Troger's Base Molecular Scaffolds in Dicarboxylic Acid Recognition

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Artificial receptors (**1**−**5**) have been designed and synthesized from simple precursors. The chain length selectivity studies of dicarboxylic acids within the cavities of new fluorescent Troger's base molecular frameworks (**1**−**3**) have been carried out with a critical examination of their role of rigidity as well as flexibility in selective binding in comparison to receptor **5**. The chiral resolution of the racemic Troger's base receptors (**1** and **2**) by chiral recognition with (+)- camphoric acid using hydrogen-bonding interactions has been studied.

Introduction

The understanding of noncovalent intermolecular interactions has become the central focus in the diverse field of chemistry. The design and synthesis of model receptors to recognize substrates of biochemical significance utilizing these weak forces to mimic biological events are of keen interest in molecular recognition research.[1,2] During the last decade, a lot of synthetic receptors for dicarboxylic acids have been designed and synthesized on the basis of the use of a relatively small number of interacting H-bonding groups. The most striking feature of the recognition of dicarboxylic acids is their selective binding by synthetic receptors.[3−7] The precise selectivity between two purely aliphatic dicarboxylic acids (close homologues) is more difficult. This happens owing to the fact that the bond rotation in an open chain can occur freely and the two carboxyl ends in the extended chain compromise to come closer for hydrogen-bonding contacts with the receptor binding groups if they demand so. However, the binding constant may be less if the receptor having the same binding groups is at a more separated distance. Having a desire to create new spacers, we designed novel Troger's base receptors (**1**−**3**), which have conformationally well-defined geometry for the selective recognition of dicarboxylic acids. Receptor **4** was synthesized for binding studies with the metal ions. 2-Aminopyridine, a hydrogen-bonding motif for the carboxylic acid group[3,4] has been used by Hamilton et al. to design dicarboxylic acid receptors by a common strategy that involved the linking of the 2-aminopyridine groups through rigid and semirigid spacers. Use of transition metals as templates for the assembly of hydrogen-bonding sites to create a functioning receptor for the selective recognition of dicarboxylic acids[5] is also of particular interest. In this context, selective binding of glutaric acid has been reported by a resorcinol−aldehyde (dodecyl aldehyde and pentyl aldehyde) cyclotetramer[6] and of dibutylmalonic acid by a receptor having a benzophenone spacer between the two urea or phosphonamide type binding moieties in the cavity for the carboxylic acid.[7]

In search for a conformationally and geometrically well-defined linker to hold the hydrogen-bonding groups, we report here Troger's base as a unique structural feature for the construction of novel molecular receptors for dicarboxylic acid recognition studies. The chiral complexation of (+)-camphoric acid with the (±)-Troger's base receptors is also reported.

Results and Discussion

During the course of our attempts to construct molecular recognition sites on rigid as well as semirigid templates of different topography, we felt that the Troger's base unit could potentially serve as a rigid molecular building block for the construction of artificial receptors for dicarboxylic acids.[8] Accordingly, we designed and synthesized the receptors **1**, **2**, and **3**, where aminopyridine heterocyclic units are the hydrogen-bonding sites for the formation of strong multihydrogen-bonded complexes with dicarboxylic acids. The other semirigid receptor **5**, where the binding arms are assembled by a flexible methylene bridge, was also synthesized and studied to investigate its role in the selective binding of dicarboxylic acids.

In all the designed receptors **1**, **2**, and **3**, the two pyridine units are angularly disposed by the Troger's base

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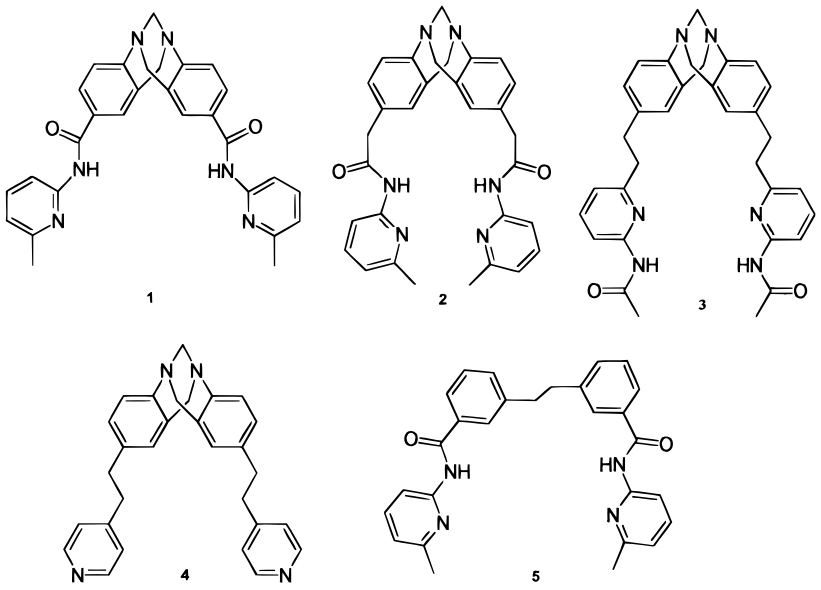
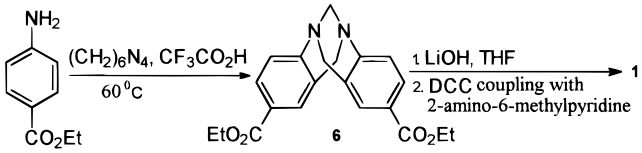
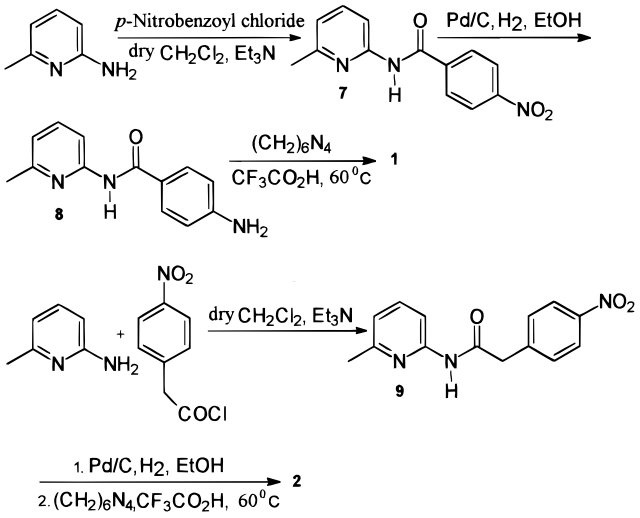


Scheme 1



Scheme 2



spacer. The hydrogen-bonding groups are well arranged in a concave face to bind dicarboxylic acids, and all of them can have three possible conformations of comparable energy values (in−in, in−out, out−out) in the solution phase. MMX calculations[9] on the "in−in" conformation of **1** (E_{min} = 52.85 kcal/mol) indicates a distance of separation of 12.99 Å between the two pyridine ring nitrogen atoms. In receptor **3** (E_{min} = 44.44 kcal/mol), the N−N (pyridine ring nitrogens) and N*H*−N*H* distances are ca. 11.60 and 10.56 Å, respectively. Thus the cavity of the receptors **1** and **3** can accommodate dicarboxylic acids of various chain lengths, and the selective binding occurs only when the cavity dimensions and the chain lengths of dicarboxylic acids are matched together. Similarly, **2** and **5** having different cavity dimensions can also bind dicarboxylic acids of different chain lengths.

Troger's base analogues **1** and **2** have been synthesized from the reaction of the corresponding amines with hexamethylenetetramine in the presence of trifluoroacetic acid (Scheme 2). It was initially planned to synthesize both receptors **1** and **2** according to Scheme 1. But this approach was frustrating owing to the very low yield of the intermediate **6**, which on subsequent hydrolysis by lithium hydroxide followed by coupling with 2-amino-6-methylpyridine gave also the desired receptor **1** but in very poor yield and which could not be isolated in pure form. Thus this route was abandoned.

Compound **1** was synthesized by Scheme 2 where 2-(4-aminobenzoyl amino)-6-methylpyridine **8** was subjected to react with hexamethylenetetramine in the presence of trifluoroacetic acid (nonaqueous medium).[10] Compound **1** was isolated in 15−20% yield by careful purification (PTLC) from a mixture of other undesirable side products. Compound **2** was obtained in the same way in 62% yield. Formation of both **1** and **6** in poor yields is ascribed to the presence of electron-withdrawing groups in the para position of aniline derivatives.[11]

The other Troger's base analogues **3** and **4** were synthesized according to Scheme 3. 2-Acetylamino-6-methylpyridine was coupled with 4-nitrobenzaldehyde in the presence of Ac_2O at 180 °C to give **10** (cis and trans mixtures; major trans).[12a] Both nitro and ethylenic double bonds in **10** were reduced by hydrogenation to give the corresponding amine **11**, which was then reacted with hexamethylenetetramine in the presence of trifluoroacetic acid to generate **3**. In a similar way, the other analogue **4** was synthesized in 70% yield. The synthetic Schemes 2 and 3 show an elegant approach to attach the heterocyclic units to the Troger's base molecular frameworks, which could potentially serve as hydrogen-bonding

(9) Energy minimization was carried out by MMX (PC Model Serena Software 1993). Molecular modeling was done using standard constants, and the dielectric constant was maintained at 1.5.

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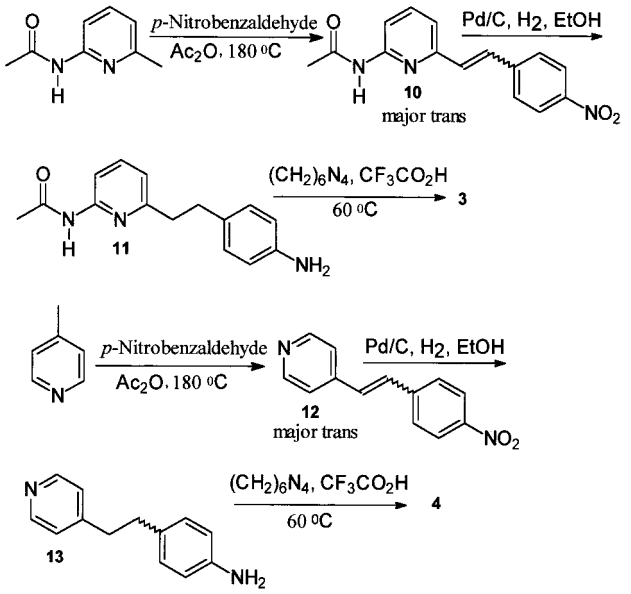
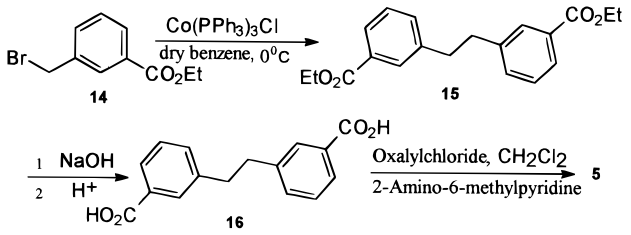
Scheme 3



Scheme 4



receptors for various guests. Compound **5** was synthesized according to Scheme 4. The C−C bond formation in **15** was achieved by the cobalt coupling method.[12b] Then alkaline hydrolysis of **15** afforded the acid **16**, which on coupling with 2-amino-6-methylpyridine amine gave the receptor **5** in good yield.

Complexation Studies. Complexation studies of dicarboxylic acids (both chiral and achiral) were carried out using NMR and fluorescence experiments.

(a) NMR Titration. In NMR titrations, hosts were dissolved in dry $CDCl_3$ and the stock solutions of guests were prepared by dissolving aliphatic dicarboxylic acids in dry $CDCl_3$. But owing to the partial solubility of the diacids into $CDCl_3$, a few drops of dry d_6-DMSO (2% in each case) were added to the stock solution to prepare a homogeneous solution of the guest. On addition of the guest to the host solution under controlled conditions, a downfield chemical shift of the amide protons of host was observed. The chemical shift values were noted as a function of the concentration of the variable component. Foster−Fyfe analysis[13a] of the titration data at 25 °C gave binding constant values. The binding constant values for **1** with dicarboxylic acids of various chain lengths are given in Table 1. The binding study shows that the cavity of Troger's base analogue **1** is selective for suberic acid. Integration of the proton signals of the 1:1 complex in the ^{1}H NMR spectrum as well as the break in the titration curve ($\Delta\delta$ vs C_{guest}/C_{host})[13b] at 1.0 establishes a 1:1 stoichiometry for the dicarboxylic acids with **1** (Table 1). In the NMR spectrum of a 1:1 complex of **1** (in $CDCl_3$) with suberic acid (dissolved in $CDCl_3$ with 2% d_6-DMSO), the considerable downfield shift of the pyridine amide protons ($\Delta\delta$ 1.68 ppm) and of the phenyl ring as well as the methylene bridge protons of the Troger's base frame ($\Delta\delta$ 1.03 ppm) indicates the formation of a stronger complex (Figure 1). The dilution of the 1:1 complex of suberic acid with the receptor **1** in $CDCl_3$ ($\Delta\delta$ 2.0 ppm of pyridine amide protons) also shows negligible change in the ^{1}H NMR spectrum. This supports a strong complexation. The other receptors **2** and **3** were explored in complexation study with the same dicarboxylic acids, used for **1** (Table 1) to see the chain length selectivity by their cavities. The comparative binding constant values are presented in Table 2. Figure 2A (E_{min} = 41.98 kcal/mol) and Figure 2B (E_{min} = 39.88 kcal/mol) are the energy-minimized forms of the complexes of suberic acid with receptors **1** and **3**, respectively. In receptor **1**, suberic acid is well-arranged (Figure 2A) with the hydrogen-bonding sites into the cavity compared to receptor **3** in Figure 2B.

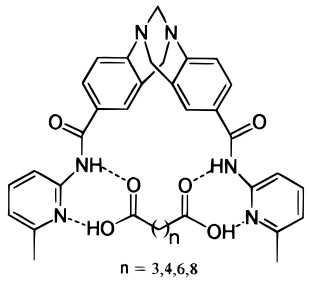


Figure 1. Mode of complexation of **1** with dicarboxylic acids.

Table 1. Binding Constants of Receptor 1 with Dicarboxylic Acids

diacids	K_a (at 25 °C), M^{-1}	ΔG (at 25 °C), kcal/mol
glutaric	1.0×10^3	−4.09
adipic	1.69×10^3	−4.40
suberic	1.5×10^4	−5.69
sebacic	3.10×10^3	−4.76
benzene-1,4-diacetic	2.88×10^2	−3.35

Table 2. Binding Constant Values K_a (M^{-1}) of Receptors 2 and 3 with Dicarboxylic Acids

	glutaric	adipic	suberic	sebacic
receptor **2**	2.4×10^2	3.5×10^2	4.8×10^2	5.8×10^2
receptor **3**	1.10×10^2	5.3×10^2	1.01×10^3	6.54×10^3

Both **2** and **3** bind adipic acid with a 1:1 stoichiometry. Receptor **2** also makes a 1:1 complex with glutaric acid. The receptor **2** shows a marginal increase in K_a with suberic acid giving a 1:2 (guest:host) stoichiometry whereas **3** binds with long chain sebacic acid with a 1:2 (guest:host) stoichiometry. All the stoichiometries of the complexes are determined from the break in the titration curves ($\Delta\delta$ vs C_{guest}/C_{host}). The reduced values of K_a with **2** and **3** are explained in terms of their flexibility. The attachment of the pyridine amide binding moieties of receptors **2** and **3** with the tetrahedral methylene carbons instead of being directly bonded to the benzene ring (as in receptor **1**) into the respective cavity may also have a steric influence on the stoichiometry of the complex. Owing to the presence of the $-CH_2-$ group between pyridine and Troger's base benzene ring, the energy barrier between the conformations is reduced and the pyridine ring can adopt different orientations in solution

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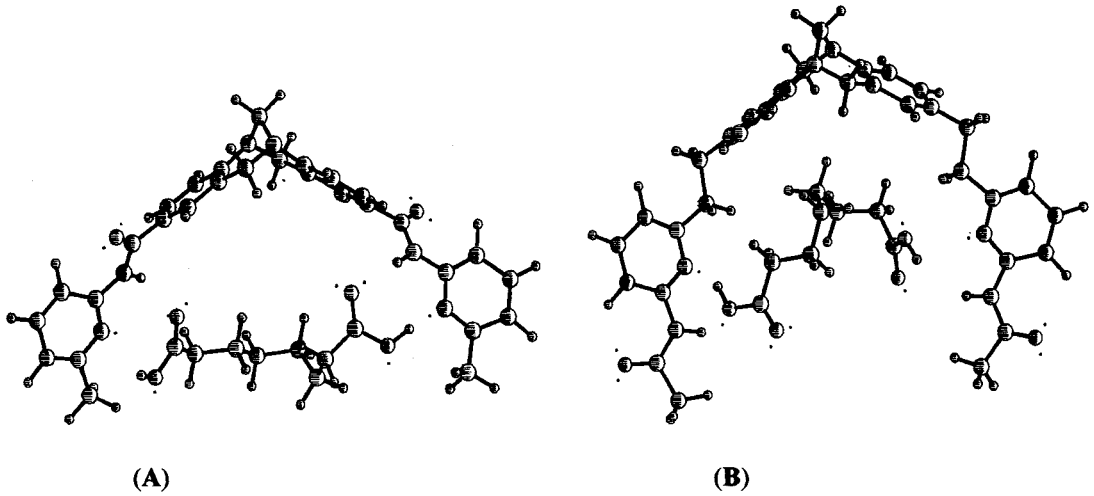

Figure 2. Energy-minimized structures of (A) **1** with suberic acid and (B) **3** with suberic acid.

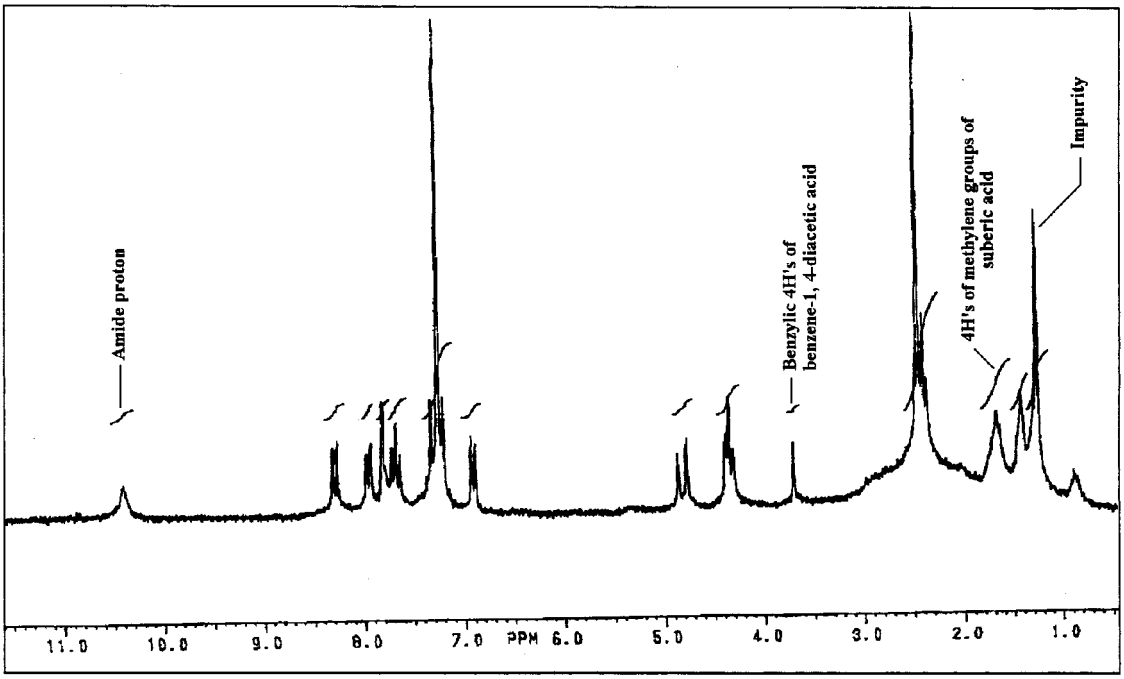

Figure 3. NMR spectrum of 1:1:1 mixture of receptor **1**, benzene-1,4-diacetic acid, and suberic acid.

phase very easily. In the case of **1**, the higher association constant for suberic acid compared to glutaric and adipic acids, respectively, is due to the complementary cavity size with suberic acid but not with shorter dicarboxylic acids. The selectivity of suberic acid was proved by its exclusive extraction (~90%) in pure $CDCl_3$ from its 1:1 mixture with benzene 1,4-diacetic acid as predicted by comparison of their NMR integrations in Figure 3.

The other receptor **5** shows weaker binding with suberic and adipic acids. In the energy-minimized structure (Figure 4A) of **5** (E_{min} = 33.51 kcal/mol), the N−N (ring nitrogens) distance is ca. 11.22 Å and N*H*−N*H* distance is ca. 9.50 Å. Although these distances are comparable with the values of the receptor **1**, it does not bind suberic acid strongly ($K_a = 3.0 \times 10^2$ M^{-1}). More interestingly, during NMR titration of **5** with suberic acid, the amide peak was broad and split into a doublet. This situation indicates the different chemical environments of amide protons, which probably arise from the loss of symmetry present in **5** owing to the C−C bond rotation in the bridge during complexation. This is also evident from the energy-minimized structure of suberic acid with receptor **5** (Figure 4B; E_{min} = 23.37 kcal/mol) where one phenyl ring at the bridge is more tilted from the plane of the other phenyl ring compared to receptor **5** itself in Figure 4A. This simple experiment thus indicates that the flexibility introduced in the design of the receptor does not have a significant role in the strong as well as selective binding of dicarboxylic acids until rigid or semirigid spacers are considered as the building blocks for the construction of such molecular receptors.

In all the cases, binding constants are sacrificed to some extent using DMSO as it is a competitive guest. This was evidently proved by taking glutaric acid as a representative with the receptor **1**. The value of binding constant measured in $CDCl_3$ for glutaric acid ($K_a = 6.01 \times 10^3$ M^{-1}) was found to be higher than the value in $CDCl_3$ containing 2% d_6-DMSO ($K_a = 1.0 \times 10^3$ M^{-1}) given in Table 1. We are now using the Troger's base

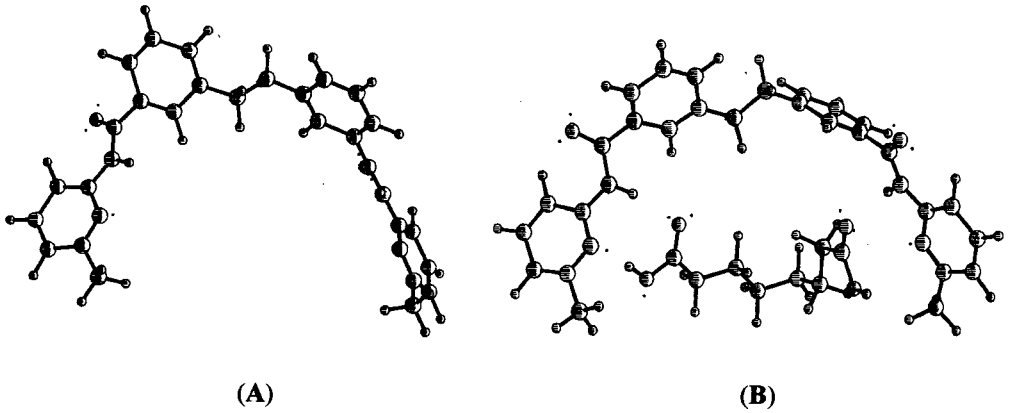

Figure 4. Energy-minimized structure of (A) receptor **5** and (B) receptor **5** with suberic acid.

analogue **4** in metal binding.[14] Its strong affinity with Cu^{II}-metal ion was noted from the broadening of the peaks of the complex in the NMR spectrum.

Fluorescence Experiments. Fluorescence experiments were carried out on the Troger's base receptors with suberic acid, which showed strong complexation characteristics in the NMR titration data of suberic acid with receptor **1**. Receptors were dissolved in $CHCl_3$, and guests were taken in $CHCl_3$ containing 2% DMSO.

Interestingly, here also a great affinity for suberic acid was noted with a considerable change in fluorescence intensity. The excitation wavelength was at 330 nm for this set of studies with the emission being monitored at 404 nm. Figure 5A shows the enhancement of fluorescence intensity with subsequent additions of the guest suberic acid. A plot of emission intensity against the concentration of suberic acid showed a nonlinear increase in the fluorescence emission as a function of suberic acid concentration. This gives a binding constant (K_a)[15a] of 2.54 $\times$ 10^4 M^{-1}, which is close to the predicted value in ^{1}H-NMR titration experiments. The experiment was repeated with receptor **2**. In the case of receptor **2**, a significant enhancement is not observed (Figure 5B) as in the case of receptor **1**. Excitation in the second case was also at 330 nm with the emission wavelength being set at 384 nm. The hydrogen bonding perturbs the lowest excited singlet state of nπ* type of the receptor **1** in a destabilizing manner.[15b] This increases the energy of the nπ* excited state of receptor **1** to such an extent that the fluorescence quantum yield increases to a considerable extent. Such perturbation is less in the case of receptor **2**, thereby indicating its poor complexation through hydrogen bonding with suberic acid. The lower affinity for the guest suberic acid in this case can be attributed to the flexibility of the arms of the Troger's base receptor that cannot take part in effective complexation as is possible in the case of receptor **1**. Studies with sebacic acid for the receptor **1** did not reveal any significant enhancement of fluorescence intensity.

Such hydrogen-bonding studies of dicarboxylic acids of various chain lengths with the Troger's base analogues

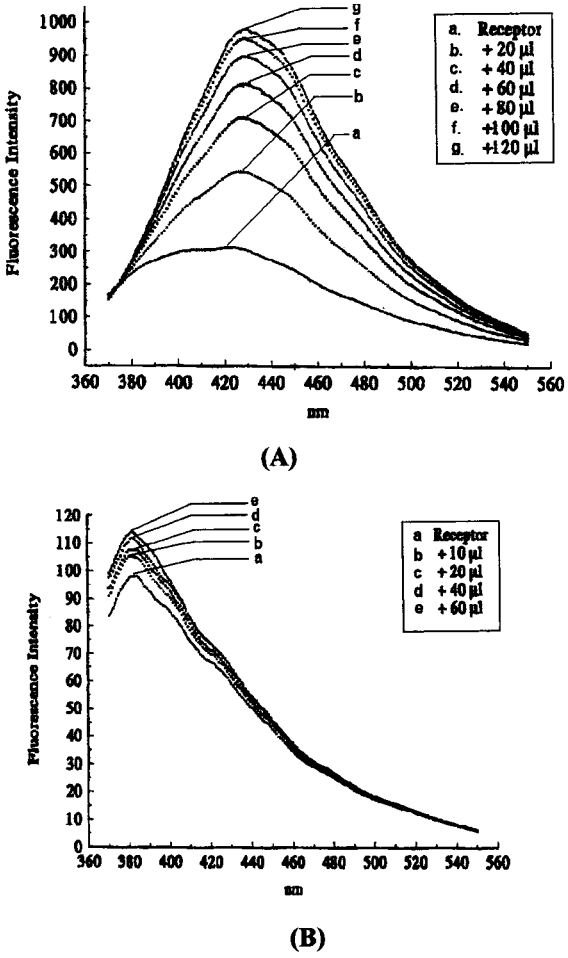

Figure 5. Fluorescence titration spectra of (A) **1** (2.04×10^{-4} M) and (B) **2** (2.57×10^{-4} M) at excitation at 330 nm with suberic acid (4.14×10^{-3} M) at 25 °C.

tempted us to study the chiral recognition process by using chiral acid guests with racemic Troger's base receptors. We studied the binding process of (±)-Troger's base receptors **1** and **2** with (+)-camphoric acid. (+)-Camphoric acid was used as the chiral dicarboxylic acid guest because of its approximate size matching with the cavity dimension of **1** and **2**. During the complexation study, the amide protons of **1** (Figure 6A) and **2** (Figure 6B) are split into two sets as shown by NMR studies, establishing an equilibrium between the two diastereomeric complexes in solution. The separation between the

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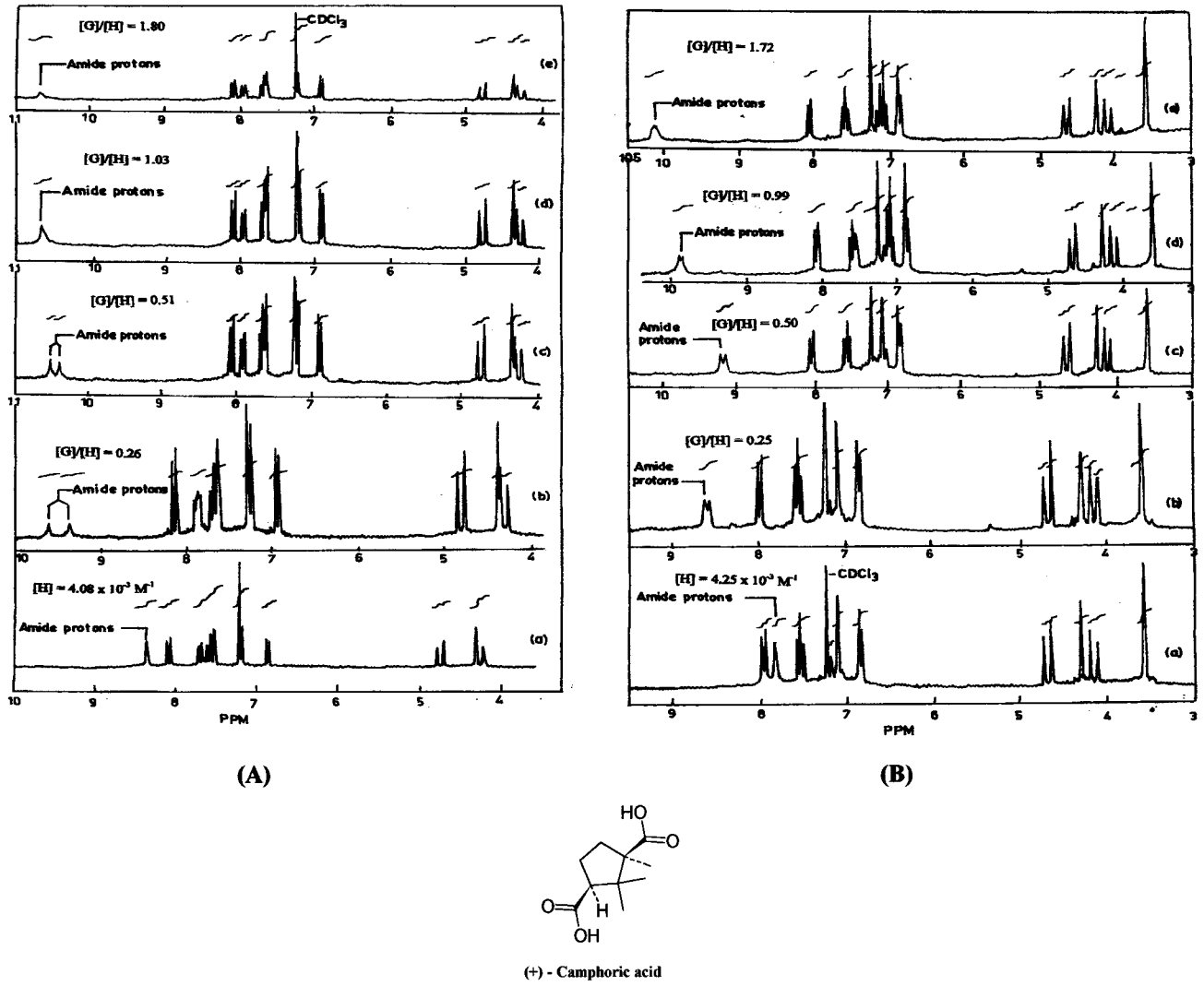

Figure 6. Variation of chemical shift of (A) **1** and (B) **2** on gradual addition of (+)-camphoric acid solution.

two sets slowly decreased on gradual addition of guest solution during the course of titrations at 25 °C. At 1:1 stoichiometry, the two peaks are merged and broadened. The two different sets of amide protons are obtained from the generation of intrinsically nonidentical chemical shifts in some of the sensor nuclei of the diastereomeric complexes that are found between (+)-camphoric acid and (±)-Troger's bases **1** and **2**.

The chemical shift variation can be explained by assuming the different stability of diastereomeric associates of the enantiomers in solution. The variation of chemical shift has been plotted (Figure 6A, B) as a function of the overall host and guest concentrations at 25 °C. In the case of **1**, two diastereomeric complexes with (+)-camphoric acid [(+)-Troger's base receptor **1**·(+)-camphoric acid and (−)-Troger's base receptor **1**·(+)-camphoric acid] differ by a binding constant (ΔK_a) value of $2.5 \times 10^2 - 3.0 \times 10^2$ M^{-1} whereas for **2** [(+)-Troger's base receptor **2**·(+)-camphoric acid and (−)-Troger's base receptor **2**·(+)-camphoric acid] the value of ΔK_a is reduced to 1×10^2 M^{-1}. Thus the NMR resolution of (±)-Troger's base analogues (**1** and **2**) proceeds from a difference between the stability constants for the diastereomeric cavities, rather than from a purely spectroscopic effect on the induced chemical shift ($\delta_0 - \delta_\alpha$).[16] We thus expected that a chromatographic resolution[17] of **1** and **2** using chiral dicarboxylic acid [(+)-camphoric acid] as a chiral stationary phase may be feasible. But practically it was very difficult to resolve the racemic Troger's bases (**1** and **2**) using (+)-camphoric acid both by crystallization as well as by chromatographic technique. This may be due to the fast equilibration between the diastereomeric complexes formed in the solution phase.

Conclusion

We have been able to readily construct a family of Troger's base molecular frameworks with heterocyclic binding motifs for the carboxyl group that selectively binds dicarboxylic acids of various chain lengths with moderate association constants. These Troger's base receptors can be used as fluorescent probes in monitoring the carboxylic acid recognition process. In the design, the flexible receptors show weak binding with dicarboxylic acid guests in spite of having comparable chain lengths with the cavity dimensions. This demonstrates that the rigidity of the host molecule has a special role in the selective binding of dicarboxylic acids.

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We have also studied the chiral resolution process of racemic Troger's base receptors by using a chiral acid through hydrogen bonding interaction, which is thus a manifestation of chiral recognition. Though the chiral resolution was successful in NMR studies, chromatographically it did not work. This is likely to be of practical interest when interactions with various chiral guest molecules are to be investigated.

Experimental Section

General. All reactions were conducted under dry nitrogen and stirred magnetically. The following solvents were freshly distilled prior to use: tetrahydrofuran (THF) from sodium and benzophenone, ethanol from CaO, magnesium turnings and, I_2, and methylene chloride and triethylamine from calcium hydride. All other solvents and reagents were of reagent-grade quality and used without further purification. All chemical shift values shown are in δ scales. Melting points were recorded in open capillaries and are uncorrected. Elemental analyses were done on a CHN analyzer. Mass spectral data are given as *m*/*z* (% abundance). Infrared spectra were taken in $CHCl_3$ and as a thin film on KBr plates. The NMR titrations were carried out in $CDCl_3$ at 25 °C. Error limits of binding constant values are within ±5%.

2,8-Bis(ethoxycarbonyl)-6*H*,12*H*-5,11-methanodibenzo-[*b*,*f*][1,5]-diazocine 6. A mixture of *p*-aminoethylbenzoate (0.1 g, 0.60 mmol) and hexamethylenetetramine (0.08 g, 0.60 mmol) in trifluoroacetic acid (TFAA) (2 mL) was stirred at 60−70 °C. After 60 h, TFAA was removed by distillation. The residue was taken in 5 mL of water, poured into a separatory funnel, and basified by the addition of concentrated NH_4OH. The aqueous layer was then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude product as a yellow glass foam. The crude product was purified by column chromatography using EtOAc in $CHCl_3$ (10%) as the eluent to give the product **6** (0.05 g, 23%), mp 126−128 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 8 Hz), 7.62 (s, 2H), 7.16 (d, 2H, J = 8 Hz), 4.73 (d, 2H, J = 16 Hz), 4.38 (m, 4H), 4.28 (s, 2H), 4.24 (d, 2H, J = 16 Hz), 1.34 (t, 6H, J = 6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 165.7, 152.3, 128.7, 127.2, 126.1, 124.7, 66.6, 60.5, 58.6, 14.3; IR ($CHCl_3$) ν_{max} 2891, 2913, 1712, 1609, 1281, 1180, 1110, 1022 cm^{-1}; UV λ_{max} (log ϵ) 284 (4.33), 250 (4.39) [*c* 1.09 × 10^{-4} M, $CHCl_3$].

2-(4-Aminobenzoylamino)-6-methylpyridine 8. To a stirred solution of 4-nitrobenzoyl chloride (1.71 g, 9.24 mmol) in dry dichloromethane (30 mL) was added dropwise 2-amino-6-methylpyridine (1 g, 9.24 mmol) and triethylamine (1.3 mL) at room temperature under nitrogen. The mixture was left overnight under stirring, and water (20 mL) was added. The organic layer was washed with water followed by saturated sodium bicarbonate solution and dried (Na_2SO_4). After evaporation of the solvent, the product **7** was further purified by silica gel chromatography (2.14 g, 90%), mp 128 °C. A mixture of compound **7** (0.52 g, 2.02 mmol), Pd/C (10%) (0.1 g), and ethanol (15 mL) was next stirred at room temperature under hydrogen atmosphere overnight. The catalyst was filtered off, and the solvent was evaporated in vacuo to give product **8** (0.41 g, 89%), which was almost pure for the next run. ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.40 (s, 1H, NH), 8.16 (d, 1H, J = 8 Hz), 7.77 (d, 2H, J = 8 Hz), 7.60 (t, 1H, J = 8 Hz), 6.86 (d, lH, J = 8 Hz), 6.69 (d, 2H, J = 8 Hz), 4.02 (bs, 2H, $-NH_2$), 2.46 (s, 3H).

2,8-Bis(2-carboxamido-6-methylpyridine)-6*H*,12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine 1. A solution of compound **8** (0.4 g, 1.76 mmol) and hexamethylenetetramine (0.25 g, 1.76 mmol) in 5 mL of trifluoroacetic acid (TFAA) was stirred at 60−70 °C. After 70 h the TFAA was removed by distillation. The residue was taken up in 20 mL of H_2O, poured into a separatory funnel, and basified by the addition of NH_4OH. The aqueous layer was then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to give a yellow brown glass foam, which was first purified by column chromatography. Further purification was done by PTLC using EtOH in C_6H_6 (5%) to give the pure product **1** (0.09 g, 15%), mp 110−2 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.46 (s, 2H, NH), 8.12 (d, 2H, J = 8 Hz), 7.72 (d, 2H, J = 8 Hz), 7.60 (t, 2H, J = 8 Hz), 7.56 (s, 2H), 7.23 (d, 2H, J = 8 Hz), 6.90 (d, 2H, J = 8 Hz), 4.78 (d, 2H, J = 16 Hz), 4.35 (s, 2H), 4.29 (d, 2H, J = 16 Hz), 2.45 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 164.9, 156.8, 156.6, 151.8, 138.7, 129.9, 127.9, 126.7, 126.4, 125.3, 119.3, 110.9, 66.7, 58.7, 29.7; IR ($CHCl_3$) ν_{max} 3361, 2924, 2857, 2327, 1670, 1603, 1528, 1390, 1297, 1201, 1090 cm^{-1}; UV λ_{max} (log ϵ) 293 (4.40), 254 (4.14) [*c* 1.43 × 10^{-4} M, $CHCl_3$]; mass (EI, *m*/*e*) 490.2 (M^+, 69%), 383.3 (100%); Anal. calcd for $C_{29}H_{26}N_6O_2$: C, 71.00; H, 5.34; N, 17.13. Found: C, 70.65; H, 5.25; N, 16.93.

6-Methyl-2-(4-nitrophenylacetylamino)pyridine 9. To a solution of 4-nitrophenylacetylchloride (0.505 g, obtained by heating 4-nitrophenylacetic acid with an excess of thionyl chloride at 60 °C for 12 h) in dry CH_2Cl_2 (10 mL), 2-amino-6-methylpyridine (0.302 g, 2.8 mmol) and triethylamine (1 mL) were added dropwise at room temperature under nitrogen atmosphere. The mixture was stirred overnight. The organic layer was washed with water followed by saturated sodium bicarbonate solution and dried over Na_2SO_4. After evaporation of the solvent, the product was purified by silica gel chromatography (0.58 g, 81%) mp 118 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.23 (d, 2H, J = 8 Hz), 7.95 (d, 1H, J = 8 Hz), 7.85 (s, 1H, $-NHCOCH_3$), 7.58 (t, 1H, J = 8 Hz), 7.52 (d, 2H, J = 8 Hz), 6.89 (d, 1H, J = 8 Hz), 3.80 (s, 2H), 2.42 (s, 3H).

2,8-Bis((2-carboxamido-6-methylpyridine)methyl)-6*H*,-12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine 2. The nitro compound **9** was reduced to the corresponding amine by hydrogen in presence of Pd/C as catalyst. The catalyst was filtered off, and the solvent was evaporated in vacuo to give the amine. The crude amine (0.14 g, 0.58 mmol) was next dissolved in trifluoroacetic acid (TFAA) (4 mL). Hexamethylenetetramine (0.08 g, 0.58 mmol) was added, and the mixture was stirred at 60 °C for 60 h under N_2 atmosphere. TFAA was removed by distillation, and the residue was diluted with water. The mixture was basified by the addition of NH_4OH, and the aqueous layer was extracted with three 50 mL portions of CH_2Cl_2. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to give a brown glass foam. After initial purification by column chromatography, final pure product **2** (0.20 g, 62%) was obtained by PTLC using EtOH in C_6H_6 (5%) as the eluent, mp 70 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.97 (d, 2H, J = 8 Hz), 7.81 (s, 2H, NH), 7.54 (t, 2H, J = 8 Hz), 7.12 (s, 4H), 6.87 (s, 2H), 6.85 (d, 2H, J = 8 Hz), 4.68 (d, 2H, J = 16 Hz), 4.30 (s, 2H), 4.16 ((d, 2H, J = 16 Hz), 3.58 (s, 4H), 2.38 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.4, 156.6, 150.4, 147.4, 138.6, 129.6, 128.8, 128.4, 127.8, 125.7, 119.3, 110.8, 66.7, 58.5, 44.4, 29.6; IR (KBr): ν_{max} 3530, 2920, 1669, 1600, 1574, 1538, 1451, 1391, 1296, 1150 cm^{-1}; UV λ_{max} (log ϵ) 280 (4.29), 241 (4.45) [*c* 7.72 × 10^{-5} M, $CHCl_3$]; mass (EI, *m*/*e*) 519.4 (MH^+, 26%), 518.2 (M^+, 7%), 384.2 (4%), 275.2 (12%), 135.2 (36%), 109.1 (100%); Anal. calcd for $C_{31}H_{30}N_6O_2$: C, 71.79; H, 5.83; N, 16.20. Found: C, 71.50; H, 5.70; N, 16.32.

2-Acetylamino-6(4-nitrostyryl)pyridine 10. A solution consisting of 2-acetylamino-6-methylpyridine (0.8 g, 5.33 mmol), 4-nitrobenzaldehyde (0.805 g, 5.33 mmol), and 5 mL of acetic anhydride was heated 175 °C for 50 h. The cooled solution was poured onto ice, and the mixture was then made alkaline with 40% aqueous NaOH. The product was then extracted from water mixture with ethyl acetate. Evaporation of the solvent in vacuo gave the impure desired product. Purification by column chromatography [using benzene:ethyl acetate (3:1)] gave the product **10** (0.72 g, 48%), mp 175−178 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (d, 2H, J = 8 Hz), 8.13 (t, 1H, J = 8 Hz), 7.98 (bs, 1H, NH), 7.75−7.64 (m, 4H), 7.60 (d, 1H, J = 16 Hz), 7.11 (d, 1H, J = 8 Hz), 2.25 (s, 3H).

2-Acetylamino-6-[(4-aminophenyl)ethyl)]pyridine 11. Compound **10** (0.3 g, 1.06 mmol) and 10% Pd/C (0.1 g) were taken in ethanol and stirred overnight at room temperature under hydrogen atmosphere. The catalyst was filtered off, and the solvent was evaporated in vacuo to give compound **11**

(0.19g, 70 %): ^{1}H NMR (200 MHz, d_6-DMSO) δ 9.58 (s, 1H, NH), 8.14 (d, 1H, J = 8 Hz), 7.63 (t, 1H, J = 8 Hz), 6.92 (d, 2H, J = 8 Hz), 6.82−6.71 (m, 3H), 4.53 (bs, 2H, $-NH_2$), 2.94 (bs, 4H), 2.20 (s, 3H).

2,8-Bis((2-acetylamino-6-methylpyridine)ethyl)-6*H*,-12*H*-5,11-methanodibenzo[*b*,*f*][1,5]-diazocine 3. A mixture of the amine **11** (0.09 g, 0.35 mmol), hexamethylenetetramine (0.05 g, 0.35 mmol), and TFAA (3 mL) was initially stirred at room temperature (25 °C) for 3 h and then heated at 60 °C with stirring for 50 h under N_2 atmosphere. The mixture was cooled to rt, diluted with water, and neutralized by NH_4OH. The mixture was extracted with CH_2Cl_2 (4 × 5 mL). The organic layer was next washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The volatiles were removed, and the crude product was initially purified by column chromatography on silica gel using EtOH in C_6H_6 (6%) as the eluent. The final purification by PTLC using the same solvent as the developing agent afforded the product **3** (0.12 g, 63%), mp 80 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.98 (d, 2H, J = 8 Hz), 7.86 (bs, 2H, NH), 7.54 (t, 2H, J = 8 Hz), 7.06−6.99 (m, 4H), 6.78 (d, 2H, J = 8 Hz), 6.71 (s, 2H), 4.64 (d, 2H, J = 18 Hz), 4.29 (s, 2H), 4.09 (d, 21, J = 18 Hz), 2.86 (s, 8H), 2.19 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) 166.6, 160.4, 150.6, 146.0, 139.5, 136.9, 127.6, 127.3, 126.6, 124.9, 118.6, 111.0, 66.8, 58.5, 39.5, 35.1, 25.1; IR (KBr) ν_{max} 3400, 3040, 2915, 1682. 1595, 1572, 1537, 1449 cm^{-1}; UV λ_{max} (log ϵ) 281 (4.22), 240 (4.35) [*c* 9.16 × 10^{-5} M, $CHCl_3$]; mass (EI, *m*/*e*) 547.5 (MH^+, 27%), 339.1 (14%), 55.0 (100%); Anal. calcd for $C_{33}H_{34}N_6O_2$: C, 72.25; H, 6.26; N, 15.37. Found: C, 71.98; H, 6.19; N, 15.23.

4′-Nitro-4-stilbazole 12. A mixture of 4-nitrobenzaldehyde (0.80 g, 5.29 mmol), 4-picoline (0.49 g, 5.30 mmol), and 10 mL of acetic anhydride was refluxed at 180 °C, poured onto ice, and neutralized with 50% sodium hydroxide solution. The mixture was extracted with EtOAc and dried (Na_2SO_4). The solvent was evaporated in vacuo, and the crude product was purified by column chromatography using EtOAc in petroleum ether (20%) as the eluent to give the pure compound **12** (0.80 g, 57%), mp 140−142 °C: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.63 (m, 2H), 8.25 (d, 2H, J = 8 Hz), 7.68 (d, 2H, J = 8 Hz), 7.41 (m, 2H), 7.28 (d, 1H, J = 10 Hz), 7.17 (d, 1H, J = 16 Hz).

2,8-Bis((4-pyridyl)ethyl)-6*H*,12*H*-5,11-methanodibenzo-[*b*,*f*][1,5]-diazocine 4. The crude amine **13** (0.10 g, 0.40 mmol), obtained from catalytic hydrogenation (H_2, Pd/C) of **12**, was dissolved in TFAA (3 mL). Hexamethylenetetramine was added, and the mixture was heated at 60 °C with stirring under N_2 atmosphere for 40 h. The mixture was cooled to rt, diluted with water, and neutralized by NH_4OH. The mixture was extracted with CH_2Cl_2 (4 × 5 mL). The organic layer was thoroughly washed with saturated $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo, and the crude product was purified by column chromatography on silica gel using EtOH in C_6H_6 (6%) as the eluent to afford the low-melting yellowish solid (0.12 g, 70%). ^{1}H NMR (200 MHz $CDCl_3$): δ 8.44 (d, 4H, J = 8 Hz), 7.07−6.94 (m, 8H), 6.68 (s, 2H), 4.64 (d, 2H, J = 18 Hz), 4.29 (s, 2H), 4.09 (d, 2H, J = 18 Hz), 2.80 (bs, 8 H); ^{13}C NMR (50 MHz, $CDCl_3$) 150.3, 149.6, 146.3, 136.3, 127.7, 127.3, 126.6, 125.0, 123.8, 66.9, 59.8, 36.9, 35.9; IR ($CHCl_3$) ν_{max} 3420, 3015, 2890, 1669, 1595, 1552, 1487, 1410 cm^{-1}; UV λ_{max} (log ϵ) 294 (3.79), 251 (4.16) [*c* 1.39 × 10^{-4} M, $CHCl_3$]; mass (EI, *m*/*e*) 432.3 (M^+, 30%), 340.3 (22%), 281.3 (50%), 188.2 (100%).

3,3′-Ethylenedibenzoic Acid 16. 1-Carboethoxy-3-bromomethylbenzene **14** (1 g, 4.1 mmol) was treated with Co(PPh_3)$_3$Cl (4.34g, 4.9 mmol) in degassed dry benzene (70 mL) at room temperature for 20 min under Ar atmosphere. The reaction mixture was filtered, and the filtrate was washed with water and concentrated to dryness. The crude **15** was hydrolyzed by 60 mL of 3N NaOH for 3-4 h at 80 °C. The solution was then neutralized by dilute HCl and extracted by EtOAc. The solvent was evaporated, and the residue was chromatographed on a silica gel column using EtOAc:MeOH (3:1) to give the pure product **16** (0.86 g, 78%), mp 218 °C: ^{1}H NMR (200 MHz, d_6-DMSO) δ 7.98−7.90 (m, 4H), 7.58 (s, 2H), 7.42 (d, 2H, J = 4 Hz), 3.07 (s, 4H).

1,2-Bis[3-(2-carboxamido-6-methylpyridine)phenyl]-ethane 5. To a stirred solution of diacid **16** (0.3 g, 1.1 mmol) in dry CH_2Cl_2, oxalyl chloride (0.19 mL, 2.2 mmol) and a drop of dry DMF (catalytic) were added. The mixture was stirred at room temperature under N_2 atmosphere for 1.5 h. The solvent was evaporated under N_2 atmosphere. The solid mass was redissolved in dry CH_2Cl_2, and a solution of 2-amino-6-methylpyridine (0.19 g, 1.74 mmol) containing Et_3N (0.5 mL) was added dropwise. The mixture was stirred overnight. The usual workup and purification by silica gel column chromatography using petroleum ether in $CHCl_3$ (30%) gave the title compound as semisolid (0.53 g, 68%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.60 (bs, 2H, −NH), 8.20 (d, 2H, J = 8 Hz), 7.91 (m, 2H), 7.87−7.54 (m, 6H), 7.32 (d, 2H, J = 6 Hz), 6.92 (m, 2H), 2.48 (s, 4H), 2.43 (s, 6H); IR (KBr) ν_{max} 3295, 3050, 2920, 1673, 1598, 1573 cm^{-1}.

Experimental Procedure for NMR Titration. As a specific example, the control titration experiment of Troger's base receptor **1** with suberic acid will be described here. A 3.46 × 10^{-3} M solution of **1** in dry $CDCl_3$ was taken in an NMR tube. A 1.33 × 10^{-2} M solution of suberic acid in dry $CDCl_3$ containing 2% d_6-DMSO was prepared. An initial NMR spectrum of **1** was taken, and the initial chemical shift of the amide proton was determined to be 8.38 ppm. The solution of guest suberic acid was then progressively added, in 10, 20, 30, 40 μL proportions, etc., to the solution of **1**, and the chemical shift of amide proton was recorded after each addition. Such addition was continued until no further change in the chemical shift of the amide proton was observed. The chemical shift of the amide proton at the saturation point was 1.70 ppm. The temperature of the NMR probe was 25 °C. The change in chemical shift ($\Delta\delta$) values were calculated by subtracting the chemical shift at each titration point from the chemical shift value of pure host. Thus, a titration curve of $\Delta\delta$ vs concentration ratio of guest/host and a binding constant curve of $\Delta\delta/C_{guest}$ vs $\Delta\delta$ were plotted. Foster−Fyfe analysis of the titration data at 25 °C gave the value of K_a (1.5 × 10^4 M^{-1}), and ΔG was calculated from the relation $\Delta G = -RT \ln K_a$.

Fluorescence Experiment. Stock solutions of receptors were prepared in $CHCl_3$, and guests were dissolved in $CHCl_3$ containing 2% DMSO. The titrations were carried out by successive addition of guest solution to the solution of receptor placed in a fluorescence curette. Fluorescence spectra were measured using a PERKIN ELMER LS 50B spectrofluorimeter, using a conventional 1 × 1 cm quartz cell at 25 °C with the excitation and emission slits of 6 nm width. The solution of receptor **1** at a concentration of 2.04 × 10^{-4} M was excited at 330 nm with the emission being monitored at 404 nm, and the fluorescence intensity at the emission maximum was used to determine the complex stability constant. A similar experiment was done with receptor **2** (2.57 × 10^{-4} M) at excitation and emission wavelengths 330 and 384 nm, respectively, with suberic acid (4.14 × 10^{-3} M) at 25 °C.

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Supporting Information Available: Spectral data for **1**−**6** and binding constant curve, titration curve, and fluorescence binding curve for suberic acid with **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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