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Anti-Neoplastic Agents of Marine Origin:
Identification, Synthesis, and Structure-Activity Studies

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemistry

by

Theodore Paul Evans

Committee in charge:
Professor D. John Faulkner, Chair
Professor Jay S. Siegel
Professor William H. Fenical
Professor Trevor C. McMorris
Professor William S. Allison

1996
The dissertation of Theodore Paul Evans is approved, and it is acceptable in quality and form for publication on microfilm.

Theodore

W. L. Allison

William Genical

Jan Sing

D. John Faulkner

Chair

University of California, San Diego

1995
This dissertation is dedicated to my father, Raymond D. Evans M.D.

1923 - 1996
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<tbody>
<tr>
<td>AIBN</td>
<td>2,2'-azobis(2-methylpropionitrile)</td>
</tr>
<tr>
<td>BOC</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>CDI</td>
<td>carbonyldiimidazole</td>
</tr>
<tr>
<td>CI</td>
<td>chemical ionization</td>
</tr>
<tr>
<td>COLOC</td>
<td>correlated spectroscopy for long range couplings</td>
</tr>
<tr>
<td>COSY</td>
<td>correlated spectroscopy</td>
</tr>
<tr>
<td>DCC</td>
<td>dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCI</td>
<td>direct insertion chemical ionization</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DDQ</td>
<td>dichlorodicyanoquinone</td>
</tr>
<tr>
<td>DKP</td>
<td>2,5-piperazinedione</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylacetamide</td>
</tr>
<tr>
<td>DMAP</td>
<td>N,N-dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DQ COSY</td>
<td>double quantum coherence correlated spectroscopy</td>
</tr>
<tr>
<td>EIMS</td>
<td>electron impact mass spectrometry</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>FABMS</td>
<td>fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography-mass spectrometry</td>
</tr>
<tr>
<td>HCT</td>
<td>human colon tumor</td>
</tr>
<tr>
<td>HPLC</td>
<td>high pressure liquid chromatography</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>-------------</td>
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</tr>
<tr>
<td>HRFABMS</td>
<td>high resolution fast atom bombardment mass spectrometry</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>MTPA</td>
<td>2-methoxy-2-trifluoromethyl-2-phenylacetic acid</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>pyr</td>
<td>pyridine</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
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<td>TLC</td>
<td>thin layer chromatography</td>
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<tr>
<td>TMS-CN</td>
<td>trimethylsilylcyanide</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>XHCorr</td>
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Journal of Natural Products 1989, 52, 207.
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ABSTRACT OF DISSERTATION


by

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Doctor of Philosophy in Chemistry

University of California, San Diego 1996

Professor D. John Faulkner, Chair

This dissertation is divided into three freestanding chapters. Chapter 1 describes the total synthesis of the cytotoxic marine natural product trans-2,5-bis(6'-bromo-3'-indolyl)piperazine (1), as well as racemic cis-2,5-bis(6'-bromo-3'-indolyl)piperazine (78). Also described are the syntheses of two additional analogs of the natural product 1; trans-2,5-bis(5'-bromo-3'-indolyl)piperazine (79) and racemic cis-2,5-bis(5'-bromo-3'-indolyl)piperazine (80). These piperazines were prepared by electrophilic addition of the indole to cis-3,6-dibromo-1,4-bis(tert-butoxycarbonyl)-2,5-piperazinedione, followed by isomerization and reduction of the resulting 3,6-bis(indolyl)-2,5-piperazinediones with borane-tetrahydrofuran. The piperazine products 1 and 78-80 were all found to be cytotoxic. In collaboration with the National Cancer Institute (NCI), in vivo testing was performed. The piperazine compounds 78, 80 and 1 have been identified as selectively cytotoxic to colon tumors. NCI has determined that the maximally tolerated doses in mice of 1 and 78 are 400 and 450 mg/kg, respectively. Efforts are underway to provide more of 1, 78 and 80 for further in vivo testing by the NCI. In addition the piperazine products were found to inhibit phorbol-induced inflammation in the Mouse Ear Edema
Assay at levels ranging from 44% for 78, 69% for 80 and 71% for 1 to a maximum of 80% for 79.

Chapter 2 describes the isolation and stucture elucidation of the marine natural product (2R, 3R, 10R)-(Z)-2-amino-1,3,10-trihydroxy-11-octadecene (81). The stucture was determined by a combination of spectral analyses and derivatizations. The Modified Mosher Method was used to determine the absolute configurations of C-3 and C-10. The compound was determined to possess broad spectrum anti-microbial properties, and was also found to inhibit phorbol-induced inflammation in the Mouse Ear Edema Assay at a level of 43%.

Chapter 3 describes the isolation and synthesis of a glycine derivative of the cytotoxic marine natural product ilimaquinone. The structure of the new compound, called glycinyllilimaquinone (91) was determined by spectral methods and by partial synthesis. Compound 91 was also found to be cytotoxic (eg. HCT-116, IC\textsubscript{50} = 7.8 \(\mu\text{g/mL}\)) and evaluated against the P388 murine lukaemia model \textit{in vivo}, but at a maximally tolerated dose showed no anti-tumor activity. Also described are structure-activity relationships for some ilimaquinone analogs tested for Golgi vescication and microtubule depolymerization in rat kidney cells.
Chapter 1
Synthesis of the Cytotoxic Marine Natural Product
Trans-2,5-bis(6'-bromo-3'-indolyl)piperazine and Structure-Activity Studies of Some Analogs.

Introduction

Despite decades of cancer research, there is still a need for new pharmaceuticals for the treatment of cancer.\textsuperscript{1} About half of the medicines in use today are of natural origin, and many of the synthetic drugs are based on naturally occurring substances. Although knowledge of the molecular basis of disease has allowed rational drug design in a few cases, most new drugs are still developed as a result of random screening of substances for specific pharmacological activities, such as cytotoxicity for the treatment of cancer.

The marine environment is a particularly rich and diverse source of compounds for screening, but is one of the least well explored. Given the enormous diversity of organisms in the ocean, one must be careful to select those which are most likely to produce compounds of pharmacological interest.

In this work, we have chosen to restrict our search to the sponges and tunicates. These organisms usually lack effective physical protection against predation, and are thought to produce noxious chemicals in order to deter potential predators. It is these "defensive" chemicals which we seek, in the expectation that some will possess cytotoxic activity.

Existing drugs used for treatment of cancer fall into several mechanistic classes.\textsuperscript{2} The first class is the DNA alkylating agents, such as mitomycin and \textit{cis}-platin, that cross-link DNA and so prevent the formation of RNA which is necessary to produce proteins.
Another class of drugs is the topoisomerase inhibitors, such as doxorubicin and camptothecin, that function by either direct binding to a topoisomerase enzyme, or by interfering with the binding of the enzyme to DNA. Since topoisomerase activity is required to form RNA, these drugs inhibit RNA synthesis.

The RNA/DNA antimetabolites can slow production of ribonucleotides by inhibiting purine/pyrimidine synthesis, or can act as analogs of nucleosides or nucleotides and the corresponding RNA equivalents. Examples of drugs in this group include methotrexate which inhibits purine ring biosynthesis, 5-fluorouracil which inhibits dTMP synthesis and 5-aza-2'-deoxycytidine which inhibits DNA synthesis by placing a terminal group (lacking a 5'-OH, required for continuation of the chain) on single stranded DNA during it's synthesis.

The last group consists of the antimitotics, of which the best known are the *Vinca* alkaloids vincristine and vinblastine (which are produced by the periwinkle), and taxol (which was isolated from the bark of the Pacific yew tree). These compounds interact with microtubules in cells, thereby preventing cell division.

The one thing that all these classes of drugs share is their non-specific action. That is, all of them are to some extent also toxic to normal cells, especially those drugs that function at the lower levels of cell function and biosynthesis, which are common to all cells and not just cancerous ones. It is the lack of specificity that so limits the use of these drugs. One way to avoid this drawback is to find drugs with new mechanisms of actions that are specific to one type of cancer cell. This can be done by first ruling out all compounds which fall into the existing drug classes, and then selecting those compounds that have unknown mechanisms of action for further screening.

The National Cancer Institute Drug Discovery Program has developed a panel of 60 cell lines, that is further divided into sub-panel types of carcinomas consisting of
lukemia, colon, breast, prostate, CNS, lung, melanoma, ovarian, and renal carcinomas. The National Cancer Institute (NCI) has also shown that the in vitro activity patterns (eg. LC₅₀) of drugs from a particular class are all similar across the cell panel. They have developed a computer program called COMPARE, that uses a database of known activity-profiles of existing drugs in each of the mechanistic classes.³⁴ This was used to identify new compounds with similar activity-profiles to those in the database, and thus "identify" the mechanism of action of the potential drug with high reliability. Implicit in this is also the identification of compounds whose activity profiles do not match any of the constructs in the database.

It is reasonable then, to use the COMPARE program to identify new mechanistic classes of drugs. This is, however, only one criterium imposed on the potential drug. In addition to the requirement of novel mechanistic action, it must also be selective. Selective in this case means that the compound shows good activity in only one cell panel, such as leukemia, and diminished activity in all others. The chance for specificity to a cancer cell as opposed to a normal cell is thus increased. Only after passage though these two criteria are compounds considered for in vivo testing.

In this way, NCI hopes to discover new treatments for cancer that are fundamentally different from the existing modes of treatment. Although this screening process may not provide new cancer treatments directly, it will at least uncover new lead compounds. These lead compounds are an important part of the drug discovery process.

Academic chemists can contribute to this process in two main ways: first by discovery of novel compounds either from natural or synthetic sources, and second by synthesizing natural products that are available in insufficient quantity for thorough screening and evaluation. This dissertation combines these two approaches by first identifying compounds of interest from marine natural products, and then producing those that are the most promising in quantity for further study. Also included in this
thesis are structure-activity studies of synthetic and semi-synthetic analogs of marine natural products that may be useful in elucidation of the mechanistic basis of activity of the natural products.

In 1987, Faulkner et al. reported the natural products isolated from two specimens of a thin encrusting tunicate that were collected from the Gulf of California, Mexico.\(^5\) The first specimen (collection #87-061, SIO Benthic Invertebrate Collection #AS139), collected by SCUBA from submerged rock faces (-3 m) near Isla Carmen was found to contain the related metabolites trans-2,5-bis(6'-bromo-3'-indolyl)piperazine (1, 0.01% dry weight) and 2,2-bis-(6'-bromo-3'-indolyl)ethylamine (2, fig. 1). The second specimen (collection #87-045, SIO Benthic Invertebrate Collection #AS138) was found encrusting on submerged mangrove roots at Isla San Jose. Bioactivity guided fractionation of the methanol extract gave the antibacterial/antifungal compound 2-(6'-bromo-3'-indolyl)ethylamine (6-bromotryptamine, 3). Both specimens were subsequently identified as *Didemnum candidum* by Dr. Ralph Lewin of SIO. The only apparent difference between these two tunicates was the color. Specimen 87-045 was off-white in color, while specimen 87-061 was blue-gray.

In a structure based search of the literature, the NCI identified 1 as a potentially interesting candidate for their Developmental Therapeutics Program and requested a sample for screening. The compound was screened against 60 human carcinoma lines, and displayed moderate activity overall in the micromolar range of concentrations. However, 1 was selectively active against NCI's colon tumor cell lines (e.g. COLO 205, \(LC_{50} = 6.2 \mu M\)), and NCI requested a re-supply for further *in vivo* testing. In a personal communication with Dr. Dan Lednicer of NCI, I requested that the data on 1 be run through the COMPARE program. The results of this program for 1 indicated no correlation to known agents.
trans-2,5-bis(6'-bromo-3'-indoly) piperazine (1) 
$C_1$ symmetry

2,2-bis(6'-bromo-3'-indoly) ethylamine (2)

2-(6'-bromo-3'-indoly) ethylamine (6-bromotryptamine, 3)

Figure 1. Metabolites of the Tunicate *Didemnum candidum*. 
A reasonable interpretation of this result is that 1 has an unknown mechanism of action. The selectivity of 1 for colon cancer cells is very unusual, and given that 1 probably has a unique mode of action, further study was required.

The first step for in vivo assays was an acute lethality study, for which NCI required an initial amount of between 20 mg and 2 g. As we had already given our entire supply of 1 to NCI, we had to determine how we could best obtain the necessary amount of 1 to allow further study. Academic interests notwithstanding, one solution to the supply problem would be a recollection of large amounts of the tunicate that produces 1. Recollection of a specific marine organism is often difficult, as populations vary from place to place. In addition, identification of the organism can be difficult, especially if there are many color-morphs as with Didemnum candidum. In order to have enough tunicate to isolate 2 g of 1, we would have to recollect approximately 20 kg of material.

Synthesis offers certain advantages over recollection. First, there would be none of the aforementioned uncertainties associated with recollection. Second, not only would large amounts be available for testing, but there is also the opportunity to synthesize related compounds which can be used in structure-activity studies. Synthesis would also allow confirmation of the assigned structure, and provide a challenging target molecule. With these thoughts in mind, we decided to initiate a total synthesis of 1, along with some analogs for primary screening.

In preparation for synthesis, the structure of the target molecule must be defined. This work was done by Fahy, Potts and Faulkner using extensive studies of 1 incorporating derivatives and low temperature NMR. Although most of the data fit precisely with the proposed structure, there was one inconsistency with the coupling constants of the piperazine ring protons which required further explanation.

The previously reported dragmacidin A (4), which is the mono-N-methyl derivative of 1, has been assigned the structure shown in fig. 2. The couplings of the
dragmacidin A (4)  \( W = Z = H \) \( X = Y = \text{Br} \) \( R_1 = \text{CH}_3 \) \( R_2 = H \)

5 \( W = Z = H \) \( X = Y = \text{Br} \) \( R_1 = R_2 = \text{Ac} \)

dragmacidin (6) \( W = \text{OH} \) \( X = Y = \text{Z = Br} \) \( R_1 = \text{H} \) \( R_2 = \text{CH}_3 \)

dragmacidin B (7) \( W = \text{H} \) \( X = Y = \text{Br} \) \( R_1 = R_2 = \text{CH}_3 \)

topsentin (8) \( X = \text{H} \) \( Y = \text{OH} \)

bromotopsentin (9) \( X = \text{Br} \) \( Y = \text{OH} \)

deoxytopsentin (10) \( X = Y = \text{H} \)

Figure 2. Marine Natural Products Structurally Related to 1.
piperazine ring protons are $J_{2,2} = 11$ Hz, $J_{2ax,3ax} = 10.5$ Hz and $J_{2eq,3ax} = 3$ Hz. However, 1 does not display the large $J_{2ax,3ax}$ coupling constant consistent with that of dragmacidin A. These differences could be due to conformational variation.

The diacetamide 5 was prepared by Faulkner et al. in an attempt to clarify the conformation of 1 (fig. 2). A low temperature $^1$H NMR of this derivative showed four sets of signals due to the piperazine ring protons (diastereomeric compounds resulting from hindered amide bond rotation). A low temperature COSY experiment allowed the spin systems of all four sets of signal to be traced. In each case the coupling constants were consistent with a piperazine ring in the chair conformation, and 1 was assigned the structure trans-2,5-bis(6'-bromo-3'-indolyl)piperazine. This structure gives the molecule $C_i$ symmetry, and it is achiral.

Other structurally related compounds, the dragmacidins (6,7) and the nortopsentins (8-10) with cytotoxic and anti-inflammatory activities have also been isolated and are shown in fig. 2.6,7 Unfortunately, none of these compounds had been synthesized at the outset of this work in 1990, leaving little literature precedent to aid in design of a synthesis of 1.

The current literature reveals only two examples of syntheses of bis(indolyl)piperazines. The first one, published by Whitlock and Cava in 1994 describes the synthesis of the natural product dragmacidin B (7, fig. 3).8 Sarcosine anhydride (11) is brominated with N-bromosuccinamide (NBS) in refluxing CCl$_4$ using a radical initiator to give 3,6-dibromo-1,4-dimethyl-2,5-piperazinedione (12) as the critical intermediate. To this is added 6-bromoindole (13), which substitutes for bromine under acidic conditions in DMF to give the piperazinedione 14. Interestingly, they report that the use of base to neutralize liberated HBr results in no reaction. The sequence is completed by reduction of the 14 with borane-tetrahydrofuran (borane-THF) to form the piperazine 7. The authors report that the spectral data of the product is identical to that previously
Figure 3. Synthesis of Dragmacidin B (7) by Whitlock and Cava. The product was isolated in 12% yield from the dibromide (12).
reported for 7, but they provide neither spectral data nor a formal experimental section on
the product or its intermediates.

A better account of the synthesis of racemic dragmacidin (6, fig. 4-6) was
reported in 1994 by Jiang et al. The group initially tried a scheme which required
synthesis of two amino acids, to be followed by cyclization to the piperazinedione and
reduction to the piperazine. Unfortunately, their attempt to form the required amino acid
failed, and the initial plan was altered.

In the successful synthesis 6-bromoindole-3-carboxaldehyde (15) was reacted with
Trimethylsilylcyanide (TMSCN) and methylamine in refluxing methanol to give the
unstable compound 2-methylamino-2-(6'-bromo-3'-indoly1)ethanenitrile (16). Without
purification, 16 was coupled with the acid chloride 17 in dichloromethane
(DCM)/triethylamine (TEA) at room temperature to give 18. Oxidation of 18 with
ammonia/hydrogen peroxide/n-Bu₄HSO₄/dioxane at room temperature provided the
carboxamide 19. Cyclization was then performed in aqueous ethanolic ammonia at reflux
for 24 hours. The cyclized intermediate 20 was reduced with borane-THF at 0°C to room
temperature for 2 days. This sequence gave racemic 4'-methoxy-dragmacidin (21) along
with the racemic cis-analog 22. The O-methyl group was removed from 21 using BBr₃
in dichloromethane, completing the synthesis of racemic dragmacidin (6).
Figure 4. Synthesis of Intermediate 19 by Jiang et al.⁹
Figure 5. Synthesis of Intermediate 21 by Jiang et al,\textsuperscript{9}
Figure 6. Synthesis of Racemic Dragmacidin by Jiang et al.$^9$


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Figure 7. Retrosynthesis of 1: Disconnection A.
Figure 8. Acid and Heat Catalyzed Equilibrium Between 25 and 26.
material, assuming a workable equilibrium distribution. Racemization of peptides and
diketopiperazines is well studied and can be induced with catalytic amounts of base or
acid to give equilibrium mixtures of isomers.\textsuperscript{13}

Another disconnection (B) is shown in figure 9. This approach is better in terms
of ability to vary the structure of the indole units and the configuration of the indole
moieties about the piperazine ring. For example one could easily synthesize
unsymmetrical compounds having differing substitution on the indole moieties.
Beginning with enantiomerically pure amino acids, any of the possible stereoisomers
could be formed. The most likely candidate for this synthon would be the amino acid 2-
amino-2-(6'-bromo-3'-indolyl)ethanoic acid (6-bromonortryptophan, 27 and 28) amino
acid, easily obtained by reaction of 6-bromoindole with an electrophilic glycine
equivalent.\textsuperscript{14-16} Coupling of the two amino acid subunits followed by cyclization to the
diketopiperazine has good literature precedent.\textsuperscript{17,18} Subsequent reduction to the
piperazine is also well studied.\textsuperscript{19}

All of these factors combine to make this approach one of the better choices for a
flexible synthesis that would allow easy synthesis of analogs with indole substitution and
configuration about the central piperazine ring that are different from the natural product
1. However, when one disconnects the two subunits of 1 as in fig. 9, it is found that two
amino acids are produced 27 and 28. The chiral centers of these molecules are of
opposite configuration. But the piperazine product 1 has \( C_i \) symmetry and is achiral. In
terms of the synthesis of 1, it would be somewhat inefficient to create two chiral centers
of opposite configuration, then in effect destroy them in the final product. If strategy (B)
were used for the synthesis of 1, it would probably be best to start with racemic materials
and separate the isomers at the piperazine stage.

The third disconnection (C, fig. 10) would be a more biomimetic one. This would
involve dimerization of tryptamines, and has the great advantage of providing the
Figure 9. Retrosynthesis of 1: Disconnection B.
Figure 10. Retrosynthesis of 1: Disconnection C.
required product directly, without the need for a reduction step. The starting materials could be prepared in the correct oxidation state at the outset. This approach would utilize an inherent ability of the indole to stabilize a positive charge at the C-2 position of 6-bromotryptamine.\textsuperscript{20} With this in mind, it is possible that only a "benzylic" oxidation of 6-bromotryptamine to the alcohol \textbf{29} would required to activate 6-bromotryptamine for dimerization to \textbf{1}. The stereochemical outcome of the reaction is not under direct control, and the product would most likely be a mixture of \textit{cis}- and \textit{trans}- isomers about the piperazine ring. This approach seems the most risky, since there is no literature precedent for this type of reaction.

A fourth strategy (D, fig. 11) involves \textit{de novo} synthesis of the indole moieties by reaction of \textit{ortho}-iodoanilines with terminal alkynes. In this sequence 4-bromo-2-iodoaniline (\textbf{30}) is reacted with \textit{trans}-2,5-bis(2-trimethylsilyl-1-ethynyl)piperazine (\textbf{31}) using palladium (II) as a catalyst. Larock and co-workers have published results showing that indoles can be formed in a one-step procedure by reaction of \textit{ortho}-iodoanilines with silyl protected terminal alkynes in the presence of palladium (II). The more bulky substituent on the alkyne (the silyl group) always resides on C-2 of the indole product, giving the reaction excellent regiocontrol. By choice of an appropriate silyl protection, it should be possible to orient the piperazine ring in the desired C-3 position of the indole. The silyl group can then be removed in a variety of ways to give the natural product \textbf{1}. The alkyne starting material is listed in the literature as a polymer intermediate, and it may possibly be obtained in pure form.

Of the three retrosynthetic strategies, (B) seemed to have the best hope for controlling stereochemistry, as well as providing for ease of model testing, and had an excellent chance of success based on literature precedent. Strategies (A), (C) and (D) were less attractive alternates that could be explored if strategy (B) met with failure.
Figure 11. Retrosynthesis of 1: Disconnection D.
Results and Discussion

Work on approach (B) began by proposing a synthesis for the previously unknown racemic 6-bromonortryptophan (32, fig. 12). The synthesis could start with indole-3-carboxaldehyde (33). Bromination would then give aldehyde 15. Formation of the amino nitrile 34 via a Strecker type synthesis followed by basic hydrolysis would then give the desired racemic 6-bromonortryptophan (32). Protection of the amine as the tert-butoxycarbamate 35 could be followed by the formation of the 4-nitrophenyl ester 36. Deprotection of 36 (fig. 13) would provide intermediate 37 that could be dimerized to 25. Reduction with borane-THF would then give 1. The desired starting material 6-bromoindole-3-carboxaldehyde was not commercially available, however 5-bromoindole-3-carboxaldehyde (38) could be obtained in pure form, and was used as a model for some of the exploratory chemistry.

Indole-3-carboxaldehyde (33) can be brominated (fig. 14) in acetic acid with bromine to yield a mixture of 5-bromoindole-3-carboxaldehyde (38) and 6-bromoindole-3-carboxaldehyde (15). The $^1$H NMR (DMSO-$d_6$) analysis of the crude reaction mixture showed two sets of signals. The first set was assigned to the known 5-bromoindole-3-carboxaldehyde (38). A second set of signals due to 6-bromoindole-3-carboxaldehyde (15) was also observed at approximately one fourth the intensity of those due to the 5-bromo isomer. However, not only is the yield for this conversion low (ca. 10%), but the 5- to 6-bromo ratio is about 3:1 in favor of the 5-bromo product. Although the 5- and 6-bromo adducts can be easily separated on silica gel (ethyl acetate-hexanes 7:3) separation of the unbrominated starting material from the 6-bromo product is difficult. This liability could be overcome by complete conversion of the starting material to brominated products.
Figure 12. Original Synthetic Scheme for the Activated Ester 36.
Figure 13. Original Synthetic Scheme for 1.
Figure 14. Bromination of Indole-3-carboxaldehyde. Conversion was solvent-dependent with TFA giving 95% conversion and acetic acid giving 10% conversion.
The low conversion of starting material to brominated product was the first problem to be solved. Various catalysts such as mineral acid (sulfuric) and/or Lewis acids (AlCl3, FeBr3) with heating were tried, but no conditions could be found to increase the yield in acetic acid solvent. Unexpectedly, use of trifluoroacetic acid (TFA) proved effective (fig. 9). Bromination of indole-3-carboxaldehyde in TFA at room temperature with a slight excess of molecular bromine gave excellent conversions. The reaction was monitored using two-dimensional TLC on silica gel. The plates were first developed with diethyl ether, then rotated 90° and developed with acetonitrile-benzene (15:85). Although this system gave good separation, it could not be used on a preparative scale due to the insolubility of the carboxaldehydes in these solvents. As mentioned before, the mixture could also be separated by HPLC on silica using ethyl acetate-hexanes (3:1) as eluent. Again, the low solubility of the carboxaldehydes precluded use of this method for preparative scale chromatography.

Large amounts (ca. 20 g) of the brominated indole-3-carboxaldehyde mixture could be partially purified by fractional recrystallization. The crude product from the bromination was dissolved in a minimum amount of boiling ethyl acetate. The mixture was then be seeded with the less soluble 5-bromoindole-3-carboxaldehyde (38). The 5-bromoindole-3-carboxaldehyde crystallized out of this solution at room temperature in nearly pure form, leaving a mixture that was enriched in 6-bromoindole-3-carboxaldehyde. Unfortunately, no conditions could be found to preferentially recrystallize the desired 6-bromoindole-3-carboxaldehyde. After two crystallizations, the ratio of 5- to 6-bromoindole-3-carboxaldehyde could be lowered to ca. 1:1.

With the bromination problem at least partially solved, the synthesis of the amino acid was pursued. A classic Strecker type synthesis (fig. 12) was attempted using indole-3-carboxaldehyde as a model substitute for 6-bromoindole-3-carboxaldehyde. This was uniformly unsuccessful under a wide range of conditions. Solvents such as methanol or
ethanol were used as reaction media. Ammonium chloride and potassium cyanide were used to generate ammonium and cyanide ion respectively. Initial experiments were run at room temperature overnight, but did not show any evidence of reaction occurring. By using methanolic ammonia or acetic acid to modify the apparent pH, a range of pH values from 4.5 to 8 were tried without success. Heating the reaction mixture resulted in benzoin condensation. When ammonia was substituted with a primary amine such as n-butyramine, some reaction did occur to form the amino nitrile, but not to a synthetically useful extent.

A modified Strecker synthesis (fig. 12) which often gives higher yields of amino nitrile (especially with aromatic aldehydes) using trimethylsilylcyanide (TMSCN)/R-NH₂/methanol did provide the amino nitrile 34 (where Br = H).²⁶-³⁰ Unfortunately, this product could not be isolated and proved to be unstable to the acidic or basic hydrolysis conditions needed to convert the nitrile to a carboxylic acid. The instability of the amino nitrile 34 may have been enhanced by the ability of indole to stabilize a cation α to the C-3 position. Under the basic conditions of nitrile hydrolysis, elimination of cyanide ion via base abstraction of the indole NH proton is possible. Loss of the indole NH proton would give an anion that could then eliminate cyanide to form the imine, and eventually the aldehyde. The amino nitrile could also lose the elements of HCN (eg. formation of dichlorocarbene by base induced decomposition of chloroform) by alpha elimination to yield a carbene, which would then perform an α-insertion, leading to the aldehyde product under the aqueous base conditions of nitrile hydrolysis. Protection of the indole nitrogen with an electron withdrawing substituent would be expected to increase the stability of the amino nitrile in two ways: indole NH abstraction would be impossible, and the electron donating ability of the indole would be lessened.

Indole-3-carboxyaldehyde (33) was employed as a model compound to explore the effect of indole nitrogen substitution on the formation and subsequent hydrolytic
indole-3-carboxaldehyde (33)

![Chemical reaction diagram showing the transformation from indole-3-carboxaldehyde (33) to 39 via RCl, Et₃N/DME, RT, and TMSCN/n-BuNH₂/MeOH, reflux.]

33

![Chemical structure of 40 showing n-BuNH-CN.

40

![Chemical structure of 41 showing n-BuNH-COOH.

41 expected product

Figure 15. Effect of Indole Protection as a Sulfonamide on Amino Nitrile Hydrolysis.
stability of 40 (fig. 15). Reaction of indole-3-carboxaldehyde with 2,4,5-trimethyl-benzenesulfonylchloride under mildly basic conditions produced the N-sulfonyl derivative 39 in high yield. When this material was subjected to TMSCN/n-butylamine/MeOH the amino-nitrile 40 formed in good yield as evidenced in the $^1$H NMR of the crude product by the replacement of the aldehyde signal by a new signal at 4.7 (d, 1H) ppm. Upon exposure to aqueous base, the compound again reverted back to indole-3-carboxaldehyde, not the expected product 41.

Substitution of the indole nitrogen with alkyl groups to form the benzyl or para-methoxybenzyl adducts 42 and 43 was also performed (fig. 16). Treatment of these alkyl derivatives with TMSCN/n-butylamine/MeOH produced the imines 44 and 45 as the major products, with only small amounts of amino nitrile.

The previously discussed results of Jiang et al. demonstrate that amino nitrogen protection (carbamate) of compounds such as 34 aids in the stability of the amino nitrile, but not enough to allow nitrile hydrolysis to the amino acid. Their results and my own, suggest that elimination of cyanide ion probably occurs by formation of the more stable imine, in a process which is not dependent on abstraction of the indole nitrogen proton. Of course, substitution of the amino nitrogen with an electron withdrawing substituent would probably improve the situation, however this modification was not explored. At this point we began to explore other methods to obtain the required amino acid.

Reductive amination of pyruvic acids has been utilized as a general method to produce amino acids. This is usually done with ammonium salts and sodium cyanoborohydride. The reaction is performed in methanol at room temperature, but careful attention must be paid to the pH of the reaction mixture. The reaction is two-step, with the first being imine formation. The imine must then be protonated in order to be reduced by the cyanoborohydride. The pH of the reaction must be adjusted to near neutral with acid or base, as a compromise to allow imine formation, and provide an
Figure 16. Effect of Indole Nitrogen Alkyl Substitution on Amino Nitrile Production.
equilibrium amount of iminium ions for reduction to the amino acid product. These factors combine to limit yields of amino acid to about 50%, even with large excesses of reagents.

The compound 3-indoleglyoxylic acid (46, available from Aldrich) can be reductively aminated to produce racemic 2-amino-2-indolylethanoic acid (47, nortryptophan, fig. 17). Since the carbonyl is in conjugation with the electron-rich indole it is not as activated as in an alkyl-substituted pyruvic acid, the traditional conditions (ammonium chloride/sodium cyanoborohydride/methanol, room temperature) are not effective. However, we determined that bringing the mixture to reflux in methanol overnight gave low (25%) yields of nortryptophan. The amino acid was most conveniently purified by the use of cation exchange resin. Both the reaction and purification could be done on a large scale quite easily. The purified product is a solid that displayed physical properties identical to the literature values. 16 Now possessing the knowledge to produce nortryptophan, testing of the rest of the proposed synthesis was begun.

Initially (S)-tryptophan (48) was used as a model compound to test the original dimerization-reduction sequence. (S)-tryptophan can be most efficiently dimerized by simple reflux in 1,2-ethanediol (fig. 18).18 Chromatography or recrystallization gives the trans-diketopiperazine 49 and the racemic cis-diketopiperazines 50. These compounds were then used as authentic standards. Subsequent reduction of the trans-diketopiperazine 49 with borane-THF to the trans-piperazine 51 also proved successful.15 The product was purified by HPLC [silica, methylene chloride-methanol-triethylamine (83:17:1)]. The 1H NMR analysis (pyridine-d5) showed the expected aromatic signals with additional signals due to the piperazine moiety at δ 3.10 (H-2ax, H-5ax), 3.01 (H-3eq, H-6 eq) and 2.50 (H-3ax, H-6ax). The signals displayed coupling constants consistent with a trans-disubstituted piperazine ring in the chair conformation.
3-indoleglyoxylic acid (46) \[ \xrightarrow{\text{NH}_4\text{OAc/NaBH}_3\text{CN, MeOH-reflux}} \] racemic nortryptophan (47) 25%
Figure 18. Cyclization of (S)-Tryptophan. Reaction produces a mixture of 49 and 50 [49:50 (4:1)]. The combined yield is approximately 90%.
Successful synthesis of a *trans*-substituted piperazine was not the only part of the sequence that required testing using models. The dimerization of nortryptophan would probably require milder conditions than refluxing 1,2-ethanediol for efficient dimerization. Again using (*S*)-tryptophan, the amino acid was first protected as the tert-butoxycarbamate (BOC) derivative. The acid **52** (fig. 19) was then esterified with a variety of phenolic leaving groups.

Peptide synthesis literature suggested the use of 4-nitrophenol, the esters of which react with primary amines at room temperature. Esterification of **52** with 4-nitrophenol produced a product displaying $^1$H NMR signals consistent with the expected 4-nitrophenyl ester, as a yellow glass which slowly decomposed on exposure to moisture. Depletion of the amine with TFA/DCM/anisole gave the TFA salt of the amine. After evaporation of excess solvents, the product was treated with four equivalents of triethylamine in ethyl acetate, and allowed to react at room temperature. This procedure gave very little DKP, and analysis of the reaction mixture by $^1$H NMR suggested that large amounts of polymer were formed in exclusion to the desired diketopiperazine.

Various other solvents and bases were tried. For example pyridine/imidazole, DMF, acetonitrile and DCM-DMF mixtures. However, no solvent or base changes radically improved the yield of DKP at room temperature. However, when the reaction mixture was heated to 80°C, the DKP was formed in better yield, especially in the EtOAc/TEA solvent system, but there was still much polymerization as a side reaction. This unwanted polymerization was probably due to a combination of slow amide bond rotation, and excessive reactivity of the 4-nitrophenyl ester.

In order for the DKP to form the intermediate dipeptide must adopt the unfavorable cis-conformation. Apparently in this case the reaction kinetics leading to the two products (cyclic dipeptide vs. linear polymer) were comparable. These kinetics could be altered by using a less labile leaving group, so phenols of higher $pK_a$ were
Figure 19. Cyclization of (S)-Tryptophan via the Activated Ester. Cyclization occurs with complete retention of configuration.
investigated. Of the esters tested, (3-nitrophenyl, 4-chlorophenyl and phenyl) the 4-
chlorophenol ester proved to give the best compromise between total reaction time and
enhancement of DKP production. Using this leaving group allowed dimerization to the
DKP in about 2 hours, with little concomitant production of polymeric material.

The 4-chlorophenyl ester of the BOC protected (S)-tryptophan was prepared using
standard coupling with dicyclohexylcarbodiimide (DCC). The purified ester 53 showed
$^1$H NMR (CDCl$_3$) signals consistent with the expected product, with the carbamate NH
appearing as a broad doublet at 6.15 ppm and $H_\alpha$ as a multiplet at 2.86 ppm. The $\beta$
protons appeared as an unresolved multiplet at 3.42 ppm. Deprotection of 53 with TFA
and subsequent dimerization were accomplished in a 2-step one-pot reaction. First the
activated ester 53 was treated with excess TFA in DCM to give 54. After evaporation of
excess reagents, a fourfold excess of triethylamine (TEA) was added along with ethyl
acetate as solvent, and the mixture was then heated at 80°C overnight. A $^1$H NMR
(DMSO-$d_6$) analysis of the crude reaction mixture indicated the expected signals for the
indole moiety, along with signals at $\delta$ 7.30 (br s, 2H) and 3.85 (br m, 2H). The signal at
7.30 ppm is assigned to the amide NH of the cis-diketopiperazine 55 with the signal at
3.85 ppm assigned to the symmetrical protons H-3 and H-6 in the diketopiperazine ring.
The $\beta$-protons shifted to $\delta$ 2.69 (dd, $J = 14.3$, 3.7 Hz) and 2.14 (dd, 2, $J = 14.3$, 6.6 Hz)
becoming highly diastereotopic as evidenced by the large (14.3 Hz) geminal coupling.

The yield of 55 was approximately 80% based on integration of H-3/H-6 of the
DKP ring and H-2 of the liberated 4-chlorophenol signals. Complete retention of (S)-
configuration was observed, as none of the corresponding trans-diketopiperazine 49 was
observed.

With model studies completed, the application of the method to the nortryptophan
series was begun. The compound 3-indoleglyoxylic acid can be brominated using
similar conditions to those used for indole carboxaldehyde (fig. 20). The glyoxylic acid
Figure 20. Synthesis of the Activated Esters 62 and 63.
is much less soluble in TFA, so a mixed solvent of chloroform-TFA (4:1) was used to carry out the bromination. The yield is comparable to that of the indole carboxaldehyde bromination, as is the ratio of products obtained. The $^1$H NMR spectrum of the crude reaction mixture indicated nearly complete consumption of starting material, with two new sets of signals in a 3:1 ratio assigned to the 5- and 6-bromo-3-indoleglyoxylic acids (56, 57), respectively. Without isolation, reductive amination of this brominated mixture with ammonium acetate and sodium cyanoborohydride in refluxing methanol gave low (25%) yields of amino acids 58 and 59. However, the procedure was easily scaled up to multi-gram quantities as purification using sulfonic acid resin proved extremely efficient. With the brominated amino acids in hand, testing of the rest of the approach was started.

Following the same sequence as for (S)-tryptophan, the brominated nortryptophan mixture was protected as the BOC derivatives 60 and 61 and esterified with 4-chlorophenol to produce the activated esters 62 and 63. The two positional isomers 62 and 63 could be easily separated by silica gel chromatography [ethyl acetate-hexanes (55:45)] at this point.

Using the pure 5-bromo compound 62, the protecting group on nitrogen was removed with TFA to give 64 and cyclized in ethyl acetate/TEA to a mixture of the cis- and trans-diketopiperazines 65 and 66 (fig. 21).

Unfortunately, the overall yield for this sequence was distressingly low, due in part to the unforeseen instability of the nortryptophan activated esters to heat. Reaction at lower temperature resulted in the production of large amounts of polymer. The use of phenol in place of 4-chlorophenol had the effect of extending the reaction time to about 24 hr, with little improvement in the DKP yield. The reaction temperature was critical to the success of the reaction. Lower temperatures at which the starting material was more stable resulted in polymers. With this result, it seemed best to move on to the other synthetic strategies.
Figure 21. Dimerization of the Activated Nortryptophan Ester 62.
The highly risky but more elegant oxidative dimerization of tryptamine was attempted. Using N-acetyltryptamine as a model compound, various "benzylic" oxidation conditions were tried. Chromium (VI) catalyzed oxidation using tert-butylhydroperoxide was attempted, as were oxidations with Ce(IV) and DDQ.\textsuperscript{34-36} In addition, chromium hexacarbonyl catalyzed oxidation with tert-butylhydroperoxide gave no reaction.\textsuperscript{37} None of these conditions were successful in producing any desired oxidation products, cyclized or not.

Initial investigation of retrosynthesis (D) was begun by attempting the synthesis of 6-bromo-2,3-bis(trimethylsilyl)indole. It was hoped that the use of 1,2-bis(trimethylsilyl)ethyne (67) would crudely approximate the steric requirements of the disubstituted alkyne (31) to be used in the synthesis of the natural product 1.

Following the literature procedure, 4-bromo-2-iodoaniline (30) was combined with 1,2-bis(trimethylsilyl)ethyne (67) in the presence of palladium acetate and sodium carbonate in DMF (fig. 22).\textsuperscript{21} After heating at 100°C for 8 hours, the reaction mixture was worked-up to give 6-bromo-2-(trimethylsilyl)indole (68) in low (8%) yield. It was expected that the other silyl group would have been retained, but this was not the case. Loss of the C-3 silyl group may have been due to the electron-rich nature of the C-3 position of indole, and the silyl group was probably lost on aqueous work-up by a facile basic hydrolysis. It was not the loss of the silyl group which was of concern for the proposed synthesis, but rather that the yield of indole was so low. This may have been due to the rather large steric constraints imposed by having two (instead of one, as in Larock's work) silyl groups on the alkyne.

Given that the alkyne to be used in the synthesis of the natural product 1 was at least as bulky as the model alkyne, it was decided that this route was not feasible, especially since no commercial source of the alkyne precursor to 67, could be located.
Figure 22. Synthesis of 6-bromo-2-(trimethylsilyl)indole (68) Using the Methodology of Larock et al.\textsuperscript{21}
Following a literature review, several papers relevant to approach A (fig. 23) were found. In several articles Armstrong reported that various N-protected glycine anhydride derivatives (69, with various R groups) were converted almost exclusively into the cis-dibromo compounds (70, with various R groups) under radical bromination conditions.38, 39 This was established directly by X-ray crystallographic studies of one of the dibromo adducts.

Using Armstrong's information, a synthetic scheme was proposed for 1 beginning with glycine anhydride (fig. 24). Protection of the nitrogen as the 4-methoxybenzyl amide 71, and subsequent bromination using Armstrong's procedure were performed to give cis-3,6-dibromo-1,4-bis(4-methoxybenzyl)-2,5-piperazinedione (72). Addition of 5-bromoindole to this compound at room temperature in DMF gave a 20% yield of 73. The product could be purified by flash chromatography on silica gel in ethyl acetate-hexanes. Analysis by 1H NMR (CDCl3) gave the usual signals for the aromatic moieties, along with a signal at δ 5.7 (s, 2H) assigned to H-2. However, attempted deprotection to remove the 4-methoxybenzyl group with Ce(IV) or DDQ oxidants gave moderate yields of an orange crystalline compound 74 shown in figure 25. The structure was established using 1H NMR, 13C NMR, XHcorr, COLOC and HRFABMS experiments. The carbon-detected experiments could be used because of the large amounts of 74 available, and all NMR work was done on a 200 MHz NMR spectrometer. The XHcorr and COLOC experiments provide the same information as the more sensitive proton-detected experiments HMQC and HMBC, that are more commonly employed with modern equipment. The 1H NMR spectrum in DMSO-d6/acetone-d6 clearly established the proton spin-systems of the 4-methoxyphenyl group as well as the 5-bromoindole moieties. The XHcorr experiments allowed assignment of all carbons directly bonded to hydrogen. Long range 13C-1H couplings (2-3 bond, J = 8 Hz) were established using the COLOC experiment. The aromatic C-5' signal was assigned the
Figure 23. N-Protection and Bromination of Glycine Anhydride by Armstrong et al.\textsuperscript{38,39} In all cases cis-dibromide was obtained except for \( R = \text{CH}_2\text{Ph}-4\text{-OMe} \). With this protecting group a mixture of the cis- and trans-dibromides was obtained.
Figure 24. Synthesis of 73.
Figure 25. Reactions of 73.
chemical shift of 165.9 ppm by long range correlation to the C-6' methyl protons. Analysis of the COLOC correlations in the indole and 4-methoxyphenyl groups established the assignment of the remaining carbon signals, except for a broad singlet at 169.4 ppm. This carbon must be the cationic center (C-1') of the molecule.

HRFABMS confirmed the molecular formula, however negative ion FABMS was unable to establish the identity of the counter-ion due the inability to observe ions of less than \( m/z = 50 \) amu. Given that removal of the 4-methoxybenzyl group of 73 by DDQ in DCM-water or Ce(IV) in DCM-water produces 74, the counter-ion may well be hydroxide. This would mean that 74 exists as an "ionized" triarylc arbinol. Reduction of 74 with sodium borohydride produces a nearly colorless compound 75 bearing an additional hydrogen signal at 5.86 ppm in acetone-\( d_6 \). HRFABMS confirmed the addition of an extra hydrogen, and the aromatic signals of 75 had returned to more normal values. Assignment of the cationic structure for 74 is strongly supported by these data.

In hindsight, it is perhaps not surprising that attempted cleavage of the 4-methoxybenzyl group of 73 with trimethylsilyliodide failed.\(^{40}\) Catalytic hydrogenation using palladium also failed to cleave the protecting group.\(^{33}\) In the hope of removing the protecting group after the reduction step, borane-THF reduction was tried. This gave no hint of any reaction. At this point it was decided to try other nitrogen protecting groups.

Two carbamate protecting groups, the benzyloxycarbamate and the tert-butoxycarbamate, were chosen for investigation (fig. 26). Of the two, only the BOC derivative 76 could be synthesized. Synthesis of the BOC derivative 76 was accomplished by first pulverizing the insoluble glycine anhydride in N,N-dimethylformamide (DMF) using extended sonication. Sonication/pulverization of the glycine anhydride is critical to the success of the reaction, because crystalline glycine anhydride does not provide sufficient surface area to allow the two-phase reaction to
Figure 26. Synthesis of the Diketopiperazines 25 and 26. Reaction at 110°C produces exclusively the cis-diketopiperazine 26. Reaction at 150°C gives a mixture of 25 and 26, but with extensive decomposition.
proceed at an appreciable rate. The slurry was then treated with di-\textit{tert}-butyl dicarbonate, triethylamine, and 4-(dimethylamino)pyridine (DMAP) at room temperature. After precipitation of the product from the reaction mixture by the addition of water, the crude solid was rinsed sequentially with water and then hexanes. Recrystallization of the solid from a minimum amount of warm toluene gave the pure product 76, which had $^1$H NMR (CDCl$_3$) signals at $\delta$ 4.41 (s, 2H) and 1.51 (s, 9H). The reaction has been done many times, and reliably gives a 50% recrystallized yield of product, on a scale up to ca. 0.2 mole.

Bromination of 76 using Armstrong's procedure proved very efficient, and $^1$H NMR analysis of the crude reaction mixture showed virtually quantitative conversion to 77 using a small excess of brominating agent. The conditions used were identical to those used by Armstrong except that the reaction had to be refluxed significantly longer for complete reaction. Reflux in carbon tetrachloride for nine hours proved to be the optimal time for best yields. Cooling, filtration of succinamide and excess NBS followed by evaporation of solvent, gives a syrup that slowly crystallizes. The product can be used in crude form after removal of solvent, or recrystallization from toluene-hexanes can be used for purification.

The material had the expected $^1$H NMR (acetone-$d_6$) spectrum with resonances at $\delta$ 6.84 (s, 1H) and 1.57 (s, 9H). The product 77 is isolated in about 70% yield by recrystallization, and is also amenable to large scale reaction. The crystalline material is stable, and can be stored under refrigeration for several months.

The well known Batcho-Leimgruber indole synthesis was utilized to convert the expensive, but commercially available 4-bromo-2-nitrotoluene (available from Lancaster Synthesis Inc.) to 6-bromoindole (13) in two steps at about 70% isolated yield (fig. 27).$^{9,41,42}$ The crystalline product had spectral properties identical to those reported in the literature.$^{41}$
Figure 27. Synthesis of 6-Bromoindole (13) Using the Batcho-Leimgruber Method.\textsuperscript{9,41,42}
Although reaction of 6-bromoindole with the dibromide 77 does not occur at room temperature, heating the mixture to 110 °C in DMF causes rapid reaction to form the cis-diketopiperazine 26 (fig. 26). The nitrogen protecting group is also removed in this step. Yields are usually in the range of 50%. This procedure gave almost exclusively the cis-bis-indolyl diketopiperazines. The compounds are difficult to work with in the sense that they are insoluble in most organic solvents except DMF, DMSO and acetic acid. They are not soluble in alcohols or aqueous organic mixtures, so purification consisted of water precipitation from DMF solution followed by a rinse with methanol. At this point various ways of isomerizing the cis-DKP 26 to the desired trans-DKP 25 were tried.

Any attempt to employ strong bases, in aqueous or organic solution resulted in decomposition of starting material. Acidic or neutral conditions could be employed to advantage. One way of inducing the isomerization was to conduct the diketopiperazine formation at higher temperature (150 °C). Although this procedure gave an equilibrium mixture of the cis- and trans- isomers, it also caused extensive decomposition. The isomerization could also be done in 30% HBr/acetic acid at room temperature. This process had to be conducted at high dilution as the cis-DKP 26 crystallizes from this solution, shifting the equilibrium to the crystalline form.

Thermal equilibration in the absence of acid catalyst could also be performed in refluxing N,N-dimethylacetamide (DMA). Heating created an equilibrium mixture of the two isomers, but also caused some decomposition, liberating the brominated indole. No conditions could be found to separate 25 and 26 on a large scale, so the mixture was used in subsequent experiments.

A modification of the aforementioned procedure, can be used to synthesize the cis-diketopiperazine 26 under milder conditions and in higher yield. Deprotection of the dibromide 77 with TFA-chloroform (1:1) overnight at room temperature gives essentially
a quantitative yield of a white solid (fig. 28). Evaporation and further drying under high vacuum gives a solid material that is the dibrominated glycine anhydride 24. The $^1$H NMR analysis of 24 in DMSO-$d_6$ shows two pairs of doublets at $\delta$ 9.6 and 4.5, assigned to H-1(NHCO) and H-2, respectively. The $^1$H NMR spectrum of 24 is consistent with a cis-dibromide in slow dynamic equilibrium between two conformational isomers. Compound 24 reacts with 6-bromoindole at room temperature in DMF to give exclusively the cis-diketopiperazine 26.

Unfortunately, the deprotected dibromide 24 is unstable, and can't be stored for any length of time, and must be used immediately after it has been synthesized. Overall, the yield and purity of the diketopiperazine product is increased over the more harsh conditions used to directly react the BOC protected dibromide 77 with indoles. Given these facts, the extra step of deprotection seems warranted.

The DKP mixture of 25 and 26 was reduced using borane-THF at reflux in THF overnight or at room temperature for 3 days (fig. 29). The reaction was then quenched with 30% HBr/acetic acid whereupon the piperazines precipitated as the dihydrobromide salts. Partitioning of this solid between ethyl acetate and 1M sodium carbonate resulted in a three phase mixture with the trans-piperazine 1 in the organic phase and the cis-piperazine 78 precipitating out of solution as a solid in nearly pure form. Silica gel chromatography using a short column and a gradient elution (5-20% methanol-chloroform) completed purification of the natural product 1. The compound was isolated as a glass and had spectral properties identical to those reported in the literature.\textsuperscript{5}

In general, starting with pure cis-diketopiperazines, reduction with borane-THF gave the corresponding cis-piperazines as racemates. Purification of these compounds was most conveniently accomplished by first precipitating the piperazine from the reaction mixture as the dihydrobromide, followed by recrystallization from ethanol-water. In this manner it is possible to produce gram quantities of the cis-piperazines in pure form.
Figure 28. Alternative Synthesis of the Racemic Diketopiperazine 26.
Figure 29. Synthesis of the Natural Product Trans-2,5-bis(6'-bromo-3'-indolyl)-piperazine (1) and the Analogs 78 - 80.
after one recrystallization. Attempts to purify the *trans*- isomers by recrystallization under similar conditions were unsuccessful.

Using 5-bromoindole as the nucleophile, the 5-bromo analogs of 1 and 78, 79 and 80 respectively were also produced (fig. 29). Compounds 78, 79, 80 and the cationic compound 74 all proved to be cytotoxic, and were submitted for investigation by the NCI.
Conclusions

In conclusion, this general method of synthesis can be used to produce quantities of 1 for further study. It also confirms the previous structural assignment of 1. In addition the general nature of the synthesis allows for the production of related symmetric analogs such as 78, 79 and 80 which may be used in structure-activity studies. The analogs 78, 79 and 80 could be helpful in the determination of both the molecular target of the drug, as well as in the design of other agents of similar activity.

Bioassay Results and Structure Activity-Relationships

The results from NCI on 1, indicate that the maximally tolerated dose of 1 is 400 mg/kilogram in mice. The next step is in vivo testing using the hollow fiber assay both subcutaneously and intraperitoneally in nude mice. NCI has requested an additional 400 mg of 1 for the in vivo assays. This newly developed assay uses hollow fibers (1 mm internal diameter) that contain human carcinoma cells. Up to three cell lines can be tested simultaneously, and if the results are positive, a xenograft of the appropriate human carcinoma is selected for further in vivo testing. None of the active compounds produced show correlation in activity to known agents in the NCI database, presumably because they possess a novel mechanisms of action.

The piperazine analogs 78 and 80 and 1 have been identified as being selective for colon tumors, and appear to share the same mechanism of action based on the Pearson coefficients in the COMPARE program. NCI has determined that the maximally tolerated dose of the 78 (cis- isomer of 1) in mice is 450 mg/kg, and 78 along with 80 have been selected for in vivo testing by the NCI. Efforts are underway to provide more of 1, 78 and 80 for further in vivo testing.
Interestingly, the cationic compound 74, and the seemingly unrelated compound 79 also seem to have the same mechanism of action based on the Pearson coefficient in the COMPARE program. NCI has determined that these two compounds are not suitable for further in vivo testing.

In addition to cytotoxicity assays, the piperazine compounds were also tested for anti-inflammatory activity in the laboratories of Dr. Robert Jacobs (University of California, Santa Cruz). The natural product 1 was active in the Mouse Ear Edema Assay and inhibited phorbol ester induced inflammation by 71%, at a dose of 50 micrograms per ear. In the assay, one mouse ear is treated with phorbol ester and solvent as a control. The other ear is treated with the compound, phorbol ester and solvent. At the conclusion of the experiment, a circular portion of each ear is excised, and the percent inhibition of inflammation is calculated based on the masses of the control and "experimental" portions of the ears. This assay is rather general and does not indicate where in the inflammatory cascade the compound is exerting its anti-inflammatory action. However, some piperazine compounds have been shown to inhibit diacylglycerol induced activation of Protein Kinase C (PKC), an enzyme which plays a major role in the inflammatory process. Thus a mechanism based assay of the piperazines produced in this study in the PKC assay would be a logical place to start if one wished to explore the anti-inflammatory mechanism of these compounds.

The most potent anti-inflammatory effect was seen with 79 at 80%. The other two piperazines exhibited somewhat lower inhibition with 78 at 44% and 80 at 69% respectively.
**Experimental**

**Instrumentation.** UV and IR spectra were recorded on Perkin-Elmer Lambda 3B and 1600 series spectrometers, respectively. All IR samples were processed using 3M Type 61 Disposable IR Cards. These cards contain a window made of microporous polyethylene which can be impregnated with samples in solution, then dried under vacuum to remove solvent. NMR spectra were recorded on a Bruker WP200SY spectrometer. All solvents used were distilled prior to use or purchased in dry form from Aldrich and used without further purification. Chemicals were purchased from Aldrich/Sigma or Lancaster and used without further purification.

1-(2,4,6-trimethylbenzenesulfonyl)-indole-3-carboxaldehyde (39): Indole-3-carboxaldehyde (144 mg, 1.0 mmole) was suspended in 1,2-dimethoxyethane (5 mL) along with triethylamine (5 mL) and 2,4,6-trimethylbenzenesulfonyl chloride (244 mg, 1.11 mmole) and stirred at room temperature overnight. The solvents were then evaporated under reduced pressure, and the residue partitioned between dichloromethane (20 mL) and water (10 mL). The organic extract was evaporated and purified by flash chromatography [silica, hexanes-dichloromethane (7:3)] to give 39 (290 mg, 88%) as a glass: $^1$H NMR (CDCl$_3$) $\delta$ 10.11 (s, 1H), 8.32 (m, 2H), 7.31 (m, 3H), 6.99 (s, 2H), 2.51 (s, 6H), 2.25 (s, 3H).

2-amino-2-indolyl-1'-(2,4,6-trimethylbenzenesulfonyl)ethanenitrile (40): 39 (200 mg, 0.61 mmole) was suspended in methanol (5 mL) and cooled in an ice bath with magnetic stirring. Trimethylsilyl cyanide (89 $\mu$L, 1.1 eq.) was added dropwise followed by $n$-butylamine (66 $\mu$L, 1.1 eq.). The mixture was then refluxed overnight. The $^1$H NMR of the crude reaction mixture indicated almost complete conversion. Excess solvents were evaporated and the residue was dried under high vacuum overnight to give 40 as a glass: $^1$H NMR (CDCl$_3$) $\delta$ 7.76 (m, 2H), 7.26 (m, 3H), 6.97 (s, 2H), 5.02 (d, 1H,
$J = 9.3 \text{ Hz}$, 2.84 (m, 2H), 2.54 (s, 6H), 2.30 (s, 3H), 1.5 (m, 5H), 0.93 (t, 3H, $J = 7.2 \text{ Hz}$).

Without purification the residue was suspended in 6% NaOH in methanol-water (3:1) with stirring. TLC analysis of the reaction mixture [EtOAc-hexanes (7:3)] indicated complete conversion to 5-bromoindole-3-carboxaldehyde within a few minutes.

**2-amino-2-indolyethanoic acid (nortryptophan, 47):** 3-indoleglyoxylic acid (46, 399 mg, 2.0 mmole) was dissolved in methanolic ammonia (12.5 mL of 2M soln., 25 mmole). Acetic acid (1.09 mL, 19 mmole) was added along with sodium cyanoborohydride (65 mg). The mixture was refluxed overnight and the solvent was removed under reduced pressure. Aqueous hydrochloric acid (20 mL of 1M soln., 20 mmole) was added and the mixture was stirred for 1 hour. The insoluble precipitate was filtered off, and the remaining solution was adjusted to pH 4.0 with 5N sodium hydroxide soln. The resulting solution was again filtered and applied to a 20 mL bed of AG50W-X8 (Hydrogen form). The resin was then washed with 300 mL distilled water, and eluted with ammonium hydroxide solution 1M. Fractions giving a positive ninhydrin test were combined and evaporated under reduced pressure to give nortryptophan 47 (102 mg, 25%) as an amorphous powder: $^1\text{H NMR} (\text{DMSO-d}_6) \delta 11.42$ (br s, 1H), 8.03 (d, 1H, $J = 7.5 \text{ Hz}$), 7.67 (d, 1H, $J = 8.0 \text{ Hz}$), 7.64 (s, 1H), 7.40 (m, 1H), 7.30 (m, 1H) 4.81 (s, 1H).

**2-(5'-bromo-3'-indoly)-2-(tert-butoxycarbamyl)-4-chlorophenyl ethanoate (62) and 2-(6'-bromo-3'-indoly)-2-(tert-butoxycarbamyl)-4-chlorophenyl ethanoate (63):** 3-indoleglyoxylic acid (5 g, 26.4 mmole) was dissolved in TFA-chloroform (1:1) 400 mL. With vigorous magnetic stirring, bromine (2.4 mL, 1.8 eq.) was added. The reaction was allowed to stir for 3 days at room temperature, then evaporated under reduced pressure to give 7.1 g (100%) of an orange solid. The $^1\text{H NMR}$ analysis of the crude product indicated a mixture of the 5- and 6-bromo adducts 56 and 57, with nearly complete consumption of starting material. Without purification, the brominated adducts (1.00 g, 3.73 mmole) was combined with ammonium acetate (2.9 g, 10 eq.) and sodium
cyanoborohydride (234 mg, 1 eq.). The mixture was brought to reflux in methanol (30 mL) and allowed to react overnight. The solvent was evaporated under reduced pressure and dissolved in water (14 mL) and brought to pH = 0 with 3M HCl (aq.). After stirring for one hour at room temperature the mixture was filtered, adjusted to pH = 4 and refiltered. The filtrate was then applied to sulfonic acid resin (AG50WX-8, hydrogen form) and the resin rinsed with 500 mL deionized water. The resin was then eluted with ammonium hydroxide 1M and fractions giving a positive ninhydrin reaction were combined and evaporated to give the brominated nortryptophans 58 and 59 (185 mg, 18%). Without purification this mixture was combined with triethylamine (106 mL, 1.1 eq.) in methanol (5 mL) and di-tert-butyldicarbonate (175 mL, 1.1 eq.) was added dropwise while the mixture was brought to reflux for ten minutes. After evaporation of solvent, the residue was partitioned between ethyl acetate (25 mL) and 1M phosphate buffer at pH = 2. The organic layer was then dried with portions of saturated brine (3 x 10 mL) and then further dried over sodium sulfate. The solvent was evaporated under reduced pressure and then placed on high vacuum overnight to give the N-BOC protected amino acids 60 and 61 (250 mg, 100%). Without purification the mixture (60, 61, 127 mg, 0.34 mmole) was combined with DCC (85 mg, 1.2 eq.), DMAP (5 mg, 0.1 eq.) and 4-chlorophenol (55 mg, 1.2 eq.). The mixture was dissolved in ethyl acetate (5 mL) and allowed to stir overnight at room temperature. After filtration to remove dicyclohexylurea and evaporation of solvent, chromatography [silica HPLC, EtOAc-hexanes (2:3)] gave the 5-bromo product 62 (54 mg, 42%) as a glass: $^1$H NMR (CDCl$_3$) δ 7.92 (br s, 1H), 7.33-7.28 (mult, 6H), 6.97 (d, 2H, $J = 8$ Hz), 5.75 (d, 1H, $J = 7.1$ Hz), 5.42 (br d, 1H, $J = 7$ Hz), 1.48 (s, 9H). The 6-bromo compound 63 was not isolated in pure form, and was contaminated with the corresponding unbrominated product.

**Trans-3,6-bis(5'-bromo-3'-indolyl)-2,5-piperazinedione (66) and cis-3,6-bis(5'-bromo-3'-indolyl)-2,5-piperazinedione (65):** 62 (40.8 mg, 0.085 mmole) was
dissolved in dichloromethane (2 mL) containing anisole (18.6 µL) and then TFA (2 mL) was added at once. The mixture was allowed to react at room temperature for 20 minutes, at which time the solvents were evaporated. The residue was placed on high vacuum to remove any residual solvents for 2 hr. The deprotected product was transferred to a 1 mL vial using 2 x 0.5 mL portions of ethyl acetate. Triethylamine (47 µL, 4 eq.) was then added and the vial was sealed with a teflon lined cap and heated to 80 °C for 16 hours. The 1H NMR (DMSO-δ6) analysis showed that the diketopiperazines 65 and 66 were produced in low yield (ca. 5%).

**N-tert-butoxycarbonyl-(S)-tryptophan (52):** (S)-tryptophan (684 mg, 3.35 mmole) was combined with triethylamine (490 µL, 1.05 eq.) and suspended in methanol (7 mL). Di-tert-butyldicarbonate (1.46 mL, 2 eq.) was added dropwise and the mixture brought to reflux for 5 minutes. The solvent was evaporated under reduced pressure and the residue partitioned between ethyl acetate (25 mL) and ice cold HCl (10 mL of 0.4M solution). The organic layer was then washed with 2 x 10 mL portions of water, then dried over sodium sulfate. The solvent was evaporated under reduced pressure to yield N-tert-butoxycarbonyl-(S)-tryptophan 52 (1017 mg, 100%) as a glass: 1H NMR (CDCl3) δ 8.11 (br s, 1H), 7.60 (m, 2H), 7.32 (s, 1H), 7.1 (m, 2H), 6.98 (s, 1H), 5.08 (br d, 1H), 4.65 (m, 1H), 3.32 (m, 2H) 1.45 (s, 9H).

**N-tert-butoxycarbonyl-(S)-tryptophan-4-chlorophenyl ester (53):** N-tert-butoxycarbonyl-(S)-tryptophan (52, 456 mg, 1.50 mmole) was combined with DCC (340 mg, 1.1 eq.), 4-chlorophenol (208 mg, 1.0 eq.) and DMAP (18 mg, 0.1 eq.). The mixture was dissolved in ethyl acetate (5 mL) and stirred at room temperature for 2 hours. Dicyclohexylurea was filtered off, and the filtrate was evaporated under reduced pressure. Flash chromatography [silica, EtOAc-hexanes (2:3)] gave the 4-chlorophenyl ester 53 (458 mg, 72%) as a glass: 1H NMR (CDCl3) δ 8.14 (br s, 1H), 7.62 (d, 1H, J = 7.6 Hz),
7.39 (d, 1H, J = 7.6 Hz) 7.3-7.0 (mult, 5H), 6.80 (d, 2H, J = 8.6 Hz), 6.15 (br d, 1H), 2.86 (m, 1H), 3.42 (m, 2H), 1.45 (s, 9H).

**Cyclo-(S)-trp-(S)-trp (55):** 53 (10 mg) was treated with TFA-DCM (1:1) 0.2 mL for 5 minutes. The solvents were then evaporated under dry nitrogen and the residue placed on high vacuum for 15 minutes. Ethyl acetate (0.5 mL) and triethylamine (18 µL, 4 eq.) were then added to the vial which was then sealed with a teflon lined screw-cap. The mixture was heated to 80 °C for 18 hours, at which point the solvents were evaporated under nitrogen and the sample again placed on high vacuum overnight. Analysis of the crude reaction mixture by 1H NMR (DMSO-d6) showed only the expected cis-diketopiperazine (55). Identity was established by comparison to an authentic standard. There was no evidence of any racemization, as no trans-diketopiperazine (49) was seen in the spectrum of the mixture.

**Cyclo-(S)-trp-(R)-trp (49) and the racemic mixture cyclo-(S)-trp-(S)-trp and cyclo-(R)-trp-(R)-trp (50):** (S)-tryptophan (410 mg) was refluxed in dry 1,2-ethanediol (4 mL) for 24 hours. The solution was cooled and partitioned between water (50 mL) and chloroform (50 mL). The chloroform layer was drawn off and the aqueous phase was then re-extracted with ethyl acetate (50 mL). Evaporation of the chloroform extract gave 186 mg of a solid which was contained the trans-diketopiperazine 49 in nearly pure form. This compound could be further purified by flash chromatography [silica, EtOAc-acetonitrile (3:2)] to yield the pure trans-diketopiperazine 49 as a glass: 1H NMR (DMSO-d6) δ 10.85 (br s, 2H), 7.84 (br s, 2H), 7.49 (d, 2H, J = 7.6 Hz), 7.28 (d, 2H, J = 7.6 Hz), 7.1-6.8 (mult, 6H), 3.38 (br m, 2H), 3.09 (dd, 2H, J = 14.5, 3.7 Hz), 2.82(dd, 2H, J = 14.5, 4.1 Hz). The ethyl acetate fraction was evaporated to a reduced volume, whereupon the racemic cis-diketopiperazine 50 crystallized: 1H NMR (DMSO-d6) δ 10.85 (br s, 2H), 7.30 (m, 4H), 7.00 (m, 4H), 6.57 (s, 2H), 3.85 (br m, 2H), 2.69 (dd, 2H, J = 14.3, 3.7 Hz), 2.14(dd, 2H, J = 14.3, 6.6 Hz).
Reduction of cyclo-(S)-trp-(R)-trp (49): 49 (30 mg, 0.1 mmole) was combined with borane-THF solution (1 mL of a 1M solution) at room temperature with stirring under dry nitrogen. After evolution of gas ceased, the mixture was brought to reflux for 7 hours, then cooled to room temperature. The reaction was then carefully quenched with acetic acid-methanol (9:1, 2 mL). The solution was then evaporated under reduced pressure to a viscous liquid, and partitioned between sodium carbonate (1M, 10 mL) and ethyl acetate (20 mL) the organic layer was dried and evaporated then chromatographed [HPLC DCM-MeOH-TEA (83:17:1)] to give the trans-piperazine 51 (2.3 mg, 10%) as a glass: $^1$H NMR (pyridine-d$_5$) $\delta$ 11.76 (s, 2H), 7.70 (d, 2H, $J$ = 8 Hz), 7.42 (d, 2H, $J$ = 8 Hz), 7.3-7.0 (m, solvent obscured), 3.10 (m, 2H, H-2$_{ax}$), 3.01 (dd, 2H, $J$ = 11.6, 10.6 Hz, H-3$_{eq}$) 3.01 (m, 4H, H$_{p}$), 2.50 (dt, 2H, $J$ = 11.6, 10.6 Hz, H-3$_{ax}$).

1,4-bis(tert-butoxycarbonyl)-2,5-piperazinedione (76): Glycine anhydride (11.9 g, 104 mmole) in DMF (75 mL) was sonicated in a septum sealed 500 mL round bottom flask equipped with a stir bar for 3 days. After the sonication treatment the insoluble glycine anhydride was pulverized to a fine powder which could be suspended in the DMF on gentle swirling. The flask was then placed in a heating mantle, equipped with a condenser and flushed with dry nitrogen. DMAP (400 mg), triethylamine (16 mL) and di-tert-butyl dicarbonate (50 g, 229 mmole) were added at once. Gentle heating (50 °C) and vigorous stirring initiated the reaction (evolution of gas) and when the solids had gone into solution, the mixture was cooled to room temperature and stirring continued for 2 hr. After evaporation of the TEA under reduced pressure, phosphate buffer (pH 2, 50 mL of a 1M solution) was added, and the mixture further diluted with water (300 mL). The light orange precipitate was then vacuum filtered in a large Buchner funnel and rinsed with portions of water (3 x 100 mL) and then hexanes (3 x 100 mL), air dried and placed on high vacuum overnight. Recrystallization from hot toluene (100 mL) at room temperature to -20 °C gave 76 (18 g, 50%) as a crystalline solid: IR (thin film) 1789,
1737, 1369, 1301, 1145 cm\(^{-1}\); \(^1\)H NMR (CDCl\(_3\), 200 MHz) \(\delta\) 4.41 (s, 2H), 1.51 (s, 9H); \(^{13}\)C NMR (50 MHz, CDCl\(_3\)) \(\delta\) 164.4 (s), 149.4 (s), 84.9 (s), 49.4 (t), 27.8 (q); HRMS (DCI, NH\(_3\)): observed \(m/z = 332.1822\) (MNH\(_4\))^+; C\(_{14}\)H\(_{26}\)N\(_3\)O\(_6\) requires \(m/z = 332.1809\).

Cis-3,6-dibromo-1,4-bis(tert-butoxycarbonyl)-2,5-piperazinedione (77): 76
(12.56 g, 40 mmole), NBS (15.66 g, 88 mmole, 2.2 eq.) and a catalytic amount of AIBN (66 mg) were combined in CCl\(_4\) (400 mL) and brought to reflux under dry nitrogen for 9 hours. After cooling, the reaction was filtered to remove succinamide, then evaporated under reduced pressure. The viscous liquid was then recrystallized from toluene-hexanes (1:1) 100 mL by first dissolving the residue in hot toluene, and then adding the warm hexanes. The crystals were allowed to grow slowly at RT to -20 °C. After vacuum filtration and a hexane rinse of the crystals, the product was first air dried then further dried under high vacuum overnight to give 77 (15 g, 80%) as a crystalline solid: IR (thin film) 1790, 1732, 1372, 1240, 1142 cm\(^{-1}\); \(^1\)H NMR (acetone-\(d_6\)) \(\delta\) 6.84 (s, 1H), 1.57 (s, 9H); \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 159.2 (s), 147.5 (s), 87.5 (s), 52.8 (d), 27.7 (q); HRMS (DCI, NH\(_3\)): observed \(m/z = 488.0032\) (MNH\(_4\))^+; C\(_{14}\)H\(_{24}\)N\(_3\)O\(_6\)Br\(_2\) requires \(m/z = 488.0034\).

Cis-2,5-bis(6'-bromo-3'-indoly)piperazine (78) and trans-2,5-bis(6'-bromo-3'-indoly)piperazine (1): 6-bromoindole (5.88 g, 30 mmole) was combined with 77 (7.08 g, 15 mmole) in a 50 mL round bottom flask. The flask was equipped with a condenser and flushed with dry nitrogen, and held under nitrogen while dry DMF (15 mL) was transferred into the flask. With magnetic stirring, the reaction was rapidly brought to 110 °C in an oil bath. As the reaction heated gas began to evolve rapidly and the mixture darkened. Heating was continued for 10 minutes following cessation of gas evolution, 20 minutes total reaction time. The reaction was divided into two centrifuge tubes and each were diluted with 40 mL D.I. water. The precipitate was centrifuged out, and
resuspended in fresh water 3 times. After the last water wash, the pellets were resuspended in methanol (50 mL) and again centrifuged. Purification in this manner gave pure cis-diketopiperazine 26 after drying overnight on high vacuum: UV (MeOH) 285, 277, 268 nm; IR (thin film) 3251, 1731, 1612, 1440, 1198 cm\(^{-1}\); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 11.26 (br s, 2H), 8.58 (br s, 2H), 7.57 (d, 2H, \(J = 8.5\) Hz), 7.38 (m, 2H), 7.16 (d, 2H, \(J = 8.5\) Hz), 5.30 (s, 2H); \(^13\)C NMR (DMSO-\(d_6\)) \(\delta\) 167.3 (s), 137.7 (s), 125.6 (d), 124.8 (s), 121.9 (d), 121.3 (d), 114.4 (d), 114.3 (s), 112.6 (s) 52.7 (d); HRFABMS: observed \(m/z = 502.9537\) (MH\(^+\), C\(_{20}\)H\(_{15}\)N\(_4\)O\(_2\)\(^{79}\)Br\(^{81}\)Br requires \(m/z = 502.9541\). Without purification the above solid 26 was dissolved in N,N-dimethylacetamide (30 mL) and refluxed overnight. The \(^1\)H NMR analysis indicated isomerization had taken place to give a 26:25 ratio of 2:1. Also produced as a decomposition product was 6-bromoindole. The DMA solution was poured onto water (100 mL) and centrifuged to remove the precipitate. The precipitate was resuspended and washed with fresh water three times, and finally with methanol (100 mL) to give (3.4 g, 45%) of the cis\(\text{cis}/\)trans-diketopiperazine mixture 26 and 25. The \(^1\)H NMR data was also obtained for the trans-diketopiperazine 25: \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) 11.12 (br s, 2H), 8.58 (br s, 2H), 7.66 (d, 2H, \(J = 8.5\) Hz), 7.59 (s, 2H), 7.11 (d, 2H, \(J = 8.5\) Hz), 6.91 (br s, 2H), 5.30 (s, 2H). After drying under high vacuum, the cis\(\text{cis}/\)trans- mixture 26 and 25 (1.7 g, 3.38 mmole) was placed in a 100 mL round bottom flask and flushed with dry nitrogen. Borane-THF (50 mL of 1M solution in THF) was added with stirring via cannula. After 3 days of stirring at room temperature the reaction was quenched with HBr in acetic acid (8 mL of a 20 M solution) whereupon the piperazines precipitated as the dihydrobromide salts. The suspension was transferred to two 50 mL centrifuge tubes and the precipitate was settled out of solution by centrifugation at 2000 rpm. The resulting pellet was sequentially washed with 50 mL portions of chloroform, ethyl acetate and THF. After drying overnight on high vacuum, the solid (1.0 g, 44%) was partitioned between ethyl acetate (30 mL) and aqueous
sodium carbonate (30 mL of a 1M solution). The undissolved solid was centrifuged out of the mixture, and the ethyl acetate layer separated. The solid consisted of nearly pure cis-piperazine 78 (126 mg, 8%). The ethyl acetate fraction was applied to a short column (silica, gradient of 5-20% methanol-chloroform) and those fraction containing the trans-piperazine 1 were combined to give (60 mg, 4%).

**Trans-bis(6'-bromo-3'-indoly)piperazine (1), glass:** $^1$H NMR (acetone-$d_6$) $\delta$

10.24 (br s, 2H), 7.67 (d, 2H, $J$ = 8.5 Hz), 7.57 (d, 2H, $J$ = 1.8 Hz), 7.56 (d, 2H, $J$ = 1.6 Hz), 7.09 (dd, 2H, $J$ = 8.5, 1.7 Hz), 4.30 (dd, 2H, $J$ = 9.3, 4.0 Hz), 3.27 (dd, 2H, $J$ = 12, 9.3 Hz), 3.14 (dd, 2H, $J$ = 12, 4.0 Hz). **Compound 1•2HBr, amorphous solid:** $^1$H NMR (DMSO-$d_6$) $\delta$ 11.73 (br s, 2H), 9.95 (br s, 2H), 9.40 (brs, 2H), 7.92 (s, 2H), 7.78 (d, 2H, $J$ = 8.5 Hz) 7.66 (s, 2H), 7.28 (d, 2H, $J$ = 8.5 Hz), 5.34 (m, 2H), 3.80 (m, 2H), 3.73 (m, 2H);

**Cis-bis(6'-bromo-3'-indoly)piperazine (78), amorphous solid:** IR (thin film) 3416, 1650, 1613 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.08 (br s, 2H), 7.68 (d, 2H, $J$ = 8.5 Hz), 7.52 (s, 2H), 7.30 (d, 2H, $J$ = 1.3 Hz), 7.10 (dd, 2H, $J$ = 8.5, 1.3 Hz), 4.08 (dd, 2H, $J$ = 8.3, 1 Hz), 3.15 (dd, 2H, $J$ = 11.6, 1 Hz), 2.88 (dd, 2H, $J$ = 11.6, 8.3 Hz). **Compound 78•2HBr, amorphous solid:** UV (MeOH) 284 ($\varepsilon$ 8 x 10$^3$), 276 ($\varepsilon$ 8 x 10$^3$), 268 ($\varepsilon$ 7 x 10$^3$) nm; IR (thin film) 3416, 1650, 1613 cm$^{-1}$; $^{13}$C NMR (DMSO-$d_6$) $\delta$ 137.0 (s), 127.1 (d), 124.8 (s), 122.9 (d), 120.7 (d), 115.2 (s), 115.0 (d), 106.8 (s), 47.9 (d), 45.8 (t); HRFABMS: Observed $m/z$ = 474.9956 (MH)$^+$, C$_{20}$H$_{19}$N$_4$Br$^{79}$Br$^{81}$Br requires $m/z$ = 474.9956.

**Trans-bis(5'-bromo-3'-indoly)piperazine (79) and Cis-bis(5'-bromo-3'-indolyl)piperazine (80):** In an exactly analogous manner to the preparation of 1 and 78, the 5-bromo analogs were prepared using 5-bromoindole.

**Trans-bis(5'-bromo-3'-indoly)piperazine (79), glass:** UV(MeOH) 280 ($\varepsilon$ 5 x 10$^3$), 271 ($\varepsilon$ 6 x 10$^3$), 260 ($\varepsilon$ 6 x 10$^3$) nm; IR (thin film) 3250, 1648, 1459 cm$^{-1}$; $^1$H
NMR (Acetonitrile-\(d_6\)) \(\delta\) 9.51 (br s, 2H), 7.87 (d, 2H, \(J = 1.5\) Hz), 7.87 (br s, 2H) 7.33 (d, 2H, \(J = 8.6\) Hz), 7.19 (dd, 2H, \(J = 8.6, 1.9\) Hz), 4.39 (t, 2H, \(J = 4.8\) Hz), 3.18 (d, 4H, \(J = 4.8\) Hz). HRFABMS: observed \(m/z = 474.9948\) (MH\(^+\)). \(\text{C}_{20}\text{H}_{19}\text{N}_{4}\text{Br}^{81}\text{Br}\) requires \(m/z = 474.9956\). **Compound 79•2HBr, amorphous solid:** \(\text{\textit{\textsuperscript{1}H NMR (DMSO-\(d_6\)) \(\delta\) 11.67 (br, 2H), 9.82 (br, 2H), 7.77 (br, 2H), 7.71 (d, 2H, \(J = 7.2\) Hz), 7.31 (d, 2H, \(J = 8.5\) Hz), 5.45 (br m, 2H), 3.94 (br m, 2H), 3.79 (br m, 2H).}

**\text{\textit{C\textsuperscript{\textit{i\textit{s}}}\text{-bis(5\textsuperscript{\textit{-bromo-3\textsuperscript{-indoly})piperazine (80), amorphous solid:**** IR (thin film) 3296, 1713, 1632, 1446, 1295 cm\(^{-1}\) \(\text{\textsuperscript{1}H NMR (DMSO-\(d_6\)) \(\delta\) 11.27 (br s, 2H), 7.96 (s, 2H), 7.40 (s, 2H), 7.35 (d, 2H, \(J = 8.7\) Hz), 7.18 (d, 2H, \(J = 8.7\) Hz), 4.23(d, 2H, \(J = 8\) Hz), 3.20 (d, 2H, \(J = 15\) Hz), 3.04 (dd, 2H, \(J = 15, 8\) Hz). **Compound 80•2HBr, amorphous solid:** UV (MeOH) 287 (\(\epsilon\) 7 x 10\(^3\)), 287 (\(\epsilon\) 9 x 10\(^3\)), 270 (\(\epsilon\) 9 x 10\(^3\)) nm; \(\text{\textit{\textsuperscript{1}H NMR (DMSO-\(d_6\)) \(\delta\) 11.80 (d, 2H, \(J = 1.9\) Hz), 9.82 (br m, 4H), 8.04 (d, 2H, \(J = 1.2\) Hz), 7.83 (d, 2H, \(J = 2.5\) Hz), 7.47 (d, 2H, \(J = 8.7\) Hz), 7.32 (dd, 2H, \(J = 8.7, 1.6\) Hz), 5.23 (br dd, 2H, \(J = 12.4, \sim 1\) Hz), 4.04 (br dd, 2H, \(J = 16.5, \sim 1\) Hz); \(\text{\textsuperscript{13}C NMR (DMSO-\(d_6\)) \(\delta\) 134.9 (s), 127.8 (d), 127.6 (s), 125.1(d), 121.3 (d), 114.5 (d), 112.7 (s), 106.2 (s), 47.9 (d), 45.6 (t); HRFABMS: observed \(m/z = 474.9956\) (MH\(^+\)), \(\text{\textit{\textsuperscript{C}_{20}\text{H}_{19}\text{N}_{4}\text{Br}^{81}\text{Br}\) requires \(m/z = 474.9956.}}\)

3,6-bis(5'-bromo-3'-indoly)-1,4-bis(4-methoxybenzyl)-2,5-piperazinedione (73): \(72\) (1.02 g, 2 mmole) was prepared according to the procedure of Armstrong et al.\(^{38,39}\) and combined with 5-bromoindole (823 mg, 2.2 eq) in DMF (5 mL). The solution was allowed to stand overnight at room temperature. The reaction mixture was partitioned between ethyl acetate (30 mL) and water (50 mL). The organic layer was separated and back-extracted with water (2 x 50 mL) and saturated brine (50 mL), then dried with sodium sulfate. After evaporation of solvent, the residue was chromatographed [silica, hexanes-ethyl acetate (1:1)] and appropriate fractions were.
combined to give 73 (296 mg, 20%) as a glass: $^1$H NMR (CDCl$_3$) $\delta$ 10.22 (br s, 2H), 7.48 (d, 2H, $J = 1.7$ Hz), 7.36 (d, 4H, $J = 8.6$ Hz), 7.28(d, 2H, $J = 8.7$ Hz), 7.16 (dd, 2H, $J = 8.6, 1.6$ Hz), 6.86 (m, 10H), 5.86 (s, 2H).

**Bis(5'-bromo-3'-indoly)-(4-methoxybenzyl)carbinol (74):** 73 (148 mg, 0.2 mmole) was dissolved in dichloromethane (5 mL) and stirred with a solution of Cerium (IV) ammonium nitrate (548 mg, 1 mmole) in a two-phase mixture in water (10 mL). Immediately, a brick-red precipitate appeared. The precipitate was collected on a glass frit, then washed with water and a small portion of dichloromethane, to give 74 (47 mg, 47%) as a red crystalline solid: UV (MeOH) 464 ($\epsilon$ 1 x $10^4$), 430 ($\epsilon$ 1 x $10^4$), 283 ($\epsilon$ 1 x $10^4$), 210 ($\epsilon$ 5 x $10^4$) nm; IR (thin film) 3104, 1598, 1504, 1435, 1202 cm$^{-1}$; $^1$H NMR [DMSO-$d_6$/acetone-$d_6$ (1:1)] $\delta$ 14.0 (br s, 2H, H-1), 8.56 (s, 2H, H-2), 7.72 (d, 2H, $J = 8$ Hz, H-7), 7.70 (d, 2H, $J = 8$ Hz, H-3'), 7.52 (dd, 2H, $J = 8$, 1.6 Hz, H-6), 7.29 (d, 2H, $J = 8$ Hz, H-4'), 6.98 (d, 2H, $J = 1.6$ Hz, H-4), 4.00 (s, 3H, H-6'); $^{13}$C NMR [DMSO-$d_6$/acetone-$d_6$ (1:1)] $\delta$ 169.4 (s, C-1'), 165.5 (s, C-5'), 147.0 (d, C-2), 138.8 (s, C-2'), 137.1 (br d, C-7), 130.2 (br s, C-3), 128.9 (s), 128.7 (d, C-6), 123.9 (d, C-4), 120.6 (s, C-9), 117.3 (s, C-5), 116.8 (d, C-3'), 115.6 (d, C-4'), 56.4 (q, C-6'). HRMS (Cl, NH3): observed m/z = 506.9723(M)$^+$, C$_{24}$H$_{17}$N$_2$OBr$_2$ requires 506.9708.

**Bis(5'-bromo-3'-indoly)-(4-methoxybenzyl)methane (75):** 74 (10 mg, 0.02 mmole) was dissolved in a mixture of DCM (1 mL) and methanol (1 mL). Sodium borohydride (22 mg) was added and the mixture was stirred for 1 hr. The pale yellow-green solution was quenched with HCL (1 M) and partitioned into ethyl acetate (10 mL) and water (10 mL). The organic phase was separated and dried with sodium sulfate, evaporated and chromatographed (silica, dichloromethane) to give 75 (3 mg, 30%) as a glass: UV (MeOH) 380 ($\epsilon$ 2 x $10^3$), 272 ($\epsilon$ 1 x $10^4$) nm; IR (thin film) 3250, 3427, 1508, 1457, 1243 cm$^{-1}$; $^1$H NMR (acetone-$d_6$) $\delta$ 10.2 (br s, 2H), 7.49 (d, 2H, $J = 1.9$ Hz), 7.36 (d, 2H, $J = 8.6$ Hz), 7.28 (d, 2H, $J = 8.6$ Hz), 7.16 (dd, 2H, $J = 8.6, 1.9$ Hz), 6.85 (m, 2H),
6.84 (m, 2H), 5.86 (s, 2H), 3.75 (s, 3H); HRMS (CI, NH3): observed m/z = 508.9866
(MH)+, C24H19N2OBr2 requires m/z = 508.9864.

6-Bromoindole (13): 4-Bromo-2-nitrotoluene (5.40 g, 25 mmole), N,N-
dimethylformamide dimethylacetal (4.00 mL, 1.2 eq.), pyrrolidine (2.50 mL, 1.2 eq.) and
dry DMF (25 mL) were combined under dry nitrogen. The mixture was heated to 110 °C
in an oil bath for 3 hr. After evaporation of the solvent under reduced pressure, the red
liquid was placed on high vacuum overnight to remove DMF. The red oil was
resuspended in THF-methanol (1:1) 50 mL, and Raney nickel (6.6 mL) was added under
nitrogen, with vigorous mechanical stirring. Hydrazine hydrate (2 mL) was added
dropwise at such a rate as to maintain a gentle reflux. The red color of the enamine was
discharged after addition of hydrazine was complete and the mixture was allowed to stir
for an additional 30 minutes. The reaction mixture was filtered through celite (Caution,
do not allow celite/Raney nickel to become exposed to atmospheric oxygen. The reduced
Raney nickel is very pyrophoric!) and rinsed with methanol-DCM (1:9).
Chromatography [silica, EtOAc-hexanes (1:3)] was performed and those fractions having
UV activity at Rf = 0.5 were combined. The combined fractions were evaporated under
reduced pressure, then recrystallized from toluene-hexanes (2:1) to yield 6-bromoindole
(3.41 g, 70%) in two crops.
References and Notes


(10) Compound 16 was formed by reaction of 6-bromotryptamine-3-carboxaldehyde with Trimethylsilyl cyanide (TMSCN) and methylamine in methanol. Purification consisted of evaporation of excess solvent. No other purification was possible due to the unstable nature of this amino nitrile. The Merck group's experience with these amino nitriles is similar to our own in terms of the hydrolytic instability of compounds like 16.


(24) Aldrich #S40616-3


(40) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. Trimethylsilyl Iodide as a Peptide Deblocking Agent. *Journal of the Chemical Society, Chemical Communications* 1979, 495.

Chapter 2
Isolation and Structure Elucidation of the Major Anti-Microbial Metabolite of the Marine Sponge *Haliclona* Sp.

*Introduction*

In 1992, I collected a sponge of the genus *Haliclona* in the La Jolla kelp beds near Marine Street using SCUBA (~20 m). The crude extract of the sponge was active against Gram-negative and Gram-positive bacteria, as well as *Candida albicans* and had moderate cytotoxic activity. Because of our interest in cytotoxic and anti-microbial compounds, the sponge was deemed suitable for further study. The goal of the research was to isolate the compound(s) responsible for the bioactivity observed in the crude extract, and determine the structure(s). Following structure determination, bioactivity testing in as many appropriate assays as possible was planned.

*Isolation and Structure Elucidation*

Isolation of 81 was begun by extraction of the frozen sponge with methanol. The extract was then concentrated to an aqueous slurry and extracted with ethyl acetate. Following chromatography on LH-20, those fractions which displayed activity against *E. coli* were recombined and chromatographed on C-18 reversed phase in acetonitrile-water-TFA (50:50:0.05) to give the natural product 81, (4%, wet weight) shown in figure 30.

High resolution mass spectral analysis (Cl, NH₃) of the natural product gave the molecular formula C₁₈H₃₈NO₃. Due to extreme overlap of signals in the ¹H NMR spectrum of 81,
Figure 30. Synthesis of the Acetonide 83. The product is a mixture of epimers at C-12, which are separable on silica gel.
it was decided to prepare the peracetylated derivative 82 for further NMR analyses. The 
$^{1}$H NMR of the peracetylated derivative showed the presence of an allylic alcohol 
moiety, accounting for the one unsaturation required by the molecular formula of the 
natural product. The alkene protons displayed a 11 Hz coupling, consistent with a cis-
double bond. Also observed were four methyl singlets at $\delta$ 1.73, 1.68, 1.69, and 1.51.
$^{13}$C NMR of 82 displayed four carbonyl signals around 170 ppm, as well as signals at $\delta$
72.4(d), 70.1(d), 63.3(t), and 50.1(d). Combined, these data reveal the presence of three 
hydroxyls and a primary amine.

COSY analysis of 82 (table 1) suggested that the molecule was a linear $C_{18}$
compound with one end group consisting of an ethyl moiety, and the other end group a
moiety that contained hydroxyls at C-1 and C-3, with an amine at C-2 and a methylene at 
C-4. The allylic alcohol moiety was positioned somewhere between the two endgroups,
in a methylene envelope which was unresolved. Thus, one of the major problems was 
determination of the location, position and orientation of the allylic alcohol moiety along 
the $C_{18}$ chain.

Analysis of the 20 eV EIMS fragmentation of 81 revealed a peak at $m/z = 230$,
which is interpreted as loss of the terminal hexyl group by fragmentation of the C-12/C-
13 bond. Although this may seem unlikely, the allylic alcohol moiety has been observed
to be easily rearranged, as in the case of 83. It is likely that similar processes take place 
in the mass spectrometer. With the similar fragmentation produced in the MS of 83, it 
seems most likely that the placement of the allylic hydroxyl is at C-10

Attempts to cleave the double bond of 81 in order to better define its position 
were attempted. Potassium periodate-osmium tetraoxide (cat.) in dioxane-water at room 
temperature failed to yield any isolable amount of oxidation products. Similar negative 
results were obtained using potassium permanganate as the stoichiometric oxidant. The 
periodate-osmium tetraoxide oxidation was also conducted at elevated temperature, and
Table 1. Partial $^1$H NMR (C$_6$D$_6$, 500MHz) Assignments and COSY Correlations of 82.

<table>
<thead>
<tr>
<th>H</th>
<th>$\delta$ $^1$H (ppm)</th>
<th>COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>4.04 (dd, $J = 11.0$, 7.0, 1H)</td>
<td>4.59, 3.93</td>
</tr>
<tr>
<td>1b</td>
<td>3.93 (dd, $J = 11.0$, 7.0, 1H)</td>
<td>4.59, 4.04</td>
</tr>
<tr>
<td>2</td>
<td>4.59 (m, 1H)</td>
<td>5.11, 4.04, 3.93</td>
</tr>
<tr>
<td>3</td>
<td>5.14 (m, 1H)</td>
<td>4.59, 1.49, 1.38,</td>
</tr>
<tr>
<td>10</td>
<td>5.41 (dd, $J = 11.0$, 9.5, 1H)</td>
<td>5.84, 5.51 (weak)</td>
</tr>
<tr>
<td>11</td>
<td>5.84 (dt, $J = 9.0$, 6.5, 1H)</td>
<td>5.41, 1.67, 1.43</td>
</tr>
<tr>
<td>12</td>
<td>5.51 (dt, $J = 11.0$, 7.5, 1H)</td>
<td>5.41 (weak), 2.29, 2.18</td>
</tr>
<tr>
<td>13a</td>
<td>2.29 (1H, m)</td>
<td>5.51, 2.18, 1.30</td>
</tr>
<tr>
<td>13b</td>
<td>2.18 (1H, m)</td>
<td>5.51, 2.29, 1.30</td>
</tr>
<tr>
<td>NHAc</td>
<td>5.11 (br d, $J = 10.0$, 1H)</td>
<td>4.59</td>
</tr>
<tr>
<td>Ac</td>
<td>1.73 (3H, s)</td>
<td></td>
</tr>
<tr>
<td>Ac</td>
<td>1.69 (3H, s)</td>
<td></td>
</tr>
<tr>
<td>Ac</td>
<td>1.69 (3H, s)</td>
<td></td>
</tr>
<tr>
<td>Ac</td>
<td>1.51 (3H, s)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5-1.1 (18H, multiple unresolved abs.)</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.87 (3H, t, $J = 7.5$)</td>
<td>1.22</td>
</tr>
</tbody>
</table>
reaction did occur. Unfortunately, GC-MS analysis of the reaction products did not give any interpretable information as to the position of the double bond. Selective oxidation of the allylic alcohol in an attempt to produce and unsaturated ketone (with the hope that this derivative would provide better MS fragmentation data) with MnO₂ also met with failure. Oxidation with purple benzene [KMNO₄/cat. (n-Bu)₄NCl/benzene] did not produce any isolable oxidation products. Iodine catalyzed addition of dimethyl disulfide to the double bond did not show any interpretable fragments in the LRMS.

Treatment of the peracetylated compound 82 with sodium methoxide in methanol provided the N-acetyl derivative. Further treatment of this compound with dimethoxy propane in refluxing benzene with a catalytic amount of p-toluenesulfonic acid provided compound 83, as a mixture of epimers about the allylic carbinol center.

Apparently, the allylic alcohol underwent exchange with methanol as well as isomerization of the double bond to the more favorable trans-configuration during formation of the ketal. The ¹H NMR confirmed the presence of a methoxyl group, and an increase in the vicinal coupling of the two alkene protons coupling from 11 Hz (82, cis-double bond) to 15 Hz, which is consistent the trans-configuration of the alkene in 83. The molecular ion of 83 was observed at m/z = 411, with another fragment at m/z = 326. The m/z = 326 peak is interpreted as loss of the terminal hexyl group of 83, as with 81.

The relative configurations of C-2 and C-3 were determined by analysis of the proton-proton coupling constants of 83 obtained by decoupling experiments, assuming a chair configuration. The NH proton of 83 was coupled to H-2 with J = 9.0 Hz. H-2 was in turn coupled to H-1ax and H-1eq with J = 2 Hz. JH₂/H₂, H₁/H₁ was too small to be determined. The magnitude of these couplings indicate that only axial-equatorial and equatorial-equatorial couplings are present. Based on these couplings, 83 must have the structure as shown, leading to the relative configurations of C-2 and C-3.
With the gross structure of the \textbf{81} defined, our attention turned to the
determination of the absolute configurations of C-2, C-3 and C-10. Because of our
interest in determinations of absolute configuration by chemical and spectroscopic
methods, we did not pursue X-ray crystallography as a method to define the structure and
absolute configuration of \textbf{81}. The large amounts of \textbf{81} that were available also played a
role in our decision to pursue the chemical/spectroscopic means of determination of
absolute stereochemistry. Because the molecule is acyclic and has a secondary chiral
allylic alcohol, application of the Sharpless-Nakanishi exiton chirality method to C-10
was possible.\textsuperscript{43} A more general approach, the modified Mosher method has also been
shown to reliably predict the absolute configurations of acyclic secondary alcohols.\textsuperscript{44,45}
We selected the modified Mosher method based on the more general applicability in that
both the absolute configurations of C-3 and C-10 could be obtained using this method.
To use either method, selective protections of C-1 and C-2 and C-10 (as well as C-3, if
the exiton chirality method were applied to C-10) would be required. Because each
individual molecule requires specialized treatment, the reader is advised to consult the
literature on determination of absolute configuration for further information.

In order to employ the modified Mosher method to determine the absolute
configuration of the chiral centers, a selective protection was employed to prepare the
hydroxyls at C-3 and C-10 for derivitization. \textit{D-erythro}-sphingosine (\textbf{84}) was employed
as the model compound. Treatment of sphingosine with excess carbonyl diimidazole
(DCI) followed by hydrolysis with aqueous base provided the primary alcohol \textbf{85} and the
secondary alcohol \textbf{86} (fig. 31). Compound \textbf{86} was treated with \textit{R} and \textit{S}-MTPA (2-
methoxy-2-trifluoromethyl-2-phenylacetic acid) using standard coupling with DCC to
form the diastereomeric esters \textbf{86R} and \textbf{86S}. Application of the modified Mosher method
to this case correctly predicted the absolute configuration at C-3 as \textit{R} (table 2). This
method assumes a model conformation of the ester, in which the CF\textsubscript{3}, carbonyl and the
**D-erythro-sphingosine (84)**

1. Carbonyldiimidazole/DCM
2. MeOH/H$_2$O/NaOH 2hr

**85**

Ca. 1:1 mixture

**86**

*R or S-MTPA acid/DCC/DMAP/EtOAc*

**86R**  *R*-MTPA

**86S**  *S*-MTPA

Figure 31. Synthesis of 86R and 86S.
Table 2. Partial $^1$H NMR (CDCl$_3$, 500 MHz) Assignments, Chemical Shifts (ppm), and Shift Differences for the MTPA Derivatives (86$R$, 86$S$), (88$R$, 88$S$), and (90$R$, 90$S$).

<table>
<thead>
<tr>
<th>H</th>
<th>$R$-MTPA</th>
<th>$S$-MTPA</th>
<th>($R$-$S$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>la</td>
<td>4.41</td>
<td>4.34</td>
<td>0.07</td>
</tr>
<tr>
<td>1b</td>
<td>4.15</td>
<td>4.05</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>4.05</td>
<td>3.99</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>5.39</td>
<td>5.41</td>
<td>-0.02</td>
</tr>
<tr>
<td>4</td>
<td>5.25</td>
<td>5.39</td>
<td>-0.14</td>
</tr>
<tr>
<td>5</td>
<td>5.91</td>
<td>6.03</td>
<td>-0.12</td>
</tr>
<tr>
<td>6</td>
<td>2.02</td>
<td>2.08</td>
<td>-0.06</td>
</tr>
<tr>
<td>NH</td>
<td>4.99</td>
<td>4.88</td>
<td>0.11</td>
</tr>
</tbody>
</table>

| la | 4.40     | 4.33     | 0.07      |
| 1b | 4.12     | 4.04     | 0.08      |
| 2  | 3.99     | 3.94     | 0.05      |
| 3  | 5.04     | 5.04     | 0.00      |
| 4  | ?        |          |           |

| la | 4.34     | 4.45     | -0.11     |
| 1b | 4.22     | 4.13     | 0.09      |
| 2  | 3.677    | 3.684    | -0.007    |
| 3  | 4.208    | 4.187    | 0.022     |
| 4a | 1.66     | 1.68     | -0.02     |
| 4b | 1.54     | 1.57     | -0.03     |
| 9a | 1.64     | 1.74     | -0.10     |
| 9b | 1.47     | 1.55     | -0.08     |
| 10 | 5.76     | 5.72     | 0.04      |
| 11 | 5.34     | 5.21     | 0.13      |
| 12 | 5.60     | 5.57     | 0.03      |
| 13 | 2.176    | 2.162    | 0.014     |
| 14 | 1.35     | 1.33     | 0.02      |
hydrogen attached to the chiral center are in a plane (fig. 31-33). In order to determine
the absolute configuration, chemical shift assignments (as many as possible) on both
sides of the chiral center are made for both the R-ester and the S-ester. The two data sets
are then subtracted, in this case the R-ester shifts minus the corresponding S-ester shifts.
This gives a set of differences which reflect shielding by the phenyl group of the R-ester
relative to the S-ester. Relative shielding of the R-ester shifts is then indicated by
negative differences, while deshielding is indicated by positive differences. The R-ester
is then drawn, giving the carbinol center an arbitrary absolute configuration (R or S)
while adhering to the atom positions in the model. If the choice is correct, those protons
on the side of the chiral center having the phenyl group will have negative (R-S) chemical
shift differences. If not, the correct absolute configuration is opposite that first assumed.

Now having a "basis set", we first synthesized the oxazalindones of \textbf{81} in an
analogous way as those of sphingosine (fig. 32). Compound \textbf{81} was treated with CDI,
then subjected to a brief hydrolysis. This produced \textbf{87} and \textbf{88}. To determine absolute
configuration of C-3, \textbf{88} was then derivatized to the R-and S-MTPA esters \textbf{88R} and \textbf{88S}
respectively. Using the method previously described for sphingosine, C-3 was assigned
absolute configuration of R (Table 2). Treatment of \textbf{81} with CDI and extending the
hydrolysis time produced, \textbf{89} and \textbf{90}. Compound \textbf{90} was derivatized to produce \textbf{90R} and
\textbf{90S} (fig. 33). The shift differences (table 2) indicated that the absolute configuration of
C-10 was also R.

The relative (and thus the absolute) configuration of C-2 was confirmed by nOe
difference spectroscopy on \textbf{90R} (Fig. 33). Irradiation of H-3 gave a four percent
enhancement of H-1a, establishing that both protons resided on the same side of the five
membered oxazalindone ring. The relative stereochemistry of C-2 and C-3 was \textit{threeo} -, unlike that of natural sphingosine. C-2 was assigned absolute configuration of R.
Figure 32. Synthesis of $88^R$ and $88^S$. 
Figure 33. Synthesis of the DiMTPA Esters 90\textit{R} and 90\textit{S}. NOe results (90\textit{R}): Irradiate H-2, enhancements in NH (4.3%), H-1\textit{a}, H-1\textit{b} (1.5, 2.4%), H-2 (1.7%). Irradiate H-3, enhancements in H-4\textit{a}, H-4\textit{b} (1.5, 2.0%), H-1\textit{a} (3.9%), H-2 (1.5%).
Conclusions and Bioassay Results

The structure assignment given for 81 is well supported by the spectroscopic data. In addition, formation of derivatives allowed assignment of the absolute sterochemistry of C-2 (R), C-3 (R) and C-10 (R) using the modified Mosher method. The data on the position of the double bond along the C18 chain are primarily based on mass spectral fragmentation. More work needs to be done on this aspect of the structure determination before we can be fully confident of the double bond position and orientation.

Of note is the striking similarity of 81 to sphingosine, a naturally occurring lipid in mammalian cells. It is interesting to note that sphingosine has no anti-microbial properties, unlike the closely related molecule 81. Sphingosine and related compounds have attracted great interest because of their inhibition of the Protein Kinase C (PKC) enzyme system. PKC is involved in inflammatory processes as well as the regulation of cellular growth, so sphingosine type compounds are potentially useful in the study of normal cellular function, as well as the treatment of cancer and inflammation. Although 81 was found to be cytotoxic, it did not have sufficient activity for further consideration in this area. Compound 81 was tested for anti-inflammatory properties by Professor Robert Jacobs of University of California-Santa Barbara, and found to inhibit phorbol-induced inflammation at a level of 43% in the Mouse Ear Edema Assay at a dosage level of 50 µg. Given that other compounds like 81 have been shown to inhibit diacylglycerol binding to PKC, mechanism based assays using this enzyme would be a logical place to start if one wished to further explore the activity of 81.
**Experimental**

**Extraction and isolation.** The frozen sponge (200 g) was extracted with methanol (1.5 L) overnight. The extract was evaporated under reduced pressure to an aqueous slurry (100 mL) and extracted with ethyl acetate (3 x 50 mL). The organic extracts were combined and dried over sodium sulfate, then evaporated under reduced pressure. The extracts were shown to inhibit the growth of *Escherichia coli* (*E. coli*). The aqueous portion showed no anti-microbial activity. Subsequent fractionations were monitored using *E. coli* as the test organism.

A portion of the organic extract (0.8 g) was applied to an LH-20 gel filtration column (2.5 cm x 80 cm) and fractionated in dichloromethane-methanol (1:1). Those fractions displaying activity against *E. coli* were combined and evaporated to give 0.5 g of an oil. An aliquot of this oil (150 mg) was chromatographed by HPLC [C-18, 0.9 cm x 55 cm, acetonitrile-water-trifluoroacetic acid (50:50:0.05)] to yield (2R, 3R, 10R)-(Z)-2-amino-1,3,10-trihydroxy-11-octadecene (81, 28 mg, 4 % wet wt.) as the trifluoroacetate after evaporation and lyophilization. The $^1$H NMR of this compound was virtually uninterpretable in various solvents due to the overlap of signals. It was decided at this point to work with derivatives for elucidation of the structure. Compound 81, oil: $^{13}$C NMR (methanol-$d_4$, 50 MHz) δ 134.98 (d), 133.20 (d), 70.08 (d), 69.19 (d), 61.47 (t), 60.06 (d), 37.7 (t), 35.85 (t), 33.86 (t), 31.78 (t), 31.67 (t), 31.58 (t), 31.05 (t), 29.66 (t), 27.47 (t), 27.25 (t), 24.67 (t), 15.44 (q); LRMS: $m/z = 236$; HRMS (CI, NH3): observed $m/z = 316.2850$ (MH)$^+$, C$_{18}$H$_{39}$NO$_3$ requires $m/z = 316.2852$.

**Acetylation of 81:** Compound 81 (31.5 mg, 0.1 mmole) was added to a mixture of dry pyridine (1 mL) and acetic anhydride (1 mL) with a catalytic amount of dimethylaminopyridine. The reaction was allowed to stand a ambient temperature overnight, then evaporated under high vacuum at room temperature. The glass obtained
was chromatographed by HPLC [C-8, 0.9cm x 30 cm, acetonitrile-water (4:1)] to yield the tetraacetyl compound 82 (30 mg, 62%) as a glass: $^1$H NMR (C$_6$D$_6$, 500 MHz) δ 170.15 (s), 169.70 (s), 169.56 (s), 168.96 (s), 134.25 (d), 128.86 (d), 72.40 (d), 70.08 (d), 63.26 (t), 50.10 (d), 35.26 (t), 32.05 (t), 31.54 (t), 29.90 (t), 29.48 (t), 29.28 (t), 28.30 (t), 25.37 (t, extra tall), 23.00 (q), 22.60 (q), 20.92 (q), 20.43 (q), 20.28 (q), 14.29 (q).

**N-acetyl derivative of 81**: Compound 82 (5 mg) was dissolved in methanol (1 mL). A solution of sodium methoxide in methanol (0.2M, 50 µL) was added and the solution was stirred at room temperature for 2 hr. The reaction was quenched with acetic acid (0.25 mL) and diluted with water (10 mL). This was then extracted with ethyl acetate (3 x 10 mL), the organic extracts combined, dried (sodium sulfate) and evaporated under reduced pressure. Flash chromatography [silica, EtOAc-MeOH (95:5)] gave N-acetyl-81 (2 mg) as a glass: Partial $^1$H NMR (CDCl$_3$, 200 MHz) δ 6.26 (d, 1H, $J$ = 8.2 Hz, NHAc), 2.02 (s, 3H, H-18).

**Acetonide (83)**: N-acetyl-81 (2 mg) was combined with benzene (6 mL), dimethoxypropane (2 mL), and a catalytic amount of p-toluenesulfonic acid. The mixture was refluxed for 2 hr, then neutralized with pyridine (25 ml). The resulting solution was evaporated under reduced pressure and then triturated with ethyl acetate. After evaporation, HPLC [silica, 1cm x 30 cm, EtOAc-Hexanes (7:3)] gave the two epimers (C-10) of 83 as glasses, there were no discernable differences in chemical shifts between the two epimers: $^1$H NMR (C$_6$D$_6$, 500 MHz) δ 5.82 (d, 1H, $J$ = 9.0 Hz), 5.54 (dt, 1H, $J$ = 15.0, 7.5 Hz), 5.36 (dd, 1H, $J$ = 15.0, 8.0 Hz), 3.84 (dd, 1H, $J$ = 9.0, 2.0 Hz), 3.61(dd, 1H, $J$ = 12.0, 2.00 Hz), 3.58 (m, 1H), 3.56 (dd, 1H, $J$ = 12.0, 2.0 Hz), 3.46 (dt, 1H, $J$ = 8.0, $-$1 Hz), 3.23 (s, 3H), 2.0 (m, 2H), 1.75 (m, 1H), 1.48 (s, 3H), 1.42 (s, 3H), 1.16 (s, 3H), 0.88 (t, 3H, $J$ = 8 Hz), 1.6-1.0 (multiple unresolved abs.).
Oxazalindones of Sphingosine (85,86): D-erythro-sphingosine (Sigma, 9 mg, 0.03 mmole) was dissolved in dichloromethane (1.5 mL). To this stirred solution was added carbonyldiimidazole (9.8 mg, 0.06 mmole) as a solution in dichloromethane (1 mL). After reaction for 16 hr, the solution was evaporated under reduced pressure, resuspended in methanol-1M NaOH (2:0.1, 2 mL), stirred for 2 hr at room temperature, and the solvent evaporated to give an aqueous slurry. This suspension was then acidified with phosphate buffer (1M, pH 2.0, 10 mL) and extracted with ethyl acetate (3 x 10 mL). The combined organic extracts were then dried (sodium sulfate) and evaporated. Chromatography by HPLC [silica, 1 cm x 30 cm EtOAc-hexanes (9:1)] gave two products. Compound 86 (1.7 mg, 17%) glass: \(^1\text{H NMR (CDCl}_3, 200 MHz\) \(\delta 5.99 \text{ (m, } 1\text{H}), 5.42 \text{ (m, } 1\text{H}), 5.14 \text{ (m, } 1\text{H}), 4.95 \text{ (m, } 1\text{H}), 4.48 \text{ (m, } 1\text{H}), 4.34 \text{ (m, } 1\text{H}), 2.10 \text{ (m, } 2\text{H}), 1.6-1.0 \text{ (multiple unresolved abs.), } 0.86 \text{ (m, } 3\text{H}).\) Compound 85 (0.9 mg, 9%) glass: \(^1\text{H NMR (CDCl}_3, 200 MHz\) \(\delta 5.88 \text{ (m, } 1\text{H}), 5.49 \text{ (m, } 2\text{H}), 5.06 \text{ (m, } 1\text{H}), 3.86 \text{ (m, } 1\text{H}), 3.64 \text{ (m, } 2\text{H}), 2.05 \text{ (m, } 2\text{H}), 1.6-1.0 \text{ (multiple unresolved abs.), } 0.85 \text{ (m, } 3\text{H}).\)

**MTPA esters of 86 (86R, 86S):** Compound 86 (0.9 mg, 0.003 mmole) was combined with dicyclohexylcarbodiimide (2.5 mg, 0.012 mmole, 4 eq.), R-MTPA (2.8 mg, 0.012 mmole, 4 eq.) and DMAP (0.04 mg, 0.0003 mmole, 0.1 eq) in DCM (0.5 mL) (The amounts of reagents were measured using microliter syringes from solutions of known concentration in DCM and injected directly into the 1 mL septum-capped reaction vial) and left to react overnight. After filtration to remove dicyclohexylurea, the solution was evaporated and chromatographed by HPLC [silica, 1 cm x 30 cm, EtOAc-hexanes (1:1)] to yield 86R (0.9 mg, 55%) as a glass. The S-MTPA ester was prepared in an analogous manner to give 86S (0.9 mg, 55%) as a glass. \(^1\text{H NMR (CDCl}_3, 500 MHz\) see table 2.

**Oxazalindones of 81:** Compound 81 (7 mg, 0.022 mmole) was stirred in DCM (2 mL) with carbonyldiimidazole (18 mg, 0.11 mmole, 5 eq.) at room temperature for 3
hr. The reaction was evaporated and resuspended in methanol-1M NaOH [(2:0.1), 2 mL] and stirred for 2 hr. The reaction mixture was then worked-up as for the sphingosine oxazalindones, and chromatographed by HPLC [silica 1 cm x 30 cm, EtOAc-MeOH (98:2)] to give 88 (3.5 mg, 39%) and 87 (2.5 mg, 28%).

**MTPA esters of 88 (88R, 88R):** Compound 88 (1.8 mg, 0.0045 mmole) in DCM (1 mL) was treated with DCC (3.7 mg, 0.018 mmole, 4 eq.), R-MTPA (4.2 mg, 0.018 mmole, 4 eq.) and DMAP (0.05 mg, 0.1eq) in an analogous manner as the preparation and work-up of 86R. The products were purified by HPLC [silica, 1 cm x 30 cm, EtOAc-hexanes (3:2)] to yield 88R (1.4 mg, 50%) and 88S (1.4 mg, 50%) as glasses. $^1$H NMR (CDCl$_3$, 500 MHz) see table 2.

**Compounds 89 and 90:** Compound 81 (35 mg, 0.111 mmole) and CDI (90.1 mg, 0.556 mmole, 5.0 eq.) were dissolved in DCM (5 mL) and stirred at room temperature for 2 hr. The reaction mixture was evaporated and dissolved in methanol-water-1M NaOH [(2:0.4:0.2), 10 mL] and stirred at room temperature for 2 days. The reaction was evaporated to an aqueous slurry and acidified with 1M sodium phosphate (pH 2.0, 10 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layer was dried (sodium sulfate) and evaporated. HPLC [silica 1 cm x 30 cm, EtOAc-MeOH (97:3)] yielded two compounds. Compound 89 (4.3 mg, 11%) glass: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 6.31 (br s, 1H), 5.40 (m, 2H), 4.42 (m, 1H), 4.17 (m, 1H), 3.72 (m, 1H), 3.51 (m, 1H), 3.20 (br, 1H), 2.01 (m, 2H), 1.6-1.0 (multiple unresolved abs.), 0.87 (m, 3H). Compound 90 (22.4 mg, 59%) glass: $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$ 5.74 (m, 2H), 5.34 (m, 1H), 4.40(m, 1H), 4.30 (m, 1H), 3.67 (m, 1H), 3.57 (m, 2H), 2.23 (br, 1H), 2.05 (m, 2H), 1.76 (m, 1H), 1.6-1.0 (multiple unresolved abs.), 0.87 (m, 3H).

**MTPA esters of 90 (90R, 90S):** Compound 90 (11.2 mg, 0.033 mmole), DCC (27.1 mg, 0.132 mmole, 4 eq.), R-MTPA (30.9 mg, 0.132 mmole, 4 eq.) and DMAP (0.4 mg, 0.1eq) were combined in DCM (1 mL). The reaction was left at room temperature
overnight, filtered and subjected to HPLC [silica, 1 cm x 30 cm, EtOAc-hexanes (45:55)] to yield \(90R\) (10.6 mg, 42%). The \(S\)-MTPA ester was prepared in an analogous way to give \(90S\) [10.6 mg, 42%]: \(^1\)H NMR (CDCl\(_3\), 500 MHz) see table 2.
References and Notes


Chapter 3
Isolation and Synthesis of a Glycine Derivative of
Ilimaquinone and Structure-Activity Studies of Some Derivatives

Introduction

JCC63 is a Dictyoceratid sponge that was collected in the Philippines as part of a program to find cytotoxic agents from marine organisms with novel modes of action. The cooperative effort was funded by the National Cancer Institute and was comprised of both industrial and academic researchers, in this case, Bristol Myers Squibb and Scripps Institution of Oceanography. Primary screening results of crude extracts of this sponge indicated cytotoxic activity against the HCT-116 cell line, as well as broad spectrum antimicrobial activity against both bacteria and fungi. The $^1$H NMR spectrum of the ethyl acetate soluble portion of the extract indicated the presence of ilimaquinone (92, fig. 34) a known and quite common cytotoxic sponge metabolite. Also present were other resonances of much lower intensity that those of ilimaquinone, but with similar chemical shifts and multiplicities. This suggested that the sponge contained a second, minor metabolite that was structurally similar to ilimaquinone.

Results and Discussion

Extraction of 27 g of the dried sponge with methanol gave the new compound called glycinyllilimaquinone (91, 0.055% dry wt.) that was 1000 times more active than the crude extract in the in vitro HCT-116 cytotoxicity assay. In addition, the known compounds ilimaquinone (92, 0.48%) and smenosponge (93, 0.013%) were also
glycinyllimaquinone (91) $R = \text{NHCH}_2\text{COOH} \quad X = \text{OH}$

Ilimaquinone (92) $R = \text{OMe} \quad X = \text{OH}$

sminosponging (93) $R = \text{NH}_2 \quad X = \text{OH}$
diaminoilimaquinone (94) $R = \text{NH}_2 \quad X = \text{NH}_2$

Figure 34. Derivatives of Ilimaquinone (92).
isolated (fig. 34).\textsuperscript{52,53} Glycinyllilimaquinone (91) was isolated as an amorphous red powder of molecular formula C\textsubscript{23}H\textsubscript{31}NO\textsubscript{4}. The major fragment ions in the mass spectrum were at \(m/z = 210\) and 191, which result from cleavage of the C-9/C-15 bond. The IR spectrum contains bands at 3300, 3175, 1725, 1587, and 1575 cm\(^{-1}\) due to the hydroxyl, acid, and quinone groups. The UV spectrum consisted of absorptions at 492 (\(\varepsilon = 2 \times 10^3\)), 454 (\(\varepsilon = 2 \times 10^3\)), 320 (\(\varepsilon = 1 \times 10^4\)), and 203 (\(\varepsilon = 2 \times 10^4\)) nm, which were similar, but not identical to the UV absorptions reported for the amino-quinone smenosponge (93). On addition of base, the absorption at 320 nm underwent a bathochromic shift to 334 nm, indicating the presence of a hydroxyl group on the quinone ring. The \(^1\)H NMR spectrum showed a certain similarity to that of iliamaquinone (92) in the upfield region, but lacked a methoxyl signal. In its place were signals at \(\delta 7.2\) (br t, 1H, \(J_{1/2} = 20\) Hz) and 4.09 (m, 2H) that were assigned to a glycine residue that must be linked to the quinone ring through nitrogen. The \(^{13}\)C NMR signals at \(\delta 44.1\) and 169.9 were also typical of a glycine residue. The NMR spectral data (table. 3) were completely compatible with the structure of glycinyllilimaquinone (91).

In order to perform \textit{in vivo} screening of glycinyllilimaquinone (91), it was necessary to obtain a greater quantity of the compound than was available from the natural source. Treatment of iliamaquinone (92), which is readily available from several Dictyoceratid sponges, with a slight excess of glycine in 0.01M sodium hydroxide solution gave, after purification, a 70\% yield of glycinyllilimaquinone (91) together with recovered starting material. This general method has been used to prepare both the monoamino and diamino (94) derivatives of iliamaquinone and some alkyl amino derivatives.

As iliamaquinone is a readily available starting material, I was able to synthesize the amount of glycinyllilimaquinone (91) required for the \textit{in vivo} testing. Glycinyllilimaquinone (91) inhibits the HCT-116 cell line with an IC\textsubscript{50} of 7.8 \(\mu\)g/mL and
Table 3. $^{13}\text{C}$ and Partial $^1\text{H}$ NMR Data for Smenosponge (93) and Glycinyllilimaquinone (91).

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was subsequently evaluated against the P388 murine leukemia tumor model \textit{in vivo}, but at a maximally tolerated dose showed no anti-tumor activity.\textsuperscript{54} However, additional testing of this and other derivatives of ilimaquinone proved to be useful in the study of their effects on Golgi membranes and microtubules.
Structure-Activity Studies of Some Ilimaquinone Derivatives

The work on the structure-activity studies of ilimaquinone derivatives was performed in collaboration with Dr. Vivek Malhotra of the UCSD department of Biology. Ilimaquinone is known to cause vesiculation of Golgi membranes and depolymerize microtubules in normal rat kidney cells.\(^{55}\) This occurs in a reversible manner, in that if the cells which have been exposed to the ilimaquinone are freed of it by dialysis, the Golgi reassemble. Our part in this collaboration was to provide compounds in addition to ilimaquinone which displayed selective activity in their assay. The ultimate goal of the collaboration was to use ilimaquinone derivatives as a probe to understand the Golgi vesiculation and microtubule depolymerization at the molecular level. It was hoped that through this process, a better understanding of these normal cellular processes could be discovered.

Initially we desired to create an ilimaquinone derivative that was radiolabeled, or one that could be used in an affinity column. The proposed molecular interaction site, and possibly a protein responsible for triggering the vesiculation and microtubule depolymerization could be identified using such derivatives. In the case that ilimaquinone initially binds to a protein, a radiolabel could allow the isolation and thus identification of the protein responsible for beginning the process. An affinity column, a solid support with an active covalently linked ilimaquinone analog, is another possibility. This would selectively bind the purported protein and allow it to be isolated in a purified form for analysis.

Treatment of ilimaquinone with iodine in carbon tetrachloride solution at room temperature gave a mixture of two compounds in which the terminal double bond had isomerized. Inspection of the crude reaction mixture by \(^{1}H\) NMR revealed that the terminal methylene of ilimaquinone had been replaced by two new compounds with
alkene signals at δ 5.40 and 5.15. These compounds could not be separated on silica gel or C-18 reversed phase and were first acetylated with pyridine/acetic anhydride and then chromatographed by HPLC in acetonitrile-water (9:1). The compound with the alkene signal at ca. δ 5.15 (95) could now be seen to also possess a methyl group attached to a double bond, leading to the structural assignment shown in figure 35. Interestingly, 95 causes Golgi vesiculation, but unlike ilimaquinone does not cause microtubule depolymerization. Deacetylation of 95 with sodium methoxide/methanol proceeded as expected to give 96 which had the same activity profile as 95. The other isomerization product with an alkene signal at δ 5.40 apparently underwent additional rearrangement, since a compound with this alkene signal was not recovered following the acetylation. Instead, a new compound 97 was isolated. This compound also had an extra methyl signal, but in the aliphatic region of the spectrum, leading to the structural assignment shown in figure 35. Compound 97 was found to have the same activity profile of the other double-bond isomer. Deacetylation of 97 using the same conditions as for 95, produced compound 98 (figure 35). Interestingly, compound 98 displayed the same activity as ilimaquinone. Apparently the drimane moiety is important in determining selectivity, but modifications are possible.

With this in mind we tried hydroboration of the terminal alkene, which would be expected to give a primary alcohol, that could allow other attachments onto the bicyclic ilimaquinone skeleton. Hydroboration of ilimaquinone with borane-THF is accompanied by reduction of the quinone to the hydroquinone, which can be followed visually as the bright yellow color of ilimaquinone fades. Reoxidation with basic aqueous hydrogen peroxide returns the quinone (in this case purple due to deprotonation of the phenol) as well as completing the alcohol formation. No conditions could be found to separate the two epimers, so the mixture was treated with pyridine/acetic anhydride and
Figure 35. Isomerization of Ilimaquinone (92) with Iodine.
Figure 36. Hydroboration of Ilimaquinone (92)
chromatographed on C-18 reversed phase to give 99 and 100 shown in figure 36. Both compounds cause Golgi vesiculation but do not show microtubule depolymerization. The added hydroxyl function could allow easy linkage to a solid support for affinity chromatography, or the linkage of a radio-labeled moiety. Further work in this area is being pursued, but as of yet no suitable derivative has been found.
**Experimental**

**Isolation of Glycinyllilimaquinone (91):** A specimen of Fasciospongia sp. (JCC 063, 27 g dry wt.) was collected near Dumaguette, Philippines. The frozen sponge was lyophilized and extracted with methanol (3 x 400 mL) using sonication to obtain, after evaporation of solvent, a dark purple residue. This extract was partitioned between water (100 mL) and hexanes, dichloromethane, and ethyl acetate, successively (60 mL each). All organic extracts were similar by TLC and $^1$H NMR. and thus were combined to give an extract (0.80 g). The organic extract was subjected to gel filtration on Sephadex LH-20, to obtain several colored antimicrobial fractions. One fraction was chromatographed on silica gel to obtain ilimaquinone (92, 130 mg, 0.5% dry wt.). The second fraction was chromatographed on C-18 reversed phase support [acetone-water (7:3)] to obtain smenospongine (93, 3.5 mg 0.01% dry wt.). The column washes were combined with additional aqueous methanol extracts of the sponge and chromatographed [acetone-0.1M sodium acetate, pH 3.5 (3:2)] to yield glycinyllilimaquinone (91, 15 mg, 0.6% dry wt.) as an amorphous red powder: UV (MeOH) see text; IR (thin film) see text; $^1$H NMR (acetone-$d_6$ 200 MHz) see Table 3; $^{13}$C NMR (acetone-$d_6$ 50 MHz) see Table 3; LRFABMS: $m/z = 402$ (MH)$^+$, 210, 191; HRMS: observed $m/z = 402.2276$, $C_{23}H_{32}NO_5$ requires $m/z = 402.2280$.

**Diaminoilimaquinone (94):** Ilimaquinone (92, 45.3 mg, 0.13 mmole) was dissolved in methanolic ammonia (2 ml of 2M solution, 4 mmole) by vigorous shaking. The purple solution was allowed to stand overnight whereupon diaminoilimaquinone crystallized as purple needles (94, 24.3 mg, 56%). The product was rinsed with MeOH, and displayed spectral data identical to the natural product. The remaining solution consisted of a mixture of mono-amino derivatives of ilimaquinone, including some smenospongine (93).
Isomerization of Ilimaquinone: Ilimaquinone (92, 33 mg, 0.1 mmole) was combined with iodine (35 mg, 0.1 mmole) in carbon tetrachloride (5 mL). The reaction mixture was then stirred overnight. After evaporation of solvent, the residue was placed on high vacuum overnight, (some iodine remained after this procedure) and the residue was then treated with [pyridine-acetic anhydride (1:1), 1 mL] overnight.

Chromatography [HPLC, C-18 1 cm x 50 cm, acetonitrile-water (9:1)] gave two compounds. Compound 95 (7.6 mg, 23%) yellow glass: $^1$H NMR (CD$_2$Cl$_2$, 200 MHz) $\delta$ 5.87 (s, 1H), 5.12 (br, 1H), 3.81 (s, 3H), 2.63 (d, 1H, $J = 13$ Hz), 2.39 (d, 1H, $J = 13$ Hz), 1.51 (br s, 3H), 0.99 (s, 3H), 0.90 (d, 3H, $J = 6$ Hz), 0.83 (s, 3H). Compound 96 (9.2 mg, 28%) yellow glass: $^1$H NMR (CD$_2$Cl$_2$, 200 MHz) $\delta$ 5.88 (s, 1H), 3.81 (s, 3H), 2.72 (d, 1H, $J = 13$ Hz), 2.45 (d, 1H, $J = 13$ Hz), 2.30 (s, 3H), 2.2-1.7 (multiple overlapping abs.), 1.7-1.2 (multiple overlapping abs.), 1.00 (s, 3H), 0.95 (s, 3H), 0.79 (d, 3H, $J = 6$ Hz).

Hydroboration of ilimaquinone: A solution of ilimaquinone (92, 99 mg, 0.3 mmole) in DME (5 mL) was treated with a solution of borane-THF (0.5 mL of a 1M soln., 0.5 mmole) and allowed to stir under dry nitrogen for 0.5 hr. During this period the reduction of the quinone moiety was visually indicated by the disappearance of the yellow color. Addition of excess H$_2$O$_2$ (1 mL of a 50% soln.) and sodium hydroxide (1 mL of 1 M soln.) reoxidized the reaction mixture returning the quinone and completing the hydroboration. The mixture was evaporated to an aqueous slurry, then HCl solution (3 mL of a 1M soln.) was added. The aqueous phase was extracted with ethyl acetate, and the organic layer was then separated and dried (Na$_2$SO$_4$) and evaporated. Pyridine-acetic anhydride [1 mL of a (1:1) soln.] was then added and the mixture was allowed to stand overnight. Removal of solvents under high vacuum followed by chromatography [HPLC, C-18, 1 cm x 50 cm, acetonitrile-water (95:5)] gave two compounds. Compound 99 (3.1 mg, 7%) yellow glass: $^1$H NMR (CDCl$_3$, 200 MHz) $\delta$ 5.93 (s, 1H), 4.19 (dd, 1H, $J = 11$, 2 Hz), 3.87 (s, 3H), 3.67 (dd, 1H, $J = 11$, 8Hz), 2.58 (d, 1H, $J = 13$ Hz), 2.40 (d,
1H, J = 13 Hz), 2.34 (s, 3H), 0.90 (d, 3H, J = 6 Hz), 0.86 (s, 3H), 0.82 (s, 3H).
Compound 100 (2.2 mg, 5%) yellow glass: 1H NMR (CDCl3, 200 MHz) δ 5.94 (s, 1H), 4.19 (dd, 1H, J = 11, 5 Hz), 3.92 (dd, 1H, J = 11, 8 Hz), 3.87 (s, 3H), 2.56 (d, 1H, J = 13 Hz), 2.40 (d, 1H, J = 13 Hz), 2.35 (s, 3H), 2.03 (s, 3H), 1.9-1.1 (multiple overlapping abs.), 1.07 (s, 3H), 0.88 (d, 3H, J = 6 Hz), 0.79 (s, 3H).

Deacetylation of 95 and 97. Deacetylation of 95 and 97 was accomplished using sodium methoxide in methanol (1 mL, 0.2M) generated by dissolving metallic sodium in dry methanol. Each compound was treated with an excess of this solution for 0.5 hr, then acidified with HCL soln. and evaporated. The residue was then partitioned between ethyl acetate and water. The organic layer was separated and dried (Na2SO4), and evaporated to give essentially quantitative yields of 96 and 98. Compound 96, yellow amorphous solid: 1H NMR (CDCl3, 200 MHz) δ 7.50 (br, 1H, OH), 5.81 (s, 1H), 5.09 (br, 1H), 3.84 (s, 3H), 2.63 (d, 1H, J = 13 Hz), 2.44 (d, 1H, J = 13 Hz), 1.53 (br, 3H), 0.98 (m, 6H), 0.84 (s, 3H). Compound 98 yellow amorphous solid: 1H NMR (CDCl3, 200 MHz) δ 7.30 (br, 1H, OH), 5.82 (s, 1H), 3.84 (s, 3H), 2.70 (d, 1H, J = 13 Hz), 2.57 (d, 1H, J = 13 Hz), 2.3-1.0 (multiple overlapping abs.), 0.99 (s, 3H), 0.92 (s, 3H), 0.83 (s, 3H), 0.76 (d, 3H, J = 6 Hz).
References and Notes


