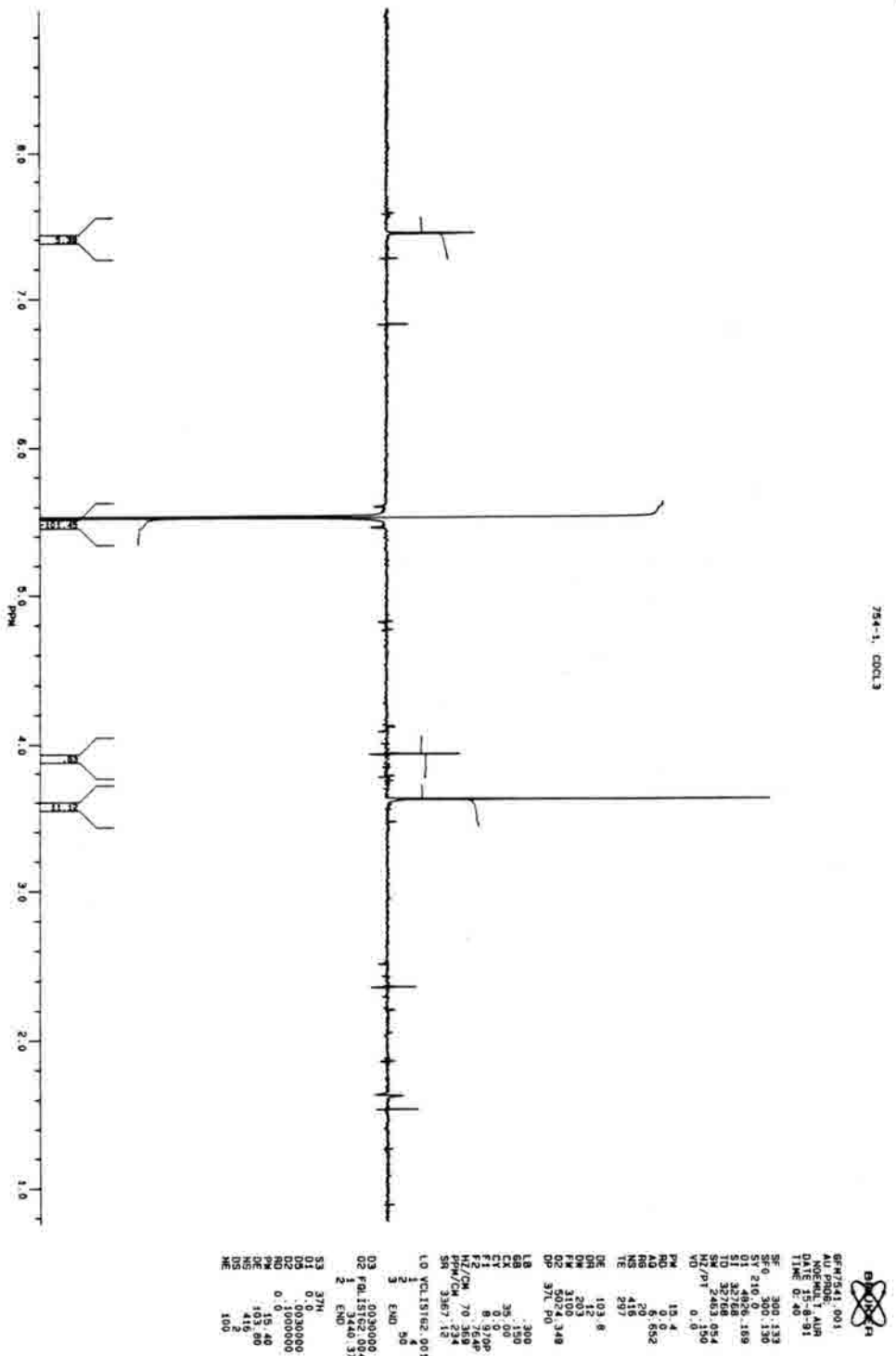


Figure 8. NOE of trans alcohol 346a-irradiation of N-methoxyl

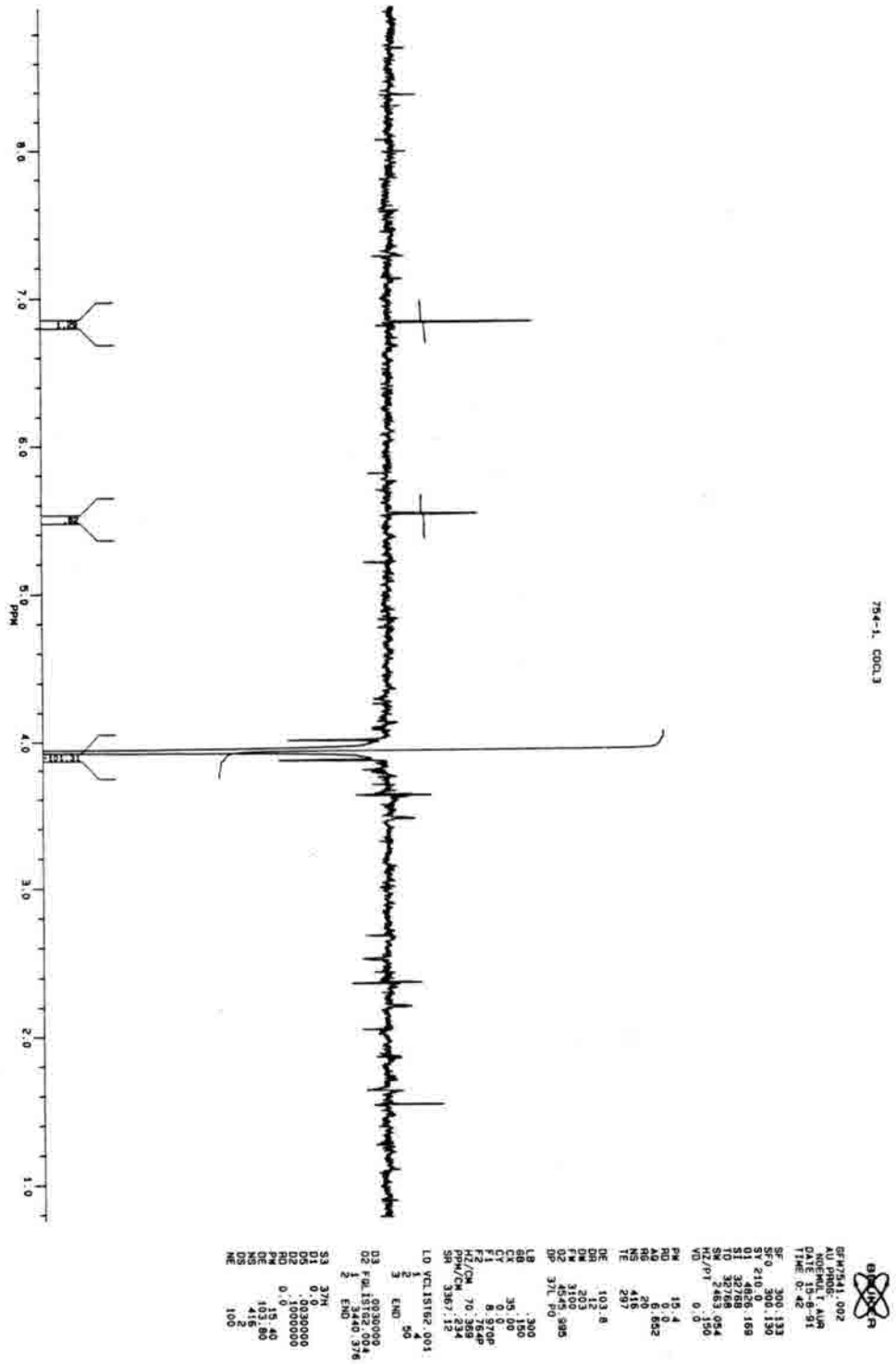


Figure 8. NOE of trans alcohol 346a-irradiation of benzylic O-methyl

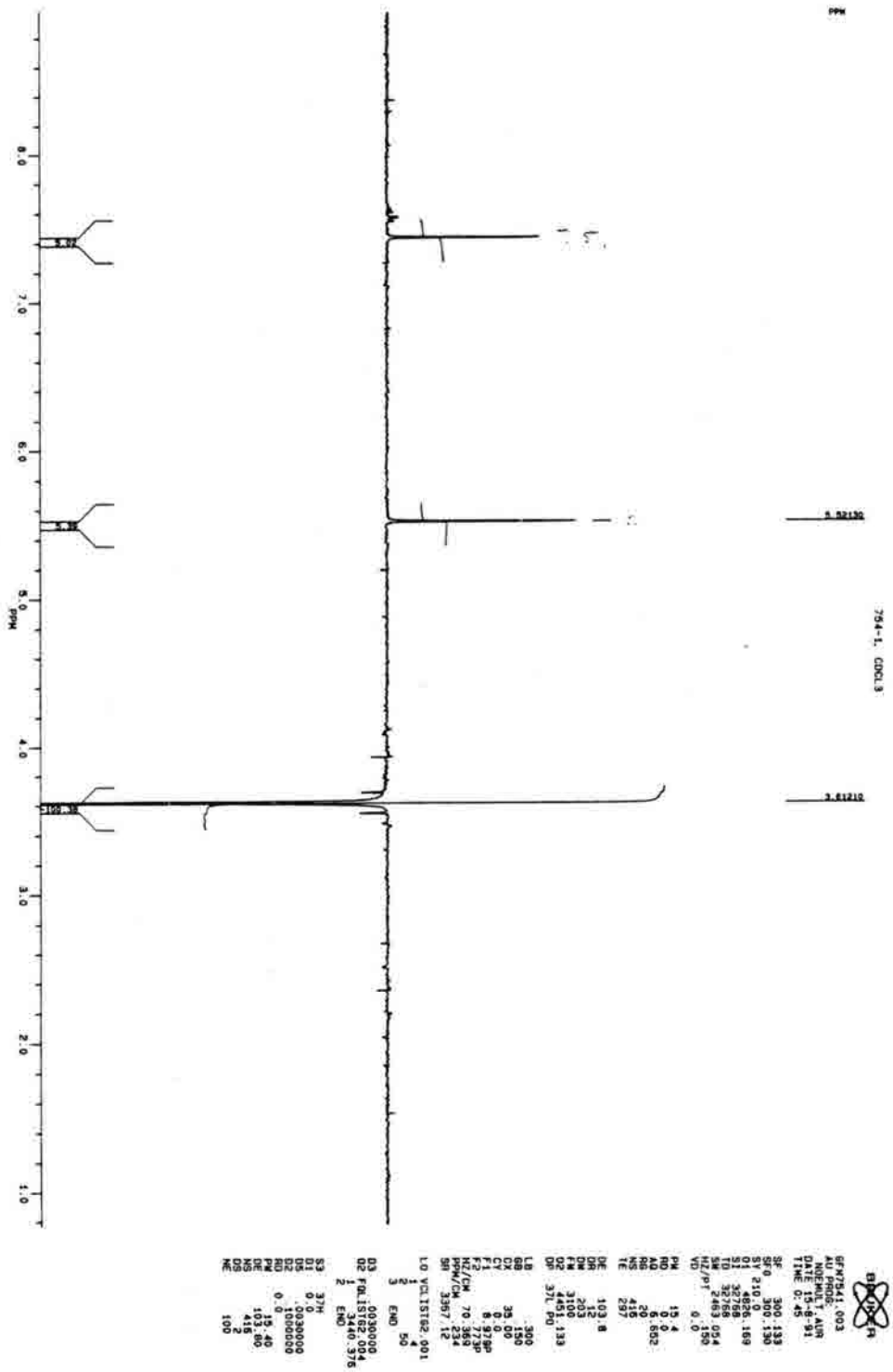


Figure 9. NOE of cis alcohol 346b-CONTROL

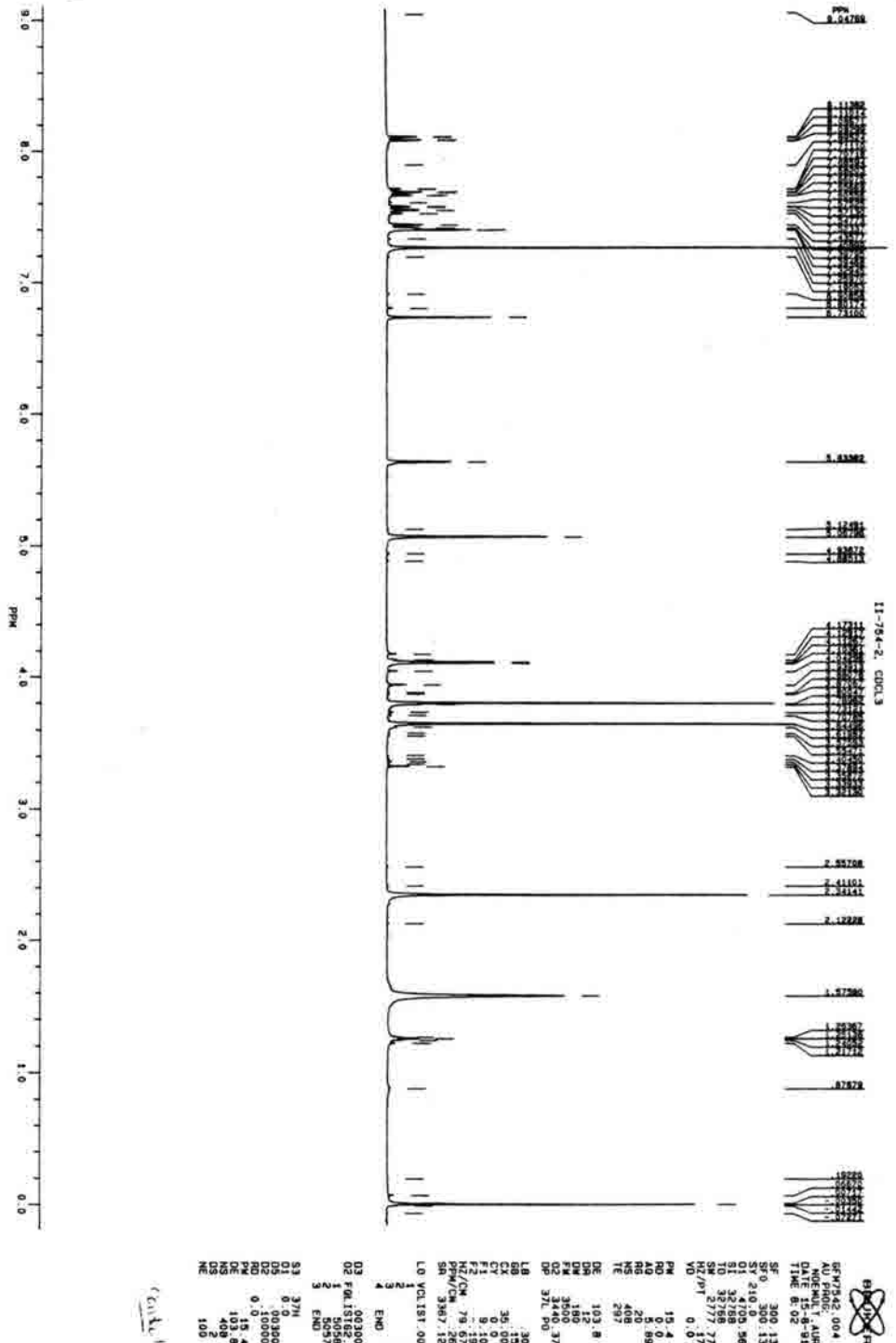


Figure 9. NOE of cis alcohol 346b-irradiation of benzylic methine

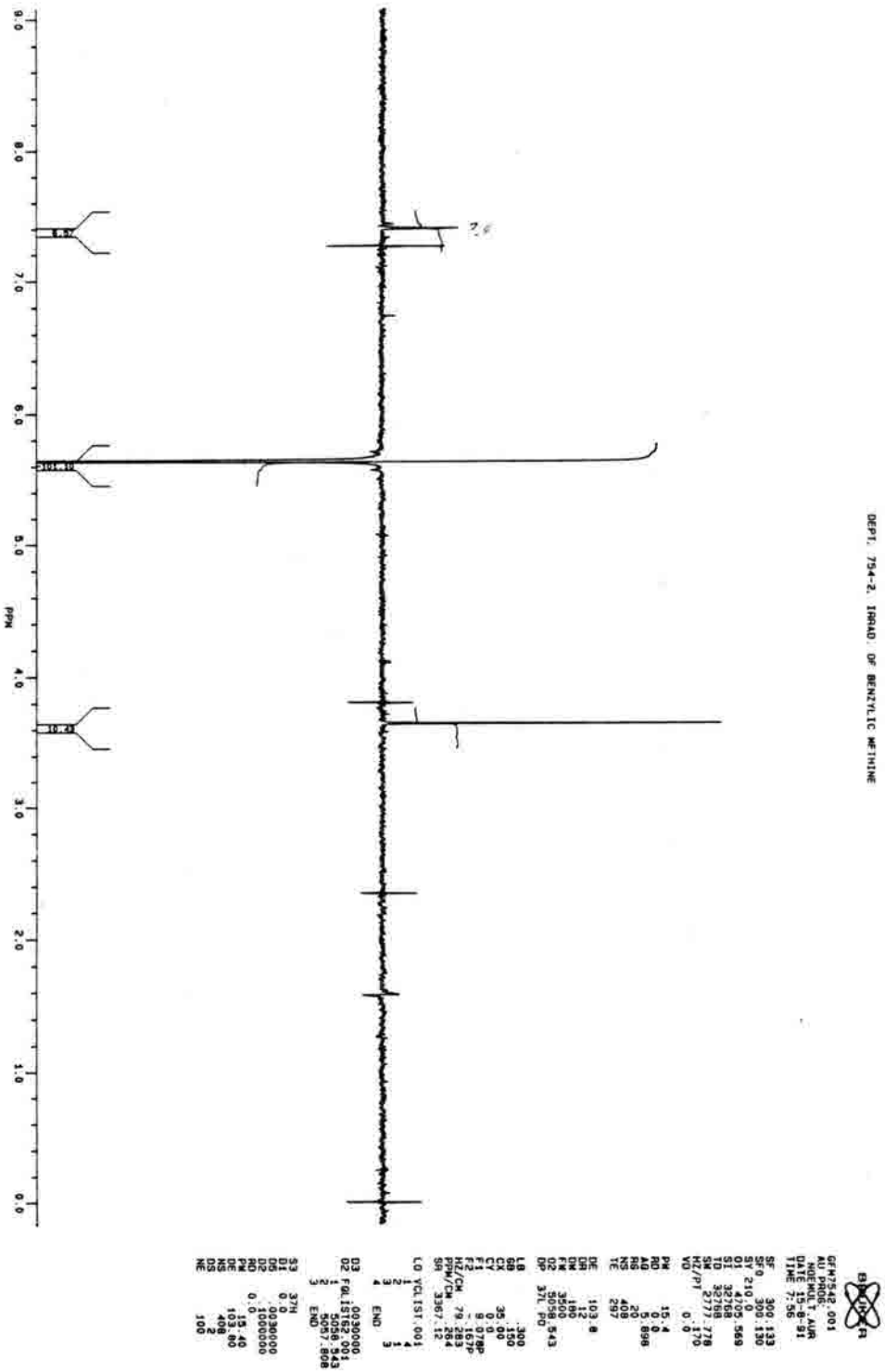


Figure 9. NOE of cis alcohol 346b-irradiation of N-methoxyl

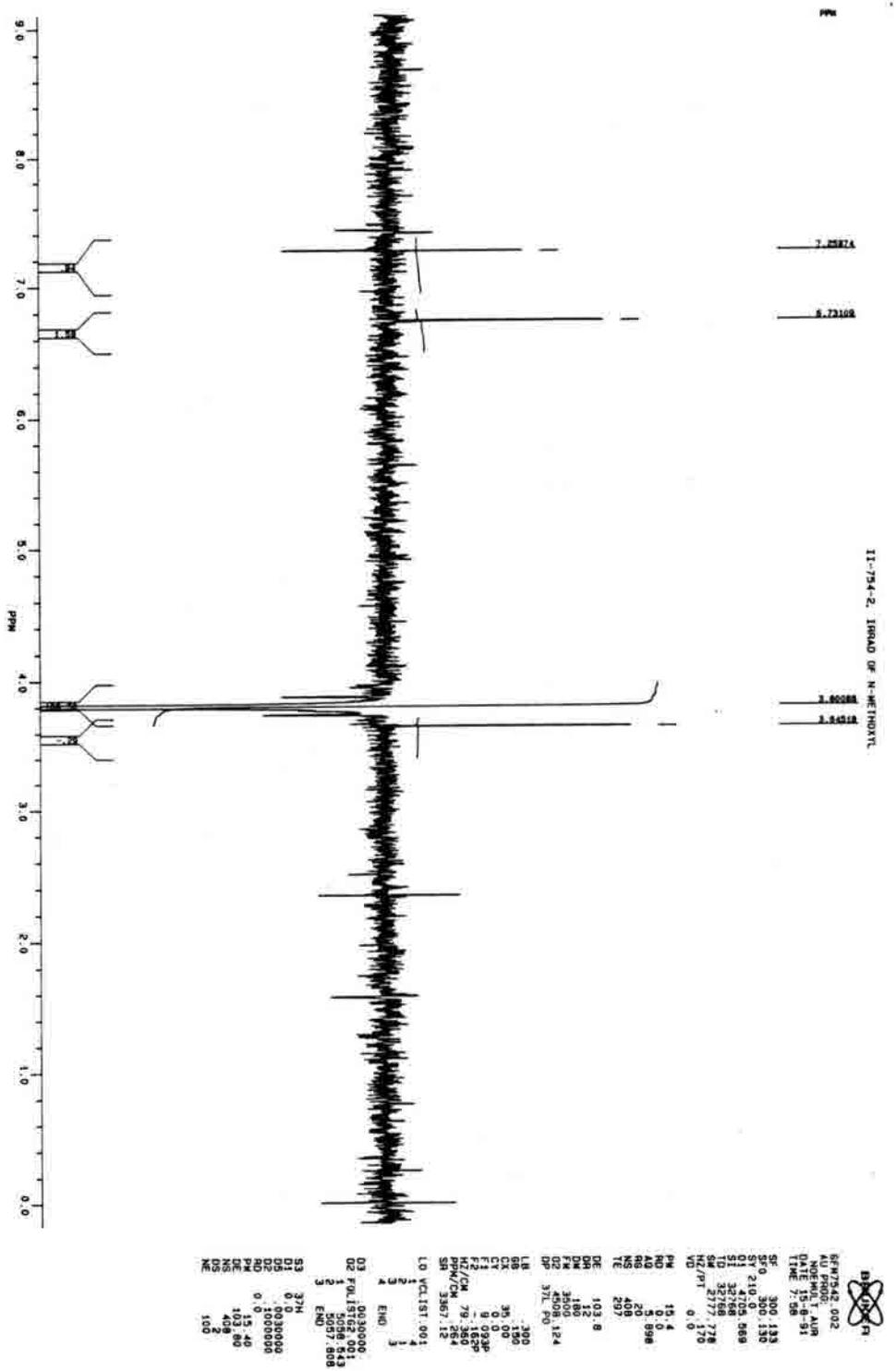


Figure 9. NOE of cis alcohol 346b-irradiation of O-methyl

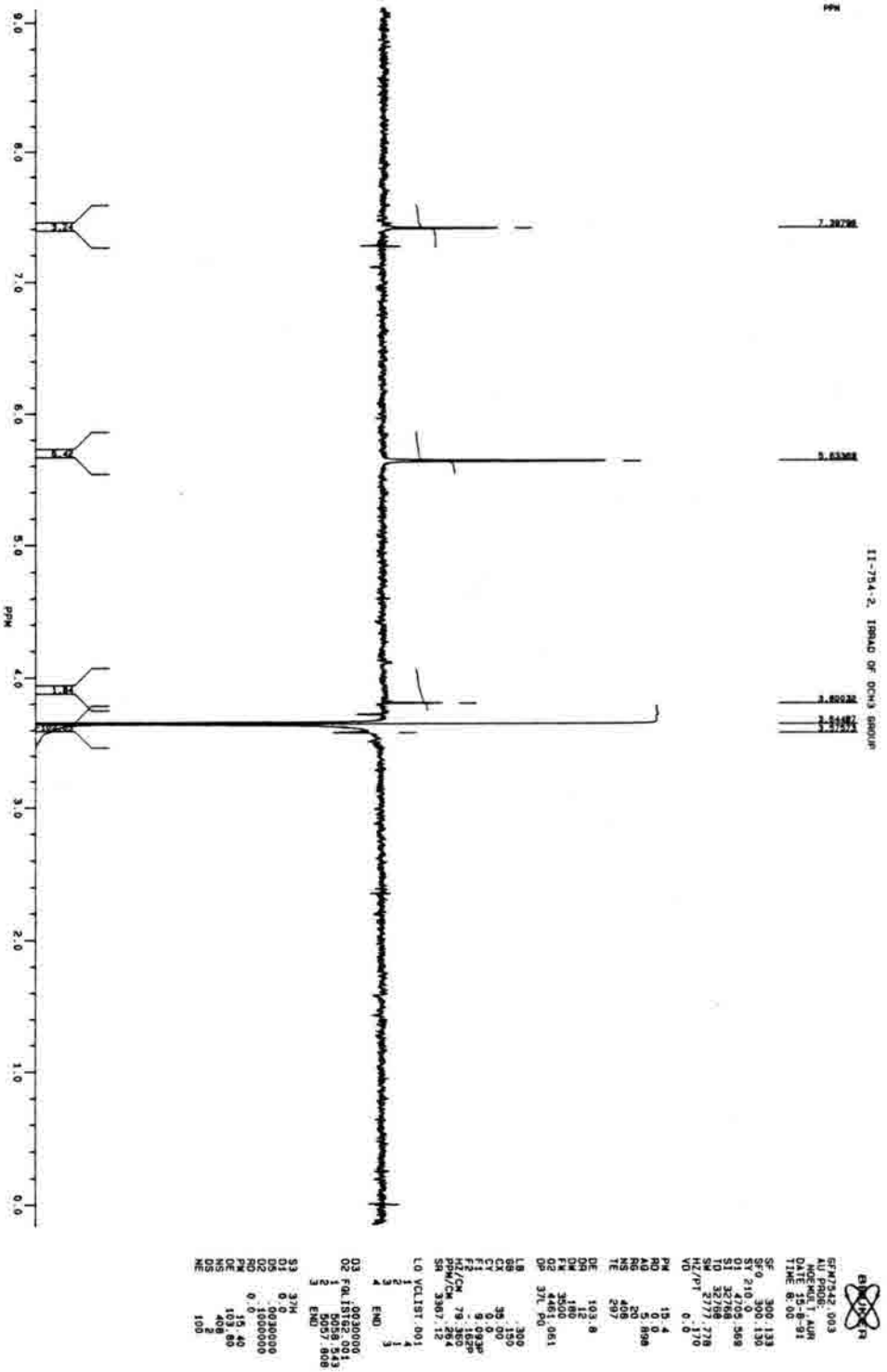


Figure 10. Mass spectral data for monosulfide 351.

RW802.36 RT 0: 4: 6 06-AUG-84 TIC=603 AUTO-GAIN=1 1. 1
CAL15 BASE INT.=1244 B/G SCAN=19 STATUS:1
G. MIKNIS GFM-1091 DIP EI 328

MASS	%HT. MOD.	%HT. BASE	ABS HT.	MASS	%HT. MOD.	%HT. BASE	ABS HT.	MASS	%HT. MOD.	%HT. BASE	ABS HT.
26.3	3.2	1.7	40	59.9	3.9	2.0	48	102.0	1.0	0.5	13
27.2	10.7	5.6	133	61.0	10.4	5.4	129	103.1	1.1	0.6	14
27.2	14.1	7.4	176	62.0	2.7	1.4	34	104.1	1.2	0.6	15
28.1	192.3	100.0	2392	63.1	6.4	3.3	79	105.1	1.4	0.8	18
29.0	53.1	27.6	661	64.0	8.4	4.4	105	105.1	1.2	0.6	15
29.0	24.1	12.5	300	64.1	2.2	1.1	27	107.0	1.3	0.7	16
29.8	14.3	7.4	178	65.1	1.9	1.0	24	107.1	1.0	0.5	13
29.8	1.7	0.9	21	66.0	1.1	0.6	14	108.0	1.6	0.8	20
30.9	64.1	33.3	797	66.1	1.0	0.5	12	109.0	0.8	0.4	10
32.0	42.4	22.0	527	67.1	3.2	1.7	40	109.0	1.0	0.5	12
32.0	44.5	23.1	553	68.0	1.8	1.0	23	111.1	1.3	0.7	16
33.1	6.5	3.4	81	68.9	6.9	3.6	86	113.0	0.7	0.4	9
34.1	2.1	1.1	26	69.0	5.6	2.9	70	115.1	1.4	0.7	17
35.1	12.4	6.4	154	69.9	2.9	1.5	36	118.0	0.6	0.3	7
36.1	76.0	39.5	946	70.0	8.2	4.3	102	122.0	0.9	0.5	11
37.1	5.8	3.0	72	70.9	1.2	0.6	15	123.0	1.1	0.6	14
37.1	1.4	0.8	18	71.0	3.6	1.9	45	124.0	1.0	0.5	13
38.0	24.5	12.8	305	72.1	0.9	0.5	11	125.0	1.1	0.6	14
38.0	1.8	0.9	22	73.1	7.2	3.7	89	126.0	1.6	0.8	20
38.9	1.6	0.8	20	74.1	3.9	2.0	48	127.0	0.8	0.4	10
39.0	8.3	4.3	103	75.1	4.2	2.2	52	129.0	5.0	2.6	62
39.8	3.4	1.8	42	76.1	2.3	1.2	29	130.0	2.3	1.2	28
39.9	1.7	0.9	21	77.1	3.4	1.8	42	130.9	1.1	0.6	14
40.9	1.0	0.5	12	78.1	1.3	0.7	16	131.0	0.8	0.4	10
41.0	22.7	11.8	283	79.0	2.2	1.1	27	135.0	1.4	0.7	17
42.0	9.7	5.1	121	81.0	3.3	1.7	41	139.0	2.6	1.3	32
42.1	8.3	4.3	103	82.0	0.7	0.4	9	139.9	1.1	0.6	14
43.1	100.0	52.0	1244	82.1	1.8	0.9	22	141.0	1.3	0.7	16
43.1	17.4	9.1	217	83.0	1.2	0.6	15	142.0	1.0	0.5	12
44.1	24.0	12.5	299	83.1	3.5	1.8	44	144.0	1.0	0.5	12
44.2	1.3	0.7	16	84.1	1.4	0.7	17	146.0	0.9	0.5	11
45.1	8.1	4.2	101	85.0	1.0	0.5	13	149.0	1.8	1.0	23
45.2	14.7	7.7	183	85.1	2.0	1.0	25	153.0	2.7	1.4	33
46.1	1.0	0.5	13	86.0	1.5	0.8	19	154.0	1.8	0.9	22
46.1	1.0	0.5	12	86.1	2.7	1.4	33	155.0	1.9	1.0	24
47.1	1.0	0.5	12	87.0	1.5	0.8	19	156.0	0.8	0.4	10
49.9	3.2	1.7	40	88.0	3.3	1.7	41	158.0	0.9	0.5	11
51.0	3.9	2.0	49	88.9	2.3	1.2	28	165.0	0.9	0.5	11
52.0	1.0	0.5	12	89.9	1.4	0.8	18	166.0	1.0	0.5	12
53.1	4.8	2.5	60	91.0	2.3	1.2	28	167.0	1.4	0.7	17
53.1	2.1	1.1	26	92.0	0.6	0.3	8	168.0	1.0	0.5	13
54.1	1.3	0.7	16	93.0	0.7	0.4	9	169.0	0.7	0.4	9
54.1	2.0	1.0	25	93.1	1.5	0.8	19	169.9	3.4	1.8	42
55.1	1.8	0.9	22	95.0	1.6	0.8	20	171.0	3.0	1.5	37
55.2	10.3	5.4	128	95.1	2.0	1.0	25	171.1	1.3	0.7	16
56.1	0.9	0.5	11	96.0	0.9	0.5	11	172.0	2.3	1.2	28
56.2	10.8	5.6	134	96.1	1.4	0.7	17	173.0	1.7	0.9	21
57.1	1.7	0.9	21	97.0	1.4	0.7	17	181.0	2.8	1.5	35
57.1	19.5	10.2	243	97.1	1.6	0.8	20	182.0	4.3	2.3	54
58.1	3.5	1.8	44	98.0	1.2	0.6	15	183.0	3.7	1.9	46
58.1	1.4	0.8	18	98.9	2.4	1.3	30	184.0	2.4	1.3	30
59.0	1.8	0.9	22	99.9	1.4	0.8	18	185.0	1.2	0.6	15
59.9	6.9	3.6	86	101.0	1.8	1.0	23	186.0	1.4	0.8	18

Figure 10 continued

RW802.36 RT 0: 4: 6 06-AUG-84 TIC=603 AUTO-GAIN=1
CAL15 BASE INT.=1244 B/G SCAN=19 STATUS:1
G. MIKNIS GFM-1091 DIP EI 328

MASS	%HT. MOD.	%HT. BASE	ABS HT.
187.0	8.7	4.5	100
188.0	3.5	1.8	44
189.0	3.7	1.9	46
189.9	1.4	0.7	17
195.0	3.3	1.7	41
196.0	1.6	0.8	20
197.0	1.8	0.9	22
198.0	1.4	0.7	17
199.0	9.0	4.7	112
199.9	23.3	12.1	290
201.0	11.4	5.9	142
202.0	9.2	4.8	114
203.0	3.4	1.8	42
204.0	1.0	0.5	12
209.0	2.3	1.2	29
210.0	1.7	0.9	21
211.0	1.7	0.9	21
212.0	4.1	2.1	51
213.0	1.6	0.8	20
214.0	3.8	2.0	47
215.0	1.7	0.9	21
216.0	1.4	0.7	17
225.0	0.7	0.4	9
226.0	4.1	2.1	51
227.0	1.8	1.0	23
228.0	3.5	1.8	43
229.0	1.1	0.6	14
230.0	1.0	0.5	12
236.0	1.2	0.6	15
237.0	1.2	0.6	15
238.0	0.8	0.4	10
239.0	0.9	0.5	11
240.0	0.8	0.4	10
241.0	0.8	0.4	10
253.0	1.4	0.7	17
254.0	3.0	1.5	37
255.0	1.7	0.9	21
256.0	1.2	0.6	15
298.1	1.0	0.5	13
328.1	3.6	1.9	45
329.1	1.9	1.0	24
330.1	1.5	0.8	19
331.1	0.7	0.4	9
354.3	1.4	0.8	18

Figure 10 continued

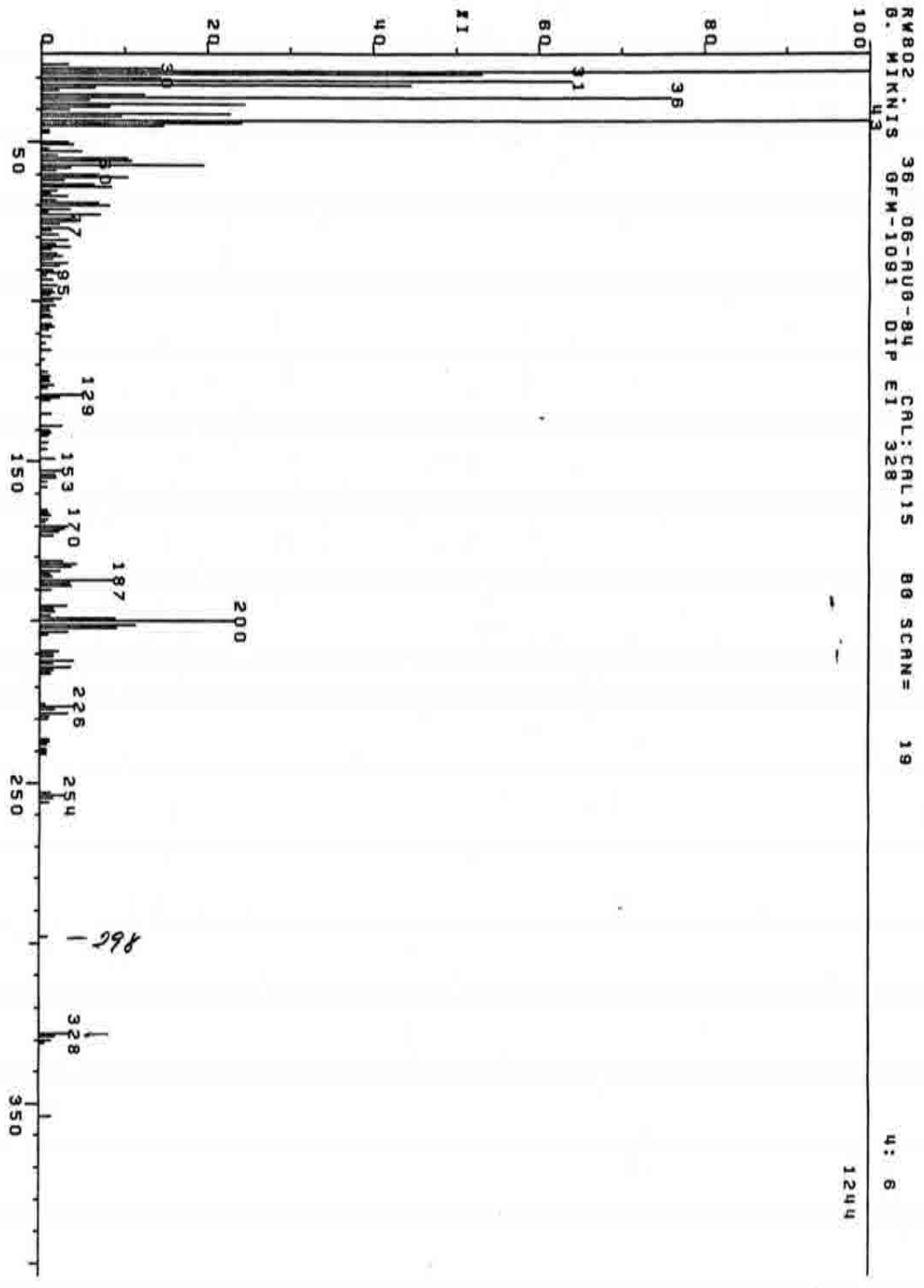


Figure 11. 300 mHz ^{1}H NMR of monosulfide 351.

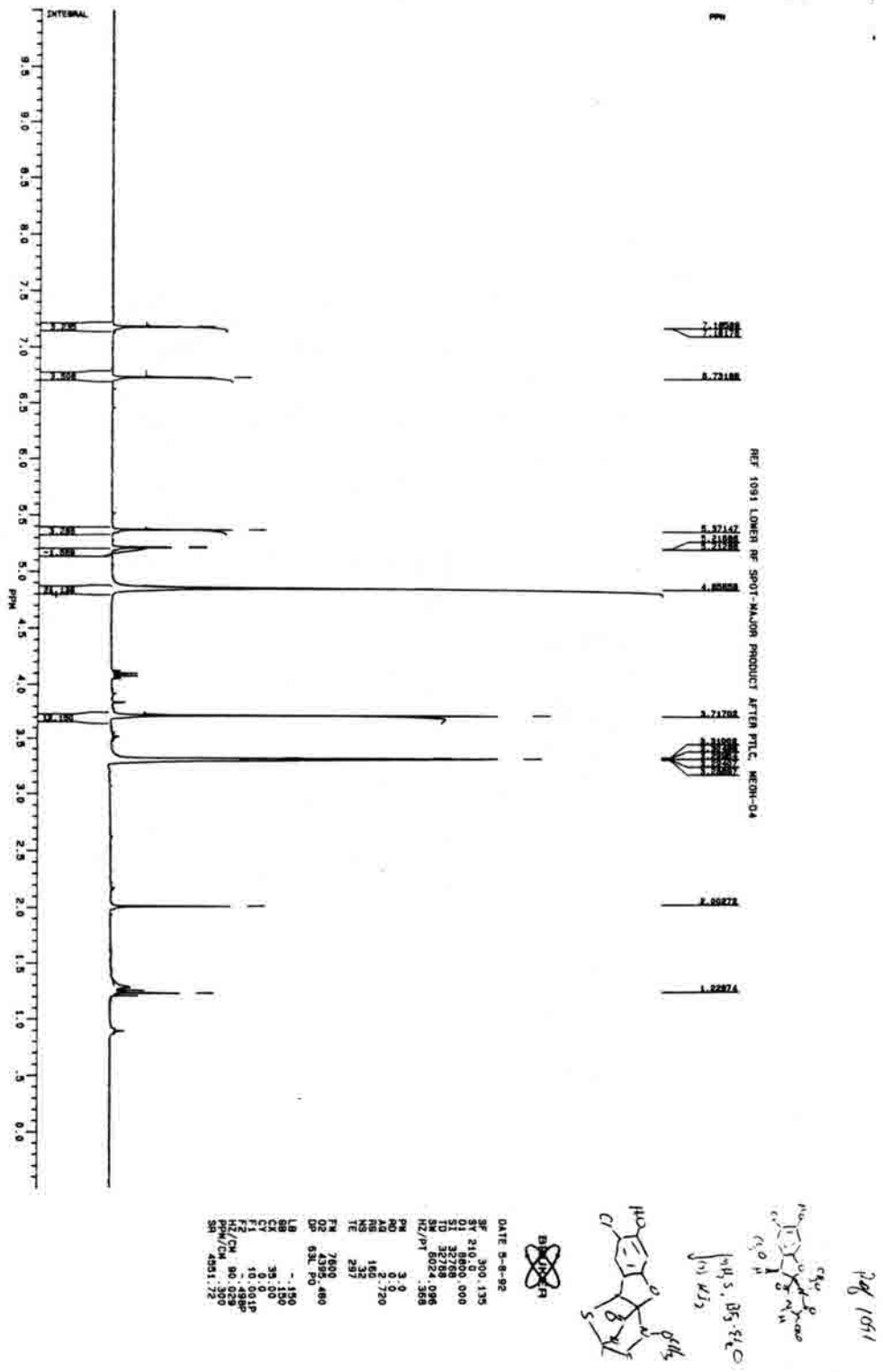
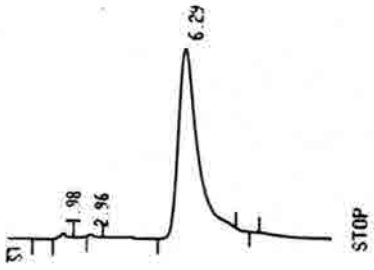


Figure 12. HPLC retention times for (±) and (+)-aspirochlorine

Conditions
2% methanol/chloroform, 2 mL/min flow rate
Waters silica 10 micron 8x10 cm radial pack compression column,
absorbance detected at 254 nm

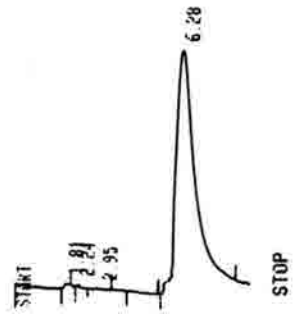
Natural aspirochlorine, 4 mg/mL stock solution, 10 µL injection



RUN # 18

AREA% RT	AREA	TYPE	AR/HT	AREA%
1.98	23513	PB	0.229	0.863
2.96	10971	BB	0.221	0.403
6.29	2690900	PB	0.766	98.735

synthetic aspirochlorine, 4 mg/mL stock solution, 10 µL injection

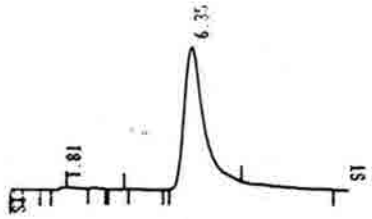


RUN # 15

AREA% RT	AREA	TYPE	AR/HT	AREA%
1.81	7190	PB	0.115	0.296
2.24	5922	BP	0.121	0.170
2.95	6496	PB	0.275	0.187
6.28	3464400	PB	0.773	99.437

Co injections

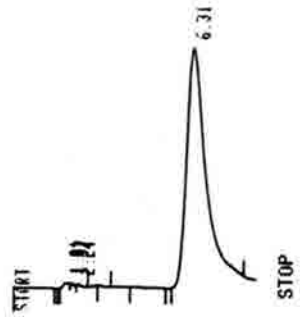
10 µL Natural aspirochlorine plus 5 µL synthetic aspirochlorine



RUN # 11

AREA%	AREA	TYPE	AR/HT	AREA%
RT				
1.81	0	PB	0.000	0.000
6.35	2088300	PB	0.794	100.000

10 µL synthetic aspirochlorine plus 5 µL natural aspirochlorine



RUN # 14

AREA%	AREA	TYPE	AR/HT	AREA%
RT				
1.81	14631	BV	0.154	0.419
1.97	17045	VV	0.197	0.488
2.24	10659	VB	0.179	0.304
6.31	3462200	PB	0.781	98.789

Appendix 2
Publications

Appendix 2 contains a list of the research articles published on various aspects of the total synthesis of aspirochlorine. In addition a congratulatory personal communication from Dr. Sera from Kobe University is included.

Publication List:

1. *Total Synthesis of (±)-Aspirochlorine*, Gregory F. Miknis and Robert M. Williams, . *J. Am. Chem. Soc.*, (1993), **115**, 536.

2. *Synthetic studies on Aspirochlorine (A30461)*, Robert M. Williams, and Gregory F. Miknis, *Tetrahedron Lett.*, (1990), **31**, 4297.

KOBE UNIVERSITY

NADA-KU, KOBE 657, JAPAN

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE

7th Aug. 1990

Professor Robert M. Williams

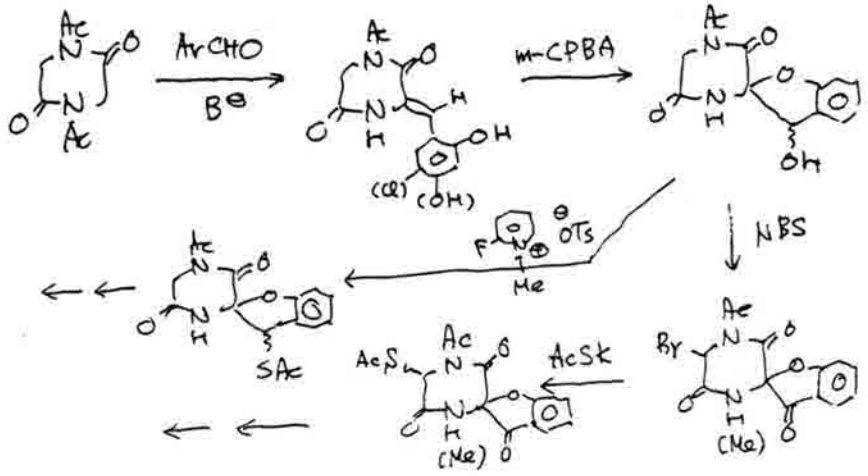
Department of Chemistry
Colorado State University
Fort Collins
Colorado 80523, U.S.A.

Dear professor Williams;

I have read your interesting paper concerning aspirochlorine synthesis (Tetrahedron Lett., 31, 4297 (1990)), and I was hartily disappointed because we have also been trying to synthsize the same compound. Anyways your synthetic study is beautiful and I have to say "congratulations" to you for your elegant success.

Now our approach to aspirochlorine synthesis (see below) seems to be meaningless, and we are planning to turn up other targets.

Sincerely yours,



Akira Sera

Tetrahedron Letters, Vol.31, No.30, pp 4297-4300, 1990
Printed in Great Britain

0040-4039/90 $3.00 + .00
Pergamon Press plc

SYNTHETIC STUDIES ON ASPIROCHLORINE (A30641)

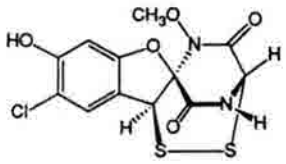
*Robert M. Williams * and Gregory F. Miknis*
Department of Chemistry, Colorado State University
Fort Collins, Colorado 80523

ABSTRACT: The main skeleton of Aspirochlorine is synthesized using a nucleophilic cycloaddition reaction of an acyclic hydroxamic ester.

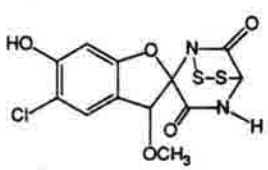
Aspirochlorine (A30641) **1**, is a unique epidithiapiperazinedione first isolated from *Aspergillus tamarii* in 1976.[1] This substance has also been isolated from *Aspergillus flavus*,[2] and is believed to be the antimicrobial principle of oryzachlorin, isolated from *Aspergillus oryzae*.[3] Aspirochlorine is the first example of an epidithiapiperazine-2,5-dione derived from glycine and contains an unusual N-methoxyl substituted piperazine ring system.[4]

Aspirochlorine displays good *in vitro* activity against Gram-positive bacteria and fungi, (~0.1-50ug/ml), but unlike the majority of epidithiapiperazine 2,5-diones, displays only mild antiviral activity.[1] The low antiviral activity of **1** may, in part, be attributable to the attenuated strain and reduction potential[5] in the seven-membered (bicyclo [3.2.2]) disulfide linkage compared to the more common bicyclo [2.2.2] disulfides which has been well-documented to be an obligate functional array for antiviral activity in this class of compounds.[6] One of the more unusual biological activities displayed by **1** is the formation of hyphal swellings produced on *Phytophothora*..[3]

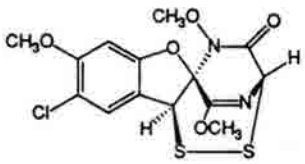
The structure was originally proposed as containing a novel N-1,3 disulfide linkage **2** based on chemical and



1, *ASPIROCHLORINE (A30641)*



2

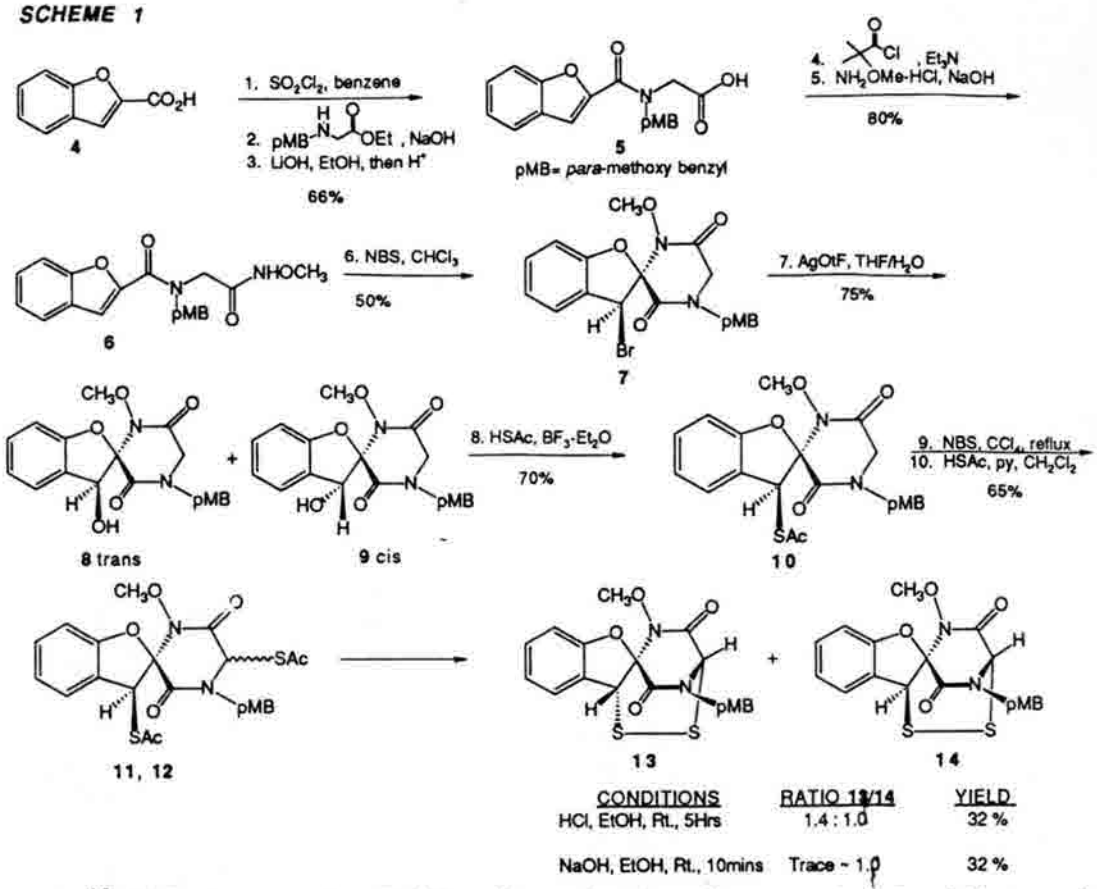


3

spectral data,[2] but the structure was later revised to **1** based on a single crystal x-ray structure of the semi-synthetic dimethyl analog **3**.[4] The synthesis of aspirochlorine has several features which render the molecule a synthetic challenge. Among these are the N-methoxyl substituted amide in the piperazine ring; the 7-membered disulfide ring; and establishing control of the relative stereochemistry at the spiro center. Furthermore, the bicyclo [3.2.2] disulfide-containing nucleus has heretofore never been prepared, despite numerous methods to synthesize the bicyclo [2.2.2] ring system.[7,14,15] In addition, this is the only known example of an epipolythiapiperazinedione with a secondary (NH) amide α- to the disulfide moiety. In this paper we wish to describe the synthesis of a model compound which embraces the tetracyclic nucleus of the correct stucture of aspirochlorine.

The model system, outlined in Scheme 1, involves a key intramolecular cycloaddition reaction to form the spiro fused benzofuran-piperazinedione ring system.[8] Starting from commercially avaliable coumarilic acid **4**,[9] acid chloride

SCHEME 1



preparation[10] ($SOCl_2$, benzene, reflux, 5h) followed by condensation with a protected glycine ethyl ester results in formation of the coupled ester product. The ester was carried on crude undergoing saponification (LiOH, EtOH, Rt. 24h) followed by hydrolysis with 1M HCl to give the free acid **5** in 66% yield from coumarilic acid. **5** was most efficiently converted into the hydroxamic ester **6** via mixed anhydride formation (pivaloyl chloride, Et_3N) followed by amide formation under Schotten-Bauman conditions using NH_2OMe-HCl and 1M NaOH as the base.

In a fashion related to the cycloaddition reactions involving piperazinediones performed by Shin,[11] **6** underwent ring closure (NBS, EtOH-free $CHCl_3$) to form the bicyclic cycloaddition adduct **7**. Fortuitously, upon dissolving the crude reaction mixture in EtOAc, the product precipitates and can be collected by filtration. Repeated recrystallizations of the crude mixture results in a 50% yield of the cycloaddition product. The relative stereochemistry of the N-OCH_3 group and Br in **7** were deduced from a single crystal x-ray analysis shown in Figure 1.

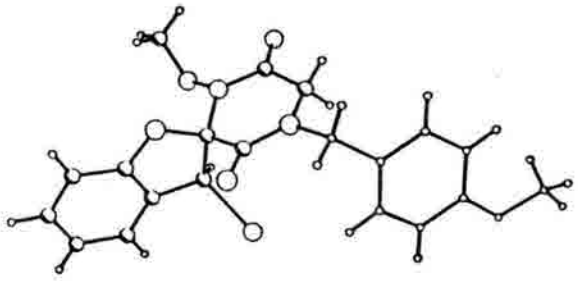


Figure 1. X-ray structure of cycloaddition product **7**. Spheres are of fixed arbitrary radii.

Attempts to functionalize **7** with sulfur nucleophiles under standard conditions[12] (eg. NaSR, ETOH) despite the apparent simplicity have failed. A possible explanation is that the N-methoxyl moiety is bulky enough such that direct S_N2 displacement is difficult. Compound **7** was subsequently converted into a mixture of alcohols **8** and **9** (AgOTf, THF/H_2O) which could be easily separated by chromatography (1.6:1.0 trans:cis). The relative stereochemistry of each of the diastereomers could be deduced using NOE techniques, observing the benzylic proton and the N-methoxyl signals.

Conversion of **8** and **9** either separately or as a mixture, into the mono-thioacetate **10** was best accomplished using excess BF_3-Et_2O (6eq) and thiol acetic acid (12eq). The relative stereochemistry of the thioacetate is as shown based on NOE experiments.

The α-position of the diketopiperazine ring in **10** can be functionalized using a modification of the method developed by Trown.[13] In a one-pot reaction sequence bromination (1.5 eq. NBS, CCl_4, reflux) followed by treatment with thiol acetic acid and pyridine results in formation of a mixture of diastereomeric bis-thioacetates **11** and **12** in essentially a 1:1 ratio presumably epimeric at the α– position. Compounds **11** and **12** can be separated by flash column chromatography or can be carried on as a mixture, since oxidative conversion of *trans*- dithiols to disulfides has been observed.[14]

Acid hydrolysis (HCl /degassed EtOH) of either **11** or **12** followed by oxidation of the intermediate dithiols using KI_3 in CH_2Cl_2[15] results in the formation of two disulfide products **13** and **14** which can be separated by chromatography. X-ray analysis of the less polar compound **14** (Figure 2) reveals the desired relative stereochemistry. Based on another crystal structure the more polar isomer **13** exhibits the undesired relative stereochemistry, indicating that under the reaction conditions the benzylic position undergoes epimerization.[16]

Hydrolysis of **11** or **12** under basic conditions (0.2N NaOH, EtOH, Rt. 10min.) followed by oxidation gives **14** in 32% yield and only a trace of the other diastereomer **13**. Despite the modest yield in the last step, this facile 12-step synthesis demonstrates the potential utility of this approach. Application of this model study to the total synthesis of aspirochlorine will be forth coming.

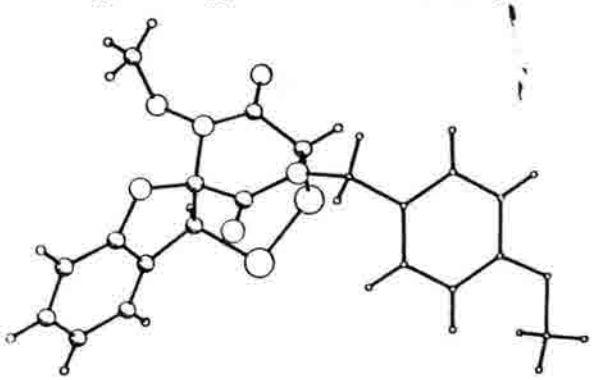


Figure 2. X-ray structure of disulfide **14**. Spheres are of fixed arbitrary radii.

Acknowledgements: We thank the National Institutes of Health, the National Science Foundation and the Petroleum Research Fund for financial support. R.M.W. also acknowledges additional fellowship support from the Alfred P. Sloan Foundation. X-ray analyses were provided by O.P. Anderson, K.A. Andersen, M. Thomson, and M.M. Miller.

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2. Sakata, K.; Masago, H.; Sakurai, A.; Takahashi, N.; *Tetrahedron Lett.*, (1982), **28**, 5607.
3. Sakata, K, Kuwatsuka, T.; Sakurai, A.; Takahashi, N.; Tamura, G.; *Agric. Biol. Chem.*, (1983), **47**, 2673.

4. Sakata, K.; Maruyama, M.; Uzawa, J.; Sakurai, A.; Lu, H.S.M.; Clardy, J.; *Tetrahedron Lett.*, (1987), **28**, 5607.

5. a) Houk, J.; Whitesides, G.M.; *J. Am. Chem. Soc.*, (1987), **109**, 6825 b) Szajewski, R.P.; Whitesides, G.M.; *ibid.*, (1980), **102**, 2011.

6. a) For a review of the biological properties of epipolythiapiperazine 2,5-diones see a) Waring, P.; Eichner, R.D.; Mullbacher, A.; *Med. Res. Rev.*, (1988), **8**, 499 b) Eicher, R.D.; Waring, P.; Geue, A.M.; Braithwaite, A.W.; Mullbacher, A.; *J. Biol. Chem.*, (1988), **263**, 3772 c) Braithwaite, A.W.; Eichner, R.D.; Waring, P.; Mullbacher, A.; *Molec. Immun.*, (1987), **24**, 47.

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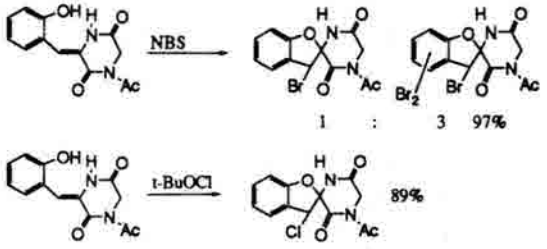
8. All new compounds displayed satisfactory spectroscopic and analytical data consistent with the assigned structures.

9. Coumarilic acid can be prepared from salicylaldehyde see a) Suzuki, T.; Horaguchi, T.; Shimizu, T.; Abe, T.; *Bull. Chem. Soc. Jpn.*, (1983), **56**, 2762, b) Tanaka, S.; *J. Am. Chem. Soc.* (1951), **73**, 872, or from coumarin; Fuson, R.C.; Kneisley, J.W.; Kaiser, E.W.; *Org. Syn.*, (1955), **3**, 209.

10. The reaction is followed by IR υ_{CO} shifts from 1684 to 1755 cm^{-1}.

11. A alternative oxidative ring closure forming spiro fused halo-benzofuran-piperazines was used to synthesize the spiro skeleton of Aspirochlorine **before** the structural revision.



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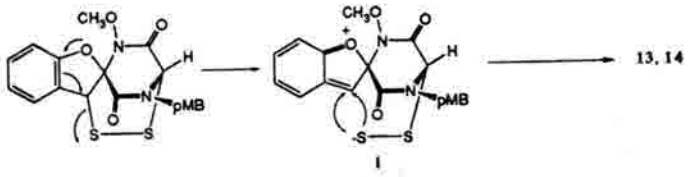
14. Leigh, C.; Taylor, A., *Adv. Chem. Ser.*, (1976), **149**, 228 and references cited therein.

15. Poisel, H.; Schmidt, U.; *Angew. Chem. Int. Ed. Engl.*, (1971), **10**, 130.

16. The mechanism of epimerazation of **13**, **14** can be rationalized as occuring via the stabilized cation **i** :



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Total Synthesis of (±)-Aspirochlorine

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Contribution from the Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523. *Received August 14, 1992*

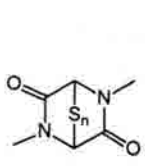
Abstract: (±)-Aspirochlorine was synthesized in a diastereoselective fashion from commercially available 5-chlororesorcinol in 13 steps. The synthesis involves an efficient stereoselective cycloaddition reaction of a hydroxamic ester to form the parent spiro[benzofuran-2(3*H*),2′-piperazine] ring system. In addition the synthesis employs a 2-nitrobenzyl group as an amide protecting group which is easily removed under photolytic conditions.

Introduction

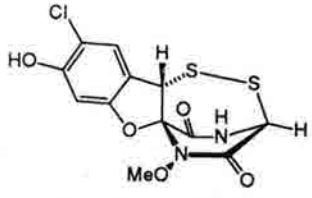
The epipolythiapiperazine-2,5-dione (**1**) containing compounds comprise a diverse and interesting class of fungal metabolites. Historically, these compounds have been the focus of a tremendous amount of investigation not only as attractive synthetic targets[1] but also for their ever increasing array of biological properties which range from potent antiviral/antifungal activities, immunosuppressive activities such as the inhibition of phagocytosis, oxidative damage to DNA, and more recently have been shown to be potent inhibitors of histamine release.[2] Furthermore, compounds which contain the epipolythiapiperazine-2,5-dione moiety have been shown to be effective inhibitors of the enzyme reverse transcriptase,[3] a key enzyme in the life cycle of retro viruses which is currently an attractive target for drug design.[4]

In all cases the disulfide bridge is required for biological activity.[5] Despite the possible therapeutic uses, no drug containing this ring system as a pharmacophore has been developed due to the high mammalian toxicity exhibited by most of these compounds.[6] Aspirochlorine (A30641, **2**) is a novel seven-membered epidithiapiperazine-2,5-dione isolated from *Aspergillus tamari* in 1976.[7] Since its isolation, aspirochlorine has also been identified as the major active constituent from extracts of *Aspergillus flavus* and *Aspergillus oryzae*.[8,9] The structure originally assigned to aspirochlorine (**3**) was proposed to have a unique bicyclo [3.2.1] N-1,3 disulfide bridge, but the structure (correctly shown as **2**) was revised in 1987 from a crystal structure of a semisynthetic derivative.[10] Aspirochlorine is the only known epipolythiapiperazine-2,5-dione-containing natural product derived from glycine, which places a free amide (i.e., NH) adjacent to the S–S bridge. In addition, it is a rare example of an amino acid metabolite to incorporate the unusual *N*-methoxyl moiety in a diketopiperazine ring.

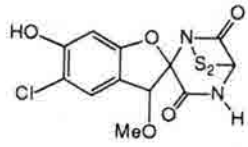
Aspirochlorine *does not* display the same potent antiviral activity which is characteristic of six-membered disulfide containing compounds and exhibits only mild antifungal properties. The relative lack of activity displayed by aspirochlorine might be attributed to differences in ring strain and redox potential for the potent six-membered bicyclo [2.2.2] disulfide ring systems versus the seven-membered bicyclo [3.2.2] disulfide ring system found in **2**.[11]



1 n = 1,2,3,4



2 Aspirochlorine (A30641)



3

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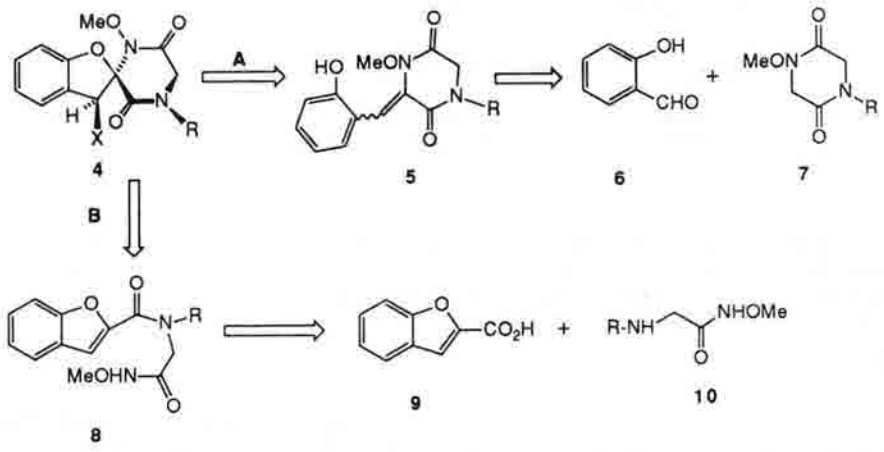
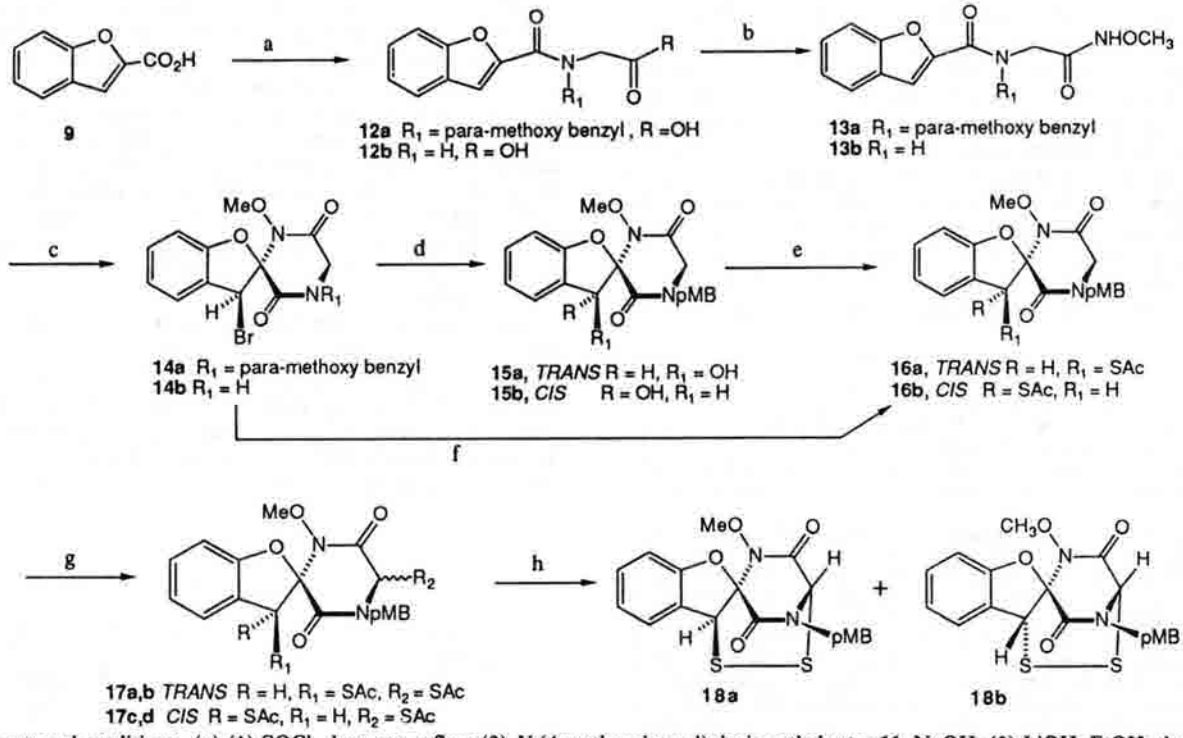
Scheme I



Scheme II[a]



[a] Reagents and conditions: (a) (1) $SOCl_2$, benzene, reflux; (2) *N*-(4-methoxybenzyl)glycine ethyl ester **11**, NaOH; (3) LiOH, EtOH; three steps 60%; (b) pivaloyl chloride, Et_3N followed by NH_2OCH_3; 80–95%; (c) NBS, $CHCl_3$, room temperature, 50%; (d) silver triflate, THF/H_2O; 75%; (e) 12 equiv of HSAc, 6 equiv BF_3–Et_2O, CH_2Cl_2, 70%; (f) excess thiolacetic acid, $ZnCl_2$ benzene; 32–69%; (g) NBS, CCl_4 reflux, followed by thiolacetic acid, pyridine; 65% (h) HCl/EtOH, room temperature 5 h or NaOH, EtOH, room temperature 10 min; followed by aqueous KI_3, CH_2Cl_2, 32%.

Our interest in aspirochlorine is 2-fold; the first objective was to develop an efficient synthesis of this unusual natural product and secondly to explore the mechanism of action/biological activity of **2** and similar compounds as it relates to the physical properties of respective disulfides. In a preliminary communication, we outlined our synthetic approach to aspirochlorine in a model system.[12] In this paper we would like to describe the details of that model study and report its successful application to the first total synthesis of (±)-aspirochlorine.

Results and Discussion

A major goal of the model system was to develop an efficient methodology to construct the spiro[benzofuran-2(3*H*),2′-piperazine] moiety **4** which encompasses the core structure. Two possible retrosynthetic routes are shown in Scheme I. The spiro center could be created by coupling an appropriate piperazine-2,5-dione **7** with a 2-hydroxybenzaldehyde **6** in a convergent approach as depicted in path A. A similar approach was used by Shin and co-workers in the synthesis of the skeleton of aspirochlorine before the structural revision appeared in the literature.[13] Applying this approach toward the synthesis of aspirochlorine had several potential drawbacks which made it less attractive. The foremost concern would be the difficult task of controlling the regiochemical outcome in the aldol condensation/dehydration between an unsymmetrically substituted piperazinedione **7** and the 2-hydroxybenzaldehyde derivative **6**. In

(11) (a) Burns, J. A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6296. (b) Singh, R.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 1190. (c) **1990**, *112*, 6304. (d) Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825. (e) Whitesides, G. M.; Houk, J.; Patterson, M. A. K. *J. Org. Chem.* **1983**, *48*, 112. (f) Szajewski, R. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 2011. (g) Whitesides, G. M.; Lilburn, J. E.; Szajewski, R. P. *J. Org. Chem.* **1977**, *42*, 332. (h) Allinger, N. L.; Hickey, M. J.; Kao, J. *J. Am. Chem. Soc.* **1976**, *98*, 741.

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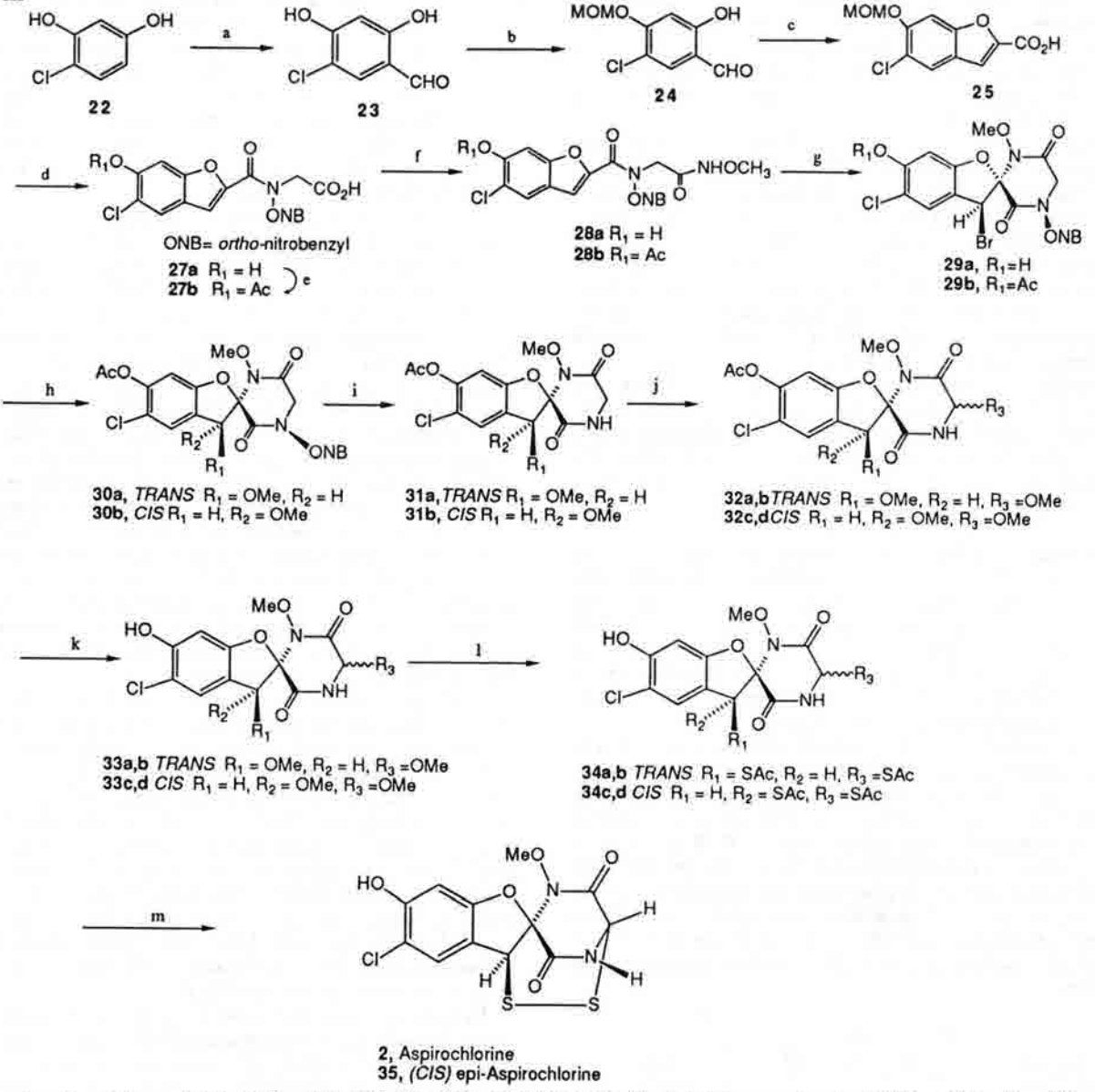
Scheme III[a]



[a] Reagents and conditions: (a) $Zn(CN)_2$, HCl; 40%; (b) MOM-Cl, Et_3N, 50%; (c) diethyl bromomalonate, K_2CO_3, MEK reflux, followed by KOH/EtOH, reflux, 72%; (d) oxalyl chloride, followed by *o*-nitrobenzyl glycine ethyl ester **26**, $NaHCO_3$, followed by aqueous HCl/dioxane reflux, 84%; (e) excess acetic anhydride, pyridine, 98%; (f) isobutyl chloroformate, *N*-methylmorpholine, THF, followed by methoxylamine, 70%; (g) NBS, EtOH-free $CHCl_3$, room temperature, 67%; (h) silver triflate, MeOH/THF, reflux, 80%; (i) *hν*, 10% H_2O/THF, 72%; (j) *tert*-butyl hypochlorite, NaOMe, 0 °C to room temperature, 56–72%; (k) NaOEt, EtOH, 0 °C, 30 min, 74%; (l) excess thiolacetic acid, BF_3–Et_2O, 65%; (m) excess methoxylamine, CSA, THF, 20–34%.

addition the desired aldol condensation/dehydration reaction did not appear likely due to the well documented rearrangement of *N*-alkoxy amides to α-alkoxy amides in the presence of base.[1,14] In addition, a potentially difficult problem would be to control the stereochemistry in the subsequent electrophilic cyclization (**5** → **4**) leading up to the parent tricyclic system.

Based on these potential problems, we envisioned the desired tricyclic system **4** could be created in a stereospecific manner via an intramolecular cyclization reaction of a suitable hydroxamic ester **8** as depicted in path B. The requisite hydroxamic ester **8** could easily be obtained from simple peptide coupling of coumarilic acid **9** and the appropriate N-protected glycine ester **10**. The successful application of this approach is shown in Scheme II.

Commercially available coumarilic acid **9** was coupled with *N*-(*p*-methoxybenzyl)glycine ester **11** to give the desired benzofuranylglycine ethyl ester which was saponified (LiOH, EtOH) to give coumarilic glycinate **12a** in 66% overall yield for the two steps. A variety of methods was used to prepare *O*-methyl hydroxamic ester **13a**; the most satisfactory procedure utilized mixed anhydride formation (pivaloyl chloride, triethyl amine) followed by treatment with methoxylamine to give **13a** in 80–95% yields. Treatment of **13a** with 1.2 equiv of NBS in ethanol-free chloroform resulted in formation of the desired tricyclic bromide **14a** in 50% isolated yield as a single diastereomer possessing the desired relative stereochemistry (i.e., trans). Confirmation of the relative stereochemistry shown for **14a** was obtained from a single crystal X-ray analysis.[12]

Having firmly established an efficient methodology to construct the spirocyclic skeleton of aspirochlorine (31% overall yield of **14** from **9**), we further investigated the scope of the facile spirocyclization reaction by carrying out the NBS oxidation on the analogous system utilizing the unprotected amide **12b** which was obtained by coupling glycine ethyl ester with **9**. To our dismay

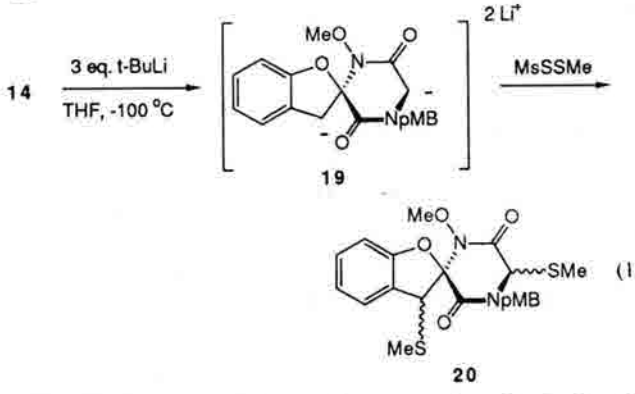
(14) For a review of *N*-alkoxy amino acids, see: Ottenheijm, H. C. J.; Herscheid, J. D. M. *Chem. Rev.* **1986**, *86*, 697. For related work, see: (a) Otteheijm, H. C. J.; Plate, R.; Noordik, J. H.; Herscheid, J. D. M. *J. Org. Chem.* **1982**, *47*, 2147. (b) Herscheid, J. D. M.; Nivard, R. J. F.; Tijhuis, M. W.; Ottenheijm, H. C. J. *J. Org. Chem.* **1980**, *45*, 1885. (c) Herscheid, J. D. M.; Nivard, R. J. F.; Tijhuis, M. W.; Scholten, H. P. H.; Ottenheijm, H. C. J. *J. Org. Chem.* **1980**, *45*, 1880.

none of the desired tricyclic bromide **14b** was obtained from treating **13b** with NBS even when reactions were conducted in refluxing CCl_4. The only products obtained were a mixture of α-brominated acyclic compounds and acyclic compounds and acyclic N-brominated compounds as determined from analysis of the ^1H NMR spectra.

The lack of any spirocyclized material from reactions involving **13b** can be readily explained by considering the conformation about the central amide bond in **13** as illustrated in Figure 1. Compounds such as **13b** containing a secondary amide should adopt the lower energy extended conformation (s-trans form) forcing the benzofuran and hydroxamic ester moieties to be distal. Conversely, cyclization of the tertiary amide-containing compound **13a** can be attributed to the amide significantly populating the s-cis conformation (the *p*-methoxylbenzyl moiety can be considered to be sterically larger than the hydroxamic ester group), allowing the benzofuran and hydroxamic ester functionalities to lie in close proximity allowing cyclization to readily occur. Based on the experimental observation that **13b** does not undergo cyclization, it is apparent that an efficient cyclization reaction requires the central amide to be capable of adopting the requisite folded conformation (assuming the hydroxamate moiety also adopts the s-cis conformation).

The cyclization of **13a** to **14a** proceeds via *anti*-addition resulting in the net desired relative stereochemistry between the *N*-methoxyl moiety and benzylic bromide. Based on the well established reactivity of benzylic halides as good leaving groups in S_N2 reactions, it was anticipated that the natural stereochemistry at the benzylic position could be established using a double inversion protocol involving initial formation of an alcohol or ether followed by a subsequent displacement by thiolate.[15]

An attractive alternative approach to directly convert **14a** to a dithiol containing compound was predicated on the assumption that halogen–metal exchange may proceed with net retention of configuration. This approach was attempted and involved treating **14a** with 3 equiv of *tert*-butyllithium at −100 °C followed by trapping of the intermediate dianion **19** with methyl methanethiolsulfonate to give the disulfenylated compound **20** (eq 1). In the event, only a trace of the desired compound was isolated from the reaction mixture under the conditions shown in eq 1, and synthetically useful amounts of **20** were never realized by this approach.



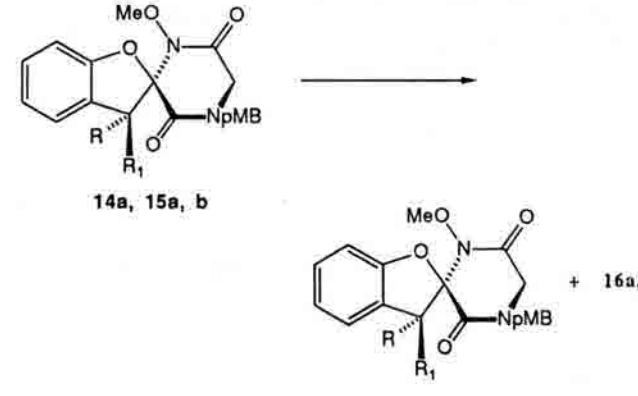
Functionalization of **14a** was ultimately achieved using Lewis acid (S_N1-type) conditions. Formation of alcohols **15a,b** was achieved simply by treating **14a** with a slight excess of silver triflate in aqueous THF. The reaction proceeds in a nonselective fashion giving a 1.6:1 (trans/cis, **15a/15b**) ratio of diastereomers in 75% overall yield; fortunately the epimeric alcohols were easily separated by column chromatography and carried on individually.

The relative configurations at the benzylic position for alcohols **15a,b** and subsequent related compounds were determined by ^1H NMR/NOE experiments. In the case of the trans diastereomer **15a**, irradiation of the benzylic methine proton at 5.80 ppm resulted in a positive NOE enhancement of the *N*-methoxyl signal at 3.88 ppm. Likewise irradiation of the *N*-methoxyl group exhibited a positive NOE enhancement of the signal assigned to the benzylic proton. Analogous NOE experiments involving the cis diastereomer **15b** did not reveal significant NOE enhancements for either signal (benzylic methane 5.59 ppm, *N*-methoxyl 3.81 ppm). The slight preference for the formation of the trans diastereomer as the major isomer is attributed to steric interactions between the *N*-methoxyl group and the approaching nucleophile on the incipient benzylic oxonium ion.

In our preliminary account, it was reported treatment of **15a,b** with 6 equiv of BF_3–Et_2O in the presence of 12 equiv thiolacetic acid resulted in the exclusive formation of the *trans*-thioacetate **16a**. However, more careful examination of the crude reaction revealed a mixture of benzylic thioacetates **16a** and **16b**. Surprisingly, along with thioesters **16a,b** a mixture of the O-acetylated compounds **21a,b** was also isolated from the reaction mixture and characterized. The identity of the O-acetylated compounds **21a,b** was confirmed by acetylation of **15a** and **15b** with acetyl chloride and comparison of the ^1H NMR spectra. The NMR of the O-acetylated compounds exhibited a downfield shift of the benzylic methines to 6.42 and 6.33 ppm for the trans and cis, while the signals due to the acetate methyl group were shifted upfield to 1.86 and 2.14 ppm for the trans and cis diastereomers, respectively. The formation of the O-acetylated compounds can be rationalized to occur via complexation of the thiol group of thiolacetic acid with the Lewis acid rendering the carbonyl oxygen more prone to react in a nucleophilic capacity. The incipient thionoacetates presumably undergo S to O exchange upon workup, although the intermediacy of such species proved elusive.

Subsequent experiments utilizing individual alcohols **15a,b** demonstrated that the product ratio of **16a,b** is dependent upon the stereochemistry of the starting alcohol. Treatment of *trans*-**15a** under the reaction conditions (12 equiv thiolacetic acid, 6 equiv BF_3–Et_2O) gave a 1.4:1 ratio of **16a/16b** in 70% yield as determined by integration of the benzylic methine signals at 5.74 and 5.81 ppm for the trans and cis diastereomers, respectively, in the crude NMR. Analogous reactions using *cis*-**15b** gave rise to a 3:1 ratio of **16a/16b** in 64% yield. Interestingly, in each case the major thioacetate diastereomer possessed the desired natural stereochemistry (i.e., *trans*-**16a**). The relative stereochemistry in **16a,b** was verified using the same NOE experiments as previously described for alcohols **15a,b**.

Despite the moderate success using BF_3–Et_2O, more stereoselective conditions to convert alcohols **15** to the thioacetates **16** was sought, and a variety of examples are shown in Table I. Treatment of **15a** or **15b** with 3 equiv of thiolacetic acid in the presence of excess anhydrous zinc chloride in refluxing benzene gave almost exclusive formation of the *O*-acetyl compounds **21a,b**



(entries 1 and 5). Analysis of the crude ^1H NMR spectra showed complete disappearance of starting material, and the mixture of *O*-acetates **21a,b** could be isolated in 50–54% yield. Again the formation of **21a,b** presumably arises from a Fisher-type transesterification process in which the zinc chloride forms a S–Zn complex with the thiolacetic acid.

(15) Wardell, J. L. In *The Chemistry of the Thiol Group*; Patai, S., Ed.; John Wiley and Sons: New York, 1974; Chapter 4.

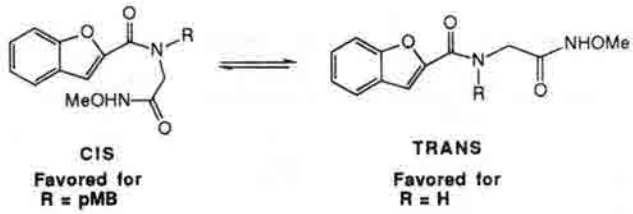


Figure 1.

Treatment of alcohols **15a,b** under a variety of conditions (entries 2–9) routinely gave mixtures of the O- and S-acetylated material. Only reactions using an excess of both thiolacetic acid and BF_3–Et_2O favored formation of the *S*-acetates.

Treatment of bromide **14a** with an excess of thiolacetic acid (>30 equiv, essentially solvolysis conditions) in the presence of zinc chloride in various solvents at room temperature resulted in exclusive formation of a mixture of **16a,b** as determined by crude ^{1}H NMR under a variety of conditions (entries 10–13). Surprisingly, treatment of bromide **14a** under the same conditions routinely gave higher ratios of **16a,b** (e.g., compare entry 2 and 12) and in every case the major diastereomer possessed the desired trans relative stereochemistry.

To determine if the product ratio of **16a/16b** might reflect an intrinsic thermodynamic stability for the trans configuration versus cis, both **16a** and **16b** were resubjected to the reaction conditions using excess thiolacetic acid/$ZnCl_2$ in benzene. Analysis of the crude NMRs showed epimerization did not take place under the reaction conditions indicating the product ratio reflects kinetic, not thermodynamic factors.

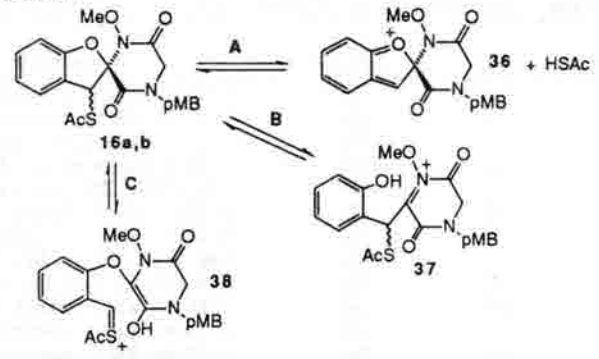
Introduction of the thiol group at the α-position of the diketopiperazine was achieved by bromination with NBS in refluxing carbon tetrachloride followed by treatment of the unstable bromides with thiolacetic acid in the presence of pyridine. The reaction gives essentially a 1:1 mixture of bisthioacetates **17a,b** in 65% combined yield presumably epimeric at the α-position only. Treatment of **16b** under the same conditions gave **17c,d** in 50% isolated yield as a 1:1 mixture of epimers. Attempts to firmly establish the stereochemistry at the α-position in each of the individual diastereomers **17a–d** by NOE experiments were ambiguous. Furthermore, since the conversion of *trans*-dithiols to disulfides has been observed previously,[1,16] the exact determination of the stereochemistry at the α-position in **17a–d** was not critical for subsequent transformations. Although the individual diastereomers could be separated, it was more convenient to carry each pair on as the mixture of epimers.

Deprotection of the thiol groups in **17a,b** under acidic conditions (HCl saturated ethanol, room temperature 6 h) followed by oxidation of the intermediate dithiols with aqueous KI_3/CH_2Cl_2 system resulted in a 1:1.4 ratio of the natural and unnatural disulfides **18a** and **18b** in a modest 32–44% combined yield. The ratio of **18a,b** was the same regardless of which diastereomeric bisthioacetate (e.g., **17a** or **b**) was used as the starting material. The relative configurations of both disulfide diastereomers were deduced from single X-ray crystal structural analysis.[12] Deprotection of the diastereomeric bisthioacetates **17c,d** containing the cis configuration under the same acidic conditions gave a surprisingly high 1:5 to 1:11 ratio of **18a/18b** in 30–49% yield.

In an attempt to gain further insight into when epimerization occurred and apparent stability of the cis thioacetates **17c,d**, the individual monothioacetates **16a,b** were subjected to the acid hydrolysis conditions, and the intermediate thiols were reacetylated with acetic anhydride. Analysis of the NMR for the *trans*-thioacetate **16a** revealed a 3:1 ratio of trans/cis in 40% combined yield, while **16b** gave a 1:5.6 trans/cis ratio in 60% isolated yield. These results indicate the cis diastereomer is in fact more stable to acidic conditions than the trans isomer.

A possible explanation for the stability of the cis configuration versus the trans may involve stereoelectronic effects. In the trans diastereomer, the *S*-acetyl bond can adopt an antiperiplanar orientation with respect to the C–N bond of the diketopiperazine ring which can lead to a weakening of the S–C bond (analogous

Scheme IV



to the anomeric affect). The cis diastereomers do not possess this type of interaction; therefore, the S–C bond is stronger and less readily cleaved leading to epimerization. Alternative mechanisms which cannot be ruled out given the available experimental evidence are the possibility of C–O or C–N bond cleavage followed by reclosure to generate the epimeric thioacetate.[17] Further investigation of this unusual isomerization reaction is currently underway.

The acid-catalyzed epimerization was avoided by conducting the final sequence under basic, anaerobic conditions. Deprotection of either **17a,b** under basic conditions (0.2 M sodium hydroxide in aqueous ethanol followed by oxidation with KI_3) resulted in formation of the desired disulfide **18a** in 32% yield and only a trace of the unnatural isomer. The same result could be obtained using NH_4OH/methanol; formation of disulfide **18a** was instantaneous as evidenced by the crude NMR prior to KI_3 oxidation, but the overall yield dropped to 18%. Comparable results were obtained using the diastereomeric thioacetates **17c,d**.

Having demonstrated the feasibility of the model study for the synthesis of aspirochlorine, we undertook the task of applying this approach toward the total synthesis. The strategy which emerged included several modifications foremost of which was the incorporation of a new amide protecting group and incorporation of both sulfur atoms in a single step.

A shortcoming of the model system was the inability to remove the glycine amide protecting group. As discussed above, a tertiary amide was required for the spirocyclization reaction to proceed. Our initial choice of the *p*-methoxybenzyl protecting group was predicated on the assumption strongly acidic media would be capable of removing this group, and literature precedent indicated epipolythiapiperazinediones were reasonably stable to strong acid. Unfortunately, we were unable to remove the *N*-*p*-methoxybenzyl group of **18** (or related spirocyclic precursors) using CAN or concentrated acids such as H_2SO_4 or TFA.[18] The lack of a good experimental procedure to remove the *p*-methoxybenzyl group in the model study thus precluded the use of this group for the total synthesis. Furthermore, the benzofuran moiety in the natural product contains substantially more electron density due to the presence of an additional hydroxyl group para to the benzylic position. This net increase in electron density around the aromatic ring might increase the likelihood of competing electrophilic aromatic substitution reactions in any type of oxidative deprotonation sequence. In order to avoid this problem we decided to incorporate a new protecting group which could be removed under

(16) Eguchi, C.; Kakuta, A. *J. Am. Chem. Soc.* **1974**, *96*, 3983. Ohler, E.; Tataruch, F.; Schmidt, U. *Chem. Ber.* **1973**, *106*, 165. Kishi, Y.; Fukuyama, T.; Nakatsuka, S. *J. Am. Chem. Soc.* **1973**, *95*, 6490.

(17) The epimerization of thioacetates **16a,b** was thought to occur via path A involving the benzofuranyl cation **36**. The absence of any products which resemble trapping of **36** with solvent (i.e., ethanol) suggests that another mechanism(s) may be occurring. Reasonable possibilities include path B which involves hydrolysis of the hemiaminal function resulting in the formation of intermediate **37**. Subsequent bond rotation followed by ring closure would allow epimerization to take place. A similar mechanism is illustrated by path C and involves formation of an incipient thionoacetate **38** as a possible intermediate.

(18) Smith, A. B.; Rano, T. A.; Chida, N.; Sulikowski, G. A. *J. Org.* 4Chem. **1990**, *55*, 1136. Yoshimura, J.; Yamaura, M.; Suzuki, T.; Hashimoto, H. *Chem. Lett.* **1983**, 1001.

Table I. Conversion of **14a** and **15a,b** to Thioacetates **16a,b**

entry	substrate	conditions[a]	ratio O vs S acetylation[b]	ratio of trans/cis[c]	combined yield (%)
1	**15a**	3 eq. HSAc, $ZnCl_2$, benzene, reflux 6 h	1:trace		50
2	**15a**	ex HSAc, $ZnCl_2$, benzene, rt, 24 h	9:1	2.5:1	77
3	**15a**	ex HSAc, $ZnCl_2$, benzene, rt, 6 days	4:1	2:1	75
4	**15a**	12 eq. HSAc, 6 eq. BF_3–Et_2O, CH_2Cl_2	1:3	1.5:1	70
5	**15b**	3 eq. HSAc, $ZnCl_2$, benzene, reflux 6 h	1:trace		54
6	**15b**	ex HSAc, $ZnCl_2$, benzene, rt, 24 h	9:1	3:1	63
7	**15b**	ex HSAc, $ZnCl_2$, benzene, rt, 6 days	1:1	2:1	80
8	**15b**	3 eq. HSAc, 0.5 eq. BF_3–Et_2O, CH_2Cl_2	1:trace		50
9	**15b**	12 eq. HSAc, 6 eq. BF_3–Et_2O, CH_2Cl_2	1:3	3:1	64
10	**14a**	ex HSAc, $ZnCl_2$, $CHCl_3$, rt, 24 h	trace:1	3:1	52
11	**14a**	ex HSAc, $ZnCl_2$, acetone, rt, 24 h	trace:1	5:1	32
12	**14a**	ex HSAc, $ZnCl_2$, benzene, rt, 24 h	trace:1	3.6:1	62
13	**14a**	ex HSAc, $ZnCl_2$, benzene, rt, 36 h	trace:1	3:1	83

more mild, selective conditions.

After reviewing the various moieties which have been used as amide protecting groups,[19] we decided to try the *o*-nitrobenzyl (oNB) group which has the distinct advantage of being photolabile under mild conditions. The oNB group has been used extensively in peptide chemistry as an amine and carboxyl protecting group[20] and has been used as an alcohol protecting group as well. The use of oNB as an *amide protecting group* has not been widely used,[21] and our synthesis demonstrates the potential utility of oNB as an amide protecting group.

The total synthesis summarized in Scheme III commences with the synthesis of 2,4-dihydroxy-5-chlorobenzaldehyde **23**. Compound **23** can be obtained from 2,4-dihydroxybenzaldehyde via electrophilic chlorination using NaOCl[22] or via formylation of 4-chlororesorcinol **22**.[23] Both methodologies were investigated with varying degrees of success.

Attempted chlorination of 2,4-dihydroxybenzaldehyde with basic NaOCl did not generate the desired 5-chloro isomer **23** as originally reported.[24] Benzaldehyde **23** was obtained selectively via Gatterman formylation of commercially available 4-chlororesorcinol **22** using zinc cyanide in the presence of HCl. Selective protection of the 4-hydroxyl to give **24** was achieved with moderate success (49%) using chloromethyl methyl ether (MOM chloride) in the presence of triethylamine. As expected, the major side product in this reaction is the over-protected product which is easily removed during workup.

Conversion of **24** to the corresponding coumarilic acid derivative was performed using Tanaka's procedure although the yields obtained did not match those reported for similar systems.[25] Treating **24** with diethyl bromomalonate and potassium carbonate followed by hydrolysis of the intermediate diester under basic conditions resulted in formation of the desired coumarilic acid **25** in 72% yield along with a small amount of the deprotected acid as a side product. The unprotected compound arises from the acidic workup required and could be suppressed by careful control of the pH.

Schotten–Baumann coupling of acid chloride **25** with *N*-(2-nitrobenzyl)glycine ethyl ester **26** (prepared from 2-nitrobenzyl bromide and glycine ethyl ester) went smoothly. The crude ester was routinely carried on without purification, immediately being subjected to acid hydrolysis (aqueous HCl, dioxane, reflux) to remove both the ethoxy and methoxymethyl groups giving carboxylic acid **27a** in 84–95% overall yield from **25**. Conversion of **27a** to the desired hydroxamic acid **28a** was achieved in a modest 41% yield by diimide coupling (1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride, methoxylamine, aqueous THF).

Attempted ring closure of **28a** did not result in any of the desired spiro[benzofuran-piperazine] compound **29a** under identical conditions (NBS, $CHCl_3$), and starting material was recovered unchanged. The lack of ring closure for the free hydroxyl containing **28a** compared to the facile ring closure for the hydroxamic ester **13a** in the model study strongly suggests that the 6-hydroxy group has a pronounced influence on the electronic nature of the 2,3 double bond presumably via a resonance effect. In order to minimize the effects of the distant hydroxy group on the double bond, the 6-hydroxy group was protected with an electron-withdrawing ester moiety. Initial reactions were conducted with pivalate, but this group proved too difficult to remove in subsequent transformations and was abandoned (data not shown). The desired transformation was obtained using acetate as the protecting group which was efficiently introduced by treating **27a** with excess acetic anhydride. Compound **27b** was further converted into hydroxamic ester **28b** in 70% yield via mixed anhydride formation (isobutyl chloroformate, *N*-methylmorpholine, methoxylamine). Treatment of **28b** with NBS gave the desired tricyclic compound **29b** in 57–63% isolated yield after workup.

Conversion of **29b** into a mixture of alcohols using the procedure developed in the model study (silver triflate, aqueous THF, 73%, ~1:1 ratio) was straightforward, but we opted for converting **29b** into a protected form of the alcohols, the analogous methyl ethers. Conversion of **29b** into **30a,b** was achieved in 73% yield by simply performing the reaction in the presence of methanol instead of water. Much to our surprise, the conversion of **29b** to **30a,b** proceeds in a stereoselective fashion resulting in a 4:1 ratio of **30a**:**30b**. If the reaction was conducted at reflux the ratio of **30a**/**30b** dropped to 2:1, but the overall yield was increased to 80%. Methyl ethers **30a,b** could be separated by careful column chromatography and carried on independently. Determination of the stereochemistry for the methyl ethers was carried out by NOE experiments and comparison of the ^1H NMR spectra with NMR data obtained from the model study.

The photolytic deprotection of **30a,b** was examined under a variety of conditions (Table II). Using a variety of aqueous THF solutions resulted in the desired **31a,b** in 40–50% yield. As the deprotection proceeds the solution darkens, and it was assumed the change in color may be quenching the reaction. Addition of Pyrex beads to the solution (entry 2) as a means of increasing the transmittance through the solution improved the yield to 58%. Further improvements in the yield were obtained by decreasing

(19) Smith, M. B.; Wand, C.; Keusenkothen, P. F.; Dembofsky, B. T.; Fay, J. G.; Zezza, C. A.; Kwon, T. W.; Sheu, J.; Son, Y. C.; Menezes, R. F. *Chem. Lett.* **1992**, 247 and references cited therein. See, also: Nakatsuka, S.; Miyazaki, H.; Goto, T. *Tetrahedron Lett.* **1980**, *21*, 2817. Kishi, Y.; Nakatsuka, S.; Fukuyama, T.; Havel, M. *J. Am. Chem. Soc.* **1973**, *95*, 6493.

(20) Review of photolabile protecting groups, see: Pillai, V. N. R. *Synthesis* **1980**, 1. For related work using *o*-nitrobenzyl groups see: (a) Walker, J. W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, *110*, 7170. (b) Kalbag, S. M.; Roeske, R. W. *J. Am. Chem. Soc.* **1975**, *97*, 440. (c) Patchornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333. (d) Barltrop, J. A.; Schofield, P. *J. Chem. Soc.* **1965**, 4758.

(21) Milburn, T.; Matsubara, N.; Billington, A. P.; Udgaonkar, J. B.; Walker, J. W.; Carpenter, B. K.; Webb, L. W.; Marque, J.; Denk, W.; McCray, J. A.; Hess, G. P. *Biochemistry* **1989**, *28*, 49.

(22) Hopkins, C. Y.; Chisholm, M. J. *Can. J. Res.* **1946**, *24B*, 208.

(23) Chakravarti, D.; Ghosh, B. *J. Ind. Chem. Soc.* **1935**, *12*, 791. Adams, R.; Levine, I. *J. Am. Chem. Soc.* **1923**, *45*, 2373.

(24) The regiochemical assignment was made based on ^1H NMR coupling constants for the 3- and 5-chloro regio isomers. The 200 MHz ^1H NMR of **23** exhibits two singlets at 7.61 and 6.59 ppm which is consistent with *para*-substitution in aromatic rings. The ^1H NMR of 3-chlororesorcylaldehyde, on the other hand, exhibits two doublets at 7.57 and 6.68 ppm (J = 8.69 Hz) consistent for *ortho* protons indicating chlorination must have taken place at the 3-position.

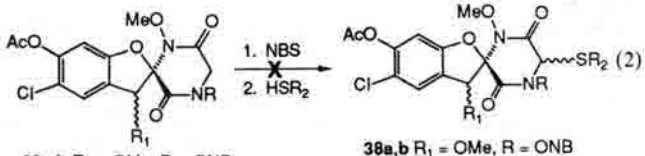
(25) Tanaka, S. *J. Am. Chem. Soc.* **1951**, *73*, 872.

Table II. Photolysis Conditions for the Conversion of **30a,b** to **31a,b**[a]

entry	solvent, conditions	time (h)	% yield **30a,b**[b]
1	30% H_2O/THF	18	40–50
2	30% H_2O/THF, beads	18	58
3	10% H_2O/THF, beads	18	66
4	10% H_2O/THF, beads	5	72
5	10% H_2O/THF, beads 10 eq. semicarbazide-HCl	5	68

[a] All reactions were carried out at 10 mmol concentrations in a quartz tube. Photolysis was carried out using a 450-W Conrad-Hano-via medium-pressure mercury vapor lamp at 37 °C. [b] Reported yields are isolated yields.

the amount of water from 30% to 10% in the reaction mixture resulting in the yield increasing to 66%. A final change in the duration of the photolysis from 18 h to 5 h increased the yield to an optimal 72%. Addition of an aldehyde trapping agent (10 equiv semicarbazide hydrochloride, entry 5) did not affect the overall yield for the photolysis. Under the reaction conditions no epimerization at the benzylic position was observed in either case. As an interesting side note, the photolytic deprotection could also be carried out efficiently using direct sunlight.



30a,b R_1 = OMe, R = ONB
31a,b R_1 = OMe, R = H

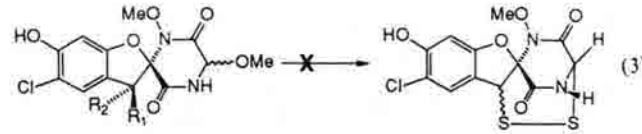
38a,b R_1 = OMe, R = ONB
39a,b R_1 = OMe, R = H

Attempted functionalization of the α-position of the diketopiperazine ring of both the *o*-nitrobenzyl protected substrate **30a,b** or the deprotected compounds **31a,b** under a variety of conditions did not give satisfactory results. Attempted bromination (NBS, benzoyl peroxide carbon tetrachloride, reflux) of **30** followed by trapping the unstable bromide with thiolacetic acid did not result in any of the desired functionalized compounds **38** in appreciable yields (eq 2). Likewise **31** was resistant to NBS under a variety of conditions which have been used to brominate α to NH groups in dipeptides (e.g., NBS/CCl_4 or NBS/$CHCl_3$)[26,27] and did not give any of the desired **39**.

The oxidation of the diketopiperazine could be carried out efficiently via N-chlorination using *tert*-butyl hypochlorite.[28] Treatment of **31a,b** with *tert*-butyl hypochlorite followed by sodium methoxide gave the bismethyl ethers *trans*-**32a,b** and *cis*-**32c,d** each as a mixture of epimers at the α-position. The reaction displays a pronounced solvent dependency; reactions carried out in chlorinated solvents gave the best synthetic yields (50–58%). The N-chlorination/rearrangement reaction proceeds with slight stereoselectivity giving a 1.6:1 ratio of **32a**/**32b** when the reaction were carried out in methylene chloride and a 3:1 ratio of **32a**/**32b** when the reaction was carried out in chloroform. Diastereomers **32a,b** could be easily separated by column chromatography, while epimers **32c,d** were inseparable and carried on as a mixture.

The acetate protecting group in **32** was efficiently removed by treating the individual diastereomers (in the case of **32a,b**) or the mixture **32c,d** with sodium ethoxide in absolute ethanol at 0 °C to give the free phenols **33a–d** in 72–74% yields. All the various diastereomers of **33** could be separated by chromatography. Direct conversion of **31** to **33** could be carried out using excess base, but better overall yields were obtained if the reactions were carried out separately.

Incorporation of the sulfur moieties into **33a–d** was anticipated to lead directly to aspirochlorine (eq 3). Unfortunately this was not borne out experimentally. Treating **33a,b** with H_2S in the presence of $ZnCl_2$ or BF_3–Et_2O under numerous conditions followed by KI_3 oxidation resulted in complex mixtures and only trace amounts of the natural product. Treatment of **33c,d** with H_2S, $ZnCl_2$ followed by oxidation gave aspirochlorine in a disappointing 5–10% isolated yield.



33a-d **2, 35**

Conversion of methyl ethers **33a,b** or **33c,d** to the corresponding bisthioacetates **34a–d** was achieved (12 equiv of thiolacetic acid, 6 eqiv of BF_3–Et_2O, CH_2Cl_2 reflux, 8 h) yielding a mixture of diastereomers in 65% combined yield. The compounds could be separated into two sets of two diastereomers: the nonpolar minor group (ca. < 20%) and the more polar major set of thioacetates. Based on the precedence observed in the model study, the major diastereomers were tentatively assigned the desired trans stereochemistry at the benzylic position and carried on. This stereochemical assignment was verified by reduction of an authentic sample of aspirochlorine (excess methyl mercaptan, pyridine) followed by trapping of the dithiol intermediate with acetyl chloride to generate **34**. Comparison of the ^{1}H NMR spectra of the products from this reaction with the ^{1}H NMR of the major thioacetates obtained via BF_3–Et_2O catalysis established that these thioacetates were identical.

Conversion of thioacetates **34a–d** into aspirochlorine also proved to be problematic. Basic conditions such as NaOMe/MeOH, aqueous $NaHCO_3$/EtOH, NaSH/EtOH, or NH_4OH/MeOH under either anaerobic or aerobic conditions resulted in disappearance of starting material but did not produce any aspirochlorine upon workup. Deprotections using a nonbasic nucleophile such as cyanide anion[29] (0.1 equiv of aqueous NaCN, MeOH, reflux) only gave traces of the natural produce by ^{1}H NMR, while reactions using chloroaniline, which has been used to cleave base sensitive thioacetates, gave no reaction at all.[30]

Removal of the acetate protecting groups under acidic conditions was achieved but with limited success. Treatment of thioacetates **34** with saturated ethanolic HCl followed by oxidation resulted in a complex mixture of products from which aspirochlorine could be isolated in 5–15% yield. Numerous attempts at varying the conditions (aqueous HCl/EtOH, catalytic HCl, aqueous acid, neat TFA/thioanisole, etc.) did not substantially improve the yield.

The low yield for the desired product along with the substantial amount of decomposition which was being observed lead us to believe the thioacetates were either unstable to the reaction conditions and/or the oxidation to the disulfide did not proceed readily. To test whether the dithiol/disulfide oxidation was contributing to the low yield, a sample of natural aspirochlorine was reduced ($NaBH_4$, EtOH, 0 °C), and the intermediate dithiols immediately oxidized with aqueous KI_3. Although the oxidation appeared to be clean by TLC, aspirochlorine was only isolated in 38% from the reaction, indicating the oxidation does not take place as readily as anticipated.

The substantial decomposition in the deprotection reactions must be due to the instability of the thioacetates under the various conditions examined. The elimination of 2 equiv of thiolacetic acid from **34** could readily occur due to the presence of strategically located activating groups (i.e., the OH activates the benzylic position, and the NH activates the α-position of the diketopiperazine ring). Evidence for this type of elimination/addition was observed in the model study and used to account for the epimerization of thioacetates **16a,b** and the formation of the mixture of disulfides **18a,b**. Thus, acid catalyzed loss of 1 equiv of thiolacetic acid could generate either a quinone methide species or an *N*-acyl iminium ion both of which are reactive intermediates,

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which can undergo further loss of thiolacetic acid and decomposition.

To take advantage of the possibility that elimination was indeed occurring, the deprotection was carried out using saturated H_2S in a nonnucleophilic solvent in order to increase the amount of sulfur nucleophile in solution. Therefore if the elimination occurred, the probability of the intermediate being quenched by thiol would be increased. Saturating a benzene solution of thioacetate **34a,b** and camphor sulfonic acid (CSA) with H_2S resulted in *clean* conversion to aspirochlorine in 28% isolated yield (51% based on recovered starting material). Unfortunately this reaction proved to be rather unpredictable and could not be repeated with any consistency. Determination of whether transesterification or elimination/addition of hydrogen disulfide is the mechanism is not clear. Treatment of methyl ethers **33c,d** under the same conditions resulted in no reaction, and only starting material was evident by TLC.

After several more attempts, we were finally able to cleave the thioacetates under mild conditions using aminolysis. Treating a solution of **34a–d** with excess methoxylamine in the presence of camphor sulfonic acid (CSA) under aerobic conditions routinely gave aspirochlorine in 20–34% yield. The synthetic aspirochlorine possessed identical ^1H NMR and IR spectral characteristics and HPLC retention time when compared with the natural substance. At the present time, none of the epimeric disulfide **35** has been positively identified from either reactions involving the methyl ethers or thioacetates. The lack of evidence for **35** does not rule out the formation of such a compound and may reflect problems associated with stability.

The first total synthesis of (±)-aspirochlorine has been achieved in 13 steps from 4-chlororesorcinol. The synthesis proceeds with moderate stereoselectivity and exemplifies the use of the *o*-nitrobenzyl group as a photolabile amide protecting group. Efforts to study the redox properties of the aspirochlorine disulfide bridge as compared to other disulfides is currently under study in these laboratories.

Experimental Section

^1H NMR spectra were recorded on a Bruker 300 MHz FT NMR or on an IBM WP270 MHz FT NMR in $CDCl_3$ or DMSO-d_6 and chemical shifts are reported relative to TMS. NMR data collected in methanol-d_4 are reported relative to the methanol peak at 3.30 ppm. IR were collected on a Perkin-Elmer 1600 FT IR. Melting points were obtained using a Mel Temp apparatus and are uncorrected. Elemental analysis were performed by MHW labs, Phoenix, AZ. Analytical thin-layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} glass plates. Preparative flash column chromatography was performed using Grade 60 230–400 mesh silica gel purchased from Aldrich, and radial chromatography was carried out on a Chromatotron Model 7924 using 1,2 or 4 mm silica plates as needed. HPLC separation of aspirochlorine was carried out using Waters 6000 pump equipped with a 254-nm fixed wavelength detector and a 8 × 10 cm silica radial compression cartridge, using 2% methanol/chloroform at a flow rate of 2 mL/min.

Unless otherwise stated, all reactions were carried out in flame-dried flasks under a N_2 or argon atmosphere. Compounds containing *o*-nitrobenzyl group were stored in flasks wrapped in aluminum foil to minimize photodecomposition. Tetrahydrofuran and diethyl ether were dried over sodium/benzophenone ketyl, while methylene chloride was dried over calcium hydride. Transfer of solvents were carried out via flame-dried syringe. Ethanol-free chloroform was obtained by first washing with water, drying over magnesium sulfate distillation from phosphorus pentoxide, and storing in an amber bottle. Triethylamine, pyridine, and acyl chlorides were first filtered through alumina prior to use. TLCs of thiol and thioacetate-containing compounds were visualized either using ethanolic I_2/NaN_3 stain or 5% Ellmans' reagent in DMF. Photolysis reactions were conducted using a 450-W Conrad-Hanovia 7825 medium-pressure lamp in a Pyrex well at 37 °C. Coumarilic acid was obtained from Lancaster Synthesis, and 5-chlororesorcinol was obtained from Aldrich. Methoxylamine hydrochloride was converted into the free base using the published procedure.[31] Methoxylamine was stored over KOH at 0 °C and filtered through a plug of alumina prior to use. *tert*-Butyl hypochlorite was prepared fresh and stored in an amber bottle over $CaCl_2$ at 0 °C.[32] All other reagents used were of commercial purity (Aldrich) unless otherwise stated.

Caution. Reactions involving the highly toxic H_2S and zinc cyanide/HCl mixtures were very carefully performed in a well ventilated fume hood. In addition, reactions carried out using the highly toxic carcinogen chloromethyl methyl ether (MOM-Cl) were conducted using appropriate lab attire (lab coat, rubber gloves, safety glasses) in a well-ventilated fume hood.

***N*-(4-Methoxybenzyl)glycine Ethyl Ester (11).** To a stirred solution of *p*-methoxybenzylamine (68.6 g, 0.5 mol, 1.0 equiv) and triethylamine (101 g, 1.0 mol, 2.0 equiv) in 300 mL of THF was added dropwise ethyl bromoacetate (87.7 g, 0.525 mol, 1.05 equiv) at 0 °C under N_2, and the mixture stirred at room temperature for 12 h. The reaction was filtered and evaporated, and the product was distilled under reduced pressure (bp 135–140 0 °C, 0.3 mmHg): obtained 94.4 g of a light yellow oil; 85% yield; ^1H NMR (270 mHz), $CDCl_3$) δ = 1.27 (3 H, t), 1.90 (1 H, s), 3.39 (2 H, s), 3.74 (2 H, s), 3.80 (3 H, s), 4.19 (2 H, qt), 6.87 (2 H, d), 7.25 (2 H, d); IR (NaCl, neat film) ν = 3340, 2975, 2930, 1735, 1610 cm^{-1}.

***N*-[Benzofuranyl-2-(3*H*)]-*N*-(4-methoxybenzyl)glycine (12a).** A solution of coumarilic acid (1.98 g, 12.2 mmol, 1.0 equiv) and thionyl chloride (3.1 mL, 38.9 mmol, 3.0 equiv) in 150 mL of dry benzene was refluxed for 4 h. The solution was concentrated. The crude acid chloride was taken up in methylene chloride and added to a vigorously stirred aqueous solution containing *N*-(4-methoxybenzyl)glycine ethyl ester **11** (3.0 g, 13.0 mmol, 1.1 equiv) and $NaHCO_3$ (1.1 g, 13.0 mmol, 1.1 equiv). The solution was stirred at room temperature for 1 h. The layers were separated, and the organic phase was washed with water, dried over $MgSO_4$, and filtered to give a yellow oil. The crude ester was taken up in 100 mL of ethanol, cooled to 0 °C, and saponified using LiOH (15 mL, 15.0 mmol, 1.2 equiv). The solution was warmed to room temperature and stirred for 24 h. The white solid was collected by filtration and taken up in water. After acidification with 2 M HCl, the acid was extracted into methylene chloride, dried with $MgSO_4$, filtered, and concentrated to a light yellow solid. The product was recrystallized from boiling ethyl acetate/hexanes: 66% yield; mp 153–154 °C; ^1H NMR (300 MHz, DMSO-d_6, T = 370 K) δ = 7.71 (1 H, d, J = 7.7 Hz), 7.54 (1 H, d, J = 8.1 Hz), 7.44–7.37 (2 H, m), 7.32–7.24 (3 H, m), 6.89 (2 H, d, J = 8.6 Hz), 4.75 (2 H, s), 4.17 (2 H, s), 3.74 (3 H, s); IR (NaCl, film) ν = 2938, 1738, 1611, 1248, 1176 cm^{-1}. Anal. Calcd for $C_{19}H_{17}NO_5$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.19; H, 5.11; N, 4.18.

***N*-[Benzofuranyl-2-(3*H*)]-*N*-glycine (12b).** Coumarilic acid (1.0 g, 6.16 mmol, 1.0 equiv) was dissolved in 100 mL of dry benzene and thionyl chloride (1.45 mL, 18.5 mmol, 3.0 equiv) was added, and the solution was refluxed for 3 h. The reaction was cooled, and the solvent was removed to give the crude acid chloride as an off-white colored solid. The acid chloride was taken up in CH_2Cl_2 and added to a vigorously stirred suspension of glycine ethyl ester hydrochloride (947 mg, 6.78 mmol, 1.1 equiv) and $NaHCO_3$ (569 mg, 6.78 mmol, 1.1 equiv) in 100 mL of water. The reaction was stirred for 1 h, the layers separated, and the organic phase was collected, washed with water, dried over $MgSO_4$, filtered, and concentrated to a white crystalline material.

The ester was dissolved in 50 mL of ethanol cooled to 0 °C. An aqueous solution of LiOH (2.8 mL, 1.1 equiv) was added, and the solution was warmed to room temperature and stirred for 24 h. The solvent was removed, and the residue was taken up in water, washed with diethyl ether, and acidified with 1 M HCl. The white precipitate was extracted into CH_2Cl_2, washed with water, dried over $MgSO_4$, filtered, and concentrated to a white solid which was recrystallized from ethyl acetate/hexanes: mp 189–191 °C; 65% yield; ^1H NMR (300 MHz, DMSO-d_6) δ = 12.71 (1 H, brs), 9.0 (1 H, t, J = 5.9 Hz), 7.79 (1 H, d, J = 7.6 Hz), 7.67 (1 H, d, J = 8.4 Hz), 7.58 (1 H, s), 7.51–7.45 (1 H, m), 7.37–7.32 (1 H, m), 3.94 (2 H, d, J = 6.0 Hz); IR (KBr) ν = 3267, 3057, 1725, 1660, 1567, 1228, 748 cm^{-1}. Anal. Calcd for $C_{11}H_9NO_4$: C, 60.27; H, 4.14; N, 6.39. Found: C, 60.25; H, 4.25; N, 6.28.

***N*-Benzofuranylglycine Hydroxamate (13a).** To a solution containing 1 g **12a** (2.94 mmol, 1.0 equiv) dissolved in 250 mL of THF cooled to 0 °C was added triethylamine (616 mL, 4.42 mmol, 1.5 equiv). Pivaloyl chloride (400 mL, 1.1 equiv) was added causing the solution to turn bright yellow. The reaction was stirred at 0 °C for 90 min before added methoxylamine (500 mL, 9.4 mmol, 3.2 equiv). Solution stirred at 0 °C until the yellow color dissipated (about 2 h). The reaction was poured into ethyl acetate, washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and concentrated to a thick, colorless oil which left standing crystallized. The hydroxamic ester was purified by recrystallization from boiling ethyl acetate/hexanes: obtained 1.03 of a white solid; typical yields 80–95%; ^1H NMR (300 MHz, DMSO-d_6, T = 370

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K) δ = 10.78 (1 H, brs), 7.48 (1 H, d, J = 7.7 Hz), 7.55 (1 H, d, J = 8.2 Hz), 7.44–7.37 (2 H, m), 7.33–7.24 (3 H, m), 6.89 (2 H, d, J = 8.6 Hz), 4.74 (2 H, s), 4.0 (2 H, s), 3.75 (3 H, s), 3.58 (3 H, s); IR (NaCl, film) ν = 3203, 2999, 2936, 1677, 1613, 1513 cm^{-1}. Anal. Calcd for $C_{20}H_{20}N_2O_5$: C, 65.20; H, 5.47; N, 7.61. Found: C, 65.25; H, 5.61; N, 7.69.

***N*-Benzofuranylglycine Hydroxamate (13b).** To an aqueous THF solution of **12b** (1.6 g, 7.3 mmol, 1.0 equiv) and 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (2.79 g, 14.6 mmol, 2.0 equiv) was added methoxylamine (686 mg, 14.6 mol, 2.0 equiv). The solution was stirred at room temperature for 48 h. The reaction was poured into water, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and concentrated to a white solid. The solid was recrystallized from ethyl acetate/hexanes to give 976 mg of a crystalline material: mp 181 °C; 53% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.23 (1 H, brs), 8.93 (1 H, brt), 7.78 (1 H, d, J = 7.6 Hz), 7.67 (1 H, d, J = 8.4 Hz), 7.58 (1 H, s), 7.50–7.45 (1 H, m), 7.37–7.32 (1 H, m), 3.80 (2 H, d, J = 5.7 Hz), 3.6 (3 H, s); IR (KBr) ν = 3275, 3196, 3001, 1684 (shoulder), 1648, 1572, 1305, 747 cm^{-1}. Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.26; H, 4.99; N, 11.25.

Trans Spiro Cyclic Bromide (14a). To a solution of glycine hydroxamate **13a** (1 g, 2.71 mmol, 1 equiv) in 50 mL of EtOH-free $CHCl_3$ was added NBS (578 mg, 3.25 mmol, 1.2 equiv) in one portion, and the solution was stirred at room temperature overnight. The reaction was diluted with additional $CHCl_3$ and extracted with saturated $Na_2S_2O_3$ and twice with H_2O, and the organic layer was dried over Na_2SO_4, filtered, and evaporated to give a thick yellow oily residue. The residue was taken up in ethyl acetate, and the white precipitate which subsequently forms collected. The mothor liquor was chromatographed using 3:2 hexane/ethyl acetate, and the product was recrystallized from boiling ethyl acetate/hexanes: 50% combined yield; mp 142–143 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) ∂ = 7.33–7.26 (4 H, m), 7.28 (1 H, d, J = 8.6 Hz, super imposed over multiplet), 7.07–7.02 (1 H, m), 6.97–6.95 (1 H, m), 6.86 (1 H, d, J = 8.6 Hz), 6.01 (1 H, s), 4.69 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.50 (1 H, $^1/_2$ABq, J = 14.3 Hz), 5.99 (2 H, d, J = 1.4 Hz), 3.92 (3 H, s), 3.80 (3 H, s); ^{1}H NMR (DMSO-d_6, 270 MHz) ∂ = 7.34–7.29 (5 H, m), 7.06–7.02 (2 H, m), 6.92 (1 H, d, J = 8.6 Hz), 6.24 (1 H, s), 4.69 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.38 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.19 (1 H, $^1/_2$ABq, J = 18.3 Hz), 4.04 (1 H, $^1/_2$ABq, J = 18.3 Hz), 3.74 (3 H, s); ^{13}C NMR ($CDCl_3$, 75.47 MHz) ∂ = 162.1 (C), 160.93 (C), 159.8 (C), 157.9 (C), 131.18 (CH), 131.08 (CH), 130.63 (CH), 125.93 (C), 125.44 (CH), 122.68 (CH), 114.28 (CH), 109.69 (CH), 100.68 (C), 65.71 (CH_3), 55.28 (CH_3), 50.15 (CH), 49.24 (CH_2), 47.96 (CH_2); IR (neat, NaCl) ν = 1693, 1613, 1513, 1245 cm^{-1}. Anal. Calcd for $C_{20}H_{19}N_2O_5Br$: C, 53.70; H, 4.28; N, 6.26; Br, 17.87. Found: C, 53.62; H, 4.22; N, 6.26; Br, 18.00.

Trans and Cis Spiro Cyclic Alcohols (15a,b). **14a** was dissolved in a solution of 1:1 THF/H_2O at room temperature. Silver triflate (1.2 equiv) dissolved in THF was added, and the resulting solution was stirred for 30 min. The solution was diluted with ethyl acetate, and saturated NaCl solution added. The solution was filtered through a plug of Celite to remove the silver salts. The filtrate was washed with brine and water, dried over $MgSO_4$, filtered, and afterwards evaporated to a colorless oil which upon standing solidifies. The residue was taken up in ethyl acetate and chromatographed using 1:1 ethyl acetate/hexanes. Both isomers were recrystallized from ethyl acetate/hexanes: 80% combined yield.

***trans*-15a**: R_f 0.17, 1:1 ethyl acetate/hexanes; ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.39–7.19 (4 H, m), 7.06–7.01 (1 H, m), 6.91–6.87 (3 H, m), 5.80 (1 H, d, J = 12.3 Hz, singlet in D_2O), 4.60 (1 H, $^1/_2$ABq, J = 14.4 Hz), 4.49 (1 H, $^1/_2$ABq, J = 14.4 Hz), 3.98 (2 H, s), 3.88 (3 H, s), 3.80 (3 H, s), 3.55 (1 H, d, J = 12.4 Hz, exchangeable in D_2O); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 161.49 (C), 160.99 (C), 159.73 (C), 158.47 (C), 130.65 (CH), 129.97 (CH), 127.19 (C), 125.81 (C), 125.0 (CH), 122.44 (CH), 114.46 (CH), 109.86 (CH), 99.92 (C), 77.50 (CH), 65.30 (CH_3), 55.29 (CH_3), 48.95 (CH_2), 47.99 (CH_2); IR (NaCl, neat film) ν = 3407, 1683 cm^{-1}; mp 135–136 °C. Anal. Calcd for $C_{20}H_{20}N_2O_6$: C, 62.49; H, 5.24; N, 7.29. Found: C, 62.49; H, 5.25; N, 7.26.

***cis*-15b**: R_f 0.1, 1:1 ethyl acetate/hexanes); ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.36–7.28 (3 H, m), 7.24 (1 H, $^1/_2$ABq, J = 8.6 Hz), 7.07–6.96 (3 H, m), 6.98 (1 H, $^1/_2$ABq, J = 8.6 Hz), 5.59 (1 H, d, J = 12.6 Hz, singlet in D_2O), 4.69 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.48 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.01 (2 H, s), 3.81 (3 H, s), 3.78 (3 H, s), 3.0 (1 H, d, J = 12.8 Hz, exchangeable in D_2O); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 162.95 (C), 161.78 (C), 159.75 (C), 157.46 (C), 130.79 (CH), 130.16 (CH), 126.27 (C), 126.20 (C), 124.57 (CH), 122.58 (CH), 114.47 (CH), 109.76 (CH), 98.28 (C), 79.08 (CH), 65.54 (CH_3), 55.33 (CH_3), 49.28 (CH_2), 48.11 (CH_2); IR (NaCl, neat film) ν = 3392, 1683 cm^{-1}; mp 140–142 °C. Anal. Calcd for $C_{20}H_{20}N_2O_6$: C, 62.49; H, 5.24; N, 7.29. Found: C, 62.44; H, 5.21; N, 7.20.

Trans and Cis Spiro Cyclic Thioacetates (16a,b). **14a** (75 mg) was dissolved in 4 mL of benzene, and excess thiolacetic acid (0.5 mL) was added followed by a spatula tip of anhydrous zinc chloride. The solution was stirred for 36 h at room temperature. The reaction was filtered, diluted with ethyl acetate, washed three times with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and concentrated to a foul-smelling yellow film. The products were purified via column chromatography using 10% acetone in CCl_4 as the eluent: yield 69–80%.

***trans*-16a**: R_f 0.3, 20% acetone/CCl_4; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.28–7.24 (4 H, m), 7.14 (1 H, d, J = 7.5 Hz), 6.97–6.85 (3 H, m), 5.74 (1 H, s), 4.87 (1 H, $^1/_2$ABq, J = 14.1 Hz), 4.16 (1 H, $^1/_2$ABq, J = 14.1 Hz), 3.94 (1 H, $^1/_2$ABq, J = 18 Hz), 3.91 (3 H, s), 3.83 (1 H, $^1/_2$ABq, J = 18.2 Hz), 3.79 (3 H, s), 2.14 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 195.2 (C), 161.48 (C), 160.58 (C), 159.7 (C), 158.1 (C), 130.69 (CH), 129.91 (CH), 126.41 (C), 124.31 (CH), 123.91 (C), 122.17 (CH), 114.25 (CH), 109.36 (CH), 101.3 (C), 65.4 (CH_3), 55.31 (CH_3), 50.11 (CH), 49.24 (CH_2), 48.45 (CH_2), 29.62 (CH_3); IR (NaCl, neat film) ν = 1694, 1513, 1462, 1244 cm^{-1}; white crystals from ethyl acetate/petroleum ether; mp 164–165 °C. Anal. Calcd for $C_{22}H_{22}N_2O_6S$: C, 59.71; H, 5.01; N, 6.33; S, 7.25. Found: C, 59.93; H, 5.18; N, 6.07; S, 7.48.

***cis*-16b**: R_f 0.4, 20% acetone/CCl_4; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.29–7.28 (4 H, m), 7.14 (1 H, d, J = 7.5 Hz), 7.11–6.88 (3 H, m), 5.81 (1 H, s), 4.81 (1 H, $^1/_2$ABq, J = 14.3 Hz), 4.47 (1 H, $^1/_2$ABq, J = 14.3 Hz), 3.91 (1 H, $^1/_2$ABq, J = 18 Hz), 3.81 (3 H, s), 3.77 (3 H, s) superimposed over 3.73 (1 H, $^1/_2$ABq, J = 18 Hz), 2.36 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 195.61 (C), 162.9 (C), 160.32 (C), 159.67 (C), 157.19 (C), 130.33 (CH), 129.78 (CH), 126.14 (C), 123.97 (CH), 123.59 (C), 122.33 (CH), 114.31 (CH), 109.26 (CH), 99.50 (C), 65.04 (CH_3), 55.29 (CH_3), 52.74 (CH), 49.45 (CH_2), 48.16 (CH_2), 29.91 (CH_3); IR (NaCl, neat film) ν = 1706, 1691, 1612, 1513, 1461, 1239 cm^{-1}; white plates from ethyl acetate/petroleum ether; mp 102–104 °C. Anal. Calcd for $C_{22}H_{22}N_2O_6S$: C, 59.71; H, 5.01; N, 6.33; S, 7.25. Found: C, 59.61; H, 5.23; N, 6.11; S, 7.00.

***trans*-*O*-Acetate 21a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.27–7.25 (4 H, m), 7.03–7.02 (2 H, m), 6.87 (2 H, d, J = 8.6 Hz), 6.42 (1 H, s), 4.63 (1 H, $^1/_2$ABq, J = 14.1 Hz), 4.32 (1 H, $^1/_2$ABq, J = 14.1 Hz), 3.99 (2 H, d, J = 1.8 Hz-collapsed AB quartet), 3.86 (3 H, s), 3.79 (3 H, s), 1.86 (3 H, s); IR (NaCl, neat film) ν = 2941, 1739, 1687, 1513, 1248 cm^{-1}.

***cis*-*O*-Acetate 21b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35–7.26 (4 H, m), 7.06–6.98 (2 H, m), 6.9 (2 H, d, J = 8.6 Hz), 6.33 (1 H, s), 4.72 (1 H, $^1/_2$ABq, J = 14.4 Hz), 4.53 (1 H, $^1/_2$ABq, J = 14.4 Hz), 3.87 (2 H, d, J = 1.2 Hz), 3.81 (3 H, s), 3.78 (3 H, s), 2.13 (3 H, s); IR (NaCl, neat film) ν = 2941, 1739, 1685, 1513, 1243 cm^{-1}.

Spiro Cyclic Bisthioacetates (17a–d). **16a** or **16b** (600 mg, 1.35 mmol, 1.0 equiv) and NBS (362 mg, 2.03 mmol, 1.5 equiv) were dissolved in 50 mL of CCl_4 under argon, and the solution was heated and refluxed for 3.5 h. The solution was cooled, and the solvent was removed. The orange residue was taken up in CH_2Cl_2, and a CH_2Cl_2 solution of pyridine (408 μL, 5.42 mmol, 4.0 equiv) and thiolacetic acid (387 μL, 5.42 mmol, 4.0 equiv) was added. The combined solution was stirred at room temperature for 30 min. The solution was extracted with saturated $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and concentrated to an obnoxious smelling foam. The products were purified using flash column chromatography using 5% acetone in CCl_4: obtained 493 mg of both diastereomers; 65% combined yield. Same experimental procedure was used for **17c,d** in 55% yield.

17a: R_f 0.4, 1:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.32 (2 H, d, J = 8.5 Hz), 7.26–7.21 (1 H, m), 7.13–7.10 (1 H, m), 7.00–6.95 (1 H, m), 6.90–6.85 (3 H, m), 6.12 (1 H, s), 5.74 (1 H, s), 5.19 (1 H, $^1/_2$ABq, J = 14.5 Hz), 3.91 (3 H, s), 3.80 (3 H, s), 3.74 (1 H, $^1/_2$ABq, J = 14.5 Hz), 2.50 (3 H, s), 2.18 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 195.18 (C), 192.31 (C), 161.64 (C), 161.13 (C), 159.72 (C), 157.35 (C), 130.97 (CH), 129.60 (CH), 126.52 (C), 125.14 (C), 124.05 (CH), 122.38 (CH), 114.14 (CH), 109.54 (CH), 101.52 (C), 64.85 (CH_3), 60.04 (CH), 55.29 (CH_3), 48.95 (CH), 46.73 (CH_2), 30.45 (CH_3), 29.64 (CH_3); IR (NaCl, neat film) ν = 1695, 1612, 1513, 1459, 1243 cm^{-1}; white crystals from ethyl acetate/hexanes; mp 204–205 °C. Anal. Calcd for $C_{24}H_{24}N_2O_7S_2$: C, 55.80; H, 4.68; N, 5.42; S, 12.41. Found: C, 55.64; H, 4.76; N, 5.24; S, 12.51.

17b: R_f 0.32, 1:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.30–7.21 (3 H, m), 7.13–7.11 (1 H, m), 7.01–6.87 (4 H, m), 5.89 (1 H, s), 5.88 (1 H, s), 5.14 (1 H, $^1/_2$ABq, J = 14.4 Hz), 3.95 (3 H, s), 3.88 (1 H, $^1/_2$ABq, J = 14.4 Hz), 3.81 (3 H, s), 2.48 (3 H, s), 2.46 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 193.41 (C), 191.96 (C), 161.9 (C), 160.01 (C), 159.74 (C), 157.85 (C), 130.57 (CH), 129.79 (CH), 126.49 (C), 125.18 (C), 124.28 (CH), 122.37 (CH), 114.2 (CH), 109.35 (CH), 101.0 (C), 65.55 (CH_3), 59.83 (CH), 55.28 (CH_3), 49.28 (CH),

47.05 (CH_2), 30.27 (CH_3), 30.17 (CH_3); IR (NaCl, neat film) ν = 1706, 1691, 1612, 1513, 1461, 1239 cm^{-1}; white solid from ethyl acetate/petroleum ether; mp 168–170 °C dec. Anal. Calcd for $C_{24}H_{24}N_2O_7S_2$: C, 55.80; H, 4.68; N, 5.42; S, 12.41. Found: C, 55.68; H, 4.84; N, 5.58; S, 12.16.

Cis Bisthioacetate Diastereomers. 17c: R_f 0.42, 1:1 ethyl acetate/hexanes; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.27–7.25 (3 H, m), 7.15–7.13 (1 H, m), 7.05–6.87 (2 H, m), 6.85 (2 H, d, J = 8.54 Hz), 6.23 (1 H, s), 5.36 (1 H, s), 5.28 (1 H, $^1/_2$ABq, J = 8.54 Hz), 3.99 (1 H, $^1/_2$ABq, J = 14.5 Hz), 3.81 (3 H, s), 3.68 (3 H, s), 2.44 (3 H, s), 2.37 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 194.24 (C), 192.23 (C), 162.45 (C), 159.96 (C), 159.7 (C), 156.75 (C), 130.60 (CH), 129.47 (CH), 126.23 (C), 124.73 (C), 123.60 (CH), 122.56 (CH), 114.15 (CH), 109.31 (CH), 99.73 (C), 64.16 (CH_3), 60.09 (CH), 55.27 (CH_3), 52.45 (CH); 47.61 (CH_2), 30.48 (CH_3), 29.96 (CH_3); IR (NaCl, neat film) ν = 2940, 1702, 1612, 1513, 1239 cm^{-1}.

17d: R_f 0.32, 1:1 ethyl acetate/hexanes; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.32 (2 H, d, J = 8.5 Hz), 7.26–7.22 (1 H, m), 7.13 (1 H, d, J = 7.4 Hz), 7.03–6.89 (4 H, m), 5.94 (1 H, s), 5.83 (1 H, s), 5.27 (1 H, $^1/_2$ABq, J = 14.47 Hz), 3.82 (1 H, $^1/_2$ABq, J = 14.47 Hz), 3.83 (3 H, s), 3.77 (3 H, s), 2.51 (3 H, s), 2.46 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 194.57 (C), 192.02 (C), 163.23 (C), 159.79 (C), 159.72 (C), 156.92 (C), 130.56 (CH), 129.7 (CH), 126.58 (C), 124.19 (C), 123.92 (CH), 122.52 (CH), 114.23 (CH), 109.16 (CH), 99.0 (C), 65.34 (CH_3), 60.03 (CH), 55.27 (CH_3), 51.75 (CH), 47.24 (CH), 30.24 (CH_3); IR (NaCl, neat film) ν = 2942, 1699, 1513, 1238 cm^{-1}; light yellow crystals from ethyl acetate/hexanes; mp 168–169 °C. Anal. Calcd for $C_{24}H_{24}N_2O_7S_2$: C, 55.80; H, 4.68; N, 5.42, S, 12.41. Found: C, 55.83; H, 4.73; N, 5.25; S, 12.14.

Spiro Cyclic Disulfides (18a,b). Acid Hydrolysis Procedure. Twenty-five milligrams (0.048 mmol, 1 equiv) of a mixture of **17a–d** was suspended in 10 mL of absolute ethanol and cooled to 0 °C. Dry HCl gas was bubbled through the solution for 30 min and stirred at room temperature for an additional 5 h. The ethanol was removed under vacuum, and the yellow residue was taken up in CH_2Cl_2. The CH_2Cl_2 layer was shaken with aqueous 2% KI_3 until the organic layer was a persistent pink/purple. The organics were washed with water, dried over Na_2SO_4, filtered, and concentrated to a dark brown film which was purified via preparative thin-layer chromatography using 3:2 hexane/ethyl acetate as the eluent. The two bands which stain white with I_2/NaN_3 were isolated: obtained 6.7 mg of white solid; combined yield 32%.

Basic Hydrolysis Procedure. Twenty milligrams (0.041 mmol, 1 equiv) of a mixture of **17a–d** was suspended in 7 mL of absolute ethanol, and argon was bubbled through the solution for 20 min. NaOH (1.5 mL, 0.2 N) was added with continued degassing, and the solution was stirred for 10 min. The reaction was poured into a separatory funnel containing CH_2Cl_2, and aqueous 2% KI_3 was added until the CH_2Cl_2 layer was purple. The organic layer was washed with water, dried over Na_2SO4, filtered, and concentrated to a brown film which was PTLC'd using 3:2 hexane/ethyl acetate (or radial chromatography using 3:1 hexane/ethyl acetate): isolated 5.4 mg of **18a** or **b** as a white solid; yield 32%.

18a: R_f 0.38, 2:3 ethyl acetate/hexanes; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.34–7.18 (4 H, m), 7.09 (1 H, d, J = 8.2 Hz), 7.04–6.99 (1 H, m), 6.89 (2 H, $^1/_2$ABq, J = 8.7 Hz), 5.27 (1 H, $^1/_2$ABq, J = 14.4 Hz), 5.00 (1 H, s), 4.89 (1 H, s), 3.96 (1 H, $^1/_2$ABq, J = 14.4 Hz), 3.96 (3 H, s), 3.81 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 165.53 (C), 164.24 (C), 159.97 (C), 157.98 (C), 131.23 (CH), 130.66 (CH), 125.25 (C), 124.79 (CH), 122.68 (CH), 121.19 (C), 114.56 (CH), 110.48 (CH), 101.62 (C), 66.44 (CH_3), 62.29 (CH), 55.33 (CH_3), 52.45 (CH), 47.44 (CH_2); IR (NaCl, neat film) ν = 2942, 1712, 1612, 1513, 1232 cm^{-1}; colorless crystals from diethyl ether/pentane; mp 188–189 °C.

18b: R_f 0.29, 2:3 ethyl acetate/hexanes; ^1H NMR (300 MHz, $CDCl_3$) δ = 7.26–7.16 (4 H, m), 7.08–7.03 (2 H, m), 6.90 (2 H, $^1/_2$ABq, J = 8.6 Hz), 5.20 (1 H, s), 5.14 (1 H, s), 4.88 (1 H, $^1/_2$ABq, J = 14.6 Hz), 4.39 (1 H, $^1/_2$ABq, J = 14.6 Hz), 3.87 (3 H, s), 3.82 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 166.09 (C), 162.97 (C), 160.05 (C), 158.1 (C), 130.78 (CH), 130.45 (CH), 125.94 (C), 123.9 (CH), 123.07 (CH), 121.48 (C), 114.76 (CH), 109.88 (CH), 100.21 (CH), 66.35 (CH_3), 64.64 (CH), 56.27 (CH), 55.34 (CH_3), 48.47 (CH_2); IR (NaCl, neat film) ν = 2943, 1708, 1513, 1458, 1249, 1030 cm^{-1}; mp 163–164 °C.

Chlororesorcylaldehyde (23). Prepared following the procedure of Chakravarti and Ghosh.[22] In a flame-dried, 1-L, three-necked flask equipped with a condensor and a mercury seal stirrer under an inert atmosphere was added 4-chlororesorcinol (25 g, 0.17 mol, 1 equiv) and anhydrous zinc cyanide (40 g, 0.34 mol, 2.0 equiv). Anhydrous diethyl ether (200 mL) was added, and the reaction was cooled to 0 °C. Dry HCl gas was passed through the rapidly stirred solution for 2 h until a solid mass formed. The ether was decanted, and 250–300 mL of water was added. The reaction was heated to reflux, and any residual ether was distilled off. The reaction was refluxed until the solid mass dissolved entirely. Upon cooling the crude chlororesorcylaldehyde separates as a red solid and was collected. Recrystallization from water gave the desire product in sufficient purity to carry on. Repeated recrystallizations gave chlororesorcylaldehyde as yellow needles in 40% yield: ^1H NMR (300 MHz, DMSO-d_6) δ = 11.39 (1 H, brs, exchangeable), 10.89 (1 H, brs, exchangeable), 9.98 (1 H, s), 7.60 (1 H, s), 6.58 (1 H, s); IR (KBr) ν = 3378, 1630, 1495, 1266, 723 cm^{-1}.

2-Hydroxy-4-(methoxymethyl)-5-chlorobenzaldehyde (24). A solution containing 20 g (116 mmol, 1.0 equiv) of chlororesorcylaldehyde **23** and triethylamine (16.2 mL, 116 mmol, 1.0 equiv) in 750 mL of THF was cooled to 0 °C. Chloromethyl methyl ether (13.2 mL, 173.8 mmol, 1.5 equiv) was added subsurface, and the solution was warmed to room temperature and stirred for 3 h. The solution was filtered through a Celite plug diluted with diethyl ether and extracted three times with 0.2 M NaOH. The etheral layer (containing dialkylated material) was discarded, while the aqueous layer was carefully acidified to pH 4–5 with cold 0.1 M H_2SO_4 and extracted with ethyl acetate. The ethyl acetate was washed with water, dried over $MgSO_4$, filtered, and concentrated to an orange solid which was taken up in a 3:1 hexane/ethyl acetate solution and filtered through a plug of 25% alumina in silica gel. The colorless filtrate was concentrated, and the product was recrystallized from hexane: obtained 12.5 g of a white crystalline solid; 49% yield; mp 68–70 °C; ^1H NMR (300 MHz, $CDCl_3$) δ = 11.29 (1 H, s, exchangeable in D_2O), 9.70 (1 H, s), 7.35 (1 H, s), 6.76 (1 H, s), 5.31 (2 H, s), 3.52 (3 H, s); IR (NaCl film) ν = 2962, 2839, 1647, 1487 cm^{-1}. Anal. Calcd for $C_9H_9O_4Cl$: C, 49.90; H, 4.19; Cl, 16.36. Found: C, 50.15; H, 4.29; Cl, 16.42.

5-Chloro-6-(methoxymethyl)coumarilic Acid (25). A slurry consisting of 4 g (18.46 mmol, 1.0 equiv) of **24**, diethyl bromomalonate (3.5 mL, 20.31 mmol, 1.1 equiv), and potassium carbonate (5.1 g, 36.9 mmol, 2.0 equiv) were heated to reflux in 15 mL of acetone. After 5 h the solution was concentrated to a thick yellow oil which was taken up in water, acidified to pH 5 with cold 0.1 M H_2SO_4, and extracted with ethyl acetate. The ethyl acetate was washed with water and concentrated, and the residue was taken up in 20 mL of ethanol, and an alcoholic solution of KOH (3.0 equiv) was added and refluxed for 2 hours. The solution was cooled and concentrated, and the residue was taken up in dilute NaOH and extracted with diethyl ether which was discarded. The aqueous layer was acidified to pH 3–4 with 0.1 M H_2SO_4 and the precipitate which gradually forms collected. The carboxylic acid was further purified by recrystallization from ethyl acetate/hexanes: obtained 3.4 g of a fine white solid; mp 193–194 °C; 72% yield; ^1H NMR (300 MHz, DMSO-d_6) δ = 13.55 (1 H, br s, exchangeable in D_2O), 7.89 (1 H, s), 7.59 (1 H, s), 7.56 (1 H, s), 5.39 (2 H, s), 3.44 (3 H, s); IR (KBr) ν = 3415, 2907, 2554, 1684, 1571 cm^{-1}. Anal. Calcd for $C_{11}H_9O_5Cl$: C, 51.48; H, 3.53; Cl, 13.81. Found: C, 51.38; H, 3.54; Cl, 13.64.

***N*-(2-Nitrobenzyl)glycine Ethyl Ester (26).** Glycine ethyl ester hydrochloride (52 g, 370.3 mmol, 4.0 equiv) was dissolved in 1.5 L of 95% ethanol. $NaHCO_3$ (38.8 g, 462.8 mmol, 5.0 equiv) was added, and after 5 min 2-nitrobenzyl bromide (20 g, 92.5 mmol, 1.0 equiv) was added and the solution refluxed for 20 h. The solution was cooled, filtered, and concentrated to a viscous yellow oil which was taken up in ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to a thick yellow/brown oil. Product (14.5 g) (yellow oil) was obtained from flash column chromatography using 3:2 hexane/ethyl acetate as the eluent along with 2 g of dialkylated side product: 65% yield; ^1H NMR (300 MHz) ($CDCl_3$) δ = 7.97 (1 H, d, J = 8.0 Hz), 7.66–7.57 (2 H, m), 7.45–7.40 (1 H, m), 4.18 (2 H, qt, J = 7.1 Hz), 4.10 (2 H, s), 3.44 (2 H, s), 1.27 (3 H, t, J = 7.1 Hz); IR (NaCl, neat film) ν = 3354, 1736, 1526 cm^{-1}.

***N*-Benzofuranylglycine (27a).** 6-Chloro-7-(methoxymethyl)coumarilic acid **25** (9 g, 35.1 mmol, 1.0 equiv) was suspended in 500 mL of CH_2Cl_2 under N_2. THF was added to the solution until all the starting material was completely dissolved. DMF (1 mL) was added followed by 3.5 mL of oxalyl chloride (38.57 mmol, 1.1 equiv). After approximately 5 min the solution turned cloudy and was stirred at room temperature for 1 h. The acid chloride solution was concentrated to approximately half the original volume and was added to a vigorously stirred solution containing *N*-(2-nitrobenzyl)glycine ethyl ester **26** (8.35 g, 35.1 mmol, 1.0 equiv) and sodium bicarbonate (4.4 g, 52.6 mmol, 1.5 equiv) in 100 mL of water. The resulting solution was stirred for 45 min. The layers were separated, and the organic layer was washed with 0.1 M K_2CO_3, acidified with 10% HCl, washed with water, dried over $MgSO_4$, filtered, and concentrated to a thick yellow oil which solidifies on standing: obtained 16.5 g of crude ester (>95% by TLC). The crude ester was taken up in 500 mL of dioxane, 55 mL of 2 M HCl was added, and the solution was refluxed for 36 h. The solution was cooled, made basic with 0.2 M NaOH, and washed with diethyl ether. The aqueous solution reacidified with aqueous HCl. The crude acid was extracted into ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to a

light yellow colored solid: obtained 12.9 g of crude acid which was carried on without further purification; ^{1}H NMR (300 MHz, DMSO-d_6, T = 357 K) δ = 12.5 (1 H, br s, exchangeable in D_2O), 10.5 (1 H, br s, exchangeable in D_2O), 8.04 (1 H, d, J = 8.1 Hz), 7.74–7.54 (3 H, m), 7.27 (1 H, s), 7.01 (1 H, s), 5.11 (2 H, s), 4.31 (2 H, s); IR (KBr) ν = 3413, 3141, 1733, 1608, 1556, 1525 cm^{-1}.

***N*-Benzofuranylglycine (27b).** To 12.9 g of crude **27a** (31.97 mmol, 1.0 equiv) dissolved in 30–50 mL of pyridine was added excess acetic anhydride (30 mL at least 10 equiv), and the solution was stirred at room temperature for 4 h. The majority of the solvent was removed in vacuo, and the residue was taken up in ethyl acetate, washed with 10% HCl followed by water, dried over $MgSO_4$, filtered, and concentrated to 14 g of a yellow/orange foam (>95% by TLC) which was also carried on without further purification: ^{1}H NMR (300 MHz, DMSO-d_6, T = 370 K) δ = 7.97 (1 H, d, J = 8 Hz), 7.85 (1 H, s), 7.67–7.47 (4 H, m), 7.34 (1 H, s), 5.03 (2 H, s), 3.95 (2 H, s), 2.32 (3 H, s); IR (KBr) ν = 3430, 3105, 2941, 1772, 1615 (br), 1525, 1196 cm^{-1}.

***N*-Benzofuranylglycine Hydroxamate (28a).** To a solution of **27a** (250 mg, 0.617 mmol, 1.0 equiv) dissolved in 60 mL of THF was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (178 mg, 0.926 mmol, 1.5 equiv). (A few milliliters of water was added to help dissolve the carbodiimide.) The yellow solution was stirred for 10 min before methoxylamine (96 mL, 1.85 mmol, 3.0 equiv) was added causing the color to dissipate. The solution was stirred for 16 h before dilution with ethyl acetate, extracted with saturated $NaHCO_3$, and dried over Na_2SO_4. The residue was purified using 20% methanol/chloroform: obtained 172 mg of a white solid which was recrystallized from ethyl acetate/hexanes; 64% yield; ^{1}H NMR (300 MHz, T = 357 K, DMSO-d_6) δ = 10.81 (1 H, br s), 8.21 (1 H, s), 8.05 (1 H, d, J = 7.9 Hz), 7.7–7.52 (4 H, m), 7.29 (1 H, s), 7.06 (1 H, s), 5.10 (2 H, s), 4.24 (2 H, s), 3.59 (3 H, s); IR (KBr) ν = 3171, 2975, 1665, 1610, 1523, 1338, 1142 cm^{-1}.

***N*-Benzofuranylglycine Hydroxamate (28b).** To a solution containing 2.77 g of **27b** (6.2 mmol, 1.0 equiv) dissolved in 200 mL of THF cooled to 0 °C was added *N*-methylmorpholine (750 mL, 6.82 mmol, 1.1 equiv). After 10 min isobutyl chloroformate (880 mL, 6.82 mmol, 1.1 equiv) was added causing the solution to turn yellow. After 45 min the ice bath was removed and methoxylamine (630 mL, 12.4 mmol, 2.0 equiv) was added causing the intense yellow color to dissipate, and the reaction was stirred for an additional 45 min at room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The ethyl acetate was washed with 10% HCl followed by water, dried over $MgSO_4$, filtered, and concentrated to a yellow/orange oil. The hydroxamic ester was purified from column chromatography using 2:1 ethyl acetate/hexanes: obtained 2.1 g of a white foam which was further purified by recrystallization from ethyl acetate/hexanes; mp 151–152 °C; 70% yield; ^{1}H NMR (300 MHz, DMSO-d_6, T = 370 K) δ 10.81 (1 H, br s), 8.02 (1 H, d, J = 8 Hz), 7.91 (1 H, s), 7.73–7.27 (4 H, m), 7.0 (1 H, s), 5.10 (2 H, s), 4.23 (2 H, s), 3.57 (3 H, s), 2.32 (3 H, s); IR (NaCl film) ν = 3210, 2998, 1771, 1677, 1637, 1526 cm^{-1}. Anal. Calcd for $C_{21}H_{18}N_3O_8Cl$: C, 53.01; H, 3.81; N, 8.83; Cl, 7.45. Found: C, 52.92; H, 3.88; N, 8.68; Cl, 7.25.

Trans Spiro Cyclic Bromide (29b). NBS (4.0 g, 22.69 mmol, 1.2 equiv) was added in one portion to a solution of **28b** (9.0 g, 18.9 mmol, 1.0 equiv) in 600 mL of ethanol-free chloroform under N_2 at room temperature, and the reaction was stirred at room temperature for 20 h. The reaction was poured into a separatory funnel containing additional $CHCl_3$, washed with saturated sodium thiosulfate followed by water, dried over $MgSO_4$, filtered, and concentrated to a thick, light yellow colored oil which if let standing solidifies. The oily product was immediately purified by column chromatography using 3:2 hexane/ethyl acetate as the eluent: obtained 6.67 g of a white solid which was recrystallized from ethyl acetate/hexanes; mp 160–162 °C; 67% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.04 (1 H, d, J = 8.0 Hz), 7.67–7.60 (2 H, m), 7.55–7.50 (1 H, m), 7.35 (1 H, s), 6.79 (1 H, s), 6.01 (1 H, s), 5.05 (1 H, $^1/_2$ABq, J = 15.7 Hz), 5.01 (1 H, $^1/_2$ABq, J = 15.7 Hz), 4.18 (2 H, s), 3.95 (3 H, s), 2.35 (3 H, s); ^{13}C NMR (75.47 (MHz, $CDCl_3$) δ 168.02 (C), 161.33 (C), 161.22 (C), 156.68 (C), 148.88 (C), 148.81 (C), 133.9 (CH), 130.59 (CH), 129.42 (C), 129.30 (CH), 126.36 (CH), 125.19 (CH), 124.41 (C), 120.71 (C), 105.92 (CH), 101.75 (C), 65.81 (CH_3), 49.06 (CH_2), 48.18 (CH), 46.49 (CH_2), 20.55 (CH_3); IR (NaCl film) ν = 2992, 1772, 1697, 1527 cm^{-1}. Anal. Calcd for $C_{21}H_{17}N_3O_8ClBr$: C, 45.47; H, 3.09; N, 7.57; total halogen content (calculated as 2Cl) 12.78. Found: C, 45.55; H, 3.15; N, 7.58; total halogen 12.67.

Trans and Cis Spiro Cyclic Methyl Ethers (30a,b). A solution of silver triflate (735 mg, 4.36 mmol, 1.2 equiv) in 15 mL of THF was added in 1 portion to a rapidly stirring solution of 2.0 g of **29b** (3.6 mmol, 1.0 equiv) dissolved in 150 mL of THF in which 30 mL of methanol had been added. The resulting solution was stirred at room temperature for 45 min. The reaction was diluted with ethyl acetate followed by the addition of saturated brine solution. The reaction was filtered through a plug of celite, and the filtrate washed with brine, water, dried over $MgSO_4$, filtered, and concentrated to a white solid. The diastereomers were purified by column chromatography using 2% acetone in $CHCl_3$. 950 mg of the trans diastereomer (nonpolar) and 315 mg of the cis diastereomer (polar) were obtained in 69% combined yield (81% based on recovered starting material). Each diastereomer was recrystallized from ethyl acetate/hexanes.

***trans*-30a**: R_f 0.3, 5% acetone/chloroform; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (1 H, d, J = 8.1 Hz), 7.62–7.49 (3 H, m), 7.43 (1 H, s), 6.82 (1 H, s), 5.52 (1 H, s), 5.26 (1 H, $^1/_2$ABq, J = 16.2 Hz), 4.88 (1 H, $^1/_2$ABq, J = 16.2 Hz), 4.12 (2 H, s), 3.93 (3 H, s), 3.61 (3 H, s), 2.35 (3 H, s); ^{13}C NMR (75.47 (MHz, $CDCl_3$) δ = 168.07 (C), 161.97 (C), 161.15 (C), 158.03 (C), 148.67 (C), 148.65 (C), 133.85 (CH), 129.97 (C), 129.62 (CH), 128.98 (CH), 126.02 (CH), 125.19 (CH), 124.12 (C), 119.93 (C), 106.37 (CH), 101.15 (C), 84.62 (CH), 65.69 (CH_3), 59.28 (CH_3), 49.24 (CH_2), 46.56 (CH_2), 20.56 (CH_3); IR (NaCl film) ν = 2943, 1773, 1697, 1527 cm^{-1}; white crystals from ethyl acetate/hexanes; mp 132–134 °C. Anal. Calcd for $C_{22}H_{20}N_3O_9Cl$: C, 52.23; H, 4.98; N, 8.31; Cl, 7.01. Found: C, 52.13; H, 4.20; N, 8.17; Cl, 6.92.

***cis*-30b**: R_f 0.2, 5% acetone/chloroform; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.09 (1 H, d, J = 8.1 Hz), 7.86 (1 H, t, J = 7.5 Hz), 7.54 (1 H, t, J = 8.1 Hz), 7.43–7.39 (2 H, m), 6.73 (1 H, s), 5.63 (1 H, s), 5.0 (2 H, s), 4.13 (1 H, $^1/_2$ABq, J = 18.1 Hz), 4.09 (1 H, $^1/_2$ABq, J = 18.2 Hz), 3.80 (3 H, s), 3.64 (3 H, s), 2.34 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 168.0 (C), 162.89 (C), 162.37 (C), 156.05 (C), 148.74 (C), 148.07 (C), 134.1 (CH), 129.54 (CH), 129.52 (C), 129.45 (CH), 125.43 (CH), 125.12 (CH), 124.44 (C), 120.1 (C), 105.84 (CH), 98.2 (C), 84.6 (CH), 64.58 (CH_3), 60.13 (CH_3), 49.17 (CH_2), 47.04 (CH_2), 20.57 (CH_3); IR (NaCl film) ν = 2946, 1772, 1688, 1527 cm^{-1}; white needles from ethyl acetate/hexanes; mp 163–164 °C. Anal. Calcd for $C_{22}H_{20}N_3O_9Cl$: C, 52.23; H, 3.98; N, 8.31; Cl, 7.01. Found: C, 52.49; H, 4.20; N, 8.39; Cl, 7.09.

Trans and Cis Spiro Cyclic Methyl Ethers (31a,b). Methyl ether **30a** (250 mg, 0.494 mmol) was dissolved in 50 mL of 10% water in THF in a quartz tube containing Pyrex beads. The solution was photolyzed at 37 °C for 5 h using a 450-W Conrad-Hanovia medium-pressure mercury vapor lamp. The reaction was filtered into a separatory funnel, containing ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to a brown film which was chromatographed using 15% acetone/chloroform: obtained 131 mg of product **31a**; 72% yield. Analogous reaction using the cis diastereomer **30b** gave **31b** as a white solid in 71% yield.

***trans*-31a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.41 (1 H, s), 7.30 (1 H, s, exchangeable in D_2O), 6.80 (1 H, s), 5.44 (1 H, s), 4.15 (1 H, $^1/_2$ABq, J = 18.4 Hz, broadened by NH, d, J = 2.8 Hz), 4.07 (1 H, $^1/_2$ABq, J = 18.4 Hz), 3.90 (3 H, s), 3.60 (3 H, s), 2.35 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 168.12 (C), 162.59 (C), 162.28 (C), 157.92 (C), 148.69 (C), 126.13 (CH), 124.19 (C), 120.04 (C), 106.3 (CH), 100.93 (C), 84.79 (CH), 65.71 (CH_3), 59.5 (CH_3), 44.0 (CH_2), 20.62 (CH_3); IR (NaCl film) ν = 3273, 2942, 1772, 1708 cm^{-1}; white needles from ethyl acetate/petroleum ether; mp 181–182 °C. Anal. Calcd for $C_{15}H_{15}N_2O_7Cl$: C, 48.59; H, 4.08; N, 7.56; Cl, 9.56. Found: C, 48.46; H, 4.21; N, 7.35; Cl, 9.46.

***cis*-31b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39 (1 H, s), 6.73 (i H, s), 6.57 (1 H, s, exchangeable in D_2O), 5.5 (1 H, s), 4.19 (1 H, $^1/_2$ABq, J = 2.0 Hz), 4.28 (1 H, $^1/_2$ABq, J = 1.3 Hz), 3.79 (3 H, s), 3.65 (3 H, s), 2.34 (3 H, s); ^{13}C NMR (75.47 MHz, DMSO-d_6) δ = 168.13 (C), 162.79 (C), 162.73 (C), 156.14 (C), 147.64 (C), 125.29 (CH), 125.19 (C), 118.5 (C), 105.83 (CH), 97.71 (C), 83.33 (CH), 63.70 (CH_3), 59.70 (CH_3), 43.21 (CH_2), 20.35 (CH_3); IR (NaCl film) ν = 3274, 2942, 1770, 1703 cm^{-1}; white solid from ethyl acetate/petroleum ether; mp dec >230 °C. Anal. Calcd for $C_{15}H_{15}N_2O_7Cl$: C, 48.59; H, 4.08; N, 7.56. Found: C, 48.61; H, 4.19; N, 7.31.

Spiro Cyclic Bismethyl Ethers (32a–d). General Procedure. Methyl ether **31** (65 mg, 0.176 mmol, 1 equiv) was dissolved in 5 mL of dry ethanol-free at room temperature. Freshly prepared *tert*-butyl hypochlorite (25 μL, 0.193 mmol, 1.2 equiv) was added followed by the dropwise addition of a freshly prepared solution of 0.1 M sodium methoxide (1.5 equiv). The solution was stirred at room temperature for 1 h. The reaction was quenched with 10% aqueous HCl, extracted with ethyl acetate, washed with water, dried over Na_2SO_4, filtered, and concentrated to a yellow film. In the case of **31a**, the individual diastereomers **32a,b** were purified by column chromatography using 20% acetone/$CHCl_3$ as the eluent: obtained a white solid for a combined yield of 56%. Analogous reactions using **31b** gave **32c,d** as an inseparable mixture of diastereomers which was carried on directly.

***trans*-32a**: R_f 0.4, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39 (1 H, s), 7.32 (1 H, br s, exchangeable in D_2O), 6.75

(1 H, s), 5.6 (1 H, s), 5.00 (1 H, d, J = 2.2 Hz), 3.91 (3 H, s), 3.57 (3 H, s), 3.49 (3 H, s), 2.35 (3 H, s); ^{13}C NMR (75.47 (MHz, $CDCl_3$) δ = 168.15 (C), 161.91 (C), 160.0 (C), 157.52 (C), 148.51 (C), 126.0 (CH), 124.49 (C), 120.10 (C), 106.23 (CH), 101.16 (C), 84.7 (CH), 80.82 (CH), 65.34 (CH_3), 60.0 (CH_3), 55.56 (CH_3), 20.59 (CH_3); IR (NaCl, film) ν = 3268, 2943, 1772, 1715, 1604, 1478, 1198, 1145 cm^{-1}; white needles from ethyl acetate/hexanes; mp 132–133 °C. Anal. Calcd for $C_{16}H_{17}N_2O_8Cl$: C, 47.95; H, 4.28; N, 6.99; Cl, 8.85. Found: C, 47.89; H, 4.40; N, 6.77; Cl, 9.03.

***trans*-32b**: R_f 0.3, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.72 (1 H, br s, exchangeable in D_2O), 7.42 (1 H, s), 6.83 (1 H, s), 5.21 (1 H, s), 4.90 (1 H, d, J = 4.2 Hz), 3.86 (3 H, s), 3.57 (3 H, s), 3.53 (3 H, s), 2.35 (3 H, s); ^{13}C NMR (75.47 (MHz, $CDCl_3$) δ = 168.02 (C), 163.17 (C), 160.66 (C), 158.12 (C), 148.77 (C), 126.0 (CH), 124.32 (C), 120.15 (C), 106.7 (CH), 100.33 (C), 85.9 (CH), 80.66 (CH), 65.92 (CH_3), 59.19 (CH_3), 56.35 (CH_3), 20.58 (CH_3); IR (NaCl, film) ν = 3281, 2942, 1772, 1716, 1604, 1476, 1197, 1147 cm^{-1}; white plates from ethyl acetate/hexanes; mp 178–180 °C. Anal. Calcd for $C_{16}H_{17}N_2O_8Cl$: C, 47.95; H, 4.28; N, 6.99; Cl, 8.85. Found: C, 47.88; H, 4.39; N, 6.71; Cl, 8.90.

Cis Diastereomers (32c,d). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.87 (1 H, d, J = 3.4 Hz), 7.60 (1 H, d, J = 2.36 Hz), 7.41 (1 H, s), 7.37 (1 H, s), 6.77 (1 H, s), 6.72 (1 H, s), 5.60 (1 H, s), 5.22 (1 H, s), 5.08 (1 H, d, J = 2.79 Hz), 4.91 (1 H, d, J = 3.6 Hz), 3.82 (3 H, s), 3.77 (3 H, s), 3.66 (3 H, s), 3.61 (3 H, s), 3.57 (3 H, s), 3.53 (3 H, s), 2.35 (3 H, s), 2.34 (3 H, s).

Spiro Cyclic Bismethyl Ethers (33a–d). General Procedure. A suspension of 44 mg (0.11 mmol, 1 equiv) of **32a,b** or **32c,d** in 2 mL of absolute ethanol was stirred at 0 °C. Exactly 1.1 equiv of 0.1 M sodium ethoxide at 0 °C was added dropwise. The resulting solution was stirred at 0 °C for 30 min before quenching the reaction by addition of 10% aqueous HCl. The reaction was poured into ethyl acetate, washed with saturated sodium chloride, dried over Na_2SO_4, filtered, and concentrated to a light yellow film. Crude NMR shows >90% product. The product was purified by column chromatography using 2:1 ethyl acetate hexanes or 15% acetone/$CHCl_3$: obtained 29 mg of a white solid. The individual diastereomers could be obtained routinely in 74% isolated yield.

***trans*-33a**: R_f 0.28, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.28 (1 H, s), 6.49 (1 H, s), 5.44 (1 H, s), 5.08 (1 H, s), 4.86 (2 H, br s), 3.83 (3 H, s), 3.51 (3 H, s), 3.49 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 163.54 (C), 162.36 (C), 160.04 (C), 156.53 (C), 127.06 (CH), 118.66 (C), 115.03 (C), 102.16 (C), 99.30 (CH), 86.77 (CH), 82.12 (CH), 65.67 (CH_3), 59.65 (CH_3), 55.76 (CH_3); IR (NaCl, film) ν = 3273 shoulder 3148, 2943, 1705, 1627.6, 1299, 1097, 1032 cm^{-1}; mp decomposes—no clean melting point (180–202 °C); white crystals from diethyl ether at −11 °C. Anal. Calcd for $C_{14}H_{15}N_2O_7Cl$: C, 46.87; H, 4.21; N, 7.81. Found: C, 47.00; H, 4.42; N, 7.69.

***trans*-33b**: R_f 0.18, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.35 (1 H, s), 6.56 (1 H, s), 5.03 (1 H, s), 4.89 (1 H, s), 4.83 (2 H, br s), 3.79 (3 H, s), 3.50 (3 H, s), 3.48 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 164.78 (C), 162.99 (C), 160.65 (C), 156.78 (C), 127.12 (CH), 119.78 (C), 118.66 (C), 115.16 (C), 99.24 (CH), 87.81 (CH), 82.04 (CH), 66.35 (CH_3), 58.71 (CH_3), 56.57 (CH_3); IR (KBr) ν = 3342 shoulder 3225, 2945, 1710, 1631, 1497, 1186, 1099, 1041 cm^{-1}; mp decomposes—no clean melting point; white solid from acetone/petroleum ether. Anal. Calcd for $C_{14}H_{15}N_2O_7Cl$: C, 46.87; H, 4.21; N, 7.81. Found: C, 46.95; H, 4.32; N, 7.7.

***cis*-33c**: R_f 0.32, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.21 (1 H, d, J = 1.1 Hz), 6.46 (1 H, s), 5.48 (1 H, s), 5.12 (1 H, s), 4.84 (2 H, br s), 3.72 (3 H, s), 3.55 (3 H, s), 3.49 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 166.41 (C), 163.57 (C), 158.19 (C), 156.06 (C), 125.96 (CH), 118.86 (C), 115.09 (C), 99.16 (CH), 99.03 (C), 87.22 (CH), 81.59 (CH), 64.59 (CH_3), 60.40 (CH_3), 55.71 (CH_3); IR (NaCl, film) ν = 3281, 2941, 1700, 1627, 1487, 1437, 1104 cm^{-1}; white crystals from diethyl ether/petroleum ether at 0 °C; mp 158–159 °C. Anal. Calcd for $C_{14}H_{15}N_2O_7Cl$: C, 46.87; H, 4.21; N, 7.81. Found: C, 46.66; H, 4.37; N, 7.63.

***cis*-33d**: R_f 0.23, 2:1 ethyl acetate/hexanes; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.29 (1 H, s), 6.49 (1 H, s), 5.10 (1 H, s), 4.93 (1 H, s), 4.85 (2 H, br s), 3.77 (3 H, s), 3.59 (3 H, s), 3.53 (3 H, s); ^{13}C NMR (75.47 MHz, $CDCl_3$) δ = 167.66 (C), 162.64 (C), 158.73 (C), 156.40 (C), 126.66 (CH), 118.7 (C), 115.2 (C), 99.1 (C), 99.03 (CH), 86.07 (CH), 82.48 (CH), 65.24 (CH_3), 60.57 (CH_3), 57.37 (CH_3); IR (NaCl, film) ν = 3272, 3171 (shoulder), 2934, 1700, 1627, 1440, 1303, 1157, 1034 cm^{-1}; white crystals from carbon tetrachloride/methanol 0 °C; dec 198–200 °C. Anal. Calcd for $C_{14}H_{15}N_2O_7Cl$: C, 46.87; H, 4.21; N, 7.81. Found: C, 46.63; H, 4.33; N, 7.57; Cl, 9.84.

Spiro Cyclic Bisthioacetates (34a–d). Representative Procedure. BF_3–Et_2O (170 μL, 1.36 mmol, 8.0 equiv) was added to 60 mg of bismethyl ether **33a** (1.0 equiv, 0.167 mmol) in 15 mL of CH_2Cl_2. Thioacetic acid (150 μL, 2.07 mmol, 12.0 equiv) was added, and the solution was heated and refluxed for 8 h. The solution was cooled, diluted with additional CH_2Cl_2, washed once with saturated $NAHCO_3$, saturated NH_4Cl, and water, dried over Na_2SO_4, filtered, and concentrated to a foul-smelling yellow film. The products were purified by column chromatography using 3% MeOH/$CHCl_3$ or 10% acetone/$CHCl_3$: 47 mg of a mixture of inseparable diastereomers were obtained which were carried on without further purification; 65% yield.

Major Diastereomers. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.08 (1 H, s), 7.07 (1 H, s), 6.61 (2 H, s), 6.50 (1 H, br s), 5.92 (1 H, d, J = 2.9 Hz), 5.83 (1 H, s), 5.70 (1 H, br s); 5.66 (1 H, d, J = 2.2 Hz), 3.98 (3 H, s), 3.93 (3 H, s), 2.48–2.41 (12 H, m); IR (NaCl, film) ν = 3401, 2938, 1707, 1367, 1197, 1145 cm^{-1}.

Methanethiol Reduction and Acetylation of Aspirochlorine. Aspirochlorine (15 mg) was dissolved in 1 mL of dry pyridine and cooled to 0 °C. Excess methanethiol was added, and the solution was stirred under argon for 15 min and at room temperature for 9 h. The solution was concentrated under vacuum, and the residue was taken up in 1 mL of fresh pyridine followed by the addition of 2 equiv of acetyl chloride. The reaction was stirred for 15 min before diluting with ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to a yellow film. ^{1}H NMR of the crude film showed the presence of thioacetates **34**.

Sodium Borohydride Reduction and KI_3 Oxidation of Aspirochlorine. Aspirochlorine (8 mg) was dissolved in 2 mL of absolute ethanol at 0 °C and the solution degassed by passing argon through the solution for 20 min. A small spatula tip of $NaBH_4$ was quickly added, and the solution was stirred for 30 min. The solution was concentrated under reduce pressure, and the residue was taken up in H_2O/$CHCl_3$ and acidified to pH 5–6 with 10% H_2SO_4. Aqueous KI_3 was added dropwise until the organic phase was a light pink color. The organics were washed with water, dried over Na_2SO_4, filtered, and concentrated to a brown film which was purified using PTLC. Aspirochlorine (3 mg) (38% yield) was obtained.

Aspirochlorine (2). **34** (10 mg) and a small amount of CSA were dissolved in 4 mL of THF, cooled to 0 °C, and saturated with oxygen. Methoxylamine (100 μL) was added, and the solution was warmed to room temperture and stirred for 6.5 h. The reaction was concentrated, and the yellow film was immediately purified by PTLC using 5% methanol/chloroform. A light yellow noncrystalline solid (2 mg) was obtained. On repeated attempts, yields of aspirochlorine routinely ranged from 20 to 34%. The synthetic and natural substances were identical by ^{1}H NMR, IR, TLC, and HPLC: R_f 0.2, 5% methanol/chloroform; 0.5 in 6:4 chloroform/acetone and 0.4 in 1:1 benzene/ethyl acetate; ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.15 (1 H, s), 6.77 (1 H, s), 6.73 (1 H, br s, exchangeable, concentration dependent), 5.78 (1 H, br s, exchangeable, concentration dependent), 5.16 (1 H, d, J = 5.5 Hz), 4.90 (1 H, s), 3.96 (3 H, s); IR (NaCl, film) ν = 3268, 2996, 1715, 1624, 1482, 1338, 1174, 1042, 754 cm^{-1}.

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Supplementary Material Available: ^{1}H NMR, IR, and HPLC analyses of natural and synthetic aspirochlorine (3 pages). Ordering information is given on any current masthead page.