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Molecular Recognition by Cyclodextrin Dimers

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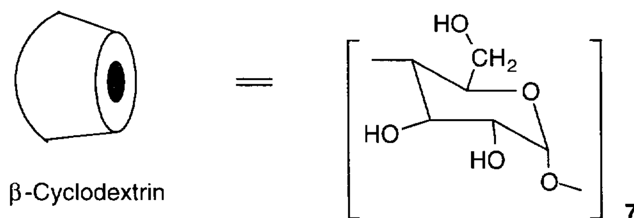
Abstract: *Cyclodextrin dimers strongly bind substrates with the correct geometry in water solution. Studies with antihydrophobic agents help clarify the factors involved.*

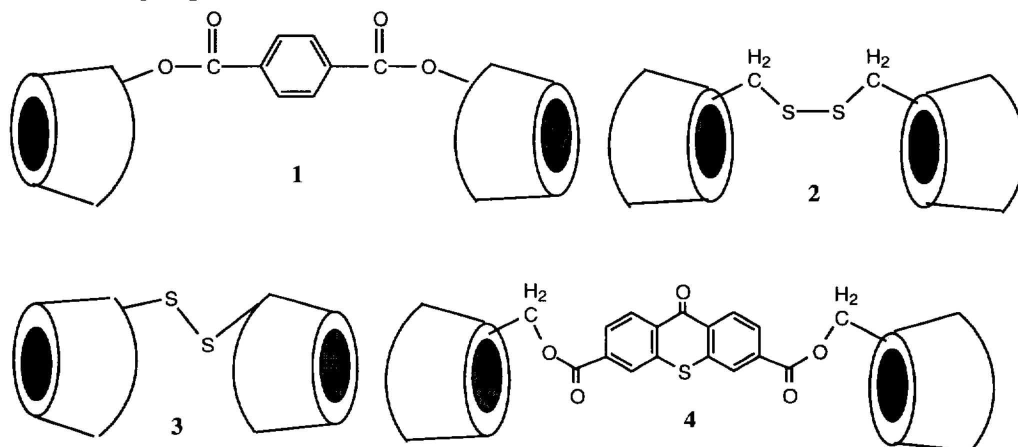
Cyclodextrins are cyclic oligomers of glucose whose interior cavity can bind substrates. The binding is chiefly hydrophobic, so it is strongest in water solution.[1] Thus cyclodextrins have been the basis of many mimics of antibodies (binding) or of enzymes (binding and catalysis). However, the binding constants are too weak to allow simple cyclodextrins to be good antibody mimics, and the complexes are often too flexible to enable simple cyclodextrin derivatives to be ideal enzyme mimics. Both of these problems can be solved with cyclodextrin dimers. When two cyclodextrin units are linked, the binding of substrates that can occupy both cavities is generally much stronger, and the geometry of the resulting complex is better defined since the substrate is grasped at both ends and held across the linker that ties the two cyclodextrins together. This should lead to better catalysts. Experimental results with cyclodextrin dimers fully support these predictions.



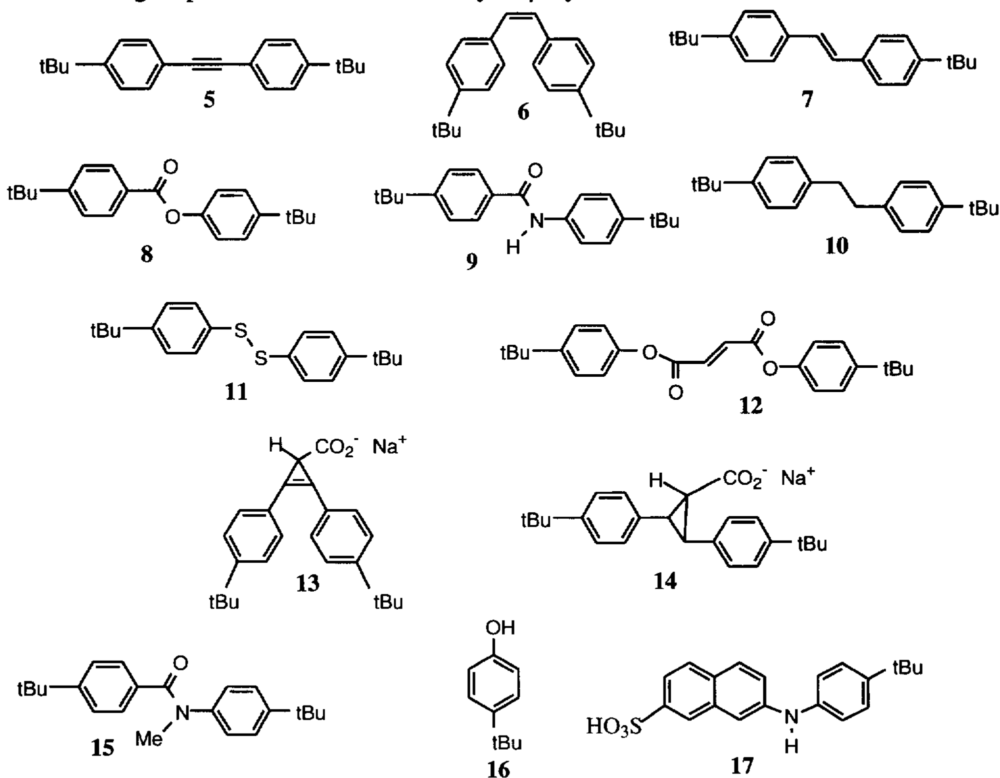
Strong binding.

Our first study involved a cyclodextrin dimer **1** with a terephthalate ester link on the secondary face of the β-cyclodextrin. This work, discussed in various talks but published only in a 1972 Ph.D. thesis,[2] showed that there was particularly effective acylation of the cyclodextrin host by substrates that could occupy both cavities. Subsequent to this, several other groups in Japan prepared cyclodextrin dimers and examined them briefly.[3-5] We took this area up seriously in the 1980's and examined a number of cyclodextrin dimers, including the β-cyclodextrin-6,6'-disulfide **2** that was first reported by Fujita.[5] In our study[6,7] we also

looked at a related β-cyclodextrin disulfide **3** linked on the secondary face at carbons 3 and 3' that we had prepared earlier.[8] Furthermore, we synthesized and studied a diester **4** carrying a thioxanthone group in the linker.



As guests we used a series of compounds **5-17** in which t-butylphenyl groups were present; such groups bind well into the cavity of β-cyclodextrin. We saw that the monomeric



substrate t-butylphenol (**16**) bound into the dimeric host **2** in water at 25 °C with a K_a of 1.6 x 10^4 M^{-1}, a normal binding constant for a t-butylphenyl group into a single β-cyclodextrin cavity. However, the ester **8** with two t-butylphenyl groups that can both insert into the cavities of host **2** had a binding constant of 1 x 10^8 M^{-1}, corresponding to almost a doubling of the binding free energy. The cyclopropene **13** had a binding constant of 3.5 x 10^8 M^{-1}, again doubling the free energy when two groups can bind. The cis-stilbene derivative **6**—closely related to the cyclopropene, but with the phenyl groups too close together because of the difference in phenyl attachment angles in **6** compared with **13**—had a very low binding constant, <3 x 10^3 M^{-1}, and several other substrates (e.g. **12**) whose geometry did not permit good binding into both cavities of **2** also showed poor binding constants.

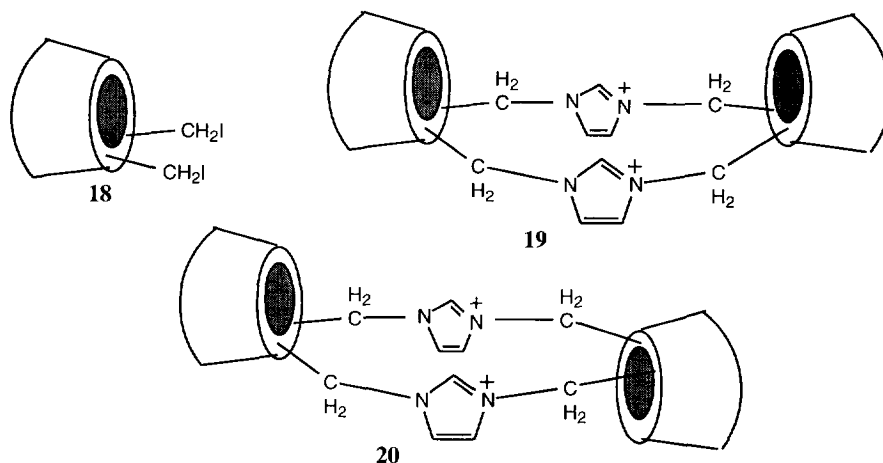
We also saw very strong binding of the cyclopropene **13** to the dimeric host **4**, consistent with molecular models that showed that both t-butylphenyl groups could bind into the cavities of **4**. However, with the disulfide linkage on the secondary face in host **3**, we saw only weak binding of all guests examined. Apparently with this linkage the two cavities are partly occupied by elements of the other cyclodextrin. We have seen similar behavior with some dimers of synthetic hosts, not just cyclodextrins.[9] Not all dimers automatically bind strongly, and those that do are still selective for their guests.

The determination of binding constants cannot always be done directly, since binding of a guest (e.g. **13** and **14**) may be too strong to permit direct observation of a modified spectroscopic signal at host concentrations low enough to prevent full saturation binding. In these cases we used the fact that binding of **17** leads to a significant increase in fluorescence, with a signal strong enough that its binding constant (5 x 10^6 M^{-1}) can be directly determined by titration. Then we competed substrates such as **13** with **17** for a limited amount of host **2**. From such competition experiments, the binding constant of **13** is related to that of **17**, which we had determined directly. This competition technique was frequently used, especially in cases—such as those we will discuss—with very strong binding.

Of course hosts such as **2**—in which there is a single link between two cyclodextrin units—have many conformations other than that required for binding a double-ended substrate. For this reason, we were sure that even better binding would be observed in cyclodextrin dimers with two linkers. For instance, if the links went from two neighboring glucose units of one cyclodextrin to two neighboring glucoses of another cyclodextrin, the result would be a hinge or strap. It would permit some motions of the two rings, but would prevent the twisting around a linker that was possible in **2** or **4**. Building on the work of Tabushi,[10] we had already learned how to selectively functionalize two neighboring glucose units in β-cyclodextrin, producing[11] what we called the 6A, 6B diiodide **18** (we label the glucose units A-G, counting clockwise when facing the primary side of the cyclodextrin). Using this compound, we have prepared several cyclodextrin dimers with double linkages involving the A and the B glucose units of the cyclodextrins.

One such doubly-linked dimer is **19** (and its isomer **20**), prepared by displacing the iodines of **18** with imidazole, then alkylating the product with another mole of **18**.[12] Such an

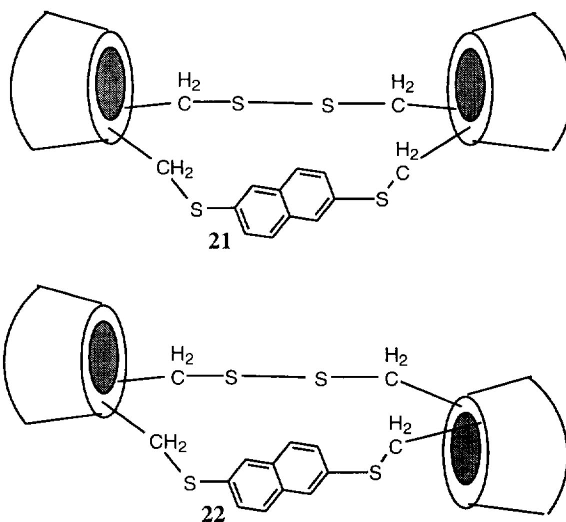
alkylation produces two isomers, the occlusive structure **19** that can cooperatively bind a double-ended substrate into both cavities and the aversive isomer **20** that cannot. They differ in symmetries as well as in binding properties. The occlusive isomer **19** has each imidazolium link attached to an A residue of one cyclodextrin and a B residue of the other. Thus the two imidazolium rings are equivalent in the NMR spectrum. By contrast, in the aversive isomer **20** one imidazole links the 6A position of one cyclodextrin with the 6A position of the other, while the second imidazole is linked to the two B positions. Thus they are not equivalent, and show different signals in the NMR. This has been used to demonstrate the structure of the two isomers.[12]



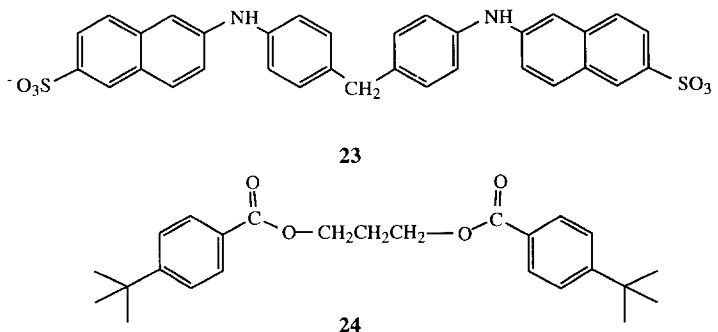
As expected, **19** can cooperatively bind double-ended substrates, but **20** cannot. However, the effects were not as striking as had been hoped, or as were found with the dimeric system to be discussed next. We found[12] that **19** binds cyclopropene **13** in water at 25 °C with an association constant of 2.8×10^6 M^{-1}. It binds the fluorescent substrate **17** with a constant of 4.4×10^6 M^{-1}, while **17** binds to **20** with a constant of only 6×10^4 M^{-1}. Thus the occlusive clamshell isomer **19** is the stronger binder—as expected—but the binding is not as strong as that to the singly linked dimer **2**! Furthermore, **32**—a singly-linked analog of **19** (and **20**)—has a K_a of ca. 10^9 M^{-1} with **13**. It is not yet clear why the double linkage in **19** leads to lower affinity.

We have also prepared a doubly-linked cyclodextrin dimer **21** in which there are two neutral links, but of different length, along with its geometric aversive isomer **22**.[13] Here the binding results were very striking. The aversive dimer **22** cannot use both cyclodextrins in cooperative binding to a single substrate, and it shows binding constants very similar to those of simple cyclodextrin. However, the occlusive dimer **21** shows two kinds of geometric recognition effects: it binds appropriate substrates with very high binding constants, and it

prefers substrates with a bent geometry so they fit well into the dimer—which is itself bent because of the different lengths of the two linkers.

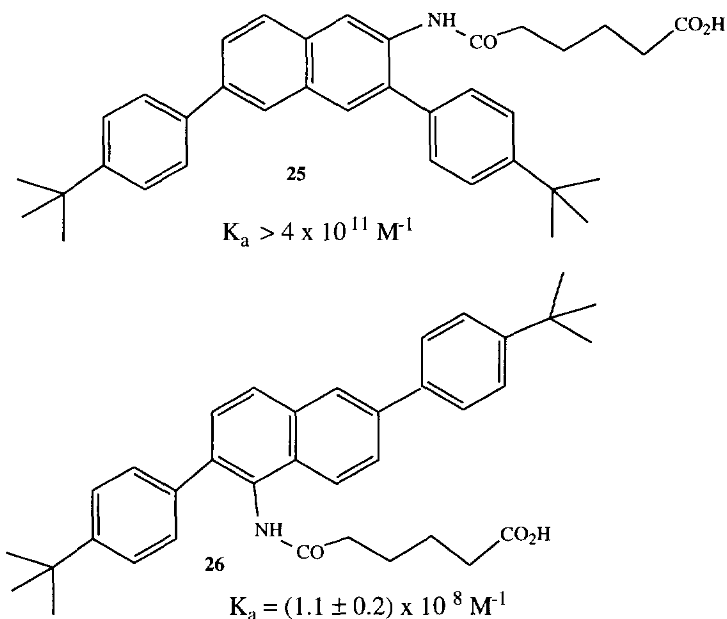


Binding into **21** was so strong that competition fluorescence methods had to be used to determine the association constants.[13,14] Thus we first synthesized the fluorescent dimer **23**, and determined—by direct titration—a K_a with **21** in water at 25 °C of $(4.0 \pm 0.2) \times 10^8$ M^{-1}. Then we competed **23** with the flexible substrate **24** for a limited amount of **21**, and found that **24** has a K_a of $(1.0 \pm 0.1) \times 10^{10}$ M^{-1}. This puts the binding constant for **24** into **21** in the range of strong antigen-antibody binding.



Shape recognition was probed by using substrates **25** and **26** (carrying solubilizing sidechains), both synthesized in standard ways.[14] In **25** the two t-butylphenyl binding groups are arranged in a bent geometry, while in the isomer **26** they are more or less colinear. Thus we expected—as confirmed by model building—that **25** would fit the bent geometry of host

21 much better than **26** does. The experimental results—using competition with the fluorescent indicator **23**—confirm this idea.



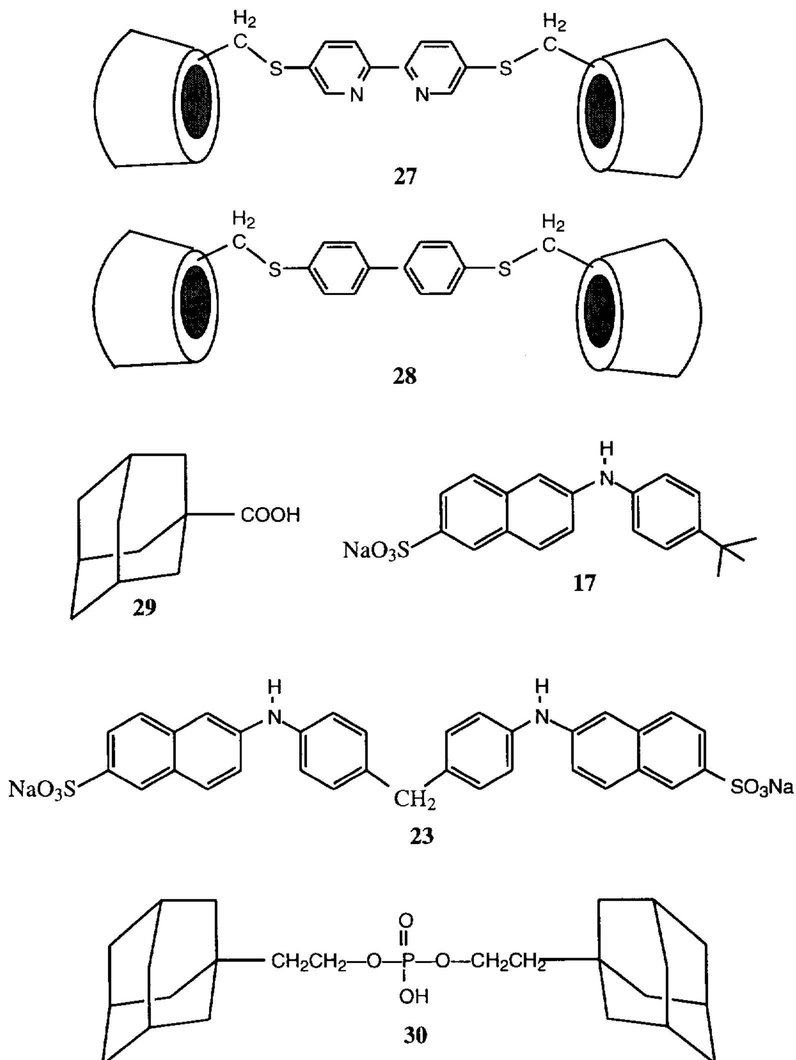
The linear isomer **26**, which does not fit **21** as well, showed a K_a of $1.1 \times 10^8 M^{-1}$, still pretty strong. However, binding of **25** into **21** was so strong that we could not displace it by any accessible concentration of **23**. From this result, we fix the lower limit of K_a for **25** into **21** as $4 \times 10^{11} M^{-1}$. The bent geometry of **21** leads to a shape preference between **25** and **26** of at least 4000-fold, and perhaps more. We are still trying to find methods to determine the binding constant of **25**, not just a lower limit. It is at least as large as that of some of the best antigen-antibody complexes.

Thermodynamic studies.

We have studied some other dimeric host-ditopic substrate binding pairs, using a titration calorimeter that furnishes both the binding constant and also the enthalpy and entropy contributions to it.[15] By examining the results at different temperatures, we can also determine the $\Delta C_p°$ for binding. The results are at first sight quite surprising.

The hosts examined were simple β-cyclodextrin, and also dimers **2**, **27**, and **28**. As guests we used a simple adamantane derivative **29**, the previously discussed BNS **17** and the related dimer **23**, and the ditopic substrate **30**. Since the details have been published,[15] we will simply review the most striking findings. First of all, the binding of **29** into β-cyclodextrin in water at 25 °C had $\Delta G°$ of -6.27 kcal/mole, made up of -5.21 kcal/mole of $\Delta H°$ and 1.06 kcal/mole of $T\Delta S°$. Similar figures were found for the binding of **17**, **23**, and **30**.

Thus simple binding of a t-butylphenyl group (or a naphthyl group) into β-cyclodextrin is enthalpy driven, and the entropy is unfavorable. This is consistent with previous studies of simple binding into the cavity of cyclodextrins,[16-19] even though hydrophobic binding is often driven by entropy.[20]



Binding of the ditopic substrates **23** and **30** into the dimeric cyclodextrins **2**, **27**, and **28** was stronger, with ΔG°'s that were almost the sum of the ΔG°'s for binding of two different β-cyclodextrins to the substrates, but the striking finding was that the ΔH°'s were considerably greater than the sums for two independent cyclodextrins, and they made up for a quite unfavorable ΔS° (over 20 e.u.) in the dimeric cases.

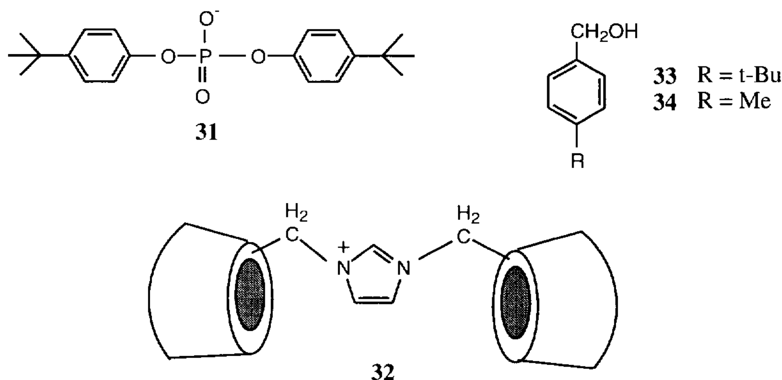
It is normally believed that the chelate effect is due to the favorable entropy of binding relative to two independent ligands, because with attached ligands there is not need to pay twice for translational entropy.[21] Our examples show clearly that in the cases we have studied the preferred binding of dimeric cyclodextrins to ditopic substrates comes in spite of an unfavorable entropy contribution in the dimeric case. This does not mean that the classical explanation is wrong—it probably means that there are compensating solvation effects whose enthalpy/entropy consequences dominate the observations. We are examining further systems to see how widespread this experimental situation is.

Quantitative studies with antihydrophobic agents.

Substances such as urea and guanidinium chloride are well-known denaturing agents for proteins.[20] They work by diminishing the hydrophobic effect that causes proteins to fold so as to place non-polar segments away from contact with water. The antihydrophobic effect of these compounds—and also of many others such as lithium perchlorate and guanidinium thiocyanate—can also show up in their ability to solubilize hydrocarbons in water.[22-24] It was widely believed that they acted by breaking up the structure of water, but our surface tension studies[24] indicate that this is not how they work. Instead, they apparently act to disperse non-polar materials in water by bridging between the solute and the water.

Regardless of their precise mode of action, we have used their antihydrophobic effect to detect hydrophobic packing in the transition states of Diels-Alder reactions[25] and the benzoin condensation[26]—especially by contrast with the effect of prohydrophobic agents such as lithium chloride. The change in rates in the presence of members of one class or the other demonstrated that hydrophobic packing in the transition states was a major factor in the fast rates of these reactions in water.

We have recently shown that these agents can also be used to obtain quantitative information.[27] The magnitude of their effect on solubilities or on binding constants into the hydrophobic cavity of cyclodextrin seems to be related to the amount of hydrocarbon surface that is exposed to water.



We examined[27] the effects of urea and of guanidinium chloride on the binding constants in water of BNS (**17**) and of bis-p-t-butylphenyl phosphate (**31**) into the cavities of simple β-cyclodextrin and of the cyclodextrin dimer **32**. We also examined the effect of these agents on the water solubilities of p-t-butylbenzyl alcohol **33**, whose non-polar segments mirror those of **31** to some extent, and of p-methylbenzyl alcohol **34**. The quantitative correlations were striking.

The data have been published in detail,[27] so we will simply summarize the results. First of all, the effects on solubilities paralleled the hydrophobic areas of the solutes. Urea at 8 M increased the solubility of **33** in water by a factor of 3.3, and of **34** by a factor of 2.5. A calculation of the van der Waals surface area of toluene shows that it is 71% that of t-butylbenzene, and 2.5 is 74% of 3.4. Of course the solubility measurements must be interpreted with caution, since the second phase is a liquid (**34**) or low-melting solid (**33**) that could be contaminated by the water or the solute urea. Solubility is ideally defined only in terms of equilibrium with the pure solute. However, we believe that the perturbation in this case is probably small. With 6 M guanidinium chloride, much less likely than urea to partition into the organic solute phase, the solubility of **33** increased by a factor of 3.9, and that of **34** by a factor of 2.8. The ratio of the effects is 72%, consistent with the urea data and with the surface area calculations. We also looked at the effect of ethanol on the water solubilities of **33** and **34**, but here the changes were almost the same for the two solutes. Ethanol might well partition appreciably into the solute phase, nullifying the solubility measurements. Such partitioning will decrease the thermodynamic activity of the solute phase, and thus diminish the apparent solubility.

When a solute such as **17** or **31** in water solution binds into a cyclodextrin cavity, it escapes from water contact much as it does by leaving the solution as a separate phase. Thus one might hope that the quantitative effects of antihydrophobic agents on the binding constants could be related to those of solubilities. This is what we observed.[27] The binding of **17** to simple β-cyclodextrin was decreased by a factor of 2.4 in 8 M urea, and by a factor of 4.4 in 6 M guanidinium chloride. Since it is clear that it is the t-butylphenyl group of **17** that binds, these numbers should be compared with the 3.3-fold effect of urea on the solubility of **33**, and the 3.9-fold effect of guanidinium chloride. The effects are not identical, but of similar magnitude. In particular, they indicate that there is no extra effect of the antihydrophobic agents on the cavity of the cyclodextrin itself, or the binding constant should have been modified more strongly than the solubility. This is consistent with our proposal[24,28] that such antihydrophic effects involve bridging by the urea or guanidinium ion between the hydrophobic surface and the water medium, which is geometrically difficult for the cyclodextrin cavity.

The effects on binding of two t-butylphenyl groups should be the square of those for monomeric binding, if the change in binding free energy is simply doubled. We saw effects that were somewhat larger than expected from this simple idea. For instance, the binding

constant of **31** into **32** decreased by a factor of 20 with 8 M urea, and of 83 with 6 M guanidinium chloride. This certainly supports the idea that both t-butylphenyl groups of **31** bind into the two cavities of **32**, as the strong binding constant itself also indicates. However, the factor of 20 is larger than 6 (2.4^2), so some new factor must be involved. Also 83 is larger than 18 (3.9^2), again reflecting an additional factor. There may be an interaction with the linker group in the dimeric case—possibly electrostatic in nature—and the antihydrophobic materials, especially the salt guanidinium chloride, may also be decreasing that interaction, not just the cyclodextrin binding interaction.

We saw similar effects larger than expected for binding of **17** into **32** and we also saw that ethanol decreased the binding of **17** and of **31** into **32** by a larger amount than it did for **17** into β-cyclodextrin, although now with almost the predicted square relationship. Thus the quantitative relationships are not yet fully established with these limited data, but it is clear that there are indeed correlations with the amount of hydrophobic surface that is buried in a binding event. Further work should test and extend these generalizations.

Catalysis. Some of these dimers show striking catalytic accelerations. For example, the Cu^{2+} complex of compound **27** catalyzes the hydrolysis of a doubly bound nitrophenyl ester by 220,000-fold,[29] and in unpublished work we find that the La^{3+} complex of **27** catalyzes the hydrolysis of bis-p-nitrophenyl phosphate with hydrogen peroxide by a factor of over 10^7. This is an area of chemistry in which much new progress can be expected.

Experimental section.

The experimental details for the studies with compounds **31-34** have been reported elsewhere.[27]

General. ^1H-NMR spectra were recorded on a Varian VXR 200 or 400 MHz spectrometers. ^{13}C-NMR spectra were recorded on a 75 MHz spectrometer. All spectra were with the residual solvent peaks as reference signals. Mass spectra were recorded on a Nermag R-10-10 instrument (for chemical ionization(CI) or electron impact ionization(EI) spectra) or a Jeol JMS-DX-303 HF instrument (for FAB spectra). Fluorescence measurements were obtained on a Perkin-Elmer LS 50 spectrometer. Calorimetric titrations were performed on an OMEGA calorimeter. pH values were measured on an Orion 701A ionalyzer with a glass electrode after calibration to standard buffer solutions.

Calorimetric Titrations. The calorimetric titrations were carried out using the OMEGA titration calorimeter from MicroCal, Inc. (Northampton, MA). The guest solution was transferred to the titration cell. The reference cell of the calorimeter serves only as a thermal reference to the sample cell and it was filled with water. A 100-μl injection syringe was filled with the host solution, 25-30 times higher in concentration than the initial guest solution. All solutions were made with 50 mM phosphate or Hepes buffer (pH 7.0). After an initial equilibration period of approximately 10 min, stirring at 400 rpm was begun. After another period of 10 min—when equilibration was finished as judged by base-line stability—3-6 μl injections were performed at intervals of approximately 3-5 min. Injections were

continued until all of the host had reacted, and the heat q_i evolved at each injection step i was measured. Analysis of calorimetric data was carried out with the Origin software provide with the OMEGA instrument. K, ΔH and molar ratio n were determined by this program using a nonlinear least-squares curve fitted to the calorimetric data points.[30]

Synthesis:

2,2'-Bipyridyl-β-cyclodextrin dimer, 27. Into 5 ml of saturated NH_3/CH_3OH solution under argon, 30 mg (0.098 mmol) of 2,2'-bipyridyl-5,5'-bisthioacetate[30] was dissolved and the solution was stirred for one h at room temperature to effect deacetylation. The solvents were evaporated under vacuum, and a solution of 400 mg (0.32 mmol) of 6-iodo-6-deoxy-β-cyclodextrin dissolved in 1.5 ml anhydrous DMF was added. The mixture was stirred at 60-65 ^oC for 3 hr., then diluted with 20 ml of DMF and stirred for 10 min. The DMF was evaporated and the crude product was purified by reverse-phase chromatography (eluted with a gradient ranging from water to 40% methanol-water). The pure white bipy-β-CD-dimer **27** was obtained in a yield of 112 mg (46%). R_f = 0.3 (silica, 5/7/7/4 H_2O/CH_3COOEt/i-PrOH/NH_4OH). ^{1}H-NMR (400 MHz, DMSO-d_6): δ3.10-4.20 (m, H_2, H_3, H_4, H_5 and H_6 on cyclodextrin), 4.50 (m, 12H, primary hydroxyl), 4.82 (m, 14H, anomeric), 5.75 (m, 28H, secondary hydroxyl), 7.93 (dd, J = 8.4 and 2.4 Hz, 2H, pyridine), 8.23 (d, J = 8.4 Hz, 2H, pyridine) and 8.58 (d, J = 2.0 Hz, 2H, pyridine). FAB-MS: m/z 2453.7 $(M + H)^+$; M = $C_{94}H_{144}O_{68}N_2S_2$: 2452.7

Biphenyl-β-cyclodextrin dimer, 28. The procedure was the same as in the synthesis of **27**, but using biphenyl-bis-thioacetate. The product was obtained in a yield of 31%. R_f = 0.27 (silica, 5/7/7/4 = H_2O/CH_3COOEt/i-PrOH/NH_4OH). ^{1}H-NMR (400 MHz, DMSO-d_6): δ3.12-4.20 (m, H_2, H_3, H_4, H_5 and H_6 on cyclodextrin), 4.55 (m, 12H, primary hydroxyl), 4.89 (m, 14H, anomeric), 5.68 (m, 28H, secondary hydroxyl), 7.41 (d, J = 8.0Hz, 4H, biphenyl) and 7.55 (d, J = 8.0 Hz, 4H, biphenyl). FAB-MS: m/z 2452 $(M + H)^+$; M = $C_{96}H_{146}O_{68}S_2$: 2450.7

Bis-2(1-adamantyl)ethyl phosphate, 30. Anhydrous pyridine (8.0 ml) was cooled in an ice bath. Methyl dichlorophosphate (0.55 ml, 5.5 mmol) was added dropwise, and the mixture was kept in the cold for a further 15 min. During this period a ppt of N-methylpyridium dichlorophosphate formed. 2-(1-Adamantyl)ethanol (2.0 g, 11 mmol) was added and the sealed mixture was stirred overnight at room temperature. It was then poured into 10% aq. $NaHCO_3$ (50 ml) and the pyridine was evaporated in *vacuo*. The slight yellow solid was dissolved in 800 ml of water and extracted with ether (three 150 ml partions). The aqueous phase was acidified with 2N HCl to pH=1, then extracted with three 150 ml portions of $CHCl_3$: n-BuOH (7:3). The organic layer was washed with water. The slightly yellow precipitate was formed in the mixture solvent; the solvents were evaporated in *vacuo*. A slightly yellow solid was formed, which was recrystallized with acetone/hexane. The solid product was dried: m.p 198-199 °C, yield 60%. ^{1}H-NMR: δ1.45-1.75 (m, 24H, $-CH_2-$, adamantyl), 1.95 (m, 6H, C-H, adamantyl), 4.07 (m, 8H, $-CH_2CH_2-$) and 8.60 (br, 1H,

POOH). CI-MS: 423 $(M + H)^+$; $C_{24}H_{39}O_4P$: 422.26. The ^{13}C-NMR was also consistent with the assigned structure.

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