Convergent Functional Groups. 5. Ternary Complexes in the Molecular Recognition of β-Arylethylamines

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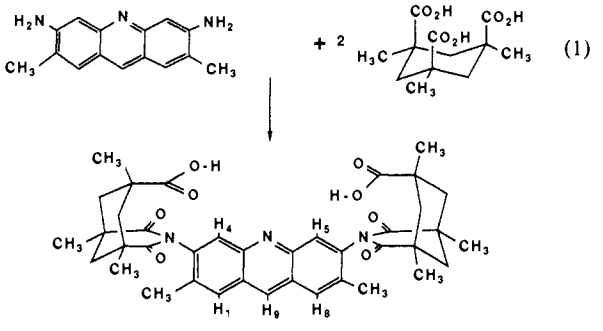
Abstract: A new class of synthetic receptors is described for binding β-arylethylamines. The structures feature a molecular cleft in which two carboxyl groups converge to create the binding site. Ionic and aromatic stacking forces combine to result in specific recognition of β-arylethylamines, and NMR titrations show that 2:1 (receptor to amine) complexes are formed. Geometric features of the complexes are established by intermolecular NOE techniques.

We recently introduced a synthetic molecular cleft and showed its general applicability to problems of molecular recognition.[1] The cleft represents a departure in molecular shape from the classical macrocyclic model compounds of bioorganic chemistry such as crown ethers of cyclodextrins. The new shape offers a number of advantages. The molecules are rapidly assembled from the Kemp[2] triacid (eq 1) and appropriate spacer groups such as acridine yellow. The carboxyl groups of structure **1** are con-



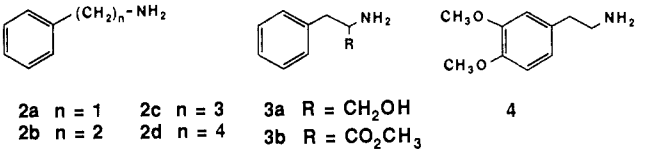
(1)

1

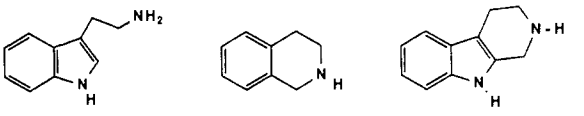
strained[3] to the convergent arrangement shown, a feature that resembles the convergence or "focusing" of functional groups at active sites of enzymes and natural receptors. The convergence is also the key to the selectivity observed in binding of smaller molecules having complementary size, shape, and functionality. The highly polar microenvironment presented by the functional groups in the cleft is also capable of binding and transporting zwitterionic amino acids such as phenylalanine and tryptophan across simple liquid membranes.[4] In this report we describe the binding of amines in general and show that the affinity of **1** to aralkylamines involves selective stacking interactions between the aromatic nuclei of the receptor and the substrate.

The complexation reactions of the model receptor **1** are conveniently monitored by NMR spectroscopy. Treatment of the diacid with conventional bases such as triethylamine causes deprotonation that can be monitored by the gradual downfield shifts[5] of the protons lining the cleft H_4 and H_5. However, with certain phenethylamines such as β-phenylethylamine **2b**, the titration causes first upfield shifts of these protons then downfield shifts as shown in Figure 1. The break in the curve when 0.5 equiv of an amine is added establishes the existence of a 2:1 complex, two receptors to one amine. The maximum downfield shift observed in these protons occurs after about 2 equiv of amine is added. This indicates a 1:2 complex (receptor to amine) at high amine concentrations. It is assumed that at intermediate stoichiometries, i.e., equimolar amounts of amine and receptor, a 1:1 complex is also present.

The 2:1 complexation appears quite similar to that established for the binding of amino acids such as phenylalanine and tryptophane within these molecules.[4] Figure 2 shows the downfield portion of the NMR spectra (300 MHz) as the diacid **1** is titrated with **2b**. When ≤0.5 mol equiv of amine is present, the phenyl



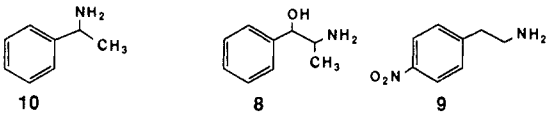
2a n = 1 **2c** n = 3 **3a** R = CH_2OH **4**
2b n = 2 **2d** n = 4 **3b** R = CO_2CH_3



5 **6** **7**

protons are shifted upfield to such a degree that simple first-order spectra result (Figure 2a). In the range 0.5–1.5 equiv of amine, these signals gradually move downfield (Figure 2b,c), and when excess amine is present, they eventually merge to a typical multiplet. Identical behavior is seen with phenylalanine derivatives such as the alcohol **3a**, the methyl ester **3b**, the dopamine derivative **4**, or tryptamine (**5**). In the latter case, the upfield chemical shifts persist even when excess tryptamine is present.

Amines in which the β-aryl function was part of a cyclic system were also examined for their interactions with **1**. Specifically, tetrahydroisoquinoline **6** and the carbazole derivative **7** were used in the titration protocol. In these cases the aromatic protons of the amines were not resolved, but the changes in chemical shift followed the trend of other β-phenylethylamines, i.e.; upfield shifts followed by downfield drift as excess amine is added. The spectra were also broadened at room temperature, suggesting that exchange of free and bound amines was slowed with these more rigid systems. The ephedrine **8** showed similar broadened spectra, but the *p*-NO_2 derivative **9** behaved like its unsubstituted parent (**2b**). The aryl–aryl interactions betweeen **1** and **9** are probably of the van der Waals or dipole–dipole sort and do not involve charge transfer, since both aromatics are π acceptors.



10 **8** **9**

In contrast, γ-phenylpropylamine (**2c**) or δ-phenylbutylamine (**2d**) show conventional aromatic multiplets throughout this titration protocol, i.e., these amines show no specific interactions with the diacid receptor other than that anticipated by simple deprotonation. Derivatives of benzylamine were also examined, and their behavior in contact with **1** indicated some, but reduced, aryl–aryl interactions. Both **2a** and α-phenethylamine **10** showed

(1) Rebek, J. *Science (Washington, D.C.)* **1987**, *235*, 1478–1484.
(2) Kemp, D. S.; Petrakis, K. S. *J. Org. Chem.* **1981**, *46*, 514.
(3) Rebek, J., Jr.; Marshall, L.; Wolak, R.; Parris, K.; Killoran, M.; Askew, B.; Nemeth, D.; Islam, N. *J. Am. Chem. Soc.* **1985**, *107*, 7476–7481.
(4) Rebek, J., Jr.; Askew, B.; Nemeth, D.; Parris, K. *J. Am. Chem. Soc.* **1987**, *109*, 2432–2434.
(5) Rebek, J., Jr.; Askew, B.; Killoran, M.; Nemeth, D.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, *109*, 2436–2431.

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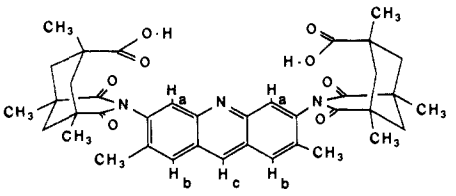
Table I



1

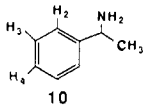
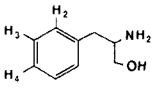
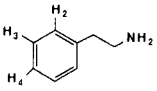
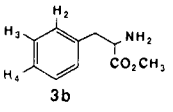
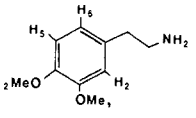
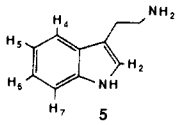
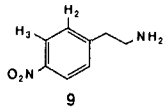
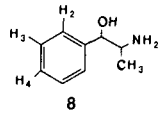
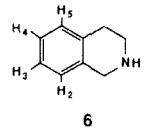
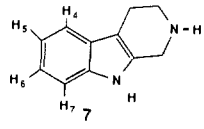
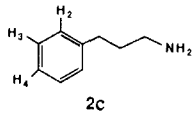
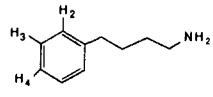
substrate	receptor/ substrate ratio	chemical shift,[a] ppm	
		receptor	substrate
 10	2:1	(a) 8.15 (0) (b) 7.72 (−0.04) (c) 8.48 (0.15)	(2) 7.11 (−0.1) (3) 6.90 (−0.3) (4) 6.79 (−0.4)
	1:1	(a) 8.18 (+0.03) (b) 7.72 (−0.04) (c) 8.46 (−0.17)	(2) 7.14 (−0.05) (3) 6.99 (−0.2) (4) 6.90 (−0.3)
 3a	8:1	(a) 8.12 (−0.3) (b) 7.76 (0) (c) 8.62 (−0.01)	(2) 6.85 (−0.4) (3) 6.13 (−1.12) (4) 5.89 (−1.36)
	2:1	(a) 8.08 (−0.07) (b) 7.76 (0) (c) 8.55 (−0.08)	(2) 6.86 (−0.39) (3) 6.13 (−1.12) (4) 5.91 (−1.34)
	1:1	(a) 8.16 (−0.01) (b) 7.76 (0) (c) 8.55 (−0.08)	(2) 6.91 (−0.34) (3) 6.66 (0.59) (4) 6.54
 2b	2:1	(a) 8.06 (−0.06) (b) 7.76 (0) (c) 8.60 (−0.03)	(2) 6.89 (−0.36) (3) 6.31 (−1.0) (4) 6.25 (−1.0)
	1:1	(a) 8.17 (+0.03) (b) 7.76 (0) (c) 8.58 (−0.05)	(2) 7.08 (−0.17) (3) 6.81 (−0.50) (4) 6.65 (−0.70)
 3b	3:1	(a) 8.13 (−0.02) (b) 7.76 (0) (c) 8.60 (−0.03)	(2) 6.83 (−0.42) (3) 6.34 (−0.93) (4) 6.15 (−1.10)
	2:1	(a) 8.08 (−0.07) (b) 7.76 (0) (c) 8.60 (−0.03)	(2) 6.83 (−0.42) (3) 6.40 (−0.88) (4) 6.18 (−1.08)
	1:1	(a) 8.15 (+0.02) (b) 7.76 (0) (c) 8.55 (−0.08)	(2) 7.08 (−0.17) (3) 6.88 (−0.42) (4) 6.79 (−0.51)
 4	3:1	(a) 8.10 (−0.05) (b) 7.76 (0) (c) 8.62 (−0.02)	(2) 6.01 (−0.74) (5) 5.89 (−0.87) (6) 6.42 (−0.43) (OMe_2) 3.11 (−0.77) (OMe_1) 3.47 (−0.42)
	2:1	(a) 8.04 (−0.11) (b) 7.76 (0) (c) 8.54 (−0.1)	(2) 6.01 (−0.74) (5) 5.89 (−0.87) (6) 6.42 (−0.43) (OMe_2) 3.11 (−0.77) (OMe_1) 3.47 (−0.42)
	1:1	(a) 8.13 (−0.02) (b) 7.76 (0) (c) 8.53 (−0.1)	(2) 6.17 (−0.57) (5) 6.18 (−0.69) (6) 6.49 (−0.30) (OMe_2) 3.32 (−0.56) (OMe_1) 3.42 (0.42)
 5	2:1	(a) 8.15 (0) (b) 7.63 (−0.13) (c) 8.35 (−0.28)	(2) 6.80 (−0.90) (4) 6.64 (−1.0) (5) 6.64 (−0.57) (6) 6.24 (−0.89) (7) 6.35 (−1.02)
	1:1	(a) 8.15 (0) (b) 7.58 (−0.18) (c) 7.90 (−0.73)	(2) 6.91 (−0.79) (4) 6.74 (−0.89) (5) 6.64 (−0.57) (6) 6.35 (0.78) (7) 6.45 (0.92)
	1:2	(a) 8.15 (0) (b) 7.35 (−0.41) (c) 7.65 (−0.98)	(2) 6.81 (−0.89) (4) 6.96 (−0.67) (5) 6.85 (−0.36) (6) 6.60 (−0.53) (7) 6.72 (−0.67)

Table I (Continued)

substrate	receptor/substrate ratio	chemical shift,[a] ppm receptor	chemical shift,[a] ppm substrate
 9	5:1	(a) 8.17 (+0.02) (b) 7.76 (0) (c) 8.55 (−0.08)	(2) 7.09 (−0.27) (3) 7.32 (−0.83)
	2:1	(a) 8.16 (+0.01) (b) 7.75 (−0.01) (c) 8.54 (−0.08)	(2) 7.05 (−0.31) (3) 7.32 (−0.83)
	1:1	(a) 8.16 (+0.01) (b) 7.72 (−0.04) (c) 8.43 (−0.20)	(2) 7.02 (−0.34) (3) 7.42 (−0.73)
 8	5:1	(a) 8.20 (+0.05) (b) 7.74 (−0.02) (c) 8.58 (−0.05)	(2) (3) 6.85 (br, m) (4)
	2:1	(a) 8.24 (+0.09) (b) 7.74 (−0.02) (c) 8.50 (+0.13)	(2) (3) 6.95 (br, m) (4)
	1:1	(a) 8.32 (+0.17) (b) 7.76 (0) (c) 8.50 (−0.13)	(2) (3) 7.15 (br, m) (4)
 6	1:2	(a) 8.15 (−0.01) (b) 7.74 (−0.02) (c) 8.18 (0.45)	*b*
	1:1	(a) 8.26 (+0.11) (b) 7.72 (−0.04) (c) 8.11 (−0.52)	
 7	1:2	(a) 8.15 (0) (b) 7.60 (−0.16) (c) 8.23 (−0.4)	*b*
	1:1	(a) 8.52 (+0.37) (b) 7.58 (−0.18) (c) 8.10 (−0.53)	
 2c	2:1	(a) 8.19 (+0.04) (b) 7.76 (0) (c) 8.53 (−0.10)	7.09 (br, m) (−0.06)
	1:1	(a) 8.25 (+0.11) (b) 7.76 (0) (c) 8.52 (−0.11)	7.10 (br, m) (−0.05)
 2d	2:1	(a) 8.20 (+0.05) (b) 7.76 (0) (c) 8.51 (−0.12)	7.09 (br, m) (−0.06)
	1:1	(a) 8.23 (+0.13) (b) 7.76 (0) (c) 8.50 (−0.13)	7.12 (br, m) (−0.03)

[a] (+) = downfield shift. [b] Aromatic region of substrate is too broad to determine shifts at room temperature. They do however follow the trend in that they are shifted upfield and then start to shift downfield after 5 equiv of amine is added.

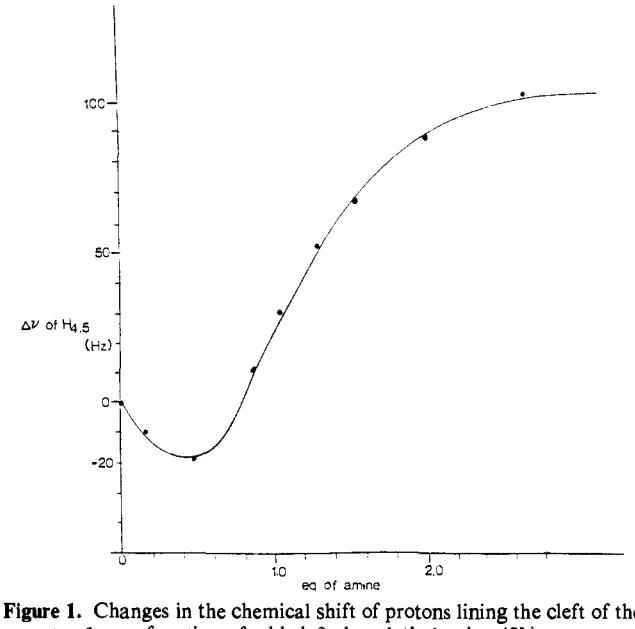


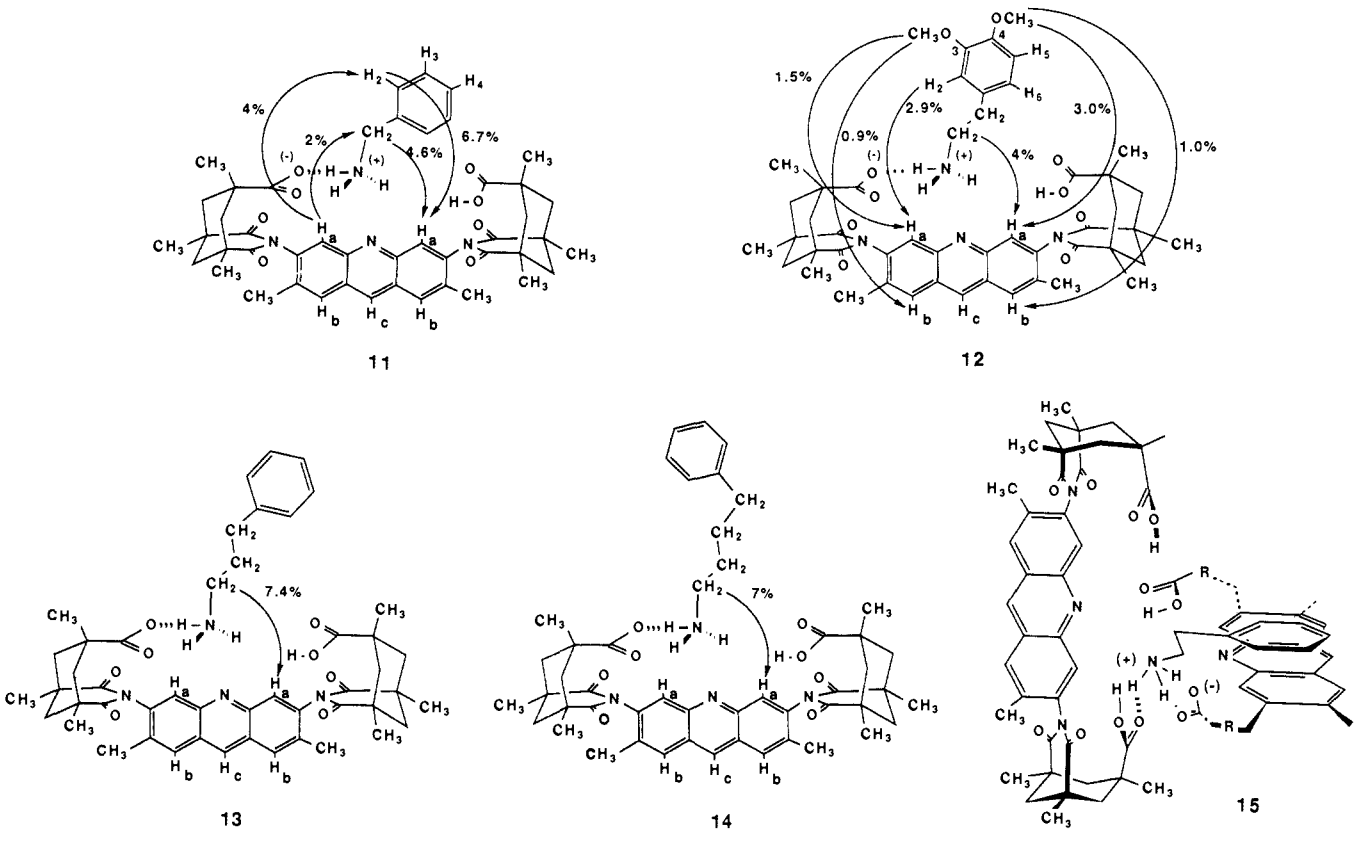
Figure 1. Changes in the chemical shift of protons lining the cleft of the receptor **1** as a function of added β-phenylethylamine (**2b**).

sufficient upfield shifts of their aromatic protons to present a first-order spectrum. The largest upfield shift in **10** was the para proton (0.4 ppm), but the corresponding shift in the β-phenyl **2b** was 1.0 ppm. Competition studies with chiral amines[6] support the relationship between upfield shifts and binding ability. These show that β-arylethylamines are able to displace α- or γ-aryl analogues from their contact with the acridine surface of **1**.

For the β-phenethylamines, then, the upfield shifts are in accord with stacking interactions between aromatic subunits in the artificial receptor and substrates in these complexes. Quite coincidently, a recent proposal[7] concerning the geometry of a natural receptor features a similar arrangement of ammonium and aromatic binding sites. The distance between the carboxylate at one side of the cleft of **1** and the pyridine nucleus matches that of the proposed quite nicely. Some refinement of the structure of the 2:1 complexes is desirable. Unfortunately, the alkyl protons of the amines are broadened to such a degree that no useful[8] coupling information and hence conformational information can be gleaned from them.

(6) Rebek, J., Jr.; Askew, B.; Ballester, P.; Doa, M. *J. Am. Chem. Soc.* **1987**, *109*, 4119–4120.
(7) Lloyd, E. J.; Andrews, P. J. *J. Med. Chem.* **1986**, *29*, 453–462.
(8) See for example: Colucci, W. J.; Junk, S. J.; Gandour, R. D. *Magn. Reson. Chem.* **1985**, *23*, 335–343.

Chart I



To date, none of the complexes have shown suitable crystallinity for X-ray diffraction studies. Additional qualitative information concerning the structures of the complexes could, however, be obtained from NOE experiments. Using pulse sequences that have been developed in other studies[9] of weak intermolecular interactions, we were able to observe a number of intermolecular NOEs within the complexes. These are summarized in structures **11–14** (Chart I) for α-, β-, γ-, and δ-aralkylamines, respectively. These were all obtained at 2:1 (diacid **1** to amine) stoichiometry. In the figures the tails of the arrows indicate which protons were irradiated and the heads of the arrows indicate which protons' resonances were enhanced (no other intramolecular NOEs were observed).

Given the qualitative nature of the structural data, a detailed geometry for these 2:1 complexes cannot be mapped out. A reasonable proposal is given in **15**. In such a structure the stacking interactions of the α- and β-arylamines can be rationalized by their ability to span the distance between the carboxyl function and the acridine nucleus. For the γ or δ derivatives, stacking would require reduced conformational freedom in the alkyl chain, and the entropic price for such rotational restrictions is apparently not met by the binding force of the stacking interaction. This structure is quite similar to that proposed for the 2:1 complexes of the acridine **1** with zwitterionic amino acids that bear β-aryl side chains.[4] The similarity in NMR spectra of phenylalanine or phenylalaninol in contact with **1** suggests a close relationship between the structures.

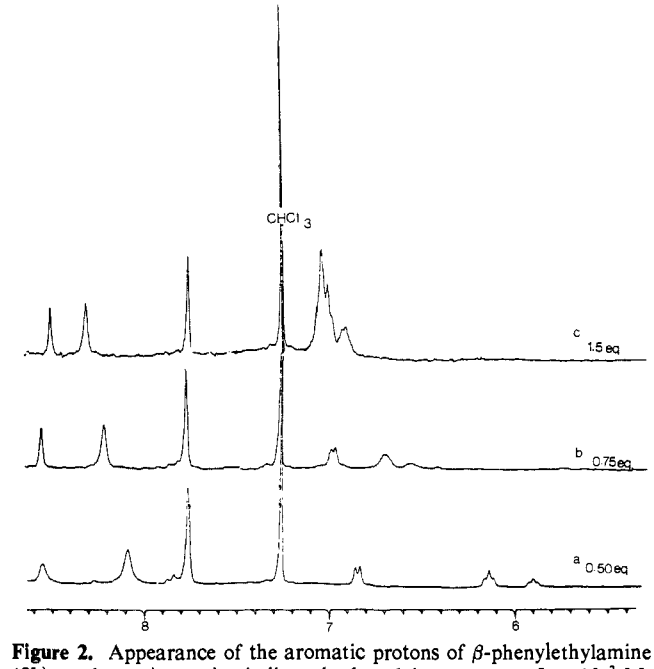


Figure 2. Appearance of the aromatic protons of β-phenylethylamine (**2b**) at the amine ratios indicated when **1** is present at 5×10^{-3} M.

Experimental Section

^{1}H NMR experiments were performed at 300 MHz on either a Bruker WH-300 with a dedicated Aspect-2000 computer or an IBM AF-300 with a dedicated Aspect-3000 computer. In the NOE experiments the decoupler was set to the desired frequency and turned on for 4 s prior to signal acquisition. Following a 0.1-s switching time a 90° pulse was applied, and the free induction decay was acquired with the decoupler off. The sequence is repeated following a 5-s delay to allow the spins to revert to their equilibrium populations. Reference spectra were obtained in a similar manner with the decoupler frequency set to irradiate an empty region of the spectrum. Four transients were acquired with the decoupler on resonance followed by four reference transients. Typically 1000–2000 transients were collected. Percent NOE enhancements were determined by integration of the difference spectra obtained by subtracting the reference fid from the enhanced fid; the smallest NOE reported in this paper is 0.9%, but the experimental method permits observation of NOEs as low as 0.5%.[10]

(9) Pirkle, W. H.; Pochapsky, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 5627–5628.

(10) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703–5711.

(11) Boissonnas, R. A.; Guttmann, S.; Jaquenoud, P. A.; Waller, J. P. *Helv. Chim. Acta* **1955**, *38*, 1491.

NMR Titration Experiments. Most amines used in these studies were commercially available (Aldrich) and were used as received. The amino acid ester **3b** was prepared by standard methods[11] and liberated from HCl just prior to use. Typically, 500 μL of a 5.0×10^{-3} M $CDCl_3$ solution of **1** prepared as described[3,12] was treated with small aliquots (1-5 μL) of a 0.5 M $CDCl_3$ solution of amine, and the spectrum was recorded after each addition. Chemical shift values for **1** and various amines at selected stoichiometries are reported in Table I. Experiments with the β-arylethylamines in $CD_3OD/CDCl_3$ (1:1, v/v) also showed the upfield shifts characteristic of stacking interactions. Spectra at low temperatures were complex but generally indicated that two different acidine subunits are present in the complexes.

Acknowledgment. We thank the National Institutes of Health for support of this research and the Spanish State of Valencia for a fellowship granted to A.C.

Registry No. 1, 98800-49-8; **2b**, 64-04-0; **2c**, 2038-57-5; **2d**, 13214-66-9; **3a**, 16088-07-6; **3b**, 2577-90-4; **4**, 120-20-7; **5**, 61-54-1; 6, 91-21-4; 7, 16502-01-5; **8**, 48115-38-4; **9**, 24954-67-4; **10**, 98-84-0.

(12) Rebek, J., Jr.; Askew, B.; Killoran, M.; Nemeth, D.; Lin, F.-T. *J. Am. Chem. Soc.* **1987**, *109*, 2426-2431.

UV-Visible and Carbon NMR Studies of Quinine Binding to Urohemin I Chloride and Uroporphyrin I in Aqueous Solution

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Abstract: Quinine binding by urohemin I and uroporphyrin I in aqueous solution at pH 6.0-6.4 has been studied by UV-visible and natural-abundance carbon NMR spectroscopies. Our results show that the soluble uroporphyrin-quinine complex has 1:1 stoichiometry characterized by an apparent overall association equilibrium constant $K_A = (4.2 \pm 0.2) \times 10^4$ M^{-1} at a uroporphyrin concentration of 10^{-6} M (22 ± 1 °C). From a combination of optical methods (Job, Scatchard, and Hill formulations) and carbon NMR spectroscopy, the complex is best formulated as a cofacial π-π dimer. In contrast, quinine interacts cooperatively with urohemin, yielding a Hill parameter of 2. Also, different from the uroporphyrin complex, the urohemin complex has a stoichiometry shown by Job's method to be 2:1 (urohemin to quinine). It is characterized by an apparent overall $K_A = (3.8 \pm 0.4) \times 10^8$ M^{-2} at 10^{-6} M urohemin and 22 ± 1 °C. NMR spectra of the quinine-urohemin I complex are consistent with iron ion coordination by the quinine 9-position hydroxyl group accompanying π-π type bonding between the heme ring and quinoline ring. For the uroporphyrin, where metal coordination is precluded, a different geometry involving primarily a π-π complex formation is indicated.

The interaction of certain malaria drugs with hemes has been studied in both aqueous and nonaqueous media[1-6] as a consequence of suggestions that protoheme IX may function as the receptor for antimalarials in *Plasmodium*-infected erythrocytes.[3,7] In this view, protoheme IX, originating from protease-degraded hemoglobin in the parasitized cell, may become available for complex formation with malaria drugs, resulting in the formation of malaria pigment clumps.[7,8] Indeed, it has been postulated that protoheme IX liberated from hemoglobin, in association with the erythrocyte membrane, may even act as the specific malaria drug receptor.[3,9]

In view of the seeming relevance of heme-malaria drug interactions, we have undertaken a study of quinine interaction with urohemin I [(uroporphyrin I)iron(III) chloride] and free base uroporphyrin I in aqueous solution. These structures are shown in Figure 1. Our motivation for this study is twofold. First, previous studies of this type in aqueous solution have utilized protohemin IX, a heme known to aggregate to varied extents in aqueous media depending upon pH and concentration,[11,12] so that with one exception[3] little quantitative data have been derived from such studies. Second, although an aqueous environment is, perhaps, more relevant to the physiological chemistry of malaria drug chemotherapy, an extremely interesting recent study was performed in nonaqueous solution.[5] The results of that work suggested that quinine is capable of axially coordinating the dimethyl ester of protohemin IX [chloroiron(III)protoporphyrin IX dimethyl ester] and (tetraphenylporphinato)iron(III) cations containing different counterions. Those results were extremely significant and stand in contrast to conclusions drawn by others who performed similar experiments with protohemin IX and uroporphyrin I in aqueous solution.[4] These latter workers concluded that axial coordination by quinine in aqueous solution was not evident.

In an attempt to further clarify the aqueous chemistry of quinine in the presence of hemes we have chosen to study this drug's interaction individually with urohemin I and uroporphyrin I near neutral pH. Previous work has characterized the aggregation dynamics of both of these hemes,[12-20] making it possible to derive equilibrium constants, stoichiometries, and Hill parameters by UV-visible methods. The choice of pH 6.0 as an appropriate value

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