Planar Chirality due to a Polysulfur Ring in Natural Pentathiepin Cytotoxins. Implications of Planar Chirality for Enantiospecific Biosynthesis and Toxicity

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A low-energy pathway for pentathiepin racemization has been found using density functional theory (DFT) calculations. 3-[1,2,3,4,5]Pentathiepin-6-yl-propylamine served as a model compound for tunicate-derived pentathiepins. Pentathiepin racemization becomes a low-energy process in the presence of a thiolate ion nucleophile. It is unknown whether the biosynthetic process for pentathiepins is enantiospecific (Bentley, R. (2005) *Chem. Soc. Rev. 34*, 609) or whether toxicity differs between enantiomers. However, the ease of thiolate ion attack on the polysulfur ring suggests that nucleophiles may induce optical instability on the laboratory time scale. The DFT study predicts that enantiospecific behaviors such as toxicity differences between *P*- and *M*-pentathiepins would be difficult to determine experimentally. The computed results fit into a broader picture that nucleophiles assist in ring-opening and equilibration reactions of polysulfanes.

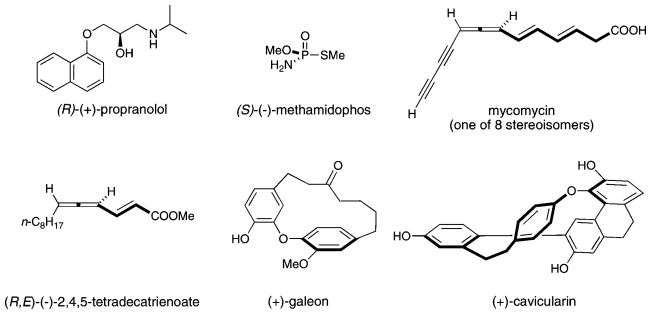
Introduction

It is well-known that physiological responses to enantiomers of a particular compound often exhibit enantiospecificity (*1*). There is, for instance a large body of literature concerning the enantiospecific behavior of drugs. For example, for the widely used propranolol (a nonselective, beta-adrenergic receptor-blocking compound), the *S*-enantiomer is the most active stereoisomer in mammals (Scheme 1). Similar enantiospecific events are known for toxins. In fact, propranolol is a toxin for aquatic organisms, such as *Daphnia magna* and *Pimephales promelas*. For the latter organism, the *S*-enantiomer of propranolol was more toxic than the *R*-stereoisomer; with *D. magna*, there was no enantiospecificity, probably because these organisms lack β-type receptors (*2*).

The widely used chiral organophosphorus pesticides are also subject to enantiospecificity. A recent example is methamidophos, where the (+) stereoisomer is 7.0 times more toxic to *D. magna* than the (−) form (*3*). Among naturally occurring toxins, the poison frog material, pumiliotoxin, is enantiospecific in its behavior to mosquitoes (*Aedes egypti*) (*4*).

Much of the work on physiological enantiospecificity has been concerned with central chirality because the structural type Cabcd occurs widely. However, other types of chirality exist in nature (for the terms used here, see ref *5*). A biaryl moiety with axial chirality is a common feature of many natural products including alkaloids, coumarins, flavanoids, lignans, polyketides, tannins, and terpenes (*6*). Axial chirality is also present in some naturally occurring allenes, abC═C═Cab; examples include the antibiotic mycomycin from *Nocardia acidophilus* (*7*) and methyl (*R,E*)-(-)-2,4,5-tetradecatrienoate, a pheromone from *Acanthoscelides obtectus* (*8*).

Scheme 1



Much less common in nature are compounds with planar chirality, and this type of chirality has consequently received only very limited attention with respect to enantiospecific behaviors. However, the natural product, (+)-galeon (from the plant, *Myrica gale*) has a chiral plane, and (+)-cavicularin (from the liverwort, *Cavicularia densa*) has both planar and axial chirality. Both of these materials contain only C, H, and O. Elements of planar chirality also occur in members of the vancomycin family: antibiotics from *Streptomyces orientalis*. However, this situation is very complex. The aglycone of these antibiotics consists of a heptapeptide (central chirality), a biaryl unit (actinoidinic acid with axial chirality), and finally two chiral planes in the so-called C-O-D and D-O-E macrocycles (*6*, *9*).

In considering the role of sulfur chirality in biology, two polysulfur natural products, varacin and lissoclinotoxin A (**1**) were described as having planar chirality (Scheme 2) (*10*, *11*, *20*, *21*). These and other pentathiepins are toxins produced by marine invertebrates such as *Lissoclinum perforatum*; toxicity depends on the presence of the polysulfur linkage (*11*−*17*). It is of interest, however, that the pentathiepins occupy a paradoxical place in planar stereochemical topics. The chair−chair interconversion barrier between 6,10-diethyl [1,2,3]trithiolo[*h*]-benzopentathiepin 8-oxide diastereomers (**2a**,**b**) was found to

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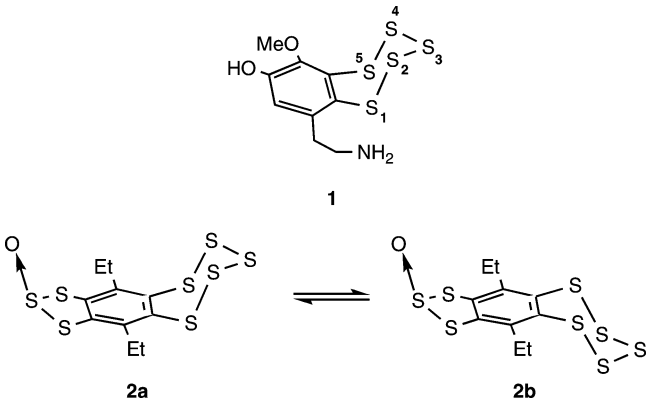
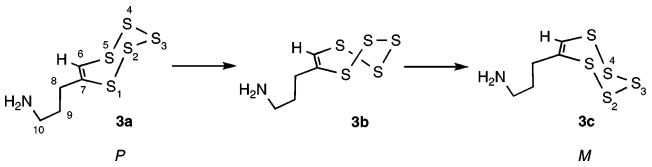
Scheme 2



Scheme 3



be ∼24 kcal/mol by Kimura et al. (*18*, *19*). This would suggest that related asymmetrical pentathiepins such as lissoclinotoxin A (**1**) would possess planar chirality because the barrier to inversion of the pentasulfane ring is high. The sulfur atoms S2, S3, and S4 are either above the plane of the benzene ring or below, and the substituents on the benzene ring of **1** eliminate a potential plane perpendicular to that ring.

Nonetheless, enantiomeric enrichment in natural pentathiepins has not been confirmed. Searle et al. report a barrier of ∼29 kcal/mol for **1**, which should lead to optical stability on the laboratory time scale (*11*). Davidson et al. also suggested a varacin derivative to be a chiral entity (*20*, *21*). However, optical stability is not observed, and there is a question about the energy barrier to polysulfane chair−chair interconversion of natural pentathiepins. The issue of chair−chair *P* and *M* interconversion (Scheme 3) is important in connection with natural pentathiepins because their biosynthesis may favor one enantiomer over the other (*10*). It is also unknown whether natural pentathiepin toxicity differs between enantiomers. The natural pentathiepins have only been isolated as the corresponding racemates.

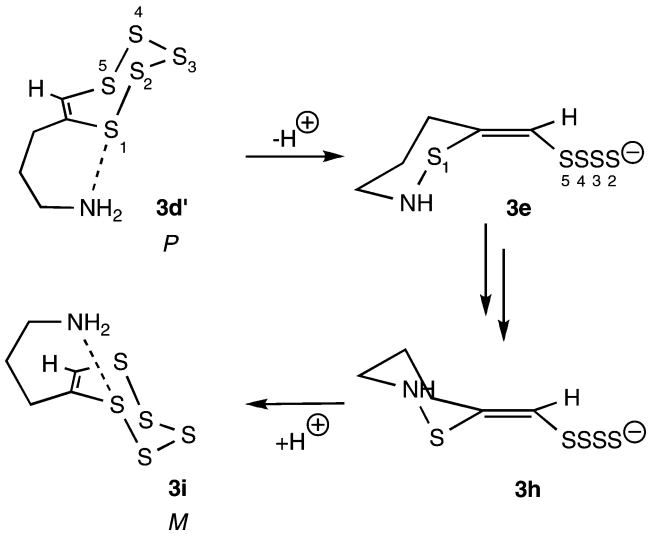
A low-energy pathway for pentathiepin racemization has so far been established neither computationally nor experimentally. It seemed desirable to investigate whether pentathiepin racemization could become a low-energy process in the presence of a nucleophile. In the present work, we studied racemization reactions for an unsymmetrical pentathiepin, 3-[1,2,3,4,5]-pentathiepin-6-yl-propylamine (**3**) with density functional theoretical (DFT) calculations. We focused on pentathiepin **3** because it is similar to natural benzopentathiepins but with an abbreviated structure due to limitations on computational resources.

Results and Discussion

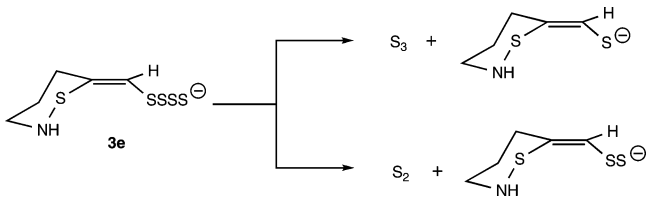
Schemes 3, 4, and 6 show proposed racemization reactions of pentathiepin **3** enantiomers. Figures 1−4 show the calculated structures and potential energy surfaces (PESs).

Unimolecular Racemization (Scheme 3 and Figure 1). Previous *ab initio*, DFT, semiempirical, and molecular mechanics studies have shown the chair−chair flipping of pentathiepins to be a high barrier process (24−29 kcal/mol) (*20*, *22*−*25*). Thus, we initially wanted to verify a similar result in the chair−

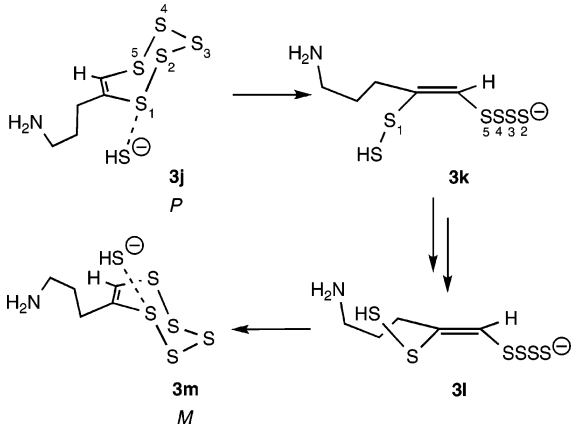
Scheme 4



Scheme 5



Scheme 6



chair flipping of pentathiepin **3a** with our theoretical method using B3LYP/6-31G(d).

Enantiomers **3a** and **3c** adopt chair conformations at the B3LYP/6-31G(d) level. The configurational assignment of pentathiepin **3a** is *P*; here, the pilot S2 atom is connected to the sulfur atom S1 in-plane with the ethylene group, located next to the propyl amine (*26*). Arbitrarily, *P* enantiomer **3a** was used as a starting point instead of *M* enantiomer **3c**. There are a number of structures clustered near the energy minimum with different propyl amine side chain conformations. The propyl amine group adopts conformations with ∼4−8 kcal/mol barriers to rotation about the dihedral angles, such as C7-C8-C9-C10 and C8-C9-C10-N. We examined the polysulfur ring inversion with a similar conformation maintained for the amine chain (*27*). Consequently, the relative energies of **3a** and **3c** in Scheme 3 are not identical ($\Delta E = 0.3$ kcal/mol).

A high-energy process is associated with the conversion of enantiomers **3a** to **3c**. Pentathiepin **3c** is formed from **3a** via a half-chair intermediate **3b** on the DFT potential energy surface

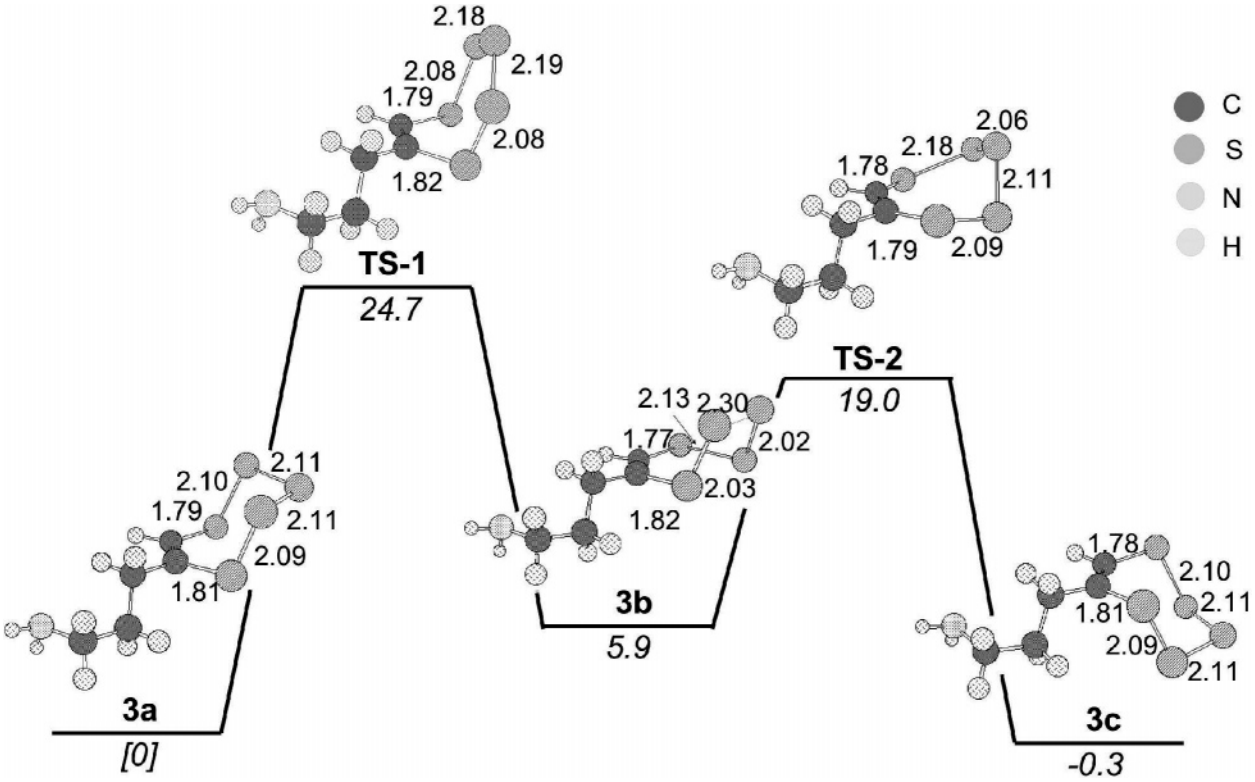
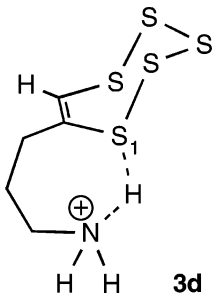


Figure 1. B3LYP/6-31G(d) calculated potential energy surface for the enantiomerization of **3a** to **3c**. (Bond distances are in Å, and energies are in kcal/mol (italics).)

(Figure 1). The magnitude of the calculated barrier for **3a** to **TS-1** is 24.7 kcal/mol. The transformation of **3a** to **3b** is endothermic by 5.9 kcal/mol. The calculated barrier of intermediate **3b** to **TS-2** is 13.1 kcal/mol. The rearrangement of **3b** to **3c** is exothermic by 6.2 kcal/mol. The rearrangement in Figure 1 is not sensitive to basis set extensions, which suggests that the 6-31G(d) basis set is sufficient for predicting the energetics of the reaction (see the Theoretical Methods section). We note that the PES corresponding to the rearrangement of **3a** to **3c** differs from the reaction pathway predicted for ethenopentathiepin $C_2H_2S_5$ carried out at the same B3LPY/6-31G(d) level. A previous study has shown that chair, half-chair, and boat structures are minima on the B3LPY/6-31G(d) PES for ethenopentathiepin (*25*). We were unsuccessful in optimizing **3a** to a boat structure. The asymmetry introduced by the propyl amine group apparently promotes half-chair formation; upon optimization, the boat structure collapses to a half-chair geometry **3b**.

Unimolecular Amination Reaction (Schemes 4 and 5, and Figures 2 and 3). Previously, we used DFT to show that coordination of an amine nitrogen with the pentathiepin ring can take place (*27*). Natural pentathiepins contain an amine group (*11*−*15*, *28*, *29*) and therefore possess a potential for an internal nucleophilic reaction (*27*).

We investigated a new aspect of the reaction, namely, whether intramolecular nucleophilic attack by the amine may facilitate pentathiepin racemization, **3a** to **3c**. At physiological pH, the amine group in natural pentathiepin will be protonated. However, hydrogen bonding of the ammonium group to the polysulfur ring is not predicted to facilitate the racemization of **3a** to **3c** (e.g., via **3d**). Indeed, the calculations predict that a high-energy (∼30 kcal/mol) process is associated with **3a** racemization via association of the internal ammonium with the polysulfur ring. We also considered a free-base mechanism in



3d

which the amine nitrogen reacts with the polysulfur ring. The lowest energy structure contains the amine nitrogen coordinated to the S1 atom (**3d′**), whereas coordination of the amine nitrogen to the S2 atom is higher in energy by 3.4 kcal/mol. Compounds **3d′** and **3i** adopt chair conformations. The configuration of **3d′** is *P*; **3i** is of the opposite configuration *M*. The internal amine group is predicted to preferentially react with the S1 atom of pentathiepin **3d′**. Deprotonation of a developing quaternary nitrogen in **3d′** gives rise to a 6-membered ring sulfenamide regioisomer, **3e**. A transition state was located (**TS-3**), which predicts that **3e** is derived from **3d′** after the loss of a proton (Figure 2). The barrier separating **3d′** or N-deprotonated **3d″** and sulfenamide ion **3e** ranges from 10 to 20 kcal/mol depending on the extent to which the proton is lost in **3d′** according to the gas-phase calculations. Our model focused on the formation of the polysulfur ion **3e**, where the perthiolate portion of the molecule is expected to exist primarily as the anion at physiological pH (*30*, *31*).

A viable pathway was found connecting **3e** to **3h**, which occurs through the intermediates **3f** and **3g**, and is associated

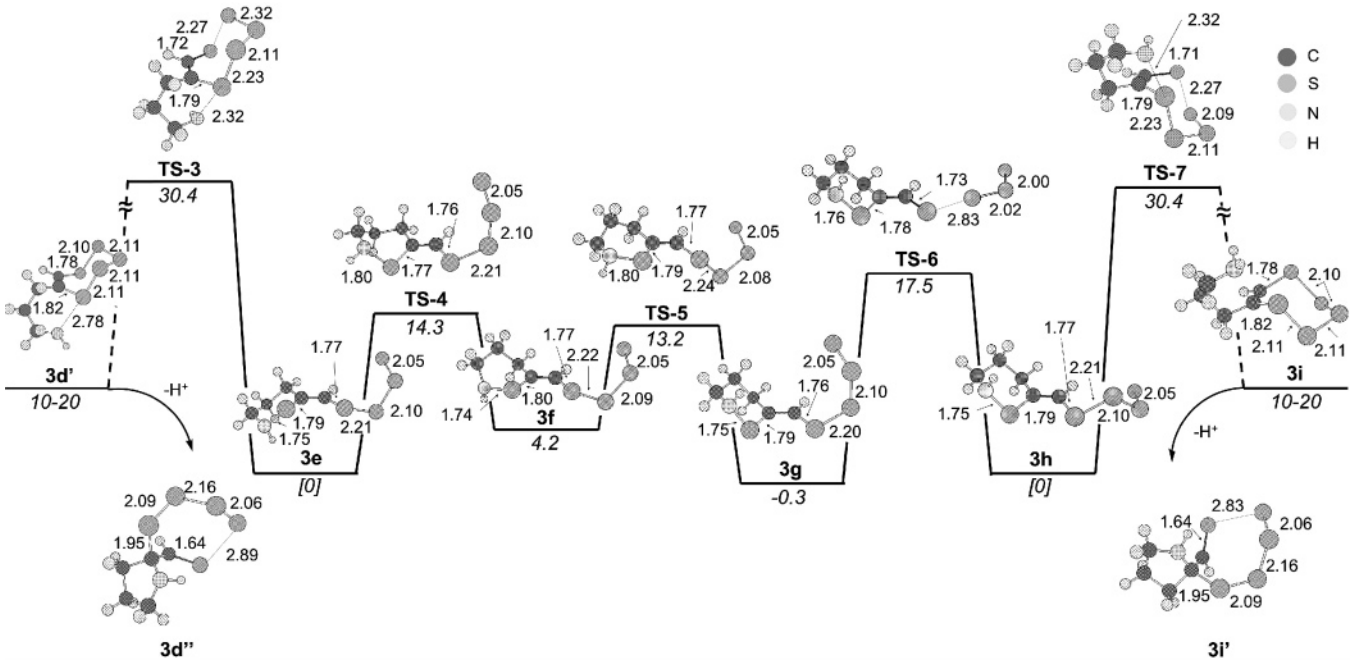


Figure 2. B3LYP/6-31G(d) calculated potential energy surface for the enantiomerization of **3d′**. (Bond distances are in Å, and energies are in kcal/mol (italics).)

with chair−chair flipping of the 6-membered ring sulfenamide, **3e**. The B3LYP half-chair transition state (**TS-4**) converts **3e** to **3f** with a barrier height of 14.3 kcal/mol. The cyclic sulfenamide boat structure **3f** rearranges to a chair structure **3g** through a half-chair transition state (**TS-5**). The energy barrier of the rearrangement **3f** to **3g** is 9.0 kcal/mol, and this process is exothermic by −4.5 kcal/mol. Intermediate **3g** proceeds to transition state **TS-6** and then to **3h** with a barrier height of 17.8 kcal/mol. Sulfenamide **3h** is essentially isoenergetic with **3g**. There is a significant barrier found (30.4 kcal/mol) for the rearrangement of **3h** to the pentathiepin *M* enantiomer **3i** because of the poor leaving ability of the amine group. Thus, the racemization of **3d′** to **3i** is not predicted to readily take place based on this cost in energy.

A similar type of high-energy process is predicted for the racemization of **3d′** to **3i**, in which the nitrogen atom of **3d′** retains its two protons throughout the reaction (Figure 3). Compound **3eH** is formed from **3d′**; the process is endothermic by 29.4 kcal/mol. The computations suggest some instability in the formation of **3eH**, possibly due to its zwitterionic character and the desire to bring together the two opposite charges. The magnitude of the calculated barrier for **3d′** to **TS-3H** is 30.7 kcal/mol. Compound **3eH** converts to **3fH**, via **TS-4H**. The barrier for this process is 15.5 kcal/mol. The remaining part of this PES was not computed because the reaction is not expected to play a role in the racemization of **3d′** to **3i**. We believe that the racemization of **3d′** to **3i** is prevented via **3eH** because of the predicted high energy of this intermediate.

The calculated DFT transition states and intermediates along the amine reaction path in Figures 2 and 3 lead us to propose that S_3-(thiozone) elimination, is preferred to the chair−chair interconversion path. Scheme 5 illustrates the cleaving process of thiozone S_3 and diatomic sulfur S_2 from **3e**. Previous computational studies have shown that S−S cleavage pathways of intermediate **3e** are minima on the B3LPY/6-31G(d) PES for **3d′** (*27*). The reaction barriers for cleavage of S_3 or S_2 from **3e** are computed to be 19.8 and 24.7 kcal/mol, respectively. The elimination of sulfur has also been shown in experimental pentathiepin counterparts, such as the reaction of diethyl amine

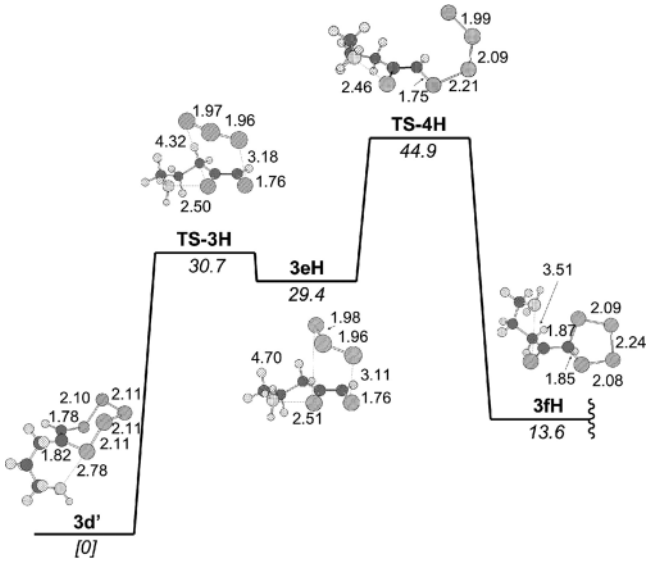


Figure 3. B3LYP/6-31G(d) calculated potential energy surface for the internal (unimolecular) reaction of **3d′** to **3fH**. (Bond distances are in Å, and energies are in kcal/mol (italics).)

with 7-methylbenzopentathiepin (*27*), 6-trifluoromethyl benzopentathiepin (*32*), and other benzopentathiepins (*33*).

Such high barriers associated with the amine-mediated reaction do not suggest a mechanism involving rapid pentathiepin *P* and *M* interconversion. This is consistent with experimental results that report the rapid decomposition of natural pentathiepins upon exposure to desalting conditions, yielding the amine free-base substituent capable of attacking the polysulfur ring (*34*, *35*). The barriers are sufficiently low to permit polysulfur ring opening, which would explain why the natural pentathiepins are unstable in the presence of free amine.

Bimolecular Nucleophilic Reaction (Scheme 6 and Figure 4). Thiols are often present in biological media, and thiolate ions are known to function as nucleophiles for attacking pentathiepin heterocycles (*36*, *37*). Thus, we investigated whether the thiolate ion could catalyze pentathiepin racemization

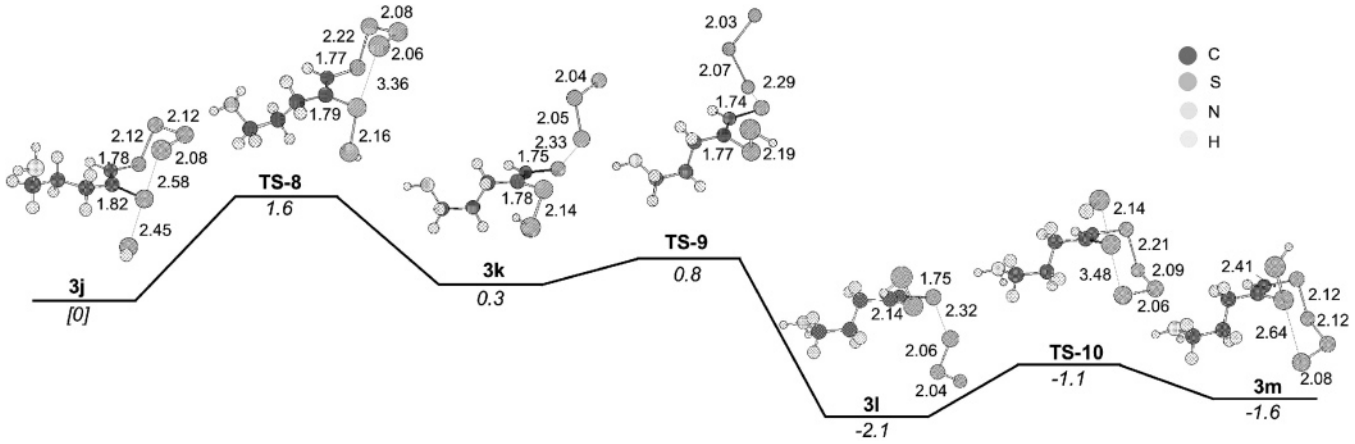


Figure 4. External nucleophile (SH^-) induced chair−chair interconversion of **3j**. (Bond distances are in Å, and energies are in kcal/mol (italics).)

3a to **3c**. The bisulfide ion, HS^-, is used here as a model for biological nucleophiles such has glutathione.

Racemization of **3a** can take place because of the low-energy nucleophilic attack of HS^- on the pentasulfane ring. A structure corresponding to the pentathiepin **3a** with HS^- coordinated to the S1 atom was found to be a bound species at the B3LYP/6-31G(d) level. The thiolate ion HS^- forms a complex with pentathiepin **3a** yielding **3j**. The HS^--complexed **3j** has the *P* configuration; HS^--complexed **3m** has the *M* configuration. The S1−SH^- bond distance in **3j** is 2.45 Å. B3LYP/6-31G(d) calculations predict that HS^- preferentially adds to the S1 atom of pentathiepin **3j**, where cleavage of the S1−S2 bond leads to the formation of the **3k** regioisomer. The lowest energy structure contains the HS^- coordinated to the S1 atom of **3a**; the coordination of HS^- to the S2 atom is higher in energy (*25*). A transition state was located (**TS-8**), which predicts that **3k** is derived from **3j**. The barrier height of **TS-8** is 1.6 kcal/mol. The reaction from **3j** to **3k** is endothermic by 0.3 kcal/mol. Transition states **TS-9** and **TS-10** involve the rotation of the HS-S1 bond and the S4−S5 bond, and lead to **3k** and **3l**, respectively. The B3LYP/6-31G(d) transition state **TS-9** converts **3k** to **3l** with a barrier height of 0.5 kcal/mol. There is a small (1.0 kcal/mol) barrier for further rearrangement of **3l** to the pentathiepin *M* enantiomer **3m** because of the good leaving ability of the HS^- group. Rotation of the linear polysulfur chain and reformation of the ring then leads to enantiomer **3m**. It is reasonable that solvent effects such as H-bonding can stabilize the separated reactants, which would yield the pentathiepin **3c** of *M* configuration.

The attack of the HS^- nucleophile on the polysulfur ring of **3a** dramatically reduces the energetics of the racemization. Coordination of HS^- with pentathiepin is predicted computationally using gas-phase B3LYP/6-31G(d) calculations. Back dissociation of the HS^-−pentathiepin complex to isolated reactants is probably a facile process in solution; nonetheless, thiolate ions react rapidly with pentathiepins experimentally (*37*). The thiolate ion−pentathiepin reaction occurs on a PES of low activation energies (Figure 4), which is in contrast to the higher energetics associated with the reactions in Figures 1−3.

Conclusions

A low-energy pathway for pentathiepin racemization has been found computationally. Pentathiepin racemization becomes a low-energy process in the presence of a thiolate ion nucleophile. The bisulfide ion, HS^-, is used here as a model for biological nucleophiles. We have evaluated that higher-energy processes are associated with the conversion of pentathiepin enantiomers via chair−chair flipping (Figure 1) and the internal amine-nucleophilic pathway (Figures 2 and 3). Importantly, a low-energy path to thiolate ion-assisted racemization suggests an easy loss of planar chirality. Consequently, the study of enantiospecific behaviors such as the toxicity difference between *P*- and *M*-pentathiepins would be difficult to determine experimentally. The ease of thiolate ion attack on the polysulfur ring suggests that other nucleophiles may also induce optical instability on the laboratory time scale.

Table 1. Single-Point Calculations for the Rearrangement of 3a → 3c Using B3LYP/6-31G(d) Optimized Geometries[a]

basis set	3a	TS-1	3b	TS-2	3c
6-31G(2df)	[0]	25.5	6.0	18.7	−0.3
6-311+G(d)	[0]	24.3	5.6	18.7	−1.1
6-311G(d,p)	[0]	23.8	5.5	18.7	−0.2

[a] Energies are in kcal/mol.

Theoretical Methods

The calculations were performed with Gaussian 03 (*38*). Geometry optimizations, vibrational frequencies, and intrinsic reaction coordinates (IRCs) were conducted by using DFT with the exchange-correlation of B3LYP along with the Pople basis set 6-31G(d) (*39*). Transition structures have been confirmed by frequency calculations and by tracing the IRCs. Analyses of the reactions in Figures 1−3 were conducted in the gas phase. In previous work, we found that temperature corrections led to minor changes in the energetics of benzopolysulfane reactions. Thus, the predicted energies involve compounds at 0 K and are uncorrected for temperature and zero-point energy corrections. We examined the quality of the B3LYP/6-31G(d) calculations. Previous reports employing B3LYP with basis sets of low computational cost can give energetic errors for molecules in which sulfur is bound to an electronegative element, such as oxygen and fluorine (*41*). Sulfur compounds with more than one fluorine or oxygen (e.g., SF_6, SO_2, and SO_3) can be problematic (*41*, *42*). We find that the qualities of the B3LYP/6-31G(d) calculations were reasonable as assessed by comparison of calculated and experimentally available geometries of pentathiepins (*22*). We calculated minor differences in energies due to the effect of *f* functions in the B3LYP method on the **3a** → **3c** potential energy surface. The B3LYP/6-31G(d) calculations were also deemed to be reasonable on the basis of a comparison to single-point calculations with 6-311+G(d) and 6-311G(dp) basis sets for the conversion of **3a** → **3c** (Table 1). The results of our DFT calculations are also consistent with earlier *ab initio* computations of pentathiepin systems (*23*, *24*).

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