BIOMIMETIC SYNTHESIS AND MECHANISTIC STUDIES OF NATURAL PRODUCT POLYSULFANES

by

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Abstract

BIOMIMETIC SYNTHESIS AND MECHANISTIC STUDIES OF NATURAL PRODUCT POLYSULFANES

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This thesis outlines an experimental and theoretical organic chemistry approach to address the structure and synthesis of polysulfane antitumor compounds and the origin of their biological activity.

The mechanism of decomposition of 1,2,3,4,5-benzopentathiepin and related polysulfanes represented by pentathiepins was studied. Theoretical and experimental studies were undertaken and revealed a possible decomposition of pentathiepin yielding thiozone (triatomic sulfur, S_3). Expulsion of S_3 is the result of intramolecular attack of the amine on the first sulfur atom of the pentathiepin ring. The decomposition of pentathiepin by expulsion of S_3 is energetically preferred over decomposition by expulsion of S_2. Theoretical studies were supported by experiments. Trapping experiments revealed higher susceptibility of penathiepins toward of S_3 transfer rather to S_2. The study may contribute to understanding the mechanism of biological activity of naturally occurring pentathiepins.

An odd-even effect in stability of polysulfanes was uncovered by theoretical methods, which helped to predict the product distribution of an *o*-benzyne—elemental sulfur reaction. DFT calculations revealed an enhanced stability of odd versus even cyclic polysulfanes likely the result of adjacent sulfur atoms lone pair electrons adopting a staggered orientation. The discovery may explain the preference for formation of odd vs even polysulfanes found in Nature. We conducted study on an elemental sulfur addition to benzyne, which revealed the formation of pentathiepin, thithiole, thianthrene and tetrathiocin, which has not been observed before.

A biomimetic synthesis of biologically active polysufanes *via* *o*-benzoquinone intermediates was also accomplished. The study was inspired by the fact that the biologically active polysulfanes isolated from ascidians may be involved in defense mechanism of tunicates where polysulfane synthesis my take place from available ingredients, elemental sulfur and dopamine. The laboratory modeled biosynthesis of polysulfanes involving catechol and sulfur in its reduced form allowed us to observe several polysulfane products with the structures reminiscent to the natural products.

Finally, the synthesis of several new substituted 1,2-dithiolan-3-one 1-oxides was accomplished.

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Most of all, I wish to express my love and gratitude to my family and life—long friends in Poland for their tremendous support and love.

To My Parents

And All the Other Wonderful People in My Life

TABLE OF CONTENTS

LIST OF SYMBOLS AND ABBRIVIATIONS

Å	angstrom
Ac	acetyl
Ac_2O	acetic anhydride
B3LYP/6-31G(d)	Becke-style-Parameter Density Functional Theory
brine	saturated aqueous sodium chloride solution
br	broad
^oC	degree Celsius
calcd	calculated
kcal	kilocalorie
^{13}C NMR	carbon-13 nuclear magnetic resonance
δ	chemical shift in ppm
d	doublet
DCM	Dichlorometane
DMAP	4-(Dimethylamino)pyridine
DFT	Density Functional Theory
DMF	N,N-dimethylformamide
ε	dielectric constant
ED_{50}	Effective Dose 50
Et_2O	diethyl ether
EtOAc	ethyl acetate
eq	equivalent

g	gram
GC	gas chromatography
GCMS	gas chromatography mass spectroscopy
h	hour
^{1}H NMR	proton nuclear magnetic resonance
HeLa 33	Henrietta Lacks (uterine cell variety) 33
HPLC	high pressure liquid chromatography
Hz	hertz
IC_{50}	50% inhibitory concentrations
IR	infra-red spectroscopy
J	coupling constant
L	liter
LAH	lithium aluminum hydride
LD_{50}	lethal dose 50
m	multiplet
MeOH	methanol
mg	milligram
min	minute
mL	milliliter
mmol	millimole
NBO	natural bond order
NBP	natural bond population

NOE	Nuclear Overhauser Enhancement
PCM	polarized continuum model
*p*H	potential of hydrogen
*p*Ka	ionization constant
ppm	parts per million
q	quartet
rt	room temperature
s	singlet
SCRF	self-consistent reaction field
t	triplet
TBAF	tetrabuthyl ammonium fluoride
THF	tetrahydrofuran
TLC	thin layer chromatography
TMEDA	tetramethyl ethylenediamine
TS	transition state
TsOH	*p*-toluenesulfonic acid
UV	ultra-violet spectroscopy

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF SCHEMES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

LIST OF TABLES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 1. Overview

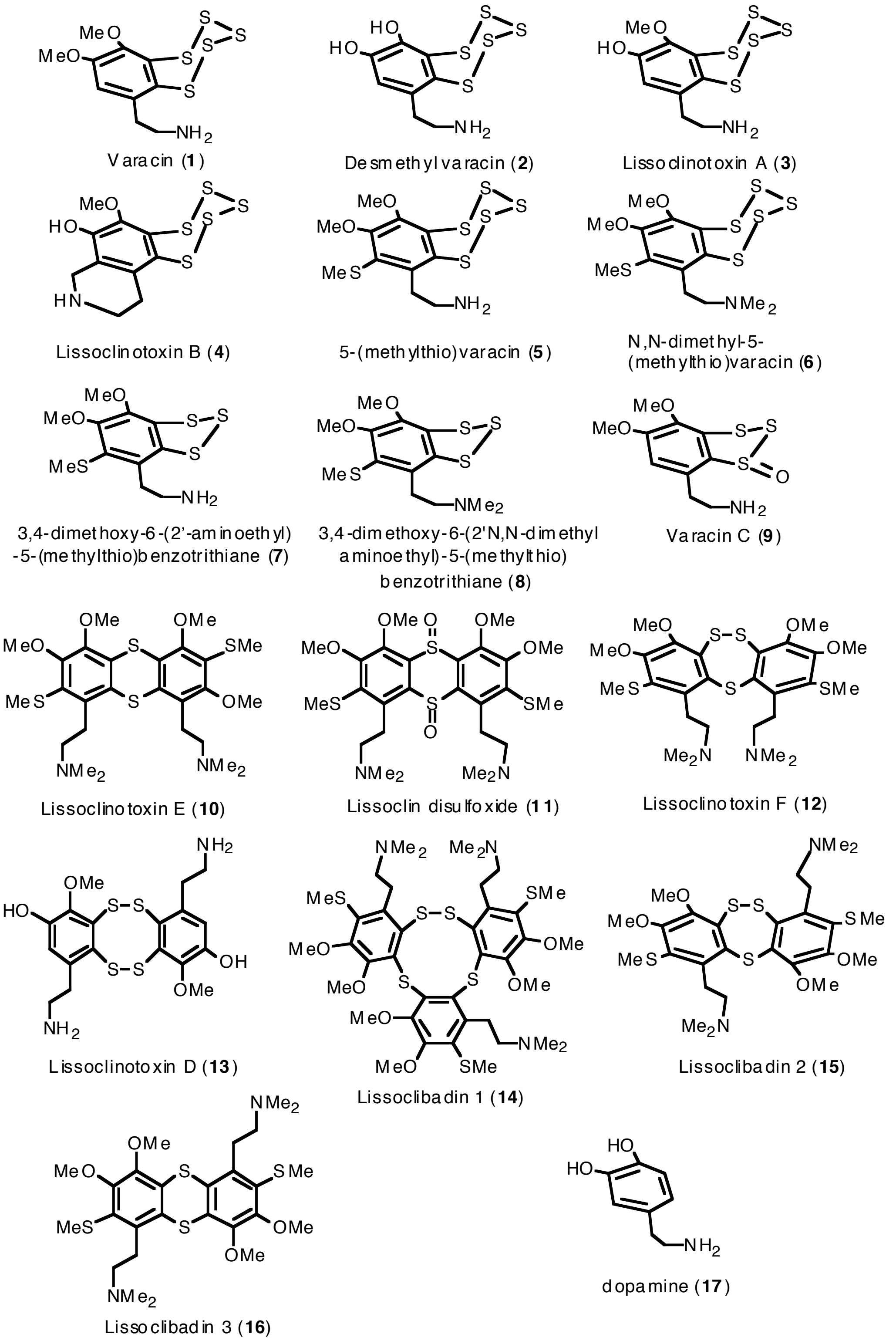
1.1 Polysulfane Natural Products.

Varacin (**1**), desmethyl varacin (**2**), lissoclinotoxin A (**3**), lissoclinotoxin B (**4**), 5-(methylthio)varacin (**5**), N,N-dimethyl-5-(methylthio)-varacin (**6**), 3,4-dimethoxy -6-(2'-aminoethyl) -5-(methylthio) benzotrithiane (**7**), 3,4-dimethoxy-6-(2' N,N-dimethylaminoethyl)-5-(methylthio)benzotrithiane (**8**), varacin C (**9**), lissoclinotoxin E (**10**), lissoclin disulfoxide (**11**), lissoclinotoxin F (**12**), lissoclinotoxin D (**13**), lissoclinbadin 1 (**14**), lissoclinbadin 2 (**15**) and lissoclinbadin 2 (**16**) (Scheme 1) are secondary metabolites obtained from organic solvent extracts of marine tunicate samples.[1-13] Naturally occurring polysulfanes **1-16** arise from tunicates of the genera *Lissoclinum*, *Aplydium*, *Eudistoma*, and *Polycitor* (Figure 1) or their closely associated cyanobacteria.[25-27] Marine compounds **1-16** were isolated and characterized fairly recently (all within the last 15 years).[1-13] The period from 1991-1994 yielded **1**-**8**, and **13**. The period from 1995-2001 did not fare as well in yielding new structures, but recently (2002-2005) polysulfanes **9-12**, and **14-16** were discovered.

1.1.1 Natural and Noxious. Polysulfanes Have a Range of Bioactivities.

The bioactivities of the naturally occurring (marine) polysulfanes **1-16** reported to date are presented in Table 1.[1-13] For example, Ireland and co-workers[1] discovered that varacin **1** exhibited cytotoxic activity. The cytotoxicity of **1** toward human colon cancer HCT 116 was promising; it possessed an IC_{50}

value of 0.05 mg/mL, which is ~100 times more active than 5-fluorouracil, which is considered as a potent antitumor agent. It was suggested that the bioactivity of varacin **1** was derived from DNA damage because of an observed difference in toxicity toward the CHO cell line EM9 (chlorodeoxyuridine sensitive) compared to BR1.[1]



Varacin (**1**)

Desmethylvaracin (**2**)

Lissoclinotoxin A (**3**)

Lissoclinotoxin B (**4**)

5-(methylthio)varacin (**5**)

N,N-dimethyl-5-(methylthio)varacin (**6**)

3,4-dimethoxy-6-(2'-aminoethyl)-5-(methylthio)benzotrithiane (**7**)

3,4-dimethoxy-6-(2'N,N-dimethyl aminoethyl)-5-(methylthio) benzotrithiane (**8**)

Varacin C (**9**)

Lissoclinotoxin E (**10**)

Lissoclin disulfoxide (**11**)

Lissoclinotoxin F (**12**)

Lissoclinotoxin D (**13**)

Lissoclibadin 1 (**14**)

Lissoclibadin 2 (**15**)

Lissoclibadin 3 (**16**)

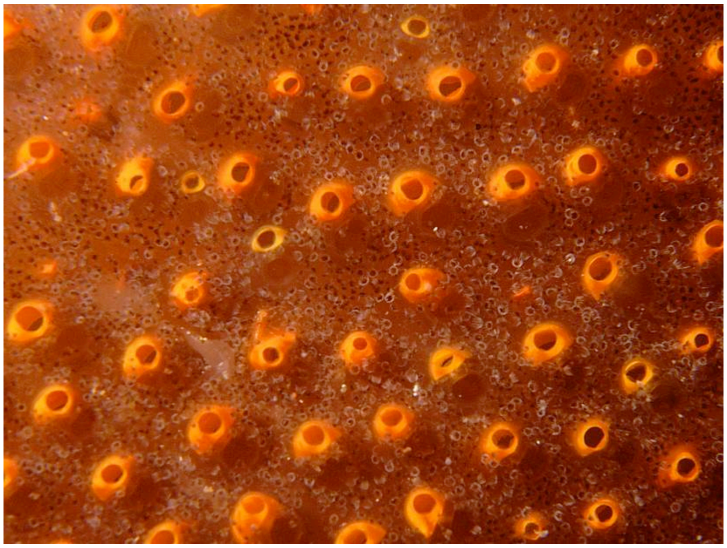
dopamine (**17**)

Scheme 1. Naturally occurring polysulfanes.

Lissoclinum spp

Aplydium spp

Figure 1. Ascidians which contain polysulfane molecules. *[photos by Boyer]*



Polycitor spp

Audistoma spp

Figure 1. (cont.)

Table 1. Biological activities of naturally occurring polysulfanes.

Compound		Activity	Ref
Varacin	**1**	Antifungal activity against *C. albicans*; toward the human colon cancer HCT 116 with an IC_{50} of 0.05 μg/mL; 1.5 differential toxicity toward the CHO cell line EM9 vs BR1	1
Desmethyl varacin	**2**	Selectively inhibited PKC with an IC_{50} of 33.0 μg/mL and PKA >25 μg/mL	4
Lissoclinotoxin A	**3**	Activity against *C. albicans*; gave zones of inhibition with loadings on 6.5 mm paper disks	2, 7
Lissoclinotoxin B	**4**	Activity against *S. aureus* 30 mm/100 μM inhibition zone and toward ichthyopathognic strains	5
5-(methylthio) varacin and 3,4-dimethoxy-6-(2'-aminoethyl) -5-(methylthio) benzotrithiane	**5+7**	Selectively inhibited PKC with an IC_{50} of 0.3 μg/mL and PKA >25 μg/mL	1
N,N-dimethyl-5-(methylthio) -varacin	**6**	Selectively inhibited PKC with an IC_{50} of 3.0 μg/mL and PKA >50 μg/mL; cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 2.9 μM	12
3,4-dimethoxy-6-(2'-N,N-dimethylaminoethyl)-5-(methylthio)benzotrithiane	**8**	Selectively inhibited PKC with an IC_{50} of 1.3 μg/mL and PKA >50 μg/mL; cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 6.2 μM	12

Table 1. (cont.)

Compound		Activity	Ref
Varacin C	**9**	Cytotoxic activity against *C. albicans* and *Bacillus subtilis*; gave acid promoted DNA cleaving activity	13
Lissoclinotoxin E	**10**	Displayed IC_{50} values of 2.3 μg/mL against the MDA-MB-468 human breast carcinoma cell line and cytotoxity against the MDA-MB-435S human breast carcinoma cell line with IC_{50} values of 2.1 μg/mL	10, 12
Lissoclin disulfoxide	**11**	Was inactive at 50 μg/mL against MDA-MB-468 human breast carcinoma cell line	13
Lissoclinotoxin F	**12**	Displayed IC_{50} values of 1.5 μg/mL against the MDA-MB-468 human breast carcinoma cell line and cytotoxity against the MDA-MB-435S human breast carcinoma cell line HL-60 at IC_{50} values of 4.2 μg/mL; cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 0.23 μM	12
Lissoclinotoxin D	**13**	Activity against *C. albicans*; gave zones of inhibition with loadings on 6.5 mm paper disks	7

Table 1. (cont.)

Compound		Activity	Ref
Lissoclinbadin 1	**14**	Inhibited the growth of *R. atlantica* strain TUF-D; cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 0.37 μM	12
Lissoclinbadin 2	**15**	Cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 0.21 μM	12
Lissoclinbadin 2	**16**	Cytotoxicity against the human leukemia cell line HL-60 at IC_{50} value 5.5 μM	12

1.1.2 Structural Features of Naturally Occurring Polysulfanes.

Polysulfane structures **1-16** have unusual chemical architectures. Compounds **1-16** possess common structural feature, which is a dopamine core (**17**) and unusual heterocyclic rings made of sulfur atoms. The unusual structural architecture may explain the low number (ca. 70) of natural product polysulfanes discovered thus far from terrestrial and marine sources combined, even though about 200,000 secondary metabolites have already been described in the literature. The polysulfane linkage RSS_xSR is less abundant than other functional groups in natural product structures. Discovery strategies of natural polysulfanes may play a role in the number that have been found. For example, many polysulfanes may not be found due to their instability. The bond dissociation energy (BDE) of an S-S bond is low, 25-30 kcal/mol, which leads to easy bond breakage and equilibration processes.

Some naturally occurring polysulfanes **10**-**13**, **15**, **16** exist as dimers.[7,12,13] Montecarlo conformation search using MM2 force field suggested that the *trans* isomer of lissoclinotoxin E **10** was by 0.4 kcal/mol energetically more preferred over *cis* isomer. It turned out that the assignment of structure of lissoclinotoxin E **10** based on result of MM2 calculation was inconsistent with experimental results.[11] It was established by NOE analysis that compound **10** exists as *trans* isomer. Furthermore, NOE analysis allowed assigning the orientation of N-alkyl chains as *cis* **10**-**12** or *trans* **13**, **15** and **16** in respect to tricyclic system. The assignment was established based on NOE correlation between -OMe and –CH_2- adjacent to aromatic ring in aminoethyl substituent, which was present in compounds **15** and **16** and absent in case of compounds **10** and **12**.[12] It was concluded, that NOE analysis is an efficient way of the elucidation of the structures of naturally occurring polysulfanes.

Naturally occurring polysulfanes **1**-**9** possess unusual rings built from 3 or 5 sulfur atoms. Interestingly, information does not exist in the literature on whether larger ring species exist such as a heptasulfane *o*-ArS_7 and octasulfane *o*-ArS_8 or on whether polysulfane mixtures arise *in vivo* (e.g., *o*-ArS_x, where x = 1-6). The result of experiments and theoretical calculations conducted in our lab provide evidence that mixtures of polysulfanes are formed based on an odd-even pattern (x = 3, 5, 7) that includes the presence of a new species, heptasulfane -

SS_5S-. This topic will be discussed later in this thesis.

1.1.3 Natural Products Polysulfanes as Evolved Chemical Warfare Agents.

Ascidians (tunicates) contain compounds with a large range of pharmacological activities (Figure 1).[15-17] With the most common of chemical ingredients, elemental sulfur and dopamine, we suggest that an ascidian has developed a means to protect itself from predators using in essence chemical warfare.[1-13] With lessons that marine organisms can provide, how might chemists seek the advent of new therapeutic substances from abundant and simple natural reagents? The problem is that we lack an understanding of the biosynthetic pathways by which marine organisms create protective or otherwise purposeful molecules from benign common precursors.

Some organisms use simple molecules for defense and protection.[18] For example, ants use formic acid and millipedes use hydrogen cyanide.[18] Others organisms use more complicated defense molecules, such as intricate protein toxins produced by spiders, or the high-temperature quinoid compounds from a bombardier beetle.[19]

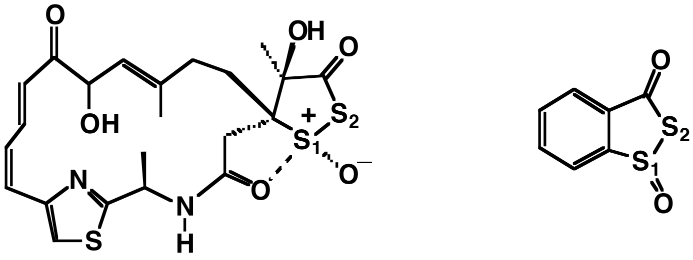
There is little data on chemical defense molecules of tunicates although some alkaloids have been implicated.[20-23] However, the polysulfanes shown in Scheme 1 are little appreciated in this regard, and may serve as a potent defense against predator fish and flatworms or pathogenic bacteria and fungi—since polysulfanes possess biocidal properties (e.g., antifungal and antibacterial activity).

Dopamine has been recently isolated in a relatively high yield (0.0022% of wet weight) from a marine sponge.[24] Sponges and tunicates are primitive animals without a nervous system; dopamine is therefore not produced as a neurotransmitter. Instead dopamine has been proposed as a grazer deterrent, although this constitutes a speculated function.

The underlying mechanism for the production of dopamine and sulfur-containing dopamine derivatives in these sessile animals remains largely unknown. Sulfur-containing dopamine derivatives (e.g., polysulfanes) or their precursors from sponges and tunicates may be produced by symbiotic microorganisms, such as cyanobacteria.[24-27] Marine invertebrates have symbiotic microorganisms that constitute about a half of their biomass. Compounds obtained from tunicates sometimes come from the symbiotic microorganisms and the biosynthesis is a bacterial process. Interestingly, *Prochloron didemni*, the endosymbiont of the ascidian *Lissoclinum patellum*, is an example of an affiliated cyanobacterium based upon evidence from a partial 16S rRNA analysis.[14]

1.2 Substituted Benzo-1,2-Dithiolan-3-one 1-Oxides.

The last chapter of the dissertation is focused on the synthesis of dithiolan-3-one 1-oxide compounds. Leinamycin (**18**) is a natural product and contains a dithiolanone-oxide heterocycle.[28]



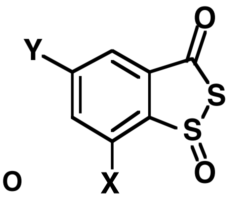
18 **19**

Scheme 2. Structure of leinamycin **18** and benzo-1,2-dithiolan-3-one 1-oxide **19**.

Leinamycin **18** causes single-strand cleavage of supercoiled double-helical DNA in the presence of thiol in vitro.[28] The lack of cytotoxicity and DNA-cleaving activity for S-deoxyleinamycin indicated dithiolanone-oxide core is key for biological activity of leinamycin. The detailed mechanism of the biological activity of leinamycin **18** was suggested Gates and co-workers.[29]

Substituted benzo-1,2-dithiolan-3-one 1-oxides might serve to uncover aspects of the thiol-activated DNA-cleavage chemistry of leinamycin. Thus, we synthesized variety of 3- and 5- substituted benzo-1,2-dithiolan-3-one 1-oxides including benzo-1,2-dithiolan-3-one 1-oxide **19** and *ortho*- (X = Cl (**20**), CH_3 (**21**), OCH_3 (**22**)) and *para*- ((X = Cl (**23**), CH_3 (**24**), OCH_3 (**25**)) in order to study substituent effects on their reactivity.

19 X=H; Y=H
20 X=Cl; Y=H
21 X=Me; Y=H
22 X=MeO; Y=H
23 X=H; Y=Cl
24 X=H; Y=Me
25 X=H; Y=MeO



Scheme 3. *ortho*- and *para*- substituted benzo-1,2-dithiolan-3-one 1-oxides.

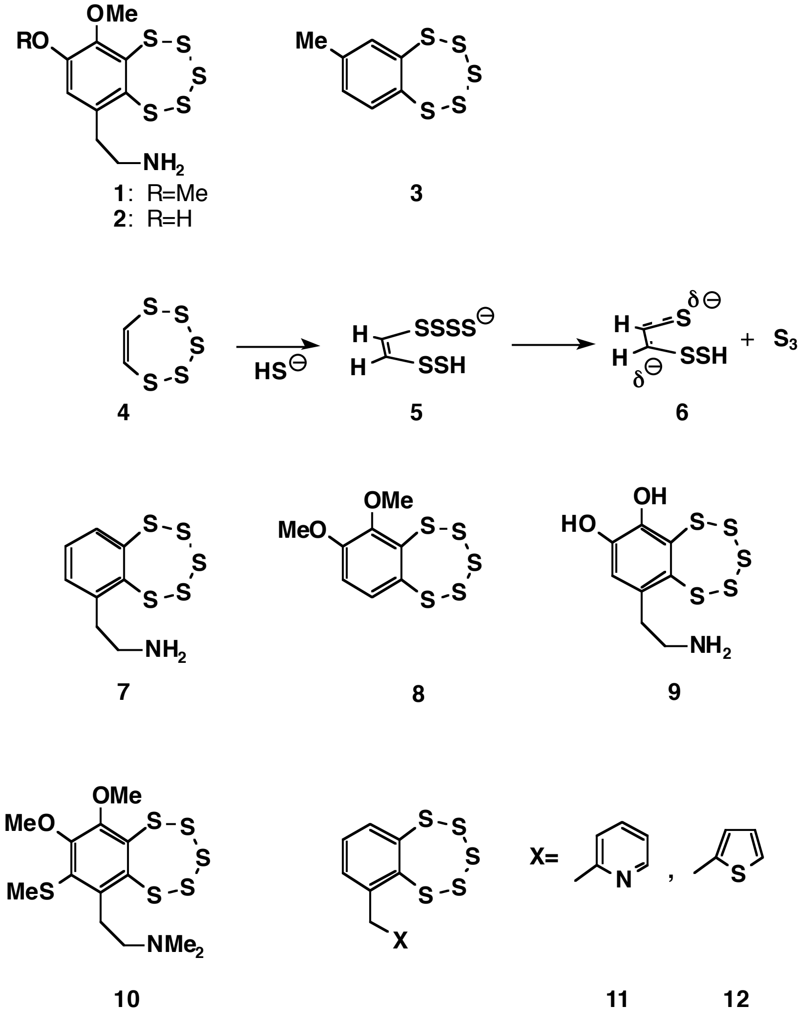
Chapter 2. Bioactive Pentathiepins. Mechanism of Decomposition

2.1 Introduction.

The 1,2,3,4,5-pentathiepin heterocycle is found in the marine natural products varacin (**1**), lissoclinotoxin A (**2**) (Scheme 1).[1,2] As was stated in Chapter 1, there is recent interest surrounding pentathiepin molecules because of their antitumor, antifungal, and antimicrobial properties.[1,3-9] Recently results from calculations were reported at the B3LYP/6-31G(d) level of theory, which revealed ring-opening of pentathiepin (**4**) with thiolate ion (HS^-).[9] Polysulfur ion (**5**) was suggested as an intermediate initially formed in the pentathiepin—HS^- reaction, which decomposed to triatomic sulfur, S_3, and a resonance stabilized carbon-sulfur ion (**6**). The unique feature of the mechanism was the suggestion that S_3-unit transfer has significance in the cytotoxicity of the natural product varacin, **1**.[9]

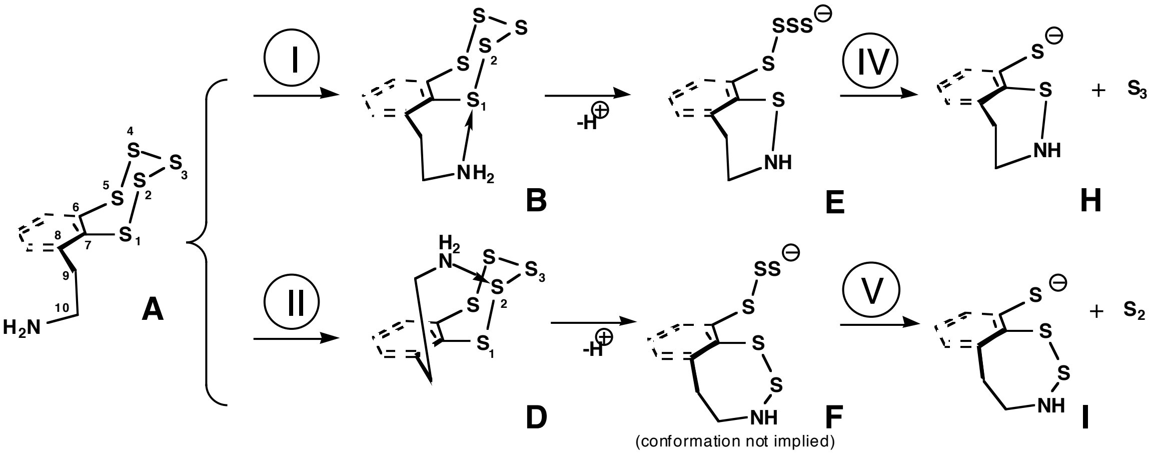
A question that concerns the reaction mechanism of natural product pentathiepins which needs to be addressed is: *Why are some pentathiepins more biologically active than others?* The polysulfur ring system is thought to be of key importance to the activity of these toxins, but the role of the remotely bonded amine has not been defined even though all natural occurring pentathiepins isolated to date bear this group.[1,2,5-7] Whether the pentathiepin amine group is protonated at physiological pH is not immediately clear. The amine basicity is expected to be a function of the solvent environment,[8,10,12,13] but as will be shown later, it is also a function of the proximity to the polysulfur ring (**2.2.3**).

There are indications that the amine functionality may influence bioactivity since the cytotoxicity to HeLa S3 cells is enhanced in 6-(2-aminoethyl)benzopentathiepin (**7**, IC_{50}=0.26 mg/mL) compared to 6,7-dimethoxybenzopentathiepin (**8**, IC_{50}=6.12 mg/mL).[3] Faulkner and co-workers[4] reported that a pentathiepin containing a primary amine (**9**) more readily inhibits protein kinase C compared to a pentathiepin containing a tertiary amine (**10**). A possible mechanism, however, that uses intramolecular promotion to explain the biological activity has not been proposed. Some synthetic investigations emphasize that pentathiepins react with external bases (e.g., NaOH or LAH).[14,15] Insight is needed on the potential intramolecular reaction of the amine in pentathiepin chemistry, which we postulate play a part in the biological mechanism of action.



Scheme 1. Lissoclinotoxin **1** and Varacin **2**. Ring opening of pentathiepin **4** with thiolate ion HS$^-$.

To address this issue, a strategy was designed (computational and experimental) to explore whether an amine has an opportunity to engage in bonding with pentathiepin to promote its decomposition. It was anticipated that a primary amine would allow for an intramolecular addition reaction to the pentathiepin ring at the nearest sulfur (Pathway I, Scheme 2). In contrast, a tertiary amine might be unable to add, or else add reversibly because the nitrogen could not lose its positive charge by deprotonation. This would preclude the promotion step. We describe our observations on this strategy, which support the above notion and assess the amine functionality as an activation element in pentathiepin decomposition. Computations predict the unimolecular destabilization of pentathiepin in those substrates bearing hydrogen(s) on amine ($-NH_2$ and –NHR), but not for the tertiary amine ($-NR_2$). The computed results are important in light of the recent suggestion that pentathiepin substitution with an amine (**7**), but not a pyridyl (**11**) nor a thienyl (**12**) group enhances the decomposition rate (Scheme 1).[16]



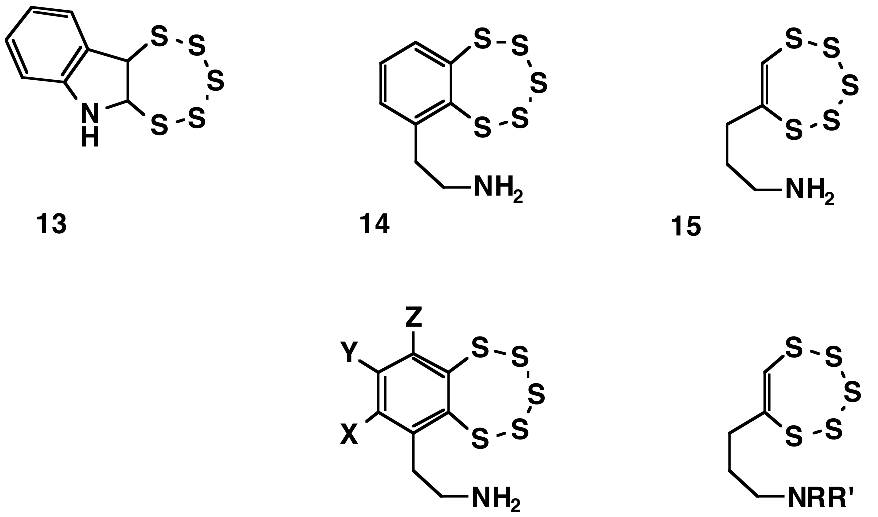
Scheme 2. Amine promoted decomposition of pentathiepins.

The present study advances an idea, the possible intermediacy of S_3 in pentathiepin desulfurization triggered by nucleophilic activation with amine (Pathway I, Scheme 2). We provide the first experimental support for S_3–transfer in the decomposition of a pentathiepin and characterize an energetically low-lying process corresponding to stepwise fragmentation. That the amine may confer an enhanced reactivity in the natural product pentathiepins, adds to the understanding of the pathway for activation, and provides new design principles that may have potential applications for this class of biocides.

2.2 Results and Discussion.

2.2.1 The Amine Dependency.

Pentathiepin crystal structures are known[22] but none are known which contain an amine group adjacent to the polysulfur ring. The crystal structure of pentathiepino(6,7-*b*)indole (**13**) represents an unusual case where a 2° amine is fused in a 1,3-position relative to sulfur S1 (Scheme 3).[26] Since little is known of the role of amine in pentathiepin natural products, we thought the research should focus on unimolecular reactions of the marine toxins **1** and **2** and a series of pentathiepin derivatives (**14-20**, Scheme 3). The computations and experiments provide the first evidence that amination underpins the production of reactive sulfur intermediates, which provides mechanistic insight on the process that gives rise to biological activity. The results for **1**, **2**, and **14-20** are discussed here using the nomenclature presented in Scheme 2. The computed results are discussed first, followed by the experimental results.



13 **14** **15**

16: (X,Y=H; Z=OMe)
17: (X,Z=H; Y=OMe)
18: (Y,Z=H; X=OMe)

19: (R=H; R'=Me)
20: (R=R'=Me)

Scheme 3. Pentathiepin derivatives.

2.2.2 Rotation of Amine in Pentathiepin.

To explore the conformational preference of amine with the pentathiepin polysulfur ring, we computed rotational energy profiles for benzopentathiepin **14** and ethenopentathiepin **15** (Figure 1). A 180° torsional constraint (ω=C6-C7-C8-C9) was introduced in **15** to mimic the dihedral angle encountered in all natural benzopentathiepin systems.[1,2,6,7] The carbon numbering scheme in which the torsions are described is shown in Figure 1. The amine energy profile is depicted in Figure 1 with rotation about the dihedral angles θ (C8-C9-C10-N) and ϕ (C7-C8-C9-C10). Conformers **14A** and **14B** are related by a 126.3° rotational arrangement about the torsion angle ϕ while rotamer **14C** is accessed from **14B** by 178.6° rotation of the CH_2NH_2 group in a counterclockwise movement about the C9-C10 bond relative to the C8-C7 bond. The barriers to rotation about θ and ϕ (4-8 kcal/mol) are not high enough to be revealed experimentally by low-temperature NMR spectroscopy,[27] but do display a conformational preference for **14A**,**B** relative to **14C**,**D** with the theoretical method B3LYP/6-31G(d). A similar situation is found with pentathiepin **15**. Here the most stable conformer is **15B** (Figure 1B).

The key issue is the computed preference for coordination of the amine for pentathiepin S1 rather than S2. The magnitude of the preference ranges from 1.0 to 3.2 kcal/mol on the B3LYP/6-31G(d) potential surface (Figure 1). The resulting apical S1—N bond distance in structure **14B** is 3.065 Å while the S2—N distance is longer in **14D** (3.435 Å). The 1-3.2 kcal/mol preference for rotamer **B** relative to **D** can be attributed to a favorable alignment for apical coordination [N-S1-S2=153.5° (**14B**) compared to N-S2-S3=152.8° (**14D**), where 180° is a perfect TBP environment] and a reduced torsional strain in **14B** (θ=63.7°) compared to **14D** (θ=66.0°), where 60° is a perfect sp^3-sp^3 bond. The difference in energy between the “coordinated” nitrogen species **14B** and **14D** and the “uncoordinated” **14A** and **14C** is small. How these interactions might affect the bioactivity provide merit for discussion.

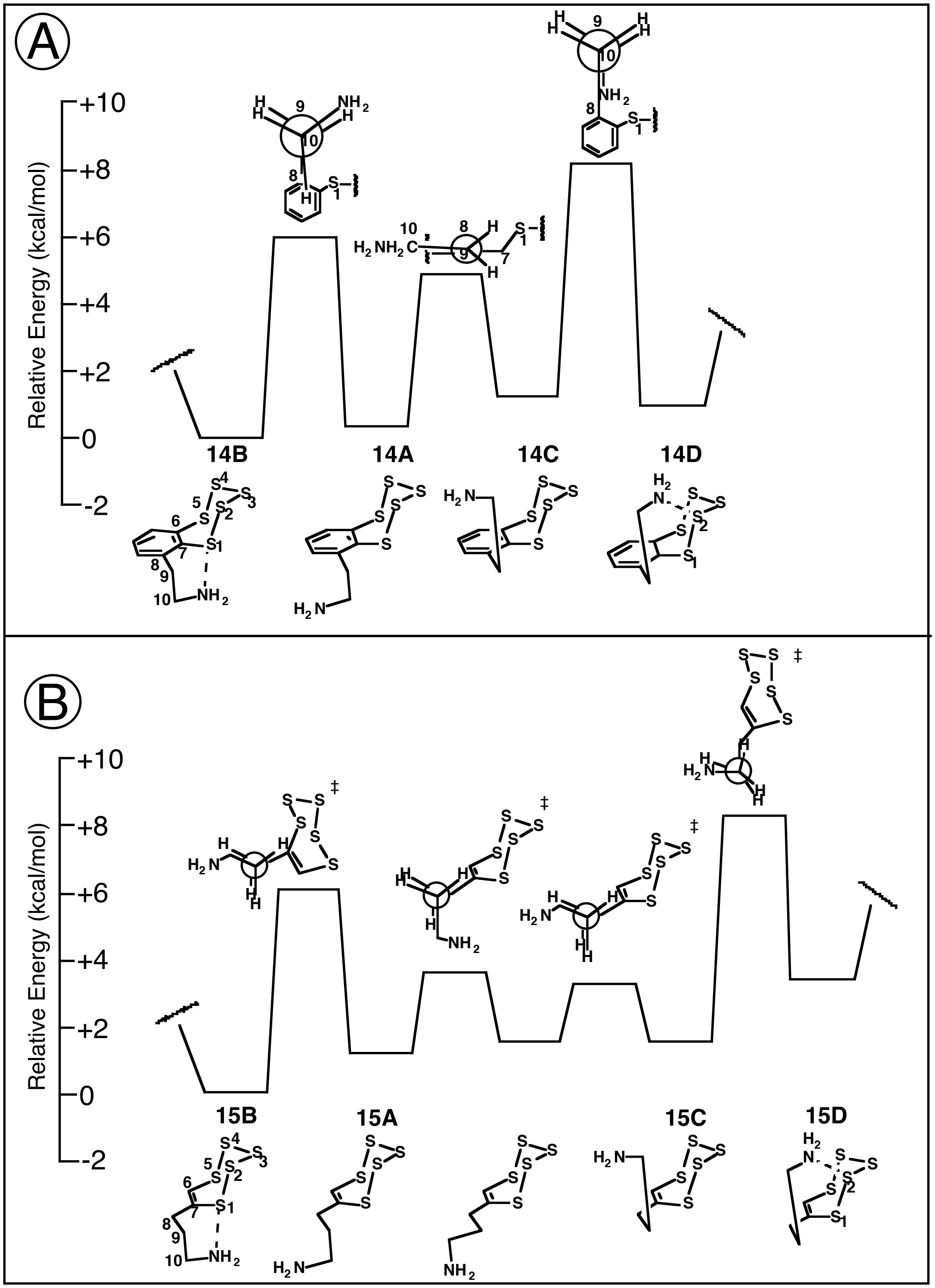
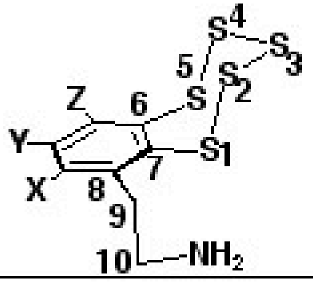


Figure 1. B3LYP/6-31G* calculated potential energy surface. The gas-phase energies are shown, and the solution-phase energies are discussed in the text. Eclipsed structures for **14** represent single point calculations, but for **15**, represent established transition state structures. Isomers shown represent rotations about sp^2-sp^3 or sp^3-sp^3 single bonds.

We believe that amine N stabilizes pentathiepin by a donor-acceptor interaction which intensifies the positive charge at S1 to stabilize the pseudo TBP geometry by an apical donating effect (Scheme 4).[28] DFT calculations predict an enhanced positive natural bond order (NBO)[29] charge at S1 relative to S2. This results in an S—N interaction where N donating is more easily accomplished with S1. Through-space interactions of the S—N and S—O type have been recognized in other biological systems.[30-34] It is the electron deficient S1 which is preferentially coordinated by amine. The pentathiepin(S1)—ligand(N) contact is accompanied by a transfer of electron density (Table 1 and 2), which is similar to donor-acceptor phenomena found in elemental sulfur-amine mixtures.[35] Subtle differences in the amine-pentathiepin conformation are observed with solvation. When the dielectric constant of water is used in the continuum solvation model calculations an enhanced stabilization is observed for **14B** relative to **14D** (3.2 kcal/mol), compared to the gas-phase value (2.5 kcal/mol). A greater solvation of **14B** may be the result of an enhanced charge separation between S1 and apical S2, which accompanies factors leading to amine nucleophilicity, proton loss, and polysulfur ring opening. In all pentathiepin derivatives examined (**1**, **2**, and **14**-**20**) coordination is favored at S1 compared to S2 with the B3LYP/6-31G(d) theoretical method.

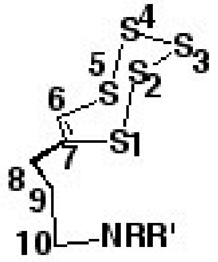
Table 1. Calculated benzopentathiepin structural parameters.[a,b]



	Substituent									NBO Charges		
	X	Y	Z	S1-N	S1-S2	S2-S3	N-S1-S2	N-S2-S3	θ[c]	N	S1	S2
1	H	OMe	OMe	3.135	2.111	2.106	151.9	122.5	63.8	-0.90	0.17	-0.04
2	H	OH	OMe	3.136	2.110	2.107	151.9	122.5	63.8	-0.90	0.17	-0.04
14A	H	H	H	4.724	2.102	2.106	138.4	129.5	62.6	-0.90	0.15	-0.02
14B	H	H	H	3.065	2.107	2.107	153.5	121.6	63.7	-0.90	0.17	-0.04
14C	H	H	H	3.726	2.100	2.110	65.5	152.8	66.0	-0.90	0.16	-0.01
14D	H	H	H	5.000	2.100	2.108	85.6	143.4	67.7	-0.90	0.15	-0.03
16	H	H	OMe	3.072	2.108	2.107	154.2	121.4	64.0	-0.90	0.17	-0.04
17	H	OMe	H	3.141	2.110	2.106	150.8	122.8	63.8	-0.90	0.17	-0.04
18	OMe	H	H	3.155	2.105	2.107	151.8	123.0	64.0	-0.90	0.17	-0.04

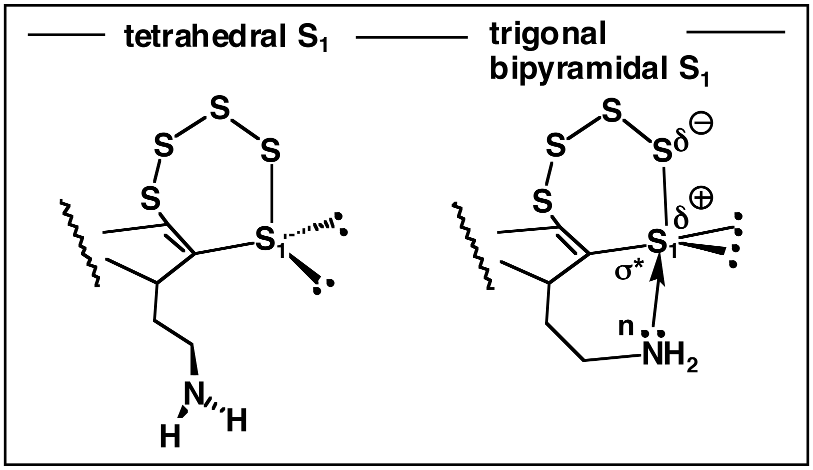
[a] Structures optimized at the B3LYP/6-31G(d) level. [b] Distances in Å, angles in deg. [c] The dihedral angle θ=C8-C9-C10-N, is positive for a counter clockwise movement from C8 to N as you look down from C9 to C10.

Table 2. Calculated ethenopentathiepin structural parameters.[a,b]



	Substituent								NBO Charges		
	R	R'	S1-N	S1-S2	S2-S3	N-S1-S2	N-S2-S3	θ[c]	N	S1	S2
15A	H	H	4.861	2.091	2.109	112.4	147.2	60.7	-0.90	0.13	-0.01
15B	H	H	2.782	2.115	2.112	172.7	109.9	60.0	-0.86	0.17	-0.05
15C	H	H	4.542	2.094	2.108	81.8	145.0	54.0	-0.91	0.15	-0.02
15D	H	H	3.361	2.095	2.107	70.3	164.6	66.2	-0.90	0.14	0.00
19	Me	H	2.880	2.111	2.112	173.6	108.0	74.0	-0.69	0.16	-0.05
20	Me	Me	2.904	2.112	2.112	174.0	107.7	74.5	-0.50	0.15	-0.04

[a] Structures optimized at the B3LYP/6-31G(d) level. [b] Distances in Å, angles in deg. [c] The dihedral angle θ=C8-C9-C10-N, is positive for a counter clockwise movement from C8 to N as you look down from C9 to C10.



Scheme 4. Stabilization of pentathiepin by amine based on donor-acceptor interaction.

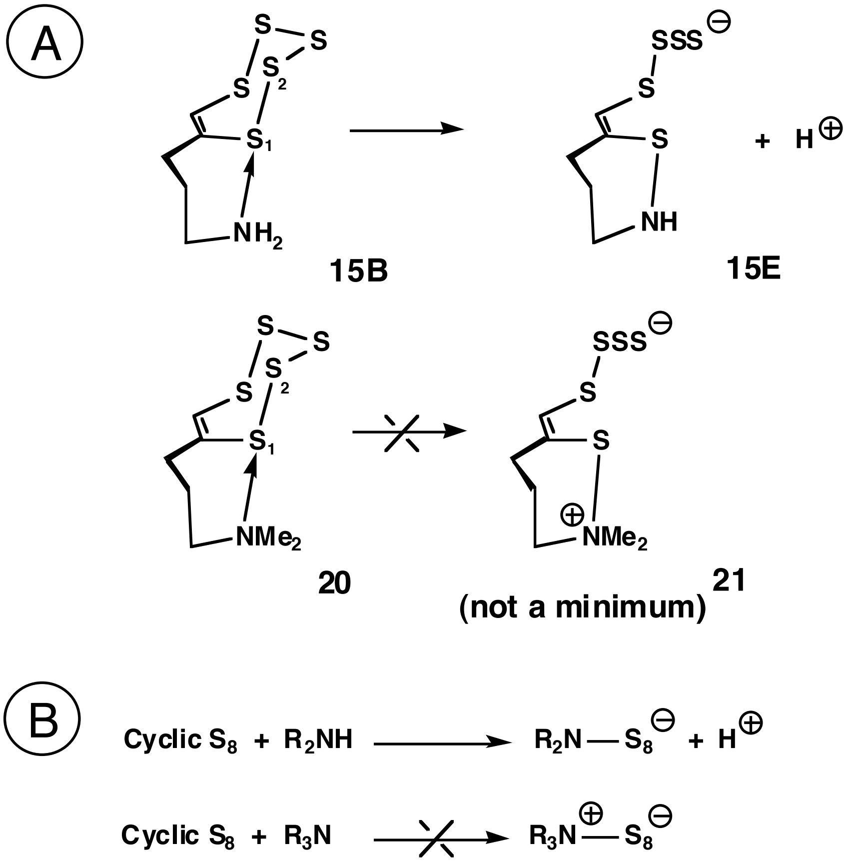
Pentathiepins **1**, **2**, and **14**-**20** are most stable when the vicinity of the S1 and N atoms are inside the Van der Waals contact distance (3.3 Å).[36] Conformer **B** places the S1 and N atoms in close proximity, e.g., 3.135 Å for varacin **1** and 3.136 Å for lissoclinotoxin A **2** (Table 1). The presence of a S1—N interaction appears to be independent to the pattern of methoxy substitution on the benzene ring (compare **16**-**18**, Table 1). That the amine N in **1**, **2**, and **14**-**20** is spatially close to S1 provides the potential for nucleophilic addition to the pentathiepin ring. While the amine N-H dissociation constant in **1** and **2** is not known and will vary from solvent to solvent, the amine side-chain has been previously suggested to influence the decomposition of varacin **1**.[13,37] Nucleophilic substitution reactions of polysulfanes are known with a wide range of nucleophiles.[38] Scales on thiophilicity rank 1° amine below that of thiolate ion and phosphine;[39] however, the weakly thiophilic ammonia molecule has been reported to attack a trisulfane.[40] Nucleophilic attack depends on the nature of the nucleophile and the substrate.[38] The preference of amine for electron-deficient S1 may be viewed as an event preceding the regioselective amination of the pentathiepin ring.

2.2.3 Amination of the Pentathiepin Ring. The Unimolecular Mechanism.

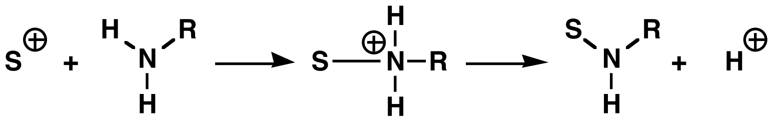
Evidence for an intramolecular (nucleophilic) reaction of amine in pentathiepin chemistry is provided. Interestingly, a number of antitumor agents are activated by nucleophilic addition.[41-43] We find the character of the substituent on the amine plays a role in the interconversion of pentathiepin to the polysulfur ion intermediate (Scheme 2).

Pentathiepins fall in two separate classes that are distinguished by the presence (**1**, **2**, **7**, **9**, and **14-19**) or absence (**10**, **11**, and **20**) of a hydrogen attached to nitrogen. The dependence on pentathiepin ring-opening was computed by comparing the substituents $-NH_2$ (**15**), -NHMe (**19**), and $-NMe_2$ (**20**). The reaction surface is different in pentathiepins bearing amine NH_2/NHMe versus NMe_2 substituents even though the S1—N bond distance is relatively insensitive to amine substitution (compare **15B**, **19**, and **20**, Table 2). The reaction is based on quaternization of amine with pentathiepin sulfur where the resulting aminium salt can only be deprotonated in those substituents bearing a hydrogen on N (e.g., **15** and **19**). The deprotonation gives rise to a destabilizing (sulfur) displacement process (Scheme 2).

Computations predict a unimolecular destabilization of pentathiepin in those substrates bearing hydrogen(s) on amine (–NH_2 and –NHMe), but not for the tertiary amine (–NMe_2) (Scheme 5). The behavior of the amine group varies with its conformation in pentathiepin and with its substitution pattern. The interconversion of pentathiepin to sulfenamide is characterized by (i) the proper orientation of the amine chain and (ii) by proton loss, which leads to a reduced magnitude of the interconversion barrier. The predicted barrier for nucleophilic displacement on the polysulfur ring for –NH^- (11 kcal/mol) is reduced compared to –NMe_2 (>40 kcal/mol) on the B3LYP/6-31(d) potential surface. To relate neutral and anionic reaction surfaces an isodesmic reaction involving HS^- and H_2S was used. In the case of **20**, B3LYP/6-31G(d) defaults do not predict the collapse to zwitterion **21**, suggesting only a reversible addition to S1 (Scheme 5A). Undoubtedly, pentathiepins **15**, **19**, and **20** are influenced differently by steric and electrostatic effects. Interconversion of pentathiepin to polysulfur ion, however, is ascribed to the necessity for deprotonation to form a new sulfenamide bond (Pathway I and II, Scheme 2). Neutral amine is suggested to lose a proton only after coordination with S1 in a symbiotic fashion where additional stabilization exists with pentathiepin ring-opening and desulfurization. This is consistent with the computed data regarding the RNH_2, RNHMe, and $RNMe_2$ derivatives. The pKa traditionally required for amine deprotonation is well above 20, which is not biologically relevant. We believe the mechanism of substitution involves a 2-step process[44] as shown in Scheme 6. The ammonium salt acquires added stability relative to separated S^+ (electrophilic pentathiepin sulfur) and RNH_2, by existing as a tetrahedral intermediate about N. The computational results provided detail about the mechanism of S—N coordination and isomerization of the amine group in pentathiepin accompanied by ring-closure to sulfenamide. The quaternization of N during substitution increases the acidity of the amine hydrogens, where the developing positive charge in the transition state is stabilized in a polar medium. Structures **15BE** and **15DF** (Figure 3) represent the transition states for conversion to polysulfur ion after the proton is lost. Loss of the positive charge is not an alternative for a pentathiepin with a 3° amine (Scheme 5A), and precludes an intramolecular displacement reaction.



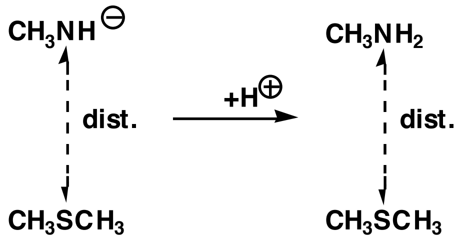
Scheme 5. Computationally predicted unimolecular destabilization of the pentathiepin ring.



Scheme 6. Mechanism of substitution illustrated as a 2-step process.

Interestingly, amine chemical reactions with elemental S_8 are similar to those with pentathiepin (Scheme 5). Davis and Nakshbendi[35] discovered that open-chain polysulfur ions can originate from a reaction of S_8 with 1° and 2° amine, but not with 3° amine (Scheme 5B). Up to now the role of amine in pentathiepin chemical reactions, let alone biochemical processes, was a poorly understood phenomenon. The new mechanism outlined here suggests deprotonation of amine makes pentathiepin sulfur susceptible to nucleophilic attack, and would be favored further in a basic environment. Work in organic synthesis has implicated basic conditions as an element for addition-elimination of higher order polysulfanes.[38] The biological conditions where nucleophilic activation proceeds are anticipated to result from a compromise among ideals for local basicity,[44] the orientation of the amine side chain, and the nature of amine substituent. Neutral or low pH conditions *in vivo* may render external nucleophiles such as glutathione more susceptible to bimolecular attack on pentathiepins (general base catalysis) compared to internal amination. In a similar vein, tertiary amines have been added in synthetic reactions as a means to control pH and to enhance thiol nucleophilicity.[45,46] When comparing the IC_{50} of amine-substituted **7** vs unsubstituted **8**, there is only about a factor of 20 difference, which may point to bimolecular activation as having significance in the mechanism. A much greater difference would be expected if the amine group was the sole determinant in the biological activity of this class of compound. At higher pH amine nucleophilicity should become competitive in the pentathiepin natural products since the proton affinity will decrease upon contact of the $-NH_2$ lone pair with the polysulfur ring (Scheme 4). Consistent with this suggestion is the fact that the proton affinity of NH_3 decreases by 30 kcal/mol when it approaches the sulfur of dimethyl sulfide at a distance of 5.0 Å compared to 2.5

Å (Scheme 7).



dist. (Å)	proton affinity (kcal/mol)
2.5	408
5.0	438

Scheme 7. Decrease of the proton affinity.

DFT calculations lead us to predict a mechanism for intramolecular attack of amine as the key step leading to sulfenamide intermediates. Two pathways may be considered for sulfenamide S-N bond formation (Pathway I and II, Scheme 2) resulting from elimination across different pentathiepin S-S bonds. Pentathiepin **15B** can convert to **15E** via transition structure **15BE** calculated to be 10.6 kcal/mol, with gas-phase B3LYP/6-31G(d) calculations (Figure 2 and 3). The computation reveals that **15B** is lower in energy than **15D** for the ring-opening process and the production of polysulfur ion. The TS which converts **15D** to the 7-membered ring **15F** is significantly higher (**15DF**, 24.8 kcal/mol). We find that an increase in dielectric constant from the gas-phase (ε=0) value to that of water (ε=80.1) has a minor effect on the energetics in formation polysulfur ion. The barrier separating **15B/E** and **15D/F** is calculated as 12.0 and 25.1 kcal/mol, respectively, with solution-phase PCM//B3LYP/6-31G* calculations.

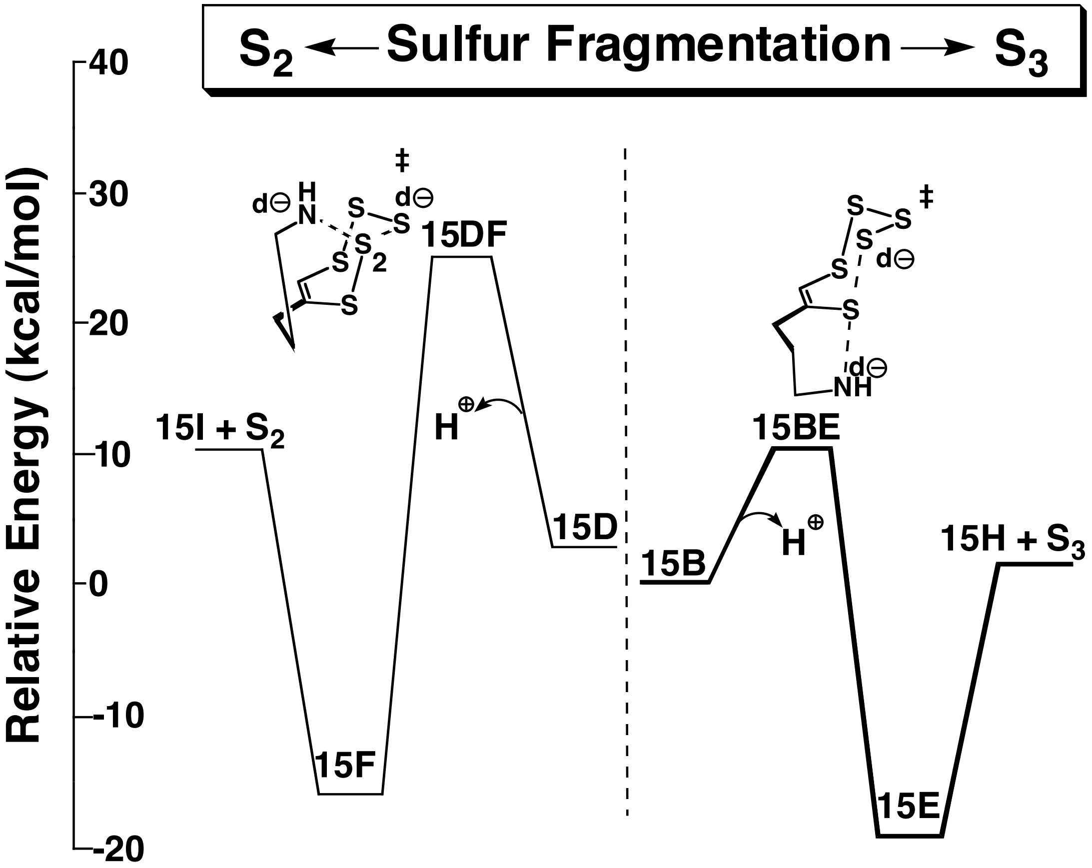


Figure 2. B3LYP/6-31G* calculated potential energy surface. The gas-phase energies are shown, and the solution phase energies are discussed in the text. Energies of **15I** + S_2 and **15H** + S_3 were optimized with the reagents separated at a 5 Å distance. Bolded line indicates the pathway with the lowest cost in energy.

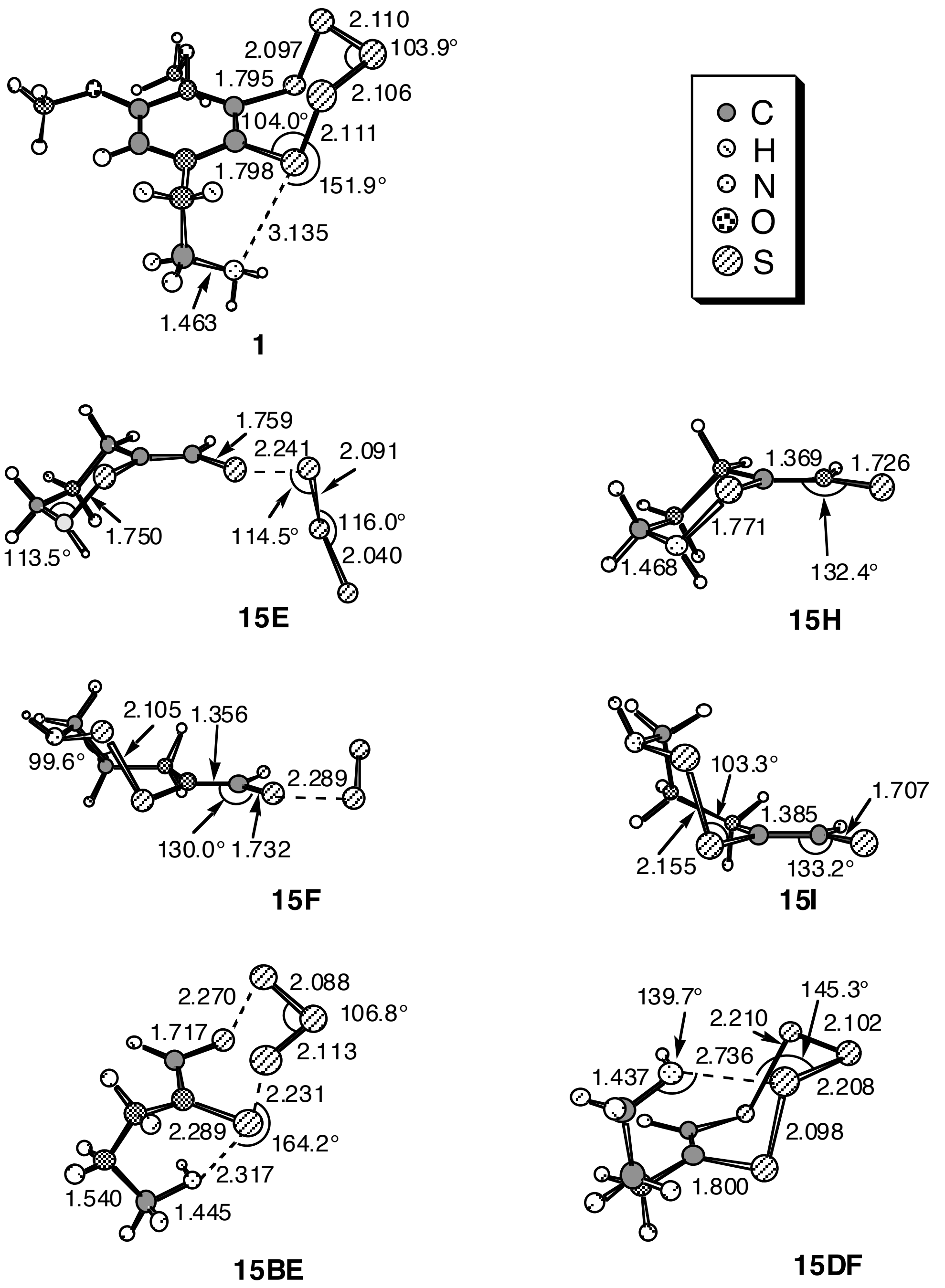


Figure 3. B3LYP/6-31G(d) optimized geometries (bond distances are in Å, angles are in degrees).

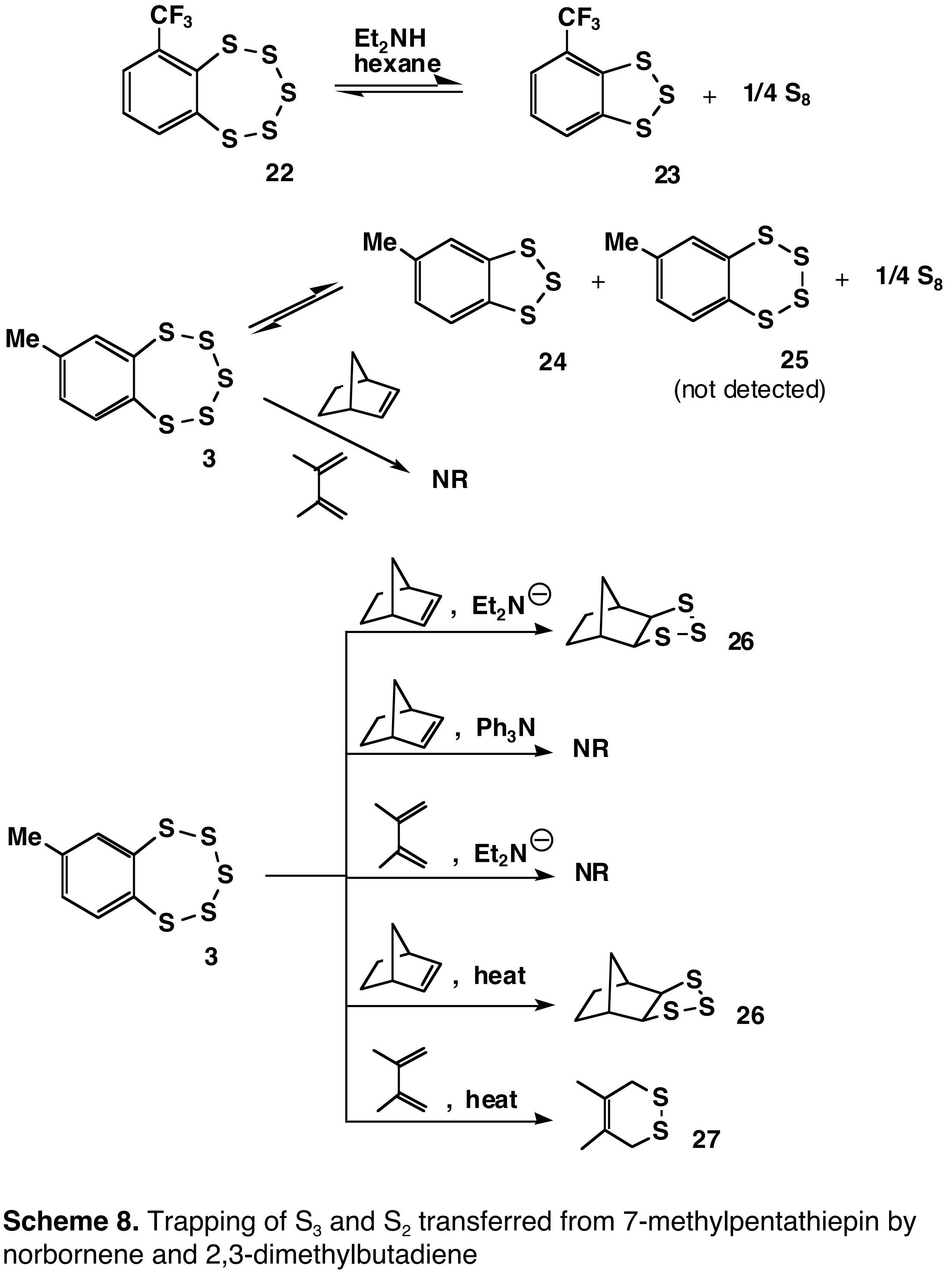
It is possible to envision pentathiepin natural products as biological precursors of triatomic sulfur, S_3. A pentathiepin containing a 1° or 2° amine is predicted to set up an apical attack on the S1 site and then eliminate the S1-S2 bond. The resulting species, such as polysulfur ion **15E**, is accompanied by a long S4-S5 bond, which may be preorganized for S_3 elimination (Figures 2 and 3). An energetically low-lying process is found on the B3LYP/6-31G(d) potential surface, which corresponds to stepwise S_3 fragmentation (**15H**, 19.8 kcal/mol). Our computational data indicate that S_3 loss has the lowest cost in energy, in particular because of the preference for attack of the amine at the S1 position of pentathiepin (Pathway I, Scheme 2). This study also predicted the formation of S_2, but in a higher-energy process that originates from polysulfur ion **15F** (24.7 kcal/mol). Our computational data reveal that amine-effects underpin the S_3 cleavage process (Pathway VI, Scheme 2). It appears that nucleophilicity of the amine group at S1 is owed in part to its decreased distance to the polysulfur ring. We are aware of established routes for generation of S_2 in a variety of organosulfur systems;[47] however, our computational data do not support the S_2 intermediate in an amine-dependant pentathiepin decomposition.

2.2.4 Amine-Pentathiepin Interaction and Activated Sulfur. Experimental Support.

Further information was desired on whether pentathiepin can lead to activated sulfur species with amine additives. Diethylamine is known to catalyze an interconversion of (trifluoromethyl)benzopentathiepin **22** with benzotrithiole

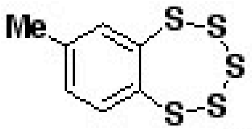
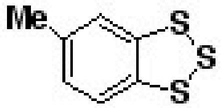
23 and cyclic S_8 (Scheme 8).[15] Because Nature provides an internal amine, we thought that natural product pentathiepins may possess a built-in parameter for equilibrating benzotrithiole and for donating an S_3 unit (vide supra). In our initial efforts, we sought to examine the effect of solvent and nitrogen additive on the equilibrium of pentathiepin **3** and trithiole **24** (Table 3).

Solvent and nitrogen additives appear to affect the equilibrium between **3** and **24**. The interplay between **3** and **24** is reduced in the nonpolar solvents benzene and chloroform, but enhanced in the polar solvents methanol and DMF (entries 1-5, Table 3). Mixtures of solvents [acetonitrile:water (95:5), methanol:water (95:5), benzene:DMF (10:3)] also yielded an enhanced conversion between **3** and **24** (entries 6-8). Analyses of the data provided evidence that an equilibrium is established in a few hours between **3** and **24** with a reasonable correlation between mass spectrometry and NMR. Data collected after 2 hours and after 24 hours with the two methods are identical to within experimental error (±5%). A GC and reversed-phase HPLC analysis was used to study the equilibrium between **3** and **24** partly dissolved in a mixture of acetonitrile:water and methanol:water. Here the experimental error between the two analytical methods is approximately ±10%. We find that insoluble material forms in all solvents examined, but to a vastly reduced extent in benzene and DMF. Based on the data in Table 3, one can suggest that pentathiepin has reduced stability in acetonitrile:water, methanol:water, and benzene:DMF mixtures compared to benzene or chloroform. Interestingly, a similar solvent effect is observed, although to a reduced extent, for equilibration of the three sulfur rings of $S_8 \rightleftharpoons S_6 + S_7$.[48] Added NH_3 or Et_2NH have an effect on the equilibrium of **3** and **24** (entry 9 and 10). Amine appears to affect the equilibrium despite a low concentration. This amine-dependent reaction is again reminiscent of that established for cyclic S_8 and the equilibrium with its cyclic allotropes S_6 and S_7.[15,48] Under our conditions related structures, such as tetrathiin **25**, were not detected with GCMS, HPLC, nor NMR.



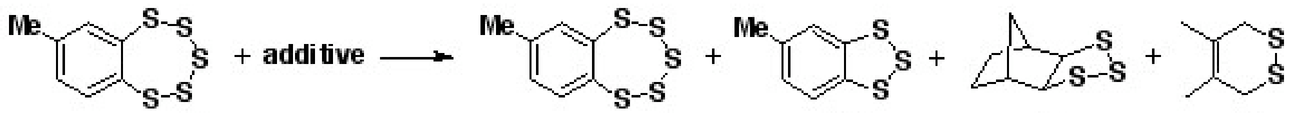
Scheme 8. Trapping of S_3 and S_2 transferred from 7-methylpentathiepin by norbornene and 2,3-dimethylbutadiene

Table 3. Effect of solvent and nitrogen additive on the equilibrium of 7-methylbenzopentathiepin (**3**) and 7-methylbenzotrithiole (**24**).

				Product Distribution		
	Reagent	Solvent	Additive	 3	 24	cyclic S_8
1.	**3**[a]	C_6H_6	-	91[b]	9[b]	c
2.	**3**[d]	C_6D_6	-	90[e]	10[e]	c
3.	**3**[d]	$CDCl_3$	-	77[e]	23[e]	c
4.	**3**[d]	CD_3OD	-	48[e]	52[e]	c
5.	**3**[a]	DMF	-	26[b]	74[b]	c
6.	**3**[f]	$CH_3CN:H_2O(95:5)$	-	64[b]	36[b]	c
7.	**3**[g]	$CH_3OH:H_2O(95:5)$	-	53[h]	47[h]	c
8.	**3**[i]	C_6H_6:DMF(10:3)	-	46[b]	54[b]	c
9.	**3**[a]	C_6H_6	$NH_{3(g)}$	83[b]	17[b]	c
10.	**3**[i]	C_6H_6	Et_2NH[j]	78[b]	22[b]	c

[a] $[\mathbf{3}]_{Init}$=0.41 M. [b] Ratio of **3**:**24** at 25°C determined by GCMS. [c] Elemental sulfur S_8 concentration not determined due to the different solubility in the solvents examined. Insoluble material is observed in all solvents, but to a reduced extent in benzene and DMF. [d] $[\mathbf{3}]_{Init}$=0.06 M. [e] Ratio of **3** : **24** at 25°C determined by NMR. [f] $[\mathbf{3}]_{Init}$=0.12 M. [g] $[\mathbf{3}]_{Init}$=0.01 M. [h] Ratio of **3** : **24** determined by HPLC. [i] $[\mathbf{3}]_{Initial}$=1.6 mM. [j] $[Et_2NH]$=1.6 mM.

Table 4. Effect of amine additive and temperature on the reaction of 7-methylbenzopentathiepin (**3**) in the presence of olefins.[a,b]



	3	**24**	**26**	**27**
1. PhH, 25°C, norbornene[c] or butadiene[d,e]	89	11	0	0
2. PhH:DMF, 25° C, norbornene[f] or butadiene[e]	45	55	0	0
3. DMF, 25°C, norbornene[g] or butadiene[e]	23	77	0	0
4. H_2O:MeCN, 25°C, norbornene or butadiene[h,i]	61	39	0	0
5. PhH:DMF, 25° C, norbornene, Et_2NH[j]	39	60	≤1	-
6. H_2O:MeCN, 25°C, norbornene, Et_2NH[h,i]	44	48	8	-
7. PhH:DMF, 25° C, norbornene, Et_2N^- (in situ)[j,k]	29	64	7	-
8. H_2O:MeCN, 25°C, norbornene, Et_2N^- (in situ)[h,l]	40	50	10	-
9. PhH:DMF, 25° C, norbornene, Ph_3N[k,m]	39	60	0	-
10. PhH:DMF, 25°C, butadiene, Et_2N^- (in situ)[j,i,n]	45	55	-	0
11. PhH, 43°C, norbornene[o]	27	18	55	-
12. PhH, 43°C, butadiene[p]	71	13	-	16

[a] Ratio of **3**:**24**:**26**:**27** determined by GC or GCMS. [b] Elemental sulfur S_8 concentration not determined due to the different solubility in the solvents examined. [c] $[\mathbf{3}]_{\text{Initial}}$=0.55 M; [norbornene]=1.0 M. [d] Butadiene represents the compound 2,3-dimethyl-1,3-butadiene. [e] $[\mathbf{3}]_{\text{Initial}}$=0.55 M; [butadiene]=0.55 M. [f] $[\mathbf{3}]_{\text{Init}}$=1.6 mM; [norbornene]=0.10 M. [g] $[\mathbf{3}]_{\text{Init}}$=0.41 M; [norbornene]=1.0 M. [h] $[\mathbf{3}]_{\text{Init}}$=0.1 M; [norbornene]=0.1 M or [butadiene]=0.1 M $[Et_2NH]$=0.1 M. [i] Insoluble material observed in significant amounts in runs conducted with water:acetonitrile. Butadiene forms some polymer in the water:acetonitrile mixture. [j] $[\mathbf{3}]_{\text{Init}}$=50 mM; $[Et_2NH]$=50 mM. [k] Runs averaged using $[n\text{Bu}_4\text{NF}]$=50 mM and [Na]=0.20 M. [l] Runs averaged using [NaOH]=0.1 M and [KF]=0.1 M. [m] $[\mathbf{3}]_{\text{Initial}}$=50 mM; $[Ph_3N]$=50 mM. [n] $[\mathbf{3}]_{\text{Initial}}$=0.10 M; [butadiene]=0.10 M. [o] $[\mathbf{3}]_{\text{Initial}}$=0.10 M; [norbornene]=0.10 M. [p] $[\mathbf{3}]_{\text{Initial}}$=0.55 M; [butadiene]=0.55 M.

That amine is integrally involved with sulfur loss from a pentathiepin was evident with an indirect study, by detecting sulfuration of an olefinic linkage (Scheme 8). Vapor-phase UV[49] and low-temperature argon IR methods[50] have observed S_3 directly, but are not compatible with our solution-phase method. Undoubtedly, challenging tasks remain in determining the structural assignment of intermediates in polysulfur reactions. Control experiments demonstrated that norbornene and 2,3-dimethyl-1,3-butadiene are inert to **3** at room temperature (entries 1-4, Table 4). A benzene:DMF solution (10:3) containing diethylamine when added to **3** and norbornene gave trace concentrations of trithiane **26** (entry 5). We then investigated the feasibility of the reaction in a more biologically relevant medium instead of benzene and DMF. Intriguingly, in water:acetonitrile (4:1) after 4 hours at 28°C, diethylamine initiated a small amount of sulfur transfer from **3** to norbonrene (entry 6). The problem of solubility precluded an analysis of efficiency for sulfur transfer from **3** to norbornene. One might suggest that the enhanced formation of **26** in water:acetonitrile (4:1) is that a developing aminium ion, $Me_2NH^{d+}{-}S$, is solvated where deprotonation can coincide with substitution. How efficient amine deprotonation would be in the presence of water may be linked to its nucleophilicity with the pentathiepin substrate. Predicting amine nucleophilicity is limited in organic displacement reactions.[51,52] The influence of additives to deprotonate diethylamine and how solvent interactions accompany reactions of **3** was explored to reveal what additives may enhance this process.

Pentathiepin data was collected under different conditions, using additives that aid in sulfur loss triggered by diethylamine. Addition of tetrabutylammonium fluoride ($n\mathrm{Bu}_4\mathrm{NF}$) or sodium metal (Na) enhanced the S_3-unit transfer in benzene:DMF (compare entry 5 and 7, Table 4). Under the conditions with added $n\mathrm{Bu}_4\mathrm{NF}$, fluoride ion appears to deprotonate diethylamine, which in turn initiates the sulfur transfer reaction. The reaction was also analyzed with NaOH and KF in a water:acetonitrile (4:1) solution (entry 8). These additives amounted to only a small enhancement in the sulfur transfer from pentathiepin **3** to norbornene (compare entry 6 and 8). Control reactions demonstrated that pentathiepin reacts with the amine base, and not KF nor NaOH under the conditions.[14,15] The low nucleophilicity of fluoride ion in water:acetonitrile may be expected to have little effect on amine deprotonation. These results suggest that amine activation and in situ generation of $\mathrm{Et}_2\mathrm{N}^-$ is in part responsible for the sulfur donor properties of pentathiepin in benzene:DMF (10:3). Desulfurization of **3** did not take place with $\mathrm{Ph}_3\mathrm{N}$ nor proceed in experiments which substituted 2,3-dimethyl-1,3-butadiene for norbornene (entries 9 and 10). Sulfur transfer, however, did increase substantially in the presence of heat. The thermal decomposition of **3** in the presence of norbornene and 2,3-dimethyl-1,3-butadiene yielded **26** as the major trapping product and **27** as the minor trapping product (compare entry 11 and 12). Pentathiepin **3** is experimentally implicated to be a source of S_3 and S_2 in the trapping experiments. The evidence for the existence of **26** and **27** was provided using mass spectrometry (Figure 4).[53]

The essential results are that a nitrogen nucleophile appears to be sufficient to open the pentathiepin ring. Experimentally it appears that loss of S_3 can occur thermally and to a small extent with diethylamide ion in benzene:DMF (10:3) or with free secondary amine in water:acetonitrile (4:1). It is tempting to reason that a high effective molarity of the internal amine may further influence factors related to pentathiepin decomposition. The effective molarity, which is relevant to the natural products **1** and **2** may not be obtainable in a bimolecular reaction[54] with free secondary amine and then compromise or minimize the promotion effect. We have not explored reactions of pentathiepin **3** with primary amine. The calculations discussed earlier suggest that such reactions would be similar. Benzopentathiepins have been noted as sulfuration agents previously in the context of sulfinate synthesis, but were not considered for the potential transfer of S_3 or S_2.[55]

Even though knowledge is limited on the existence of S_3 as a discrete intermediate in organic chemistry some interesting and potentially important questions are associated with the understanding of the nature of the pentathiepin reaction and the possibility of S_3 and S_2 transfers.[56] We are aware of the need to tread cautiously in interpreting the above trapping results. In the past 20 years there have been a significant number of papers dealing with sulfur transfer. Our evidence that amine influences an S_3-unit transfer from pentathiepin **3** is the first of its kind and may have biological implications. Sulfuration of alkene linkages adds knowledge to the amine pathway of pentathiepin activation. Nakayama and co-workers have had success in determining the presence of S_3 by interpreting results of norbornene and 2,3-dimethyl-1,3-butadiene trapping from a 1-adamantyl-tert-butyltetrathiolane 2,3-dioxide system.[57,58] Since direct experimental observation of the S_3 intermediate in reactions of pentathiepins has not been possible, our theoretical calculations yield important information to predict the geometries and energies of the intermediates. The present study brings together a set of computational and experimental results and incorporates past work in the area to unfold the possible role of amine in the mechanism of pentathiepin bioactive agents. The involvement of amine as an activation element and the resulting S_3-transfer would be associated with a new form of biological action displayed by naturally occurring pentathiepins.

2.2.5 Conclusion.

After ten years of research, an understanding of the mechanism of action of pentathiepin antitumor agents is beginning to emerge. Our computations and experiments provide the first evidence that amination underpins the production of reactive sulfur intermediates, which provides mechanistic insight on the process that gives rise to pentathiepin biological activity. Primary or secondary amine allows for an intramolecular addition to the pentathiepin ring at the nearest sulfur (S1). In contrast, tertiary amine adds reversibly to S1, because nitrogen can not lose its positive charge by deprotonation. This precludes the catalysis step. An energetically low-lying process is characterized corresponding to S_3-loss triggered by nucleophilic activation with a 1° or 2° amine. Pentathiepin desulfurization via S_3-unit transfer is supported by a trapping study with norbornene. That amine influences the reactivity of pentathiepin adds understanding to the mechanism of bioactivity. The study provides a new mechanistic view to help resolve the question posed earlier in the paper on why some pentathiepins are more biologically active than others. We anticipate the findings from this study will be useful in the generation of new pentathiepins and the manipulation of their biochemical properties.

2.3 Experimental Section.

2.3.1 General Aspect.

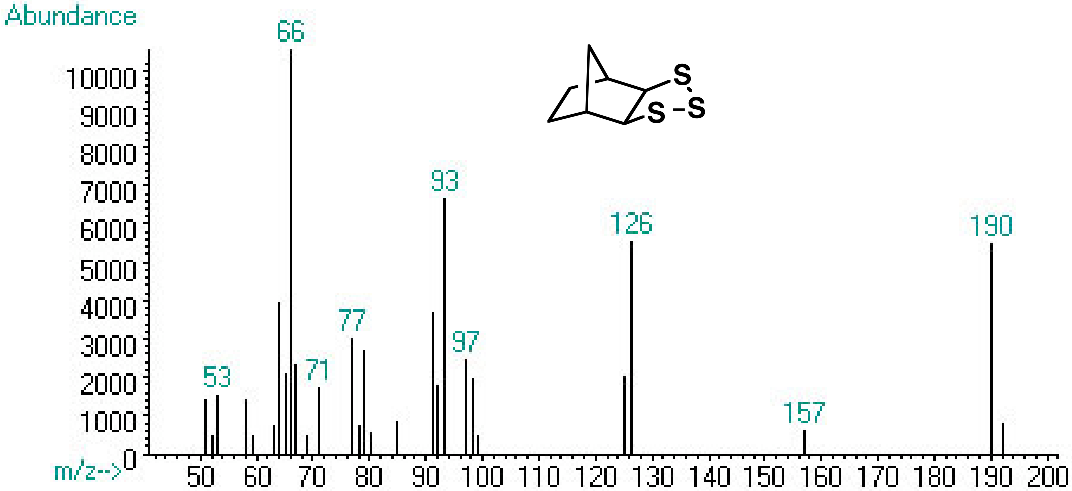
Diethylamine, triphenylamine, sodium (lump in kerosene), biphenyl, triphenylmethane, norbornene, 2,3-dimethyl-1,3-butadiene, tetrabutylammonium fluoride (1.0 M solution in tetrahydrofuran), 4-methyl-1,2-benzodithiol, toluene (anhydrous), acetonitrile (anhydrous), dimethylformamide (anhydrous), and benzene (anhydrous) were used as received. The purity of the reagents was checked by GC or GC/MS prior to use. Pentathiepin **3** was synthesized and purified using a literature method.[15] Relative concentrations of pentathiepin **3**, 7-methylbenzotrithiole (**24**), norbornene, and *exo*-3,4,5-trithiatricyclo[5.2.1.$0^{2,6}$]decane (**26**) were determined by reference to calibration curves constructed from authentic samples. Gas chromatographic data were collected on one of two gas chromatographs, a Hewlett-Packard GC/MS instrument consisting of a 5890 series GC and a 5988A series mass selective detector, or on a Shimadzu-17A auto-sampler capillary gas chromatograph.

Additional measurements were carried out on a Bruker (250 MHz) FT-NMR spectrometer and a Perkin Elmer HPLC equipped with an LC 250 pump, a C_{18} column, and a diode array detector.

2.3.2 S_3-Trapping Studies.

The S_3 trapping studies were carried out in 1-mL solutions of benzene, chloroform, methanol, dimethylformamide, and water, or mixtures there of, which contained 0.0016-0.41 M **3**, 0.10-1.0 M norbornene or 2,3-dimethyl-1,3-butadiene, and 2.5×10^{-3} M biphenyl or triphenylmethane as an internal standard. A solution containing diethylamine (0.005-9.67 M) with tetrabutylammonium fluoride (0.05 M), potassium fluoride (0.1 M), sodium hydroxide (0.1 M), or sodium (0.20 M) was added to initiate a reaction. In benzene, DMF, and methanol solvents, the chemistry was examined after 1 hour at 43°C and at ambient temperatures. Otherwise the samples were incubated for 4 hours at 28°C before analysis. Control experiments demonstrated that norbornene and 2,3-dimethyl-1,3-butadiene are inert to **3** in the absence of diethylamine at room temperature. Aliquots were removed to examine the percent conversion of the reaction components. The concentrations of products were determined by gas chromatography, NMR, and HPLC.

Mass Spectrum of 26



Mass Spectrum of 27

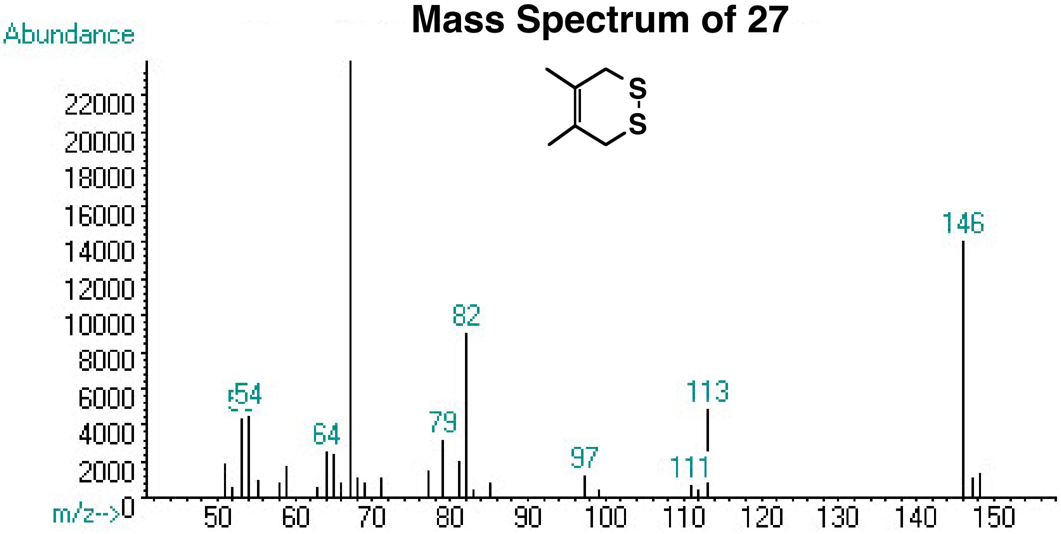


Figure 4. Mass sectrum of **26** and **27**.

2.3.3 Theoretical Methods.

Density functional theoretical (DFT) calculationswere performed using the Gaussian-94 and -98 program packages.[17,18] Geometries were optimized using the DFT exchange-correlation of B3LYP along with the 6-31G(d) basis set, which has been shown to reproduce geometries in a variety of experimental systems.[19] The geometries and energies obtained are in good agreement with calculations using a basis set with diffuse functions [6-311+G(d)]. The majority of the stationary points were examined by harmonic vibrational frequency calculations. Polarized continuum model (PCM)[20,21] and self-consistent reaction field (SCRF) single-point calculations at the B3LYP/6-31(d) level were performed on stationary points to model solvent effects. The dielectric constant of 80.10 was used to simulate an aqueous environment. The resulting DFT geometries adequately reproduce X-ray crystal structures of pentathiepins,[22,23] other sulfur natural products,[24,25] and inorganic polysulfur compounds.[22]

Descriptions of the geometries of all stationary points and absolute energies

Varacin **1**

C(1)	-0.539	1.135	-1.142
C(2)	-1.768	0.657	-0.629
C(3)	-1.763	-0.220	0.449
C(4)	-0.570	-0.630	1.067
C(5)	0.654	-0.149	0.573
C(6)	0.664	0.716	-0.557
S(7)	2.179	-0.699	1.353
S(8)	2.839	0.987	2.438
S(9)	4.143	2.025	1.150
S(10)	2.857	2.872	-0.293
S(11)	2.170	1.224	-1.393

C(12)	-0.690	-1.617	2.210
C(13)	-0.646	-3.090	1.748
N(14)	0.643	-3.424	1.142
O(15)	-0.547	2.059	-2.146
O(16)	-2.885	1.126	-1.247
C(17)	-0.838	1.564	-3.457
C(18)	-4.153	0.733	-0.739
H(19)	-2.701	-0.590	0.849
H(20)	-1.646	-1.456	2.723
H(21)	0.100	-1.451	2.948
H(22)	-1.422	-3.246	0.988
H(23)	-0.913	-3.725	2.611
H(24)	0.591	-4.319	0.661
H(25)	1.361	-3.512	1.857
H(26)	-0.820	2.434	-4.115
H(27)	-1.827	1.095	-3.495
H(28)	-0.071	0.849	-3.779
H(29)	-4.297	-0.353	-0.813
H(30)	-4.892	1.240	-1.360
H(31)	-4.278	1.045	0.306

B3LYP/6-31G* Optimization
–2585.008441

Lissoclinotoxin A **2**

C(1)	-1.409	0.985	-0.703
C(2)	-2.628	0.437	-0.254
C(3)	-2.619	-0.591	0.678
C(4)	-1.429	-1.096	1.221
C(5)	-0.205	-0.548	0.790
C(6)	-0.202	0.476	-0.197
S(7)	1.323	-1.212	1.466
S(8)	2.000	0.291	2.783
S(9)	3.297	1.506	1.652
S(10)	2.002	2.562	0.363
S(11)	1.299	1.097	-0.962
C(12)	-1.543	-2.242	2.204
C(13)	-1.508	-3.628	1.526
N(14)	-0.226	-3.871	0.866
O(15)	-1.423	2.049	-1.555
O(16)	-3.775	0.972	-0.762
C(17)	-1.763	1.765	-2.917
H(18)	-3.569	-1.004	1.017
H(19)	-2.493	-2.156	2.744
H(20)	-0.746	-2.189	2.951
H(21)	-2.291	-3.665	0.757
H(22)	-1.773	-4.385	2.285
H(23)	-0.286	-4.682	0.254

H(24)	0.497	-4.071	1.553
H(25)	-4.536	0.534	-0.350
H(26)	-1.746	2.726	-3.434
H(27)	-2.761	1.323	-2.994
H(28)	-1.020	1.096	-3.368

B3LYP/6-31G* Optimization
–2545.7017627

Compound **14A**

C(1)	-0.692	0.687	-1.847
C(2)	-1.909	0.466	-1.205
C(3)	-1.924	0.033	0.115
C(4)	-0.730	-0.200	0.830
C(5)	0.499	0.004	0.170
C(6)	0.513	0.455	-1.175
S(7)	2.018	-0.431	1.039
S(8)	2.871	1.404	1.609
S(9)	4.163	1.923	0.029
S(10)	2.850	2.458	-1.530
S(11)	2.004	0.646	-2.165
C(12)	-0.851	-0.688	2.260
C(13)	-0.849	-2.224	2.389
N(14)	-2.007	-2.811	1.707
H(15)	-0.666	1.044	-2.871
H(16)	-2.842	0.647	-1.732
H(17)	-2.870	-0.132	0.622
H(18)	-1.792	-0.319	2.681
H(19)	-0.033	-0.288	2.866
H(20)	-0.930	-2.471	3.454
H(21)	0.124	-2.613	2.044
H(22)	-2.067	-3.804	1.928
H(23)	-1.877	-2.755	0.698

B3LYP/6-31G* Optimization
–2355.9730922

Compound **14B**

C(1)	-1.190	0.822	-1.494
C(2)	-2.373	0.587	-0.798
C(3)	-2.315	0.140	0.516
C(4)	-1.093	-0.074	1.173
C(5)	0.106	0.183	0.473
C(6)	0.049	0.618	-0.876
S(7)	1.675	-0.121	1.311
S(8)	2.432	1.805	1.704
S(9)	3.666	2.290	0.067
S(10)	2.294	2.672	-1.485
S(11)	1.486	0.791	-1.945
C(12)	-1.139	-0.632	2.579

C(13)	-1.093	-2.176	2.606
N(14)	0.169	-2.682	2.066
H(15)	-1.216	1.167	-2.523
H(16)	-3.332	0.755	-1.279
H(17)	-3.238	-0.046	1.061
H(18)	-2.072	-0.314	3.060
H(19)	-0.316	-0.238	3.183
H(20)	-1.905	-2.563	1.978
H(21)	-1.302	-2.507	3.638
H(22)	0.092	-3.670	1.833
H(23)	0.915	-2.595	2.753

B3LYP/6-31G* Optimization
–2355.9732087

Compound **14C**

C(1)	-0.614	-0.383	-2.153
C(2)	-1.833	-0.533	-1.494
C(3)	-1.853	-0.857	-0.144
C(4)	-0.669	-1.046	0.587
C(5)	0.567	-0.903	-0.084
C(6)	0.587	-0.569	-1.463
S(7)	2.089	-1.262	0.815
S(8)	2.929	0.613	1.249
S(9)	4.222	1.015	-0.367
S(10)	2.915	1.401	-1.974
S(11)	2.083	-0.465	-2.457
C(12)	-0.782	-1.356	2.066
C(13)	-0.871	-0.091	2.946
N(14)	-2.133	0.620	2.722
H(15)	-0.585	-0.117	-3.205
H(16)	-2.764	-0.385	-2.034
H(17)	-2.800	-0.947	0.377
H(18)	0.066	-1.955	2.406
H(19)	-1.684	-1.959	2.234
H(20)	-0.050	0.585	2.683
H(21)	-0.710	-0.392	3.996
H(22)	-2.078	1.563	3.100
H(23)	-2.886	0.154	3.226

B3LYP/6-31G* Optimization
–2355.9716335

Compound **14D**

C(1)	-0.972	-0.294	-2.064
C(2)	-2.203	-0.518	-1.448
C(3)	-2.245	-0.882	-0.109
C(4)	-1.075	-1.044	0.654
C(5)	0.174	-0.839	0.023
C(6)	0.215	-0.456	-1.344

S(7)	1.687	-1.195	0.937
S(8)	2.423	0.691	1.494
S(9)	3.763	1.192	-0.057
S(10)	2.495	1.608	-1.689
S(11)	1.734	-0.254	-2.291
C(12)	-1.242	-1.406	2.119
C(13)	-1.682	-0.212	2.989
N(14)	-0.639	0.812	3.045
H(15)	-0.924	0.008	-3.105
H(16)	-3.123	-0.398	-2.013
H(17)	-3.205	-1.047	0.373
H(18)	-0.315	-1.805	2.531
H(19)	-2.009	-2.187	2.198
H(20)	-1.843	-0.581	4.009
H(21)	-2.660	0.158	2.627
H(22)	-0.891	1.534	3.718
H(23)	-0.552	1.279	2.144

B3LYP/6-31G* Optimization
–2355.971851

Compound **15A**

S(1)	1.464	1.051	-3.094
C(2)	0.078	0.681	-2.031
C(3)	0.083	0.286	-0.743

S(4)	1.595	0.073	0.233
S(5)	2.228	2.016	0.676
S(6)	3.477	2.590	-0.923
S(7)	2.123	2.951	-2.499
C(8)	-1.198	-0.075	-0.025
C(9)	-1.274	-1.552	0.405
C(10)	-2.540	-1.861	1.215
N(11)	-2.583	-1.062	2.444
H(12)	-0.882	0.744	-2.540
H(13)	-2.048	0.153	-0.681
H(14)	-1.307	0.550	0.869
H(15)	-0.393	-1.809	1.010
H(16)	-1.246	-2.200	-0.480
H(17)	-2.587	-2.951	1.385
H(18)	-3.424	-1.599	0.619
H(19)	-1.870	-1.391	3.094
H(20)	-3.477	-1.188	2.913

B3LYP/6-31G* Optimization
–2241.631422

Compound **15B**

S(1)	0.931	1.264	-2.412
C(2)	-0.391	0.999	-1.246
C(3)	-0.308	0.597	0.038

S(4)	1.260	0.375	0.924
S(5)	1.883	2.370	1.243
S(6)	3.061	2.890	-0.430
S(7)	1.654	3.189	-1.969
C(8)	-1.579	0.297	0.819
C(9)	-2.078	-1.161	0.716
C(10)	-1.154	-2.200	1.352
N(11)	0.163	-2.174	0.713
H(12)	-1.384	1.134	-1.674
H(13)	-2.374	0.951	0.440
H(14)	-1.429	0.558	1.872
H(15)	-2.237	-1.411	-0.343
H(16)	-3.061	-1.226	1.200
H(17)	-1.641	-3.189	1.312
H(18)	-1.009	-1.959	2.412
H(19)	0.117	-2.511	-0.247
H(20)	0.833	-2.756	1.210

B3LYP/6-31G* Optimization
–2241.6323843

S(4)	1.524	-1.078	0.466
S(5)	2.294	0.757	1.117
S(6)	3.646	1.376	-0.377
S(7)	2.385	1.969	-1.961
C(8)	-1.321	-1.030	0.008
C(9)	-1.461	-1.589	1.433
C(10)	-1.323	-0.557	2.565
N(11)	-2.253	0.568	2.377
H(12)	-0.757	-0.027	-2.337
H(13)	-1.745	-1.766	-0.688
H(14)	-1.964	-0.145	-0.076
H(15)	-0.754	-2.410	1.600
H(16)	-2.469	-2.016	1.500
H(17)	-0.273	-0.243	2.666
H(18)	-1.587	-1.057	3.504
H(19)	-1.822	1.279	1.789
H(20)	-2.422	1.024	3.271

B3LYP/6-31G* Optimization
–2241.6217788

Compound **15C**

S(1)	1.624	0.181	-2.746
C(2)	0.168	-0.182	-1.782
C(3)	0.071	-0.671	-0.529

Compound **15D**

S(1)	1.711	-0.114	-2.908
C(2)	0.190	-0.268	-1.989
C(3)	0.006	-0.594	-0.693

S(4)	1.386	-0.868	0.432
S(5)	2.128	1.052	0.821
S(6)	3.644	1.348	-0.611
S(7)	2.541	1.735	-2.372
C(8)	-1.430	-0.842	-0.209
C(9)	-1.704	-1.217	1.261
C(10)	-1.956	-0.039	2.211
N(11)	-0.773	0.807	2.389
H(12)	-0.695	-0.150	-2.614
H(13)	-1.832	-1.643	-0.842
H(14)	-2.016	0.054	-0.454
H(15)	-0.903	-1.853	1.657
H(16)	-2.612	-1.835	1.276
H(17)	-2.355	-0.443	3.159
H(18)	-2.749	0.589	1.786
H(19)	-0.081	0.317	2.953
H(20)	-1.020	1.649	2.906

B3LYP/6-31G* Optimization

–2241.6209665

Compound **15BE**

S(1)	0.892	1.195	-2.244
C(2)	-0.332	0.608	-1.193
C(3)	-0.173	-0.231	-0.088

S(4)	1.422	-0.657	0.600
S(5)	2.064	1.255	1.554
S(6)	3.151	2.325	0.092
S(7)	1.757	3.059	-1.279
C(8)	-1.412	-0.506	0.766
C(9)	-2.095	-1.807	0.312
C(10)	-0.979	-2.862	0.203
N(11)	0.203	-2.331	-0.437
H(12)	-1.345	0.963	-1.387
H(13)	-2.102	0.343	0.690
H(14)	-1.119	-0.594	1.818
H(15)	-2.552	-1.649	-0.676
H(16)	-2.900	-2.109	1.000
H(17)	-1.358	-3.757	-0.326
H(18)	-0.703	-3.193	1.216
H(19)	0.000	-2.117	-1.415

B3LYP/6-31G* Optimization

–2241.0415622

Compound **15E**

S(1)	1.199	0.670	1.842
C(2)	0.105	1.057	0.521
C(3)	-1.240	0.967	0.513
S(4)	-2.178	0.461	1.947

S(5)	4.171	-2.653	0.274
S(6)	3.421	-0.946	-0.553
S(7)	3.159	0.665	0.754
C(8)	-2.080	1.331	-0.693
C(9)	-3.057	0.207	-1.097
C(10)	-3.975	-0.223	0.059
N(11)	-3.275	-0.712	1.253
H(12)	0.608	1.419	-0.376
H(13)	-1.421	1.564	-1.539
H(14)	-2.666	2.240	-0.479
H(15)	-2.480	-0.664	-1.439
H(16)	-3.672	0.537	-1.947
H(17)	-4.65	-1.024	-0.274
H(18)	-4.601	0.622	0.375
H(19)	-2.727	-1.540	1.012

B3LYP/6-31G* Optimization
–2241.08877

Compound **15H**

S(1)	3.117	0.088	1.209
C(2)	1.805	0.306	0.110
C(3)	0.446	0.292	0.281
S(4)	-0.345	0.017	1.852
C(5)	-0.528	0.554	-0.846
C(6)	-1.570	-0.574	-1.020
C(7)	-2.353	-0.857	0.275
N(8)	-1.533	-1.229	1.434
H(9)	2.090	0.526	-0.930
H(10)	0.028	0.676	-1.788
H(11)	-1.082	1.496	-0.679
H(12)	-1.052	-1.494	-1.329
H(13)	-2.282	-0.320	-1.823
H(14)	-3.080	-1.667	0.104
H(15)	-2.924	0.037	0.561
H(16)	-1.000	-2.068	1.197

B3LYP/6-31G* Optimization
–1046.4846183

Compound **15H+S$_3$** Optimized at a 5.0 Å Fixed Distance

S(1)	1.743	2.117	2.221
C(2)	0.611	2.449	0.976
C(3)	-0.759	2.452	1.036
S(4)	-1.626	2.157	2.558
C(8)	-1.620	2.846	-0.144
C(9)	-2.815	1.903	-0.390
C(10)	-3.701	1.756	0.856
N(11)	-3.006	1.214	2.028

H(12)	1.021	2.727	-0.001
H(13)	-0.992	2.889	-1.043
H(14)	-2.021	3.862	0.014
H(15)	-2.444	0.911	-0.684
H(16)	-3.418	2.282	-1.229
H(17)	-4.551	1.093	0.642
H(18)	-4.111	2.733	1.142
H(19)	-2.664	0.277	1.810
S(5)	0.926	-4.735	-1.140
S(6)	2.324	-3.312	-1.275
S(7)	2.869	-2.403	0.405

B3LYP/6-31G* Optimization

–2241.05716

Compound **15DF**

S(1)	1.162	0.213	-2.670
C(2)	-0.133	-0.171	-1.556
C(3)	-0.027	-0.760	-0.323
S(4)	1.587	-1.069	0.414
S(5)	2.058	0.897	0.972
S(6)	3.512	1.556	-0.553
S(7)	2.187	2.085	-2.097
C(8)	-1.201	-1.465	0.318
C(9)	-1.413	-1.252	1.821
C(10)	-1.641	0.237	2.170
N(11)	-0.529	1.087	1.845
H(12)	-1.135	0.063	-1.917
H(13)	-1.068	-2.547	0.143
H(14)	-2.110	-1.182	-0.232
H(15)	-0.522	-1.598	2.358
H(16)	-2.259	-1.878	2.156
H(17)	-1.830	0.265	3.263
H(18)	-2.602	0.542	1.698
H(19)	-0.709	1.522	0.937

B3LYP/6-31G* Optimization

–2241.0031365

Compound **15F**

S(1)	2.181	-0.788	2.076
C(2)	1.268	-0.485	0.636
C(3)	-0.074	-0.474	0.439
S(4)	-1.178	-0.951	1.743
S(5)	-2.242	0.830	2.100
C(8)	-0.620	-0.045	-0.928
C(9)	-2.141	-0.023	-1.180
C(10)	-2.984	1.082	-0.506
N(11)	-3.454	0.704	0.834
H(12)	1.915	-0.226	-0.215

H(13)	-0.184	-0.705	-1.692
H(14)	-0.228	0.958	-1.155
H(15)	-2.592	-0.990	-0.932
H(16)	-2.262	0.111	-2.265
H(17)	-3.893	1.230	-1.106
H(18)	-2.425	2.034	-0.515
H(19)	-4.242	1.280	1.128
S(6)	4.403	0.370	-0.425
S(7)	4.295	-0.864	1.201

B3LYP/6-31G* Optimization

–2241.06850983

Compound **15l**

S(1)	2.862	-1.070	1.465
C(2)	1.830	-0.848	0.123
C(3)	0.453	-0.853	-0.025
S(4)	-0.635	-1.122	1.315
S(5)	-1.222	0.873	1.883
C(6)	-0.152	-0.608	-1.407
C(7)	-1.535	0.076	-1.493
C(8)	-1.732	1.418	-0.752
N(9)	-2.368	1.234	0.569
H(10)	2.345	-0.671	-0.833
H(11)	-0.256	-1.556	-1.965
H(12)	0.567	-0.010	-1.989
H(13)	-2.312	-0.604	-1.127
H(14)	-1.741	0.239	-2.563
H(15)	-2.433	2.036	-1.334
H(16)	-0.777	1.967	-0.692
H(17)	-2.806	2.111	0.857

B3LYP/6-31G* Optimization

–1444.6707609

Compound **15l+S$_2$** Optimized at a 5.0 Å Fixed Distance

S(1)	0.947	-0.578	2.232
C(2)	-0.104	-0.089	0.962
C(3)	-1.468	-0.176	0.984
S(4)	-2.166	-0.886	2.480
S(5)	-4.220	-0.606	2.167
C(8)	-2.329	0.219	-0.191
C(9)	-3.095	-0.920	-0.926
C(10)	-4.525	-1.217	-0.449
N(11)	-4.566	-1.770	0.909
H(12)	0.340	0.284	0.034
H(13)	-1.646	0.685	-0.912
H(14)	-3.049	0.999	0.096
H(15)	-2.503	-1.842	-0.883

H(16)	-3.177	-0.640	-1.986
H(17)	-4.959	-1.982	-1.109
H(18)	-5.140	-0.306	-0.557
H(19)	-5.452	-2.223	1.119
S(6)	5.608	1.233	-1.371
S(7)	5.485	0.608	0.501

B3LYP/6-31G* Optimization
–2241.02852433

Compound **16**

C(1)	-0.638	1.130	-0.412
C(2)	-1.865	0.593	-0.015
C(3)	-1.915	-0.316	1.029
C(4)	-0.765	-0.704	1.735
C(5)	0.472	-0.147	1.351
C(6)	0.546	0.747	0.249
S(7)	1.951	-0.637	2.265
S(8)	2.439	1.079	3.386
S(9)	3.818	2.170	2.226
S(10)	2.633	2.987	0.683
S(11)	2.091	1.315	-0.462
C(12)	-0.922	-1.737	2.829
C(13)	-0.716	-3.181	2.322
N(14)	0.652	-3.38	1.846
O(15)	-0.605	2.075	-1.403
C(16)	-0.752	1.580	-2.734
H(17)	-2.770	0.919	-0.520
H(18)	-2.876	-0.725	1.332
H(19)	-1.936	-1.667	3.243
H(20)	-0.227	-1.544	3.652
H(21)	-1.394	-3.358	1.478
H(22)	-1.019	-3.875	3.125
H(23)	0.716	-4.241	1.293
H(24)	1.291	-3.496	2.630
H(25)	-0.778	2.458	-3.384
H(26)	-1.683	1.010	-2.852
H(27)	0.099	0.946	-3.012

B3LYP/6-31G* Optimization
–2470.48801826

Compound **17**

C(1)	-0.828	1.443	-1.690
C(2)	-2.071	0.983	-1.233
C(3)	-2.122	0.158	-0.111
C(4)	-0.953	-0.219	0.571
C(5)	0.299	0.244	0.117
C(6)	0.342	1.077	-1.034
S(7)	1.790	-0.312	0.950

S(8)	2.448	1.366	2.047
S(9)	3.789	2.378	0.775
S(10)	2.537	3.236	-0.686
S(11)	1.864	1.615	-1.835
C(12)	-1.119	-1.164	1.743
C(13)	-1.084	-2.652	1.330
N(14)	0.215	-3.023	0.771
O(15)	-3.151	1.403	-1.943
C(16)	-4.443	0.989	-1.520
H(17)	-0.798	2.091	-2.559
H(18)	-3.073	-0.206	0.261
H(19)	-2.087	-0.972	2.222
H(20)	-0.348	-0.984	2.498
H(21)	-1.841	-2.821	0.554
H(22)	-1.385	-3.254	2.205
H(23)	0.161	-3.931	0.313
H(24)	0.911	-3.103	1.509
H(25)	-4.546	-0.103	-1.557
H(26)	-5.144	1.443	-2.222
H(27)	-4.664	1.342	-0.505

B3LYP/6-31G* Optimization
–2470.49651359

Compound **18**

C(1)	-0.807	1.775	-1.369
C(2)	-2.001	1.302	-0.831
C(3)	-1.963	0.354	0.190
C(4)	-0.732	-0.117	0.714
C(5)	0.461	0.371	0.154
C(6)	0.428	1.317	-0.907
S(7)	2.022	-0.302	0.764
S(8)	2.855	1.266	1.894
S(9)	4.102	2.340	0.578
S(10)	2.744	3.373	-0.658
S(11)	1.863	1.864	-1.831
C(12)	-0.785	-1.155	1.812
C(13)	-0.875	-2.596	1.266
N(14)	0.329	-2.952	0.512
O(15)	-3.08	-0.180	0.759
C(16)	-4.355	0.280	0.326
H(17)	-0.833	2.507	-2.169
H(18)	-2.942	1.672	-1.219
H(19)	-1.665	-0.973	2.436
H(20)	0.098	-1.071	2.452
H(21)	-1.732	-2.664	0.588
H(22)	-1.086	-3.270	2.115
H(23)	0.174	-3.798	-0.032

H(24)	1.101	-3.147	1.147
H(25)	-4.521	0.062	-0.736
H(26)	-4.473	1.357	0.499
H(27)	-5.086	-0.265	0.926

B3LYP/6-31G* Optimization
–2470.49659507

Compound **19**

S(1)	0.597	0.935	-2.555
C(2)	-0.651	0.727	-1.298
C(3)	-0.479	0.490	0.017
S(4)	1.144	0.375	0.812
S(5)	1.737	2.396	0.945
S(6)	2.786	2.794	-0.845
S(7)	1.276	2.910	-2.308
C(8)	-1.678	0.332	0.931
C(9)	-1.689	-0.920	1.831
C(10)	-1.384	-2.213	1.065
N(11)	0.042	-2.284	0.727
C(12)	0.869	-2.962	1.722
H(13)	-1.668	0.770	-1.684
H(14)	-2.584	0.338	0.311
H(15)	-1.733	1.223	1.574
H(16)	-2.676	-0.989	2.305
H(17)	-0.960	-0.805	2.641
H(18)	-1.963	-2.231	0.133
H(19)	-1.704	-3.087	1.656
H(20)	0.176	-2.710	-0.185
H(21)	0.551	-4.000	1.923
H(22)	0.836	-2.410	2.666
H(23)	1.909	-2.972	1.382

B3LYP/6-31G* Optimization
–2280.94072085

Compound **20**

S(1)	0.693	1.249	-2.446
C(2)	-0.683	0.975	-1.346
C(3)	-0.656	0.615	-0.047
S(4)	0.871	0.365	0.892
S(5)	1.516	2.339	1.274
S(6)	2.752	2.853	-0.359
S(7)	1.401	3.165	-1.944
C(8)	-1.947	0.419	0.723
C(9)	-2.082	-0.910	1.493
C(10)	-1.744	-2.135	0.636
N(11)	-0.295	-2.254	0.427
C(12)	0.058	-2.829	-0.866
C(13)	0.369	-2.954	1.522

H(14)	-1.654	1.091	-1.825
H(15)	-2.783	0.514	0.018
H(16)	-2.045	1.249	1.438
H(17)	-3.114	-0.987	1.858
H(18)	-1.439	-0.894	2.380
H(19)	-2.225	-2.032	-0.344
H(20)	-2.156	-3.052	1.094
H(21)	-0.301	-3.868	-0.989
H(22)	1.146	-2.828	-0.978
H(23)	-0.366	-2.218	-1.669
H(24)	0.075	-4.019	1.580
H(25)	0.128	-2.479	2.477
H(26)	1.454	-2.901	1.387

B3LYP/6-31G* Optimization
–2320.2510681

HS^-

S(1)	0.677	0.026	-0.077
H(2)	-0.677	-0.026	0.077

B3LYP/6-31G* Optimization
–398.8062523

PCM//B3LYP/6-31G*–398.945243254

H_2S

S(1)	0.003	0.457	-0.090
H(2)	0.974	-0.457	0.120
H(3)	-0.980	-0.456	0.060

B3LYP/6-31G* Optimization
–399.3854355

Triatomic sulfur (S_3)

S(1)	0.000	0.500	0.000
S(2)	1.675	-0.500	0.000
S(3)	-1.675	-0.500	0.000

B3LYP/6-31G* Optimization
–1194.542662;

PCM//B3LYP/6-31G* –1194.54360118

Diatomic sulfur (S_2)

S(1)	0.482	-0.002	0.835
S(2)	-0.482	0.002	-0.835

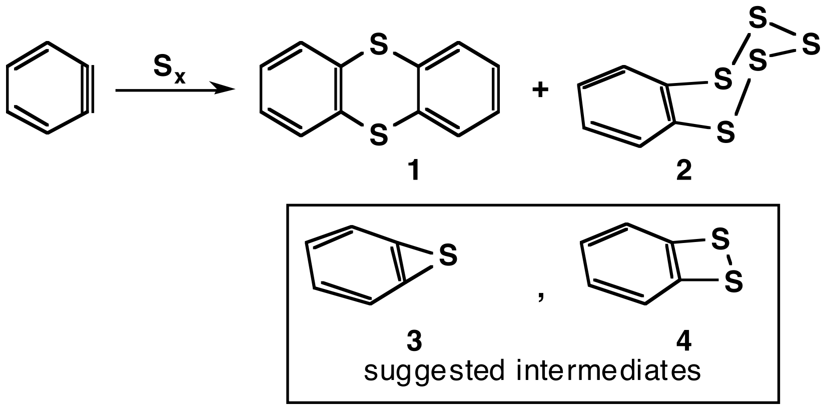
B3LYP/6-31G* Optimization

–796.325987

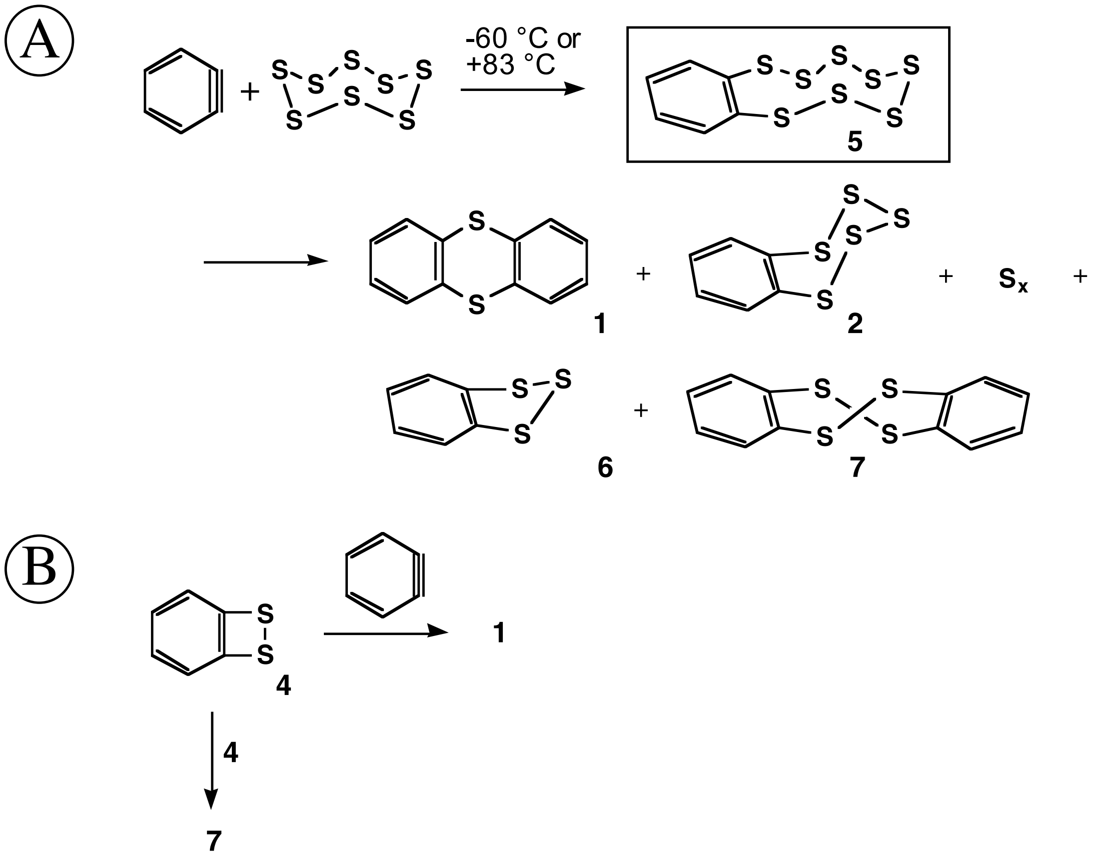
Chapter 3. Polysulfane Antitumor Agents from *o*-Benzyne

3.1 Introduction.

We present the study of a reaction that can make *ortho* fused polysulfur chemical bonds. Previous research has demonstrated that elemental sulfur can react with unsaturated chemical groups such as benzyne[1,2] and alkynes.[3-5] A reaction where thianthrene (**1**) and benzopentathiepin (**2**, *o*-$C_6H_4S_5$) are formed, which utilizes *o*-benzyne as a precursor has been established previously by Nakayama et al. (Scheme 1).[1,2] The report suggested an initial formation of benzothiirene (**3**, *o*-C_6H_4S) or benzodithiete (**4**, *o*-$C_6H_4S_2$) intermediates.[1,2] A more detailed study of the *o*-benzyne—elemental sulfur reaction has not yet appeared. The study of *o*-benzyne with elemental sulfur is presented here, which provides evidence for the decomposition of an initial *o*-$C_6H_4S_8$ intermediate[6-9] (**5**) to give thianthrene **1**, pentathiepin **2**, trithiole **6**, and tetrathiocin **7** products (Scheme 2).



Scheme 1. *o*-Benzyne reaction.



Scheme 2. Decomposition of an *o*-$C_6H_4S_8$ intermediate in a benzyne reaction.

3.2 Results and Discussion.

3.2.1 Oscillation in the Stability of *o*-Benzopolysulfanes, *o*-$C_6H_4S_x$ (x=1-8).

Various heterocycles may be expected to arise from the reaction of *o*-benzyne with cyclic S_8. B3LYP/6-31G(d) calculations were used to predict the stability of possible *ortho* fused heterocycles, *o*-$C_6H_4S_x$ (where x = 1 to 8). We identified low-energy conformers of the rings containing up to 8 sulfurs by a search of conformational space. We found that an oscillation pattern emerges in the relative stability of these heterocycles (Figure 1). The odd-membered *o*-

$C_6H_4S_x$ rings (except x = 1 which suffers from ring strain) have enhanced conformational stability compared to the even-membered rings.

Figure 1 is a measure of the stability of *o*-$C_6H_4S_x$. We compared the energy difference of *o*-$C_6H_4S_8$ **5** relative to *o*-$C_6H_4S_x$ plus a fraction of cyclic S_8's energy in an isodesmic reaction. Compounds *o*-$C_6H_4S_7$ and *o*-$C_6H_4S_5$ possess staggered lone-pair electrons in the stable crown and chair forms, respectively. The 5-membered ring molecule *o*-$C_6H_4S_3$ adopts a stable half-chair conformation.

The conformations in *o*-$C_6H_4S_x$ (x = 3, 5, 7) can better adopt gauche interactions; whereas eclipsing interactions occur in the even numbered cases. The extent of eclipsing is measured by comparison of the polysulfur torsion angle (θ = S1-S2-S3-S4) in *o*-$C_6H_4S_x$ as positive or negative to the pentathiepin θ angle of 73.0° as a reference point described as $|\Delta\theta|$. Figure 2 reveals that the conformations for the even-membered rings exhibit enhanced eclipsing strain with higher $|\Delta\theta|$ values.

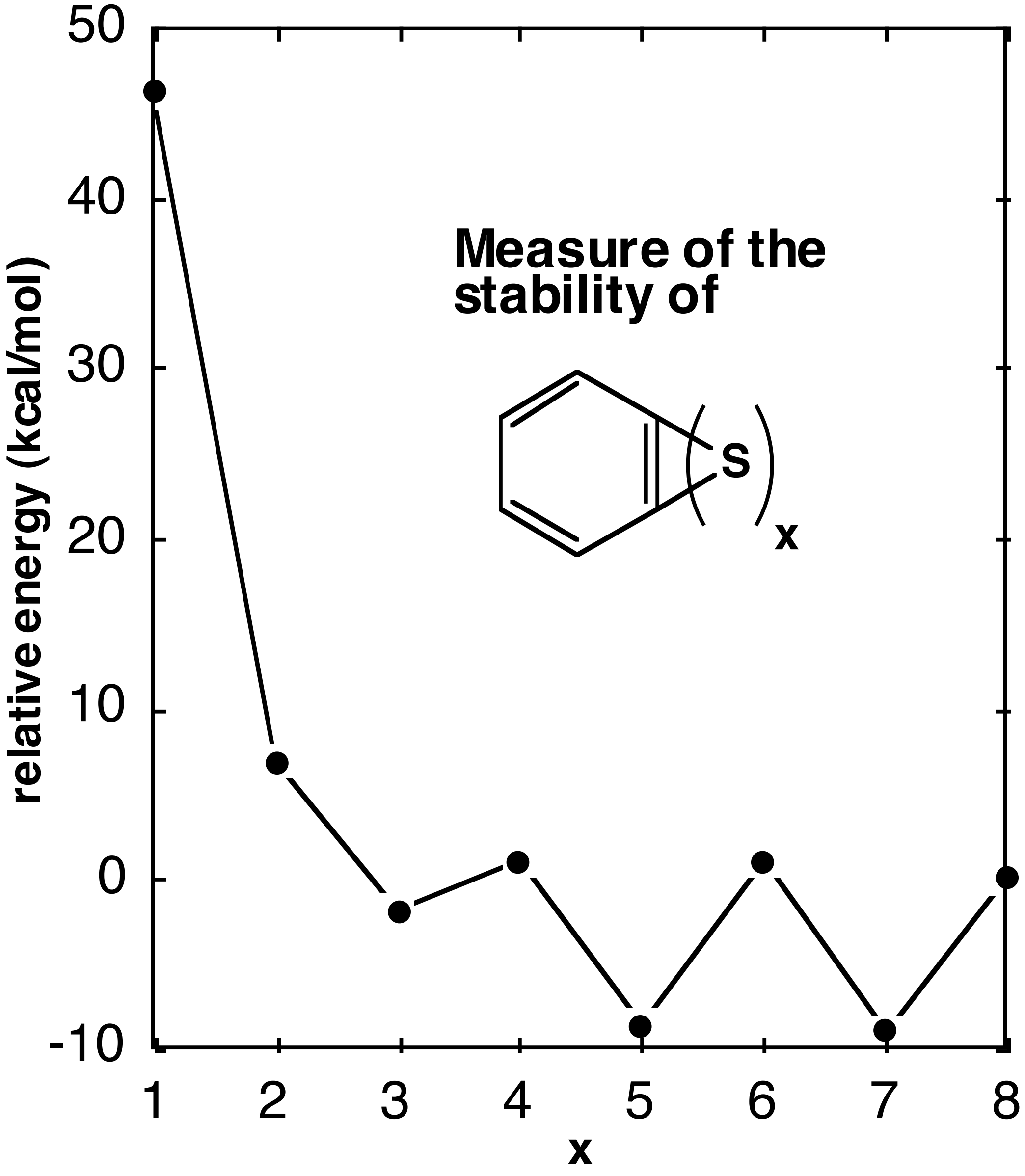


Figure 1. Oscillations in the stability of *o*-benzopolysulfanes *o*-$C_6H_4S_x$ gauged by a fraction of cyclic S_8 subtracted from *o*-$C_6H_4S_8$ in an isodesmic reaction, i.e., "relative energy" is the energy of the reaction *o*-$C_6H_4S_8 \rightarrow$ *o*-$C_6H_4S_x$ + (8-x)/8 cyclic S_8. The data shown were obtained with B3LYP/6-31G(d) gas phase calculations. The temperature sensitivity of the stability of polysulfur compounds appears to be small. The perfect linear correlation of energy for (8-x)/8 cyclic S_8, where x = 1 to 8, allowed for the study of *o*-$C_6H_4S_x$ heterocycle stability plotted here.

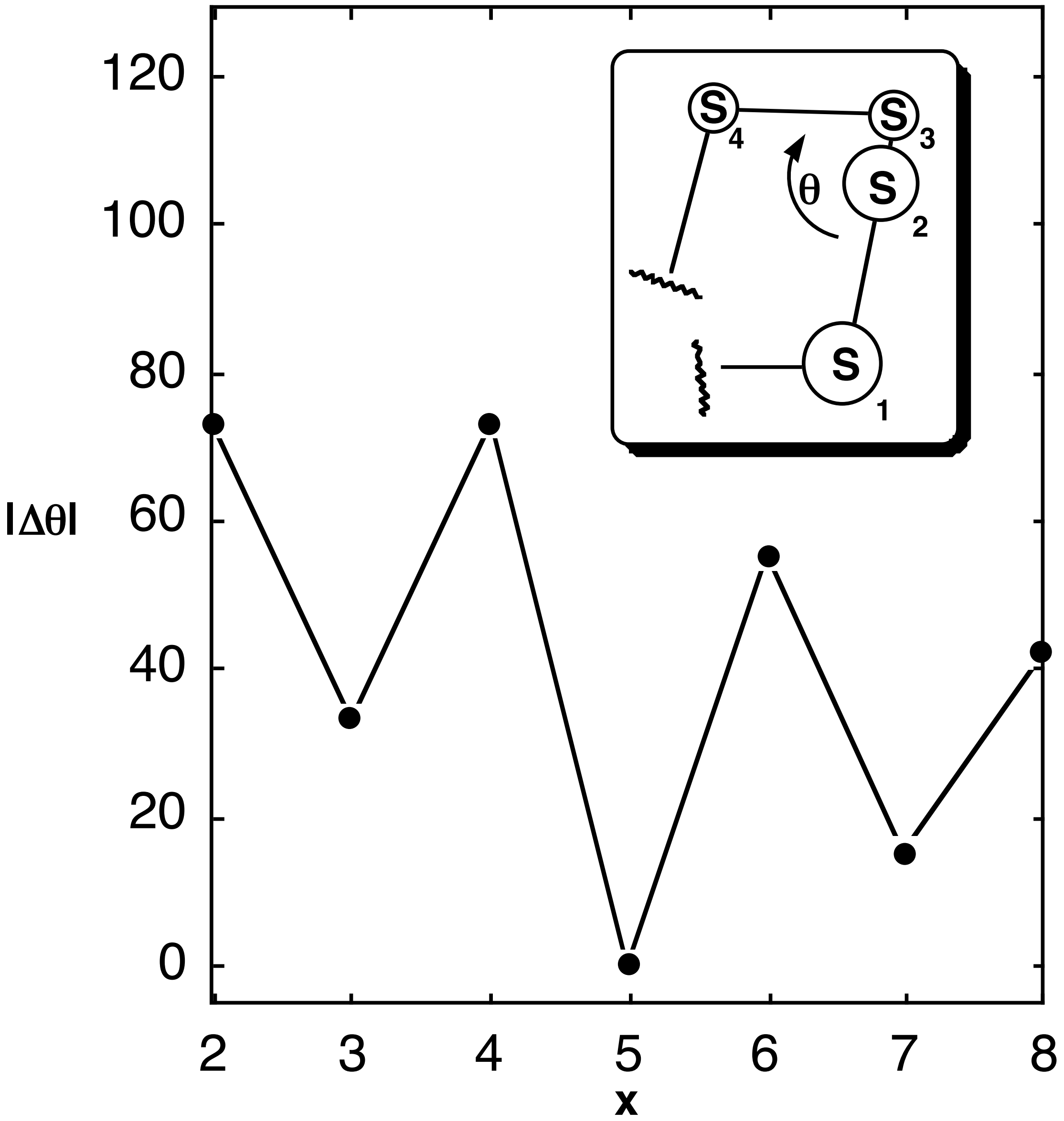
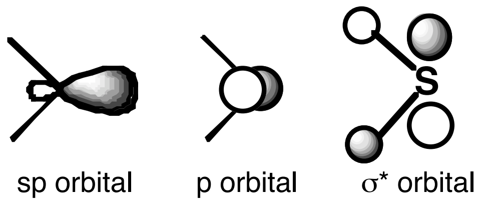


Figure 2. Odd-even alternation arising from eclipsing strain measured in *o*-$C_6H_4S_x$ heterocycles based on B3LYP/6-31G(d) calculations. The dihedral angle of pentathiepin *o*-$C_6H_4S_5$ (73.0°) is used as a reference point from which the segment S1-S2-S3-S4 in *o*-$C_6H_4S_x$ (x = 4 to 8), C-S1-S2-S3 in *o*-$C_6H_4S_3$, and C-C-S1-S2 in *o*-$C_6H_4S_2$ are compared. The difference gives rise to the IΔθI values.

3.2.2 Sulfur Lone Pair Electrons.

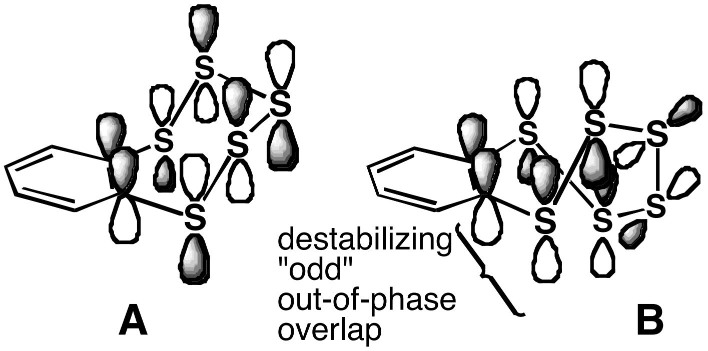
Sulfur lone pairs are in nonequivalent orbitals: one pair in the 3p orbital (axial to the plane of the molecule) and the other in an sp (equatorial) orbital. Sulfur also possesses a vacant σ* orbital (Scheme 3).



Scheme 3. The nature of sulfur orbitals.

3.2.3 Possible Analogy to Hückel and Möbius π Electron System.

The origin of the oscillating feature in Figure 1 is also likely the result of the overlap in the p-orbitals for the odd- vs even-membered rings. With *o*-$C_6H_4S_x$ (x = 3, 5, 7) an even number of out-of-phase overlaps is achieved. Whereas, with *o*-$C_6H_4S_x$ (x = 2, 4, 6, 8) an odd number of out-of-phase overlaps is achieved (e.g., compare structures **A** and **B**) (Scheme 4). Even vs odd out-of-phase overlap in *o*-$C_6H_4S_x$ (x = 1 to 8) is reminiscent of Hückel and Möbius π electron systems. Similar "odd-even" trends may apply to allotropes of elemental sulfur but are not described in the literature.



Scheme 4. Stabilizing **A** and destabilizing **B** out of phase overlaps.

3.2.4 *o*-Benzyne—Elemental Sulfur Reaction.

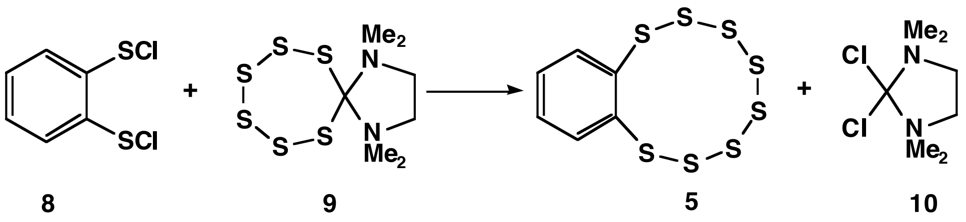
Described below is an experimental study of the *o*-benzyne—S_x reaction. The identification of *o*-$C_6H_4S_5$ **2** and *o*-$C_6H_4S_3$ **6** as products is based on an NMR and GC/MS analysis. Formation of *o*-$C_6H_4S_2$ **4** is suggested based on a secondary reaction that traps *o*-benzyne to give thianthrene **1** as well as a *o*-$C_6H_4S_2$ **4** dimerization process to give tetrathiocin **7** (Scheme 2B).

Benzyne was generated by a low temperature (-60 °C reaction of *n*-BuLi with *o*-dihalobenzene in diethyl ether)[10] and an elevated temperature method (83 °C decomposition of benzenediazonium-2-carboxylate in 1,2-dichloroethane).[11] The production of *o*-benzyne was demonstrated with furan trapping where 1,4-dihydronaphthalene-1,4-endoxide was detected in 80% yields. Elemental sulfur was added to trap the *in situ* generated *o*-benzyne and gave thianthrene **1**, pentathiepin **2**, trithiole **6**, and tetrathiocin **7** in low overall yields [**1** (17.2%), **2**

(11.9%), **6** (1.3%), **7** (2.6%)]. Acyclic polysulfanes are probably formed concomitantly. One of the byproducts obtained: *o*-C_6H_4ClSH (1%) suggests that the *o*-benzyne precursor, *o*-C_6H_4ClLi, reacts with elemental sulfur and generates thiolates, which can equilibrate polysulfides. Concentrations of **1**, **2**, **6**, and **7** are identical to within experimental error with the *o*-benzyne precursors *o*-C_6H_4ClLi or *o*-$C_6H_4CO_2^-N_2^+$, which provide evidence for thermodynamic product ratios. Pentathiepin **2** displayed a weak molecular ion peak *m/z* 236 [M^+] and a base peak at *m/z* 172 [M^+-2S] representing the loss of two sulfur atoms. The molecular ion of trithiole **6** is abundant and represents the base peak *m/z* 172 [M^+]. Mass spectrometry was also used to confirm the presence of thianthrene **1**. ^{1}H NMR data on the *o*-benzyne sulfuration reaction yielded conclusive evidence for the formation of thianthrene **1**, pentathiepin **2**, and tetrathiocin **7**.[12-14] The expected upfield chemical shifts for trithiole **6** are obscured by the reagent and byproduct peaks. The assignment of **2** is based on 2 sets of dd's; a downfield set d=7.85 (2H) and an upfield set d=7.34 (2H). Similar chemical shifts are found for **1** [d=7.58 (4H); d=7.32 (4H)] and **7** [d=8.66 (4H)].

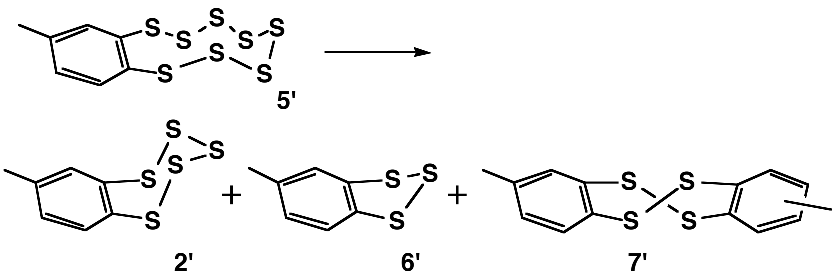
3.2.5 The Decomposition of Benzooctathiecin Intermediate.

The reaction shown in Scheme 2 stems from the proposal that benzooctathiecin **5** forms. There was no direct detection of **5** in the benzyne reaction. However, experimental evidence for the decomposition of an octathiecin **5** was obtained by synthesizing **5** and **5’** from the method of Steudel using ZnS_6(TMEDA) (**9**) (Scheme 5).[15]



Scheme 5. Synthesis of octathiecin **5**.

In the presence of heat (40 °C) or an added nucleophile *n*-PrSH in dicholormethane, gas chromatographic analyses of the reaction revealed the formation of **2**, **6**, **7** and **2'**, **6'**, **7'**, from **5** and **5'** respectively. These results provide evidence for the formation of **5** in the benzyne—S_x reaction and are consistent with the idea that heat or nucleophiles can influence the equilibrium between polysulfane compounds,[6,15-21] which offers a reason for the decomposition of o-$C_6H_4S_8$ **5** under the conditions. Formation of stable arene annulated polysulfur products from precursor **5** likely occur by reversible exchange reactions involving complex mixtures of polysulfanes.

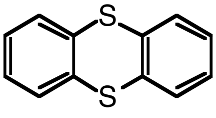
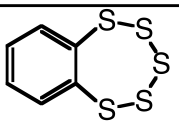
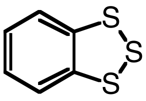
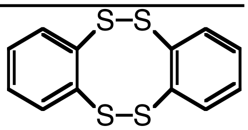


Scheme 6. Decomposition of octathiecin **5'**.

3.2.6 Product Distribution of *o*-Benzyne—S_8 Reaction.

The product distribution also bears on the solubility of elemental S_x in the benzyne system. The yield of thianthrene **1** decreased by 17% when the initial cyclic S_8 concentration is varied from 0.8 mM to 85 mM (Table 1). Such an effect between *o*-benzyne and cyclic S_8 concentration and the suppression of **1** is consistent with a reduced *o*-benzyne trapping of the dithiete intermediate **4** (Scheme 2B). It follows that the ability of **2**, **6**, and **7** to form from *o*-benzyne is enhanced with higher elemental sulfur concentrations. We find that elemental sulfur is soluble to the extent of 0.0078 g in 10 g diethyl ether at –60 °C, which leads to the conclusion that amounts of elemental sulfur above ~10 mM indicates a heterogeneous process amplifies the production of polysulfanes **2**, **6**, and **7**, but not sulfane **1**.

Table 1. Product distribution of *o*-benzosulfanes that arise from the cyclic S_8 sulfuration of *o*-benzyne.[a-c]

$[\text{Cyclic } S_8]_{\text{init}}$	 **1**	 **2**	 **6**	 **7**
0.8 mM	57	31	3	7
10 mM	52	36	4	8
52 mM	46	41	5	9
85 mM	40	48	4	8

[a] GC/MS and NMR detection of products formed in diethyl ether at –60 °C after addition of cyclic S_8 to *o*-benzyne. [b] *o*-Benzyne concentration in the reaction is approximately 200 mM. Each entry is an average of 4 runs. [c] Reactions were taken to ~20% completion.

3.2.7 Conclusion.

This study describes a reaction that can make *ortho* fused polysulfur bonds. We find that the odd-membered *o*-$C_6H_4S_x$ rings (x = 3, 5, 7) have enhanced stability compared to the even-membered rings. The acquisition of “odd-even” data can reveal patterns on stability and structure of cyclic polysulfanes. The mechanism of polysulfane product formation appears to be governed by reversible, equilibrium, exchange processes.

3.3 Experimental Section.

3.3.1 Reagents and Solvents.

Reagents and solvents were obtained commercially [*n*-BuLi (1.6 M in hexanes), 2-bromochlorobenzene, 2-bromofluorobenzene, anthranilic acid, trichloroacetic acid, isoamyl nitrile, furan, diethyl ether (anhydrous), benzene (anhydrous), tetrahydrofuran, 1,2-dichloroethane, dimethylformamide (anhydrous), elemental sulfur, biphenyl, 2-chlorobenzenethiol, 2-fluorobenzenethiol, 1,2-benzodithiol] and were used as received. Diethyl ether was distilled over sulfuric acid and stored in a bottle containing sodium metal. Relative concentrations of pentathiepin **18**, trihiole **22**, 2-chlorobenzenethiol, and 2-fluorobenzenethiol were determined by GCMS with reference to an internal standard in terms of calibration curves of authentic samples. Good-to-excellent linear correlations were observed in the calibration curves (with correlation coefficients of 0.979 to 0.999). Comparison of integrated peaks of the known compounds provided relative percent yields in the ^{1}H NMR studies. The concentration of pentathiepin **18**, trithiole **22**, tetrathiocin **23**, and naphthalene-1,4-endoxide were also determined by using ^{1}H NMR. Experiments were carried out using a Hewlett-Packard GC/MS instrument consisting of a 5890 series GC and a 5988A series mass selective detector, a Shimadzu-17A auto-sampler capillary gas chromatograph equipped with a flame ionization detector, and a Bruker 400 MHz NMR spectrometer.

3.3.2 Synthetic Procedures.

***o*-Benzyne** was generated *in situ* using two literature methods.[22,23] *o*-Benzyne was generated by a low temperature (-60 °C reaction of *n*-BuLi with *o*-dihalobenzene in diethyl ether)[22] and an elevated temperature method (83 °C decomposition of benzenediazonium-2-carboxylate in 1,2-dichloroethane).[23] Both methods involved the analysis of products at room temperature. We demonstrated an ability to generate *o*-benzyne and determined high trapping efficiency with furan, which is in accord with previous reports.[24] The influence of added *n*-BuLi to *o*-dihalobenzene at –60 °C or heating of benzenediazonium-2-carboxylate to 83 °C accompanies a reaction that leads to *o*-benzyne formation. The *o*-dihalobenzene experiments were conducted at -60 °C and carried out under N_2-atmosphere in 1- or 5-mL solutions of anhydrous Et_2O, which contained *o*-C_6H_4BrCl (0.74 M) or *o*-C_6H_4BrF (0.74 M) and *n*-BuLi (0.3 M). After 30 minutes, a diethyl ether solution of elemental sulfur (0.016 to 0.16 g) and/or furan (6.3-9.3 M) was added to trap the *in situ* generated *o*-benzyne and was allowed to stir for

1 hr. The reaction mixture was then warmed to room temperature and the hydrolysis of possible unreacted *n*-BuLi was conducted. The organic layer was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure, and replaced with $CDCl_3$ or a 1-mL benzene solution containing biphenyl as the internal standard. Use of *o*-C_6H_4BrF yielded significant quantities (19%) of the *o*-C_6H_4FSH byproduct. For this reason *o*-C_6H_4BrCl was used instead which produced only 1% of the corresponding thiol byproduct, *o*-C_6H_4ClSH. Experiments were also conducted in a 25-mL solution of THF, which contained anthranilic acid (0.1 M), trichloroacetic acid (7.2 mM), and isoamyl nitrile (1.64 M). The reaction was allowed to stir for 1 hr at 20 °C where benzenediazonium-2-carboxylate was suction filtered and washed with 1,2-dichloroethane. Elemental sulfur (6.4 g) was added to a 125-mL solution of 1,2-dichloroethane containing 0.02 M benzenediazonium-2-carboxylate and heated to 83 °C. The *o*-benzyne reactions were typically taken to 20% completion gauged by the disappearance of the *o*-dihalobenzene or benzenediazonium-2-carboxylate reagents. Cyclic S_8 is marginally soluble in Et_2O at -60 °C. This was determined by stirring S_8 in 10 g of Et_2O precooled in a Et_2O/liquid nitrogen bath. Using a precooled pipette the liquid was decanted from the insoluble sulfur. Upon evaporation of the solvent the remaining sulfur weighed.

Thianthrene 17 was prepared in a 5-mL solution of anhydrous Et_2O, which contained *o*-C_6H_4BrCl (0.74 M) and *n*-BuLi (0.3 M). After 30 minutes, a diethyl ether solution of elemental sulfur (0.0254 g) was added. Compound 17 was identified in the reaction mixture. ^{1}H NMR 400 MHz ($CDCl_3$): d 7.58 (dd, J=5.77, 3.47 Hz, 4H), 7.32 (dd, J=5.75, 3.4 Hz, 4H).[25] High resolution MS :217.0146.

Pentathiepin 18 was prepared in a 5-mL solution of anhydrous Et_2O, which contained *o*-C_6H_4BrCl (0.74 M) and *n*-BuLi (0.3 M). After 30 minutes, a diethyl ether solution of elemental sulfur (0.254 g) was added. Compound **18** was identified in the reaction mixture. ^{1}H NMR 400 MHz ($CDCl_3$): d 7.846 (dd, J=5.61, 3.2Hz, 2H), d 7.34 (dd, J=6, 4Hz, 2H). ^{13}C NMR 400 MHz ($CDCl_3$): d 130.19 (2 C), 136.13 (2 C), 144.10 (2 C). GC/MS: *m/z* (%)=236 (9.7) [M^+], 172 (100) [M^+-2S], 140 (22.5) [M^+-3S], 128 (2.6) [M^+-4S], 108 (40.8), 82 (9), 64 (33.8), 58 (4.3).[26] Pentathiepin **18** was also synthesized using a method similar to that of Chenard et al.[27] A solution of 1,2-benzodithiol (0.2 g) and THF (3 ml) was purged with NH_3. Sodium (0.0805 g) was then added the mixture was allowed to reflux 30 minutes. Ammonium chloride (0.1873 g) was then added. The resulting white slurry was diluted with THF (4 ml) and cooled to 0 °C. Sulfur monochloride (0.387 g) in 2 mL THF was added. The insoluble material was filtered through Celite, and then extracted with 5% sodium bicarbonate, water and brine and dried with $MgSO_4$.[28]

Trithiole 22 was prepared in a 5-mL solution of anhydrous Et_2O, which contained *o*-C_6H_4BrCl (0.74 M) and *n*-BuLi (0.3 M). After 30 minutes, a diethyl ether solution of elemental sulfur (0.016 to 0.16 g) was added. Compound **22** was identified in the reaction mixture. ^{1}H NMR data is not available. Expected peaks for **22** were obscured by the reagent and byproduct peaks. GC/MS: *m/z* (%)=172 (96.8), 140 (4.3) [M^+-2S], 128 (2.7) [M^+-3S], 108 (100), 96 (20.8), 82 (14), 74 (4), 69 (42), 64 (15), 51 (4.6).

Tetrathiocin 23 was prepared in a 5-mL solution of anhydrous Et_2O, which contained *o*-C_6H_4BrCl (0.74 M) and *n*-BuLi (0.3 M). After 30 minutes, a diethyl ether solution of elemental sulfur (0.016 to 0.16 g) was added. Compound **23** was identified in the reaction mixture. ^1H NMR 400 MHz (CDCl3): d 8.665 (dd, J=6.4, 4.4Hz, 4H).

3.3.3 Theoretical Methods.

Density functional theoretical (DFT) calculations were performed using the Gaussian-98 program package.[29] Geometries were optimized using the DFT exchange-correlation of B3LYP along with the 6-31G(d) basis set, which are known to reproduce experimental geometries in a variety of polysulfane systems and with molecules that possess hypervalent sulfur centers. The predicted energies involve compounds at 0 K and are uncorrected for temperature and zero point energy corrections. This simplification will not significantly influence the relative energetics for the polysulfanes considered here. Analysis of the reaction in Figure 1 and eliminating the benzene ring to get the gas phase reaction S8 = Sx* + [(8-x)/8]S8. For x=4, using the thermodynamic data in the JANAF* tables, one finds that ΔH for this reaction changes from 22.8 kcal/mol at 300 K to 22.2 kcal/mol at 600 K, and 21.5 kcal/mol at 900 K.

Description of the geometry of the stationary point of **17** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1258.465710

1	6	0	3.869912	-0.697460	-0.002149
2	6	0	2.659713	-1.384875	-0.000907
3	6	0	1.435418	-0.700104	0.000471
4	6	0	1.435419	0.700107	0.000347
5	6	0	2.659718	1.384872	-0.001200
6	6	0	3.869915	0.697452	-0.002323
7	16	0	-0.000002	-1.758582	0.002711
8	16	0	0.000007	1.758586	0.002711
9	6	0	-1.435423	0.700104	0.000480
10	6	0	-1.435421	-0.700106	0.000357
11	6	0	-2.659718	-1.384874	-0.001195
12	1	0	-2.656306	-2.472003	-0.001431
13	6	0	-3.869915	-0.697454	-0.002327
14	6	0	-3.869915	0.697457	-0.002165
15	6	0	-2.659717	1.384875	-0.000891
16	1	0	4.803562	-1.252150	-0.003075
17	1	0	2.656298	-2.472005	-0.000931
18	1	0	2.656307	2.472001	-0.001449
19	1	0	4.803566	1.252139	-0.003416
20	1	0	-4.803566	-1.252143	-0.003419
21	1	0	-4.803566	1.252147	-0.003095
22	1	0	-2.656303	2.472004	-0.000917

Description of the geometry of the stationary point of **18** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2222.0035571

C	-1.699	0.360	-1.194
C	-2.790	-0.259	-0.582
C	-2.584	-1.132	0.483
C	-1.287	-1.384	0.933
C	-0.180	-0.783	0.319
C	-0.390	0.105	-0.764
S	1.439	-1.307	0.899
S	2.172	0.319	2.005
S	3.213	1.506	0.611
S	1.681	2.396	-0.528
S	0.929	0.850	-1.733
H	-1.856	1.051	-2.016
H	-3.796	-0.048	-0.934
H	-3.426	-1.612	0.974
H	-1.121	-2.054	1.771

Description of the geometry of the stationary point of **19** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -629.1412457

1	6	0	-1.908262	-0.696912	0.000015
2	6	0	-0.707707	-1.464157	-0.000010
3	6	0	0.410542	-0.671509	-0.000039
4	6	0	0.410361	0.671609	-0.000034
5	6	0	-0.707955	1.464206	-0.000011
6	6	0	-1.908373	0.696720	0.000014

7	1	0	-2.859589	-1.222504	0.000037
8	1	0	-0.713051	-2.548262	0.000004
9	1	0	-0.713312	2.548296	0.000005
10	1	0	-2.859778	1.222186	0.000034
11	16	0	2.100881	0.000034	0.000019

Description of the geometry of the stationary point of **20** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1027.3976132

1	6	0	2.431342	-0.700808	-0.000031
2	6	0	1.236679	-1.431804	-0.000072
3	6	0	0.057566	-0.699144	-0.000031
4	6	0	0.057574	0.699145	0.000025
5	6	0	1.236688	1.431803	0.000063
6	6	0	2.431347	0.700800	0.000037
7	1	0	3.378091	-1.233443	-0.000043
8	1	0	1.240583	-2.517684	-0.000080
9	1	0	1.240599	2.517683	0.000079
10	1	0	3.378100	1.233429	0.000056
11	16	0	-1.685765	1.082120	-0.000062
12	16	0	-1.685769	-1.082117	0.000065

Description of the geometry of the stationary point of **22** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) 1425.6053865

6	-1.667615	-1.401229	0.045597
6	-2.871802	-0.696663	0.143129
6	-2.871801	0.696665	0.143136

6	-1.667614	1.401229	0.045576
6	-0.465962	0.704081	-0.067847
6	-0.465963	-0.704082	-0.067839
16	1.096430	1.552231	-0.329083
16	1.096426	-1.552227	-0.329094
1	-1.665785	-2.486980	0.064962
1	-3.805333	-1.243672	0.236844
1	-3.805330	1.243677	0.236863
1	-1.665784	2.486976	0.064942
16	2.245067	-0.000004	0.529794

Description of the geometry of the stationary point of **23** (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2054.8430524

1	6	0	-3.310946	1.264539	0.566124
2	6	0	-4.521006	0.633442	0.291221
3	6	0	-4.520996	-0.633472	-0.291344
4	6	0	-3.310919	-1.264576	-0.566173
5	6	0	-2.081444	-0.645081	-0.286693
6	6	0	-2.081446	0.645023	0.286768
7	16	0	-0.656898	-1.593281	-0.816562
8	16	0	0.656946	-1.593279	0.816607
9	6	0	2.081442	-0.645016	0.286790
10	6	0	2.081448	0.645087	-0.286673
11	16	0	0.656909	1.593297	-0.816548
12	16	0	-0.656957	1.593288	0.816601
13	6	0	3.310937	-1.264547	0.566131
14	6	0	4.521001	-0.633464	0.291218
15	6	0	4.521000	0.633448	-0.291351
16	6	0	3.310927	1.264564	-0.566172

17	1	0	-3.305327	2.263393	0.992557
18	1	0	-5.455835	1.137260	0.519414
19	1	0	-5.455815	-1.137269	-0.519622
20	1	0	-3.305272	-2.263409	-0.992651
21	1	0	3.305311	-2.263404	0.992559
22	1	0	5.455825	-1.137295	0.519402
23	1	0	5.455823	1.137233	-0.519644
24	1	0	3.305290	2.263393	-0.992660

Description of the geometry of the stationary point of *o*-$C_6H_4S_4$ (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1823.7945478

1	6	0	-1.997247	-1.403351	-0.124011
2	6	0	-3.147279	-0.699084	-0.483241
3	6	0	-3.147767	0.697759	-0.482673
4	6	0	-1.998207	1.402552	-0.122937
5	6	0	-0.849894	0.706201	0.264242
6	6	0	-0.849411	-0.706513	0.263783
7	16	0	0.615561	1.567404	0.834374
8	16	0	2.007988	1.115037	-0.649842
9	16	0	2.010140	-1.114348	-0.648580
10	16	0	0.616145	-1.567022	0.834381
11	1	0	-1.986159	-2.488900	-0.136477
12	1	0	-4.041793	-1.242909	-0.773146
13	1	0	-4.042673	1.241193	-0.772100
14	1	0	-1.987884	2.488118	-0.134588

Description of the geometry of the stationary point of *o*-$C_6H_4S_6$ (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2620.1822122

1	6	0	2.767395	-1.262879	0.578107
2	6	0	3.978380	-0.631435	0.296248
3	6	0	3.978756	0.632347	-0.290041
4	6	0	2.768031	1.262080	-0.576722
5	6	0	1.543855	0.642237	-0.289504
6	6	0	1.543567	-0.644479	0.286369
7	16	0	0.096989	1.550766	-0.869401
8	16	0	-1.051637	2.088896	0.734925
9	16	0	-2.626678	0.448904	0.893852
10	16	0	-2.637851	-0.446048	-0.882333
11	16	0	-1.054781	-2.085249	-0.741630
12	16	0	0.096953	-1.556534	0.862250
13	1	0	2.761572	-2.254119	1.021387
14	1	0	4.913766	-1.133125	0.526423
15	1	0	4.914405	1.135127	-0.516726
16	1	0	2.762429	2.253112	-1.020437

Description of the geometry of the stationary point of *o*-$C_6H_4S_7$ (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -3018.3918302

1	6	0	3.684071	-0.699079	-1.256518
2	6	0	3.684808	0.696777	-1.256450
3	6	0	2.775562	1.389973	-0.461985
4	6	0	1.831808	0.705596	0.319782
5	6	0	1.831114	-0.706089	0.319766

6	6	0	2.774133	-1.391393	-0.462082
7	16	0	-0.554736	2.574758	-0.065600
8	16	0	0.726523	1.678563	1.348814
9	16	0	-2.451982	1.686147	0.008730
10	16	0	-2.552176	0.000480	-1.247545
11	16	0	-2.452987	-1.685425	0.008546
12	16	0	-0.555968	-2.574648	-0.064964
13	16	0	0.725187	-1.678019	1.349154
14	1	0	4.399337	-1.248820	-1.861422
15	1	0	4.400676	1.245834	-1.861264
16	1	0	2.785890	2.475004	-0.443210
17	1	0	2.783371	-2.476433	-0.443334

Description of the geometry of the stationary point of *o*-$C_6H_4S_8$ (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -3416.5713018

1	6	0	3.490501	-1.132430	0.810016
2	6	0	4.702606	-0.569995	0.411186
3	6	0	4.705551	0.557364	-0.407373
4	6	0	3.496444	1.125542	-0.807249
5	6	0	2.267046	0.575683	-0.411123
6	6	0	2.264030	-0.577037	0.412545
7	1	0	3.480124	-2.018961	1.435589
8	1	0	5.637076	-1.019740	0.733957
9	1	0	5.642379	1.002879	-0.729194
10	1	0	3.490895	2.012217	-1.432679
11	16	0	0.820261	1.348646	-1.117986
12	16	0	-0.064870	2.323350	0.594728
13	16	0	-2.077964	2.385346	0.130331
14	16	0	-3.162371	0.698248	0.790575

15	16	0	-3.166116	-0.698080	-0.787612
16	16	0	-2.080685	-2.386321	-0.131930
17	16	0	-0.068647	-2.319051	-0.598416
18	16	0	0.812421	-1.342835	1.116830

Description of the geometry of the stationary point of *o*-C_6H_4 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -230.909948

1	6	0	1.461255	-0.134245	-0.000133
2	6	0	0.703969	1.058374	0.000041
3	6	0	-0.703053	1.058834	0.000018
4	6	0	-1.461242	-0.133120	-0.000127
5	6	0	-0.626132	-1.237598	0.000084
6	6	0	0.624931	-1.237636	0.000079
7	1	0	2.546535	-0.135669	-0.000045
8	1	0	1.230388	2.010438	0.000161
9	1	0	-1.228778	2.011302	0.000146
10	1	0	-2.546518	-0.133725	-0.000024

Description of the geometry of the stationary point of S-atom (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -398.0425733

1	16	0.000000000	0.000000000	0.000000000

Description of the geometry of the stationary point of S_2 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) –796.3259877

S(1)	0.482	-0.002	0.835
S(2)	-0.482	0.002	-0.835

Description of the geometry of the stationary point of linear S_3 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) –1194.542662

S(1)	0.000	0.500	0.000
S(2)	1.675	-0.500	0.000
S(3)	-1.675	-0.500	0.000

Description of the geometry of the stationary point of cyclic S_4 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1592.7020446

1	16	0	1.034670	1.035738	0.310724
2	16	0	-1.035748	1.034659	-0.310725
3	16	0	-1.034655	-1.035738	0.310729
4	16	0	1.035733	-1.034660	-0.310729

Description of the geometry of the stationary point of cyclic S_5 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -1990.937494

1	16	0	-1.522350	-0.548485	-0.458364
2	16	0	-0.014186	-1.618172	0.591450
3	16	0	1.514029	-0.574137	-0.456679
4	16	0	1.131749	1.361684	0.161075
5	16	0	-1.109242	1.379109	0.162518

Description of the geometry of the stationary point of cyclic S_6 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2389.146922

1	16	0	0.468458	0.330087	-1.877120
2	16	0	-1.500497	-0.120697	-1.259428
3	16	0	-1.206420	-1.519928	0.295496
4	16	0	-0.468458	-0.330087	1.877120
5	16	0	1.500497	0.120697	1.259428
6	16	0	1.206420	1.519928	-0.295496

Description of the geometry of the stationary point of cyclic S_7 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -2787.3449706

1	16	0	0.451965	-1.780689	0.745957
2	16	0	-1.308673	-1.685075	-0.498077
3	16	0	-2.314387	-0.001197	0.225534
4	16	0	-1.310602	1.684100	-0.497506
5	16	0	0.450385	1.781111	0.745933
6	16	0	2.014898	1.126161	-0.361283
7	16	0	2.016413	-1.124411	-0.360558

Description of the geometry of the stationary point of cyclic S_8 (cartesian coordinates) and absolute energy in hartrees. B3LYP/6-31G* gas phase (Charge=0, spin state=1) -3185.550505

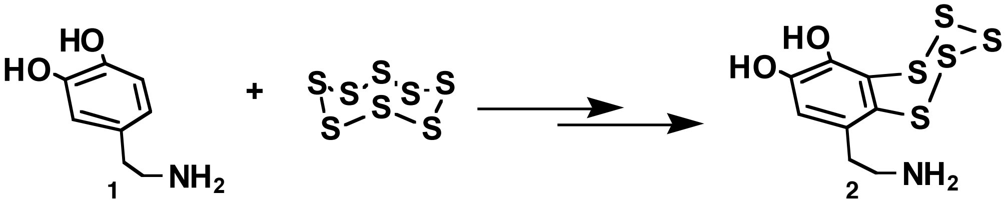
S	-1.782	-0.870	1.479
S	0.014	-1.960	1.497
S	0.605	-2.343	-0.481

S	2.038	-0.920	-1.059
S	1.091	0.616	-2.133
S	0.686	2.217	-0.835
S	-1.305	2.086	-0.182
S	-1.351	1.172	1.709

Chapter 4. Biomimetic Synthesis of *o*-Benzopolysulfanes

4.1 Introduction.

A study on the biomimetic synthesis of naturally occurring polysulfanes **1-16** (Scheme 1, Chapter 1) is presented. Compounds **1-16** exhibit a range of biological activities, which might be a consequence related to a chemical defense mechanism of the marine organisms that synthesize them.[1-13] The work presented here provides an explanation on how sulfur atoms may be incorporated into marine organic molecules biosynthetically. A dopamine—S_8 (Scheme 1) reaction is suggested as a biosynthetic pathway of naturally occurring polysulfanes **1-16** in the tunicates of the genera *Lissoclinum*, *Aplydium*, *Eudistoma* and *Polycitor* (Figure 1, Chapter 1).



Scheme 1. Biomimetic reaction yielding natural product benzopolysulfanes.

Dopamine **1** has been isolated from marine sponges.[14] Dopamine **1**, as well as epinephrine, norepinephrine, and 3,4-dihydroxyphenylalanine represent the water-soluble hormones, catechol-amines, which play a key role as neurotransmitters in terrestrial organisms.[15-17] The presence of dopamine in marine sponges is surprising due to the fact that sponges and tunicates are primitive animals without a nervous system. Dopamine has been hypothesized to play a role of a grazer deterrent rather than neurotransmitter[14], but there is no evidence at present to support this claim. Elemental sulfur may be available to ascidian and sponge organisms where dopamine is present. It is often a neglected fact that the tremendous reserves of elemental sulfur, which exist in marine environments may serve as a source for sulfur in biomolecules.[18-21] We propose, that tunicates can filter elemental sulfur and transform solid insoluble S_x into a soluble form by transferring sulfur atoms chemically into a dopamine molecule for possible use as a protecting agent (Figure 1).

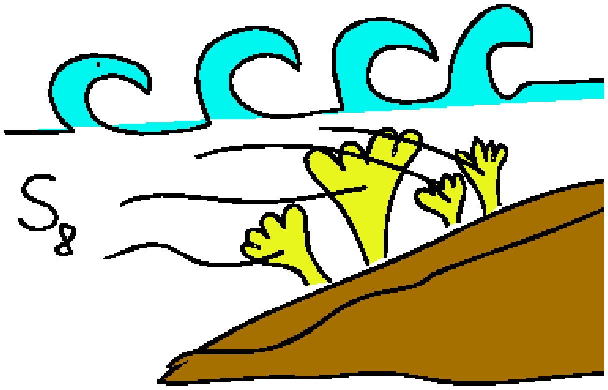
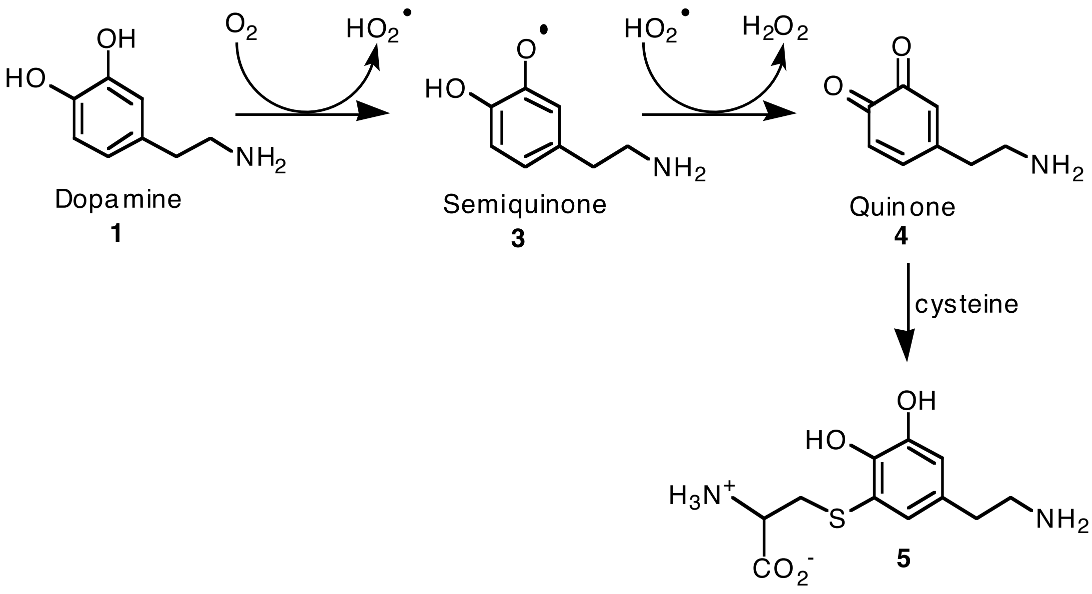


Figure 1. Picture of sulfur uptake by filter feeding animals, sponges and tunicates.

Catechol-amines are known to undergo autoxidation yielding semi-quinones (e.g., **3**), quinones (e.g., **4**), protonated superoxide ion, and H_2O_2 under aerobic conditions (Scheme 2).[22-24] Dopamine-*o*-quinone **4** is reactive because it has an electron deficient ring and can readily react with nucleophiles[25-27] such as HS^- and RS^-.[28-31]



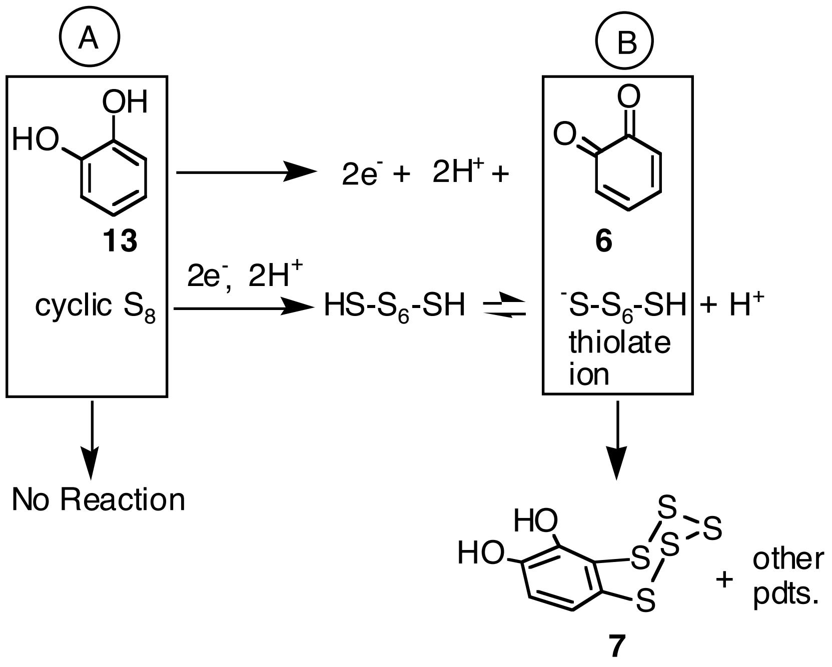
Scheme 2. Oxidation of dopamine **1**.

We chose to study catechol as the starting material, which differs from dopamine by the lack of an aminoethyl substituent. We have accomplished successfully the introduction of S atoms into catechol. The details are presented below.

4.2 Results and Discussion.

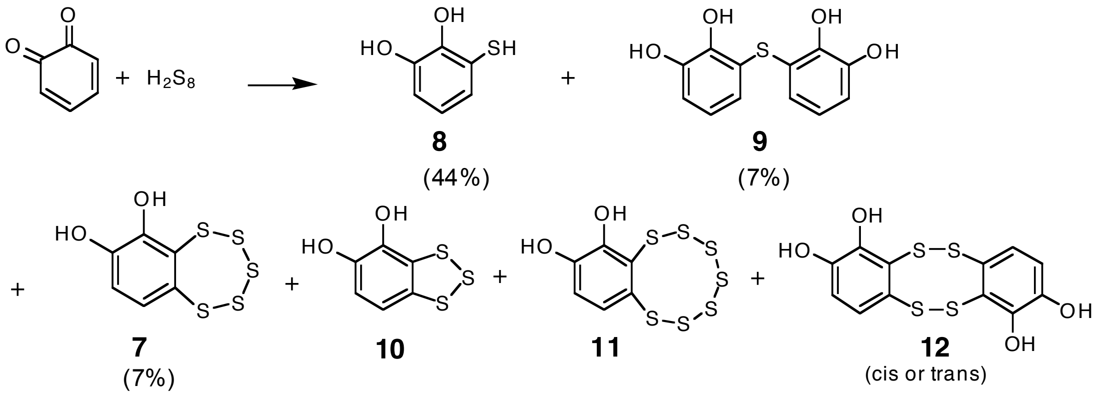
4.2.1 Biomimetic Elemental Sulfur Incorporation.

The reaction of catechol with S_x gives no reaction (Scheme 3A). Both reagents needed to be activated. HS_x^- was used as the sulfur source. The oxidation of catechol then provides *o*-benzoquinone (**6**) which can react with reduced elemental sulfur (Scheme 3B).



Scheme 3. Incorporation of sulfur into catechol.

Inorganic thiolate ions (e.g., HS_8^-) adds to **6**, and gives rise to cyclic 5,6,7,8,9-pentathia-benzocycloheptene-1,2-diol (**7**), 3-mercapto-benzene-1,2-diol (**8**), diarylsulfide (**9**), benzotrithiole-4,5-diol (**10**), 5,6,7,8,9,10,11-heptathia-benzocyclononene-1,2-diol (**11**), and diarydisulfide (**12**) (Scheme 4). Compounds **7**-**12** are formed, as well as other products whose structures have not been determined. Compounds **7**, **10**, and **12** are reminiscent to the naturally occurring polysulfanes (see Chapter 1, Scheme 1). Compounds **8** and **9** might represent precursors of the cyclic polysulfanes found in nature. The studies utilizing **8** as a precursor to compounds **7**, **10**, **11** and **12** are being conducted presently in the laboratory by Dr. David Aebisher. Interestingly, compound **8** is a new compound not previously described in the literature.



Scheme 4. Reaction of catechol and H_2S_x.

Ortho-quinone **6** was generated by a literature method in a reaction of Ag_2O with catechol **13** in acetone.[32] Inorganic polysulfanes (H_2S_x) were generated by a modified method from literature.[33] Sodium sulfide nonahydrate was heated with elemental sulfur, and upon filtration Cl_3CCO_2H was added to precipitate H_2S_x. An experimental method to generate inorganic polysulfanes in our *in vitro* H_2S_x—quinone biomimetic study is shown in eq 1.

$$2Na_2S + S_x \longrightarrow 2Na_2S_x \xrightarrow{CCl_3CO_2H} H_2S_x + CCl_3CO_2^-Na^+ \qquad (1)$$

Compounds **7-12** were prepared in acetone by slow, drop-wise addition of H_2S_x dissolved in 1 mL of CS_2 to a dark brown, vigorously stirred solution of freshly prepared *ortho*—benzoquinone until the decolorization of the reaction mixture took place. After stirring for 1 h the raw mixture was filtered and concentrated under vacuum. Attempts to purify the raw mixture by preparative chromatography and HPLC failed due to very high polarity of the products formed making separation and structure elucidation of **7-12** difficult. There are also several yet unidentified products.

GC/MS was used to analyze the raw material, which revealed evidence consistent with 5,6,7,8,9-pentathia-benzocycloheptene-1,2-diol **7** [*m/z* 64 (43), 96 (12), 110 (48), 142 (66), 204 (100), 268 (10)]. Compound **7** displays a weak molecular ion peak *m/z* 268 [M^+] and a strong base peak at *m/z* 204 [M^+-2S] representing the loss of two sulfur atoms (Figure 2, inset). Furthermore, GC/MS analysis revealed evidence for 3-mercaptobenzene-1,2-diol **8** [*m/z* 51 (6), 81 (18), 96 (17), 109 (21), 113 (10), 142 (100)]. The molecular ion of 3-mercaptobenzene-1,2-diol **8** was abundant and eluted at about 11 minutes on the GC/MS trace; compound **9** eluted at 39 minutes (Figure 2). Catechol and S_8 could also be seen in the GC/MS trace. We have obtained single ion mode (SIM) GC/MS evidence for compounds **10**, **11**, and **12**.

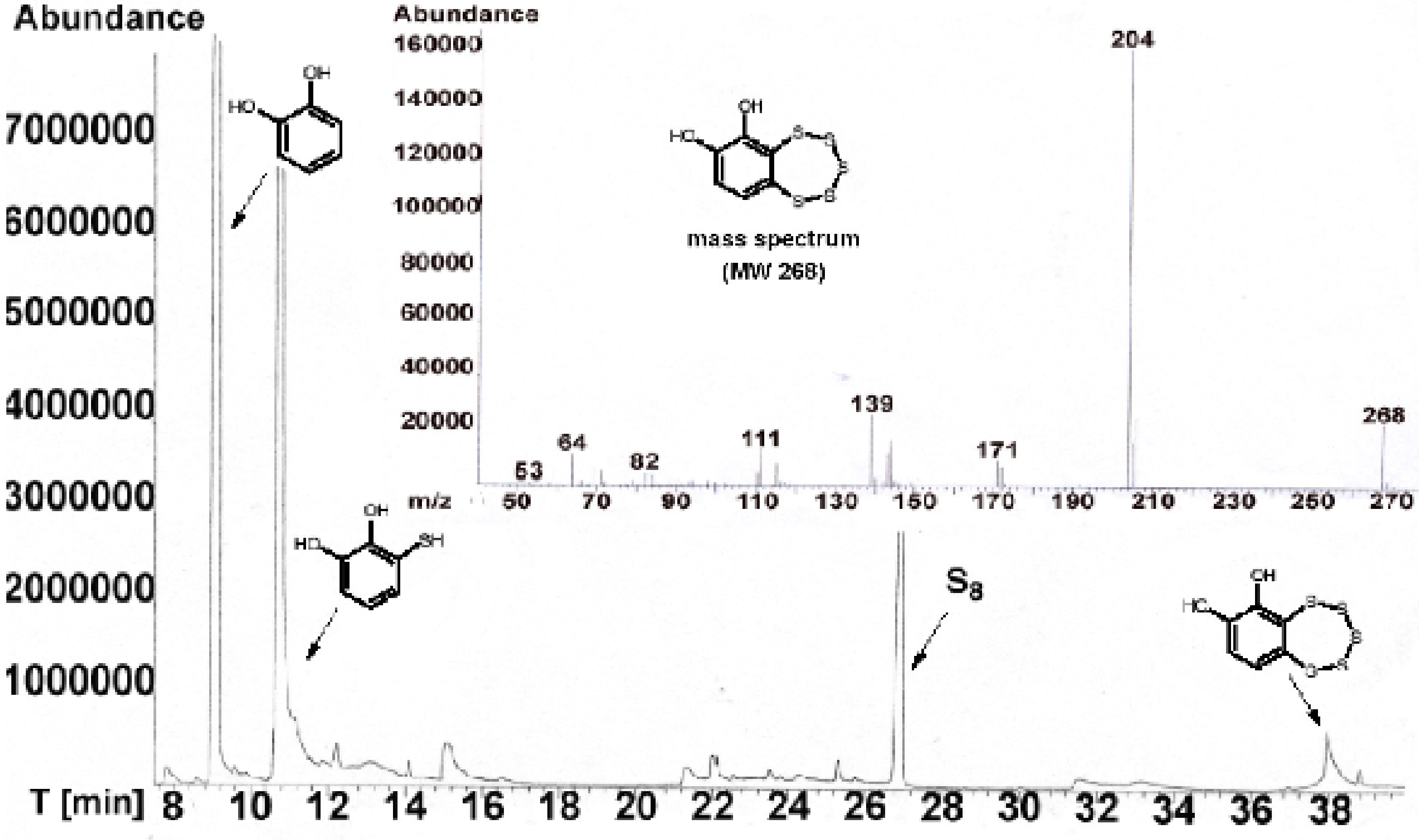
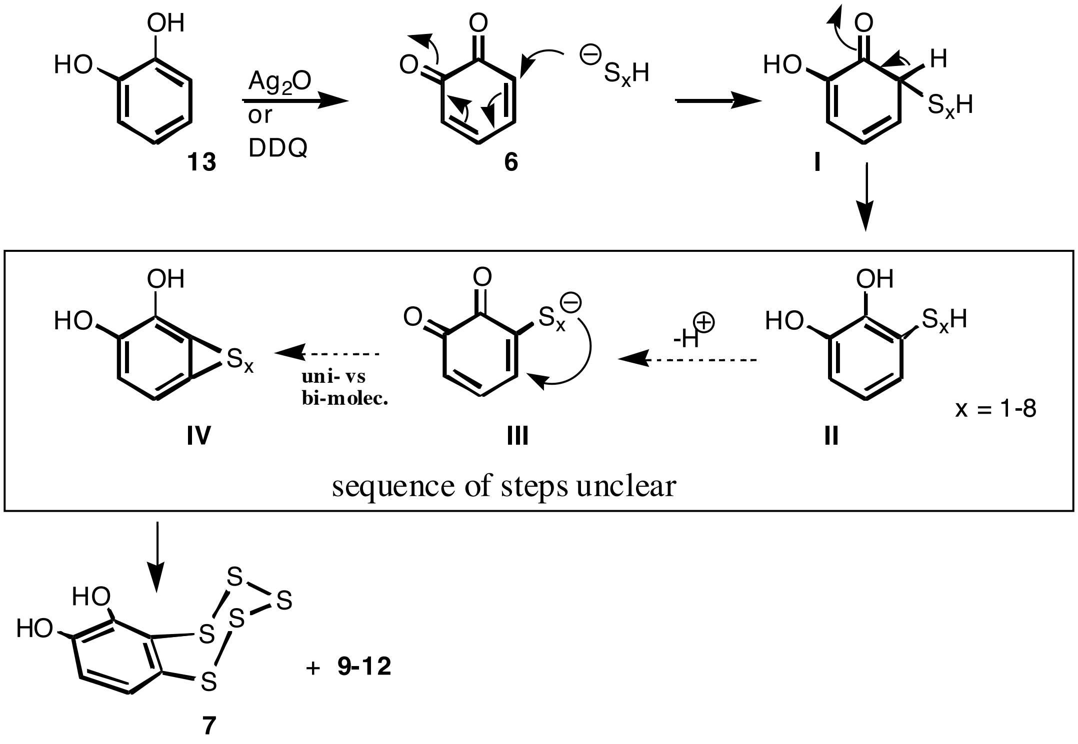


Figure 2. GC/MS trace of H2Sx + *o*-quinone reaction mixture

4.2.2 Mechanism of Sulfur Incorporation.

The data suggest a mechanism with an HS_x^- ion 1,6-conjugate addition to *o*-quinone and a second quinol oxidation-sulfuration as key processes to the formation of pentathiepin **7** (Scheme 5). We propose that benzoquinone **6** derived from catechol **13** undergoes nucleophilic addition, which gives rise to α-hydroxy ketone (**I**). α-Hydroxy ketone intermediate (x=1-8) **I** can become aromatic by rearrangement to mercaptocatechol, when x=1 or hydropolysulfidecatechol, when x>1 (**II**).



Scheme 5. Mechanism of sulfur incorporation

The fact that **7** forms in our experiments suggest that it arose from a second oxidation of **II** to quinone **III** (**II** → **III**). The oxidizing agent that enables the possible **II** → **III** interconversion is yet to be identified since Ag_2O (used to generate **6**) is not present in the sulfuration reaction. It is possible that **6** itself is serving as the oxidizing agent on **II** and this second oxidation step is connected with pentathiepin **7** formation. The reaction may follow a unimolecular mechanism where hydropolysulfidobenzoquinone ion **III** undergoes intramolecular 1,4-conjugate (Michael) addition to form cyclic polysulfanes **IV** containing from 1 to 8 sulfur atoms. Cyclic polysulfanes **IV** may then equilibrate to form thermodynamically stable **7** and other products **9-12**. A bimolecular mechanism is also conceivable for the conversion of **III** to **IV**, which involves a 1,4-conjugate addition of a second thiolate ion (HS_x^-) with **III** to give rise to an intermediate with *ortho*—polysulfide ion chains, which may subsequently equilibrate to polysulfanes **IV** (x=1-8). The equilibration to **IV** is followed by another equilibration process to form thermodynamically stable products. The proposed mechanism involving subsequent equilibration steps can explain the formation of the cyclic polysulfanes detected.

4.2.3 Protecting Groups and the Structure Elucidation of the Products of Catechol—S_8 Reaction.

A strategy of chemical protection the hydroxy and mercapto groups was conducted so that conclusive spectroscopic data could be collected for the polysulfanes generated in the quinone—S_x reaction. Benzyl, methyl, acetonide and acetyl protecting groups were tested the polarity of the raw mixture of polysulfanes would be reduced to enable their separation. During the addition of the –OH or –SH protecting group, we desired that the S-S bonds remain intact to make their separation and structure elucidation of polysulfanes possible rather than unwanted conversion of polysulfane group RSS_xSR to thiols RSH. Acetylation turned out to be the best protecting strategy. Acetylation of the crude mixture of products allowed isolating characterizing compounds **18**, **19**, **20** and **21** (Scheme 7). The detailed procedures and comments on successes and failures of protecting group strategy as well as separation and structure elucidation will be given in experimental section.

4.2.4 Conclusion.

The biomimetic synthesis of *o*-benzopolysulfanes from *o*-benzoquinone and reduced elemental sulfur had been completed. The *ortho*-benzoquinone—S_x reaction gave rise to a complicated mixture of products, which includes compounds **7**-**12**. Compounds **7**, **10** and **12** are reminiscent of the secondary metabolites isolated from ascidians (Scheme 1, Chapter 1). Compounds **8** and **9** may be envisioned as precursors in the biomimetic synthesis of more complicated polysulfanes. Enzyme involved in biosynthesis of naturally occurring polysulfanes probably provides a very oxidizing environment, thus these compounds are not seen in Nature. The possibility of the biomimetic synthesis of dimeric and trimeric polysulfanes, e.g., lissoclinobadin 1, lissoclinobadin 2 or lissoclinobadin 3 (Scheme 1, Chapter 1) will be investigated in the future. The hydroxy groups of products containing **7**-**12** were protected with benzylation, methylation, acetonidation and acetylation for the purpose of structure elucidation. Finally, products **7**, **8** and **9** were isolated and characterized in their acetylated form as acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester **18**, acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester **19** and acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester **20**.

The results of the *ortho*-benzoquinone—S_x reaction provide the evidence, that using molecules involved in defense mechanism of organisms as a template may serve as a mechanistic guide for the synthesis of naturally occurring polysulfanes and designing and synthesis of new compounds.

4.3 Experimental Section

4.3.1 Reagents and Solvents

Reagents and solvents were obtained commercially: catechol, silver oxide, acetone, sodium sulfide nonahydrate, sulfur, trichloroacetic acid, sodium sulfate anhydrous, carbon disulfide, TsOH, DMF, K_2CO_3, benzyl bromide, Et_2O, TBAF, MeI, THF, $CHCl_3$, MeOH, ethyl acetate, acetic anhydride, DMAP, sodium bicarbonate and hexanes were used as received. Experiments were carried out using TLC-Sheets Silica gel 60 F 254, Agilent Technologies GC/MS instrument consisting of a 6890N series GC and 5973 series mass detector, Perkin-Elmer HPLC, and a Bruker 400 MHz NMR spectrometer.

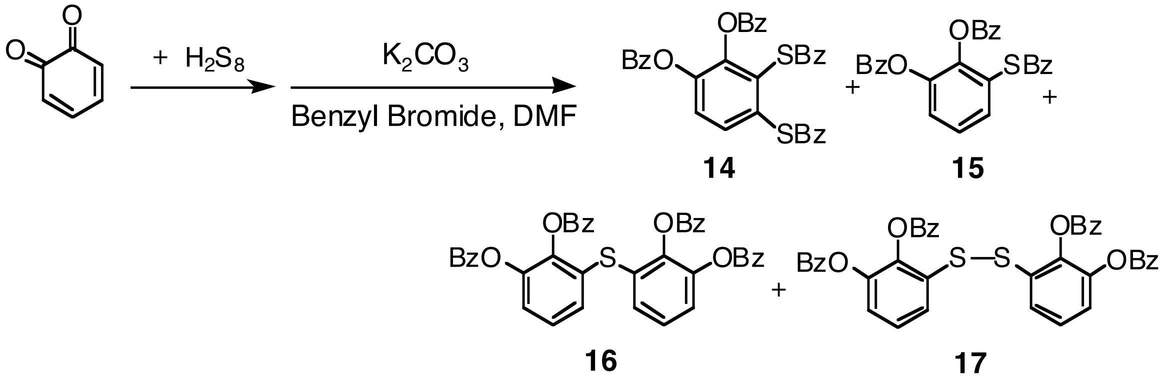
***Ortho*-benzoquinone** was prepared by a literature method.[32] To a solution of catechol (0.1g, 0.0018 mol) in acetone (5 mL), silver oxide (0.4 g) was added and a mixture was stirred for 10 min. The mixture containing benzoquinone was filtered and immediately sulfurated.

Inorganic polysulfanes H_2S_x were synthesized by modified method of Steudel.[33] Two grams of $Na_2S \times 9H_2O$ crystals were dissolved in 2 mL of water and heated with 1 g of S_8 over night. An aqueous solution of sodium polysulfanes were obtained [$NaS(S)_xSNa$], which was filtered and slowly added to an excess of aqueous CCl_3COOH (4 g in 3 mL of H_2O, 8 mM) at 0 °C. H_2S_x precipitated settling to the bottom of the flask as a yellow oil. H_2S_x was separated from the aqueous phase and dissolved in 1 mL of CS_2.

4.3.2 Use of Protecting Groups.

Benzyl Protecting Group.

We decided to use benzyl protecting groups by applying procedure used by Roush et al.[34] A raw material prepared by addition of polysulfanes to benzoquinone obtained from 0.1 g of catechol was dissolved in 5 mL of DMF. To a solution of the mixture of polysulfanes in DMF was added K_2CO_3 (2.5 g, 0.018 mmol) and benzyl bromide (2 mL, 17 mmol). This mixture was stirred at ambient temperature for 12 h. When TLC indicated completion of the reaction, 30 mL water was added and the aqueous solution was extracted three times with Et_2O•hexanes (8 : 2). The combined extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. The GC/MS analysis of the crude material revealed the presence of (**14**), (**15**), (**16**) and (**17**) (Scheme 6).



Scheme 6. Benzylated polysulfanes

Benzylated 3,4-dimercapto-benzene-1,2-diol (**14**) was derived from pentathiepin **7**. A pentasulfane ring of **7** underwent decomposition under basic condition to give rise to **14**. The decomposition of pentasulfane ring is an undesired result, however it might be considered as an indirect evidence for the formation of cyclic polysulfanes including pentathiepins. The purposeful reduction of polysulfanes was used by Barton et al. to provide evidence for biomimetic, iron catalyzed insertion of sulfur into C-H bond. The goal of the project was to obtain pure pentathiepin **7** and other cyclic polysulfanes such as **10** and **11** in order to provide the full spectral evidence for their formation.

Methyl Protecting Group.

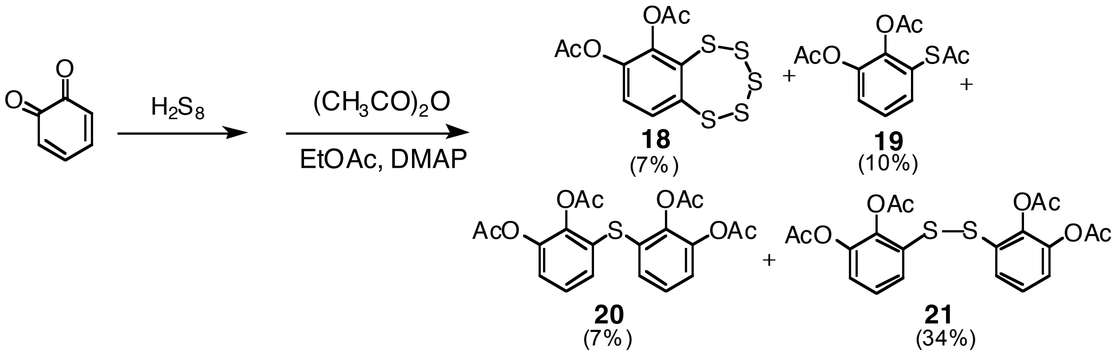
We tried to methylate hydroxy groups of the crude sample of the polysulfanes obtained in quinone—S_8 reaction.[36] TBAF (4.0 mL, 3 μmol) was added to a stirring solution of crude polysulfanes in THF (12 mL) and MeI (37 mL, 0.59 mol) to give a dark green solution. The solution turned deep red within a few minutes. The solution was allowed to stir 20 minutes then was diluted with chloroform (20 mL) and poured into 20 mL H_2O. The organic layer was separated and dried with Na_2SO_4. The TLC analysis in $CHCl_3$:MeOH (98:2) mixture of solvent of the raw material revealed, that the sample after methylation was more complicated compared to mixture before methylation. We reason that the complexity of the methylated sample can be explained since GC/MS analysis showed that methylation was not complete. Furthermore, the described method of protection of the free hydroxy groups led again to decomposition of pentathiepin ring. The result convinced us to change the method.

Acetonide Protecting Group.

We decided to convert all hydroxy and mercapto groups into acetonide functionality.[37] The mixture of polysulfanes derived from (0.1 g) catechol was dissolved in 5 mL of acetone followed by addition of 0.2 eq of TsOH (0.18 mmol, 35 mg). GC/MS analysis showed the formation of protected compounds. Interestingly, it turned out to be not possible to separate the protected compounds due to immediate deprotection of all hydroxy groups catalysed by silicagel.

Acetyl Protecting Group.

We found that acetylation is the best method to protect the hydroxy groups. Acetylation efficiently protects all hydroxy and thiol groups and, what is the most important, it does not affect the pentathiepin ring. In order to acetylate the raw material containing compounds **7-12** was dissolved in 3.6 ml of ethyl acetate, followed by addition of 0.2 eq (0.006 g, 0.18 mmol) of DMAP and (230 μL, 2.52 mmol) of acetic anhydride (Scheme 7). After stirring for 16 h the reaction mixture was diluted with 5 mL of ethyl acetate, washed 2 times with 10 mL saturated $NaHCO_3$ and brine, and dried with anhydrous Na_2SO_3. We were able to detect by GC/MS 4 compounds: acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester (**18**), acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester (**19**), acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester (**20**) acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester (**21**) (Scheme 17). Compound **21** originated from compound **8**. The formation of compound **21** provided evidence that coupling of **8** to give **21** occurred faster than its acetylation.



Scheme 7. Acetylated polysulfanes

4.3.3 Purificalion of Acetylated Sample of the Products of the Biomimetic Reaction

Purification by Using HPLC

We decided to use HPLC to purify acetylated sample of polysulfanes. We used acetonitrile and water mixture to elute the products of biomimetic reaction of polysulfanes with benzoquinone. The standard pump program used in the experiment is presented in Table 1.

Table 1. The pump program used for HPLC analysis and purification of polysulfanes from the acetylation of the products of the nucleophilic addition of polysulfanes to benzoquinone.

	Time [min]	Flow [ml/min]	%A MeCN	%B H_2O	Curve
0	3	1	10	90	0
1	10	1	40	60	1
2	10	1	60	40	1
3	10	1	90	10	1
4	10	1	100	0	1
5	5	1	100	0	0

The purification of polysulfanes by HPLC turned out to be not efficient because of the size of the column (*μ*BONDPACK C18 P/N 27324) used for the purpose of the purification.

Purification by Using Preparative Chromatography

The raw material containing acetylated polysulfanes **18**-**21** was purified by preparative chromatography in several steps. First, the mixture of acetylated polysulfanes **18**-**21** was separated into 3 fractions by using EtOAc and Hexane (1:3) as an eluent. The fraction at R_f=0.5 contained mixture of compounds including **18** and acetylated catechol. The fraction at R_f=0.4 contained compound **19**. The fraction at R_f=0.25 contained **20** and **21**. In order to separate and characterize the compounds of interest fractions 1, 2 and 3 were eluted in different eluents.

Purification of acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester 18

Acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester **18** was separated from fraction isolated at R_f=0.5 in mixture of solvents containing EtOAc and Hexanes (1:3) by its eluting in THF:hexanes 1:3 mixture, R_f=0.4. ^{1}H NMR spectrum reveals two singlets corresponding to methyls of acetyl groups protecting 2 OH groups adjacent to each other at 2.31 and 2.35 ppm and two doublets for H_1 and H_2 of aromatic ring with δ=7.18 ppm $^3J_{13}$=8.4 Hz and δ=7.76 ppm $^3J_{13}$=8.4 Hz respectively. MS spectrum is very specific for this class of compounds. It shows very low molecular ion peak at 352 and parent peak at 288 corresponding to loss of 2S fragment. Peaks at 246 and 204 indicate fragmentation by the lost of 2 fragments of the mass 42 corresponding to ketene $CH_2=C=O$. Peak at 204 is a parent peak of **7**.

Purification of acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester 19

Acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester **19** was isolated from fraction at R_f=0.4 in mixture of solvents containing EtOAc and hexanes (1:3) followed by the second separation of the fraction in EtOAc:hexanes 1:3, silica gel TLC R_f=0.42. ^{1}H NMR spectrum of the compound **19** shows three doublets corresponding to methyls of acetyl groups protecting 2 OH and SH groups adjacent to each other at 2.28, 2.30 and 2. 41 ppm respectively, td for H_2 of aromatic ring with δ=7.397 ppm $^3J_{13}$=0.01 Hz, $^3J_{23}$=0.014 Hz and trough space J = 0.003 Hz, two overlapped doublets for H_1 δ = 7.311 ppm, $^3J_{13}$ = 0.01 Hz and H_3 δ = 7.308 ppm, $^3J_{23}$ = 0.014 Hz. MS spectrum reveals parent peak at 142, molecular ion peak at 268 and fragmentation pattern, which indicates the lost of 3 fragments of the mass 42 corresponding to ketene $CH_2=C=O$.

Purification of Acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester 20 and Acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester 21

Compounds **20** and **21** were first collected together (fraction at R_f=0.25 eluted in mixture of solvents containing EtOAc and Hexanes (1:3)) and then separated by eluting in mixture of solvents containing EtOAc and Hexanes (1:3). The analysis of ^{1}H NMR of compounds **20** and **21** shows, that both compounds give almost identical spectra. There are singlets corresponding to CH_3s of both acetyl groups of **20** and **21** located up field at 2.28 ppm. The protons of aromatic ring of **20** and **21** reveal the same pattern of multiplet located between 7.12-71.16 ppm and doublet of doublets (dd) corresponding to 1H at 7.44 ppm. When ^{13}C NMR of 28 and 29 were compared it was possible to observe that chemical shifts of peaks of **20** and **21** are slightly different. Peaks corresponding to **20** are shifted up field in comparison to **21** on ^{13}C NMR.

4.3.4 Characterization of 18-21

Acetic acid 2-acetoxy-5,6,7,8,9-pentathia-benzocyclohepten-1-yl ester 18

Yield 0.0007 g; silica gel R_f = 0.4 (1:3 THF:hexanes); ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.35 (s, 3H), 7.18 (d, 1H, J = 8.4 Hz), 7.76 (d, 1H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 20.36, 20.64, 117.89, 124.83, 125.53, 133.68, 146.88, 144.55, 167.67, 167.76 mass spectrum (EI), *m/z* 64 (23), 102 (10), 131 (21), 139 (15), 172 (6), 204 (100), 246 (34), 288 (26), 352 (0.9). UV (CH_2Cl_2) l_{max} 232 nm; FT-IR (KBr) 1610.39 (C=O), 1257.45 (C-O-C), 702.82 (C-S); HRMS calcd for $C_{10}H_8O_4S_5$ $(M + H)^+$ 352.4708, found 351.9026.

Acetic acid 2-acetoxy-3-acetylsulfanyl-phenyl ester 19

Yield 0.0006 g; silica gel R_f = 0.42 (1:3 EtOAc:hexanes); ^1H NMR ($CDCl_3$) δ 2.28 (d, 3H, J = 1.17 Hz), 2.30 (d, 3H, J = 1.17 Hz), 2. 41 (d, 3H, J = 1.17 Hz) 7.30-7.41 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 20.28, 20.65, 30.14, 125.36, 126.64, 133.69, 167.48, 167.51, 167.89, 191.95; mass spectrum (EI), *m/z* 83 (2), 96 (1), 113 (2), 142 (100), 184 (55), 226 (27), 268 (8). HRMS calcd for $C_{12}H_{12}O_5S$ $(M + H)^+$ 268.2838, found 268.0405.

Acetic acid 2-acetoxy-6-(2,3-diacetoxy-phenylsulfanyl)-phenyl ester 20

Yield 0.0012 g; silica gel R_f = 0.48 (2:3 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 2.29 (s, 3H), 7.12-7.16 (m, 2H), 7.44 (dd, 1H, *J*=8.0 Hz, *J*=1.51 Hz); ^{13}C NMR ($CDCl_3$) δ 20.18, 20.67, 123.19, 124.07, 127.48, 131.26, 140.79, 142.96, 167.03, 168.03 mass spectrum (EI), *m/z* 110 (20), 142 (11), 250 (100), 292 (60), 334 (41), 376 (19), 418 (12); UV (CH_2Cl_2) λ_{max} 280 nm, 321 nm; HRMS calcd for $C_{20}H_{18}O_8S$ $(M + H)^+$ 418.4174, found 418.0722

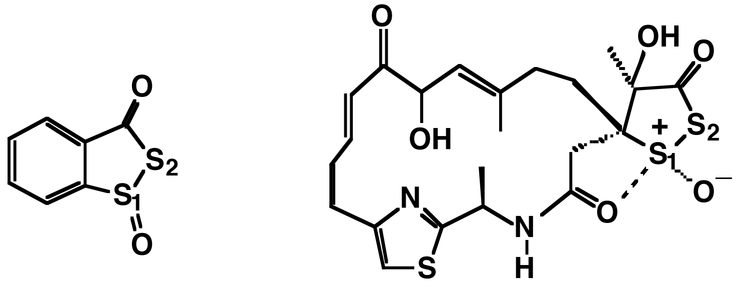
Acetic acid 2-acetoxy-3-(2,3-diacetoxy-phenyldisulfanyl)-phenyl ester 21

Yield 0.0048 g; silica gel R_f =0.42 (2:3 EtOAc:hexanes); ^{1}H NMR ($CDCl_3$) δ 2.83 (s, 3H), 2.93 (s, 3H), 7.18 (d, 1H, *J*=8.4 Hz), 7.76 (d, 1H, *J*=8.4 Hz); ^{13}C NMR ($CDCl_3$) 20.20, 20.68, 123.04, 124.08, 126.28, 126.95, 140.85 142.99, 167.60, 168.05 mass spectrum (EI), *m/z* 83 (6), 111 (10), 142 (71), 184 (22), 282 (100), 324 (60), 366 (91), 408 (29), 450 (41); UV (CH_2Cl_2) λ_{max} 280 nm, 320 nm; HRMS calcd for $C_{20}H_{18}O_8S_2$ $(M + H)^+$ 450.4774, found 450.0443.

Chapter 5. Synthesis of a Series of 3- and 5- Substituted Benzo-1,2-Dithiolan-3-one 1-Oxides

5.1 Introduction.

Benzo-1,2-dithiolan-3-one 1-oxide (**1**) contains a thiosulfinate ester heterocycle[1] and possesses DNA-cleaving activity, albeit reduced, compared to the natural product leinamycin (**2**) (Scheme 1).[2-5] DNA-cleaving activity[2-5] and antitumor activity[6-13] of leinamycin **2** and other dithiolanone-oxide compounds are thought to arise by an initial attack of thiol or another nucleophile onto the dithiolanone-oxide ring system.[14-16] We have synthesized *ortho* [X = Cl (**3**), CH_3 (**4**), OCH_3 (**5**)] and *para* [Y = Cl (**6**), CH_3 (**7**), OCH_3 (**8**)] substituted 1,2-dithiolan-3-one 1-oxides that contain different substituent groups, which also differ in being nearer or further to the sulfinate sulfur (S1) (Scheme 2). The *ortho* and *para* positions are relative to the sulfinyl sulfur (S1) and refer to the 3- and 5-positions of the fused benzene ring. A structure-activity study in which the structure of the benzene core of **1** is varied by introduction of substituents may provide insight into the factors influencing the thiol reaction with dithiolanone-oxide drugs.



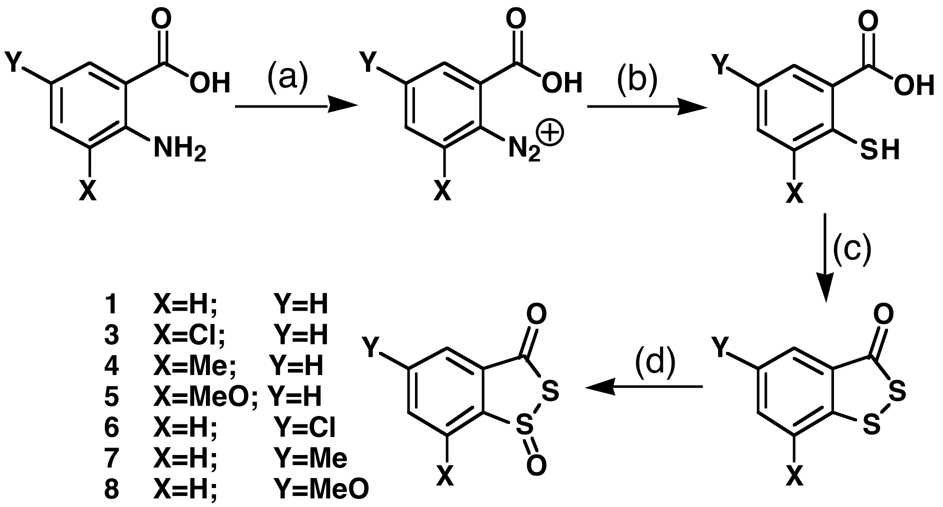
1 **2**

Scheme 1. Molecules containing the dithiolanone oxide heterocycle ring.

5.2 Results and Discussion.

5.2.1 Synthesis of Benzo-1,2-Dithiolan-3-one 1-Oxides.

Preparation of dithiolanone-oxides **1** and **3**-**8** is shown in Scheme 2 where a modification of the method of Beaucage et al.[17] was used.



Scheme 2. Synthesis of 1,2-benzodithiolan-3-one 1-oxides **3**-**8**: (a) HCl, $NaNO_2$, 5 °C; (b) (i) Na_2S, elemental S_8, NaOH, 5 °C; (ii) Zn, glacial CH_3CO_2H, reflux, 48 h; (c) H_2SO_4, CH_3COSH, 24 °C, 15 min.; (d) dimethyldioxirane, 0 °C, 2 h, 20-30%.

Treatment of a 3- or 5-substituted anthranilic acid with $NaNO_2$ yielded the diazo compound. Sodium sulfide and elemental sulfur were added to the diazo compound to give the dithiosalicylic acid derivative and after reduction thiolsalicylic acid. Thiolacetic acid was then added to the thiosalicylic acid, which after oxidation with dimethyldioxirane gave the substituted benzodithiolanone-oxides **3**-**8** in 20-30% yield (Scheme 2). The synthetic method gave higher yields with structures possessing the methyl (30%) or methoxy groups (25%) compared to the chloro substituent (20%). A likely reason is that the generation of the diazo group should depend on an electron donating ability to add stability. Compounds **3**-**8** were synthesized and characterized as described in the Experimental Section.[18] The known dithiolanone-oxide **1**[1] was prepared by oxidation of commercially available 3*H*-1,2-benzodithiolan-3-one with dimethyldioxirane.

5.2.2 Reaction of Benzo-1,2-Dithiolan-3-one 1-Oxides with Thiol.

Experiments to analyze the substituent effect were collected for the reaction of **1** and **3**-**8** with *n*-propyl thiol in a mixture of CD_3CN and D_2O. These experiments were conducted by Nahed Sawwan. Thiol was added to **1** and **3**-**8** in acetonitrile water (7:3) mixtures. The reaction is facilitated by reducing the electron density at the *para* position, or by placing substituents bearing lone pair electrons *ortho* to the dithiolanone-oxide (S1) reaction center. Through-space and through-bond effects both contribute to the conversion of polysulfane products.

Further details of the substituent effects on the reactivity of 1,2-benzodithiolan-3-one 1-oxides with *n*-PrSH will be presented in the Ph.D. thesis of Nahed Sawwan.

5.2.3 Conclusion.

In conclusion, the synthesis of a series of 1,2-benzodithiolan-3-one 1-oxides **3**-**8** has been accomplished. Studies on the substituent effects of benzodithiolanone oxides in terms of subsequent reactivity will be presented in the future.

5.3 Experimental Section.

5.3.1 Materials and Instrumentation.

Reagents and solvents were obtained commercially [2-amino-3-methylbenzoic acid, 2-amino-3-methoxybenzoic acid, 2-amino-3-chlorobenzoic acid, 2-amino-5-methylbenzoic acid, 2-amino-5-chlorobenzoic acid, 2-amino-5-methoxybenzoic acid, 1,3,5-trimethoxybenzene, sodium nitrate, sodium sulfide, elemental sulfur (S_8), sodium hydroxide, sodium bicarbonate, zinc dust, hydrochloric acid solution (12 M), sulfuric acid solution (1 M), glacial acetic acid, thiolacetic acid, magnesium sulfate, potassium bromide, acetone, ethanol, $CHCl_3$, $CDCl_3$, CH_3CN, CD_3CN, hexane, propyl thiol] and were used as received. Proton and carbon NMR data was acquired on a Bruker 400 MHz NMR spectrometer. Mass spectrometry data was collected on one of two GC/MS instruments, a Hewlett-Packard GC/MS instrument consisting of a 6890 series GC and a 5973N series mass selective detector, or a Hewlett-Packard GC/MS instrument consisting of a 5890 series GC and a 5988A series mass selective detector. IR data was collected on a Nicolet Magna FI-IR 760 spectrometer. UV data was collected on a Hitachi V-2001 spectrometer.

Dithiolanone-oxide Synthesis. The synthesis involved adding a 3- or 5-substituted anthranilic acid (6.6 mmol) to a 4 mL water solution of 2 mL of concentrated HCl and $NaNO_2$ (6.6 mmol) at 5 °C to generate the diazo compound. A separate solution was prepared by mixing $Na_2S\times(9H_2O)$ (7.3

mmol) with elemental sulfur (7.3 mmol) at 24 °C, which was heated and made alkaline by the addition of 10 M NaOH. The mixture of the two solutions resulted in a precipitate upon addition of HCl. The precipitate was collected and boiled in a saturated solution of $NaHCO_3$ (16 mL). After boiling for 15 min, the mixture was filtered to remove the insoluble material and concentrated HCl was added to the filtrate until the crude product precipitated out as a yellow solid. Excess of concentrated HCl was added to the mixture until precipitation was complete and the precipitate was isolated by filtration. The material collected by filtration was boiled in absolute EtOH (6 mL) for 15 min and filtered and the filtrate was concentrated under reduced pressure to yield the dithiosalicylic acid derivative. The dithiosalicylic acid derivative was mixed and Zn dust (0.40 g) in glacial acetic acid and refluxed for 48 hours. The mixture was then cooled and filtered. The collected solid was boiled in 5 M NaOH (12.0 mL). After boiling for 30 min, the undissolved solid was removed by filtration and the clear filtrate acidified with concentrated HCl until the crude product precipitated out as a yellow solid. The precipitate was collected, boiled in EtOH (6 mL), filtered and the filtrate was concentrated under reduced pressure to yield the thiolsalicylic acid derivative. The material was directly carried on to the next step.

Thiolacetic acid (0.110 mol) was added to thiosalicyclic acid (0.005 mol) in concentrated H_2SO_4 at 24 °C under nitrogen over a period of 10 min. The mixture was placed in an oil bath pre-heated to 60 °C and stirred for 4 hours. The dark mixture was the poured over crushed ice and allowed to stand at 24 °C for 30 min. The obtained precipitate was washed with boiling $CHCl_3$ (2 × 42 mL) and water (2 × 42 mL). The organic layer was dried over MgSO4 and the solvent was removed under reduced pressure to yield the crude benzo-substituted 1,2-dithiolanone, which was purified by preparative chromatography by using chloroform:hexanes (2:1) mixture as an eluent.

The purified benzo-substituted dithiolanone (0.2 mmol) dissolved in DCM (1 mL) was oxidized by drop wise addition of 2.5 mL dimethyldioxirane in acetone at rt. When the oxidation was accomplished the mixture of solvents was removed under reduced pressure to yield benzo-substituted 1,2-dithiolanone oxide.

5.3.2 Characterization of Dithiolanone oxides 1 and 3-8.

1-Oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 1

^{1}H NMR (CD_3CN): 7.95 (t, *J*=7.35 Hz, 1H), 7.82 (t, *J*=7.47 Hz, 1H), 8.07 (d, *J*=7.77 Hz, 1H), 8.02 (d, *J*=7.60 Hz, 1H). ^{13}C NMR 152.1, 135.7, 133.2, 131.3, 128.1, 127.0. MS m/z (% rel. intensity) 68 (26), 76 (37), 108 (28), 136 (100), 184 (M^+, 3). FT-IR (KBr) 1703 (C=O), 1071 (S=O). UV (CH_2Cl_2) λ_{max} 281 nm.

7-Chloro-1-oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 3

^{1}H NMR (CD_3CN): 7.98 (dd, J_1=1.18, J_2=7.98, 1H), 7.93 (dd, J_1=1.19, J_2=7.67, 1H), 7.86 (t, *J*=7.75, 1H). ^{13}C NMR (CD_3CN): 190.6, 148.1, 136.6, 135.2, 134.0, 133.1, 125.5. MS *m/z* (% rel. intensity) 69 (25), 75 (35), 95 (17), 110 (21), 142 (22), 170 (100), 218 (M^+, 17). FT-IR (KBr) 1690 (C=O), 1100 (S=O).

7-Methyl-1-oxo-1H-1l^4-benzo[1,2]dithiol-3-one 4

^{1}H NMR (CD_3CN): 7.68 (d, *J*=5.10 Hz, 2H), 7.84 (t, *J*=4.2 Hz, 1H), 2.78 (s, 3H, CH_3). ^{13}C NMR (CD_3CN): 191.4, 149.6, 139.1, 137.9, 133.4, 130.8, 124.5, 16.9. MS *m/z* (% rel. intensity63 (43), 90 (44), 121 (64), 150 (100), 198 (M^+, 25). FT-IR (KBr) 1690 (C=O), 1100 (S=O). UV (CH_2Cl_2) λ_{max} 290 nm.

7-Methoxy-1-oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 5

^{1}H NMR (CD_3CN): 7.55 (dd, J_1=0.87, J_2=7.61 Hz, 1H), 7.34 (dd, J_1=0.58 J_2=8.17 Hz, 1H), 7.74 (t, *J*=7.76, 1H), 4.07 (s, 3H, OCH_3). ^{13}C NMR (CD_3CN): 191.5, 158.1, 137.8, 136.0, 132.8, 118.9, 118.0, 56.6. MS *m/z* (% rel. intensity) 69 (42), 76 (45), 108 (21), 138 (23), 166 (100), 214 (M^+, 28). FT-IR (KBr) at 1710 (C=O), 1690, 1080 (S=O). UV(CH_2Cl_2) λ_{max} 323 nm.

5-Chloro-1-oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 6

^{1}H NMR (CD_3CN): 7.82 (dd, J_1=1.98, J_2=8.26 Hz, 1H), 7.90 (t, *J*=8.27 Hz, 2H). ^{13}C NMR (CD_3CN): 190.0, 150.5, 139.5, 135.6, 132.9, 129.1, 126.1. MS *m/z* (% rel. intensity): 75 (39), 95 (31), 110 (25), 130 (9), 142 (38), 170 (100), 202 (18), 218 (M^+, 31). FT-IR (KBr) 1720, 1690 (C=O), 1080 (S=O), 780, 820 (C-Cl). UV (CH_2Cl_2) λ_{max} 280 nm.

5-Methyl-1-oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 7

^{1}H NMR (CD_3CN): 7.92 (d, *J*=7.97 Hz, 1H), 7.79 (s, 1H), 7.73 (d, J=8.0 Hz, 1H), 2.56 (s, 3H, CH_3). ^{13}C NMR (CD_3CN): 191.2, 149.6, 144.8, 136.6, 131.0, 127.7 126.5, 20.0. MS *m/z* (% rel. intensity): 39 (54), 51 (49), 63 (78), 78 (54), 89 (71), 121 (92), 150 (100), 182 (55), 198 (M^+, 17). FT-IR (KBr) 1700 (C=O), 1087 (S=O). UV (CH_2Cl_2) λ_{max} 280 nm.

5-Methoxy-1-oxo-1H-1λ^4-benzo[1,2]dithiol-3-one 8

^{1}H NMR (CD_3CN): 8.01 (d, *J*=8.63 Hz, 1H), 7.51 (dd, J_1=2.57, J_2=8.65 Hz, 1H), 7.42 (d, *J*=2.5 Hz, 1H), 3.95 (s, 3H, OCH_3). ^{13}C NMR (CD_3CN): 190.8, 163.6, 143.7, 133.5, 129.0, 122.1, 109.9, 56.0. MS *m/z* (% rel. intensity): 64 (57), 95 (20), 123 (45), 138 (21), 166 (100), 214 (M^+, 14).

APPENDIX

1H

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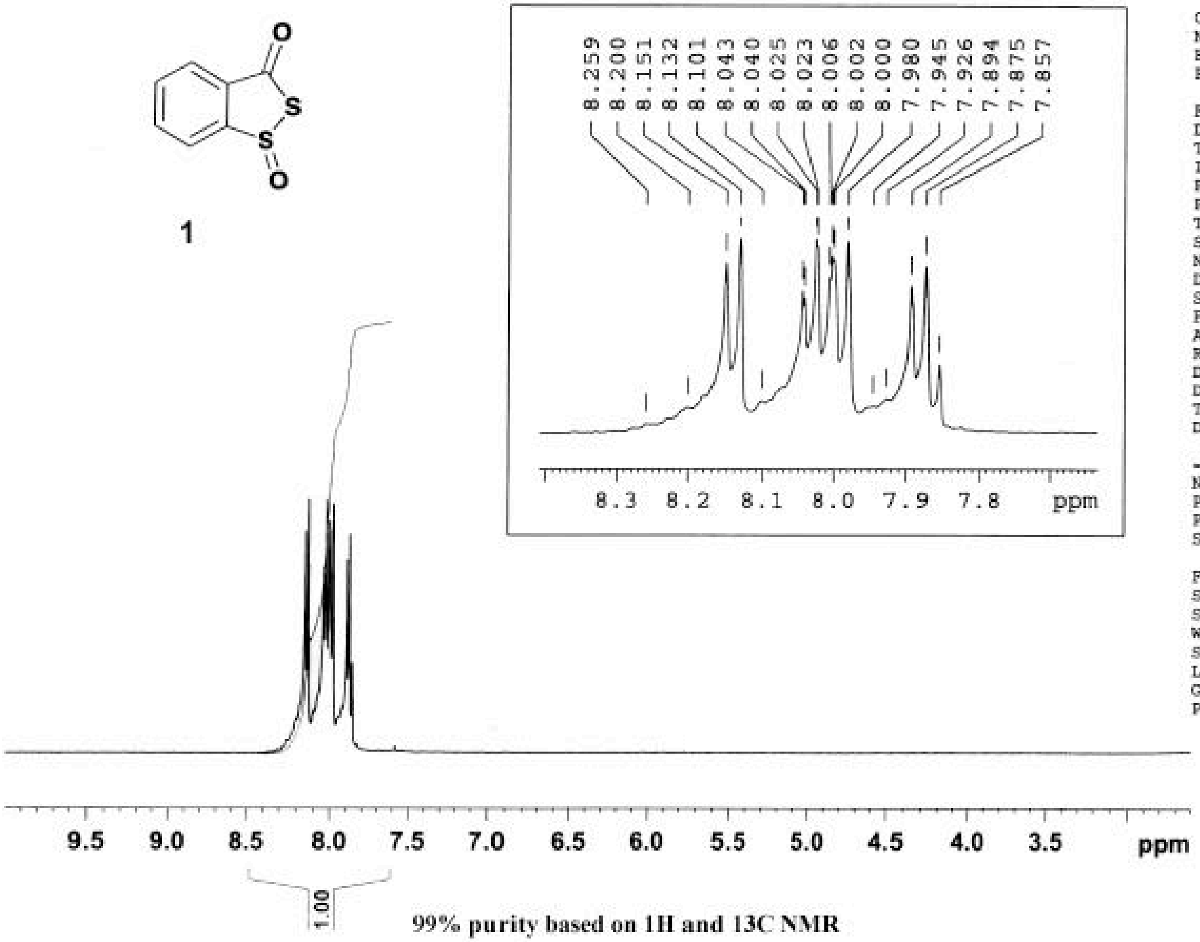

99% purity based on 1H and 13C NMR

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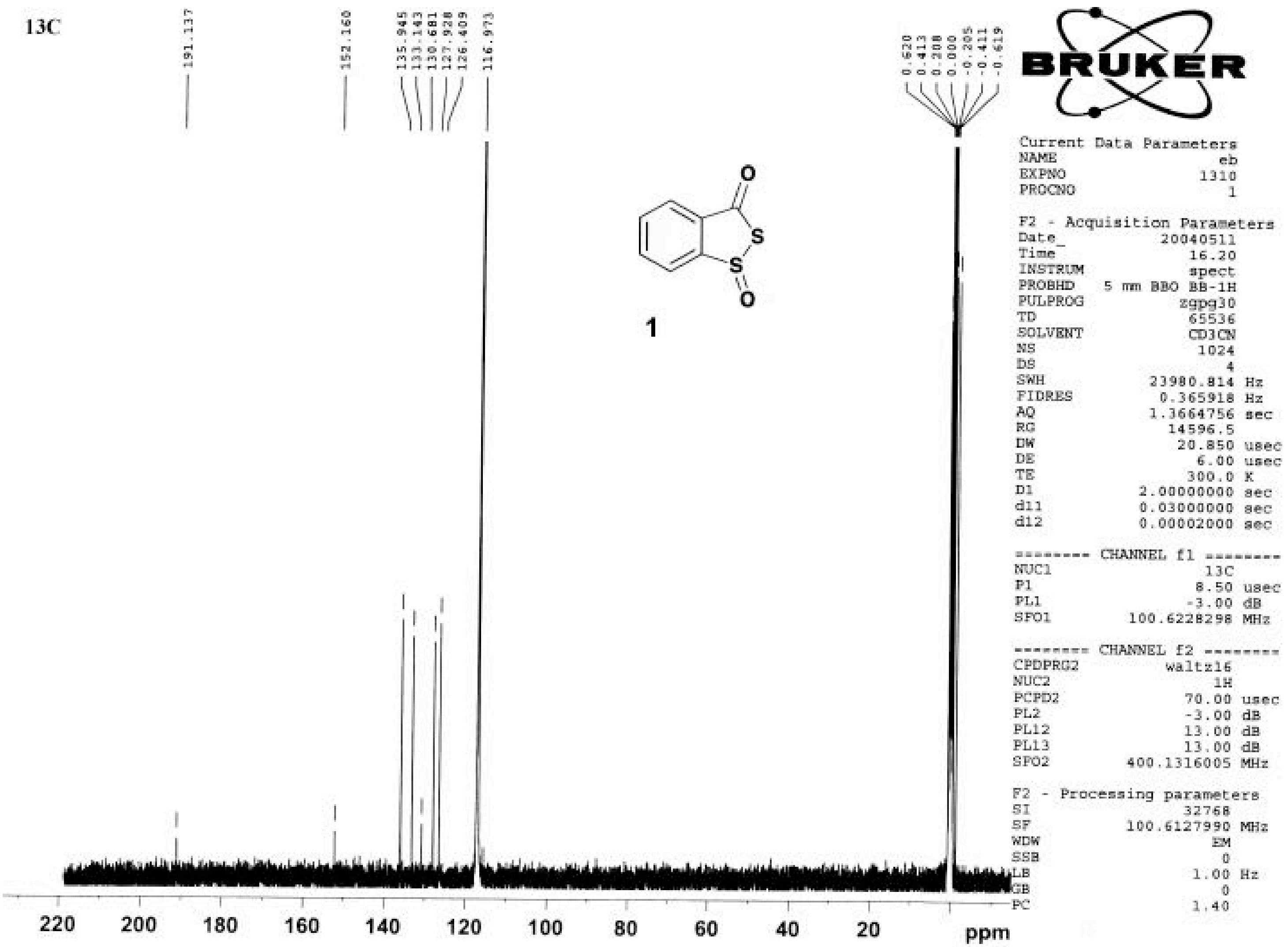

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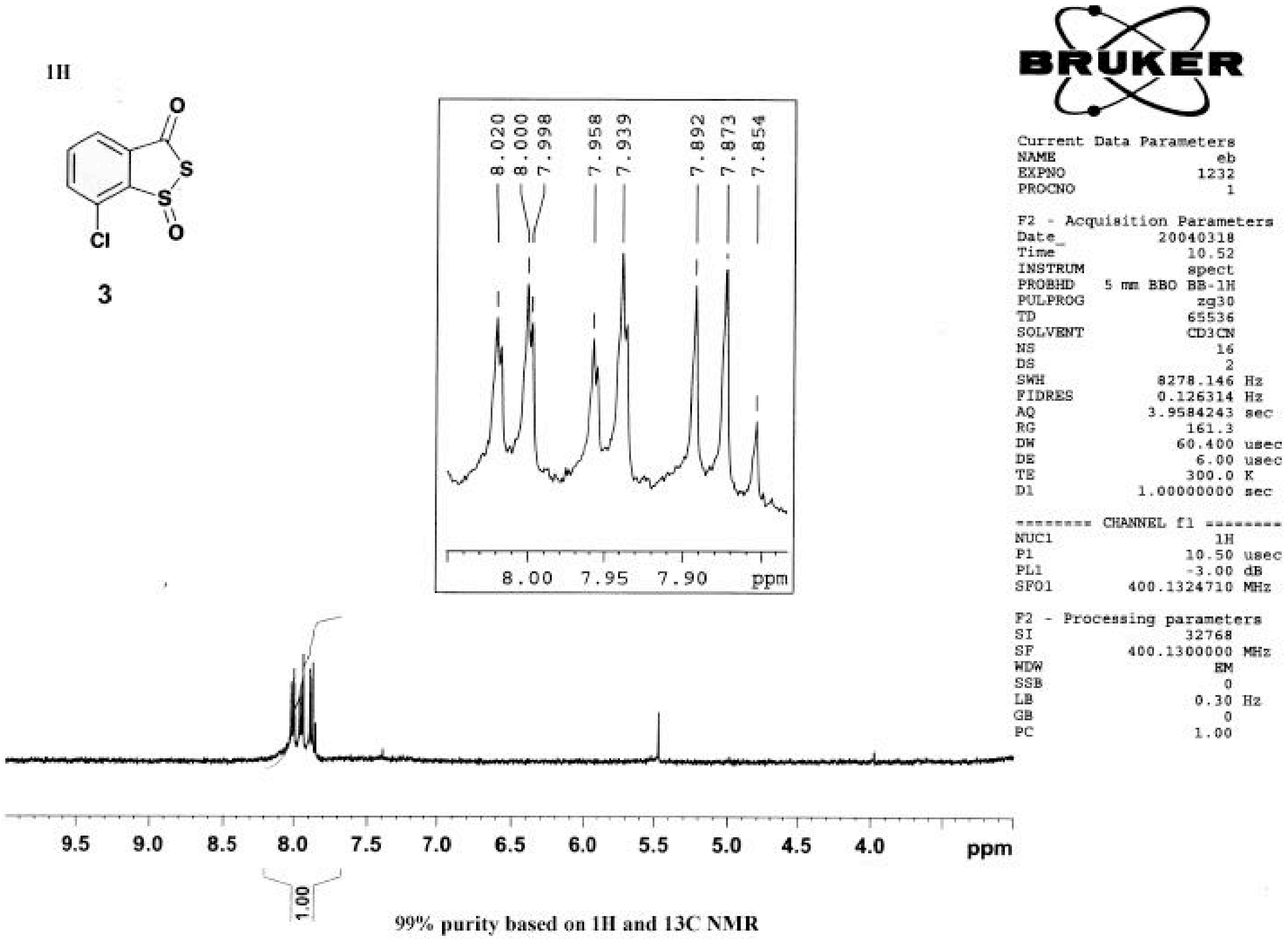
99% purity based on 1H and 13C NMR



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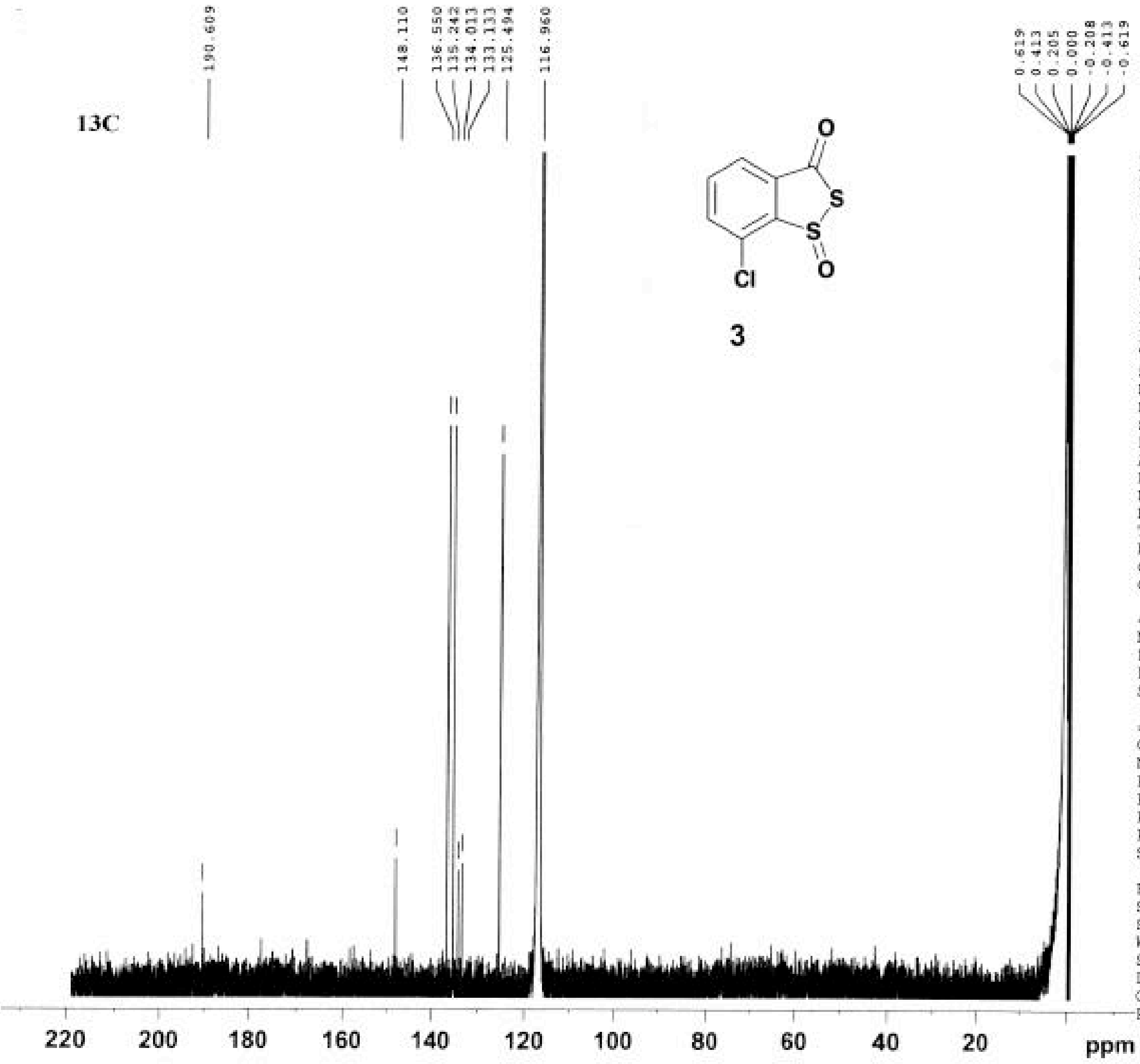
99% purity based on 1H and 13C NMR



99% purity based on 1H and 13C NMR

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d12          0.00002000 sec

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======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                 1H
PCPD2             70.00 usec
PL2               -3.00 dB
PL12              13.00 dB
PL13              13.00 dB
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                32768
SF          100.6127930 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
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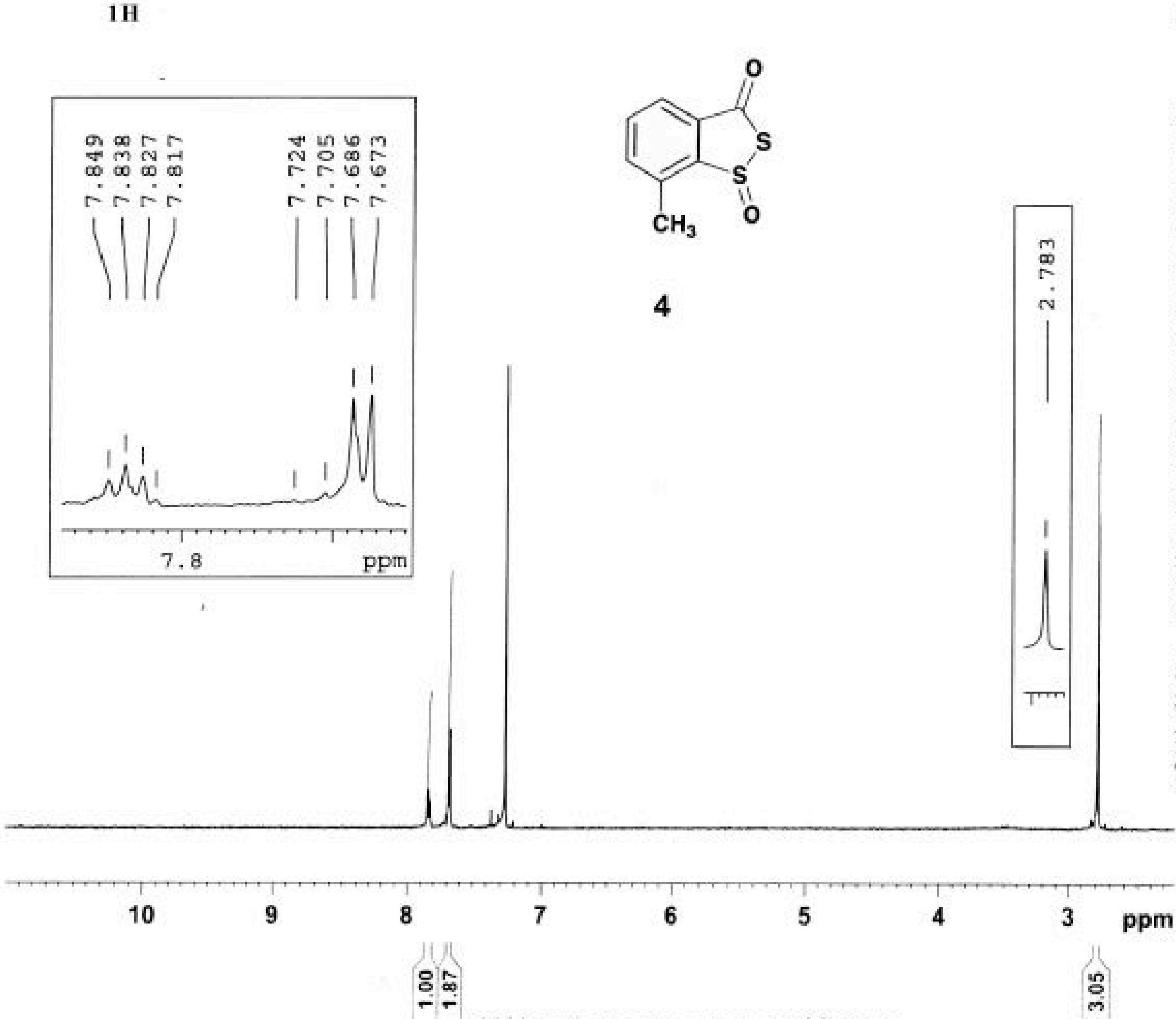


4

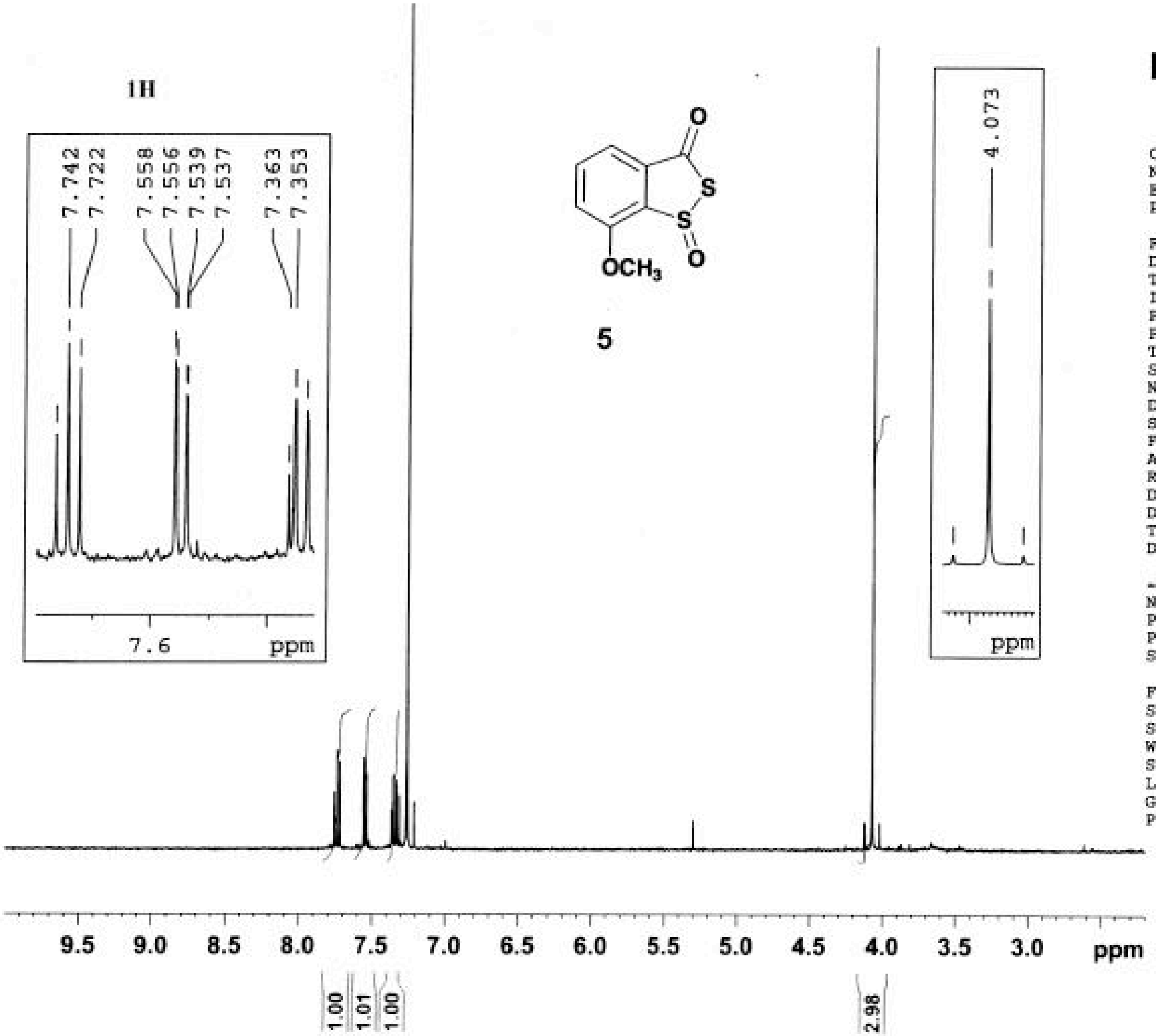
```
Current Data Parameters
NAME                 eb
EXPNO                 2
PROCNO                1

F2 - Acquisition Parameters
Date_          20030818
Time              11.56
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    2
SWH            8278.146 Hz
FIDRES         0.126314 Hz
AQ            3.9584243 sec
RG                724.1
DW               60.400 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                10.50 usec
PL1               -3.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300086 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

99% purity based on 1H and 13C NMR



5

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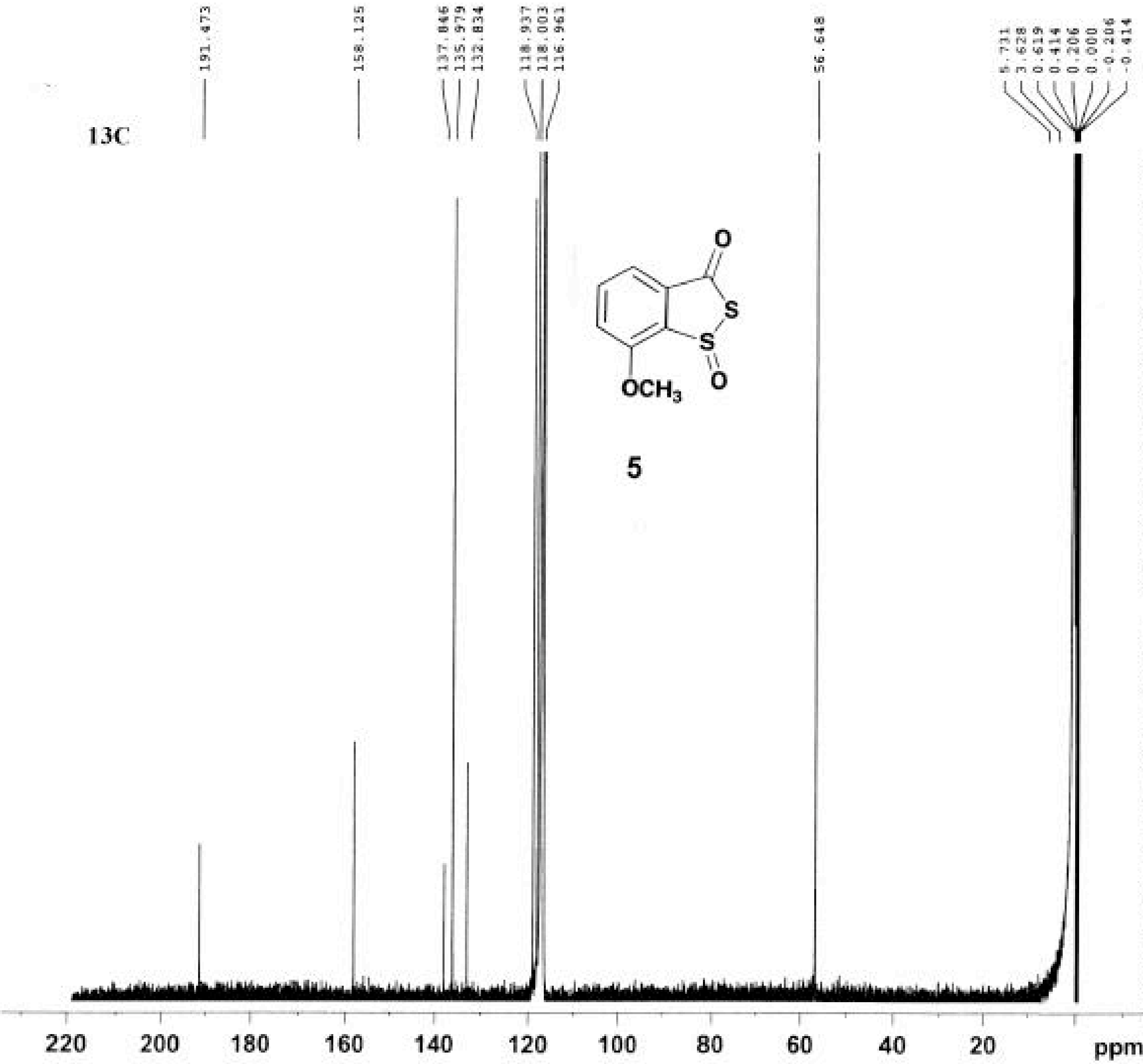
Current Data Parameters
NAME                    eb
EXPNO                   14
PROCNO                   1

F2 - Acquisition Parameters
Date_             20030813
Time                 15.02
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH               8278.146 Hz
FIDRES            0.126314 Hz
AQ               3.9584243 sec
RG                   645.1
DW                  60.400 usec
DE                    6.00 usec
TE                   300.0 K
D1              1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                  -3.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF             400.1300090 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

99% purity based on 1H and 13C NMR



99% purity based on 1H and 13C NMR

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Current Data Parameters
NAME                     eb
EXPNO                  1223
PROCNO                    1

F2 - Acquisition Parameters
Date_              20040219
Time                   4.32
INSTRUM               spect
PROBHD   5 mm BBO BB-1H
PULPROG             zgpg30
TD                    65536
SOLVENT               CD3CN
NS                    10000
DS                        4
SWH             23980.814 Hz
FIDRES           0.365918 Hz
AQ              1.3664756 sec
RG                    16384
DW                   20.850 usec
DE                     6.00 usec
TE                    300.0 K
D1               2.00000000 sec
d11              0.03000000 sec
d12              0.00002000 sec

======== CHANNEL f1 ========
NUC1                    13C
P1                     8.50 usec
PL1                   -3.00 dB
SFO1         100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2             waltz16
NUC2                     1H
PCPD2                 70.00 usec
PL2                   -3.00 dB
PL12                  13.00 dB
PL13                  13.00 dB
SFO2         400.1316005 MHz

F2 - Processing parameters
SI                    32768
SF           100.6127938 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.40
```
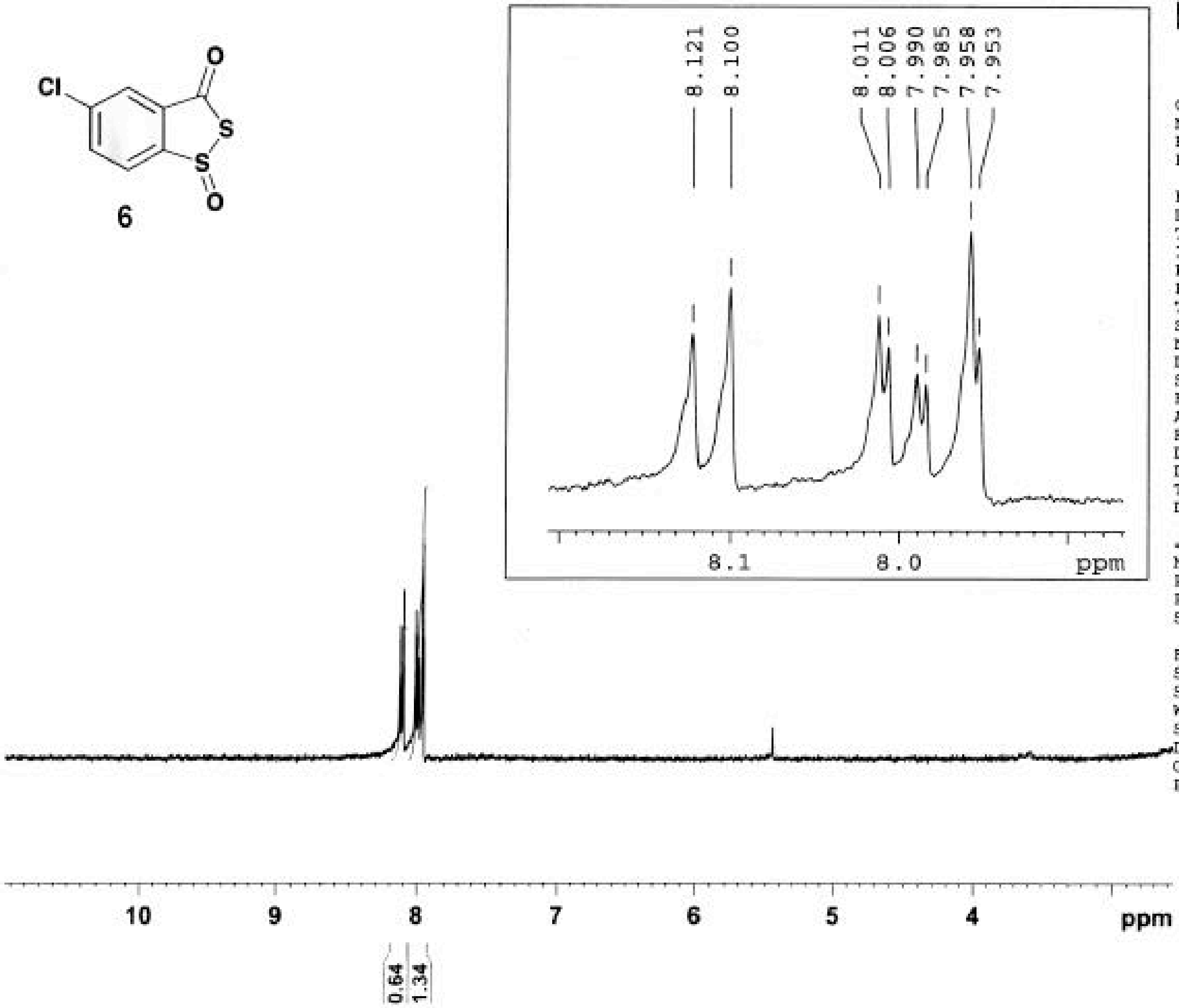

1H

6



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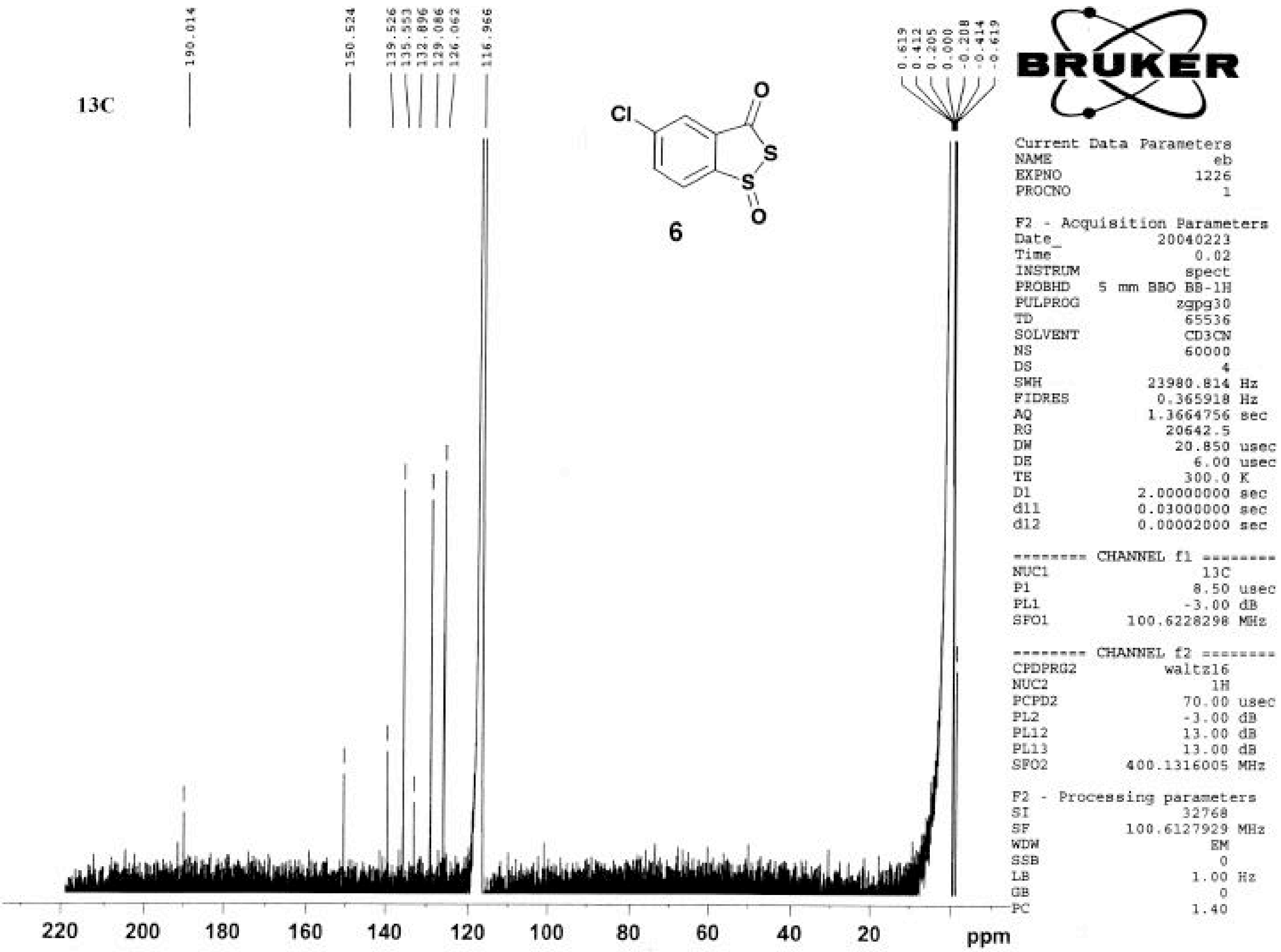
Current Data Parameters
NAME                  eb
EXPNO               1175
PROCNO                 1

F2 - Acquisition Parameters
Date_           20040203
Time               13.39
INSTRUM            spect
PROBHD   5 mm BBO BB-1H
PULPROG             zg30
TD                 65536
SOLVENT            CD3CN
NS                    16
DS                     2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9584243 sec
RG                 645.1
DW                60.400 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 10.50 usec
PL1                -3.00 dB
SFO1         400.1324710 MHz

F2 - Processing parameters
SI                 32768
SF           400.1300117 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

99% purity based on 1H and 13C NMR



6

```
Current Data Parameters
NAME                    eb
EXPNO                 1226
PROCNO                   1

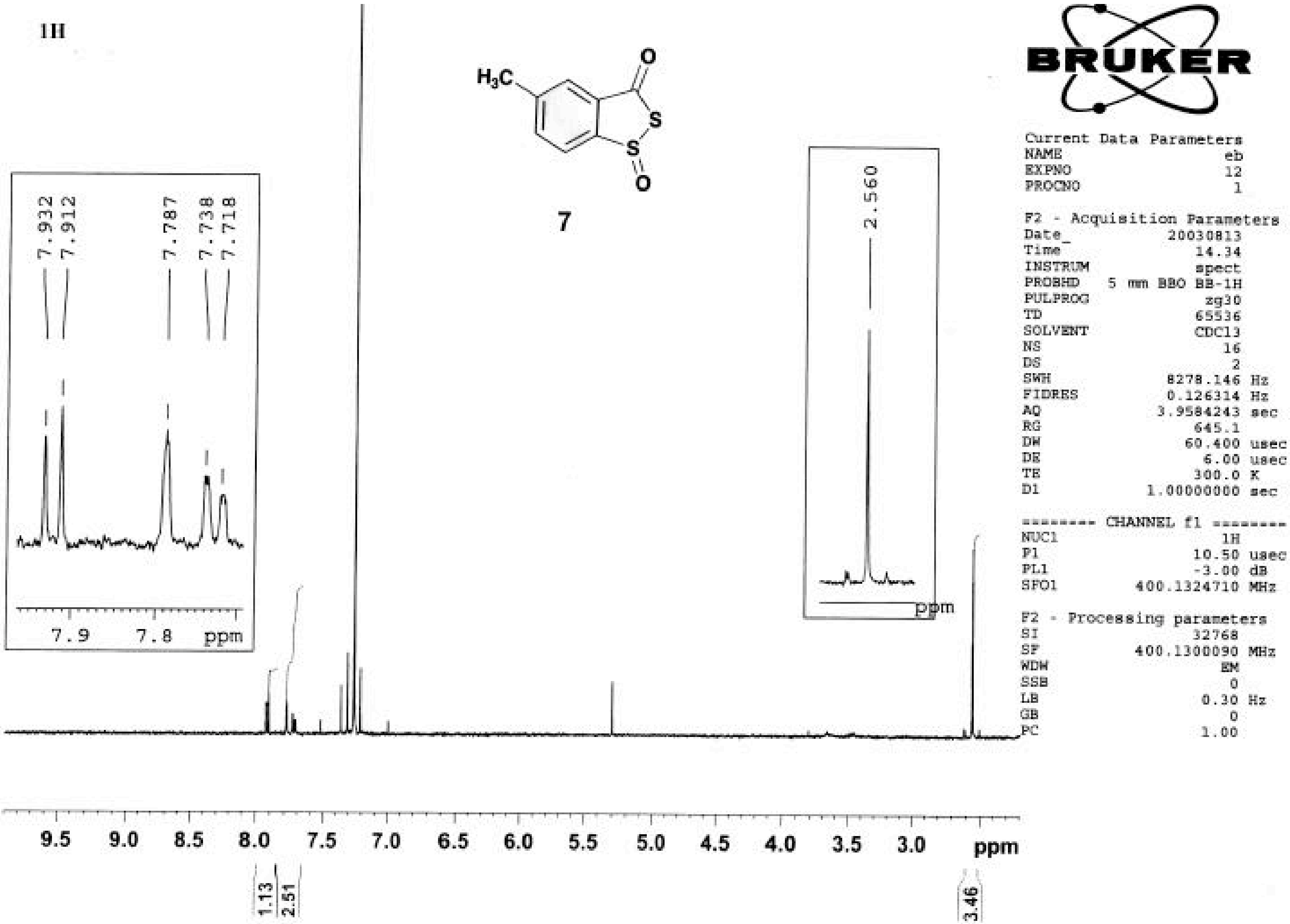
F2 - Acquisition Parameters
Date_             20040223
Time                  0.02
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG            zgpg30
TD                   65536
SOLVENT              CD3CN
NS                   60000
DS                       4
SWH             23980.814 Hz
FIDRES           0.365918 Hz
AQ              1.3664756 sec
RG                 20642.5
DW                  20.850 usec
DE                    6.00 usec
TE                   300.0 K
D1              2.00000000 sec
d11             0.03000000 sec
d12             0.00002000 sec

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -3.00 dB
SFO1          100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                70.00 usec
PL2                  -3.00 dB
PL12                 13.00 dB
PL13                 13.00 dB
SFO2          400.1316005 MHz

F2 - Processing parameters
SI                   32768
SF            100.6127929 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

99% purity based on 1H and 13C NMR



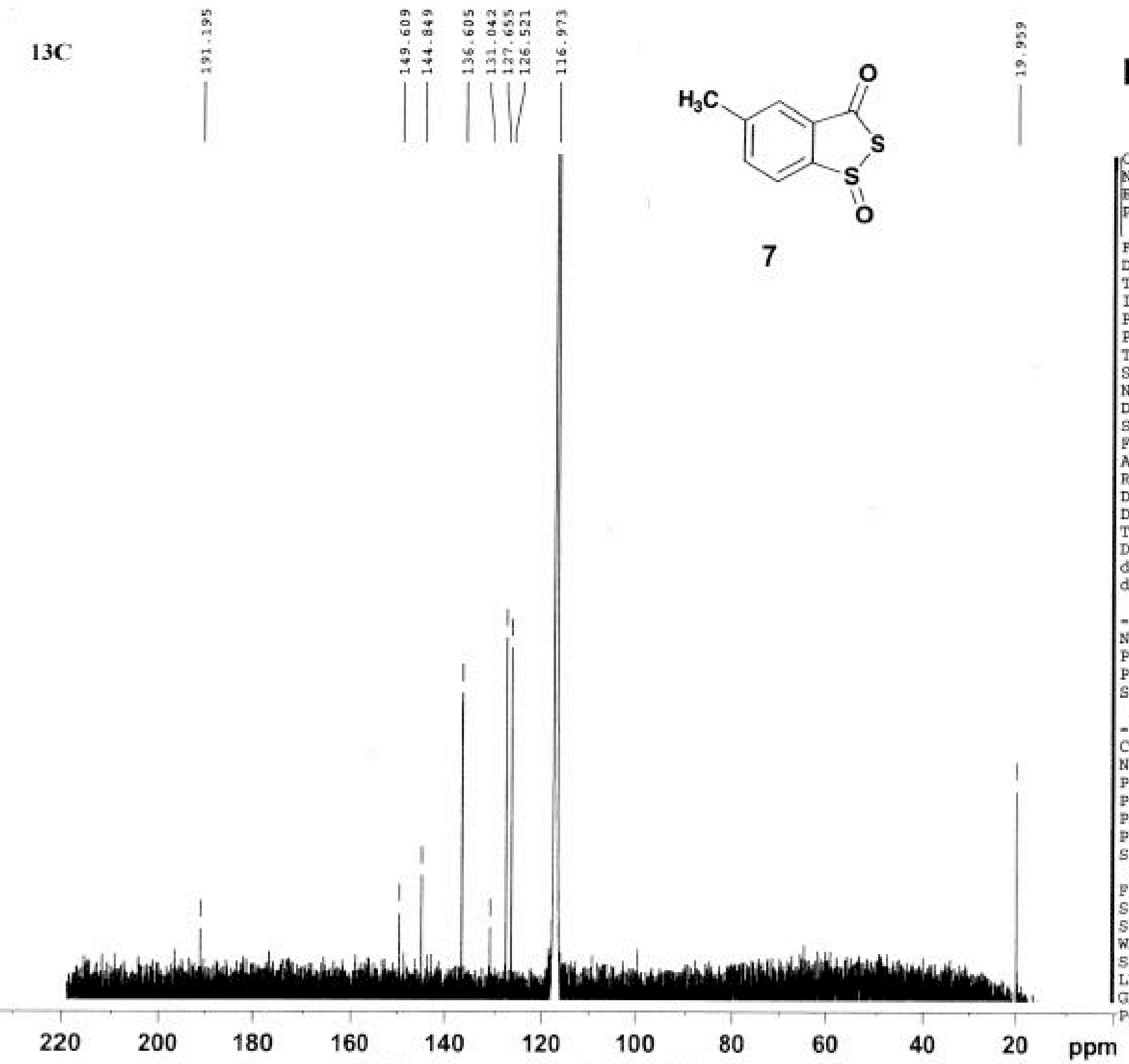
```
Current Data Parameters
NAME                    eb
EXPNO                   12
PROCNO                   1

F2 - Acquisition Parameters
Date_             20030813
Time                 14.34
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ             3.9584243 sec
RG                   645.1
DW                 60.400 usec
DE                   6.00 usec
TE                  300.0 K
D1            1.00000000 sec

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.50 usec
PL1                  -3.00 dB
SFO1           400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF             400.1300090 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

99% purity based on 1H and 13C NMR



7

99% purity based on 1H and 13C NMR

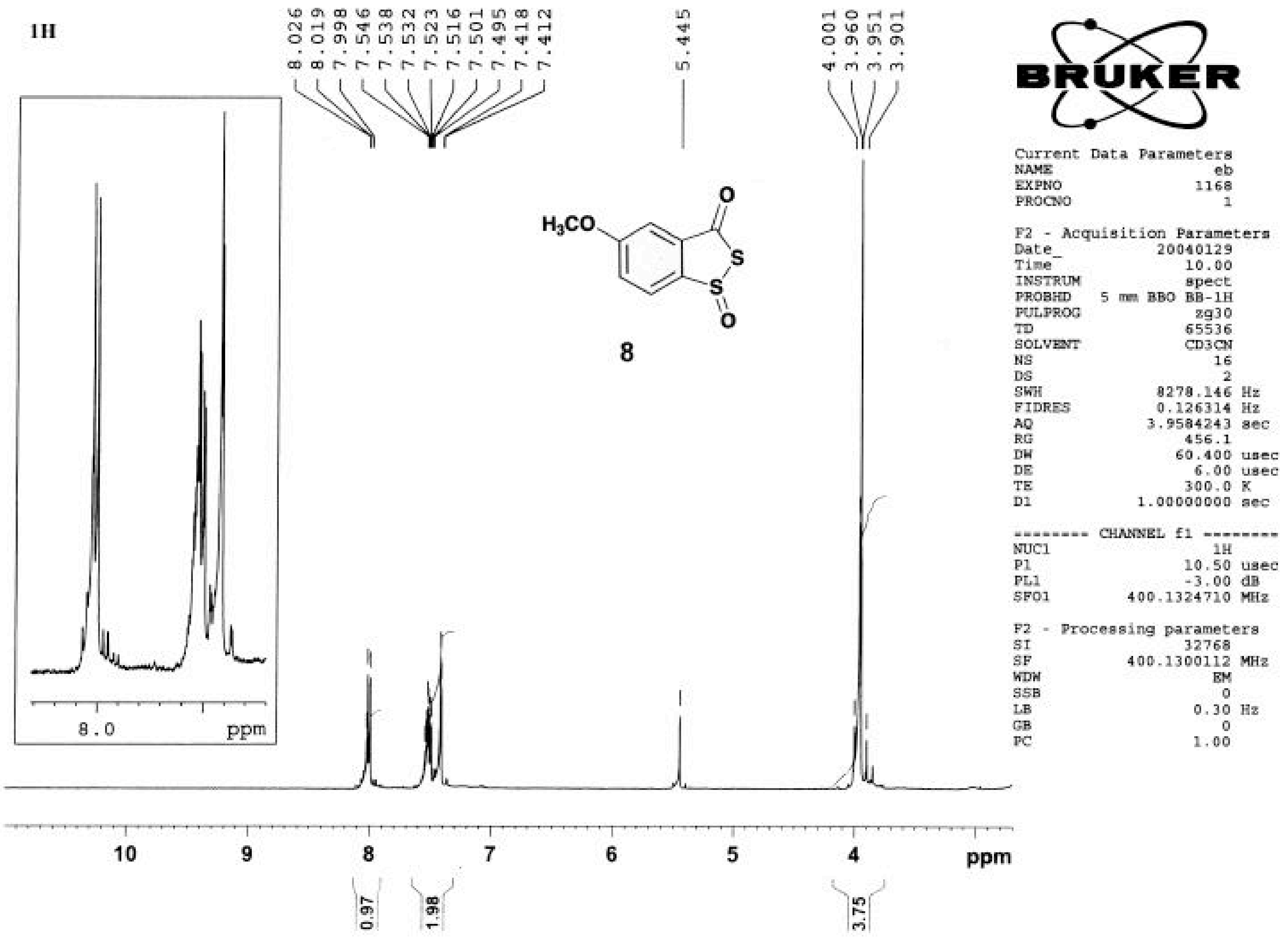
```
Current Data Parameters
NAME                 eb
EXPNO              1225
PROCNO                1

F2 - Acquisition Parameters
Date_          20040220
Time              15.34
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG         zgpg30
TD                65536
SOLVENT           CD3CN
NS                 6000
DS                    4
SWH           23980.814 Hz
FIDRES         0.365918 Hz
AQ            1.3664756 sec
RG              14596.5
DW               20.850 usec
DE                 6.00 usec
TE                300.0 K
D1           2.00000000 sec
d11          0.03000000 sec
d12          0.00002000 sec

======== CHANNEL f1 ========
NUC1                13C
P1                 8.50 usec
PL1               -3.00 dB
SFO1        100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2         waltz16
NUC2                 1H
PCPD2             70.00 usec
PL2               -3.00 dB
PL12              13.00 dB
PL13              13.00 dB
SFO2        400.1316005 MHz

F2 - Processing parameters
SI                32768
SF          100.6127922 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```



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Current Data Parameters
NAME                 eb
EXPNO              1168
PROCNO                1

F2 - Acquisition Parameters
Date_          20040129
Time              10.00
INSTRUM           spect
PROBHD   5 mm BBO BB-1H
PULPROG            zg30
TD                65536
SOLVENT           CD3CN
NS                   16
DS                    2
SWH            8278.146 Hz
FIDRES         0.126314 Hz
AQ            3.9584243 sec
RG                456.1
DW               60.400 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                10.50 usec
PL1               -3.00 dB
SFO1        400.1324710 MHz

F2 - Processing parameters
SI                32768
SF          400.1300112 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```
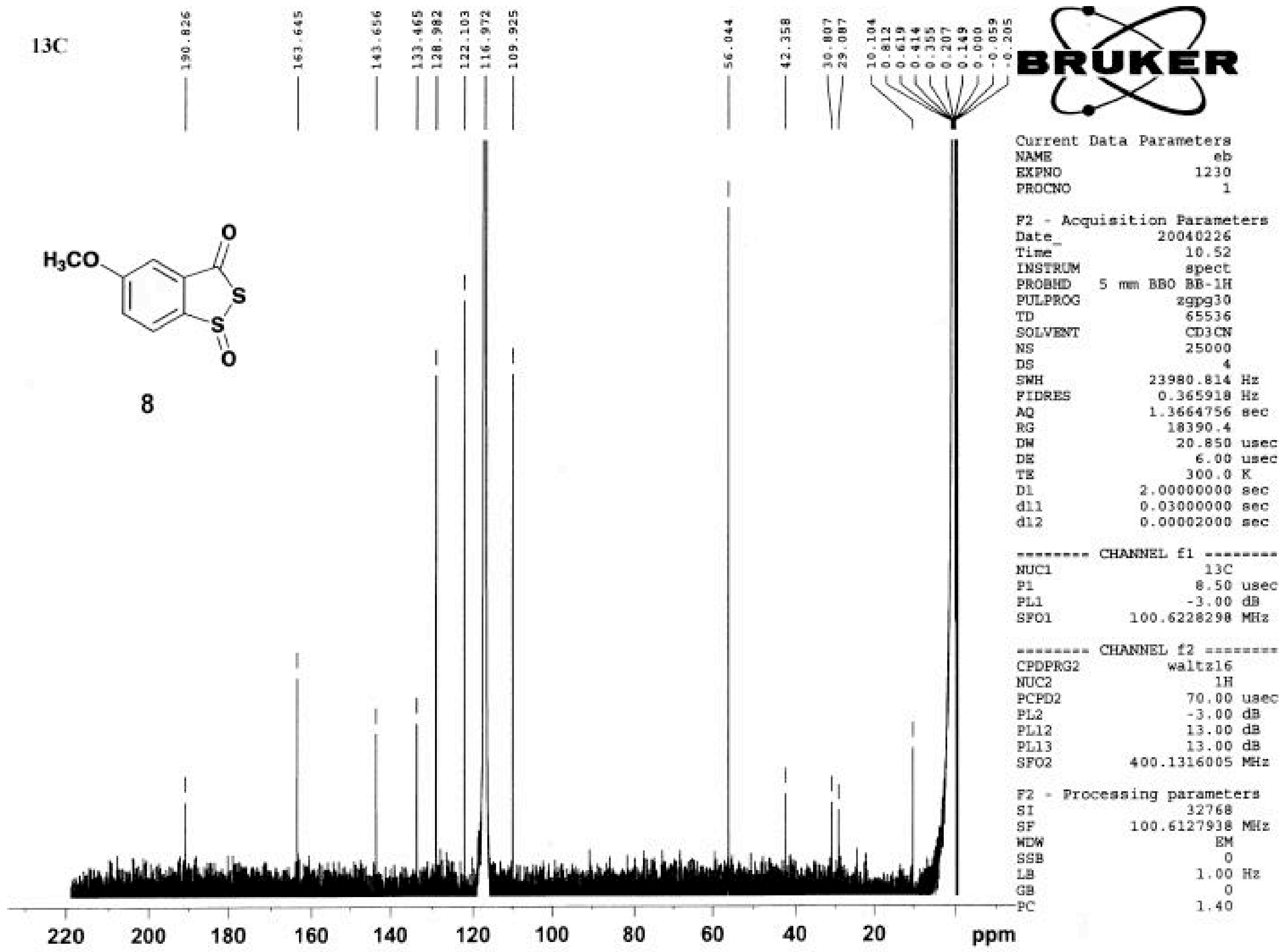

90% purity based on 1H and 13C NMR



```
Current Data Parameters
NAME                    eb
EXPNO                 1230
PROCNO                   1

F2 - Acquisition Parameters
Date_             20040226
Time                 10.52
INSTRUM              spect
PROBHD   5 mm BBO BB-1H
PULPROG            zgpg30
TD                   65536
SOLVENT             CD3CN
NS                   25000
DS                       4
SWH              23980.814 Hz
FIDRES            0.365918 Hz
AQ               1.3664756 sec
RG                 18390.4
DW                  20.850 usec
DE                    6.00 usec
TE                   300.0 K
D1              2.00000000 sec
d11             0.03000000 sec
d12             0.00002000 sec

======== CHANNEL f1 ========
NUC1                   13C
P1                    8.50 usec
PL1                  -3.00 dB
SFO1           100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2                70.00 usec
PL2                  -3.00 dB
PL12                 13.00 dB
PL13                 13.00 dB
SFO2           400.1316005 MHz

F2 - Processing parameters
SI                   32768
SF             100.6127938 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

90% purity based on 1H and 13C NMR

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