Design, Synthesis, and Evaluation of Bile Acid-Based Molecular Tweezers

Lawrence J. D'Souza and Uday Maitra*

Department of Organic Chemistry, Indian Institute of Science, Bangalore 560 012, India

Received April 24, 1996 (Revised Manuscript Received September 30, 1996[⊗])

A family of bile acid-based molecular tweezers (**7**−**9**) has been constructed readily from simple precursors. Binding experiments with various electron deficient aromatic compounds showed that tweezer **8** binds trinitrofluorenone **10e** with an association constant of 220 M^{-1} in $CDCl_3$. Single-crystal X-ray analysis of compound **8** shows aromatic−aromatic interactions producing a two-dimensional lattice of pyrene units. Tweezer **8** was immobilized on Merrifield resin, and binding studies have shown that these data compare well with those of the solution state studies.

Introduction

The past decade has witnessed an explosive growth in research involving various aspects of supramolecular chemistry.[1] A large number of molecular receptors (including chiral receptors)[2−4] of varying sizes, shapes, and functionalities have been synthesized, and their interaction with guests has been assessed. The design of all such receptors is based on the fundamental molecular interactions exhibited by biological systems, particularly enzymes.[5] Since the binding of a substrate by an enzyme is the *first* step in catalysis, the primary goal in molecular recognition research has been toward the development of *selective* synthetic receptors. Such studies have not only advanced our knowledge in understanding fundamental molecular interactions but have also led to the design of novel molecular devices, including sensors.[6] There has also been considerable interest in designing molecular units which self-assemble in solution and in the solid state.[7] Many organized solid state structures are expected to have interesting material properties, which can possibly be tailor-made through proper and optimized molecular design.

Although diverse types of molecular hosts have been designed during the past decade, one of the simplest concepts has been that of a *molecular tweezer*. First developed by Whitlock[8] and Zimmerman,[9] this class of molecules was shown to form sandwich complexes with aromatic guests through $\pi-\pi$ interaction. Whitlock's tweezers were flexible, and apart from $\pi-\pi$ interaction, hydrophobic interaction also played an important role in their tight binding to aromatic (bis-phenol)carboxylates in water. The tweezers constructed by Zimmerman, however, were more rigid and showed exceptionally high association constants with guests such as polynitroaromatics and 9-alkylated adenines in chloroform.

Among the variety of molecular scaffolds which have been employed in supramolecular design, bile acids have recently attracted the attention of a number of research groups for their ready availability and unique structural features.[4] The array of hydroxyl groups lining one surface of bile acids provides opportunities for the attachment of appropriate molecular units to generate structures with properties determined by the nature and arrangement of these units. Most of the interesting chemistry of the bile acids before the 1990s have come from their ability to form inclusion complexes and clathrates with organic guests.[10] We have been interested in building novel structures with bile acids and decided to utilize the hydroxyl groups to design a series of hosts in which structural and/or electronic perturbations could be made easily. Although a variety of host structures could be envisaged, we decided to construct a series *molecular tweezers* as the first step.[11]

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1996.

(1) (a) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304. (b) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417. (c) Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 245. (d) *Frontiers in Supramolecular Organic Chemistry*; Schneider, H.-J., Durr, H., Eds.; VCH: Weinheim, 1991 and references therein.

(2) Wilcox, C. S. *Chem. Soc. Rev.* **1993**, 383 and references therein.

(3) (a) Maitra, U.; Bag, B. G. *J. Org. Chem.* **1994**, *59*, 6114. (b) Voyer, N.; Lamothe, J. *Tetrahedron* **1995**, *34*, 9241. (c) Kikuchi, Y.; Kobayashi, K.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1351. (d) Breslow, R. *Science* **1982**, *218*, 532. (e) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 848. (f) Rebek, J., Jr.; Askew, B.; Nemeth, D.; Parris, K. *J. Am. Chem. Soc.* **1987**, *109*, 2432. (g) Garcia-Tellado, F.; Albert, J.; Hamilton, A. D. *J. Chem. Soc., Chem. Commun.* **1991**, 1761.

(4) For representative examples, see: (a) Davis, A. P. *Chem. Soc. Rev.* **1993**, 243. (b) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259. (c) Bonar-Law, R. P.; Mackay, L. G.; Walter, C. J.; Marvaud, V.; Sanders, J. K. M. *Pure Appl. Chem.* **1994**, *66*, 803. (d) Maitra, U.; Balasubramanian, S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 83. (e) Bonar-Law, R. P.; Sanders, J. K. M. *J. Chem. Soc., Perkin Trans. 1* **1995**, 3085.

(5) (a) Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1417. (b) Fersht, A. *Enzyme Structure and Mechanism,* 2nd ed.; Freeman and Co.: New York, 1985; p 293.

(6) (a) Sandanayake, K. R. A. S.; Imazu, S.; James, T. D.; Mikami, M.; Shinkai, S. *Chem. Lett.* **1995**, 139. (b) Zhao, S.; Luong, J. H. T. *J. Chem. Soc., Chem. Commun.* **1995**, 663. (c) Rojas, M. T.; Kaifer, A. E. *J. Am. Chem. Soc.* **1995**, *117*, 5883. (d) Sandanayake, K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2207. (e) Fabbrizzi, L.; Licchelli, M.; Pallavicini, P.; Perotti, A.; Sacchi, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1975. (f) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 9397. (g) Chawla, H. M.; Srinivas, K. *J. Chem. Soc., Chem. Commun.* **1994**, 2593. (h) Hamasaki, K.; Ikeda, H.; Nakamura, A.; Ueno, A.; Toda, F.; Suzuki, I.; Osa, T. *J. Am. Chem. Soc.* **1993**, *115*, 5035. (i) Teixidor, F.; Flores, M. A.; Escriche, L.; Vinas, C.; Casabo, J. *J. Chem. Soc., Chem. Commun.* **1994**, 963. (j) Marsella, M. J.; Swager, T. M. *J. Am. Chem. Soc.* **1993**, *115*, 12214.

(7) Sessler, J. S. *New J. Chem.* **1991**, *15*, 153.

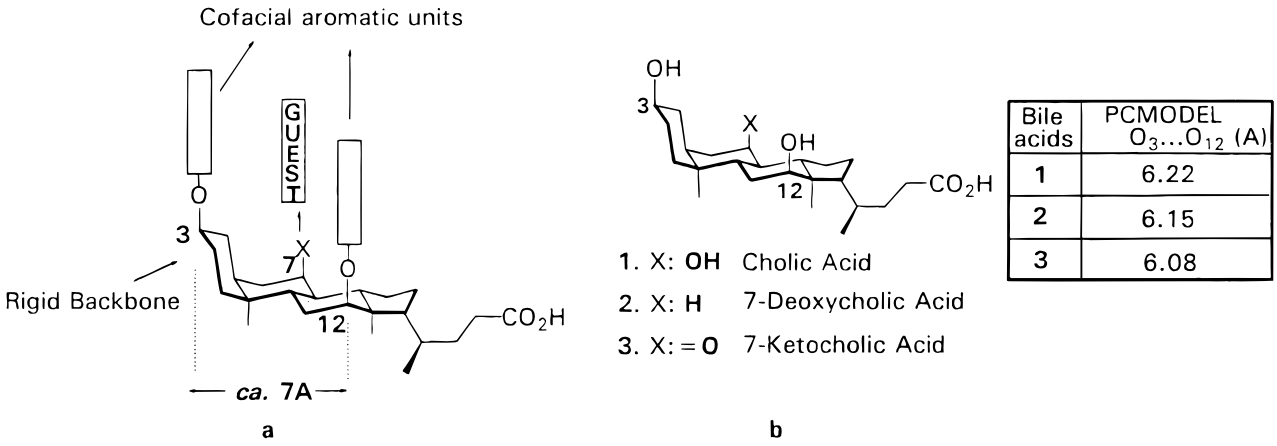
(8) (a) Chen, C. W.; Whitlock, H. W. *J. Am. Chem. Soc.* **1978**, *100*, 4921. (b) Nedar, K. M.; Whitlock, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 7269.

(9) (a) Zimmerman, S. C.; Zeng, Z.; Wu, W.; Reichert, D. E. *J. Am. Chem. Soc.* **1991**, *113*, 183. (b) Zimmerman, S. C.; Wu, W.; Zeng, Z. *J. Am. Chem. Soc.* **1991**, *113*, 196. (c) For further references see: Zimmerman, S. C. *Bioorganic Chemistry Frontiers*; Springer-Verlag: New York, 1991; Vol. 2, p 33. (d) Blacker, A. J.; Jazwinski, J.; Lehn, J.-M. *Helv. Chem. Acta* **1987**, *70*, 1.

(10) (a) Biro, R. P.; Chang, H. C.; Tang, T. P.; Shochet, N. R.; Lahav, M.; Leiserowitz, L. *Pure Appl. Chem.* **1980**, *52*, 2693. (b) Giglio, E. *Inclusion Compounds;* Atwood, J. L., Davies, J. E. D., MacNicol, D. D., Eds.; Academic Press: London, 1984; Vol. 2, p 207. (c) Mik, K.; Kasai, N.; Shibakami, M.; Takemoto, K.; Mujata, M. *J. Chem. Soc., Chem. Commun.* **1991**, 1757. (d) Caira, M. R.; Nassimbeni, L. R.; Scott, J. L. *J. Chem. Soc., Chem. Commun.* **1993**, 612.

(11) Preliminary communication: Maitra, U.; D'Souza, L. J. *J. Chem. Soc., Chem. Commun.* **1994**, 2793.

S0022-3263(96)00752-9 CCC: $12.00 © 1996 American Chemical Society



Bile acids	PCMODEL $O_3...O_{12}$ (A)
1	6.22
2	6.15
3	6.08

Figure 1. (a) Schematic representations of a bile acid-based molecular tweezer (b) O_3-O_{12} distances in bile acids **1**−**3**, from PCMODEL.

Objectives

The construction of bile acid-based molecular tweezers, involving the linkage of the rigid bile acid backbone with rigid π-surfaces, presents a general strategy by which a variety of semirigid receptors can be synthesized easily. Since a small degree of flexibility is present in these tweezers, detailed studies on a family of such molecules can lead to a better understanding of the geometrical requirements of binding. In spite of the success of Zimmerman's approach, variation of electron density of the aromatic arms required considerable synthetic effort. Our modular approach for the construction of semirigid systems, in which "sticky" arms are attached to a template through spacers by a simple reaction, is synthetically much straightforward. We initially planned to synthesize molecular tweezers based on $\pi-\pi$ interactions.[8,11,12]

Bile acids have a *rigid* backbone: the C-3 and C-12 OH groups are *ca.* 6−6.5 Å apart, and they are approximately parallel. We reasoned that these would meet the geometric requirements for synthesizing bile acid-based *tweezers* which can be easily made by attaching flat π-surfaces such as pyrene units on the 3- and 12-hydroxyl groups. We also felt that the 7-OH position can be used subsequently, after appropriate functionalization, as an additional handle to interact with a bound guest (Figure 1a). We have used both cholic and deoxycholic acids to synthesize a family of molecular tweezers, which are described in the following section.

Results and Discussion

Modeling Studies. Analysis of the PCMODEL[13] -minimized structures of cholic (**1**), 7-deoxycholic (**2**), and 7-ketocholic (**3**) acids revealed that the hydroxyl groups at the 3- and 12-positions are *ca.* 5.9−6.2 Å apart (O−O distance) (Figure 1b). The two C−O vectors, however, are not exactly parallel; rather they diverge away from the steroid. This suggested that the attachment of two large and flat aromatic surfaces to these two hydroxyl groups will lead to the formation of a deep cleft, thereby generating a new class of molecular tweezers in which structural variations could be accomplished in a straightforward manner.

Synthesis. 1-Acetylpyrene (**5a**) was prepared from pyrene (**4**) using Ac_2O/AcOH and anhyd $ZnCl_2$ in 83% yield.[14] Compound **5a** was oxidized with NaOCl in aq pyridine to acid **5b** in 75% yield, which was converted to acid chloride **5c** using either thionyl chloride or oxalyl chloride in benzene (Scheme 1).

Methyl deoxycholate **2a** was converted to **6** in 62% yield by careful esterification using Oppenauer conditions (CaH_2, toluene, $BnEt_3N^+Cl^-$, reflux) with pyrene-1-carbonyl chloride (**5c**) (Scheme 1). With the use of an excess of **5c**, and at a higher temperature, compound **7** was obtained in 86% yield from methyl deoxycholate **2a**. The C-7 hydroxyl group of **1** was selectively oxidized with NBS/aq $NaHCO_3$ to the corresponding ketone,[15] which upon esterification with methanolic HCl afforded methyl 7-ketocholate (**3a**) in 77% yield. Following the same esterification procedure as above, compound **8** was prepared from **3a** in 86% yield. The C-7 hydroxy derivative **9** was prepared in 89% yield by the reduction of the C-7 keto group of **8** with $NaBH_4$ in MeOH/THF (Scheme 2). A mixture of steroidal ester **6**, along with **5d**, was used as a control for all spectroscopic studies, including association constant measurements.

Fluorescence Spectra. Pyrene and its derivatives show intense fluorescence emission even at very low concentrations.[16] Our systems with two pyrenes placed at a distance of *ca.* 6−7 Å clearly are good substrates for studying their fluorescence properties. As the distance between the two pyrenes was close enough to sandwich a guest (which was apparent from InsightII calcula-

(12) (a) Heinemann, U.; Saenger, W. *Nature* **1982**, *229*, 27. (b) *Ibid. Pure Appl. Chem.* **1985**, *57*, 417. (c) Saenger, W. *Principles of Nucleic Acid Structure;* Springer-Verlag: New York, 1984; p 132. (d) Dahl, T. *Acta Chem. Scand.* **1994**, *48*, 95. (e) Stynes, D. V. *Inorg. Chem.* **1994**, *33*, 5022. (f) Burly, S. K.; Petsko, G. A. *Adv. Protein Chem.* **1988**, *39*, 125. (g) Burly, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23. (h) Burly, S. K.; Petsko, G. A. *J. Am. Chem. Soc.* **1986**, *108*, 7995.

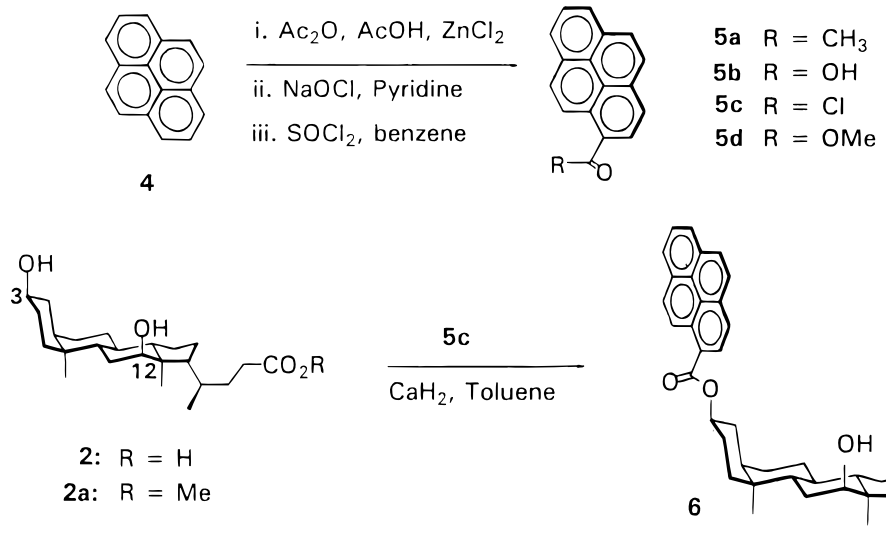
(13) PCMODEL version 4.0 and PCMODEL for Windows v. 5.13 were purchased from Serena Software, Bloomington. In general the minimizations were first carried out without hydrogens. Subsequently hydrogen atoms were put, and the resulting structure was minimized. The entire operation was repeated several times with different initial geometries until consistent results were obtained.

(14) Vollmann, H.; Becker, H.; Corell, M.; Streeck, H. *Liebigs Ann. Chem.* **1937**, 1, *531*, 108.

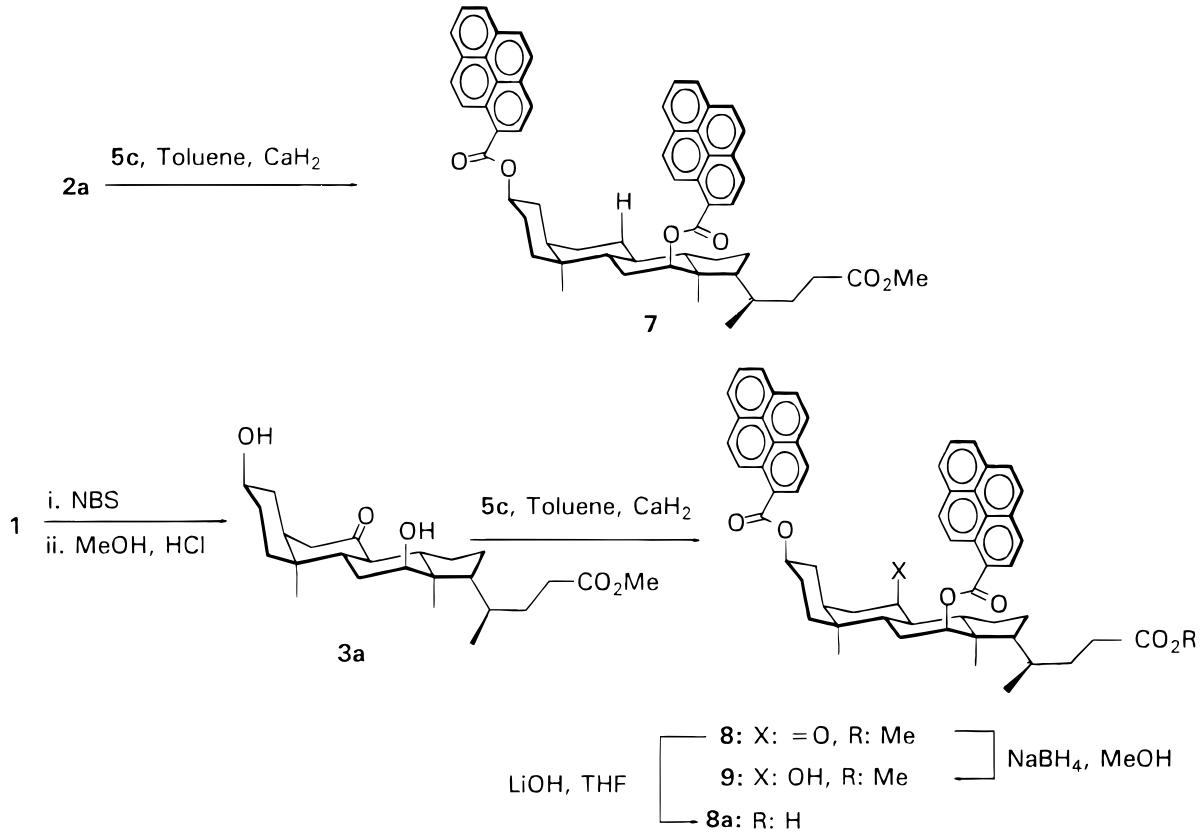
(15) Fieser, L. F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1950**, *72*, 5530; **1951**, *73*, 4133.

(16) (a) Iyoda, T.; Morimoto, M.; Kawasaki, N.; Shimadzu, T. *J. Chem. Soc., Chem. Commun.* **1991**, 1480. (b) Jin, T.; Ichikawa, K.; Koyama, T. *J. Chem. Soc., Chem. Commun.* **1992**, 499. (c) Aoki, I.; Sakaki, T.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 730. (d) Ueno, A.; Suzuki, I.; Osa, T. *J. Chem. Soc., Chem. Commun.* **1988**, 1373. (e) Sandanayake, K. R. A. S.; James, T. D.; Shinkai, S. *Chem. Lett.* **1995**, 503. (f) Aoki, I.; Harada, T.; Sakaki, T.; Kawahara, Y.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 1341.

Scheme 1



Scheme 2



tions),[17] fluorescence experiments were expected to give additional information in support of the proximity of the pyrenes in our tweezers. A strong *intra*molecular excimer emission was observed for all the tweezers (1.04 μM in 3% $CHCl_3/CH_3CN$), whereas, with the control run using **6** and **5d** each at 1.04 μM, only monomer emission was observed (Figure 2). This variation in emission behavior results in an important noticeable difference: Hosts **7**−**9** in $CHCl_3$ appear greenish, while a mixture of **6** and **5d** (control) at identical concentrations appears bluish when held in sunlight. These experiments suggested that the long-wave emission resulted from the interaction of the pyrene units from the same steroid unit and not through intermolecular interaction.

Fluorescence Spectra in the Presence of a Guest. Synergistic interaction between the two pyrenes in the hosts was further evident from fluorescence-quenching experiments. Figure 3 shows the fluorescence spectrum of host **7** (0.67 mM) with octafluoronaphthalene (**10b**; 33 mM) in $CHCl_3$. In this case the guest fluorescence was completely quenched in the presence of the host, and there was an *increase* in the monomer emission; however, there was no significant change in the intensity of excimer emission. This provides evidence that the guest sandwiches between the two pyrenes thereby increasing the monomer emission. Figure 3 also shows the fluores-

(17) InsightII minimization calculations showed that all three tweezers have a distance of separation of 5−6 Å (data not shown).

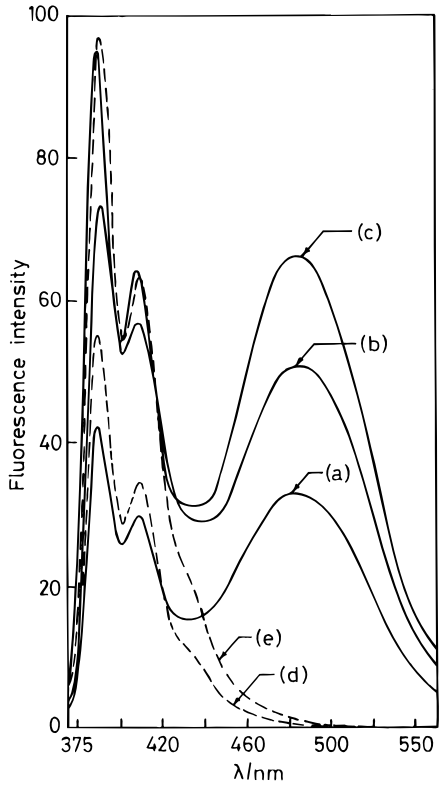


Figure 2. Fluorescence spectra (λ_{ex} = 355 nm) in 3% chloroform−acetonitrile: (a) **7**, (b) **8**, (c) **9**, and (d) **6**, each at 1.04 μmol dm^{-3}, and (e) **5d** plus **6**, each at 1.04 μmol dm^{-3}.

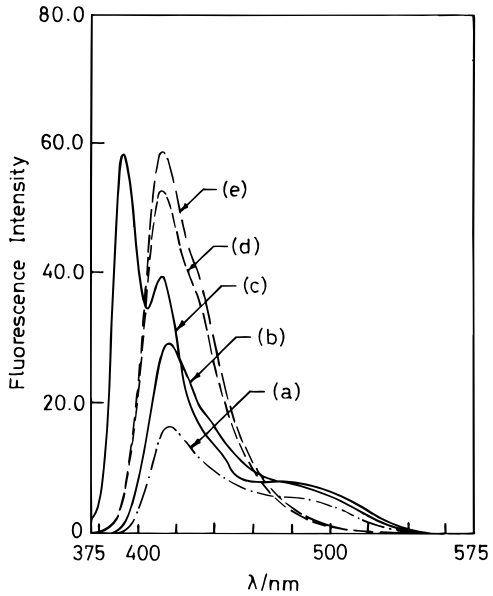


Figure 3. Fluorescence spectra (λ_{ex} = 355 nm) in chloroform: (a) **7** at 0.67 mmol dm^{-3}, (b) **7** at 0.67 mmol dm^{-3} plus **10b** at 33 mmol dm^{-3}, (c) **10b** at 33 mmol dm^{-3}, (d) **5d** plus **6**, each at 0.67 mmol dm^{-3}, and (e) **5d** plus **6**, each at 0.67 mmol dm^{-3} plus **10b** at 33 mmol dm^{-3}.

cence spectra for the controls (each at identical concentration as **7**) in the presence of guest **10b**. Although the fluorescence of the guest was quenched, there was no significant increase in the monomer emission. This clearly shows that the controls act as independent entities, and the quenching observed is due to the stacking of donor−acceptor groups which is a predominant interaction in organic solvents.

Absorption Spectra. UV−visible spectroscopic studies on these compounds indicated that the two pyrene moieties of compounds **7**−**9** did not interact in the ground state, since the spectral pattern of these compounds roughly matched the absorption of the control (*i.e.*, a mixture of **6** and **5d**). When a $CHCl_3$ solution of any of the hosts was mixed with trinitrofluorenone **10e**, trinitrobenzene **10d**, and 3,5-dinitrobenzonitrile (**10c**), deep red, red, and yellowish-red solutions were obtained, respectively. Tweezer **8** in the presence of trinitrofluorenone **10e** as the guest showed a well-defined charge-transfer band, as observed in the difference spectrum.[18] These observations support the existence of donor−acceptor interactions in our systems.

NMR Titration Experiments. Encouraged by the modeling work and fluorescence/UV experiments, we proceeded to use NMR spectroscopy as the tool to assess the binding strengths of our tweezers with different guests in $CDCl_3$. Before detailed NMR titration studies were initiated with **7**−**9**, an important control experiment was done. A *mixture* of **5d** and **6** (each at 0.031 M in $CDCl_3$) and 1,3,5-trinitrobenzene (**10d**; 0.06 M) was prepared in $CDCl_3$, and the NMR spectrum was recorded. This spectrum, when compared with that of a mixture of **5d** and **6**, showed that under these conditions there were no significant upfield shifts of the aromatic signals, suggesting the lack of any interaction under these conditions. *Interestingly, NMR titration experiments with trinitrofluorenone **10e** with the controls under conditions as stated above could not be carried out because of the poor solubility of the former in $CDCl_3$.* But this 'problem' was not encountered with tweezers **7**−**9** since all the guest (up to a maximum of 1.5 equiv) went into solution. This observation confirms that the hosts indeed increased the solubility of the guest (upon binding).

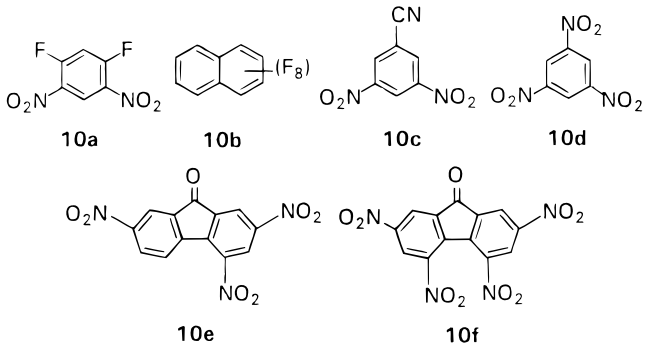
All NMR titration experiments were performed in $CDCl_3$ at 25 °C. Guests were usually titrated up to 70% saturation limits. Almost all aromatic ^{1}H NMR resonances of the hosts moved upfield (by varying degrees), but the aromatic region between δ 7 and 8.3 was quite complicated for analysis. However, one of the pyrene doublets (δ 8.91 in host **8**) moved considerably upfield and was well-separated from all other resonances, and this signal was monitored during the titration. The chemical shifts ($\Delta\delta$) were analyzed using a nonlinear curve-fitting program.[19] All experiments were repeated at least twice. When an NMR titration experiment was carried on methyl pyrene-1-carboxylate (**5d**) with guest **10c**, the estimated association constant was <5 M^{-1}. Thus, the order of magnitude difference with that of the host (K_a = 43 M^{-1}) was completely due to the presence of the other pyrene in a *preorganized* manner (Table 1). A variety of other electroneutral aromatics (pyrene, fluorene, anthracene, naphthalene, phenanthrene etc) or electron-rich aromatics (phenol, anisole, 1,4-dimethoxybenzene, etc.) showed no detectable binding.

Binding measurements were also attempted in other solvent systems, such as DMSO-d_6 and acetone-d_6. When compared to $CDCl_3$, similar shifts of the aromatic protons of the host **7** in DMSO-d_6 in the presence of guest **10b** were observed, but this study could not be pursued further because of solubility problems. The experiments

(18) Spectrum of (**8** + **10e** together in solution) − (computer sum of the spectra of **8** and **10e** taken separately). A similar difference spectrum was recorded with the controls.

(19) Macomber, R. S. *J. Chem. Ed.* **1992**, *69*, 375. The reliability of our data was assessed by the incorporation of the rms deviation of $\Delta\delta$ in the original program. rms deviation of <0.005 ppm indicated good fit of the data.

Table 1. Association Constants in $CDCl_3$ at 25 °C[a]



	guests, K_a (dm^3 mol^{-1})					
host	**10a**	**10b**	**10c**	**10d**	**10e**	**10f**
9	6	11	27	47	155	nd
7	7	15	35	65	189	nd
8	8	19	43	83	220	171

[a] nd: not determined.

done in acetone-d_6 with **7** and **10b** did not show any change in the chemical shifts, either of the host or of the guest.[20]

According to the donor−acceptor concept, more electron deficient is a group, tighter is the binding.[21] Our systems did not show this trend, as the K_a for tetranitrofluorenone **10f** was found to be *less* than that for trinitrofluorenone **10e**. These results possibly indicate that in addition to the interaction forces, the cleft dimension may also play an important role in binding.

NMR titration results mentioned earlier were analyzed by the curve-fitting method assuming a 1:1 stoichiometry of the complex formed between the host and the guest. This was proved by a separate titration of host **8** with guest **10e** to get a Job plot which showed a maximum at a mole ratio of 1.0.[22]

Crystal Structure of 8. In spite of repeated attempts, we were unable to grow crystals of host−guest complexes. Host **8**, however, was crystallized from a mixture of ethyl acetate and hexanes to yield needle-shaped crystals suitable for X-ray analysis.

The O_3-O_{12} distance in the solid state was found to be 5.78 Å (Figure 4). Although the distance is somewhat less than the ideal value, we believe that binding occurs in solution because of the flexibility of the pyrene esters to self-adjust the cleft dimensions in solution.

The packing diagram from the X-ray structure shown in Figure 5 consists of molecules of the compound packed in the unit cell.[23] There are no abnormally close contacts between the molecules. The distances and angles within the molecule appear to be normal. The two pyrene planes

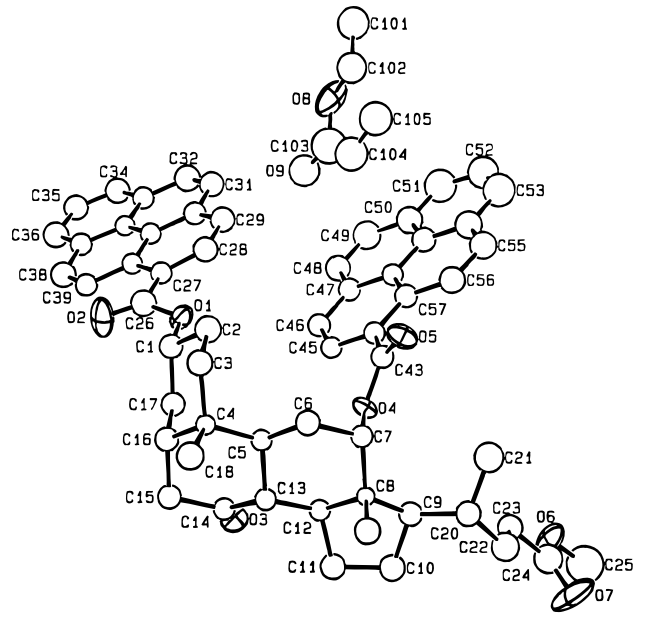


Figure 4. X-ray structure of **8**. Labeling diagram showing molecule and solvent. Hydrogen atoms are numbered according to the atom to which they are attached but are omitted from the figure for clarity. See ref 23 for information on the solvent molecule in the crystal.

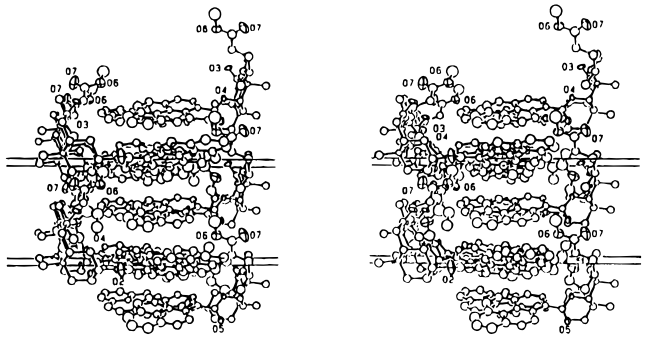


Figure 5. Stereoview of the X-ray structure of **8** (packing diagram). View down the *b* axis showing one of the aliphatic−aromatic−aliphatic bilayers in the structure. The packing of the aromatic−aromatic rings are centered on $z = 0, 1/2, 1$, etc.

are almost parallel to each other and to the *c* axis.[24] The primary interaction is that of pyrene rings interacting in a "herringbone"[25] fashion (edge to face) around the planes at $z = 0, 1/2, 1$, etc., with rings entering the interaction region from aliphatic backbones located in the positive and negative *c* direction alternately. Each pyrene unit is interacting with the adjacent four pyrenes with a edge to face manner.

The two-dimensional supramolecular organization of the pyrene moieties suggests that the bile acid backbone may possibly be used to organize other flat aromatic units to create a specific arrangement of such systems.[26] Such organization will allow one to orient aromatic residues in a predictable way. It will also be of interest to engineer such crystals and study their material properties, particularly for host−guest types of systems.

(20) At this time we are not sure of the reasons for such solvent effects.

(21) Otsuki, J.; Chiang, L.-C.; Lee, S.-H.; Araki, K.; Seno, M. *Supramol. Chem.* **1993**, *2*, 25.

(22) An attempted "intracomplex" NOE measurement between **8** and **10e** was not successful.

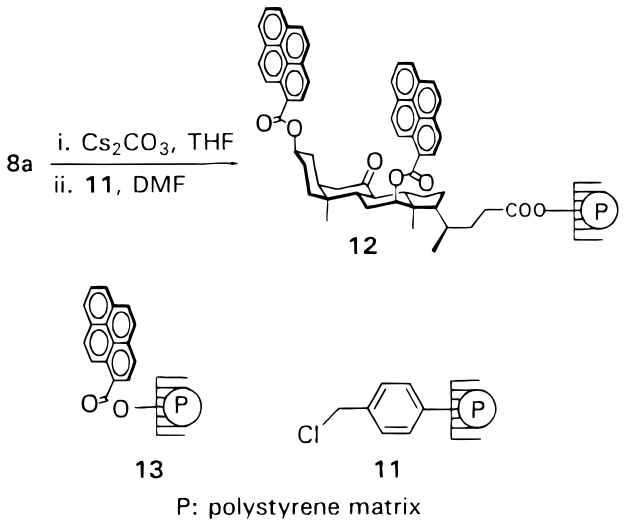
(23) Given the crystallization conditions, the composition of this solvent molecule almost certainly varies from crystal to crystal. The solvent in this case seems to be a mixture of about 65% ethyl acetate and 35% *n*-hexane. The fractional occupancy of atom O-9 is 0.65 and of atom C-105 is 0.35; O-8 is given full occupancy as an oxygen atom even though it should theoretically be 65% O and 35% C. There might also be some contribution from isohexane, which has the same basic shape as ethyl acetate. The empirical formula is based on full occupancy by ethyl acetate.

(24) (a) Desiraju, G. R.; Gavezzotti, A. *J. Chem. Soc., Chem. Commun.* **1989**, 621. (b) Kai, Y.; Hama, F.; Yasuoka, N.; Kasai, N. *Acta Crystallogr.* **1978**, *B34*, 1263. (c) Munakata, M.; Dai, T.; Maekawa, M.; Takayoshi K.-S.; Fukui, J.-T. *J. Chem. Soc., Chem. Commun.* **1994**, 2331.

(25) Burley, S. K.; Petsko, G. A. *J. Am. Chem. Soc.* **1986**, *108*, 7995.

(26) Otsuki, J.; Oya, T.; Lee, S.-H.; Ariki, K. *J. Chem. Soc., Chem. Commun.* **1995**, 2193.

Scheme 3



Immobilization of Tweezer 8. After demonstrating the binding of polynitro aromatic hydrocarbons (PAH's) by the steroidal tweezers, it was of interest to further exploit and develop these compounds as immobilized hosts.[27] The side chain of the steroid provides a built-in handle for attaching the tweezers to a polymeric support.

Compound **8a** was obtained from **8** by treating it with LiOH/THF/MeOH in 89% yield. This acid was converted into its cesium salt using aq Cs_2CO_3/THF (Scheme 3). The salt obtained was directly reacted with 1% cross-linked chloromethylated polystyrene **11** in dry DMF at 80−90 °C for 24 h to yield the polymer-bound host **12**, which was washed thoroughly with DMF, water, and EtOH to remove any unreacted starting materials present. Similarly, the same reactions done on pyrene-1-carboxylic acid (**5b**) yielded **13** which was used as the control.

The pyrene loading in the polymer was estimated by hydrolyzing known weights of **12** and **13** using NaOMe/MeOH/H_2O and weighing the pyrene-1-carboxylic acid. It was found that **12** contained 0.54 mmol of steroid units/g of functionalized polymer, and the control (**13**) contained 0.83 mmol of pyrene units/g of the functionalized polymer.

We examined the interaction of the polymer-bound tweezer with the same guests (*viz.*, **10d**−**f**), which were used for solution studies as follows. A solution of the three guests (0.5 mL of 1×10^{-4} M each) in chloroform was stirred with a known weight of the polymer-bound host and the control for 24 h, taking care to prevent the evaporation of the solvent. After filtration, a known volume of the filtrate was analyzed by HPLC (85:15 EtOAc/hexanes), and the areas under the three guest peaks were measured. The original guest solution (10−20 μL) was also analyzed by HPLC under identical conditions. The decrease in any peak area relative to the original guest solution implies its "*removal*" by the polymer. The percentage of guest bound to **12** and **13** is shown in Table 2.[28]

It is interesting to see that even the controls were removing the nitroaromatics to a small extent (practically no binding was detected by NMR, *vide infra*). However,

Table 2. Guest Binding by 12 and 13

	% guest bound	
guests	**12**	**13**
10d	52	8.2
10e	75	37
10f	68	37

the magnitude of binding with respect to that of host is important, which means that the pyrene units in **13** are acting cooperatively. The polymer-bound control **13** did not bind trinitrobenzene **10d** efficiently.

The results obtained with **10e**,**f** are interesting, and it was found that **10e** binds to tweezer **12** more tightly than **10f**. *The relative degree of "removal" of the three guests follows their relative association constants observed in $CDCl_3$ by NMR titration experiments.* Estimated binding constant from HPLC data for **12** and **10e** was 97 M^{-1}.

Conclusion

We have been able to readily construct a family of bile acid-based molecular tweezers which bind polynitroaromatic compounds with moderate association constants in $CDCl_3$. Analysis of the solid state structure of **8** indicated that *this structure represents a novel organization of the bile acid backbone which has not yet been observed in other bile acid crystals*. Binding of the guests with a polymer-bound host was demonstrated, and this is likely to be of practical interest when more efficient hosts are developed.

We believe that our simple approach toward the design of steroidal molecular tweezers has provided sufficiently interesting results to warrant further investigations, and our attractive bile acid-based molecular tweezers are likely to generate more interests, both academically as well as in applied areas.

Experimental Section

General. All reactions were conducted under dry nitrogen and stirred magnetically unless otherwise stated. Cholic acid, 7-deoxycholic acid, and pyrene were purchased from Fluka. The polynitroaromatic compounds were commercial samples or prepared according to literature procedures. All solvents were purified and distilled before use.[29] Toluene, benzene, and tetrahydrofuran were distilled from sodium/benzophenone ketyl. Methanol was distilled from magnesium methoxide. Thin layer chromatography was performed on precoated plates (silica gel 60F-254) purchased from Sigma. These plates were stained either with iodine vapor or with 5−10% phosphomolybdic acid in ethanol. Usually purification of the products was done using gravity columns. Melting points were recorded in open capillaries and are uncorrected. Proton NMR spectra were recorded on 90, 200, 270, and 400 MHz spectrometers. Unless otherwise stated ^{1}H NMR spectra were taken in $CDCl_3$ using $CHCl_3$ as the internal standard (δ = 7.270). For ^{13}C NMR spectra the peak at 77.0 ppm arising from $CDCl_3$ was used as the internal reference. All chemical shift values shown are in δ scales. Multiplicity of NMR signals is designated as s (singlet), d (doublet), t (triplet), etc. Broad unresolved lines are designated as br m (broad multiplet).

Optical rotations were measured in appropriate solvents using sodium D light. Microanalyses were done on an automated CHNS analyzer. LR mass spectral data are given as *m*/*z* (% abundance). FAB mass spectra were recorded using argon/xenon (6 kV, 10 mA) as the FAB gas using *m*-nitrobenzyl

(27) (a) Zimmerman, S. C.; Saionz, K. W. *J. Am. Chem. Soc.* **1995**, *117*, 1175. (b) Smith, P. W.; Chang, G.; Still, W. C. *J. Org. Chem.* **1988**, *53*, 1587. (c) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *16*, 373. (d) Yoon, S. S.; Still, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 823.

(28) No significant (>5% loss of signal) binding was observed with resin **11**.

(29) (a) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1988. (b) Vogel, A. I. *Textbook of Practical Organic Chemistry*, 4th ed.; Longman Group, 1978.

alcohol as a matrix. Infrared spectra were taken in $CHCl_3$, as a thin film on NaCl plates (neat),[30] or in Nujol. HPLC analysis was carried out on either (a) an ODS column (25 cm × 4.6 mm i.d.) using H_2O/MeOH or (b) a silica gel column using EtOAC/*n*-hexane as the mobile phase. Samples were detected using a variable wavelength UV detector and the resulting data analyzed on a data processor.

Methyl 7-Deoxycholate (2a). This was prepared using deoxycholic acid and methanolic HCl following a literature procedure in 90% yield.[31]

Methyl 3α,12α-Dihydroxy-7-keto-5β-cholan-24-oate (3a).[32] To a warm (*ca.* 60 °C) mixture of $NaHCO_3$ (2.5 g, 29.8 mmol) in water (80 mL) was added cholic acid (2.5 g, 6.12 mmol) till it dissolved completely. The solution was cooled to about 15 °C, and *N*-bromosuccinimide (2.18 g, 12 mmol) was added slowly over a period of 30 min. The mixture was stirred for 14 h at rt when the NBS dissolved completely. It was then heated in an oil bath (80−100 °C) for 1 h. The solution was cooled and acidified with 5 M HCl. The white precipitate was filtered off, washed thoroughly with water, and dried under high vacuum. The crude product (2.4 g) was dissolved in dry methanol (15 mL), and 2% HCl in CH_3OH (4 mL) was added. The mixture was stirred at rt for 22 h. Volatiles were removed under reduced pressure, and the crude product was purified by column chromatography (silica gel 100−200 mesh, 42 g, 12 cm × 3 cm) using 50−70% EtOAc/hexanes as the eluent. The ester was isolated in 77% yield (1.91 g): IR (neat) 3390 (s), 1710 (s), 1440 (m), 1254 (m), 1060 (m) cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 0.69 (s, 3H), 1.01 (d, 3H, J = 6.4 Hz), 1.19 (s, 3H), 1.3−2.5 (m, steroidal CH and CH_2), 2.90 (m, 1H), 3.64 (br m, 1H), 3.70 (s, 3H), 4.00 (s, 1H).

1-Acetylpyrene (5a). A homogeneous solution of pyrene (2 g, 9.9 mmol) in acetic anhydride (7.5 mL, 73.5 mmol) was added to a solution of zinc chloride (2.5 g, 18 mmol) in acetic acid (7.5 mL, 125 mmol) at 85 °C. The mixture was stirred at 80−90 °C for 1 h, and the resulting thick red precipitate was filtered off. This complex was decomposed by adding crushed ice and was left in the refrigerator for 4 h. The clear supernatant was filtered, and the residue was dried under high vacuum and then crystallized from hot MeOH. The pure product was obtained in 83% yield (2 g): mp 88−89 °C (lit.[14] mp 90 °C).

Pyrene-1-carboxylic Acid (5b). A mixture of 1-acetylpyrene (**5a**) (2.3 g, 9.38 mmol) and pyridine (1.95 mL, 24.4 mmol) was stirred at 85−90 °C. After 5 min 4% NaOCl solution (70 mL, 37.6 mmol) was added, and the mixture was heated at the same temperature for 1 h. Volatiles were distilled off; the mixture was concentrated and filtered. The white voluminous precipitate was dissolved in hot water (150 mL) and filtered through a fluted filter paper. The filtrate was cooled and acidified with dilute HCl, and the pale yellow mass was filtered, dried, and sublimed at reduced pressure. The pure product was obtained in 75% yield (1.73 g) and melted at 260−261 °C (lit.[14] mp 261−263 °C).

Methyl Pyrene-1-carboxylate (5d). Methyl ester of pyrene-1-carboxylic acid was prepared using **5b** and MeOH/concd H_2SO_4. The crude product was crystallized from MeOH: mp 75−76 °C; UV (λ_{max}, log ϵ) (3% $CHCl_3$/CH_3CN, v/v) 350 (4.33), 280 (4.42), 243 (4.64); ^1H NMR (90 MHz, $CDCl_3$) δ 4.15 (s, 3H), 8.0−8.35 (m, 7H), 8.61 (d, 1H, J = 8.1 Hz), 9.24 (d, 1H, J = 9.9 Hz).

Pyrene-1-carbonyl Chloride (5c). Pyrene-1-carboxylic acid (**5b**) (1 mmol) was refluxed with an excess of thionyl chloride (3−4 mmol) for 3 h at 80 °C. Excess thionyl chloride and volatiles were distilled off, and the residue was dried under reduced pressure for several hours. The crude acid chloride was used directly for all esterification reactions.

Methyl 3α-((1-Pyrenoyl)oxy)-12α-hydroxy-5β-cholan-24-oate (6). To a stirred solution of methyl deoxycholate (0.3 g, 0.737 mmol) in dry toluene (4 mL) were added CaH_2 (0.046 g, 1.1 mmol), $BnEt_3N^+Cl^-$ (0.041 g, 0.18 mmol), and pyrene-1-carbonyl chloride (0.254 g, 0.96 mmol). This mixture was heated in an oil bath at 60 °C for 14 h. The mixture was filtered through a bed of Celite, and the residue was washed with ethyl acetate. The combined organic layer was washed with 7% $NaHCO_3$ solution, water, and brine, finally dried over anhyd Na_2SO_4, and filtered. The solvent was removed *in vacuo* to yield the crude product, which was purified by column chromatography on silica gel (100−200 mesh, 13 g, 23 × 1.4 cm) using 10−20% EtOAc/hexanes as the eluent. The pure product was crystallized from EtOAc/hexane (1:1) and weighed 0.25 g (62%): mp 200−201 °C; IR (neat) 3010 (m), 2940 (s), 1710 (s), 1690 (m), 1440 (s), 840 (s), 950 (s) cm^{-1}; $[\alpha]_D^{21}$ +53 (*c* 5.34, $CHCl_3$); UV (λ_{max}, log ϵ) (6% $CHCl_3$/CH_3CN, v/v) 350 (4.31), 280 (4.36), 243 (4.61); ^1H NMR ($CDCl_3$, 270 MHz) δ 0.75 (s, 3H), 0.99 (m, 6H), 1.01−2.40 (m, steroidal CH and CH_2), 3.60 (s, 3H), 4.02 (s, 1H), 5.19 (br m, 1H), 8.28−8.03 (m, 7H), 8.63 (d, 1H, J = 9.5 Hz), 9.24 (d, 1H, J = 9.5 Hz); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 12.5, 17.1, 23.0, 23.4, 25.9, 26.7, 26.8, 27.2, 28.6, 29.5, 30.7, 30.8, 32.3, 33.6, 34.0, 34.8, 41.8, 46.3, 47.1, 48.1, 51.3, 72.9, 75.0, 123.9, 124.0, 124.2, 124.7, 125.8, 125.9, 126.0, 127.0, 128.1, 129.1, 129.2, 130.2, 130.7, 130.8, 133.9, 167.4, 174.4; MS: 634 (M^+), 291, 246 (100), 57 (100), 43; HRMS calcd for $C_{42}H_{50}O_5$ 634.3658, found 634.3651.

Methyl 3α,12α-Bis((1-pyrenoyl)oxy)-5β-cholan-24-oate (7). To a stirred solution of methyl deoxycholate (0.40 g, 0.98 mmol) in dry toluene (5 mL) were added CaH_2 (0.124 g, 2.95 mmol), $BnEt_3N^+Cl^-$ (0.055 g, 0.24 mmol), and pyrene-1-carbonyl chloride (0.573 g, 2.16 mmol). The resulting mixture was heated in an oil bath at 110 °C for 24 h. The reaction mixture was filtered through a pad of Celite which was subsequently washed with ethyl acetate. The combined organic layer was washed with 7% $NaHCO_3$ solution, water, and brine, finally dried over anhyd Na_2SO_4, and filtered. The solvent was removed *in vacuo* to yield the crude product which was purified on silica gel (100−200 mesh, 13 g, 13 cm × 1.8 cm) using 10−20% EtOAc/hexanes as the eluent. The pure product was crystallized from EtOAc/hexane (1:1) and weighed 0.73 g (86% yield): mp 183−185 °C; $[\alpha]^{21}_D$ +137 (*c* 1.90, $CHCl_3$); UV (λ_{max}, log ϵ) (6% $CHCl_3$/CH_3CN, v/v) 349 (4.63), 279 (4.70), 243 (4.93); IR (neat) 2910 (s), 1720 (s), 1695 (s), 1440 (s), 1250 (s), 840 (s), 750 (s) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.95 (s, 3H), 1.0 (d, 3H, J = 6.5 Hz), 1.07 (s, 3H), 1.14−2.43 (m, steroidal CH and CH_2), 3.61 (s, 3H), 5.15 (br m, 1H), 5.63 (s, 1H), 7.47 (d, 1H, J = 8.1 Hz), 7.60 (t, 1H, J = 7.6 Hz), 7.68 (d, 1H, J = 8.9), 7.75 (d, 1H, J = 7.5 Hz), 7.81 (d, 1H, J = 9.4 Hz), 7.89 (t, 2H, J = 8.8 Hz), 7.97 (d, 2H, J = 4.8 Hz), 8.04 (d, 1H, J = 8.1 Hz), 8.07 (d, 1H, J = 8.1), 8.12 (d, 1H, J = 7.0), 8.14 (d, 1H, J = 7.5 Hz), 8.20 (t, 2H, J = 4.4 Hz), 8.58 (d, 1H, J = 8.0 Hz), 8.95 (d, 1H, J = 9.4 Hz), 9.21 (d, 1H, J = 9.4 Hz); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 12.7, 17.8, 23.1, 23.6, 25.8, 26.3, 26.9, 27.4, 29.8, 30.9, 31.1, 32.5, 34.3, 34.9, 35.1, 35.9, 41.9, 45.6, 48.0, 50.1, 51.5, 74.9, 76.8, 123.9, 124.1, 124.4, 124.7, 125.4, 126.0, 126.1, 126.2, 127.0, 127.1, 127.2, 127.7, 128.0, 129.0, 129.3, 129.3, 129,4, 130.3, 130.5, 130.6, 130.8, 131.0, 133.9, 133.9, 167.7, 168.0, 174.6; MS 863 (M^+, 16), 220, 154 (100); HRMS calcd for $C_{59}H_{58}O_6$ 862.4233, found 862.4226.

Methyl 3α,12α-Bis((1-pyrenoyl)oxy)-7-keto-5β-cholan-24-oate (8). To a stirred solution of methyl 3α,12α-dihydroxy-7-keto-5β-cholan-24-oate (0.216 g, 0.515 mmol) in dry toluene (2.6 mL) were added CaH_2 (0.0864 g, 2 mmol), $BnEt_3N^+Cl^-$ (0.03 g, 0.13 mmol), and pyrene-1-carbonyl chloride (0.3 g, 1.13 mmol). This was stirred in an oil bath at 110 °C for 24 h. The reaction mixture was cooled, filtered through a bed of Celite, and washed with ethyl acetate. The combined organic layer was washed with 7% $NaHCO_3$ solution, water, and brine, finally dried over anhyd Na_2SO_4, and filtered. The solvent was removed *in vacuo* to yield the crude product. The crude product was purified by column chromatography on silica gel (100−200 mesh, 16 g, 15 × 2.4 cm) using 15−30% EtOAc/hexanes as the eluent. The pure product was crystallized from EtOAc/hexane (1:1) and weighed 0.41 g (86%): mp 161−162 °C; IR (neat) 3001 (m), 2920 (s), 2850 (s), 1740 (s), 1690 (s),

(30) Even with solids with high melting points the IR data were sometimes taken before crystallization, when the material was still in a glassy form.

(31) (a) Fieser, L.; Rajagopalan, S. *J. Am. Chem. Soc.* **1949**, *71*, 3935. (b) See also: Reigel, B.; Moffett, R. B.; Mcintosh, A. V. *Organic Syntheses;* Wiley: New York, 1955; Collect. Vol. 3, p 237.

(32) Fieser, L. F.; Rajagopalan, S. *J. Am. Chem. Soc.* **1950**, *72*, 5530; **1951**, *73*, 4133.

1590 (s), 1440 (s), 840 (s) and 740 (s) cm^{-1}; $[\alpha]^{22}_D$: +131 (*c* 1.902, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.90 (s, 3H), 0.98 (d, 3H, J = 6.5 Hz), 1.03−2.3 (m, steroidal CH and CH_2), 2.57 (m, 2H), 2.99 (m, 1H), 3.59 (s, 3H), 5.08 (br m, 1H), 5.63 (s, 1H), 7.48 (d, 1H, J = 8.1 Hz), 8.19−7.56 (m, 14H), 8.55 (d, 1H, J = 8.0 Hz), 8.90 (d, 1H, J = 9.5 Hz), 9.10 (d, 1H, J = 9.5 Hz); ^{13}C NMR ($CDCl_3$, 100.5 MHz) δ 13.0, 14.3, 18.0, 21.1, 22.8, 24.5, 26.3, 26.5, 27.6, 31.0, 31.2, 33.5, 33.9, 34.8, 34.9, 37.4, 42.9, 45.4, 45.8, 47.3, 49.6, 51.5, 60.5, 73.4, 76.0, 123.7, 123.9, 124.1, 124.2, 124.4, 124.5, 124.5, 124.7, 124.9, 126.1, 126.1, 126.2, 126.2, 127.0, 127.2, 127.9, 128.1, 128.5, 129.2, 129.4, 129.6, 130.2, 130.3, 130.8, 130.8, 130.9, 134.0, 134.2, 167.4, 167.7, 174.5, 211.6; UV (λ_{max}, log ϵ) (6% $CHCl_3/CH_3CN$, v/v) 383 (4.05), 350 (4.65), 280 (4.65), 243 (4.91); MS (FAB) 877 (100), 632 (15), 460 (22). Anal. Calcd for $C_{59}H_{56}O_7$: C, 80.79; H, 6.44. Found: C, 80.75; H, 6.44.

Methyl 3α,12α-Bis((1-pyrenoyl)oxy)-7α-hydroxy-5β-cholan-24-oate (9). Methyl 3α,12α-bis((1-pyrenoyl)oxy)-7-keto-5β-cholan-24-oate (**8**) (0.1 g, 0.11 mmol) was dissolved in THF (0.2 mL), and methanol (1 mL) was added. The reaction mixture was cooled in an ice bath, and $NaBH_4$ (6 mg, 0.17 mmol) was added. The reaction mixture was stirred at rt (*ca*. 24 °C) for 4 h. Volatiles were removed *in vacuo*, and the residue was taken up in ethyl acetate. The organic layer was washed with 7% $NaHCO_3$ solution, water, and brine, finally dried over anhyd Na_2SO_4, and filtered. The solvent was removed in vacuo to yield the crude product, which was purified by column chromatography on silica gel (100−200 mesh, 8 g, 18 cm × 1.4 cm) using 30−40% EtOAc/hexanes as the eluent. The pure product was crystallized from EtOAc/hexane (1:1) and weighed 0.09 g (89% yield): mp 172−174 °C; $[\alpha]^{22}_D$: +105 (*c* 4.904, $CHCl_3$); IR (neat) 3100 (s), 2920 (s), 2840 (m), 1690 (s), 1720 (s), 1590 (m), 750 (s) cm^{-1}; UV (λ_{max}, log ϵ) (6% $CHCl_3/CH_3CN$, v/v) 383 (4.02), 350 (4.66), 280 (4.66), 243 (4.94); ^1H NMR (400 MHz, $CDCl_3$) δ 0.91 (s, 3H), 0.99 (d, 3H, J = 6.6 Hz), 1.01 (s, 3H), 1.10−2.30 (m, steroidal CH and CH_2), 2.74 (m, 2H), 3.56 (s, 3H), 5.04 (br m, 1H), 5.67 (s, 1H), 7.44 (d, 1H, J = 8.0 Hz), 7.61 (t, 1H, J = 7.7 Hz), 8.09−7.69 (m, 12H), 8.20 (d, 1H, J = 8.7 Hz), 8.60 (d, 1H, J = 8.0 Hz), 8.98 (d, 1H, J = 9.5 Hz), 9.18 (d, 1H, J = 9.5 Hz); ^{13}C NMR (100.5 MHz, $CDCl_3$) δ 12.4, 14.1, 17.7, 22.3, 23.0, 25.5, 27.0, 27.2, 28.2, 29.2, 29.6, 30.7, 30.9, 34.4, 34.7, 34.8, 35.0, 35.4, 39.6, 41.2, 43.6, 45.3, 47.7, 51.3, 68.1, 74.8, 76.8, 123.8, 124.0, 124.0, 124.1, 124.3, 124.4, 124.6, 124.7, 125.2, 125.8, 125.9, 126.5, 126.8, 127.0, 127.9, 128.0, 128.8, 129.1, 129.2, 129.5, 130.1, 130.1, 130.4, 130.5, 130.7, 130.8, 133.7, 133.9, 167.6, 168.1, 174.4; MS (FAB) 879 (M^+, 50), 229 (100). Anal. Calcd for $C_{59}H_{58}O_7$: C, 80.61; H, 6.65. Found: C, 80.60; H, 6.68.

Experimental Procedure for NMR Titration. As a specific example, titration of methyl 3α,12α-bis((1-pyrenoyl)-oxy)-7-keto-5β-cholan-24-oate (**8**) with octafluoronaphthalene (**10b**) is described here. A 0.0758 M stock solution of host ($CDCl_3$) and a 0.187 M stock solution of guest ($CDCl_3$) were prepared. In 8 separate NMR tubes, 100 μL of the of host solution and 0, 20, 30, 40, 60, 80, 120, and 200 μL of the guest solution were added.[33] The total volume in each NMR tube was adjusted to 400 μL by adding $CDCl_3$. ^1H NMR spectra were recorded for each tube, and $\Delta\delta$ values were calculated by subtracting the chemical shift of interest (δ 8.91 doublet) in the spectrum of the mixture (δ_x) from the same resonance in the spectrum of pure host (δ_o). Thus, a titration curve of $\Delta\delta$ vs mole ratio of guest/host was plotted. The data were analyzed using a nonlinear curve-fitting program, and the output file gave the values of K_a (19 M^{-1}) and $\Delta\delta_{max}$ (0.5 ppm). We have modified the program to give the value of the rms deviation of $\Delta\delta$ ($\Delta_{calc}-\Delta\delta_{obs}$), and the calculated value ($\Delta\delta_{rms}$) in this case was 0.005 33 ppm.

Job Plot for Trinitrofluorenone 10e and 8.[34] This experiment involved the preparation of a standard solution of the guest (G = **10e**) (0.015 g, 0.03 M in $CDCl_3$) and the host (H = **8**) (0.05 g, 0.057 M in $CDCl_3$). In nine NMR tubes standard solutions of host and guest were mixed in different proportions, starting from 0.125 to 0.875 mole fraction so that the [H] + [G] was kept constant while varying [H]/[G]. The total volume of $CDCl_3$ in the NMR tube was 400 μL. ^1H NMR spectra were recorded for each NMR tube, and $\Delta\delta$ values were calculated by subtracting the chemical shift of the doublet of one of the pyrenes in the spectrum of the mixtures (δ_x) from the same resonance of the pure host (δ_o) (δ 8.91). Using host molarities and $\Delta\delta_{max}$, the actual concentration of the complex was calculated. A graph of the concentration of the complex vs mole fraction was plotted. The maximum corresponded to 0.5 mole fraction, confirming a 1:1 stoichiometry.

Fluorescence Experiments with Guest. Standard solutions of host **8** (0.66 mM) and guest **10b** (33 mM) in $CHCl_3$ were prepared. Fluorescence spectra were recorded for host, guest, and the host−guest mixture. The excitation wavelength and the measured emission ranges were 355 and 375−600 nm, respectively. A similar procedure was followed for the controls also using monopyrene **6** and methyl pyrene-1-carboxylate (**5d**) each at 0.66 mM concentration.

Determination of the Charge-Transfer Bands by the UV Method.[35] The UV spectrum of host **8** (3.52×10^{-4} M in $CHCl_3$) and guest **10e** (0.031 M $CHCl_3$) were taken separately in the 420−600 nm range using a 1 mm quartz cuvette. The sum of these two were done by the computer to give the spectrum of (H + G). Similarly, the UV spectrum of the mixture (HG) was recorded. The difference spectrum [(HG) − (H + G)] gives the charge-transfer spectrum which is usually seen in the longer wavelength region than the normal absorptions. A similar experiment was also done for the controls taking **6** and **5d** each at identical concentrations as above.

Polymer-Bound Molecular Tweezer: 3α,12α-Bis((1-pyrenoyl)oxy)-7-keto-5β-cholan-24-oic Acid (8a). Methyl 3α,12α-bis((1-pyrenoyl)oxy)-7-keto-5β-cholan-24-oate (**8**) (0.08 g, 0.093 mmol) was dissolved in THF/MeOH (0.5:1, v/v). An aqueous solution of LiOH (0.3 mL of 1.25 M, 0.38 mmol) was added, and the mixture was stirred at room temperature for 5 h. The reaction mixture was acidified with dilute HCl, and the volatiles were removed on a rotavapor. The residue was taken up in ethyl acetate (5 mL), and the organic layer was washed with water and brine, finally dried over anhyd Na_2SO_4, and filtered. The solvent was removed in vacuo to yield the crude product, which was purified by column chromatography on silica gel (100−200 mesh, 1.2 cm × 12 cm) using 5−10% EtOAC/$CHCl_3$ as the eluent. The pure product was obtained in 89% yield (0.07 g): mp 178−180 °C; IR (neat) 2940 (s), 1730 (s), 1580 (m), 1450 (m), 1250 (m), 850 (s), 750 (s) cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 0.90 (s, 3H), 0.98 (d, 3H, J = 6.3 Hz), 1.0−2.40 (m, steroidal CH and CH_2), 2.58 (m, 2H), 3.0 (m, 1H), 5.2 (br m, 1H), 5.61 (s, 1H), 7.55 (d, 3H, J = 6.5 Hz), 8.27−7.27 (m, 15H), 8.55 (d, 1H, J = 8.1 Hz), 8.91 (d, 1H, J = 9.4 Hz), 9.10 (d, 1H, J = 9.4 Hz); MS (FAB) 863 (M^+ + H, 82), 229 (100). Anal. Calcd for $C_{58}H_{54}O_7$: C, 80.72; H, 6.31. Found: C, 80.28; H, 6.24.

Preparation of Polymer-Bound Host 12.[36] 3α,12α-bis-((1-pyrenoyl)oxy)-7-keto-5β-cholan-24-oic acid (**8a**) (0.181 g, 0.21 mmol) was suspended in THF (1 mL), and Cs_2CO_3 (0.05 g in 0.3 mL of H_2O, 0.15 mmol) was added. After stirring for 3 h, the solvents were evaporated and the residue was dried in vacuum. To this residue (0.2 g, 0.3 mmol) were added DMF (2 mL) and 1% cross-linked chloromethylated polystyrene (0.18 g, 0.18 mmol). The mixture was heated in an oil bath at 80−100 °C for 24 h. The reaction mixture was filtered and washed thoroughly with dilute HCl, DMF, EtOH, THF, and dry acetone to remove any soluble material. After drying under high vacuum, the polymer weighed 0.33 g (0.025 g of **8a** was recovered). For estimating the degree of pyrene loading, the polymer-bound host (20 mg) was refluxed with an excess of

(33) For all NMR titration experiments the same gas tight microliter syringe was used.

(34) (a) Job, A. *Ann. Chim.* (*Paris*) **1928**, *9*, 113. (b) Gil, V. M. S.; Olivira, N. C. *J. Chem. Ed.* **1990**, *67*, 473.

(35) Schwartz, M. H. *J. Inclusion Phenom. Mol. Recog.* **1990**, *9*, 1.

(36) Shrerrington, D. C.; Hodge, D. *Synthesis and Separation using Functional Polymers*; Wiley: New York, 1988.

(37) The author has deposited atomic coordinates for **8** with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.

sodium methoxide in MeOH/H_2O. After 24 h, the reaction mixture was acidified (dilute HCl) and stirred for 1 h. The reaction mixture was filtered, and the residue was washed thoroughly with EtOH. Volatiles were removed from the filtrate, and the residue was thoroughly dried under vacuum. The resulting pyrene-1-carboxylic acid (**5b**) and the steroid were separated by chromatography on a short silica gel column using 50% EtOAc/hexanes as the eluent. Pure pyrene-1-carboxylic acid obtained weighed 7.5 mg (0.03 mmol). The Merrifield resin was reported to have 1 mequiv of active chlorine/g of resin. Calculation based on the amount of the pyrene-1-carboxylic acid obtained showed that **12** contained 0.54 mmol of steroid units/g of functionalized polymer.

Preparation of Polymer-Bound Control 13. From pyrene-1-carboxylic acid (**5b**) (0.1 g/1 mL of THF, 0.4 mmol) and Cs_2CO_3 (0.08 g/0.3 mL of H_2O, 0.24 mmol) the same procedure as above was followed to prepare the cesium salt of the acid. The crude cesium salt (0.15 g, 0.4 mmol) was suspended in DMF (2 mL). To this was added 1% cross-linked chloromethylated polystyrene (0.32 g, 0.32 mmol), and the coupling and purification were carried out as for **12**. The product weighed 0.32 g. Estimation of the amount of pyrene-1-carboxylic acid bound to the polymer was done by refluxing the polymeric product (20 mg) with an excess of sodium methoxide in MeOH and then hydrolyzing the methyl ester with HCl. The pure acid obtained weighed 9.5 mg (0.039 mmol). Calculation based on the amount of the pyrene-1-carboxylic acid obtained indicated that polymer **13** contained 0.83 mmol of pyrene units/g of the functionalized polymer.

Determination of Guest Selectivity of the Polymeric Materials by HPLC. Solutions (0.1 mM) of different guests (TNB-**10d**, TNF-**10e**, TENF-**10f**) in $CHCl_3$ (10 mL) were prepared. In three separate 2 mL volumetric flasks were taken 20 mg each of polymeric host **12**, control **13**, and Merrifield resin **11**; 0.5 mL of the standard guest solution was added to each, and the flasks were tightly stoppered. After stirring for 24 h, the $CHCl_3$ layer was filtered and analyzed by HPLC on a 250 mm × 4.6 mm silica gel column using 85:15 (v/v) hexane/EtOAc as a mobile phase at rt at a detector wavelength of 254 nm. The retention time (t_R) of each guest was determined prior to the binding studies. Table 2 containing data for percent guest bound to **12** and **13** is given in the Results and Discussion. From the ratios it is clear that **12** removes trinitrofluorenone most effectively. Merrifield resin **11** did not remove any of the guests under the identical conditions.

X-ray Structure Determination. Clear colorless columnar crystals of the compound were obtained by slow crystallization from petroleum ether/ethyl acetate: instrument used, Enraf-Nonius CAD-4 diffractometer, equipped with a nitrogen-flow low-temperature apparatus and controlled by a microVAX II computer; radiation, Mo Kα (λ = 0.710 73 Å); monochromator, highly oriented graphite (2θ = 12.2); detector, crystal scintillation counter, with PHA; reflections measured, $+h$, $+k$, $+l$; 2θ range, 3° → 45°; scan type, ω; scan width, $\Delta\omega = 0.60 + 0.35 \tan\theta$; scan speed, 5.49 ($\omega$, deg/min); background, measured over 0.25*($\Delta\omega$) added to each end of the scan; vertical aperture = 3.0 mm; horizontal aperture = 2.0 + 1.0 tan θ mm; no. of reflections collected, 3794; no. of unique reflections, 3763. Crystal data of **8**: Empirical formula, $C_{63}H_{64}O_9$, M_r = 965.2; orthorhombic, space group $P2_12_12_1$; a = 8.299(2) Å, b = 16.071-(2) Å, c = 38.037(6) Å, α = 90.0°, β = 90.0°, γ = 90.0°; V = 5073.10(24) Å^3, Z = 4; D_c = 1.26 g cm^{-3}; Mo Kα (λ = 0.710 73 Å); crystal size, 0.22 × 0.28 × 0.50 mm.

Acknowledgment. We are grateful to Prof. Paul A. Bartlett for help with the X-ray analysis. We thank Dr. Frederick J. Hollander for performing the X-ray analysis of **8** in the CHEXRAY facility at the University of California at Berkeley. Thanks are due to the SERC and the SIF of this institute for providing computing and high-field NMR facilities, respectively. L.J.D. thanks the UGC for a graduate fellowship. This work was supported by a grant from the Department of Science and Technology (SP/S1/G09/91), New Delhi.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **6**−**9**, ^{1}H NMR spectrum of **8a**, representative NMR titration data, two other views of the packing diagram of **8**, and charge-transfer spectrum and Job plot for the **8·10e** complex (13 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9607525