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Synthesis and properties of disulfide-bond containing eight-membered rings

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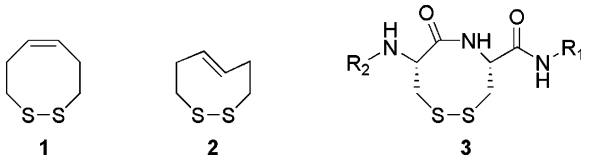
Abstract—The cyclocystine ring structure (CRS, **3**), which results from a disulfide-bond between adjacent cysteine residues, is a rare motif in protein structures and is functionally important to those few proteins that posses it. This letter will focus on the construction of CRS mimics and the determination of their respective redox potentials.

A disulfide-bond between adjacent cysteine residues (**3**, Fig. 1) is a very rare occurrence in protein structures. Currently, 32 out of ca. ~28,000 proteins structurally identified in the Brookhaven Protein Data Bank (PDB) carry this unique motif.[1] In every case the amide bond of the CRS is reported to be in a strained *trans* geometry with an average ω value of 171°.[1] Peptide bonds prefer a *trans* conformation with a torsion angle of 180° so that the nitrogen lone-pair can have maximal delocalization into the π-system, while minimizing steric repulsions from peptidyl side-chains. However, the small ring nature of the eight-membered CRS allows for multiple amide conformations to be energetically feasible. The amide bond could adopt a *cis* conformation, which still allows for delocalization of the nitrogen lone-pair, but would cause the main peptidyl-chain to have a kink in it. A 'strained' *trans* conformation allows the main chain to remain relatively unaltered, but still allows for partial delocalization of the nitrogen lone-pair into the π-system. Our model studies show that a torsion angle of 180° is not allowed for a CRS due to its inability to form the disulfide-bond. Hence, the nitrogen lone-pair must come slightly out of phase with the π-system to allow disulfide-bond formation.



1 2 3

Figure 1. Small molecule mimics of CRS.

A reasonable model for a CRS is cyclooctene. Energetically, the *cis* isomer is more stable than the *trans* as a result of the ring strain required to incorporate a *trans* double bond. This ring strain is demonstrated by the higher $\Delta H_{\text{Hydrogenation}}$ of *trans*-cyclooctene (34.4 kcal/mol) compared to the *cis* isomer (23.0 kcal/mol).[2] If the cyclooctene analogy is applied to the eight-membered CRS, one might expect *cis* amide geometry to predominate. This is not what has been observed experimentally in the PDB. Investigations into proteins that carry the CRS reveal that this motif is important for activity.[3] The central focus of this study is to assess how CRS conformation affects the redox potential of the disulfide-bond. Our central hypothesis is that a CRS with a *cis* peptide bond should be much more reducing (low redox potential) than a CRS with a *trans* peptide bond.

In order to test this hypothesis, *cis* and *trans* substrates were constructed in both oxidized (**1** and **2**) and reduced forms (**10** and **13**). Both systems have a central bond (*cis*/*trans*-olefin) with restricted geometry that mimics the 0° and 180° amide conformations of a *cis* and *trans* CRS. The synthesis of these compounds has not been reported previously, though the theoretical value for the redox potential of **1** has been calculated.[4]

Retrosynthetically, dithiocines **1** and **2** are available upon intramolecular oxidation of the appropriate

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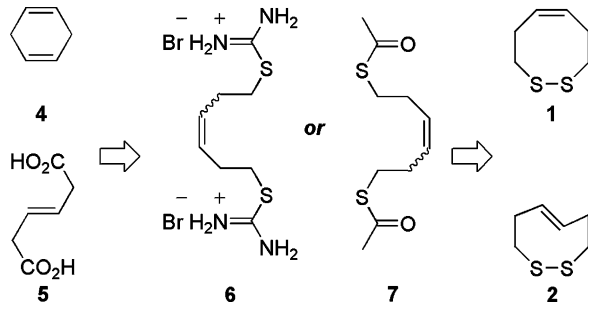


Figure 2. Retrosynthesis of dithiocines.

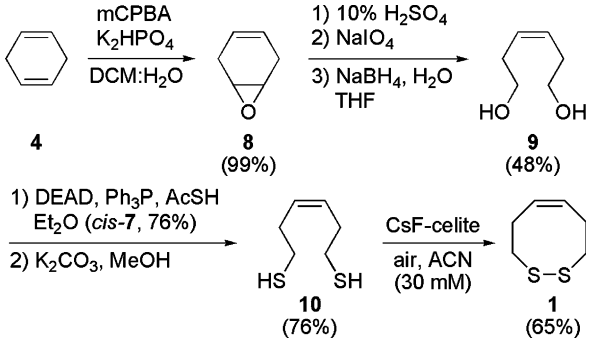
dithiol precursor. Originally the dithiol substrates were to be constructed via dithioureic salt **6**. However, while the synthesis of **6** was non-problematic, the harsh conditions necessary for the generation of the dithiol led to significant by-product formation.[5] This led to the use of dithioester **7** as the intermediary target, which could undergo saponification easily (Fig. 2).[6]

The redox properties were determined by thiol–disulfide exchange, with the varying concentrations of reduced and oxidized forms monitored by ^{1}H NMR (Fig. 3).[7] Employment of oxidized or reduced butane dithiol ($\mathbf{BDT}_{ox}$ and $\mathbf{BDT}_{red}$, respectively), a species of known redox potential ($E_{0(BDT)}$), allows for the redox potential of the CRS mimics to be determined by Eqs. 1 and 2.

$$K_{ox}^{-1} = K_{red} = [\mathbf{CRS}_{red}][\mathbf{BDT}_{ox}]/[\mathbf{CRS}_{ox}][\mathbf{BDT}_{red}] \quad (1)$$

$$E_0 = E_{0(BDT)} - 0.03\log(K_{ox}) \quad (2)$$

The synthesis of *cis*-dithiocine **1** begins with the epoxidation of 1,4-cyclohexadiene to generate **8** almost quantitatively (Scheme 1).[8] A subsequent one-pot protocol entails diol formation, followed by oxidative ring cleavage and reduction of the intermediate acyclic dial to pro-



Scheme 1. Synthesis of *cis*-dithiocine (**1**).

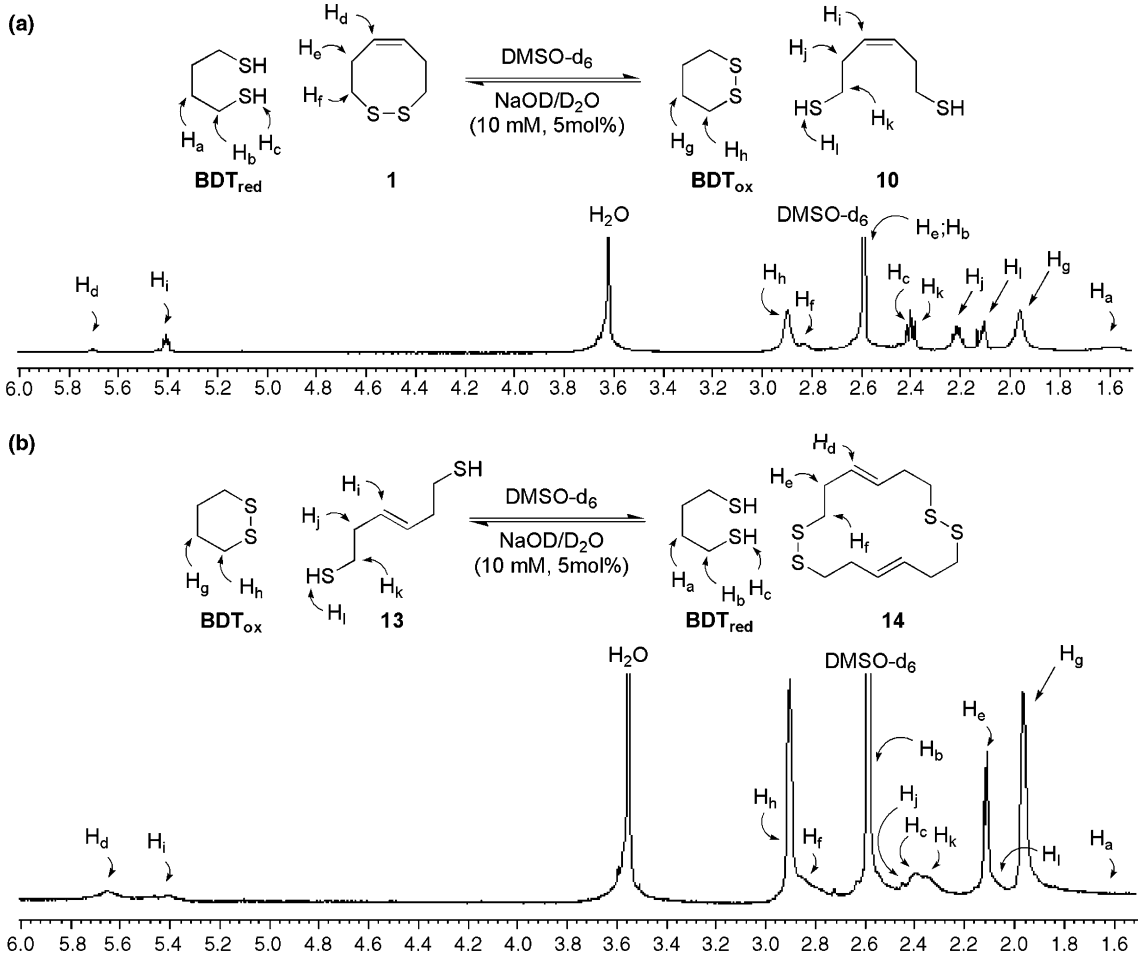
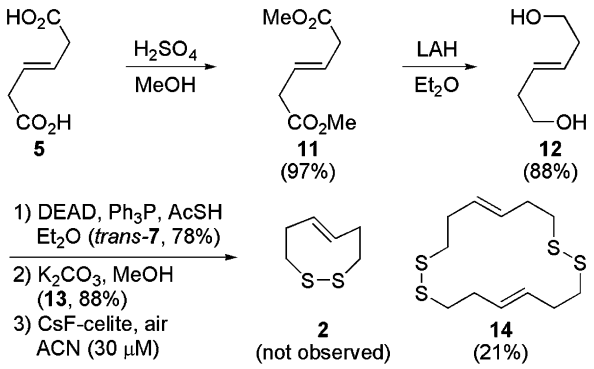


Figure 3. Proton NMR equilibrium redox experiment for *cis*- and *trans*-dithiols.



Scheme 2. Attempted synthesis of *trans*-dithiocine (**2**).

duce **9** in good overall yield.[9] The formation of dithioester *cis*-**7** occurs under Mitsunobu conditions,[10] after which mild saponification affords dithiol **10**.[6] Air-oxidation mediated by CsF impregnated celite produced the desired *cis*-dithiocine **1**.[11] Disulfide formation does occur without the CsF–celite additive; however, reaction times are significantly longer.

The synthesis of *trans*-dithiocine precursor **13** begins with *trans*-mucionic acid (Scheme 2). Methyl esterification and reduction generates *trans*-diol **12**.[12] Analogous to the construction of **1**, Mitsunobu thioesterification and mild saponification affords *trans*-dithiol **13** in good overall yield. While the formation of dithiol **13** was not troublesome, oxidative intramolecular construction of **2** was never realized even under dilute conditions. The only disulfide-bond containing compound isolated was that of cyclic-dimer **14**.

The redox potentials were then determined by equilibrium thiol–disulfide exchange. These redox experiments reached equilibrium in approximately 5 days in DMSO-d_6. Integration of olefinic signals, as well as others, allowed for the respective redox potentials to be determined (Fig. 3).[13] This resulted in a derived cyclomonomeric redox potential of -0.318 eV for dithiol **10** (Fig. 3a),[14] which is in close agreement with the predicted redox potential for this compound.[4] The cyclodimeric redox potential of *trans*-dithiol **13** was determined to be -0.329 eV (Fig. 3b).[15] The inability to determine the cyclomonomeric redox potential for the disulfide-bond of **2** alludes to its highly oxidative character.

The ease of monomeric disulfide formation for the production of *cis*-dithiocine **1** (low redox potential) is due to (i) the very high collisional frequency of two sulfur-atoms in close proximity while in the *cis* configuration, and (ii) the lack of unfavorable non-bonded interactions.[16] The inability to construct *trans*-dithiocine **2** is due to the remoteness of the sulfur-atoms in conjunction with the rigidity of the central olefinic bond. The higher monomeric redox potential of **13** is illustrated by its lack of ability to form a cyclic monomer and its propensity to dimerize as well as oligomerize. Upon disulfide-bond ring formation there is an absence of ring strain in the incorporation of a *cis*-olefin in the eight-membered ring

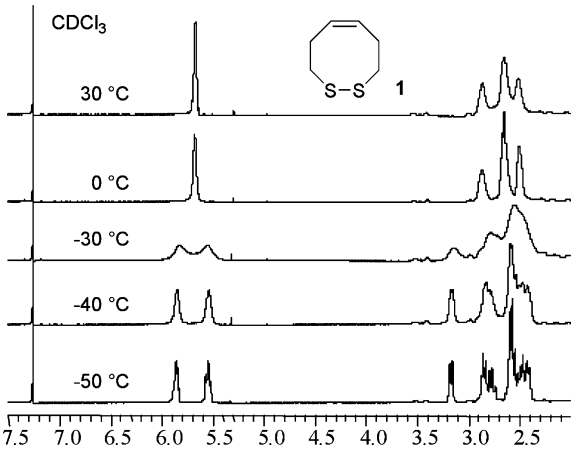


Figure 4. ^{1}H NMR temperature dependent coalescence experiment for *cis*-dithiocine (**1**).

when compared to the *trans* isomer. As a result, *cis*-dithiocine **1** is much more stable than *trans*-dithiocine **2**. The stability and absence of ring-strain in **1**, when compared to **2**, is also demonstrated via temperature dependent ^{1}H NMR coalescence experiments (Fig. 4).

It is interesting to compare *trans*-dithiol **13** to that of a CRS found in proteins. A disulfide-bond can form between nearest neighbors because the peptide bond is not as rigid as an olefin. This allows the central peptide bond to 'twist' slightly allowing disulfide-bond formation to occur with a strained *transoid* geometry. When the central torsional angle is constrained to 180°, as is the case for **13**, disulfide-bond formation is impossible. Redox enzymes, such as mammalian thioredoxin reductase, may take advantage of the low redox potential of adjacent cysteine residues in a *cis* configuration, which cycle between reduced and oxidized states.[17] This is especially true for this enzyme since the redox pair occurs at the C-terminus, which would minimize the effect of a *cisoid* peptide bond on the main chain.

Acknowledgments

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References and notes

1. Hudáky, I.; Gáspári, Z.; Carugo, O.; Čemžar, M.; Pongor, S.; Perczel, A. *Proteins: Struct. Funct. Bioinf.* **2004**, *55*, 152–168.
2. Rogers, D. W.; von Voithenberg, H.; Allinger, N. L. *J. Org. Chem.* **1978**, *43*, 360–361.
3. Carugo, O.; Čemžar, M.; Zahariev, S.; Hudáky, I.; Gáspári, Z.; Perczel, A.; Pongor, S. *Protein Eng.* **2003**, *16*, 637–639.
4. Burns, J. A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 6296–6303.
5. Lai, Y.-H.; Soo, T.-B. *Heterocycles* **1985**, *23*, 1205–1214.

6. Redman, J. E.; Sanders, J. K. M. *Org. Lett.* **2000**, *2*, 4141–4144.
7. Houk, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 6825–6836.
8. Perlman, N.; Albeck, A. *Synth. Commun.* **2000**, *30*, 4443–4449.
9. Tamoto, K.; Terashima, S. *Chem. Pharm. Bull.* **1984**, *32*, 4340–4349.
10. Dobbs, A. P.; Guesné, S. J. J.; Martinović, S.; Coles, S. J.; Hursthouse, M. B. *J. Org. Chem.* **2003**, *68*, 7880–7883.
11. Shah, S. T. A.; Khan, K. M.; Fecker, M.; Voelter, W. *Tetrahedron Lett.* **2003**, *44*, 6789–6791.
12. Gassman, P. G.; Bonser, S. M.; Mlinarić-Majerski, K. *J. Am. Chem. Soc.* **1989**, *111*, 2652–2662.
13. Redox experiments were conducted under dry Ar, with reagents and solvents of reagent grade. Solvent deoxygenation was accomplished by sparging with Ar (30 min), followed by sonication (10 min) at aspirator pressure. Standard Redox Procedure: Dithiocine **1** (1.0 equiv) and $\mathbf{BDT}_{red}$ (1.64 equiv) were combined in DMSO-d_6, after which a 10 mM NaOD/D_2O (5 mol% NaOD) was added. Residual O_2 was removed by Ar-sparging for 30 min. The redox experiment was monitored by ^{1}H NMR and reached equilibrium in 5 days. Compound **10**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.49 (t, $J = 4.7$ Hz, 2H), 2.72 (t, $J = 7.0$ Hz, 4H), 2.47 (q, $J = 7.0$ Hz, 4H), 1.25 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.9 (CH), 38.4 (CH_2), 27.2 (CH_2); HRMS (EI) m/z 148.0382 [(M^+) calcd for $C_6H_{12}S_2$: 148.0380]. Compound **1**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.67 (m, 2H), 2.87 (br s, 2H), 2.64 (br s, 4H), 2.50 (br s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.7 (CH), 39.4 (CH_2), 27.2 (CH_2); HRMS (EI) m/z 146.0225 [(M^+) calcd for $C_6H_{10}S_2$: 146.0224]. **13**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.47 (m, 2H), 2.57 (q, $J = 7.7$ Hz, 4H), 2.33 (m, 4H), 1.42 (t, $J = 7.7$ Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 128.9 (CH), 38.4 (CH_2), 27.2 (CH_2); HRMS (EI) m/z 148.0379 [(M^+) calcd for $C_6H_{12}S_2$: 148.0380]. Compound **14**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.62 (m, 4H), 2.79 (t, $J = 7.1$ Hz, 8H), 2.40 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 129.8 (CH), 39.7 (CH_2), 31.8 (CH_2); HRMS (EI) m/z 292.0446 [(M+) calcd for $C_{12}H_{20}S_4$: 292.0448].
14. The redox potential of **10** was determined by the integration of H_d, H_c, H_j and H_g ^{1}H NMR signals (Fig. 3a), which led to a ratio of 1.00:1.64:3.60:3.60 (**1**:$\mathbf{BDT}_{red}$:**10**:$\mathbf{BDT}_{ox}$). Equilibrium concentrations were then determined by comparison of the equilibrium ratio to initial concentrations of **1** and $\mathbf{BDT}_{red}$. Insertion into Eq. 2 produces
$$E_0 = -0.345 - 0.03 \log[(1.30)(3.08)/(4.69)(6.76)] = -0.318 \text{ eV}.$$
15. The redox potential of **13** was determined by the integration of H_d, H_c, H_i and H_g ^{1}H NMR signals (Fig. 3b), which led to a ratio of 1.00:2.00:1.11:1.93 (**14**:$\mathbf{BDT}_{red}$:**13**:$\mathbf{BDT}_{ox}$). Equilibrium concentrations were then determined by comparison of the equilibrium ratio to initial concentrations of **13** and $\mathbf{BDT}_{ox}$. Use of a cyclodimeric derived form of Eq. 2 produces
$$E_0 = -0.345 - 0.03 \log[(2.84)(5.30)^2/(3.14)^2(5.12)^2] = -0.329 \text{ eV}.$$
16. Zhang, R.; Snyder, G. H. *J. Biol. Chem.* **1989**, *264*, 18472–18479.
17. Sandalova, T.; Zhong, L.; Lindqvist, Y.; Holmgren, A.; Schneider, G. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 9533–9538.