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The Role of Amine in the Mechanism of Pentathiepin (Polysulfur) Antitumor Agents

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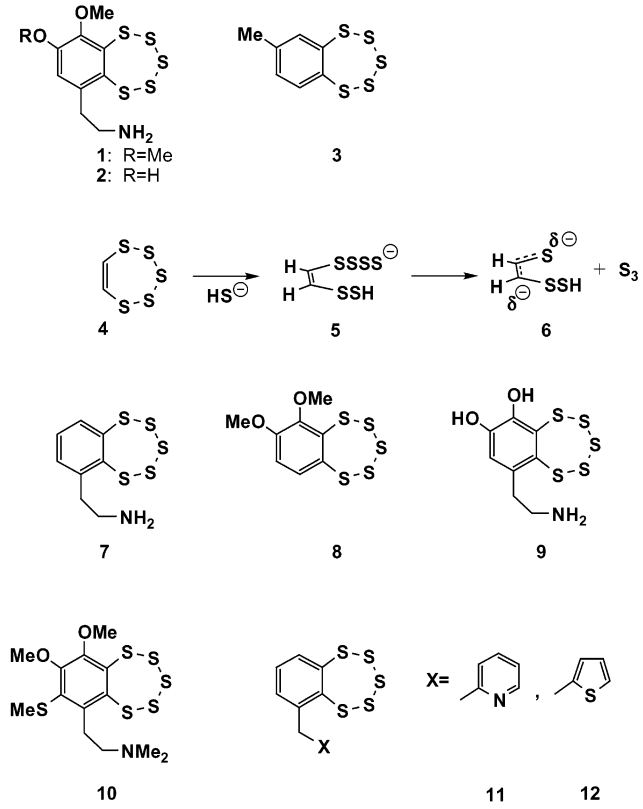
Abstract: A computational and experimental study is presented, which provides the first evidence that amine has an opportunity to engage in bonding with pentathiepin to promote its decomposition. The study provides mechanistic insight into the process that gives rise to pentathiepin biological activity. Primary or secondary amine will allow for an intramolecular addition to the pentathiepin ring at the nearest sulfur (S1). In contrast, tertiary amine adds reversibly to S1, because nitrogen cannot lose its positive charge by deprotonation. This precludes the amine promotion step. An energetically low-lying process is characterized, corresponding to S_3-loss triggered by nucleophilic activation with a primary or secondary amine. Pentathiepin desulfurization via S_3-unit transfer is supported by a trapping study with norbornene. That the amine may confer an enhanced reactivity in the natural products varacin, **1**, and lissoclinotoxin A, **2**, adds to the understanding of the pathway for pentathiepin activation and may provide new design concepts that have potential applications for this class of biocides.

Introduction

The 1,2,3,4,5-pentathiepin heterocycle is found in the marine natural products varacin (**1**) and lissoclinotoxin A (**2**) (Scheme 1).[1,2] There is recent interest surrounding pentathiepin molecules because of their antitumor, antifungal, and antimicrobial properties.[1,3−9] In 1991, Ireland and co-workers suggested that the biological activity of **1** originates from DNA damage.[1] Consistent with the in vivo data,[1] evidence for DNA damage by 7-methylbenzopentathiepin (**3**) was obtained in vitro.[10,11] We have recently reported results from calculations 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

Scheme 1



(1) Davidson, B. S.; Molinski, T. F.; Barrows, L. R.; Ireland, C. M. *J. Am. Chem. Soc.* **1991**, *113*, 4709.
(2) Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1994**, *59*, 6600.
(3) Sato, R.; Ohyama, T.; Ogawa, S. *Heterocycles* **1995**, *41*, 893.
(4) Compagnone, R. S.; Faulkner, D. J.; Carte, B. K.; Chan, G.; Hemling, M. A.; Hofmann, G. A.; Mattern, M. R. *Tetrahedron* **1994**, *50*, 12785.
(5) Makarieva, T. N.; Stonik, V. A.; Dmitrenok, A. S.; Grebnev, B. B.; Iskov, V. V.; Rebachyk, N. M. *J. Nat. Prod.* **1995**, *58*, 254.
(6) Litaudon, M.; Guyot, M. *Tetrahedron Lett.* **1991**, *32*, 911.
(7) Litaudon, M.; Trigalo, F.; Martin, M.-T.; Frappier, F.; Guyot, M. *Tetrahedron* **1994**, *50*, 5323.
(8) Ford, P. W.; Davidson, B. S.; Behar, V.; Danishefsky, S. J.; Stafford, J. A.; Feldman, P. L. *Chemtracts: Org. Chem.* **1994**, *7*, 242.
(9) Greer, A. *J. Am. Chem. Soc.* **2001**, *123*, 10379.
(10) Chatterji, T.; Gates, K. S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 535.
(11) Gates, K. S. *Chem. Res. Toxicol.* **2000**, *13*, 953.

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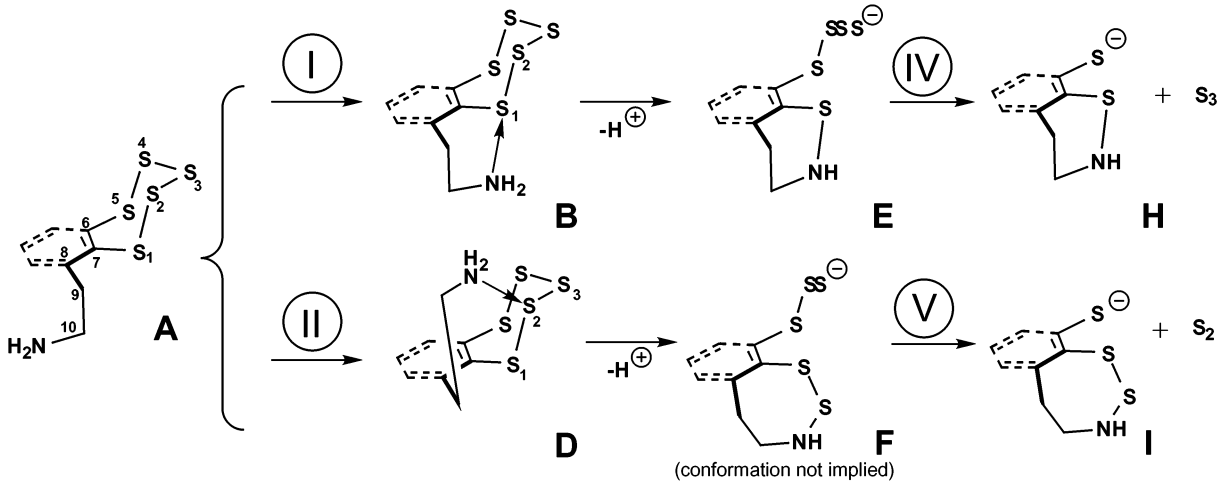
Scheme 2



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 we will show later, it is also a function of the proximity to the polysulfur ring (vide infra).

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**, $IC_{50} = 0.26$ μg/mL) compared to 6,7-dimethoxybenzopentathiepin (**8**, $IC_{50} = 6.12$ μg/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 plays a part in the biological mechanism of action.

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 either might be unable to add or might 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**) or a thienyl (**12**) group, enhances the decomposition rate (Scheme 1).[16]

The present study advances the idea of 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.

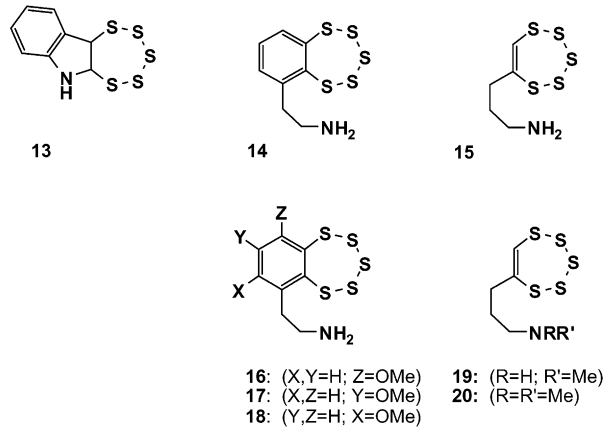
Experimental Section

General Aspects. Diethylamine (Aldrich 99.5+%), triphenylamine (Aldrich 98%), sodium (lump in kerosene, 99%), biphenyl (Aldrich 99.5%), triphenylmethane (Aldrich 99%), norbornene (Aldrich, 99%), 2,3-dimethyl-1,3-butadiene (Aldrich, 98%), tetrabutylammonium fluoride (1.0 M solution in tetrahydrofuran, Aldrich), 4-methyl-1,2-benzodithiol (Aldrich 98%), toluene (anhydrous, Aldrich 99.8%), acetonitrile (anhydrous, Aldrich, 99.8%), dimethylformamide (anhydrous, Aldrich 99.8%), and benzene (anhydrous, Aldrich 99.8%) 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.

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 h at 43 °C and at ambient temperatures. Otherwise, the samples were incubated for 4 h at 28 °C before analysis. Control experiments

(12) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*; Harper & Row: New York, NY, 1987.
(13) Behar, V.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 7017.
(14) Ogawa, S.; Wagatsuma, M.; Sato, R. *Heterocycles* **1997**, *44*, 187.
(15) Chenard, B. L.; Harlow, R. L.; Johnson, A. L.; Vladuchick, S. A. *J. Am. Chem. Soc.* **1985**, *107*, 3871.
(16) Sato, R.; Ohyama, T.; Kawagoe, T.; Baba, M. *Heterocycles* **2001**, *55*, 145.

Scheme 3



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.

Theoretical Methods. Density functional theoretical (DFT) calculations were 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]

Results and Discussion

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 secondary 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 nucleophilic attack of amine on S1 sulfur underpins the production of reactive sulfur intermediates, and provides mechanistic insight into 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.

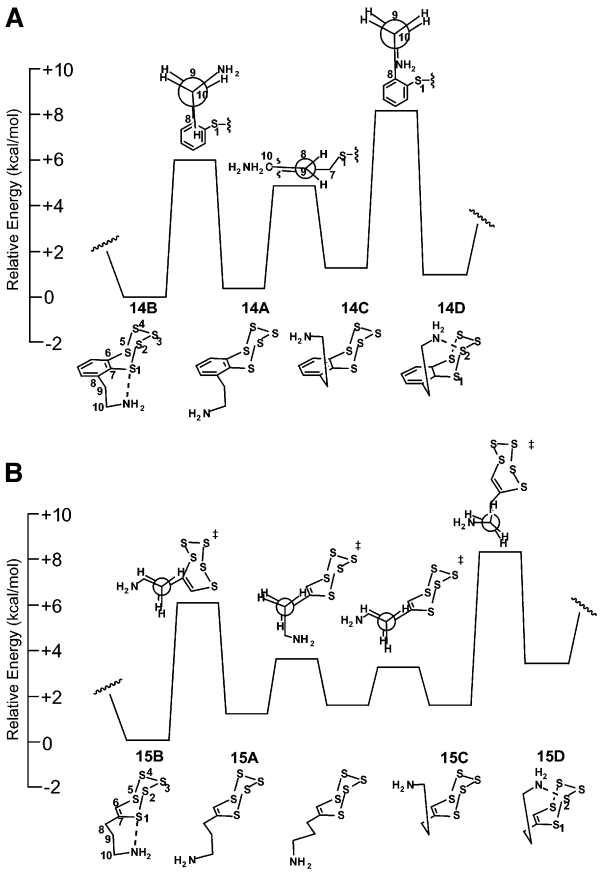
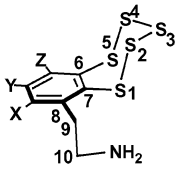


Figure 1. B3LYP/6-31G* calculated potential energy surface. The gas-phase energies are shown, and the solution-phase energies are discussed in the text. Eclipsed structures for **14** represent single-point calculations, but for **15**, represent established transition-state structures. Isomers shown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

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

(17) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*; Gaussian, Inc.: Pittsburgh, PA, 1998.
(18) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian-94*; Gaussian, Inc.: Pittsburgh, PA, 1994.
(19) Bartolotti, L. J.; Flurchick, K. *An Introduction to Density Functional Theory*; VCH: New York, 1996; Vol. 7.
(20) Miertus, S.; Scrocco, E.; Tomasi, J. *J. Chem. Phys.* **1981**, *55*, 117.
(21) Miertus, S.; Tomasi, J. *J. Chem. Phys.* **1982**, *65*, 239.
(22) Cambridge X-ray Crystallographic Data Base; Cambridge, UK, 2001.
(23) Feher, F.; Engelen, B. *Z. Anorg. Allg. Chem.* **1979**, *37*, 452.
(24) Oae, S.; Doi, J. T. *Organic Sulfur Chemistry: Structure and Mechanism*; CRC Press: Boca Raton, FL, 1991.
(25) Wu, S.; Greer, A. *J. Org. Chem.* **2000**, *65*, 4883.
(26) Bergman, J.; Stalhandski, C. *Tetrahedron Lett.* **1994**, *35*, 5279.

Table 1. Calculated Benzopentathiepin Stuctural 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

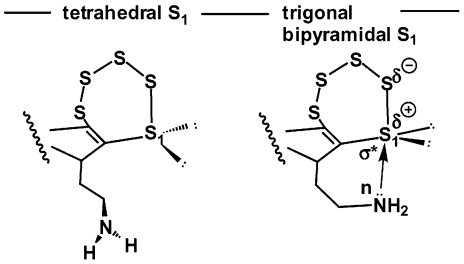
[a] Structures optimized at the B3LYP/6-31G(d) level. [b] Distances in Å, angles in deg. [c] The dihedral angle θ = C8−C9−C10−N, is positive for a counterclockwise movement from C8 to N as you look down from C9 to C10.

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 the S1 rather than S2 of the pentathiepin. 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.

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 (Tables 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.

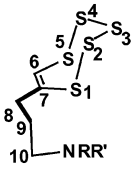
Scheme 4



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, for example, 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 of 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

(27) Davidson, B. S.; Ford, P. W.; Wahlman, M. *Tetrahedron Lett.* **1994**, *35*, 7185.
(28) Trippett, S. *Phosphorus Sulfur* **1976**, *73*, 1.
(29) Reed, A. E.; Curtiss, L. A.; Weinhold, F. *Chem. Rev.* **1988**, *88*, 899.
(30) Iwaoka, M.; Takemoto, S.; Okada, M.; Tomoda, S. *Chem. Lett.* **2001**, *2*, 132.
(31) Iwaoka, M.; Takemoto, S.; Tomoda, S. *J. Am. Chem. Soc.* **2002**, *124*, 10613.
(32) Schöneich, Ch.; Pogocki, D.; Wisiowski, P.; Hug, G. L.; Bobrowski, K. *J. Am. Chem. Soc.* **2000**, *122*, 10224.
(33) Pogocki, D.; Schöneich, Ch. *J. Org. Chem.* **2002**, *67*, 1526.
(34) Reznik, R.; Greer, A. *Chem. Res. Toxicol.* **2000**, *13*, 1193.
(35) Davis, R. E.; Nakshbendi, H. F. *J. Am. Chem. Soc.* **1962**, *84*, 2085.
(36) Bondi, A. J. *J. Phys. Chem.* **1964**, *68*, 441.

Table 2. Calculated Ethenopentathiepin Stuctural 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 counterclockwise movement from C8 to N as you look down from C9 to C10.

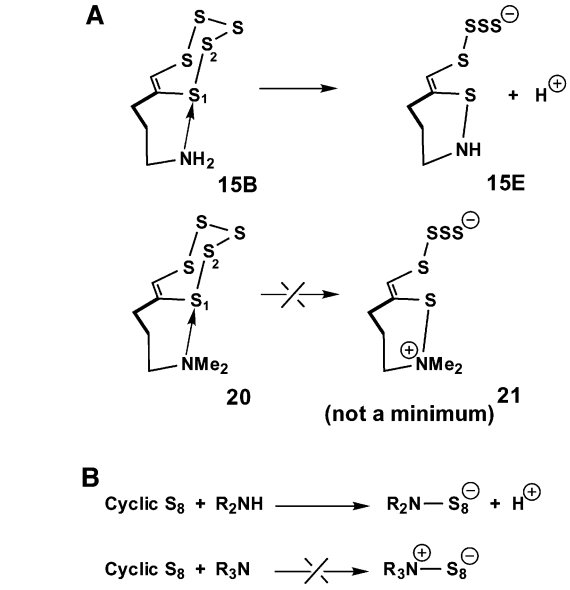
the decomposition of varacin **1**.[13,37] Nucleophilic substitution reactions of polysulfanes are known with a wide range of nucleophiles.[38] Scales on thiophilicity rank primary amine below that of thiolate ion and phosphine;[39] however, the weakly thiophilic ammonia molecule has been reported to 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.

Amination of the Pentathiepin Ring: The Unimolecular Mechanism. Here we provide the first evidence for an intramolecular (nucleophilic) reaction of amine in pentathiepin chemistry. 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).

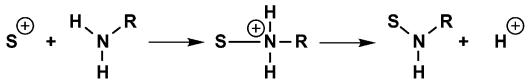
Computations predict a unimolecular destabilization of pentathiepin in those substrates bearing hydrogen(s) on amine (−NH_2 and −NHMe), but not for the tertiary amine (−NMe_2) (Scheme 5). The behavior of the amine group varies with its conformation in pentathiepin and with its substitution pattern. The 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 that for −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 (pathways 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 pK_a traditionally required for amine deprotonation is well above 20, which is not biologically relevant. We believe the mechanism of substitution involves a

Scheme 5



(37) Toste, F. D.; Still, I. W. J. *J. Am. Chem. Soc.* **1995**, *117*, 7261−2
(38) Clennan, E. L.; Stensaas, K. L. *Org. Prep. Proced. Int.* **1998**, *30*, 551.
(39) Davis, R. E. *Survey of Progress in Chemistry*; Academic Press: New York, 1964; Vol. 2.
(40) Sato, R.; Sato, T.; Takikawa, Y.; Takikawa, S. *Technol. Rep. Iwate Univ.* **1979**, *13*, 37.
(41) Wolkenberg, S. E.; Boger, D. L. *Chem. Rev.* **2002**, *102*, 2477.
(42) Smith, A. L.; Nicoloau, K. C. *J. Med. Chem.* **1996**, *39*, 2103.
(43) Lee, M. D.; Ellestad, G. A.; Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235.

Scheme 6



two-step process[44] as shown in Scheme 6. The aminium 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 tertiary amine (Scheme 5A) and precludes an intramolecular displacement reaction.

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 primary and secondary amines, but not with tertiary amine (Scheme 5B). Until 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 vain, 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** versus 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).

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 (pathways I and II,

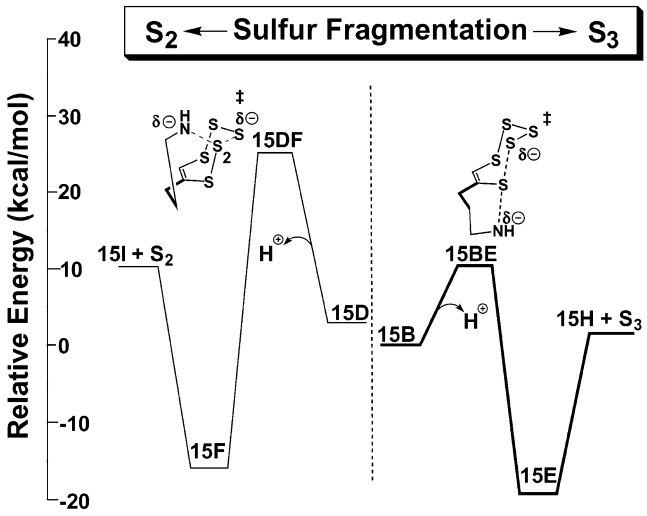
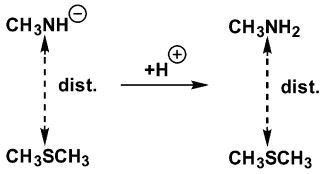


Figure 2. B3LYP/6-31G* calculated potential energy surface. The gas-phase energies are shown, and the solution-phase energies are discussed in the text. Energies of **15I** + S_2 and **15H** + S_3 were optimized with the reagents separated at a 5 Å distance. Bolded line indicates the pathway with the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

Scheme 7



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

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 (Figures 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 seven-membered ring **15F** is significantly higher (**15DF**, 24.8 kcal/mol). We find that an increase in dielectric constant from the gas phase ($\epsilon = 0$) value to that of water ($\epsilon = 80.1$) has a minor effect on the energetics in the formation of 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.

It is possible to envision pentathiepin natural products as biological precursors of triatomic sulfur, S_3. A pentathiepin containing a primary or secondary 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

(44) Patai, S. *The Chemistry of the Amino Group*; Wiley-Interscience Publishers: New York, 1968.
(45) Harpp, D. N.; Smith, R. A. *J. Org. Chem.* **1979**, *44*, 4140.
(46) Harpp, D. N.; Derbesy, G. *Tetrahedron Lett.* **1994**, *35*, 5381.

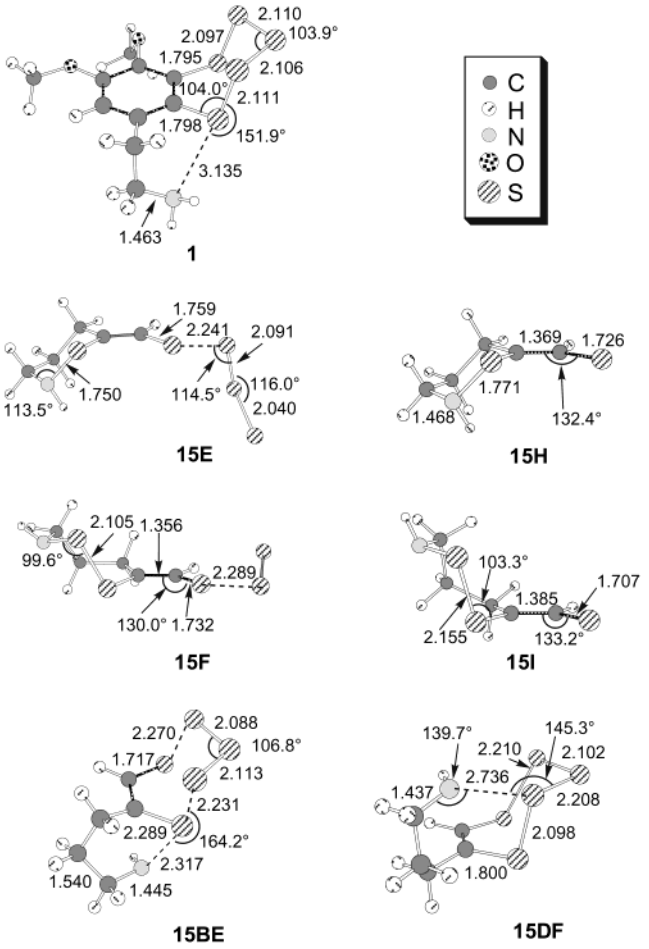


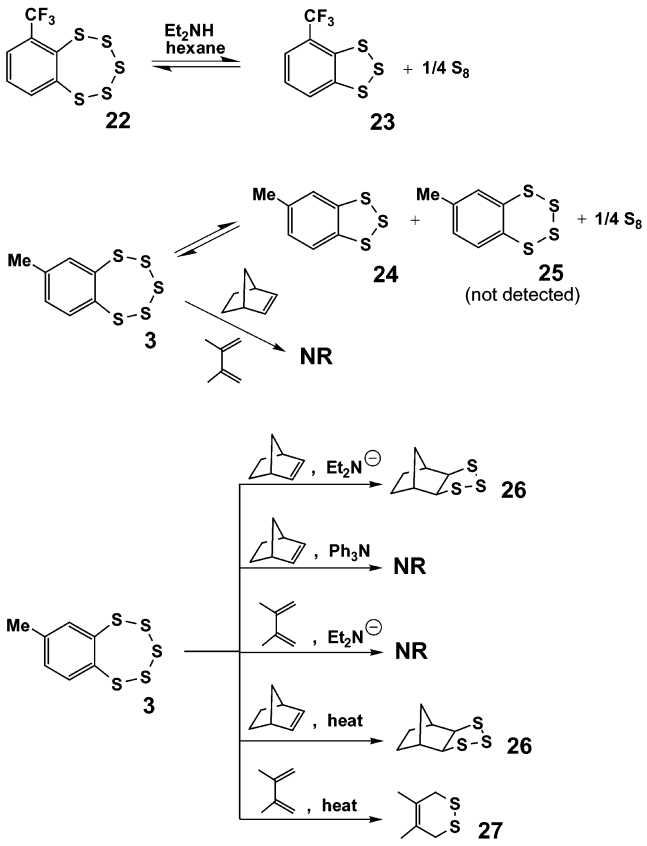
Figure 3. B3LYP/6-31G(d) optimized geometries (bond distances are in Å, angles are in degrees).

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 results in part from 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-dependent pentathiepin decomposition.

Amine−Pentathiepin Interactions 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 is 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

Scheme 8



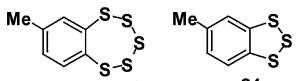
conversion between **3** and **24** (entries 6−8). Analyses of the data provided evidence that an equilibrium is established in a few hours between **3** and **24** with a reasonable correlation between mass spectrometry and NMR. Data collected after 2 and 24 h with the two methods are identical to within 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. On the basis of the data in Table 3, one can suggest that pentathiepin has reduced stability in acetonitrile:water, methanol:water, and benzene:DMF mixtures compared to that in benzene or chloroform. Interestingly, a similar solvent effect is observed, although to a reduced extent, for equilibration of the three sulfur rings of $S_8 \leftrightarrow S_6 + S_7$.[48] Added NH_3 or Et_2NH has an effect on the equilibrium of **3** and **24** (entries 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 GC/MS, HPLC, nor NMR.

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

(47) Harpp, D. N. *Phosphorus, Sulfur Silicon Relat. Elem.* **1997**, *120*, 41.

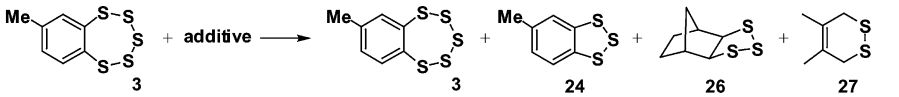
(48) Tebbe, F. N.; Wasserman, E.; Peet, W. G.; Vatvars, A.; Hayman, A. C. *J. Am. Chem. Soc.* **1982**, *104*, 4971.
(49) Billmers, R. I.; Smith, A. L. *J. Phys. Chem.* **1991**, *95*, 4242.
(50) Brabson, G. D.; Mielke, Z.; Andrews, L. *J. Phys. Chem.* **1991**, *95*, 77.

Table 3. Effect of Solvent and Nitrogen Additive on the Equilibrium of 7-Methylbenzo-pentathiepin (**3**) and 7-Methylbenzotrithiole (**24**)

	Reagent	Solvent	Additive	Product Distribution:  **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] [**3**]init = 0.41 M. [b] Ratio of **3**:**24** at 25 °C determined by GC/MS. [c] Elemental sulfur S8 concentration not determined due to the different solubility in the solvents examined. Insoluble material is observed in all so **3**:**24** at 25 °C determined by NMR. [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** at 25 °C 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 **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)[i,j,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 GC/MS. [b] Elemental sulfur S8 concentration not determined due to the different solubility in the solvents examined. [c] $[\mathbf{3}]_{initial}$ = 0.55 M; [norbornene] = 1.0 M. [d] Butadiene represents the compound 2,3-dimethyl-1,3-butadiene. [e] $[\mathbf{3}]_{initial}$ = 0.55 M; [butadiene] = 0.55 M. [f] [**3**]init = 1.6 mM; [norbornene] = 0.10 M. [g] [**3**]init = 0.41 M; [norbornene] = 1.0 M. [h] [**3**]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] [**3**]init = 50 mM; $[Et_2NH]$ = 50 mM. [k] Runs averaged using [nBu$_4$NF] = 50 mM and [Na] = 0.20 M. [l] Runs averaged using [NaOH] = 0.1 M and [KF] = 0.1 M. [m] $[\mathbf{3}]_{initial}$ = 50 mM; $[Ph_3N]$ = 50 mM. [n] $[\mathbf{3}]_{initial}$ = 0.10 M; [butadiene] = 0.10 M. [o] $[\mathbf{3}]_{initial}$ = 0.10 M; [norbornene] = 0.10 M. [p] $[\mathbf{3}]_{initial}$ = 0.55 M; [butadiene] = 0.55 M.

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 h at 28 °C, diethylamine initiated a small amount of sulfur transfer from **3** to norbornene (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, $Me_2NH^{\delta+}-S$, 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 the mechanism of solvent interactions accompanying reactions of **3** were 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 (nBu$_4$NF) or sodium metal (Na) enhanced the S_3-unit transfer in benzene:DMF (compare entries 5 and 7, Table 4). Under the conditions with added nBu$_4$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

(51) Pearson, R. G.; Sobel, H.; Songstad, J. *J. Am. Chem. Soc.* **1968**, *90*, 319.
(52) Swain, C. G.; Scott, C. B. *J. Am. Chem. Soc.* **1953**, *75*, 141.

norbornene (compare entries 6 and 8). Control reactions demonstrated that pentathiepin reacts with the amine base and not with KF or 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 Et_2N^- is in part responsible for the sulfur donor properties of pentathiepin in benzene:DMF (10:3). Desulfurization of **3** did not take place with Ph_3N 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 entries 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[53] (see Supporting Information).

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 may 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 understanding 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 reports 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-unit transfer would be associated with a new form of biological action displayed by naturally occurring pentathiepins.

Conclusions

After 10 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 into 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 cannot 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 primary or secondary 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 this work 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.

Acknowledgment. This work was supported by the Petroleum Research Fund, administered by the American Chemical Society, a grant from the City University of New York (CUNY), and by a research award from PSC-CUNY. E.M.B. is a CUNY Ginell Award recipient.

Supporting Information Available: Descriptions of the geometries of all stationary points and absolute energies and mass spectral data of trithiane **26** and disulfide **27** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA027416S

(53) Block, E.; Bentley, M. D.; Davis, F. A.; Douglass, I. B.; Lacadie, J. A. *J. Org. Chem.* **1975**, *40*, 2770.
(54) Haberfield, P.; Cincotta, J. J. *J. Org. Chem.* **1987**, *52*, 4627.
(55) Sato, R.; Akutsu, Y.; Goto, T.; Saito, M. *Chem. Lett.* **1987**, 2161.
(56) Steudel, R. *Chemistry of the Non-Metals*; De Gruyter Publishers: Berlin, 1977.
(57) Ishii, A.; Nakabayashi, M.; Nakayama, J. *J. Am. Chem. Soc.* **1999**, *121*, 7959.
(58) Ishii, A.; Nakabayashi, M.; Jin, Y.-N.; Nakayama, J. *J. Organomet. Chem.* **2000**, *611*, 127.