Bile Acid-Derived Molecular Tweezers: Study of Solvent Effects in Binding, and Determination of Thermodynamic Parameters by an Extraction-Based Protocol

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Received May 8, 2000

A family of bile acid-based molecular tweezers (**1**−**3**) were synthesized, and their binding affinities with picric acid in different solvents were evaluated using a simple extraction-based protocol. The binding affinities increased in nonpolar solvents. The size of the solvent molecule did not affect the binding constant. Thermodynamic parameters for the binding of picric acid in CCl_4 were also determined by this method. Binding constants of these tweezers with trinitrofluorenone in $CDCl_3$ were determined by NMR titration.

Introduction

The ability of certain biomolecules to recognize and bind other molecules is the key feature associated with a number of biological processes. This phenomenon is manifested in a variety of processes such as enzyme activity (enzyme−substrate complexes), antibody production (immunoglobin−antigen complexes), DNA double helix formation (Watson−Crick base pairing), and biosynthesis of nucleic acids and protein synthesis (transcription and translation), etc. The past two decades have witnessed enormous growth in the efforts of chemists to understand and mimic some of these processes using systems which are easier to synthesize, manipulate, and study.[1,2,3]

To design rigid preorganized molecular systems to create clefts, cavities, and other types of binding surfaces, a variety of molecular frameworks, including natural products, have been utilized.[1−18] In this respect, bile acids present one of the most attractive architectures—a rigid backbone with hydrophilic and hydrophobic faces, and ease of functionalization of the three hydroxyl groups.[4] These aspects are well reflected in the large number of reports which utilized bile acids for the recognition of carbohydrates,[5] anions,[6] polynitroaromatics,[7] adenine/biotin;[8] in the construction of dentritic species,[9] cyclocholates,[10] chola-crowns,[11] macrocycles;[12] and in organogels.[13]

Among the various types of molecular hosts designed so far, a class known as "molecular tweezers" has been quite popular. The term "molecular tweezer" was initially used for a host which complexed guest molecules through $\pi-\pi$ interactions. Subsequently, the usage was extended to acyclic host molecules utilizing hydrogen bonding, coordination, and other noncovalent interactions for complexation.[14] These species are characterized by two similar or dissimilar "sticky arms" for binding, which are separated by a rigid or a semirigid spacer.

Solvent effects on molecular recognition is an important aspect which has not been very extensively studied. While the effect of solvents on H-bonding is a generally well understood phenomenon,[15] similar effects on intermolecular forces involving π-stacking and donor−acceptor interactions have been less thoroughly studied.[16] Some

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(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. (d) Maitra, U. A Survey of Supramolecular Chemistry, 1993−1994. In *Perspectives in Supramolecular Chemistry*; Hamilton, A. D., Ed.; John Wiley & Sons: UK, 1996; Vol. 3, pp 41−100, and references therein.

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

(3) Zimmerman, S. C. *Top. Curr. Chem.* **1991**, *165*, 33.

(4) For recent examples of bile acid in supramolecular chemistry, see (a) Davis, A. P. *Chem. Soc. Rev.* **1993**, *22*, 243. (b) Bonar-Law, R. P.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1995**, *117*, 259. (c) Hsieh, H.-P.; Muller, J. G.; Burrows, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 12077. (d) Cheng, Y. A.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813. (e) Janout, V.; Lanier, M.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 1573. (f) Venkatesan, P.; Cheng, Y.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6955. (g) Davis, A P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793.

(5) (a) Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1407. (b) Battarai, K. M.; Bonar-Law, R. P.; Davis, A. P.; Murray, B. A. *J. Chem. Soc., Chem. Commun.* **1992**, 752.

(6) .Davis, A. P.; Perry, J. J.; Williams, R. P. *J. Am. Chem. Soc.* **1997**, *119*, 1793.

(7) (a) Maitra, U.; D'Souza, L. J.; *J. Chem. Soc., Chem. Commun.* **1994**, 2793. (b) D'Souza, L. J.; Maitra, U. *J. Org. Chem.* **1996**, *61*, 9494.

(8) Rao, P.; Maitra, U. *Supramol. Chem.* **1998**, *9*, 325.

(9) Balasubramanian, R.; Rao, P.; Maitra, U. *Chem. Commun.* **1999**, 2353.

(10) Gao, H.; Dias, J. R. *New J. Chem.* **1998**, 579.

(11) (a) Maitra, U.; D'Souza, L. J.; Vijay Kumar, P. *Supramol. Chem.* **1998**, *10*, 97. (b) Maitra, U.; Bag, B. G.; *J. Org. Chem.* **1994**, *59*, 6114.

(12) Maitra, U.; Balasubramanian, S. *J. Chem. Soc., Perkin Trans. 1* **1995**, 83.

(13) (a) Maitra, U.; Vijay Kumar, P.; Chandra, N.; D'Souza, L. J.; Prasanna M. D.; Raju, A. R. *Chem. Commun.* **1999**, 595. (b) Hishikawa, Y.; Sada, K.; Watanabe, R.; Miyata, M.; Hanabusa, K. *Chem. Lett.* **1998**, 795.

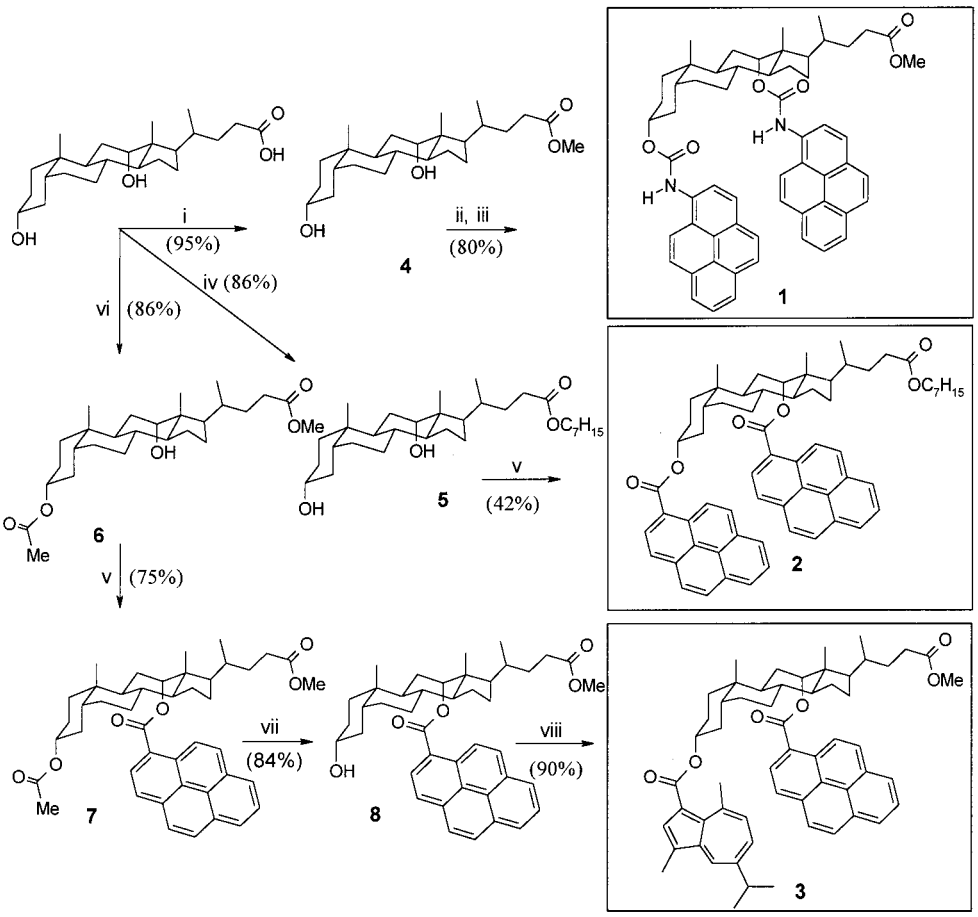
(14) Gunther, R.; Nieger, M.; Vögtle, F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 601.

(15) For an account, see: Schneider, H.-J.; Mohammad-Ali, A. K. In *Comprehensive Supramolecular Chemistry*; Vögtle, F., Ed.; Elsevier Science Ltd., Oxford, 1996; Vol. 2, p 91.

(16) For recent examples of molecular tweezers utilizing π-stacking, see (a) Otsuki, J.; Oya, T.; Lee, S.-H.; Araki, K. *J. Chem. Soc. Chem. Commun.* **1995**, 2193. (b) Lorente, A.; Fernandez-Saiz, M.; Espinosa, J.-F.; Jaime, C.; Lehn, J.-M.; Vigneron, J.-P. *Tetrahedron Lett.* **1995**, *29*, 5261. (c) Mink, D.; Deslongchamps, G. *Tetrahedron Lett.* **1996**, *37*, 7035. (d) Magnus, P.; Morris, J. C.; Lynch, V. *Synthesis* **1997**, 506. (e) Klarner, F. G.; Burkert, U.; Kamieth, M.; Boese, R.; Buchholz, J. B. *Chem. Eur. J.* **1999**, *5*, 1700. (f) Kamieth, M.; Klarner, F. G. *J. Prakt. Chem.* **1999**, *341*, 245. (g) Kurebayashi, H.; Sakaguchi, M.; Okajima, T.; Haino, T.; Usui, S.; Fukazawa, Y. *Tetrahedron Lett.* **1999**, *40*, 5545.

10.1021/jo000704r CCC: $19.00
Published on Web 10/24/2000

Scheme 1[a]



[a] i. HCl/MeOH; ii. $CO(OCCl_3)_2$/pyr/CH_2Cl_2; iii. 1-aminopyrene/pyr; iv. n-$C_7H_{15}Br$/DBU/THF; v. pyrene-1-carbonyl chloride/CaH_2/$BnEt_3N^+Cl^-$/PhMe; vi. MeOAc/TsOH; vii. K_2CO_3/MeOH; viii. 1-guaiazuloyl bromide/pyr/$CHCl_3$

of the main factors which are known to affect π-stacking are the polarity of the solvent, solvophobic effects, and competitive binding by the solvent.[17] During our study on the evaluation of bile acid-based semiflexible molecular tweezers for electron-deficient aromatic substrates, we had developed a simple biphasic extraction protocol for measuring binding constants using picric acid as the electron deficient guest.[18] In this paper we report the full details of the results of our study on bile acid-derived molecular tweezers **1**−**3** and their binding affinities toward trinitrofluorenone in chloroform (determined by NMR titration) and to picric acid in different solvents (determined by the extraction protocol and NMR titration). The thermodynamic parameters for the binding process using the extraction protocol are also reported.

Synthesis. Bis-pyrene tweezer **1** was made from methyl 7-deoxycholate **4** by reacting it with triphosgene in CH_2Cl_2 to yield the bis-chloroformate, which on reaction with 1-aminopyrene gave bis-carbamate **1** in 80% yield (Scheme 1). 7-Deoxycholic acid was converted to its *n*-heptyl ester **5** by treating it with *n*-heptyl bromide and DBU (65%). *n*-Heptyl 7-deoxycholate **5** was converted to the bis-pyrene ester **2** by Oppenauer esterification.[19] Compound **3** was made via a protection−deprotection sequence. 7-Deoxycholic acid was first converted to methyl 3α-acetoxy-12α-hydroxy-5β-cholan-24-oate **6** following a reported procedure.[20] Compound **6** was then esterified to give **7**, which was subsequently methanolyzed to give the free 3-hydroxy derivative **8**. Finally tweezer **3** was obtained by the esterification of **8** with guaiazuloyl bromide.

Extraction-Based Binding Constant Measurement. Cram had developed and extensively used an extraction-based method for determining the binding constants of alkali metal ions to crown ethers in chloroform,[21] in which alkali metal picrates were used. Among other substrates, picric acid was also chosen as an electron deficient guest to study its binding behavior with hosts **1**−**3**. We realized that a similar extraction-based procedure could be employed by using picric acid instead of metal picrates. Since picric acid in general distributes[22,23] between water and an immiscible solvent, the binding constants can be evaluated[24] by measuring the distribution of picric acid in such a pair of solvents in

(17) Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1994**, *116*, 2301. Ferguson, S. B.; Sanford, E,.; Seward, E. M.; Diederich, F. *J. Am. Chem. Soc.* **1991**, *113*, 5410.

(18) Maitra, U.; Rao, P.; Vijay Kumar, P.; Balasubramanian, R.; Mathew, L. *Tetrahedron Lett.* **1998**, *39*, 3255.

(19) Although we had earlier studied the bis-pyrene ester of methyl 7-deoxycholate (ref 7), we had to make the heptyl derivative due to the relative insoluble nature of the methyl ester in some of the solvents which we wanted to study by the extraction-based method.

(20) Ksenija, K.; Julijan, K.; Vera, C.-N.; Dusan, M. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1073.

(21) Newcomb, M.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6398.

(22) For a detailed account of distribution phenomenon, see Nernst, E. *Zeitsch. Physik. Chem.* **1891**, *8*, 105.

Table 1. K_a (M^{-1}) of Picric Acid with Hosts 1 to 3 in Different Solvents at 26 °C

host	$CDCl_3$[a]	$CHCl_3$	$(CHCl_2)_2$	CCl_4	C_6H_6
1	27	30	26	290	180[b]
2	82	120	117	1250	640
3	30	30	10	400	170

[a] These values are obtained by ^1H NMR titration. [b] ^1H NMR titration in C_6D_6 gave a value of 178 M^{-1}.

the absence and in the presence of a host. The binding constants can be calculated using eq 1 (see Experimental Section). This procedure is applicable not just for picric acid but for any guest which partitions between water and the water-immiscible organic solvent of interest. Using this method we have measured the binding constants of three tweezers in $CHCl_3$ (Table 1, column 2) and checked the validity of this procedure by the NMR titration method (Table 1, column 1). It is indeed pleasing to see that these two methods gave comparable values. Encouraged by these results, the binding behavior in $(CHCl_2)_2$, C_6H_6 and CCl_4 was also studied. Tweezer **2** showed a higher binding compared to the other two probably due to the presence of a larger π-surface (than **3**)[25] and higher rigidity (than **1**).

Solvent Effects on the Binding Constants. We have observed higher binding constants in less polar solvents such as CCl_4 and benzene, implying that the donor−acceptor interaction is stronger in these solvents. Lower affinities observed in benzene compared to carbon tetrachloride can be explained by the ability of the solvent binding competitively with the guest. The relatively polar solvents, chloroform and tetrachloroethane, decreased the binding affinity. The binding behavior is very similar in both chloroform and tetrachloroethane, and therefore the size of the solvent molecule does not seem to be important.[26] An order of magnitude increase in the K_a's from $CHCl_3$ to CCl_4 indicated an enhanced stabilization ($\Delta\Delta G_{complexation}$) of 1.3−1.5 kcal mol^{-1}.

Determination of Thermodynamic Parameters. Using this extraction based method the K_a values in CCl_4 at different temperatures were easily evaluated. The thermodynamic parameters ΔG, ΔH, and ΔS were obtained from the van't Hoff plots (Figure 1). Table 2 summarizes the results. The fact that binding takes place against entropic factors suggests that the process is not solvophobic (i.e., the host does not displace solvent molecules from the guest).[27] This considerable decrease in entropy is also typical of association processes between preorganized hosts and neutral guest molecules in organic media.[28] Binding with **2** and **3** is entropically less

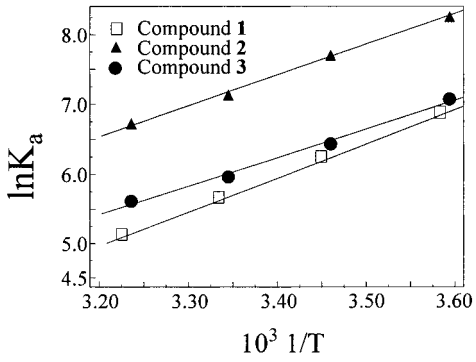


Figure 1. van't Hoff plot with best fit straight lines for tweezers **1**−**3**. Each point is the average of three measurements.

Table 2. Thermodynamic Parameters for the Binding with Picric Acid in CCl_4

parameter	**1**	**2**	**3**
ΔG at 25 °C (kcal mol^{-1})	−3.31	−4.22	−3.55
ΔH at 25 °C (kcal mol^{-1})	−9.88	−8.73	−8.56
ΔS (eu)	−22	−15	−17

disfavored than **1** possibly because the relatively flexible **1** (one extra bond between pyrene units and the steroid compared to **2** and **3**) becomes more organized in the presence of a guest ($\Delta\Delta S = -7$ eu). This point is further corroborated by the more or less similar ΔS observed for binding of **2** and **3** which are very similar in terms of flexibility.

Binding Constants with Trinitrofluorenone. All the three tweezers were also evaluated for binding affinities with trinitrofluorenone in $CDCl_3$ by ^1H NMR titrations. As expected, tweezer **2** showed the highest affinity (K_a 275 M^{-1}) toward TNF. The binding with tweezers **1** and **3** were comparable (125 M^{-1} and 74 M^{-1}, respectively), as observed with picric acid.

Stoichiometry of Complexation. The stoichiometry of complexation for **1**:picric acid in $CDCl_3$ was determined by Job plot.[29] NMR chemical shifts were recorded for solutions with different relative concentrations of **1** and picric acid at a fixed total concentration of **1** and picric acid. The concentration of complex formed at each data point was plotted against the mole fraction of **1**. A maximum at 0.5 mole fraction indicated that the stoichiometry of complexation between the host and the guest is 1:1 (Figure 2).

Conclusions

We have synthesized three bile acid-based semirigid molecular tweezers which bind electron deficient aromatic compounds. The binding constants were evaluated using a simple extraction-based protocol. The credibility of this method was confirmed by the NMR method. Thermodynamic parameters for binding were also evaluated by this method.

The simplicity in the design and synthesis of these tweezers **1**−**3** and the ease of determining the association constants and thermodynamic parameters can be utilized for systematic study in which various parameters which affect binding can be easily altered.

(23) For an exhaustive account on electron donor−acceptor (EDA) complexes in general and picric acid in particular, see (a) Briegleb, G. *Electronen-Donator-Acceptor-Komplexe*; Springer-Verlag: Göttingen, 1961. (b) Briegleb, G.; Czekalla, J.; Hauser, A. *Z. Phys. Chem. Neue Folge*. **1959**, *21*, 99−103.

(24) For biphasic extraction-based methods for determining host-mediated transport, see (a) Murray, B. A.; Whelan, G. S. *Pure Appl. Chem*. **1996**, *68*, 1561. (b) Takeuchi, M.; Koumoto, K.; Goto, M.; Shinkai, S. *Tetrahedron*, **1996**, *52*, 12931. (c) Newcomb, M.; Moore, S. S.; Cram, D. J. *J. Am. Chem. Soc.* **1977**, *99*, 6398.

(25) Rebek has shown that by increasing the π-surface by a benzene ring the binding energy increases by 0.4 kcal M^{-1}. (Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl*. **1990**, *29*, 245.)

(26) We believe the results obtained by Still and co-workers were different since their host had a rigid cavity whose solvation was affected by the size of the solvent molecule (Chapman, K. T.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 3075)

(27) William, K.; Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1090.

(28) Cram. D. J.; Stewart, K. D.; Goldberg, I.; Trueblood, K. M. *J. Am. Chem. Soc.* **1985**, *107*, 2574.

(29) Job, A.; *Ann. De Chim*. (Paris) **1928**, *9*, 133.

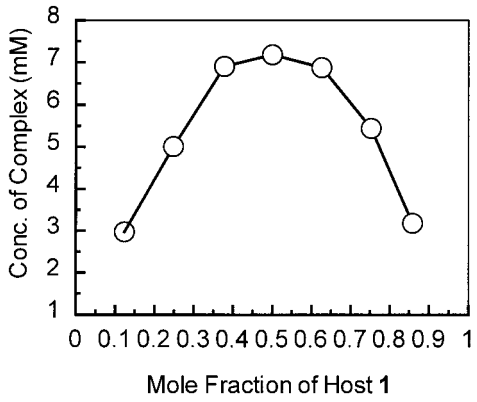


Figure 2. Job plot for the complexation of **1** with picric acid in $CDCl_3$.

We believe that this simple and straightforward method will compete with the NMR titration method (no need for deuterated solvents) and can be the method of choice for determining binding of guests which partition between water and an immiscible solvent.

Experimental Section

General. 7-Deoxycholic acid and pyrene were purchased from Fluka. Picric acid was purchased from NICE Chemicals and was used after crystallization from water. Trinitrofluoreneone was prepared from fluorenone by a reported procedure.[30] All solvents were purified and distilled before use.[31] 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 Aldrich. These plates were stained either with iodine vapor or Liebermann−Buchard reagent. Purification of the products was usually done using gravity columns. Melting points were recorded in open capillaries and are uncorrected. Proton NMR spectra were recorded on 90 and 300 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 and the multiplicity of NMR signals are shown with standard notations. Optical rotations were measured in appropriate solvents using sodium D light. Microanalyses were done on an automated CHN analyzer. LR mass spectral data are given as *m*/*z* (% abundance). Infrared spectra were taken in $CHCl_3$ as a thin film on NaCl plates (neat).[32]

Methyl 3α,12α-Dihydroxy-5β-cholan-24-oate (4). This was prepared using deoxycholic acid and methanolic HCl.[33]

Methyl Bis(3α,12α(*N*-1-pyrenyl-*N*-carbonyloxy))-5β-cholan-24-oate (1). Methyl 3α,12α-dihydroxy-5β-cholan-24-oate (0.10 g, 0.25 mmol) and bis(trichloromethyl) carbonate (0.05 g, 0.17 mmol) were dissolved in dry CH_2Cl_2 (3 mL) and cooled in an ice bath. To the cooled mixture was added pyridine (50 μL, 0.63 mmol) and the mixture stirred at room temperature for 5 h. 1-Aminopyrene (0.106 g, 0.106 mmol) and pyridine (100 μL, 1.27 mmol) were added and stirred at rt for 4 h. The reaction mixture was diluted with $CHCl_3$, washed with water, 5% HCl, and brine, and dried over anhyd Na_2SO_4, and volatiles were removed. The crude product was chromatographed on a column of silica gel (100−200 mesh, 1 cm × 25 cm) with 20% ethyl acetate/hexanes to yield the pure product in 80% yield (175 mg): mp 125 °C; $[\alpha]^{24}_D$: +94° (*c* 3.31, $CHCl_3$); UV (λ_{max}, log ϵ) (1% $CHCl_3/CH_3CN$, v/v): 242 (5.13), 276 (4.89), 341 (4.83); fluorescence: λ_{ex} 355 nm, λ_{em} 380, 410, 480 nm; IR (neat): 3450−3100 (br), 2920−2800 (br, s), 1725 (s), 1695 (s), 1600 (s), 1520 (s), cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.71 (s, 3 H), 0.93 (br, 9 H), 1.1−2.3 (26 H), 3.61 (s, 3 H), 4.88 (m, 1 H), 5.12 (br, s, 1H), 6.94 (br, 2H,), 7.6−8.4 (18H); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ 12.3, 17.7, 22.7, 23.0, 27.2, 28.0, 30.8, 33.9, 35.5, 36.2, 37.5, 38.0, 42.1, 45.1, 46.3, 48.5, 49.6, 51.5, 120.1, 122.5, 124.5, 125.7, 126.9, 128.7, 130.4, 130.9, 154.5, 154.7, 174.6. MALDI-TOF MS: 894 (M^+), 917 ($M + Na^+$). Anal. Calcd for $C_{59}H_{60}O_6N_2$: C: 79.34, H: 6.77. Found: C: 78.96, H: 7.08.

1-Heptyl 3α,12α-Dihydroxy-5β-cholan-24-oate (5). To a solution of 7-deoxycholic acid (2.0 g, 5.22 mmol) and *n*-heptyl bromide (0.8 mL, 6.41 mmol) in THF (11 mL) was added DBU (0.8 mL, 5.26 mmol), and the mixture was refluxed for 8 h. Volatiles were removed, and the residue was suspended in ethyl acetate, washed with water, 10% HCl, and water, and dried over anhyd Na_2SO_4. After the volatiles were removed, the crude product was chromatographed on a column of silica gel (60−120 mesh, 3.5 cm × 35 cm) with 50% ethyl acetate/hexanes to yield the pure product in 86% yield (2.2 g) as viscous oil: $[\alpha]^{24}_D$: +56° (*c* 4.8, $CHCl_3$); IR (neat) 3100−3600 (br), 2920−2800 (br, s), 1725 (s) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (t, J = 6.6 Hz, 2H), 3.98 (s, 1H), 3.60 (m, 1H), 1.0−2.5, 0.96 (d, J = 6.0 Hz, 3H), 0.86−0.91 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.32, 72.95, 71.46, 64.34, 48.07, 47.10, 46.40, 42.04, 36.33, 35.95, 35.23, 34.05, 33.47, 31.65, 31.35, 30.89, 30.30, 28.84, 28.58, 27.49, 27.13, 26.09, 25.83, 23.66, 23.06, 22.50, 17.13, 14.0, 12.61. LRMS: *m*/*z* M^+ 472 ($M^+ - H_2O$), 255 (100%).

1-Heptyl 3α,12α-Bis(1-pyrenoyloxy)-5β-cholan-24-oate (2). To a solution of **5** (0.1 g, 0.2 mmol) in toluene (1 mL) were added calcium hydride (0.11 g, 2.62 mmol) and benzyltriethylammonium chloride (0.01 g, 0.04 mmol), and the mixture was refluxed for 5 min. To the refluxing solution was added a solution of freshly prepared 1-pyrenoyl chloride (0.14 g, 0.53 mmol) in toluene (1 mL) and refluxed for 19 h. The reaction mixture was filtered through a pad of Celite, diluted with $CHCl_3$, and washed with aq $NaHCO_3$. The organic layer was dried over anhyd Na_2SO_4, and volatiles were removed. The crude product was chromatographed on silica gel (100−200 mesh, 2 cm × 20 cm) with 40% chloroform/hexanes to yield the pure product (80 mg, 42%). The monoesterified product was also obtained (20 mg, 14%): mp 117 °C; $[\alpha]^{24}_D$: +131° (*c* 0.9, $CHCl_3$); UV (λ_{max}, log ϵ) (2.5% $CHCl_3/CH_3CN$ v/v) 244 (4.94), 280 (4.68), 350 (4.67), 383 (4.03). fluorescence: λ_{ex} 355 nm, λ_{em} 290, 408, 485 nm; FT-IR (neat) 2927(s), 2966(s), 1733-(s), 1705(s) cm^{-1}; ^{1}H NMR, (300 MHz, $CDCl_3$) δ 9.12 (d, J = 9.3 Hz, 1 H), 8.97 d, J = 9.3 Hz, 1 H), 8.58 (d, J = 8.1 Hz 1 H), 8.25 (t, J = 4.2 Hz,1 H), 8.19 (d, J = 9.3, 1 H), 8.16 (d, J = 9.1 Hz, 1 H), 8.08 (m, 2 H), 8.00 (m, 2 H), 7.94 (m, 2 H), 7.85 (d, J = 9.6 Hz, 1 H), 7.78 (d, J = 7.2 Hz, 1 H), 7.72 (d, J = 9.3 Hz, 1 H), 7.63 (t, J = 7.8 Hz, 1 H), 7.51 (d, J = 8.1 Hz, 1 H), 5.63 (s, 1 H), 5.21 (m, 1 H), 3.97 (t, J = 6.6 Hz, 2 H), 2.2−2.1, 1.08 (s, 3 H), 0.99 (d, J = 6.6 Hz, 3 H), 0.87 (s, 3 H), 0.85 (t, J = 6.9 Hz, 3 H); ^{13}C NMR, (75 MHz, $CDCl_3$) δ 174.20, 167.93, 167.57, 133.88, 133.82, 132.47, 130.94, 130.88, 130.80, 130.56, 130.47, 130.25, 130.21, 129.36, 129.29, 129.21, 128.97, 128.81, 127.97, 127.64, 127.12, 126.93, 126.13, 126.01, 125.94, 125.91, 125.34, 124.78, 124.67, 124.62, 124.32, 124.27, 124.15,124.11, 123.92, 77.27, 74.84, 68.17, 64.41, 50.09, 47.95, 45.53, 41.87, 38.75, 35.84, 34.99, 34.82, 34.28, 32.41, 31.94, 31.67, 31.35, 30.87, 30.37, 29.71, 29.67, 29.36, 28.94, 28.87, 28.6, 27.38, 26.83, 26.27, 25.83, 25.70, 23.76, 23.59, 23.00, 22.70, 22.54, 17.72, 14.12, 14.06, 14.04, 12.64.10.97; MALDI-TOF MS: 948.2 (M^+), 971.4 ($M + Na^+$).

Methyl 3α-Acetoxy-12α-hydroxy-5β-cholan-24-oate (6). The compound was made in 86% yield following a literature method:[34] mp 121−123 °C. (lit.[34] 123−125 °C); $[\alpha]^{23}_D$: 61.1 (*c* 1.57, $CHCl_3$); FT-IR (neat) 3415, 2938, 2850, 1736, 1720 cm^{-1};

(30) Woolfolk, E. O.; Orchin. M. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, p 837.

(31) (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.

(32) Even with solids with high melting points the IR data were taken when the material was still in a glassy form.

(33) (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.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.354 (m, 1 H), 3.934 (s, 1 H),3.613 (s, 3 H), 2.38−1.97 (m, 2 H), 1.83−0.99 (m), 0.928 (d, J = 5.7 Hz, 3 H), 0.87 (s, 1 H), 0.632 (s, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 174.53, 170.53, 74.15, 72.86, 51.34, 48.09, 47.11, 46.35, 41.72, 35.85, 34.97, 34.55, 33.98, 33.47, 32.01, 30.90, 30.77, 28.61, 27.32, 26.83, 26.33, 25.89, 23.48, 22.98, 21.30, 17.16, 12.59.

Methyl 3α-Acetoxy-12α-(1-pyrenoyloxy)-5*β*-cholan-24-oate (7). To a solution of **6** (0.312 g, 0.696 mmol) in toluene (2 mL) were added calcium hydride (0.40 g, 9.52 mmol) and benzyltriethylammonium chloride (0.01 g, 0.04 mmol), and the mixture was refluxed for 5 min. To the refluxing solution was added a solution of freshly prepared 1-pyrenoyl chloride (0.264 g, 0.10 mmol) in toluene (1 mL) and refluxed for 18 h. The reaction mixture was filtered through a pad of Celite, and the solvent removed in vacuo. The crude product was chromatographed on silica gel (100−200 mesh, 2 cm × 20 cm) with 10% ethyl acetate/hexanes to give the pure product in 75% yield (355 mg): mp 112 °C; $[\alpha]^{24}_D$ +89 (*c* 1.0, $CHCl_3$); UV (λ_{max}, log ϵ) (5% $CHCl_3$/CH_3CN v/v) 383 (3.85), 350 (4.46), 280 (4.47), 244 (4.74); fluorescence (5% $CHCl_3$/CH_3CN v/v): λ_{ex} 355 nm, λ_{em} 388 and 408 nm; FT-IR (neat) 2947, 2869, 1735, 1705, 1596 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.198 (d, J = 9.6 Hz, 1 H), 8.615 (d, J = 8.1 Hz, 1 H), 8.223−8.285 (m, 4 H), 8.189 (d, J = 8.7 Hz, 1 H), 8.107 (d, J = 9.0 Hz, 1 H), 8.061 (t, J = 7.8 Hz, 1 H), 5.592 (s, 1 H), 4.361 (s, 1 H), 3.576 (s, 3 H), 2.33−1.03 (m), 1.759 (s, 1 H), 0.96−0.98 (m, 6 H), 0.870 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.49, 170.44, 167.58, 134.05, 131.04, 130.77, 130.43, 129.52, 129.36, 127.83, 127.11, 126.33, 126.23, 126.03, 124.96, 124.87, 124.82, 124.28, 124.26, 73.92, 51.38, 49.99, 47.91, 45.50, 41.74, 35.79, 34.94, 34.78, 34.14, 32.20, 30.97, 30.78, 27.35, 26.84, 26.47, 26.10, 25.81, 23.55, 23.02, 21.22, 17.64, 12.60. LRMS: *m*/*z* M$^+$ 676 (30), 246 (100).

Methyl 3α-Hydroxy-12α-(1-pyrenoyloxy)-5*β*-cholan-24-oate (8). To a solution of **7** (0.355 g, 0.525 mmol) in MeOH/THF (1:1, 4 mL) was added K_2CO_3 (0.138 g, 0.530 mL) and stirred for 3 h at room temperature. The reaction mixture was quenched with acetic acid (1.5 mL), suspended in water and extracted with ethyl acetate. The organic layer was dried over anhyd Na_2SO_4, and volatiles were removed. The crude product was chromatographed on silica gel (100−200 mesh) with 20% ethyl acetate/hexanes to yield the pure product in 285 mg (84%): mp 180−181 °C; $[\alpha]^{24}_D$ +72° (*c* 1.53, $CHCl_3$); UV: (λ_{max}, log ϵ) (5% $CHCl_3$/CH_3CN v/v): 383 (3.91), 350 (4.53), 280 (4.54), 244 (4.92). Fluorescence (5% $CHCl_3$/CH_3CN, v/v) λ_{ex} 355 nm, λ_{em} 388 and 408 nm; FT-IR (neat) 3600−3100, 2934, 1734, 1704 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.221 (d, J = 9.6 Hz, 1 H), 8.612 (d, J = 8.1 Hz, 1 H), 8.29−8.23 (m, 4 H), 8.187 (d, J = 9.0 Hz, 1 H), 8.107 (d, J = 8.7 Hz, 1 H), 8.060 (t, J = 7.8 Hz, 1 H), 5.574 (s, 1 H), 3.568 (s, 3 H), 3.52 (m, 1 H), 2.32−1.01 (m), 0.973 (s, 3 H), 0.951 (d, J = 6.6 Hz, 3 H), 0.869 (s, 3 H); ^{13}C NMR (75 MHz. CDCl3) δ 174.52, 167.49, 134.16, 131.05, 130.95, 130.43, 129.53, 129.41, 127.80, 127.18, 126.30, 126.24, 126.13, 124.93, 124.76, 124.65, 124.39, 124.28, 71.61, 51.38, 49.98, 47.94, 45.53, 41.97, 36.30, 35.90, 35.08, 35.01, 34.78, 34.15, 30.96, 30.79, 30.64, 27.38, 27.06, 26.18, 25.87, 23.59, 23.14, 17.61, 12.62.; LRMS: *m*/*z* M$^+$ 634 (50). HRMS: calcd for $C_{42}H_{50}O_5$ 634.366, found 634.365.

Methyl 3α-(1-Guaiazuloyloxy)-12α-(1-pyrenoyloxy)-5*β*-cholan-24-oate (3). To a solution of guaiazulene (0.180 g, 0.909 mmol) in toluene (0.5 mL) was added oxalyl bromide (0.300 g, 1.4 mmol) and stirred for 5 h. Excess oxalyl bromide was pumped off, and the acid bromide dissolved in $CHCl_3$ (3 mL) was added to compound **8** (0.24 g, 0.380 mmol). Pyridine (0.5 mL, 6.33 mmol)) was added, and the mixture was stirred for 12 h. The reaction mixture was diluted with $CHCl_3$ and washed with 10% HCl. The organic layer was dried over anhyd Na_2SO_4, and volatiles were removed. The crude product was chromatographed on silica gel (100−200 mesh, 2 cm × 20 cm) with 1% ethyl acetate/hexanes to yield the pure product in 90% yield (295 mg): mp 84−86 °C; $[\alpha]^{24}_D$: +124° (*c* 2.5, $CHCl_3$); UV (λ_{max}, log ϵ) (5% $CHCl_3$/CH_3CN v/v) 383 (4.11), 350 (4.48), 300 (4.56), 282 (4.65), 243 (4.86); fluorescence (5% $CHCl_3$/CH_3CN v/v) λ_{ex} 355 nm, λ_{em} 390 and 408 nm; FT-IR (neat): 2951, 2868, 1736, 1700, 1597 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.154 (d, J = 9.6 Hz, 1 H), 8.588 (d, J = 8.1 Hz, 1 H), 8.249 (d, J = 9.0.0 Hz, 1 H), 8.15−8.19 (m, 2 H), 8.089 (d, J = 8.1 Hz, 1 H), 8.048 (d, J = 9.0 Hz, 1 H), 7.987 (d, J = 7.2 Hz, 1 H), 7.902 (t, J = 7.8 Hz, 1 H), 7.815 (d, J, = 9.3 Hz, 1 H), 7.66, (s, 1 H), 7.273 (dd, J = 1.5 Hz, 10.5 Hz, 1 H), 6.695 (d, J = 10.8 Hz, 1 H), 5.620 (s, 1 H), 4.972 (m, 1 H), 3.569 (s, 3 H), 3.097 (h, J = 6.9 Hz, 1 H), 2.574 (s, 3 H), 2.340 (s, 3 H), 2.31−1.12 (m), 1.363 (d, J = 6.9 Hz, 6 H), 1.035 (s, 3 H), 0.976 (d, J = 6.6 Hz, 3 H), 0.88 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 174.51, 167.74, 167.04, 146.82, 142.88, 139.89, 139.78, 135.46, 135.30, 134.14, 133.93, 130.94, 130.63, 130.39, 129.86, 129.40, 129.19, 127.71, 127.10, 126.18, 126.08, 125.98, 125.14, 124.81, 124.69, 124.43, 124.23, 123.75, 117.69, 73.95, 51.39, 50.00, 47.87, 45.50, 41.90, 37.90, 35.82, 35.03, 34.99, 34.78, 34.27, 32.47, 30.99, 30.78, 27.43, 27.35, 26.91, 26.69, 26.17, 25.91, 24.60, 23.56, 23.07, 17.68, 12.63, 12.61.; FAB MS (*m*/*z*): 859 (M$^+$ + 1, 100). Anal. Calcd for $C_{58}H_{66}O_6$: C: 81.08, H: 7.74. Found: C: 80.96, H: 7.78.

Extraction Method for Determining K_a Values. A solution of picric acid (2 mL, ≈5.0 mM, absorbance = A_0 after diluting *n* fold with buffer) in 0.11 M HCl was stirred with a solution of the host in CCl_4 (2 mL, ≈5.0 mM, H_0) in a thermostated bath at 26 °C. After 20 min an aliquot of the aqueous layer was diluted *n* fold with phosphate buffer (pH 8.2), and the absorbance (**A**) was measured at 380 nm. Another run was made in which the solution of the host was replaced by CCl_4 alone and the absorbance A_d was measured. K_d (distribution coefficient) was calculated as $A_d/(A_o - A_d)$ (the dissociation of picric acid in the aqueous solution and its possible association in the organic solvent were ignored in the determination of K_d). Considering a 1:1 stoichiometry, together with the knowledge of the ϵ at 380 (11700) of picrate, the K_a values were calculated using eq 1.

$$\mathbf{H} + \mathbf{G} \rightleftharpoons \mathrm{HG}$$

Concentration of uncomplexed guest in organic layer is given by

$$[\mathrm{G}] = nA/K_d\epsilon$$

Concentration of host−guest complex [HG] in organic layer is given by

$$\mathrm{HG} = [(A_o - A)n/\epsilon] - [An/K_d\epsilon] = n(A_o - A - A/K_d)/\epsilon$$

where the first term represents the total concentration of guest in organic layer, and the second term represents the concentration of free guest in organic layer.

Concentration of uncomplexed host in organic layer is given by

$$[\mathrm{H}] = \mathrm{H_o} - [\mathrm{HG}] = \mathrm{H_o} - n(A_o - A - A/K_d)/\epsilon$$

and association constant K_a is defined as

$$K_a = [\mathrm{HG}]/[\mathrm{H}][\mathrm{G}]$$

Therefore

$$K_a = (A_o - A - A/K_d)/[\mathrm{H_o} - n(A_o - A - A/K_d)/\epsilon]A/K_d \quad (1)$$

^{1}H NMR Titration. For compound **1**, K_a values were determined by keeping the concentration of guest constant and incrementing the concentration of the host. For compounds **2** and **3**, K_a values were determined by keeping the concentration of the host constant and varying the concentration of guest. Guest at 9 mM was titrated with the host at 2.5−12.5 mM, and the upfield shift of ^{1}H NMR signal of the guest was followed. The binding constant was determined from these

(34) Ksenija, K.; Julijan, K.; Vera, C.-N.; Dusan, M. *Collect. Czech. Chem. Commun.* **1996**, *61*, 1073.

Table 3. δ (ppm) of Guest in the Absence and the Presence of an Equivalent Amount of Host[a]

guest	no host	**1**	**2**	**3**
TNF (3-H)	9.032	8.546 (9.40)	8.675 (3.74)	8.708 (2.75)
picric acid	9.196	8.808 (9.30)	8.570 (6.32)	8.942 (2.60)

[a] Figures in parentheses denote the concentration in mM.

data using a nonlinear curve-fitting program.[35] Given above are the δ (ppm) values of TNF and picric acid in the absence and presence of an equivalent amount of the three hosts (Table 3).

Job Plot for Picric Acid and 1. This experiment involved the preparation of standard solutions of the picric acid (5.5 mg, 0.024 M in $CDCl_3$) and the host **1** (21.2 mg, 0.024 M in $CDCl_3$). In seven NMR tubes these solutions were mixed in different proportions, 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 250 μL. ^{1}H NMR spectra were recorded for each NMR tube, and $\Delta\delta$ values were calculated by subtracting the chemical shift of the singlet from picric acid protons in the spectrum of the mixtures (δ_x) from the same resonance of the pure picric acid (δ_0 9.19). Using guest 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.

(35) Hynes, M. J. *J. Chem. Soc., Dalton Trans.* **1993**, 311.

Acknowledgment. Financial support from Department of Science & Technology, New Delhi (grant no. SP/S1/G-08/96) is gratefully acknowledged, V.K.P. thanks U.G.C. for financial support.

JO000704R