

Figure 1. Lanes 1–6 contain 20 μM streptavidin core (in monomer). Lane 2: 20 μM CuCl_2 . Lane 3: 20 μM CuCl_2 , 20 μM EDTA. Lane 4: 20 μM ZnCl_2 , 20 μM **1a**. Lane 5: 20 μM CuCl_2 , 20 μM **1a**. Lane 6: 1 mM (+)-biotin, followed by 20 μM CuCl_2 , 20 μM **1a**. Lane 7: horse heart myoglobin fragments. Cleavage experiments were carried out under aerobic conditions by complexation of FeCl_3 or CuCl_2 to **1a** followed by addition of the complex to streptavidin in 100 μL (final) 50 mM borate, pH 7.5. After 10 min, 2-mercaptoethanol was added (10% w/v), and the reactions were heated at 90 $^\circ\text{C}$ for 5 min and analyzed by gel electrophoresis.

imide ester with the appropriate triethyl ester EDTA derivative in CH_3CN ,⁸ followed by hydrolysis with LiOH and chromatography on DEAE–Sephadex with a 0.01–1.5 M NH_4HCO_3 gradient.^{9,10}

Cleavage experiments were performed by complexation of Cu^{2+} (or Fe^{3+}) with an equimolar amount of **1a** or **1b** followed by addition to 1 molar equiv of streptavidin core in the presence of oxygen (streptavidin was from Sigma and is a proteolyzed core streptavidin of approximately 14 kDa per monomer that is missing 13 N-terminal and 20 C-terminal amino acids¹¹). Mercaptoethanol was then added, the sample was heated to 90 $^\circ\text{C}$ for 5 min, and the reaction products were analyzed by gel electrophoresis¹² with silver staining.¹³ Examination of the cleavage gel (Figure 1) reveals that the biotin–EDTA– Cu^{2+} complex (**1a**– Cu^{2+}) selectively cleaves streptavidin core to produce a 7-kDa fragment (the **1a**– Fe^{3+} complex generates the same cleavage band). The streptavidin:7-kDa-fragment ratio was 3:1 by quantitation of the silver-stained bands.¹⁴ The lack of additional bands (corresponding to other cleavage fragments) may be due to comigration of two 7-kDa cleavage fragments or to secondary cleavage reactions. In contrast to cleavage by the **1a**– Cu^{2+} complex, incubation of streptavidin with Cu^{2+} –EDTA, Fe^{3+} –EDTA, or the preformed complex of **1a** with the non-redox-active metal Zn^{2+} does not afford any cleavage. In addition, cleavage of streptavidin by the **1a**– Cu^{2+} complex is completely inhibited by addition of 1 mM (+)-biotin. Cleavage of streptavidin by uncomplexed Cu^{2+} affords lower yields of two fragments that are different from those generated by **1a**– Cu^{2+} (probably due to chelation of Cu^{2+} by protein side chains or backbone⁴). Uncomplexed Fe^{3+} produces no cleavage. The complex of either Cu^{2+} or Fe^{3+} with **1b**, which has a seven-chain tether, does not cleave protein.

These results are consistent with the notion that the biotin–EDTA conjugate **1a** selectively delivers redox-active Cu^{2+} or Fe^{3+} in close proximity to the polypeptide backbone at the biotin binding site of streptavidin, resulting in selective protein cleavage at that site. Examination of the three-dimensional structure of streptavidin suggests that 7-kDa fragment(s) would result from cleavage within the sequence Trp 79–Ala 89 at the biotin binding site.⁷ Finally, pyruvic acid and alanylamine are generated by cleavage of the dipeptide Ala–Ala with EDTA– Fe^{3+} in the presence of O_2 and mercaptoethanol. These products are consistent with an oxidative cleavage mechanism involving initial oxidation of the α carbon to the α -hydroxylated product, with subsequent cleavage.^{4,15} Additional experiments into the nature and stoichiometry of the cleavage reaction are in progress.

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(15) Products were analyzed by isolation and spectral characterization of the phenylhydrazine (pyruvic acid) and dansyl (alanylamine, alanine methyl ester) derivatives.

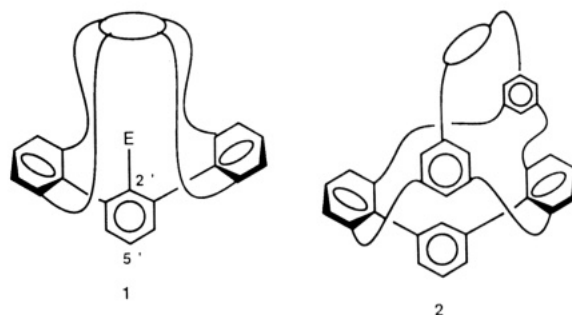
Synthesis of Two Noninterconvertible Conformers of a Single Host. Self-Filled and Vaulted Cappedophanes

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We recently described the synthesis of *m*-terphenyl-based cyclophanes **1**, for which we coined the name *cappedophanes*.¹ Our one-pot route to the *m*-terphenyl moiety of **1** permits the direct introduction of substituents E at C2'.² In our first studies, however, the tethers to the aromatic cap were too short (only two or three atoms each) to permit E to be larger than a proton.³



The aim of the present work was to construct a vaulted cappedophane of sufficient volume to accommodate a larger E, so that functional group chemistry inside and outside a cavity with a controlled microenvironment could be compared. To do this, it was essential to provide rigid cavity walls, because simply increasing the number of atoms in the tethers of **1** would make the cap collapsible and reduce the enclosed volume.⁴ Consequently, we set molecules such as **2**, with aromatic cavity walls and cap, as our target.

(1) Vinod, T. K.; Hart, H. J. *Am. Chem. Soc.* **1988**, *110*, 6574–6575. Vinod, T. K.; Hart, H. J. *Org. Chem.* **1990**, *55*, 881–890.

(2) Du, C.-J. F.; Hart, H.; Ng, K.-K. D. *J. Org. Chem.* **1986**, *51*, 3162–3165. Du, C.-J. F.; Hart, H. J. *Org. Chem.* **1987**, *52*, 4311–4314. Vinod, T. K.; Hart, H. *Tetrahedron Lett.* **1988**, *29*, 885–888.

(3) The short distance between these aryl protons and the cap was clear from their chemical shifts, which ranged from δ 3.3 to δ 4.2.

(4) For examples of cyclophanes with collapsible and noncollapsible cavities, see: Jarvi, E. T.; Whitlock, H. W. *J. Am. Chem. Soc.* **1982**, *104*, 7196–7204.

(8) (a) Chaturvedi, D. N.; Knittel, J. J.; Hruby, V. J.; Castrucci, A. M.; Hadley, M. E. *J. Med. Chem.* **1984**, *27*, 1486. (b) Schultz, P. G. Ph.D. Thesis, California Institute of Technology, 1984. (c) Takeshita, T.; Shimohara, T. A.; Maeda, S. *J. Am. Chem. Soc.* **1982**, *59*(2), 104.

(9) Fractions containing **1a** or **1b** were identified by staining with 5% *p*-(dimethylamino)cinnamaldehyde in 5% sulfuric acid/ethanol.

(10) Derivatization of the carboxyl group at biotin does not appreciably alter its association constant to streptavidin.⁸

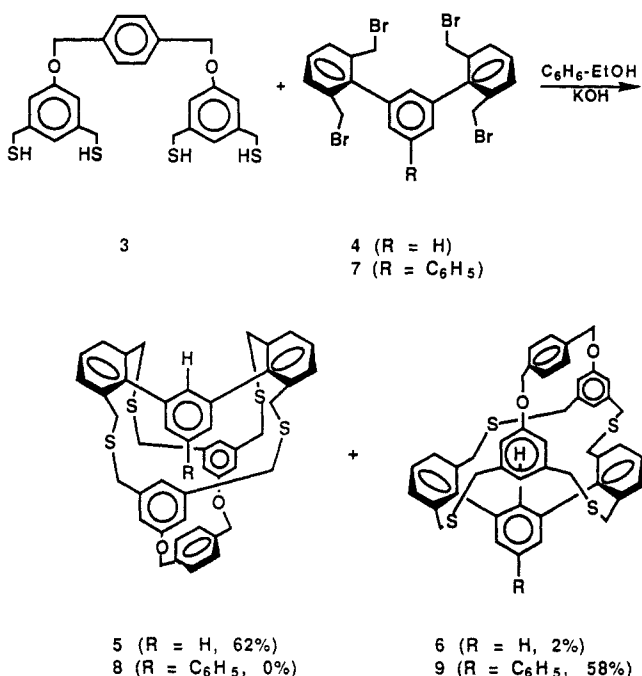
(11) Bayer, A.; Ben-Hur, H.; Hiller, Y.; Wilchek, M. *Biochem. J.* **1989**, *259*, 369.

(12) Loading buffer (4% SDS, 50 mM Tris, pH 6.8, 12% glycerol) was added to the cleavage products. The samples were denatured at 90 $^\circ\text{C}$ for 5 min, and polyacrylamide gel electrophoresis was carried out by the method of Schagger et al. with the stacking gel omitted: Schagger, H.; Von Jagow, G. *Anal. Biochem.* **1987**, *166*, 368.

(13) Heukeshoven, J.; Dernick, R. *Electrophoresis* **1985**, *6*, 103.

(14) Yuksel, K. U.; Gracy, R. W. *Ibid.* **1985**, *6*, 361.

Our first approach to **2** involved constructing a unit that included the walls and the cap and then attaching that unit in one step to the *m*-terphenyl base.⁵ Tetrathiol **3**^{6,7} reacted with tet-



rakis(bromomethyl)-*m*-terphenyl **4**¹ and base under high-dilution conditions⁸ to give two products. The desired vaulted cappedodophane **6** was formed in only 2% yield (for a better route to **6**, vide infra). To our surprise, the major product (62%) was **5**, formed as a consequence of tetrathiol **3** displacing the four bromines in **4** from below, thus encapsulating the central *m*-terphenyl ring in a cavity.

The distinction between **5** and **6** was apparent from their ¹H NMR spectra. The proton at C5' of the *m*-terphenyl unit in **5** appeared as a triplet at δ 4.31 (J = 7.7 Hz), shielded by the *p*-xylylene ring; the corresponding proton in **6** appeared at δ 7.35 (J = 7.5 Hz). This upfield shift of 3.04 ppm corresponds⁹ to a calculated distance of 2.36 Å between this proton and the *p*-xylylene ring in **5**. Consistent with this conclusion, irradiation of the δ 4.31 triplet¹⁰ resulted in a 10.4% enhancement of the signal at δ 7.62 due to the *p*-xylylene aryl protons.

The most diagnostic signal in the ¹H NMR spectrum of **6** was a narrow triplet at δ 5.70 (J = 1.7 Hz) due to the isolated proton at C2' of the *m*-terphenyl unit (weakly coupled with the C4' and C6' protons). The same proton in **5** appears at δ 6.24. Moderate shielding of this proton in **6** is due mainly to one of the aryl rings that support the cap. Calculated lowest energy conformers of **5** and **6** are shown in Figure 1.¹³

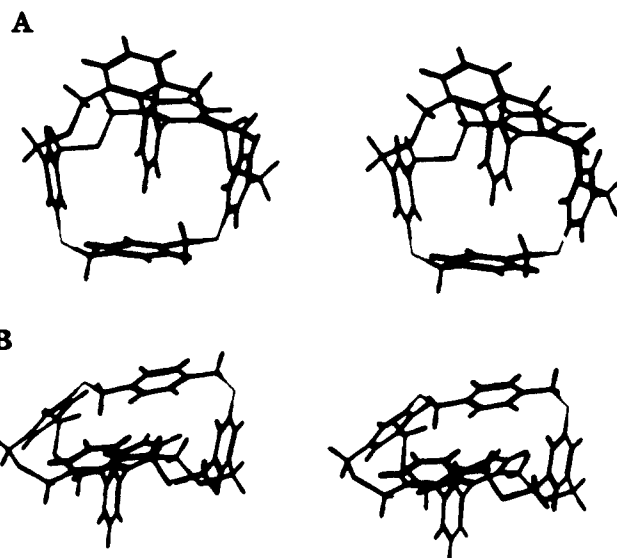
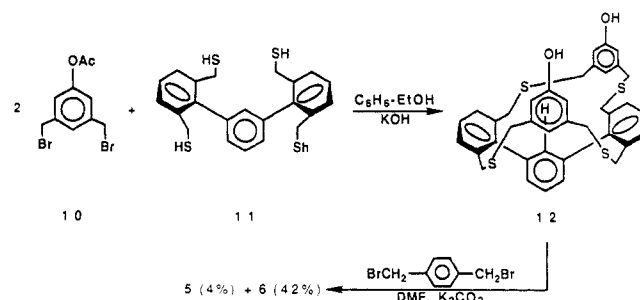


Figure 1. Stereoview of energy-minimized¹³ conformations of (A) **5** and (B) **6**.

Macrocycle **5** can be viewed as a host that acts as its own guest.^{11,12} A probable cause for such cavity-filling behavior is the favorable van der Waals interaction between the encapsulated aryl ring and the aryl rings that line the cavity,¹³ and the predominance of **5** over **6** is a reflection of this interaction in the transition state.

Formation of self-filled (autophagous¹²) cyclophanes such as **5** can be made sterically prohibitive by incorporating a bulky substituent at C5' of the *m*-terphenyl unit. Thus tetrathiol **3** and tetrabromide **7** gave (58%) vaulted cyclophane **9** as the only isolable product. The internal C2' proton in **9** appeared at δ 5.67 (J = 1.7 Hz), almost identical with the same proton in **6**.

A second approach to vaulted cyclophanes such as **6** provided a general solution to avoidance of the autophagous conformer. A functionalized cappedodophane¹ that contains the wall of the cavity was prepared first, and the cap was added in a second step. For example, coupling of dibromide **10**¹⁵ with tetrathiol **11**¹ with base under high dilution gave cappedodophane **12**.¹⁶ The capped



structure of **12** was evident from the chemical shift of the isolated proton on the central *m*-terphenyl ring (δ 6.36, br s), moderately shielded by the cofacial hydroxyl-bearing rings.¹ Coupling of **12** with *p*-xylylene dibromide gave predominantly **6** (42%), although some **5** was also formed.¹⁷

In summary, independent syntheses of **5** and **6** are described, each in moderate to good yield. We believe this to be the first example of a synthesis of two noninterconvertible conformers of a single host, one (**6**) with a substantial cavity, the other (**5**) with a self-filled cavity. We are currently extending these studies to

(5) Similar methodology has been used to construct capped porphyrins. Collman, J. P.; Brauman, J. I.; Fitzgerald, J. P.; Hampton, P. D.; Naruta, Y.; Sparapany, J. W.; Ibers, J. A. *J. Am. Chem. Soc.* **1988**, *110*, 3477-3486.

(6) All new compounds gave satisfactory analytical and spectroscopic data.

(7) Prepared from the known⁵ tetraester 1,4-bis[(3,5-dicarboethoxyphenyloxy)methyl]benzene.

(8) Vögtle, F. *Chem. Ind. (London)* **1973**, 1037-1038.

(9) Elmsley, J. W.; Feeny, J.; Sutcliffe, L. H. *High Resolution Nuclear Magnetic Resonance Spectroscopy*; Pergamon Press: Oxford, 1965; Vol. 1, pp 595-604.

(10) 1D NOE difference spectrum at 300 MHz and 298 K.

(11) For a discussion of this phenomenon, leading references, and other examples, see: (a) Rubin, Y.; Dick, K.; Diederich, F.; Georgiadis, T. M. *J. Org. Chem.* **1986**, *51*, 3270-3278. (b) Loncharich, R. J.; Seward, E.; Ferguson, S. B.; Brown, F. K.; Diederich, F.; Houk, K. N. *J. Org. Chem.* **1988**, *53*, 3479-3491.

(12) We propose for such molecules the descriptive and short term *autophagous* (Greek) meaning *self-devouring* (*Webster's Third New International Dictionary*).

(13) Preliminary molecular mechanics calculations using BIOGRAF show that the van der Waals energy contributions toward the total energies of **5** and **6** are 71.35 and 75.21 kcal/mol, respectively. The magnitude of the difference (4 kcal/mol vdW stabilization) is similar to other calculated values.^{11b,14}

(14) Miller, S. P.; Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1492-1494.

(15) Prepared from 3,5-dimethylphenyl acetate by NBS bromination.

(16) The acetoxy groups were saponified during the coupling reaction.

(17) Coupling of **12** across the "top" was expected, because an X-ray structure of an analogous cappedodophane¹ showed that the bridging aryl rings are face-to-face as drawn. The small yield of **5** is thought to originate from a small amount of a conformer of **12** in which one phenolic ring is "up" and one is "down" (detected by NMR).

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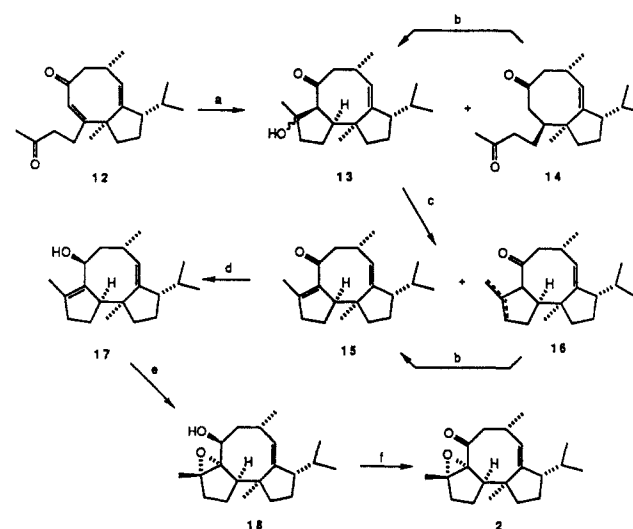
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The ready availability of aldehyde **3** from (*R*)-(+)-limonene⁴ prompted its utilization as the cornerstone of our approach. Reduction with sodium borohydride, followed by osmylation and regioselective acetone formation, led to **4** (80%, Scheme 1).⁵ Chain extension was next accomplished by sequential PDC oxidation, Wittig olefination, and hydroboration. The overall yield of **5** based on **4** after purification by silica gel chromatography was 62%. Once conversion to the carboxylic acid had been achieved, hydrolysis with 10% hydrochloric acid in THF delivered a 15:1 mixture of **6** and its epimer (80%). Consequently, osmylation of the alcohol derived from **3** proceeds with a pronounced preference for attack from that face syn to the 2-hydroxyethyl substituent.

Enantiomerically homogeneous **6** was next transformed into **7** with high efficiency (86%). The use of benzeneseleninic an-

Scheme II^a



^a (a) H₂, PtO₂, C₂H₅OH; (b) K₂CO₃, CH₃OH; (c) SOCl₂, py, CH₂-Cl₂; (d) LiAlH₄, THF, 0 °C; (e) MCPBA, CH₂Cl₂, 0 °C; (f) (COCl)₂, DMSO, Et₃N, CH₂Cl₂, -70 °C.

hydride for this purpose⁶ necessitated that the hydroxyl group be transiently protected by silylation. With arrival of **7**, we had nearly completed the *indirect* construction of ring B. Homologative generation of two additional sights of unsaturation, one with high stereocontrol, was now required to reach this major plateau of the synthesis. In fact, PDC oxidation and condensation with ethylenetriphenylphosphorane⁷ (THF, 0 °C) led uniquely to **8** (77%), the stereochemistry of which was confirmed by X-ray analysis.⁸

(5) All formulas are drawn in their proper absolute configuration. In addition, all structural assignments are in accord with individual 300-MHz ^1H NMR, 75-MHz ^{13}C NMR, and high-resolution mass spectra. Key intermediates have also given acceptable combustion analysis data. All recorded yields are based upon isolated material of >97% purity.

(8) The crystals of compound **8** belong to the space group $P2_12_12_1$ (No. 19) with $a = 8.457(1)$ Å, $b = 12.753(2)$ Å, and $c = 12.951(2)$ Å with four molecules per unit cell, $D_{\text{calcd}} = 1.114$ g cm $^{-3}$; data collected = $h, k, \neq l$, unique data = 1180, unique data with $F_o^2 > \sigma(F_o^2) = 905$, final number of variables = 155, $R(F) = 0.094$, $R_w(F) = 0.078$, $R = 0.055$, and $R_e = 0.064$.