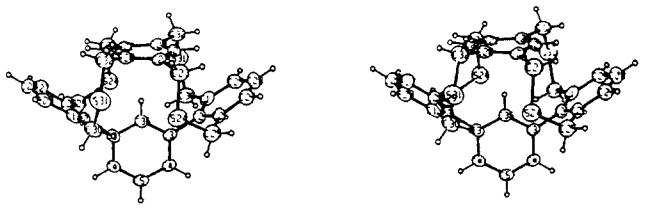
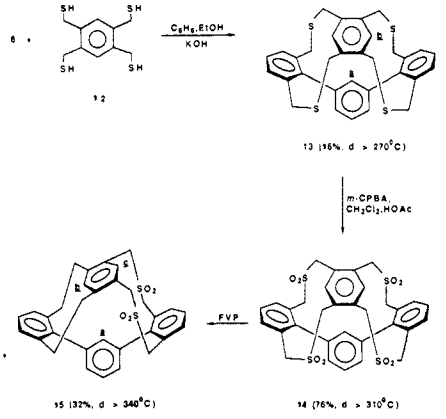


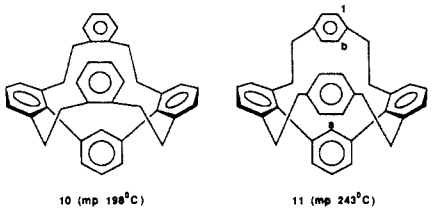
Figure 1. Stereoview of 13.

Scheme III



tetrasulfone 8 which, on flash vacuum pyrolysis,[7] gave hydrocarbon 9[8] (Scheme II).

The structures of 9 and intermediates en route to it were clear from their ^{1}H NMR spectra.[9] The *o*- and *p*-analogues of 9 (i.e., 10 and 11) were similarly prepared from *o*- and *p*-xylylenedithiols in comparable yields.[10]



A synthesis of four cappedophanes is outlined in Scheme III. Reaction of tetrabromide 6 with tetrathiol 12[11] afforded 13, plus an isomer not shown, in which the cap is turned 90° (1.9%). Oxidation of 13 with *m*-CPBA gave the tetrasulfone 14 which was pyrolyzed to a mixture of disulfone 15 and hydrocarbon 16.[12]

Space-filling molecular models of these cappedophanes suggest that the structures are compact and somewhat strained. Indeed, one can only construct a CPK model of 13 if aromatic proton *a* is omitted from the model, and it is not possible to construct such models of 15 or 16. The aromatic proton *a* in 13, 15, and 16 appears at δ 3.97, 3.31, and 3.77, respectively, highly shielded by the π cloud of the benzenoid cap. The canopy aromatic protons *b* are shielded by the outer rings of the *m*-terphenyl unit. They appear at δ 4.75 in 13, at δ 3.67 in 16, and as two singlets at δ 3.45 (b) and 5.16 (c) in the tilted canopy of 15.[13]

An X-ray structure of 13 shows it to have only a C_2 axis, perpendicular to and through the center of the capping ring (Figure 1).[14]

We are actively extending our studies of cuppedophanes and cappedophanes in the many directions that suggest themselves, such as to polar and hydrogen-bonding linking chains, to nonproton E's, to other capping groups, and to frameworks other than *m*-terphenyl.[16]

Acknowledgment. We are indebted to the National Institutes of Health (GM 15997) for financial support of this research. We are indebted to Dr. Donald L. Ward for the X-ray structure and to Dr. Kurt Loening for assistance with nomenclature.

Supplementary Material Available: Physical data for all new compounds and crystallographic data for 13 (14 pages). Ordering information is given on any current masthead page.

(7) Vögtle, F.; Rossa, L. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 515–529.

(8) CAS name for 7: 13*H*, 15*H*-1,19-(methanothiomethano[1,3]-benzenomethanothiomethano)-8,12:20,24-dimetheno-5*H*,7*H*-dibenzo[*k*,*r*]-[1,9]dithiacycloeicosin; for 9: 5,6,12,13-tetrahydro-1,17-(ethano[1,3]-benzenoethano)-7,11:18,22-dimethenodibenzo[*a*,*h*]cyclooctadecene.

(9) For example, the proton at the center of the bowl in 9 on C2′ of the *m*-terphenyl unit appeared at δ 5.92 (absent in the deuterio analogue prepared from 6D), shielded by the aryl rings; detailed descriptions and X-ray structures will be given in a full account.

(10) Models show 9 and 11 to be quite rigid, whereas 10 is fairly flexible; in accord, the NMR spectrum of 10 shows no exceptional high field aryl protons (δ 7.05–7.60, m, 18 H), whereas in 11 the proton at the bottom of the bowl (δ 6.59) as well as the "top" and "bottom" protons of the 1,4-linked benzene rings (δ 6.43, 6.27) were shielded, and the methylene protons were separated into four distinct four-proton sets (δ 2.53–2.61, 2.73–2.84, 3.05–3.14, and 3.26–3.38).

(11) Klieser, B.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 618–619.

(12) CAS name for 13: 7,12-Dihydro-14*H*-1,9:10,18-bis(methanothiomethano)-19,23-metheno-5*H*-tribenzo[*c*,*h*,*o*][1,6]dithiacycloheptadecin; for 16: 5,6,20,21-Tetrahydro-2,16:3,10-diethano-15,11-metheno-11*H*-tribenzo-[*a*,*e*,*i*]cyclopentadecene.

(13) Although the regioisomer of 13 with the canopy turned 90° with respect to the *m*-terphenyl unit also had a high field proton corresponding to proton a (δ 3.99), the canopy aromatic protons were deshielded (δ 8.30), thus allowing the structures of the two isomers to be readily distinguished.

(14) X-ray structures of selected cupped- and cappedophanes will be reported in due course in a full account. We note here, however, that the capping benzene ring in 13 deviates somewhat from planarity in a twist-boat conformation, that the sulfur-containing bridges occur in two different sets with substantially different dihedral angles, and that proton a is only 2.16 Å from the mean carbon plane of the capping ring.[15]

(15) For a recent study in which an sp^3-methine hydrogen is similarly poised with respect to a benzene ring, see: Pascal, R. A., Jr.; Grossman, R. B.; Van Engen, D. *J. Am. Chem. Soc.* **1987**, *109*, 6878–6880.

(16) Hart, H.; Harada, K.; Du, C.-J. F. *J. Org. Chem.* **1985**, *50*, 3104–3110. Harada, K.; Hart, H.; Du, C.-J. F. *J. Org. Chem.* **1985**, *50*, 5524–5528.

Molecular Recognition: Multipoint Contacts with New Sizes and Shapes

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We recently introduced new molecular shapes useful as probes for molecular recognition.[1] The structures are easily assembled from the condensation of Kemp's[2] triacid with suitable spacer groups. Their uniqueness derives from the unusual triaxial relationship that exists between the carboxyl functions; it provides an opportunity to engineer a U-turn into structures derived from it. As a consequence, the functional groups derived from the carboxylic acids tend to *converge* toward the center of the molecule. Here we describe the use of this architectural cliche with new spacer elements and multiple branch points.

Condensation of the acid chloride anhydride[2] 1a with the 3-aminobenzyl alcohols 2 followed by oxidation[3] gave the aromatic aldehydes 3 (Scheme I). These were reacted at room temperatures with pyrrole under acid catalysis (CF_3CO_2H) to give the porphyrinogen, which was then oxidized with DDQ.[4] The re-

(1) Rebek, J. Jr. *Science (Washington, D.C.)* **1987**, *235*, 1478–1484.

(2) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 5140–5143. For an improved, large scale preparation, see: Rebek, J., Jr.; Askew, B.; Killoran, M.; Nemeth, D.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, 2426–2431.

(3) Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.

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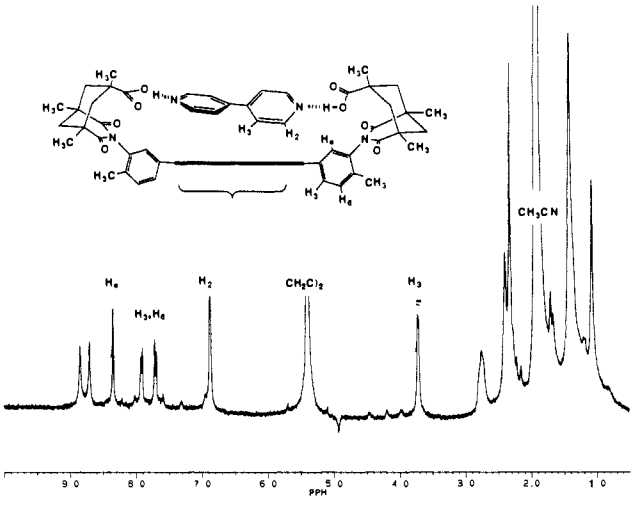
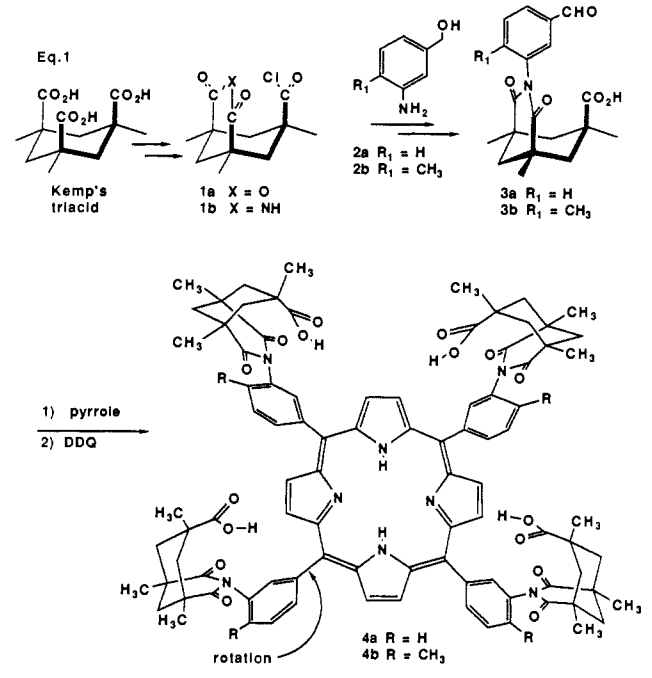


Figure 1. Proton NMR spectrum (300 MHz) of the 2:1 complex formed between 4,4′-bipyridine and **4b** in CD_2Cl_2/CD_3CN at 230 K. Only one bipyridyl is shown in the structure; the two most downfield signals represent the β-pyrrole protons of the porphyrin tautomers.

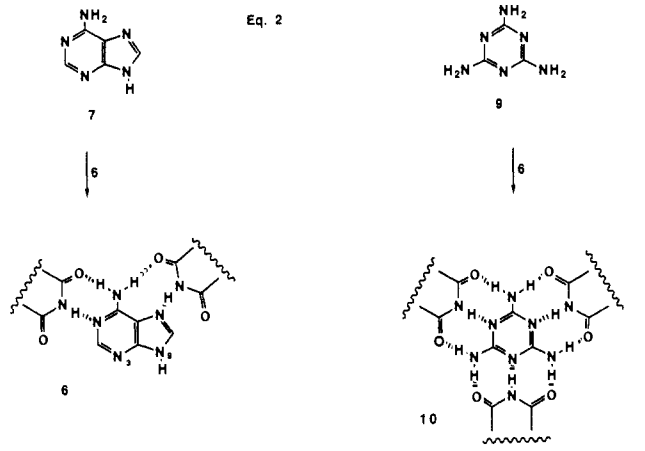
Scheme I



sulting porphyrins **4** were obtained in 20% yield and were characterized by high resolution NMR and ^{252}Cf fission mass spectrometry.[5] Such structures feature four carboxylic acid functions. The rotations about the N_{imide}–C_{aryl} bonds permit other conformations in **4a**, but the methyls of **4b** prevent those rotations and limit the conformations to those in which the OH bonds of the acids converge as shown.[6] In **4b** isomerism is a result of the rapid rotation of the aryl function.[7]

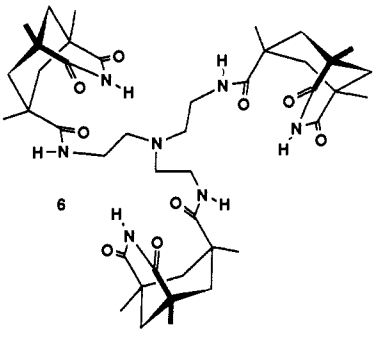
Molecular modeling indicated that a distance of ~15 Å separates opposing carboxyl oxygens in the conformation shown.

Scheme II



Accordingly, complex formation was observed with substrates able to bridge this gap with complementary function and stereoelectronics, e.g., 4,4′-bipyridine. Titrations in CD_3CN/CD_2Cl_2 (3:1, v/v) showed gradual sharpening of the signals in the NMR spectral of the porphyrins (3×10^{-3} M) as up to 2 equiv of 4,4′-bipyridine were added. At low temperatures, exchange was sufficiently slow so that a well-defined spectrum was obtained for a 2:1 complex (Figure 1). The large upfield shifts of the bipyridine hydrogens (ca. 4 ppm) establish its position over the top of the extended aromatic system.[8] Neither DABCO nor pyrimidine gave evidence of specific complexation with either **4a** or **4b**.

Conversion of Kemp's triacid to the corresponding imide acid chloride[9] **1b**, followed by its condensation with tren gave a trisimide **6**. The flexible skeleton of **6** permits its action as a promiscuous



molecular tool chuck. For example, **6** (10^{-3} M) in $CHCl_3$) extracts 1 equiv of adenine **7** from aqueous solution. Chemical shifts observed in the complex are in accord with simultaneous Watson–Crick and Hoogsteen base pairing[10] as in **8** (Scheme II). Additional contacts involving N_3 and the N_9–H of adenine may also contribute to complexation. A 1:1 complex is also formed with melamine **9** under these conditions. With either heterocycle, the organized hydrogen bonding surfaces of **6** compete effectively with water and result in complete desolvation when the complex is formed. Up to nine hydrogen bonds might be involved with melamine as suggested schematically in **10**. The actual association constant for **10** is too large to determine readily by NMR methods.[11] However, exchange rates between **6** and **10** were slow on the NMR time scale at room temperature; an activation barrier ($\Delta G_c^{\ddagger}$) of 14.2 kcal/mol was determined at the coalescence tem-

(4) Lindsey, J. S.; Schreiman, I. C.; Hsu, H. C.; Kearney, P. C.; Marguerettaz, A. M. *J. Org. Chem.* **1987**, *52*, 827–836.

(5) Chait, B. T.; Agosta, W. C.; Field, F. H. *Int. J. Mass Spectrom. Ion Phys.* **1981**, *39*, 339–366. Calcd for **4a** $C_{92}H_{90}O_{16}N_8$ ($M + H^+$) = 1564.7, found 1564.4. UV-vis absorption spectra were characteristic of a meso tetrasubstituted porphyrin: $\lambda_{abs}(CHCl_3)$ 426, 514, 548, 594, 648 nm; λ_{em} 644, 612 nm. Compound **6** was obtained in 55% yield, mp 140 °C, with appropriate high resolution spectroscopic features.

(6) Rebek, J., Jr.; Marshall, L.; Wolak, R.; Parris, K.; Killoran, M.; Askew, B.; Nemeth, D.; Islam, N. *J. Am. Chem. Soc.* **1985**, *107*, 7476–7481.

(7) For a recent discussion with leading references, see: Crossley, M. J.; Field, L. D.; Forster, A. J.; Harding, M. M.; Sternhell, S. *J. Am. Chem. Soc.* **1987**, *109*, 341–348.

(8) For related complexation of aliphatic diamines with porphyrin-crown ether derivatives see: Hamilton, A. D.; Lehn, J. M.; Sessler, J. L. *J. Am. Chem. Soc.* **1986**, *108*, 5158–5167. For complexation of dicarboxylic acids by related porphyrin structures, see: Staubli, B.; Fretz, H.; Piantini, U.; Woggon, W.-D. *Helv. Chim. Acta.* **1987**, *70*, 1173–1193.

(9) Rebek, J., Jr.; Askew, B.; Buhr, C.; Jones, S.; Nemeth, D.; Williams, K.; Ballester, P. *J. Am. Chem. Soc.* **1987**, *109*, 5033.

(10) For NOE techniques as a probe of base pairing, see: Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Costero, A.; Jones, S.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 6866–6867.

(11) Dilution of the complex in $CDCl_3$ to 6×10^{-5} M caused no changes in chemical shifts of the imide N–H signals; a lower limit for K_a of 5×10^5 M^{-1} can be set from these experiments.

perature (307 K) for the amide NH signals.

In summary, the large cleft-like shapes and variety of chemical linings presented by the new structures permit their use for the selective chelation of sizable, functionally complex molecules. Their facile assembly augurs well for the development of a new generation of medicinal agents wherein *concave synthetic surfaces are tailored to enfold smaller, convex targets.*[12] This would represent a reversal of roles for current strategies in which the smaller synthetic agent is directed at the folds of a biological macromolecule.

Acknowledgment. We thank the National Institutes of Health for financial support and Dr. J. Huff for advice. The fission fragment mass spectrometric analyses were performed by Brian Chait of The Rockefeller University Mass Spectrometric Research Resource supported by the Division of Research Resources, NIH.

(12) For amino acid targets, see: Rebek, J., Jr.; Nemeth, D. *J. Am. Chem. Soc.* **1985**, *107*, 6738–6739. For heterocyclic targets, see: Rebek, J., Jr.; Askew, B.; Islam, N.; Killoran, M.; Nemeth, D.; Wolak, R. *J. Am. Chem. Soc.* **1985**, *107*, 6736–6738. Chang, S.-K.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 1318–1319. Kelly, J. R.; Maguire, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 6549–6551. Kilburn, J. D.; MacKenzie, A. R.; Still, W. C. *J. Am. Chem. Soc.* **1988**, *110*, 1307–1308. Hamilton, A. D.; Van Engen, D. *J. Am. Chem. Soc.* **1988**, *110*, 5035–5036. For carboxylic acid targets, see: Rebek, J., Jr.; Nemeth, D.; Ballester, P.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, *109*, 3474–3475. Pant, N.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 2002–2003. For urea, see: van Staveren, C. J.; Fenton, D. E.; Reinhoudt, D. N.; van Eerden, J.; Harkema, S. *J. Am. Chem. Soc.* **1987**, 3456–3458. Bell, T. W.; Liu, J. *J. Am. Chem. Soc.* **1988**, *110*, 3673–3674.

Isolation and Structural Elucidation of the Tetrahedral Intermediate in the EPSP Synthase Enzymatic Pathway

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EPSP (5-enolpyruvoylshikimate-3-phosphate) synthase is an enzyme in the shikimic acid pathway that catalyzes the unusual transfer of an enolpyruvoyl moiety from PEP to S3P with the elimination of inorganic phosphate. The enzyme is the target of the commercially important herbicide glyphosate, *N*-(phosphonomethyl)glycine.[1,2] In this report, we describe the isolation and structure determination of the tetrahedral intermediate formed at the active site of the enzyme from the nucleophilic attack of the 5-OH of the S3P on the C-2 position of PEP as shown in Scheme I.

Our previous work has demonstrated the presence of an acid-labile intermediate in the reaction pathway.[3,4] Rapid quench kinetics afforded the complete kinetic description of the reaction showing that a single intermediate was formed within 5 ms on the enzyme and decayed to form products (EPSP and phosphate) over the next 50 ms. Although the data were strongly suggestive of a tetrahedral intermediate, the structure was not definitively established because it decomposed under acidic quench conditions to form pyruvate and S3P.

Scheme I

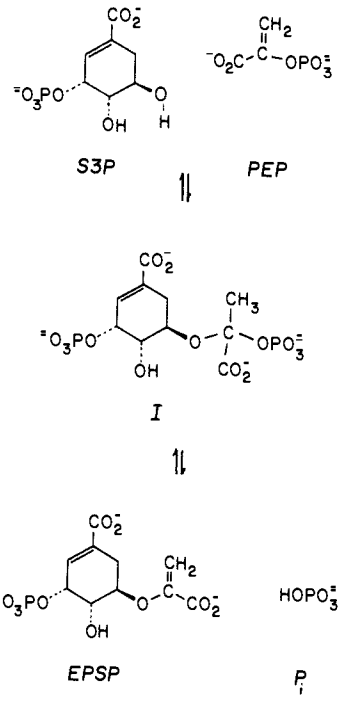

Table I. Synthesis of the Intermediate[a]

radiolabel	[S3P]	[PEP]	[P_i]	rx time	% intermediate	% yield
[^{14}C]S3P	4 μM	2 mM	10 mM	5 s	33	90
[^{14}C]PEP	100 μM	3.5 μM		10 ms	15	60
[^{32}P]PEP	100 μM	3.5 μM		10 ms	12	50

[a] The indicated concentrations of substrates were incubated with 10 μM enzyme for the specified time,[5] and then the reaction was stopped by mixing with neat triethylamine. The percentage conversion of radiolabel into intermediate was quantitated by HPLC using a Synchropak AX-100 column with a continuous flow radioactivity detector. Details of the methods for rapid mixing were described previously.[4] Rationale for design of the experiments to form the intermediate under single turnover (10 ms) or equilibrium (5 s) conditions and calculations of the expected yields were based upon previous rapid quench kinetic and equilibrium measurements.[4]

We have now discovered that if the enzyme is denatured rapidly by quenching under mildly basic conditions with neat triethylamine, the intermediate is stable and can be isolated by ion exchange HPLC.

The first test of the identity of the intermediate was conducted with [^{14}C]S3P, [^{14}C]PEP, or [^{32}P]PEP to synthesize the intermediate on the enzyme under conditions described previously.[4,5] In each case, the efficiency of the incorporation of radiolabel into the intermediate was 50–90% of the theoretical yield (Table I). These results demonstrated that the intermediate contained the shikimate ring donated by S3P and both the enolpyruvoyl and the phosphate moieties donated by PEP.

The enzyme catalyzed the decomposition of the isolated intermediate, thus reinforcing our identification of the adduct as a reaction intermediate.

The structure of the intermediate was confirmed by ^{1}H NMR, ^{31}P NMR, and ^{13}C NMR. The required quantity (300 μg) of the intermediate was synthesized enzymatically by mixing enzyme with equimolar [^{14}C]S3P and high concentrations of [^{13}C]-2-PEP

† A unit of Monsanto Company.

(1) Steinrucken, H. C.; Amrhein, N. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207–1212.

(2) Franz, J. E. In *The Herbicide Glyphosate*; Grossbard, E., Atkinson, D., Eds.; Butterworth and Co.: Boston, MA, 1985; pp 1–17.

(3) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 1604–1610.

(4) Anderson, K. S.; Sikorski, J. A.; Johnson, K. A. *Biochemistry* **1988**, *27*, 7395–7406.

(5) All enzyme reactions were performed at 20 °C in a buffer containing 50 mM HEPES and 50 mM KCl at pH 7.0.

0002-7863/88/1510-6577$01.50/0