DOI: 10.1002/ejoc.200900044

Preparation of a Reversible Redox-Controlled Cage-Type Molecule Linked by Disulfide Bonds

Yih-Chern Horng,*[a] Tzung-Lung Lin,[a] Cheng-Yi Tu,[a] Ting-Ju Sung,[a] Chang-Chih Hsieh,[a] Ching-Han Hu,[a] Hon Man Lee,[a] and Ting-Shen Kuo[b]

Keywords: Dimerization / Self-assembly / Sulfur / Preorganization

A unique trithiol macromolecule, with an intrinsic conformational propensity for dimerization (cage) instead of oligomerization upon oxidation, was prepared straightforwardly through rational design. The quantitative conversion and the reversibility between the cage and trithiols through redox reactions were assayed by ^{1}H NMR spectroscopic analysis. The X-ray structure of the synthesized cage-type molecule represents the first successful example of a redox-controlled reversible dimeric capsule linked through covalent disulfide bonds.

Introduction

Synthetic macromolecular capsules have attracted much attention because of their potential applications in biological systems, for example, in molecular recognition,[1] in drug delivery,[2] and as miniature reactors.[3] Three main methods are employed in the literature for the formation of synthetic capsules: (i) Synthesis through permanent covalent bonds under kinetic control.[4] (ii) Formation through noncovalent interactions or metal–ligand coordination under thermodynamic control.[5] (iii) Production through reversible covalent bonds under thermodynamic control.[6] Although many molecular capsules have been made by applying the first and second methods, only a few examples exist of synthetic capsules that have been formed by using the third method.[7,8] Because the reversible thiol–disulfide interchange reaction is an important factor for controlling the protein folding process, and the strong covalent S–S bonds are thought to be the key for stabilizing the structures of small proteins,[9] the utilization of dynamically reversible covalent disulfide bonds holds great potential for synthesizing macromolecular capsules.

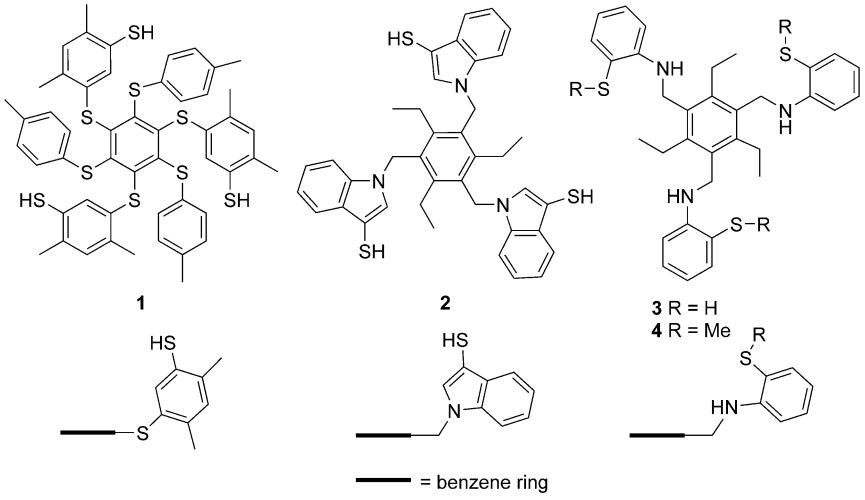
However, attempts to synthesize macromolecular capsules, especially dimeric tris(disulfide)s, have often resulted in oligomeric products to some extent.[8,10] The restricted CSSC dihedral angle of about 90°[11] thermodynamically limits the formation of an S–S-bond-linked capsule. In addition, preorganization of the thiol groups at the right geometry and orientation in the monomer is required for the favorable kinetic formation of capsules. Thus, it is extremely difficult to obtain a thermodynamically and kinetically stable synthetic capsule with tris(disulfide) bonds. In addition, there is no structural information on disulfide-bond-based synthetic capsules in the literature.

Herein we report the design, synthesis, and structural determination of a novel reversible dimeric macromolecular capsule linked by tri(disulfide) bonds.

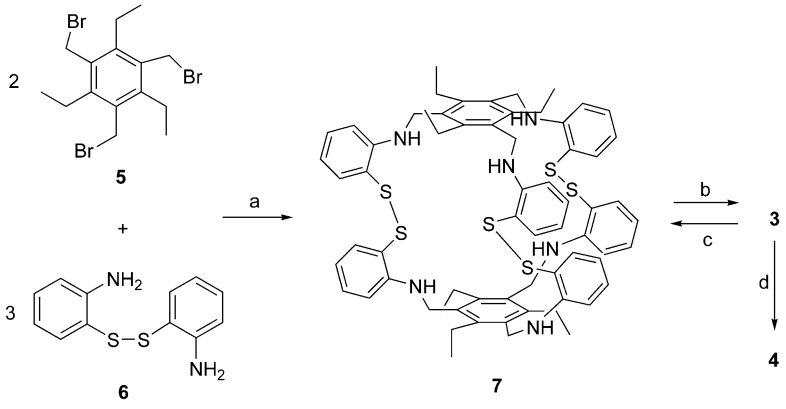
Results and Discussion

Inspired by the synthetic trithiol compounds **1**[12] and **2**[13] utilized to form a 3:1 subsite differentiated [4Fe–4S] cluster for modeling the active site of aconitase (Scheme 1), we planned to make a similar trithiol compound (i.e., **3**) by a more convenient method and convert it into a cage-like macromolecule through oxidation under kinetic or thermodynamic control. The fundamental topological features of both **1** and **2** are (i) based on a hexasubstituted benzene ring, (ii) a staggered conformation, which allows the functional groups carrying the thiol donors to orient on the same side of the aromatic base, and (iii) the thiol donors turned inward toward a common area due to the steric demands of the methyl substitutes of 4,6-dimethylphenyl group and the benzo ring of the indolyl moiety. All three features promised the formation of a dimeric molecular capsule linked through three disulfide bonds. Most importantly, the third feature of the thiol donors turned inward would prevent oligomerization due to steric hindrance. However, the synthesis of **1** requires tremendous effort and the preparation of both **1** and **2** uses mercury acetate, which is potentially hazardous to human health and the environ-

[a] Department of Chemistry, National Changhua University of Education,
1 Jin-De Road, Changhua 50058, Taiwan
Fax: +886-4-721-1190
E-mail: ychorng@cc.ncue.edu.tw
[b] Instrumentation Center, Department of Chemistry, National Taiwan Normal University,
Taipei 11677, Taiwan
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.



Scheme 1. Trithiol molecules.



Scheme 2. Syntheses of trithiol **3**, its methyl derivative **4**, and macromolecular capsule **7**. Reagents and conditions: (a) K_2CO_3, CH_3CN, 72 h, 85 °C; (b) $NaBH_4$, DMF, 10 min, 4 °C; (c) O_2 or I_2, CH_2Cl_2, r.t.; (d) NaOMe, MeI, CH_3CN, 12 h, r.t.

ment, for the deprotection of the trithiol. Thus, an alternative approach toward the creation of a new trithiol with features (i) to (iii), described above, was conducted (Scheme 2).

Before performing the experiments, the most stable conformation of **3** was evaluated by density functional theory computations. It is well known that the three substituents in 2,4,6-tris(substituent)-1,3,5-triethylbenzene tend to orient on the opposite side of the benzene plane relative to the ethyl groups.[14] The optimized conformation with all three thiol donors turned inward is more stable than the outward one by 20.4 kcal mol^{-1}, according to density functional theory (B3LYP/6-31G) computation (Figure 1). Thus, the computational results indicated a favorable preorganization of **3** for forming a dimeric capsule.

Protection reagents, required for syntheses involving highly reactive thiolates, were unnecessary for the very straightforward synthesis of **3**. When an acetonitrile solution containing commercially available 2,4,6-tris(bromomethyl)-1,3,5-triethylbenzene (**5**) and 2,2′-aminophenyl disulfide (**6**) was heated at reflux for 72 h in the presence of an excess amount of K_2CO_3, a major product was formed

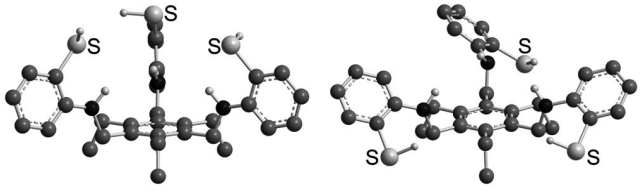


Figure 1. The optimized structure of **3** with all three thiols turned inward (**3a**, left) and outward (**3b**, right). Hydrogen atoms bound to carbon atoms are omitted for clarity.

as a precipitate. This product was then further reduced with $NaBH_4$ in a one-pot reaction to give designed trithiol molecule **3**. The identification of **3** was established by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and elemental analysis (EA).

Although we were not able to obtain crystals of **3**, the molecular structure of its methyl derivative (i.e., **4**) was determined by X-ray analysis (Figure 2), which confirmed the right geometries and orientations of the ethyl groups and S atoms, and that it was comparable to the structure of **3a** simulated by computation.

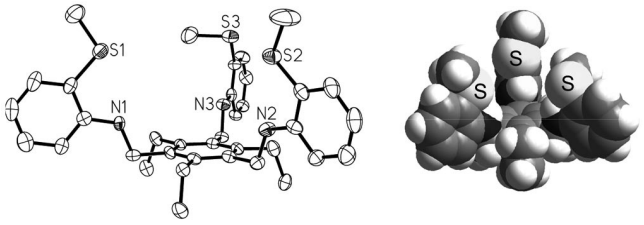


Figure 2. The molecular structure (left) and space-filled model (right) of **4**. The thermal ellipsoids are drawn at a 35% probability level. Nitrogen and sulfur atoms are represented as shaded ellipsoids. All of the hydrogen atoms are omitted for clarity.

Next, attempts were made to synthesize tris(disulfide) molecular capsules by the oxidation of **3** under thermodynamic control (O_2) or kinetic control (I_2, not under high-dilution conditions) through self-assembly. Both attempts gave product **7** with a high yield (about 90%). Surprisingly, this product was identical to the one from the reaction between **5** and **6**. Thus, the formation of **7** is thermodynamically and kinetically favored over other larger oligomers. The identity of **7** was confirmed by EA and ^1H and ^{13}C NMR spectroscopy, whereas the highly symmetric structure, as suggested by the ^1H and ^{13}C NMR spectra, was further supported by X-ray structure determination of **7**, whose crystals were obtained from the recrystallization in CH_2Cl_2 solution (Figure 3).

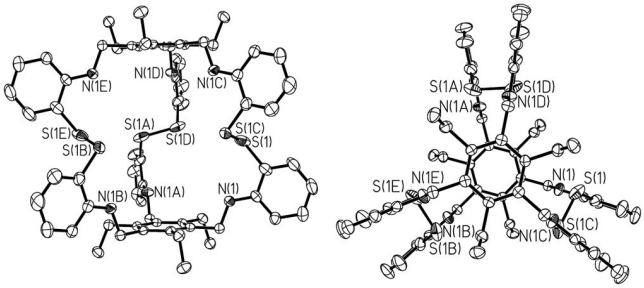


Figure 3. The molecular structure of capsule **7**; side view (left) and top view (right). The thermal ellipsoids are drawn at a 35% probability level. Nitrogen and sulfur atoms are represented as shaded ellipsoids. Severely disordered solvent molecules in the void spaces inside and outside of **7** were not identified and refined. All of the hydrogen atoms are omitted for clarity. Key bond parameters for **7**: S–S 2.065(2) Å, C–S–S–C torsion angle 96.8(2)°.

The molecular structure of dimeric capsule **7** shows an $R\bar{3}c$ symmetry, which is rare in terms of the cage molecules reported to date.[15] All six phenyl rings orient perpendicular to the benzene bases to form a staggered conformation (top view), with the ethyl groups pointing outward, and all six amino protons oriented toward the center. The CSSC dihedral angle and S–S distance of **7** are very similar to those of starting reagent **6** [89.5° and 2.061(4) Å, respectively],[16] and bis[2,6-bis(pivaloylamino)phenyl] disulfide [98.9° and 2.079(4) Å, respectively].[17] The cavity can be viewed as a cylinder in the core of **7**, with a diameter of 8.06 Å[18] and a height of 8.98 Å. If we account for the van der Waals radius of a sulfur atom (1.80 Å) and the thickness of the arene ring (1.70 Å), the van der Waals volume of the cavity for encapsulation can be calculated to be 87.1 $Å^3$ (diameter: 4.46 Å; height: 5.58 Å). With the volume of the cavity and a gate size of 2.4 Å,[19] capsule **7** can easily accommodate molecules such as dichloromethane (55 $Å^3$) and chloroform (72 $Å^3$).[20]

The quantitative conversion and the reversibility between the cage and trithiols are essential for its biological applications. Under equilibrium conditions, the conversion of **3** into **7** in C_6D_6 was followed in the presence of air by using ^1H NMR spectroscopy (Figure 4). During the entire conversion process, no precipitates were observed, which ensures that no other products were formed except the ones observed from the NMR measurements. The complete oxidation of **3** only afforded **7**; no oligomeric products were formed, and one or two intermediates appeared during the conversion process. Our DFT computation revealed that the reaction energy needed for two moles of **3** to form capsule **7** plus three moles of H_2 was 11.6 kcal mol^{-1}, which is much lower than what is needed for 2,4,6-tris(mercaptomethyl)-1,3,5-triethylbenzene (**8**) to form a dimeric molecule (36.13 kcal mol^{-1}). Entropic effects will further favor capsule formation. Experimentally, the oxidation of **8** under kinetic conditions (I_2, $CHCl_3$) only yielded the oligomeric product as a result of the highly strained CSSC dihedral angle on dimer formation.[8a]

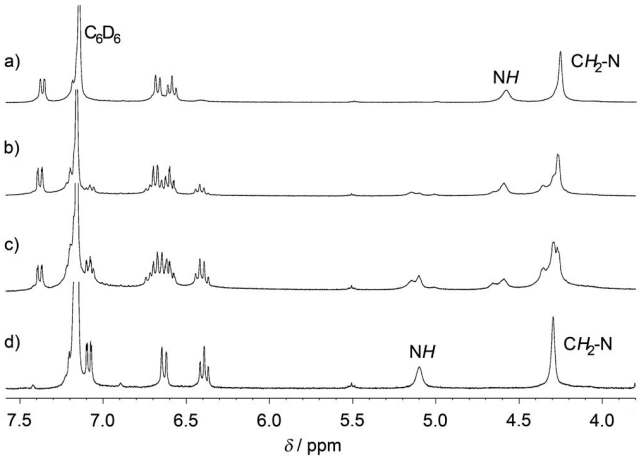


Figure 4. ^1H NMR spectra (400 MHz, 298 K, C_6D_6) of (a) purified **3**, (b) oxidation of **3** in air for 2 weeks at room temperature, (c) further oxidation of **3** from (b) in air for 1 d at 70 °C, and (d) complete oxidation of **3**.

Interestingly, when the conversion was conducted in DMF solution aerobically at room temperature, crystals of DMF-encapsulated molecule **7**·DMF were found in the precipitate. The identification of **7**·DMF was established by ^1H and ^{13}C NMR spectroscopy, EA, and X-ray structure determination (Figure 5). To the best of our knowledge, this is the first time that the X-ray structure of a reversible molecular capsule encapsulating a guest molecule using disulfide linkages has been determined. Although there is no obvious hydrogen bonding between the guest and **7**, a weak lone pair (carbonyl)···π interaction between the DMF molecule and the aromatic base of **7** was observed in the crystal structure.[21]

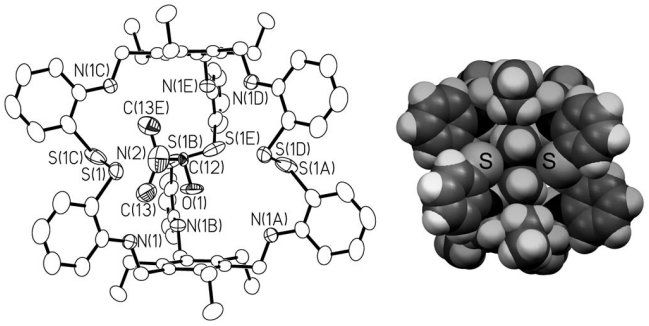


Figure 5. The molecular structure (left) and space-filled model (right) of **7**·DMF. The thermal ellipsoids are drawn at a 35% probability level. The DMF molecule is disordered with D_{3h} symmetry and only one disorder component is shown. The atoms of the encapsulated DMF molecule are represented as shaded ellipsoids. All of the hydrogen atoms are omitted for clarity. Key bond parameters for **7**·DMF: S–S 2.049(3) Å, C–S–S–C torsion angle 96.4(2)°, O(1)···centroid (aromatic base) 3.26 Å, aromatic plan-centroid–O(1) axis 76.7°.

Conclusions

We designed an extremely straightforward method to synthesize in remarkably high yield a novel dimeric capsule that can be assembled by tris(disulfide) bonds and determined its structure. We also demonstrated that the capsule is easily opened up through the reduction of disulfide bonds. The encapsulation of a neutral guest molecule in **7** during the oxidation process of **3** was observed. The information contained in the molecular structure of **7** improves our understanding of the rational design for a dimeric macromolecular capsule containing S–S bonds and the reversible opening and closing of **7** through its dynamic covalent bonds. Furthermore, the results described here help to address the problems of applications of disulfide-linked molecular capsules in biological and medicinal systems. The next challenge will be to create a reversible water-soluble molecular capsule that employs compact and rigid building blocks similar to **7** for greater biological relevance.

Experimental Section

The experimental details can be found in the Supporting Information.

CCDC-704226 (for **4**), -704227 (for **7**), and -707649 (for **7**·DMF) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting Information (see footnote on the first page of this article): Details of the syntheses of **3**, **4**, **7**, and **7**·DMF; conversion studies between **3** and **7**; crystallographic data for **4**, **7**, and **7**·DMF; computational studies of **3** and **7**.

Acknowledgments

We are grateful to the National Science Council (Taiwan) for their financial support of this work.

[1] S. M. Biros, J. Rebek Jr, *Chem. Soc. Rev.* **2007**, *36*, 93–104.
[2] K. R. West, S. Otto, *Curr. Drug Discov. Technol.* **2005**, *2*, 123–160.
[3] a) T. S. Koblenz, J. Wassenaar, J. N. Reek, *Chem. Soc. Rev.* **2008**, *37*, 247–262; b) L. R. MacGillivray, G. S. Papaefstathiou, T. Friscic, T. D. Hamilton, D. K. Bucar, Q. Chu, D. B. Varshney, I. G. Georgiev, *Acc. Chem. Res.* **2008**, *41*, 280–291.
[4] a) A. Jasat, J. C. Sherman, *Chem. Rev.* **1999**, *99*, 931–968; b) J. Sherman, *Chem. Commun.* **2003**, 1617–1623.
[5] a) J. Rebek Jr, *Angew. Chem. Int. Ed.* **2005**, *44*, 2068–2078; b) W. Y. Sun, M. Yoshizawa, T. Kusukawa, M. Fujita, *Curr. Opin. Chem. Biol.* **2002**, *6*, 757–764; c) S. Liu, B. C. Gibb, *Chem. Commun.* **2008**, 3709–3716.
[6] S. J. Rowan, S. J. Cantrill, G. R. Cousins, J. K. Sanders, J. F. Stoddart, *Angew. Chem. Int. Ed.* **2002**, *41*, 898–952.
[7] a) S. Ro, S. J. Rowan, A. R. Pease, D. J. Cram, J. F. Stoddart, *Org. Lett.* **2000**, *2*, 2411–2414; b) C. Naumann, S. Place, J. C. Sherman, *J. Am. Chem. Soc.* **2002**, *124*, 16–17; c) A. L. Kieran, S. I. Pascu, T. Jarrosson, J. K. M. Sanders, *Chem. Commun.* **2005**, 1276–1278.
[8] a) S. W. Tam-Chang, J. S. Stehouwer, J. Hao, *J. Org. Chem.* **1999**, *64*, 334–335; b) K. R. West, K. D. Bake, S. Otto, *Org. Lett.* **2005**, *7*, 2615–2618.
[9] J. D. Hartgerink, *Curr. Opin. Chem. Biol.* **2004**, *8*, 604–609.
[10] J. Houk, G. M. Whitesides, *J. Am. Chem. Soc.* **1987**, *109*, 6825–6836.
[11] D. Jiao, M. Barfield, J. E. Combariza, V. J. Hruby, *J. Am. Chem. Soc.* **1992**, *114*, 3639–3643.
[12] T. D. P. Stack, R. H. Holm, *J. Am. Chem. Soc.* **1988**, *110*, 2484–2494.
[13] C. Walsdorff, W. Saak, S. Pohl, *J. Chem. Soc., Dalton Trans.* **1997**, 1857–1862.
[14] G. Hennrich, E. V. Anslyn, *Chem. Eur. J.* **2002**, *8*, 2218–2224.
[15] Z. Xie, C. W. Tsang, F. Xue, T. C. W. Mak, *Inorg. Chem.* **1997**, *36*, 2246–2247.
[16] J. D. Lee, M. W. R. Bryant, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1970**, *26*, 1729–1735.
[17] N. Ueyama, T.-a. Okamura, Y. Yamada, A. Nakamura, *J. Org. Chem.* **1995**, *60*, 4893–4899.
[18] The diameter can be estimated as that of a sphere in contact with all the midpoints of the three S–S bonds.
[19] The distance between two nearby S atoms (6.0 Å), not bound together, minus the diameter of an S atom (3.6 Å).
[20] a) A. Bondi, *J. Phys. Chem.* **1964**, *68*, 441–451; b) Y. H. Zhao, M. H. Abraham, A. M. Zissimos, *J. Org. Chem.* **2003**, *68*, 7368–7373.
[21] M. Egli, S. Sarkhel, *Acc. Chem. Res.* **2007**, *40*, 197–205.

Received: January 16, 2009
Published Online: February 17, 2009

