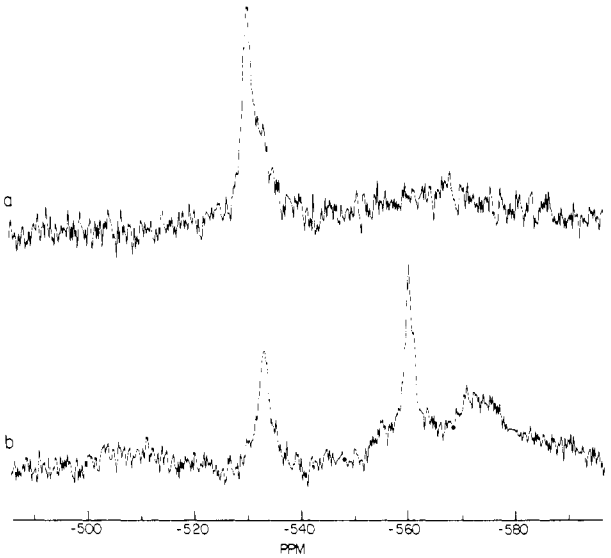

Figure 2. ^{51}V NMR evidence of selective binding of vanadium and iron: (a) Equimolar mixture of V(V) and apo-transferrin at 0.54 mM in 0.1 M HEPES buffer, pH 5.8; 87 285 scans. (b) Equimolar mixture of V(V) and Fe_C-transferrin at 0.5 mM in 0.1 M HEPES buffer, pH 5.8; 228, 590 scans. Fe_C-transferrin was prepared and identified (urea-polyacrylamide gel electrophoresis) according to published procedures.[24]

Thus we interpret the experimentally observed intensity loss as being due to extremely rapid relaxation of the other components which broadens their signal contribution beyond detectability. The remaining intensity of 15% is close to the theoretically predicted value (19%).

While the observation of two peaks with approximately equal intensity for the bound vanadate agrees well with the qualitative result obtained by UV-vis spectroscopy,[23a] the ^{51}V NMR technique is capable of distinguishing these sites according to their different chemical shifts, hence demonstrating their chemical inequivalence. Titrations carried out at pH 7.5 in 0.1 M HEPES buffer for vanadate/protein ratios between 0.1 and 2.3 confirm the large complex formation constants published previously[23] and indicate indiscriminate binding to the C- and N-terminal sites. However, Figure 2a shows that if vanadate solution is added to a HEPES buffer solution at pH 5.8 of transferrin loaded with ferric iron in the C-terminal site, no site scrambling occurs and only a single peak at −532 ppm is observed (in addition to absorptions due to free vanadate). Likewise, we have selectively observed the −529.5 ppm peak when adding vanadate solution to a HEPES buffer solution at pH 7.5 of transferrin loaded with ferric iron in the N-terminal site.[25] Thus we assign the peaks at −529.5 and −531.5 ppm to V(V) bound to the C- and N-terminal sites, respectively. In conclusion we have shown that ^{51}V NMR is a highly sensitive tool to monitor binding of vanadate to apo-human transferrin and could be a widely applicable technique to characterize V(V) binding to metalloproteins in general. Such studies as well as investigations of small molecular complexes that model protein binding sites are currently in progress.

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Registry No. Vanadium, 7440-62-2; vanadate, 14333-18-7.

(23) (a) Harris, W. R.; Carrano, C. J. *J. Inorg. Biochem.* **1984**, *22*, 201. (b) Chasteen, N. D.; Grady, J. K.; Holloway, C. E. *Inorg. Chem.* **1986**, *25*, 2754.

(24) Folajtar, D. A.; Chasteen, N. D. *J. Am. Chem. Soc.* **1982**, *104*, 5775.

(25) Fe_N-transferrin was prepared and characterized by the method outlined in ref 24.

Synthesis of Chiral Molecular Clefts. New Armatures for Biomimetic Systems

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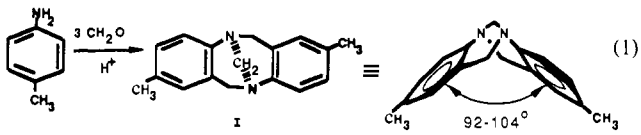
Natural receptors are often described as clefts, grooves, or depressions on protein, membrane, or polynucleotide surfaces. The essential characteristic of a small molecule receptor is *concavity*.[1] The great majority of synthetic receptors have used macrocyclic rings to enforce the formation of concave surfaces.[2,3] We report here the first syntheses of nonmacrocyclic chiral molecules which contain a deep cleft or groove large enough to bind small organic molecules. The rigid chiral grooves are maintained by conformational constraints intrinsic to the architecture of these synthetic receptors. The concave surfaces in these new molecules are produced without recourse to macrocyclic structural elements.

Analogues of Tröger's base [2,8-dimethyl-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]diazocine, I] are useful chiral structural elements for biomimetic systems.[4] Such molecules are readily prepared from aniline derivatives and formaldehyde (eq 1) and



(1)

have a folded structure wherein the three methylene carbons and two nitrogen atoms form a hingelike bridge between the two aryl units. Tröger's base analogues have been incorporated into a macrocyclic ring to provide chiral, water-soluble cyclophanes which act as receptors for benzenoid substrates.[5b] All analogues of Tröger's base examined have been found to be sharply folded. The angle formed by the least-squares planes containing the two aryl rings varies depending on the ring substituents and ranges from 92° to 104°.[5] We reasoned that two cup-shaped molecules joined in this manner would form a substantial concave surface and define an interesting new binding site.

To test this idea the well-known adduct **1**, available in quantitative yield from anthracene and dimethyl acetylenedicarboxylate, was treated with nitric acid in CH_3NO_2 to afford the mononitrate **2** in 76% yield.[6,7] This nitro compound was quantitatively reduced

(1) (a) Moran, J. R.; Karbach, S.; Cram, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 5826-5828. (b) Kyba, E. P.; Helgeson, R. C.; Madan, K.; Gokel, G.; Tarnowski, T. L.; Moore, S. S.; Cram, D. J. *Ibid.* **1977**, *99*, 2564-2571.

(2) (a) Odashima, K.; Koga, K. In *Cyclophanes*; Kheehn, P. M., Rosenfeld, S. M., Eds.; Academic: New York, 1983; Vol. 2. b) Murakami, Y.; *Top. Curr. Chem.* **1983**, *115*, 107-155. (c) Tabushi, I.; Yamamura, K. *Top. Curr. Chem.* **1983**, *113*, 145-182. (d) Tabushi, I.; Sasaki, H.; Kuroda, Y. *J. Am. Chem. Soc.* **1976**, *98*, 5727-5728. (e) Whitlock, H. W.; Jarvi, E. T. *Ibid.* **1982**, *104*, 7196-7204. (f) Diederich, F.; Dick, K. *Ibid.* **1984**, *106*, 8024-8036. (g) Cram, D. J.; Katz, H. E. *Ibid.* **1983**, *105*, 135-137. (h) Cram, D. J.; Trueblood, K. N. *Top. Curr. Chem.* **1981**, *98*, 43-106.

(3) Interesting examples of nonmacrocyclic hosts are appearing at an accelerating rate: (a) Rebek, J., Jr.; Askew, B.; Islam, N.; Killoran, M.; Nemeth, D.; Wolak, R. *J. Am. Chem. Soc.* **1985**, *107*, 6736-6738. (b) Rebek, J., Jr.; Nemeth, D. *Ibid.* **1985**, *107*, 6738. (c) Hart, H.; Lin, L.-T. W.; Ward, D. L. *Ibid.* **1984**, *106*, 4043-4045. (d) Chen, C. H.; Whitlock, H. W. *Ibid.* **1978**, *100*, 4921. (e) McKenna, J.; McKenna, J. M.; Thornwaite, D. W. *J. Chem. Soc., Chem. Commun.* **1977**, 809.

(4) (a) Wilcox, C. S. *Tetrahedron Lett.* **1985**, 5749-5752. (b) Wilcox, C. S.; Cowart, M. D. *Ibid.* **1986**, 5563-5566.

(5) (a) Larson, S. B.; Wilcox, C. S. *Acta Crystallogr., Sect. C* **1986**, *C42*, 224-227. (b) Larson, S. B.; Wilcox, C. S. *Acta Crystallogr., Sect. C* **1986**, *C42*, 253-254. (c) Sucholeiki, I.; Wilcox, C. S., manuscript in preparation.

(6) (a) Diels, O.; Alder, K. *Ber.* **1931**, *64*, 191-202. (b) Tanida, H.; Ishitobi, H. *Tetrahedron Lett.* **1964**, 807-811. (c) Ethanoanthracene derivatives were recently reported to be useful components for preparing macrocyclic hosts: Petti, M. A.; Shepodd, T. J.; Dougherty, D. A. *Ibid.* **1986**, 807-810.

(7) New compounds had elemental compositions and spectral properties in accord with the indicated structures.

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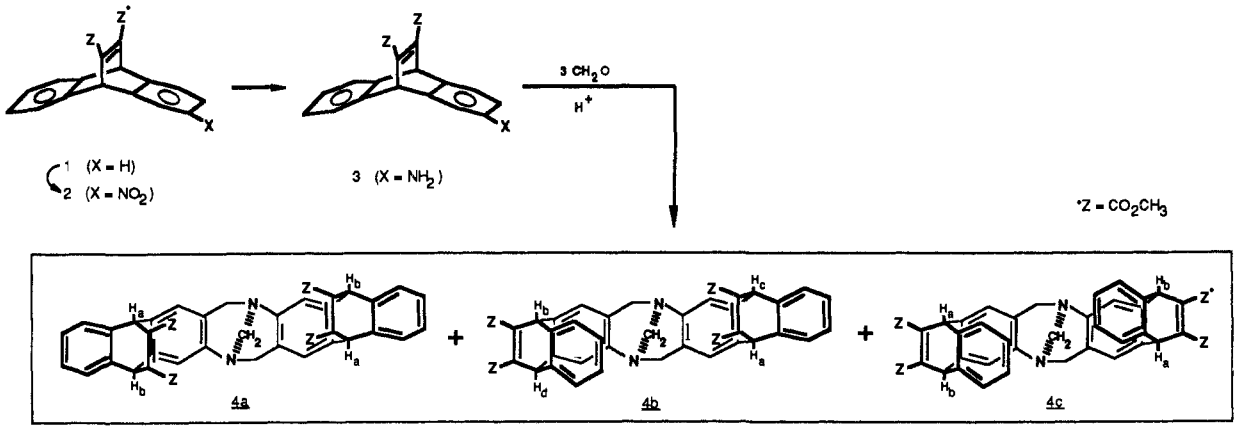
Scheme I



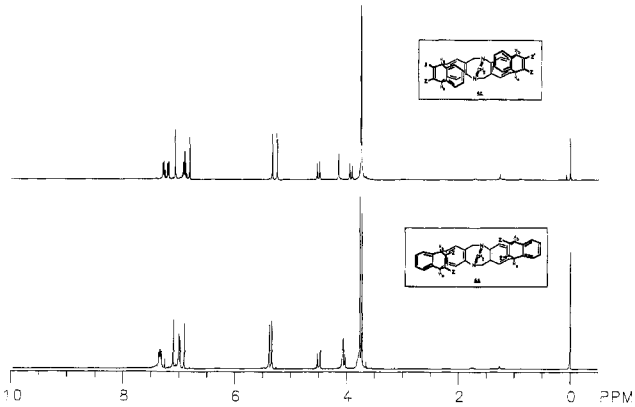


Figure 1. ^{1}H NMR data, 360 MHz, for **4a** and **4c** in $CDCl_3$. Differences between the ester inside isomer and the ester outside isomer are readily apparent.

by $SnCl_2/C_2H_5OH$.[8] The resulting racemic amine **3** was treated with formaldehyde and HCl to provide, in 42% yield, three diastereomeric Tröger's base analogues **4a**, **4b**, and **4c**, easily separable by chromatography, and consistently obtained in a 1:2:1 ratio (Scheme I).

The ^{1}H NMR and mass spectral data for these products leaves little doubt about the structural assignments (Figure 1). The most mobile isomer, **4a**, and the least mobile isomer, **4c**, are obviously the products of coupling homochiral pairs of the amine. The NMR data for isomer **4b** indicate that the four bridghead protons and four methyl groups are nonequivalent and thus **4b** can be none other than the product of heterochiral coupling. Heating of either racemic **4a** or racemic **4c** in ethanolic HCl generated the same mixture of approximately equal amounts of **4a** and **4c**. The difference in chemical shift for the two nonequivalent methyl groups in **4a** is greater than the corresponding difference in **4c**. Also, the difference in chemical shift for the bridghead protons in **4a** is less than the corresponding difference in **4c**. It was surmised that **4a** had the "ester inside" geometry shown in Figure 1 and the least mobile isomer was the "ester outside" product **4c**.

Crystals of **4a** were obtained from ethanol at room temperature and X-ray diffraction analysis confirms the assignments shown in Figure 1.[9] The structure found for **4a** (Figure 2) reveals that the dibenzodiazocine unit and the four ester groups define a concave surface of helical symmetry. The U-shaped molecule has a slight twist and the span separating one pair of equivalent esters across the cleft is shorter than that separating the other symmetry-related pair. Two ethanol molecules are located within the cleft defined by the esters. The solvent molecules are relatively disordered and precise positions cannot be obtained. A packing diagram reveals that the crystal is composed of continuous stacks of individual molecules. Guest molecules in this clathrate are held in a chamber of molecular scale: the fourth side of the chamber is defined by the next molecule in the stack.[10] The generality of this approach to clathrate design remains to be tested.

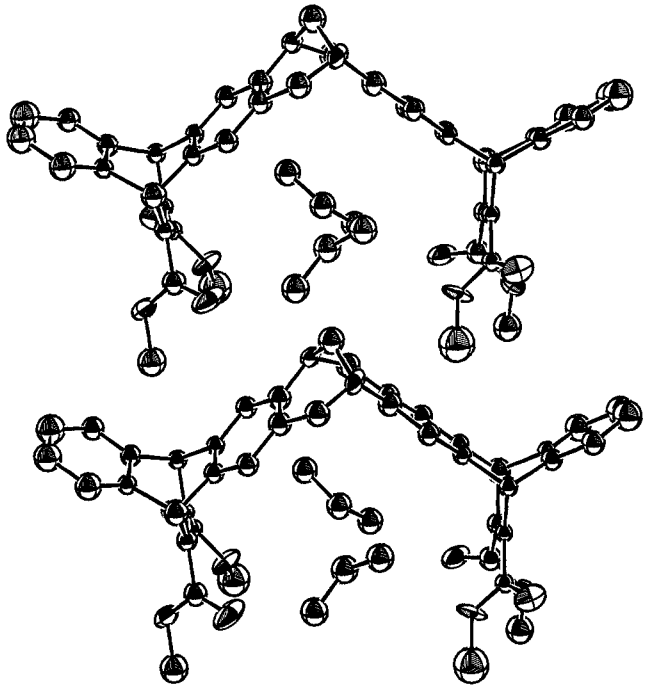


Figure 2. ORTEP drawing of **4a**·$(C_2H_5OH)_2$ and closest neighbor showing packing along the *b* axis (hydrogen atoms omitted).

The experiments described here illustrate a simple and powerful approach to a singular new class of concave molecules. Remarkably, these exciting new molecules are available in just four chemical steps and are easily prepared in multigram quantities. Such molecules are of strategic importance in efforts to create biomimetic systems, catalytic systems, or new solid-state materials. On the basis of these experiments, molecules which enforce previously unobtainable functional group spatial relationships can now easily be prepared and a new approach to clathrate design can be formulated. The catalytic capabilities, intramolecular reactivity, or substrate binding capacity of such systems will be examined in future work.

Acknowledgment. Support of this research by the National Institutes of Health (GM34846) is gratefully acknowledged.

(8) Bellamy, F. D.; Ou, K. *Tetrahedron Lett.* **1984**, 839–842.

(9) Crystallographic data: Monoclinic space group $I2/a$ with a = 18.340 (4) Å, b = 9.188 (3) Å, c = 24.673 (8) Å, β = 96.41 (2)°, V = 4132 (2) Å^3, Z = 2. Structure solved by direct methods and refined by full-matrix least squares. Final R = 0.1382, R_w = 0.09971. Details presented in the supplementary material.

(10) Wilcox, C. S.; Lynch, V., manuscript in preparation.

Supplementary Material Available: Crystallographic details for **4a**·$(C_2H_5OH)_2$, including tables of atomic coordinates, thermal parameters, bond lengths, and bond angles (10 pages); structure factor amplitude table for **4a**·$(C_2H_5OH)_2$ (17 pages). Ordering information is given on any current masthead page.

Direct Observation of Association Processes between Polymer Latex Particles

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The study of association–dissociation processes between solute species has in most cases been carried out by measuring the changes of parameters associated with the physical processes. One of the widely used techniques is spectrophotometry. In this paper, we report briefly on a kinetic investigation of two association processes of polymer latex particles with the use of a much more direct technique. We have taken advantage of latex particles being large enough to be observed by the naked eye with an ultramicroscope. To our knowledge this is the first "direct" determination of kinetic parameters. The first system studied here is the association reaction between oppositely and highly charged latex particles, and the second one is the selective association reaction between almost neutral latex particles carrying antigen and those carrying antibody on their surfaces.

An aqueous suspension of anionic latex particle G-5301 (poly(styrenesulfonic acid-*co*-styrene), diameter 3700 Å, charge density 10 $\mu C \cdot cm^{-2}$, final concentration 8.1×10^{-12} M[1], based on particles) obtained from Japan Synthetic Rubber Co. was mixed with a 10 times excess amount of a suspension of cationic latex particles MATA-2 synthesized by us (poly(3-methacryloylaminopropyltrimethylammonium chloride-*co*-styrene), diameter 3000 Å, charge density 4.0 $\mu C \cdot cm^{-2}$) in a thermostated observation cell set on a microscope (Carl Zeiss AXIOMAT, IAC). Both latices were monodispersed according to electron microscopy, and their suspensions were purified beforehand by thorough washing with mixed-bed ion-exchange resin (Amberlite MB-3) and an ultrafiltration method. The mixing was completed rapidly by using a polyethylene mixing rod, the association process was recorded on a video tape recorder, and the information was transferred onto a video disk. By replaying the video disk, the percents of dimeric particles in the suspension mixture were evaluated at appropriate intervals by using an image data analyzer (Carl Zeiss IBAS).[2] The number of particles in the visual field is large enough (>250) for statistical calculation.

Since the association in water was too fast for the microscopic observation, we carried out the study in 30% (w/v) aqueous sucrose solutions at 25 °C. Only dimeric and monomeric particles (no higher aggregates) were observed during the association (within 20 min after mixing). The time dependence of the percents of the dimeric particles in the reacting suspension are shown in Figure 1. Using the number of dimeric particles in the suspension calculated from this figure, we could obtain the reciprocal of the relaxation time of the association of the latex particles of opposite charges as 9.1×10^{-3} s^{-1}, which is nearly equal to the first-order rate constant because the backward process was hardly perceptible, and therefrom the second-order rate constant of this association reaction was calculated to be 1.1×10^8 M^{-1} s^{-1}. This suggests that the association reaction of oppositely charged latex particles is almost diffusion-controlled.[3] The spectrophotometric method gave $k_2 = 1.2 \times 10^8$ M^{-1} s^{-1}.[4] From this agreement we can conclude that the relaxation observed spectrophotometrically doubtlessly corresponds to the dimeric association.

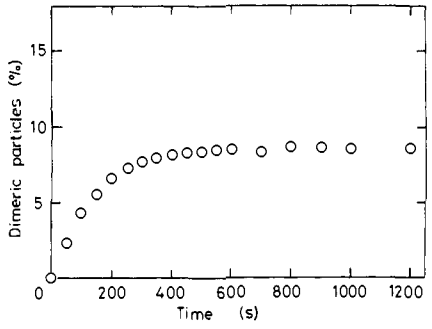

Figure 1. Time dependence of the percents of dimeric particles in the suspension at 25 °C ([MATA-2] = 8.1×10^{-11} M, [G-5301] = 8.1×10^{-12} M).

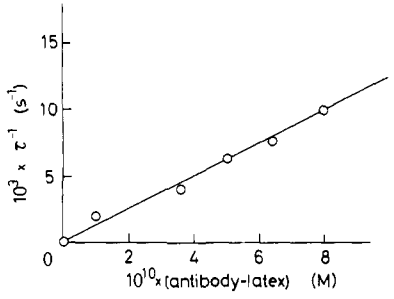

Figure 2. Influence of the concentration of the antibody–latex on the reciprocal of the relaxation time of the association of antibody–latex with antigen–latex at 25 °C.

Further, we examined an association of latex particles modified with human serum albumin (HSA) or anti-HSA-immunoglobulin G from rabbit (anti-HSA-IgG). Anti-HSA-IgG was purified by precipitation from 40% saturated ammonium sulfate, ion-exchange chromatography using a DEAE-Cellulose column, and immunological chromatography using a HSA-Sepharose column.[5,6] As a carrier of these proteins we used poly(acrolein-*co*-styrene) latex (AL-2, diameter 3750 Å, acrolein content in the latex 19%) modified with 6-aminohexanoic acid. Schiff base formed between the latex and the spacer molecule is reduced by a borane–dimethylamine complex.[7] HSA and anti-HSA-IgG were separately immobilized onto this latex particle by using a water-soluble carbodiimide, viz., 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride at pH 5. The number of proteins immobilized on the latex particle was about 16000 for both antigen–latex and antibody–latex. The association process was studied at pH 8.7, where ζ-potentials of the latex particles were almost zero.

By mixing the suspension of antigen–latex with an excess amount of the antibody–latex suspension, we could observe the dimeric association of latex particles. Figure 2 shows the plot of the reciprocal of the relaxation times vs. concentration of antibody–latex. From the slope of the figure we could determine the second-order rate constant of interlatex association as 1.3×10^7 M^{-1} s^{-1}. This k_2 value is larger than that found by us for free HSA and anti-HSA-IgG (1.9×10^6 M^{-1} s^{-1})[8] because the local concentration of the reactants is much higher for the latex-bound case than for the free systems.

The present analysis is essentially two-dimensional, but it will be valid in the three-dimensional case, provided that the particle

(1) 1 M denotes 6.02×10^{23} particles in 1 L of suspension.
(2) Ito, K.; Nakamura, H.; Ise, N. *J. Chem. Phys.* **1986**, *85*, 6136.
(3) Eigen, M.; Hammes, G. G. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1963**, *25*, 1.
(4) Kitano, H.; Iwai, S.; Ise, N.; Okubo, T., manuscript in preparation.
(5) Cuatrecasas, P.; Anfinsen, C. B. *Methods Enzymol.* **1971**, *22*, 345.
(6) Cuatrecasas, P. *J. Biol. Chem.* **1970**, *245*, 3059.
(7) Geoghegan, K.; Cabacungan, J. C.; Dixon, H. B. F.; Feeney, R. E. *Int. J. Peptide Protein Res.* **1981**, *17*, 345.
(8) Kitano, H.; Iwai, S.; Okubo, T.; Ise, N., manuscript in preparation.

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