Synthetic Bis-Metal Ion Receptors for Bis-Imidazole "Protein Analogs"

Sanku Mallik, Robert D. Johnson, and Frances H. Arnold*

Contribution from the Division of Chemistry and Chemical Engineering 210-41, California Institute of Technology, Pasadena, California 91125

Received May 24, 1994®

Abstract: We are investigating an approach to protein recognition that is based on matching a pattern of metal ions in a synthetic receptor to a complementary pattern of metal-coordinating functional groups (histidine) on a protein's surface. In this model study, target "protein analogs" were constructed by linking two imidazoles *via* organic spacers of varying lengths. By computer modeling the individual targets and receptors, bis-Hg^{2+} receptors were designed to position two metal ions to match the available nitrogen ligands of their target bis-imidazoles. While ^1H NMR studies in DMSO-d_6 show that the receptors can bind 2 equiv of 1-benzylimidazole ($K_1 \sim 10^4$ M^{-1}), a bis-imidazole is bound in a 1:1 complex with association constants as high as 3×10^6 M^{-1}. Bis-metal ion receptors are indeed selective for their target bis-imidazoles in competitive binding experiments, preferring the target over others that are both longer and shorter by ~4 Å (maximum selectivity = 11.5). A maximum selectivity of 140 was observed for the competition between a target bis-imidazole and 1-benzylimidazole. Increasing the available coordination sites on the metal ion significantly reduces selectivity, presumably by allowing the receptor to take on multiple bound conformations. Attempts to improve binding selectivity by restricting the receptors' conformational mobility reduced selectivity, primarily by introducing unanticipated unfavorable interactions with the target bis-imidazoles.

Nature creates receptors for proteins with spectacular success, as exemplified by the specificity of interactions in the immune system.[1] Precisely arranged arrays of weak interactions extending over large contact areas (>1000 Å^2) determine the strength of binding and selectivity an antibody exhibits for a complementary site on its antigen. The rational design of receptors to recognize proteins or other molecules in aqueous media presents significant challenges. Design and synthesis of a receptor incorporating a large number of weak complementary interactions[2] is difficult. To simplify receptor design and preparation, but still achieve useful selectivities and binding affinities, we have chosen strong metal-to-ligand coordination as the basis for recognition. Our approach to preparing small, stable artificial receptors for proteins and other molecules is to match arrays of metal ions in the receptor to distributions of metal-coordinating functional groups on the molecule of interest.[3]

Recognition based on metal-to-ligand interactions offers several potential advantages over schemes that rely on hydrogen bonding,[4] hydrophobic,[5] or other interactions.[6] For a receptor to form a complex with its target, the entropic cost of bimolecular association must be paid by the thermodynamic forces driving binding. One obvious attractive feature of metal-to-ligand interactions is that they are quite strong, even in aqueous media. Thus fewer individual interactions between receptor and target are required to achieve tight binding. The association constant for Cu^{2+} complexation by the imidazole side chain of histidine, $10^{3.5}$ M^{-1}, translates to a binding energy of 4.8 kcal/mol.[7] In contrast, a hydrogen bonding interaction generally contributes less than 1 kcal/mol to binding in water.[8]

A second attractive feature is the availability of a wide variety of ligands and transition metal ions, which allows us to tailor both the strength and kinetics of binding. The strength of the metal–imidazole interaction, for example, varies over a wide range, depending on the metal (pK_a = 4.6 for Cu^{2+}, 2.9 for Zn^{2+}, and 9.2 for Hg^{2+}).[7] Complexes of imidazole with exchange-inert metal ions such as Co^{3+}, Ru^{2+}, or Pt^{2+} exhibit such slow ligand exchange kinetics that they are essentially covalent for many purposes. We are exploiting this feature, for example, to prepare high-fideltiy polymer "imprints" of template bis-imidazoles[9,10] and to stabilize proteins by cross-linking dihistidine metal-binding sites.[11] Exchange-labile transition metal ions offer interactions that can be formed and broken under relatively mild conditions. Thus the target molecule can be released from the metal ion-containing receptor by adjusting the pH or with a competing ligand. Finally, the metal center, depending on the metal ion and its oxidation state, can have magnetic, spectroscopic, and chemical properties which can be used, for example, to extract structural information or for catalysis.

The affinity of the metal ion for hydroxide ion may restrict the choice of metal ion and necessitate careful pH control. Some

* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts*, September 1, 1994.

(1) Owen, M. J.; Lamb, J. R. *Immune Recognition*; IRL Press: Oxford, U.K., 1988.

(2) Schneider, H.-J. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1417–1436.

(3) Mallik, S.; Plunkett, S. D.; Dhal, P. K.; Johnson, R. D.; Pack, D.; Shnek, D.; Arnold, F. H. *New J. Chem.* **1994**, *18*, 299–304.

(4) Albert, J. S.; Hamilton, A. D. *Tetrahedron Lett.* **1993**, *34*, 7363–7366. Seto, C. T.; Mathias, J. P.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 1321–1329. Slobodkin, G.; Fan, E.; Hamilton, A. D. *New J. Chem.* **1992**, *16*, 643–645. Kikuchi, Y.; Kobayashi, K.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 1351–1358. Wang, X.; Erickson, S. D.; Iimori, T.; Still, W. C. *J. Am. Chem. Soc.* **1992**, *114*, 4128–4137.

(5) Carcanague, D. R.; Knobler, C. B.; Diederich, F. *J. Am. Chem. Soc.* **1992**, *114*, 1515–1517. Diederich, F. *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 362–386.

(6) Kobayashi, K.; Asakawa, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10307–10313. Sijbesma, R. P.; Kentgens, A. P. M.; Lutz, E. T. G.; van der Maas, J. H.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1993**, *115*, 8999–9005. Hunter, C. A.; Purvis, D. H. *Angew. Chem. Int. Ed. Engl.* **1992**, *16*, 792–795. Pieters, R. J.; Rebek, J., Jr. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 330–334. Conn, M.; Deslongchamps, G.; de Mendoza, J.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 3548–3557. Reetz, M. T.; Niemeyer, C. M.; Hermes, M.; Goddard, R. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1017–1019. Deslongchamps, G.; Galan, A.; de Mendoza, J.; Rebek, J., Jr. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 61–63.

(7) Sinha, P. C.; Saxena, P. K.; Nigam, N. B.; Srivastava, M. N. *Indian J. Chem.* **1989**, *28A*, 335–336. Martell, A. E. *Critical Stability Constants*; Plenum Press: New York, 1974; Vol. 2, pp 144–145; Vol. 3, pp 1–14.

(8) Searle, M. S.; Williams, D. H.; Gerhard, U. *J. Am. Chem. Soc.* **1992**, *114*, 10697–10704. Shirley, B. A.; Stanssens, P.; Hahn, U.; Pace, C. N. *Biochemistry* **1992**, *31*, 725–732. Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 3770–3771.

(9) Plunkett, S. D.; Sunderasan, V.; Arnold, F. H. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)*, in press.

(10) Dhal, P. K.; Arnold, F. H. *Macromolecules* **1992**, *25*, 7051–7059.

(11) Muheim, A.; Todd, R. J.; Casimiro, D. R.; Gray, H. B.; Arnold, F. H. *J. Am. Chem. Soc.* **1993**, *115*, 5312–5313.

0002-7863/94/1516-8902$04.50/0

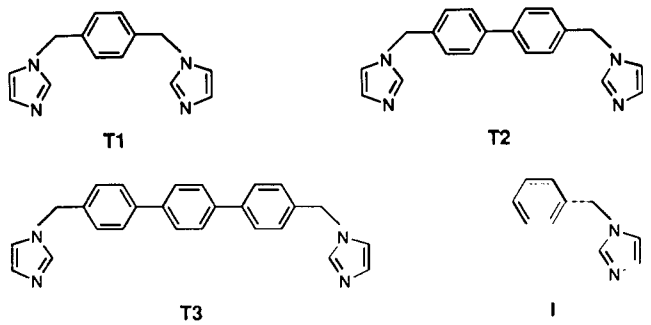


Figure 1. Bis-imidazole "protein analogs" **T** and 1-benzylimidazole (**I**).

transition metals form insoluble hydroxides in water at pH 7; Zn^{2+}–diethylenetriamine, for example, precipitates above pH 6, and Fe^{3+}–iminodiacetate precipitates at pH ~ 7. Even when the metal hydroxide is water soluble, hydroxide can compete with the target molecule for metal binding. These shortcomings can be overcome by judicious choice of the metal ion and the ligand. Surface histidine coordination to complexes of Cu^{2+}, Ni^{2+}, and Zn^{2+} provide the basis for protein separation by metal-affinity chromatography.[12]

Our approach to protein recognition exploits the protein's unique pattern of surface-exposed histidines (or other metal-coordinating functional groups), which can be recognized by a receptor displaying a complementary pattern of transition metal ions. The simplest receptor for a protein would position two metal ions so that they could simultaneously coordinate a pair of surface histidines. The specificity of binding is dictated by the degree to which two-point binding is favored over binding to single surface histidines and the probability that no other protein in the sample has a pair of histidines with the same spacing. This is aided by the fact that histidine is relatively rare: ~2% of the amino acids in globular proteins are histidines, of which ~50% are surface exposed. Metal coordination could be used in conjunction with other interactions to further increase binding specificity.

To test the validity of this simple approach to making artificial receptors for protein recognition, we have prepared several bis-metal receptors and studied their interactions with a series of bis-imidazole "protein analogs".[13] The goal of this work is to define the extent to which two metal–imidazole coordination interactions can provide selectivity in binding the target molecule. These model studies also serve to elucidate how specific features of the receptor design—distance between metal centers, mobility, and the number of available coordination sites—can influence binding selectivity.

Results

Design of Target Protein Analogs. To construct a model system for testing this concept for synthetic receptors, simple "protein analogs" were designed and synthesized[10] to position two imidazoles at predetermined distances using rigid spacers of varying lengths. The bis-imidazole targets, shown in Figure 1, are designated **T1**, **T2**, and **T3**, based on the number of phenyl rings separating the imidazole moieties. 1-Benzylimidazole (**I**) was used as a control in the binding experiments.

Because force-field parameters appropriate for metal-to-ligand interactions are not readily available, receptor designs were based on matching the conformations of energy-minimized receptors and bis-imidazoles in the separated states. To estimate the optimal distance between the two imidazole N–3 lone pairs on a target protein analog, **T1**, **T2**, and **T3** were modeled using Insight II (Version 2.3.0; Biosym Technologies, San Diego, CA). The two imidazole moieties were forced to lie on one face of the aromatic spacer (so that both N-3's could coordinate to the two metal ions of a receptor), and the energy of the structure was minimized in the vapor phase. The resulting geometries and distances between the two coordinating nitrogen atoms are shown in Figure 2. Four bonds in each target bis-imidazole (marked a and b) can freely rotate. Rotation around the C—N bond (b) changes the spacing between nitrogen ligands by ~1 Å. Optimum distances for receptor binding based on the structures shown in Figure 2 and rotation about the C—N bond are predicted to be 6.9–8.3 Å for **T1**, 11.1–12.5 Å for **T2**, and 16.3–17.9 Å for **T3**.

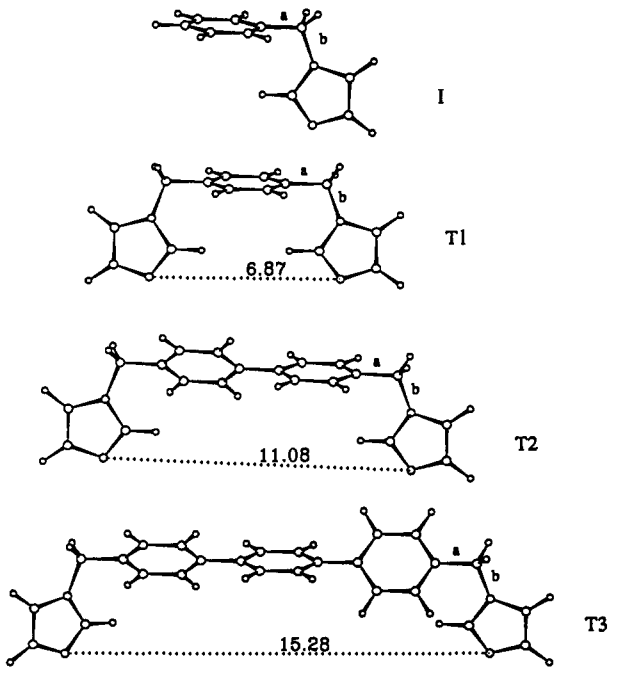


Figure 2. Energy-minimized structures of bis-imidazoles, showing distances between the imidazole N-3 (Å). Bonds a and b are capable of rotating freely.

Receptor Design and Synthesis. The bis-metal receptors shown in Figure 3 were designed to position metal ions to match the coordinating nitrogens of the bis-imidazole targets. A dimagnetic metal ion, Hg^{2+}, was used in these model studies so that binding could be monitored by ^1H NMR. The metal ions are held in place by either 1,4,8,11-tetraazacyclotetradecane (cyclam)[14] or diethylenetriamine (dien). The formation constants are 10^{23} M^{-1} for Hg^{2+}–cyclam and 10^{11} M^{-1} for Hg^{2+}–dien in water.[7] The crystal structure of Hg^{2+}–cyclam is essentially square pyramidal with the four N donors in one plane and the central metal ion displaced from the plane by 0.80 Å.[15] The large ionic radius of Hg^{2+} (1.10 Å) prevents it from remaining in plane with the four nitrogen atoms of the cyclam macrocycle. Because the macrocycle effectively blocks one face of the metal ion, each metal center in receptors $\mathbf{R_{cc}}$, $\mathbf{R_{cxc}}$, $\mathbf{R_{crc}}$ and $\mathbf{R_{cnc}}$ presents only one possible coordination site for imidazole. The bis-Hg^{2+}–dien receptor $\mathbf{R_{dxd}}$ was designed to investigate the effect on selectivity of having more than one metal coordination site available for imidazole binding. Each metal ion in a Hg^{2+}–dien complex has three vacant coordination sites.[16] Perchlorate was used as the counter anion for all of the receptors due to its low tendency to coordinate to transition metal ions.

(12) Arnold, F. H. *Bio/Technology* **1991**, *9*, 151–156. For a recent compilation of articles on applications of metal-affinity separations, see *Methods: A Companion to Methods in Enzymology* **1992**, *4*, 1–108.

(13) For preliminary communication see: Mallik, S.; Johnson, R. D.; Arnold, F. H. *J. Am. Chem. Soc.* **1993**, *115*, 2518–2520.

(14) Bradshaw, J. S., Ed. *Heterocyclic Compounds*; Wiley & Sons: New York, 1993; Vol. 51, pp 539–691.

(15) Alcock, N. W.; Curson, E. H.; Herron, N.; Moore, P. *J. Chem. Soc. Dalton Trans.* **1979**, 1987–1993.

(16) Diaz, A.; Massacesi, M.; Ponticelli, G.; Paschina, G. *J. Inorg. Nucl. Chem.* **1975**, *37*, 2469–2472.

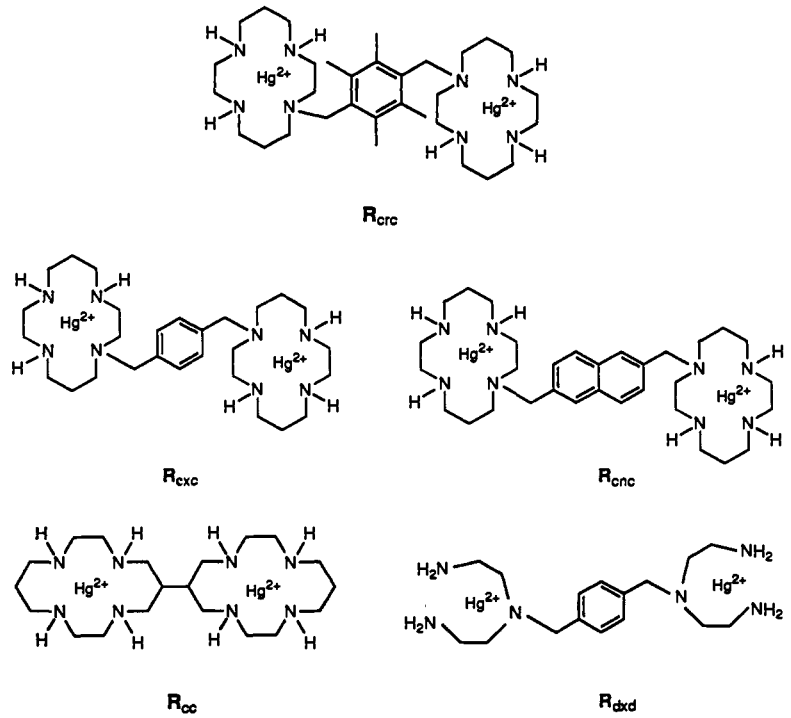


Figure 3. Structures of bis-metal ion receptors designed to recognize protein analogs.

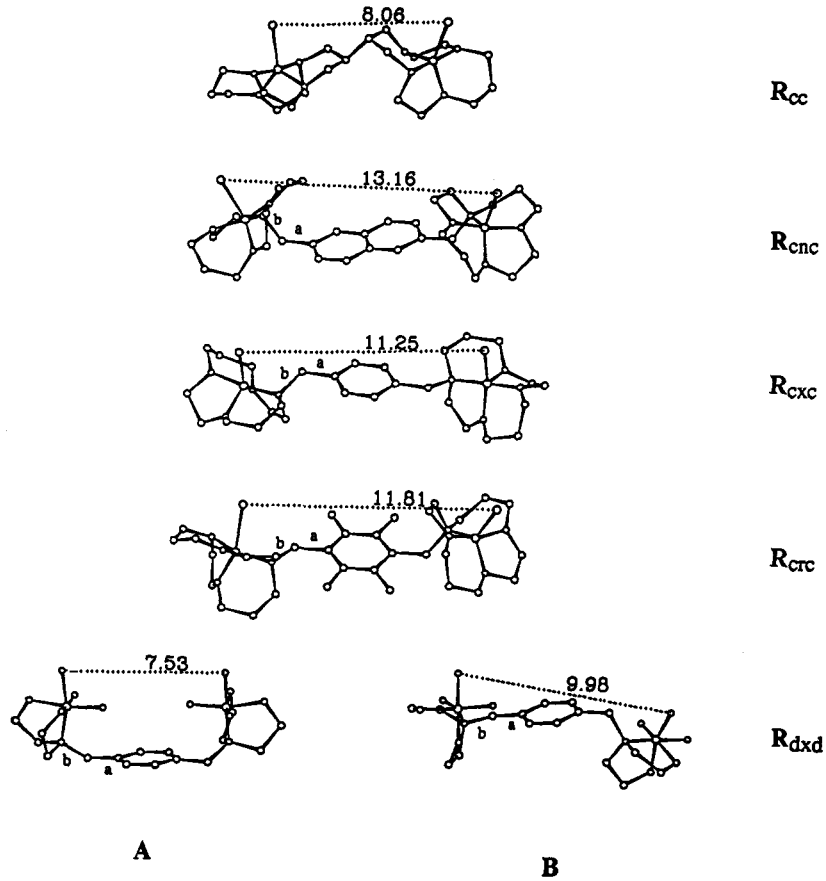


Figure 4. Energy-minimized structures for receptors R_{cc} R_{cxc}, R_{cnc}, R_{crc}, and R_{dxd}. Two possible conformations of R_{dxd} suitable for two-point binding are shown (A and B). Distances between the two metal ions are indicated (Å). Bonds a and b are capable of rotation. Hydrogen atoms are omitted for clarity.

To estimate the optimal geometry for bis-metal binding, receptor structures were generated by energy minimization in the gas phase, using the molecular modeling software Biograf (Version 3.1; Molecular Simulations, Waltham, MA). Ru^{2+} was substituted for Hg^{2+} in the modeling, since the program does not have force field parameters for Hg^{2+}. Energy minimization yielded square pyramidal geometry for the Ru^{2+}–cyclam complex (starting from planar geometry). The difference in the ionic radii of Hg^{2+} and Ru^{2+} (1.10 Å for Hg^{2+} vs 0.82 Å for Ru^{2+})[17] should only affect the displacement of the metal ion from the

plane of the four cyclam N-donor atoms and not the distance between the two metal ions in a receptor. The available coordination sites for both metal ions were placed on one face of the aromatic spacers to generate the optimal geometry for recognition of target bis-imidazoles. Distances between the two free coordination sites in the optimized receptor structures shown in Figure 4 are 13 Å for $\mathbf{R_{cnc}}$ and 11 Å for $\mathbf{R_{cxc}}$. For receptor $\mathbf{R_{dxd}}$, the dien moiety was made to coordinate facially before energy minimization. Two viable conformers were obtained for this receptor, with optimum distances of 7.5 and 10 Å. On the basis of the distance-matching argument, receptors $\mathbf{R_{cnc}}$ and $\mathbf{R_{cxc}}$ should prefer to bind **T2** over **I** and the other bis-imidazoles. The shorter conformer of $\mathbf{R_{dxd}}$ should be able to bind **T1**, favoring it over **I**, **T2**, and **T3**.

Four bonds in each of these receptors are capable of rotating (marked a and b in Figure 4). Formation of a 1:1 cyclic complex between a receptor and its target bis-imidazole freezes these rotations. Since this is entropically unfavorable (entropic cost of restricting a C—C bond in a hydrocarbon is ~0.8 kcal mol^{-1}),[18] restricting these rotations could improve the binding selectivity of a receptor. To test this hypothesis, two restricted receptors, $\mathbf{R_{crc}}$ and $\mathbf{R_{cc}}$, were synthesized. $\mathbf{R_{crc}}$ is an analog of $\mathbf{R_{cxc}}$ with four methyl groups on the spacer phenyl ring to constrain the rotation of the benzylic C—C bonds. Modeling results in a structure somewhat different from that of $\mathbf{R_{cxc}}$; the distance between the sites that would be occupied by a target bis-imidazole increases slightly (Figure 4). The two cyclam rings in $\mathbf{R_{cc}}$ are connected by a single C—C bond, and only this bond can rotate freely. Bis-metal complexes of this ligand are known,[19] although crystal structures have not been reported. The distance between the two metal centers in the energy-minimized structure for the bis-Ru^{2+} complex of $\mathbf{R_{cc}}$ (shown in Figure 4) is 8 Å, which matches bis-imidazole **T1**.

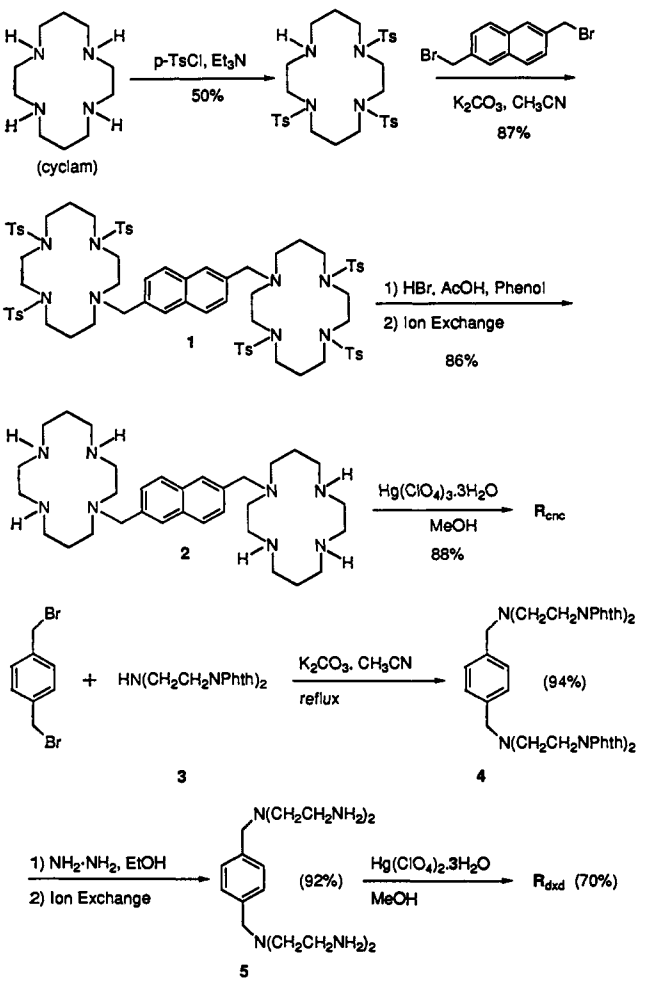
Receptor $\mathbf{R_{cxc}}$ was synthesized by literature procedure.[20] Synthesis of the compounds, $\mathbf{R_{cnc}}$ and $\mathbf{R_{dxd}}$ are shown in Scheme 1. Compound $\mathbf{R_{dxd}}$ is known in the literature.[21] A modified procedure led to a much higher yield of product (for details, see Experimental Section). The synthesis of $\mathbf{R_{crc}}$ is outlined in Scheme 2. $\mathbf{R_{cc}}$ was prepared as described in the literature.[19]

Binding Studies. Although the bis-Hg^{2+} complexes $\mathbf{R_{cnc}}$, $\mathbf{R_{cxc}}$, $\mathbf{R_{dxd}}$, and $\mathbf{R_{cc}}$ are soluble in water at pH 7.0 to a concentration of ~10 mM (the solubility of $\mathbf{R_{crc}}$ is much lower), poor water solubilities of the bis-imidazole targets required that the NMR binding studies be performed in a polar organic solvent, and dimethylsulfoxide (DMSO-d_6) was chosen.

Because 1-benzylimidazole (**I**) can form 2:1 complexes with the receptors (*vide infra*), the approach of Lenkinski[22] was used to obtain accurate estimates of the two association constants from simultaneous analysis of two types of NMR titration experiments: a solution of **I** (~5 mM) was titrated with increasing amounts of receptor (1–10 mM, type 1 titration) and a solution of the receptor (~10 mM) was titrated with increasing amounts of I (2–20 mM, type II titration). Good estimates of the first binding constant and chemical shift of the 1:1 complex can be obtained from type 1 titration data, while type II titrations determine the second binding constant and chemical shift of the 2:1 complex.[22]

Type I and type II titration curves for receptor $\mathbf{R_{cxc}}$ and **I** are shown in Figure 5a. 1-Benzylimidazole (and the bis-imidazoles)

Scheme 1

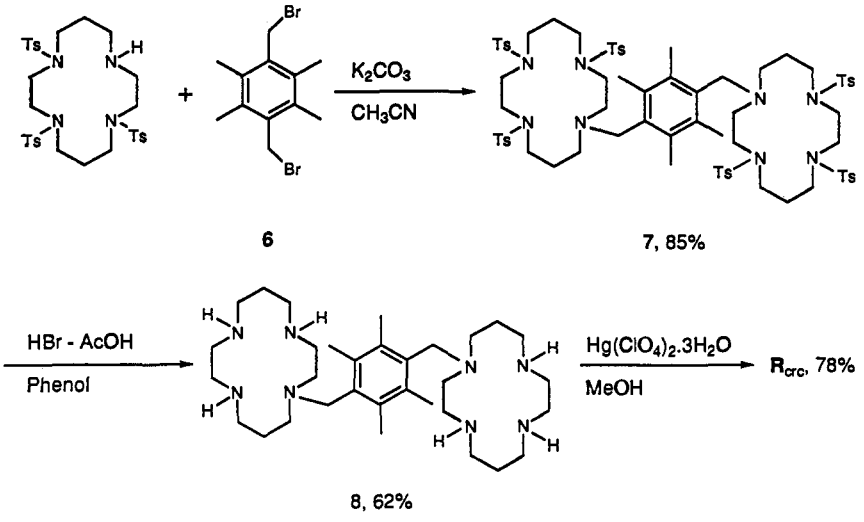


are in fast exchange with the receptors on the NMR time scale, so that an average signal is observed for the free and bound forms. Also shown in Figure 5a are the titration curves calculated for 2:1 complexes of **I** with $\mathbf{R_{cxc}}$ (see Appendix). Curves calculated for stoichiometric ratios other than 2:1 do not reproduce the measured curves. Titration data for **I** and receptors $\mathbf{R_{cnc}}$, $\mathbf{R_{crc}}$, $\mathbf{R_{dxd}}$, and $\mathbf{R_{cc}}$ are available in the supplementary material.

Bis-imidazoles (both target and nontarget) form complexes of 1:1 stoichiometry with the receptors, and only type II titration experiments were performed. Measured and calculated titration curves for $\mathbf{R_{cxc}}$ and bis-imidazoles **T1**, **T2**, and **T3** are shown in Figure 5b. Because the receptors can bind the bis-imidazoles with high affinity (association constants greater than 10^4 M^{-1}), titration experiments would have to be performed at concentrations less than 0.1 mM in order to obtain reliable values for these binding constants directly. Clearly this concentration regime is difficult to probe by NMR. Binding constants for bis-imidazoles were therefore determined indirectly, from competition experiments with **I**. Competition experiments also serve to directly determine a receptor's selectivity for binding one bis-imidazole over another. In a typical competition experiment, a 1:1 complex of a receptor and its target bis-imidazole (~10 mM) was titrated with a nontarget bis-imidazole or with 1-benzylimidazole. As seen in Figure 6 for $\mathbf{R_{cxc}}$, most of the target bis-imidazole **T2** remains bound to its receptor in the presence of stoichiometric amounts of **T3**, **T1**, or **I**, showing that $\mathbf{R_{cxc}}$ is indeed selective for its target over nontarget bis-imidazoles of **I**. Bis-imidazoles **T1** and **T3** are better competitors for **T2** than is 1-benzylimidazole. Competition data for the remaining four receptors are provided in the supplementary material.

(17) Huheey, J. E. *Inorganic Chemistry*; Harper and Row: New York, 1983; pp 73–76.
(18) Williams, D. H.; Searle, M. S.; Mackay, J. P.; Gerhard, U.; Maplestone, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1172–1178.
(19) Fabbrizzi, L.; Forlini, F.; Perotti, A.; Seghi, B. *Inorg. Chem.* **1984**, *23*, 807–813. Fabbrizzi, L.; Montagna, L.; Poggi, A.; Kaden, T. A.; Seigfreid, L. C. *J. Chem. Soc. Dalton Trans.* **1987**, 2631–2634.
(20) Ciampolini, M.; Fabbrizzi, L.; Perotti, A.; Poggi, A.; Seghi, B.; Zanobini, F. *Inorg. Chem.* **1987**, *26*, 3527–3533.
(21) Ng, C. Y.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1979**, *18*, 2982–2986.
(22) Lenkinski, R. E.; Elgavish, G. A.; Ruben, J. *J. Magn. Reson.* **1978**, *32*, 367–376.

Scheme 2



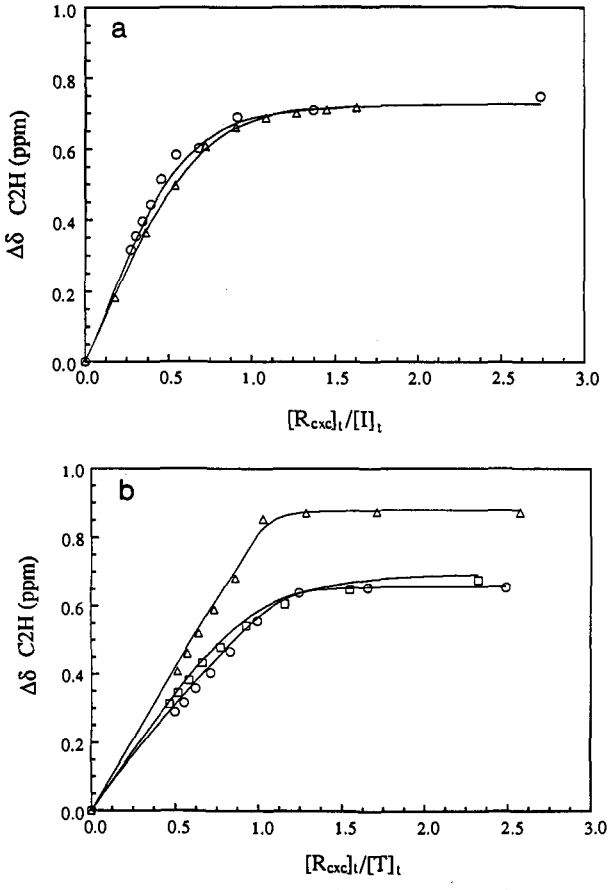


Figure 5. (a) Change in chemical shift ($\Delta\delta$) of 1-benzylimidazole (**I**) C2-H upon binding to receptor $\mathbf{R_{cxc}}$ in type I and type II titrations. Type I: solution of **I** (initial concentration = 6.3 mM) in DMSO-d_6 titrated with $\mathbf{R_{cxc}}$ (△). Type II: solution of $\mathbf{R_{cxc}}$ in DMSO-d_6 (initial concentration = 10.2 mM) titrated with of **I** (○). Solid lines are the change in chemical shifts predicted using the parameters in Table 1 and eq 16. (b) Change in chemical shift ($\Delta\delta$) of C2-H for **T1** (○), **T2** (△) and **T3** (□) in type II titration experiments with receptor $\mathbf{R_{cxc}}$. Solutions of $\mathbf{R_{cxc}}$ in DMSO-d_6 titrated with **T1** (initial concentration of $\mathbf{R_{cxc}}$ = 10.2 mM), **T2** (initial concentration of $\mathbf{R_{cxc}}$ = 10.2 mM), and **T3** (initial concentration of $\mathbf{R_{cxc}}$ = 12.5 mM). Solid lines are the change in chemical shifts predicted using the parameters in Tables 1 and 2 and eq 9.

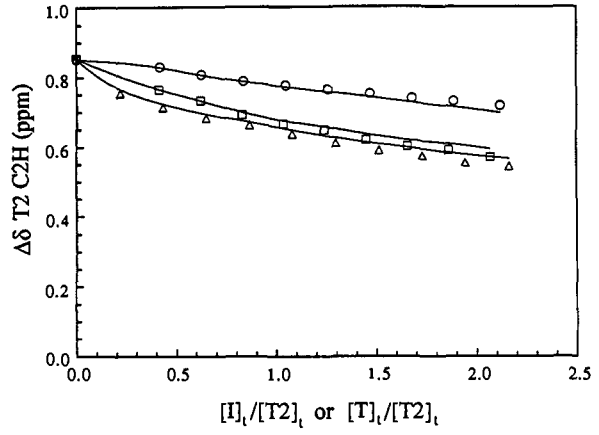


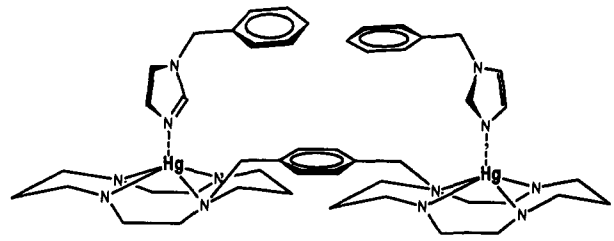
Figure 6. Change in chemical shift ($\Delta\delta$) of imidazole C2-H of **T2** (relative to unbound form) in competition experiments with **I** (○), **T1** (□) and **T3** (△). $\mathbf{R_{cxc}}$·**T2** (1:1 stoichiometry) titrated with **I** (initial concentration of $\mathbf{R_{cxc}}$ = 8.5 mM), **T1** (initial concentration of $\mathbf{R_{cxc}}$ = 8.8 mM), and **T3** (initial concentration of $\mathbf{R_{cxc}}$ = 8.3 mM). Solid lines represent the values predicted using eqs 11 and 12 for **T1** and **T3** and eqs 21–23 for **I**.

Discussion

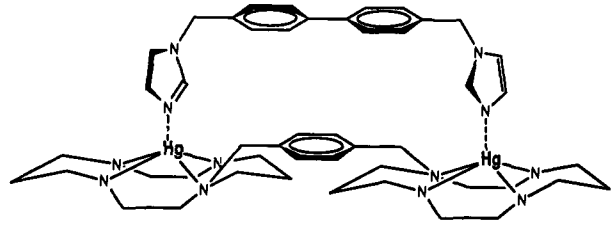
The imidazole C2-H resonances of the bis-imidazoles **T1**, **T2**, and **T3** and 1-benzylimidazole (**I**) are shifted downfield by 0.6–0.8 ppm upon binding to the receptors (Figure 5 and supplementary material), due mainly to mercury coordination at the imidazole nitrogen. The observed chemical shift changes for 1-benzylimidazole increase slightly, but consistently, with the number of aromatic rings on the receptor ($\mathbf{R_{cc}} < \mathbf{R_{dxd}}$, $\mathbf{R_{crc}}$, $\mathbf{R_{cxc}} < \mathbf{R_{cnc}}$), following the same order as aromatic ring shift currents for the spacers (no ring < benzene < naphthalene).[23] These features and the 2:1 binding stoichiometry measured in titration experiments are consistent with the schematic structure for 1-benzylimidazole binding by a receptor shown in Figure 7a. For receptor $\mathbf{R_{cxc}}$, the nontargets, **T1**, **T3**, and **I**, experience a smaller chemical shift change upon binding than does the target **T2**. The effect is even more apparent for receptor $\mathbf{R_{cnc}}$ with the naphthalene spacer (supplementary material). This apparent deshielding of **T2** by the ring current of the receptor's aromatic spacer and the measured 1:1 stoichiometry for bis-imidazole binding are consistent with the schematic structure of a 1:1 receptor:target complex shown in Figure 7b.

Titration data for binding of 1-benzylimidazole (**I**) by a receptor **R** were analyzed using a variation of Lenkinski's method for molecular complexes of 2:1 stoichiometry.[22] The first (K_1) and second (K_2) association constants for **I** binding are defined as

(23) George, P.; Trachtman, M.; Bock, C. W.; Brett, A. M. *J. Chem. Soc. Perkin Trans. 2* **1976**, 1222–1227. Haddon, R. C. *J. Am. Chem. Soc.* **1979**, *101*, 1722–1728.



(a) $I{\cdot}R_{CXC}{\cdot}I$



(b) $R_{CXC}{\cdot}T2$

Figure 7. Schematic structures of complexes of receptor (R_{cxc}) and (a) distance-matched target bis-imidazole **T2** and (b) two 1-benzylimidazoles (**I**).

$$K_1 = \frac{[R{\cdot}I]}{[R][I]} \tag{1}$$

$$K_2 = \frac{[I{\cdot}R{\cdot}I]}{[R{\cdot}I][I]} \tag{2}$$

The observed imidazole C2-H chemical shift (δ) is a function of these binding constants and the chemical shifts in the bound **R·I** and **I·R·I** species (δ_1 and δ_2). These relationships can be linearized as (see Appendix)

$$\frac{2[I{\cdot}R{\cdot}I]}{I_t\delta} = -\frac{\delta_1}{\delta_2}\frac{[R{\cdot}I]}{I_t\delta} + \frac{1}{\delta_2} \tag{3}$$

where I_t is the total concentration of **I**

$$I_t = [I] + [R{\cdot}I] + 2[I{\cdot}R{\cdot}I] \tag{4}$$

Thus, for appropriate values of K_1 and K_2 (determined by nonlinear regression of titration data using eqs 14–16 from the Appendix), the calculated concentrations of **R·I** and **I·R·I** will generate a straight line of slope $(-\delta_1/\delta_2)$ and intercept $1/\delta_2$. These plots, shown in Figure 8, were used to determine δ_1 and δ_2 of the bound complexes for the five receptors (reported in the supplementary material). The values of K_1 and K_2 resulting from this analysis for the five receptors are given in Table 1. Estimated errors were calculated relative to the "saturation fractions" defined by Lenkinski.[22,24]

Association constants K_1 for binding the first equivalent of 1-benzylimidazole are expected to be approximately the same for all the receptors; they are found to be so, within a factor of 2 (Table 1). These association constants, $\sim 10^4$ M^{-1}, correspond to a binding energy of $\sim$6 kcal/mol. The second association constants K_2 for binding a second **I** to the remaining metal ion should be more sensitive to differences among the receptors. If binding of the first and second **I** were completely independent events, K_2 would be one fourth of K_1. The K_2 values (Table 1) are in fact more widely distributed (0.14 to 0.79 $\times$ 10^3 M^{-1}) and are smaller than K_1 by at least 1 order of magnitude. The presence of one **I** on a receptor inhibits binding of the second by as much as a factor of 30 (2 kcal/mol).

(24) Deranleau, D. A. *J. Am. Chem. Soc.* **1969**, *91*, 4044–4049.

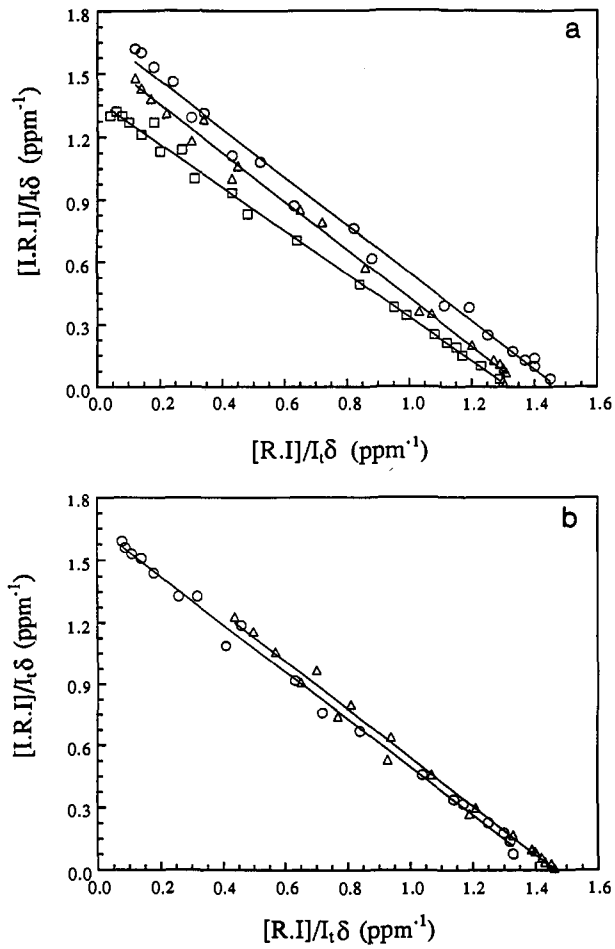


Figure 8. Linearized plots of $2[I{\cdot}R{\cdot}I]/I_t\delta$ vs $-[R{\cdot}I]/I_t\delta$. Intercept on the vertical axis is the reciprocal of chemical shift of the 2:1 complex (**I·R·I**), and the horizontal axis intercept is the reciprocal of chemical shift of the 1:1 complex (**R·I**) between the receptors and 1-benzylimidazole (**I**). (a) Series of receptors varying in the length of spacers; R_{cc} (○), R_{cxc} (△), and R_{cnc} (□). (b) Receptors R_{crc} (○) and R_{dxd} (△). Solid lines represent the calculated values from parameters of Table 1 and eqs 14–16.

Table 1. First and Second Association Constants (K_1 and K_2) for 1-Benzylimidazole (**I**) for 1:1 and 2:1 Complexes and with the Receptors. Errors are Indicated in Parentheses

receptor	K_1 (10^3 M^{-1})	K_2 (10^3 M^{-1})
R_{cnc}	16.9 (0.7)	0.79 (0.03)
R_{cxc}	22.1 (1.6)	0.42 (0.03)
R_{crc}	11.1 (0.7)	0.47 (0.03)
R_{dxd}	17.0 (0.8)	0.14 (0.01)
R_{cc}	16.8 (1.8)	0.49 (0.05)

The titration experiments (Figure 5b) indicate that a receptor **R** binds only 1 equiv of a bis-imidazole **T**. The association constant (K_T) is defined by

$$K_T = [R{\cdot}T]/[R][T] \tag{5}$$

Association constants for this strong interaction were measured relative to the first association constant of **I** (K_1) using equilibrium competition experiments. For bis-imidazole (**T**) competition with 1-benzylimidazole (**I**), parameters δ_1, δ_2, δ_T, K_1, and K_2 were obtained from the equilibrium titration experiments on **I** and **T**. The receptor selectivity for bis-imidazole over 1-benzylimidazole ($\alpha = K_T/K_1$) can then be calculated by nonlinear regression of the chemical shifts of both species (**I** and **T**) in the competition experiment (eq 24 in the Appendix). Selectivities α and bis-imidazole association constants K_T are listed in Table 2 for bis-imidazoles **T1** and **T2** in competition with **I** for the five receptors.

Table 2. Receptor Selectivities ($\alpha = K_T/K_1$) and Estimated Binding Constants ($K_1\alpha$) from Competitive Binding between 1-Benzylimidazole (**I**) and Bis-Imidazoles **T1** and **T2**. For **I**, 2:1 Complexes with the Receptors were Included in the Data Analysis. Errors are Indicated in Parentheses

receptor	K_{T1}/K_1	K_{T1} (M^{-1})	K_{T2}/K_1	K_{T2} (M^{-1})
$\mathbf{R_{cnc}}$	23 (4)	3.9×10^5	140 (30)	2.4×10^6
$\mathbf{R_{cxc}}$	10 (1)	2.2×10^5	140 (30)	3.1×10^6
$\mathbf{R_{crc}}$	24 (4)	2.7×10^5	89 (20)	9.8×10^5
$\mathbf{R_{dxd}}$	13 (1)	2.2×10^5	4.5 (0.5)	7.7×10^4
$\mathbf{R_{cc}}$	1.4 (0.2)	2.4×10^4	1.3 (0.2)	2.2×10^4

Table 3. Binding Selectivities ($\alpha = K_{T2}/K_T$) for Bis-Imidazole **T2** in Equilibrium Competition Experiments with Bis-Imidazoles **T1** and **T3**. Errors are Indicated in Parentheses

receptor	K_{T2}/K_{T1}[a]	K_{T2}/K_{T1}[b]	K_{T2}/K_{T3}
$\mathbf{R_{cnc}}$	7.6 (0.2)	6.3 (1.7)	4.9 (0.3)
$\mathbf{R_{cxc}}$	11.5 (0.4)	14.3 (3.4)	10.9 (0.5)
$\mathbf{R_{crc}}$	2.7 (0.2)	3.7 (1.0)	ND
$\mathbf{R_{dxd}}$	0.49 (0.10)	0.35 (0.1)	ND
$\mathbf{R_{cc}}$	0.91 (0.10)	1.1 (0.2)	ND

[a] Values determined from the competition experiments. [b] Values calculated by $K_{T2}/K_{T1} = (K_{T2}/K_1)/(K_{T1}/K_1)$, from Table 2.

Receptors $\mathbf{R_{cxc}}$ and $\mathbf{R_{cnc}}$ bind their target bis-imidazole **T2** with affinities greater than 10^6 M^{-1}. This large binding constant, a result of *only two* properly positioned metal-to-ligand interactions, is similar to binding constants commonly observed for monoclonal antibody–antigen interactions.[25] All the receptors except $\mathbf{R_{cc}}$ show significantly higher affinities for bis-imidazoles **T1** and **T2** than for 1-benzylimidazole.

The data analysis for competition between any two bis-imidazoles (e.g. **T1** and **T2**) proceeds similarly, using the chemical shift parameters (δ_{T1}, δ_{T2}) determined from the equilibrium titrations. The ratio of the bis-imidazole association constants (K_{T1}/K_{T2}) can be calculated by nonlinear regression of the observed chemical shifts of both bis-imidazole species in the competition experiment. The resulting selectivities for different pairs of bis-imidazoles are listed in Table 3.

The fraction of bis-imidazole T bound to a receptor compared to the fraction of 1-benzylimidazole (**I**) bound is a practical measure of the selectivity (α_{app}) of the receptor for T over **I** under the conditions of the experiment:

$$\alpha_{app(T/I)} = ([T]_{bound}/[T]_{free})/([I]_{bound}/[I]_{free}) \quad (6)$$

This apparent selectivity can be calculated without knowledge of the association constants, as long as the chemical shifts of all the bound forms are known. A receptor's selectivity for a bis-imidazole T over **I** depends strongly on the concentration, due to the shift from **R·I** to **I·R·I** complexes at increasing concentrations of **I** and because the 2:1 complex of **I** with a receptor is a better competitor for a bis-imidazole than the 1:1 complex. This can be seen in Figure 9, where the apparent selectivities of receptor $\mathbf{R_{cxc}}$ are plotted as a function of the relative competitor concentration. The apparent selectivity is predicted to increase with decreasing concentrations of competitor **I**, reaching a maximum value of K_T/K_1 (see Appendix). As indicated in Figure 9, $\alpha_{T2/I}$ increases rapidly with decreasing amounts of **I**. Extrapolating the calculated curve to zero competitor concentration yields a maximum selectivity of 140, which is consistent with the value of K_{T2}/K_1 reported in Table 2. The close match between the measured values and those calculated using eq 24 demonstrates the validity of the model.

Similarly, the fraction of bis-imidazole **T1** bound to the receptor compared to **T2** bound is the apparent selectivity of the receptor for two bis-imidazoles,

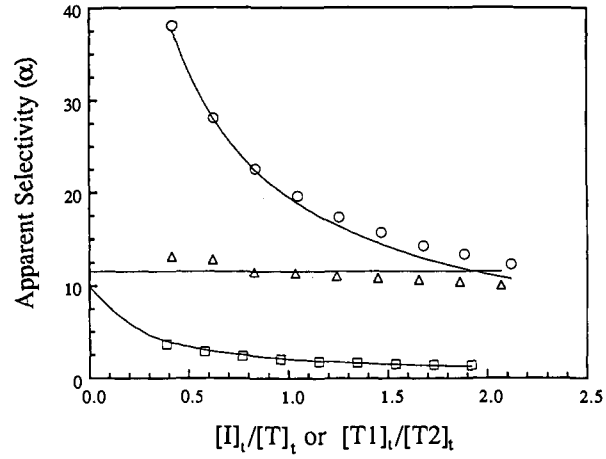


Figure 9. Apparent selectivities for receptor $\mathbf{R_{cxc}}$ as a function of competitor concentration in competition experiments from **T2** and **I** (○), **T1** and **I** (□), and **T2** and **T1** (△). Solid lines indicate the curves predicted using parameters in Tables 2 and 3.

$$\alpha_{app(T1/T2)} = ([T1]_{bound}/[T1]_{free})/([T2]_{bound}/[T2]_{free}) \quad (7)$$

As before, α_{app} can be determined if the chemical shift parameters (δ_{T1}, δ_{T2}) are known. The apparent selectivity for a pair of bis-imidazoles is predicted to be independent of the concentration because both species from 1:1 complexes with the receptor. As seen in Figure 9, this is approximately true for receptor $\mathbf{R_{cxc}}$ and bis-imidazoles **T1** and **T2** over the concentration range studied.

These competition experiments are internally consistent. According to the model assumptions (see Appendix), the ratio of bis-imidazole association constants (K_{T2}/K_{T1}) should be the same whether measured directly in a bis-imidazole competition experiment or measured indirectly in separate competition experiments with **I** ($[K_{T2}/K_1]/[K_{T1}/K_1]$). Selectivity values obtained both ways are in fact similar (Table 3).

Receptors $\mathbf{R_{cxc}}$ and $\mathbf{R_{cnc}}$ are highly selective for their target bis-imidazole, **T2**, prefering it to **I** by a factor of 140. A good receptor should also be able to discriminate its target bis-imidazole from a shorter or a longer bis-imidazole. The distances between the two coordinating nitrogens increase by approximately 4 Å along the series **T1**, **T2**, and **T3** (Figure 2). When it comes to distinguishing **T2** from the others, $\mathbf{R_{cxc}}$ is the most effective, exhibiting a selectivity of 11.5 for **T2** over shorter **T1** and 10.9 for **T2** over longer **T3** (Table 3). These selectivity values are quite attractive for applications, since adsorbents exhibiting selectivities (separation factors) of 2 or even less are routinely employed for chromatographic separations.[26]

It is useful to compare these observed binding constants and selectivities to those obtainable for two-point binding based on simple energetic arguments. For simultaneous coordination of the two imidazole moieties to the two metal ions in a receptor, the *minimum* overall binding energy should approximate the overall binding energy for binding two molecules of 1-benzylimidazole (Figure 7), and the association constant K_T would be the same order of magnitude as the product of K_1 and K_2 (Table 1). The minimum selectivity that could be obtained for ideal two-point binding over one-point binding (e.g. no strain induced in the bound complex) is of the same order of magnitude as K_2 ($\sim 10^3$). In most cases, the observed association constants for the target bis-imidazoles are considerably less than K_1K_2 (and the selectivities are considerably less than K_2), indicating that two-point binding is not particularly favored. For receptor $\mathbf{R_{cxc}}$, however, the selectivity for its target **T2** over **I** is approximately

(25) Webster, D. M.; Henry, A. H.; Rees, A. H. *Curr. Opin. Str. Biol.* **1994**, *4*, 123–129.

(26) Pirkle, W. H.; Burke, J., III. *J. Chromatogr.* **1991**, *557*, 173–185. Sellergen, B.; Lepisto, M.; Mosbach, K. *J. Am. Chem. Soc.* **1988**, *110*, 5853–5860.

one-third the value of K_2, and two-point binding of **T2** does not appear to present a severe energetic penalty, relative to binding two independent imidazoles. This penalty would be minimized if the conformation of the bound bis-imidazole can closely match the conformations of the two bound 1-benzylimidazoles (Figure 7).

The two potential binding conformations available to receptor $\mathbf{R_{dxd}}$ (Figure 4) are a consequence of having multiple vacant coordination sites in each metal center. Conformer A matches bis-imidazole **T1**, while conformer B is more suited to **T2**. The relative populations of the conformers (their relative energies) will therefore affect the binding selectivity. Receptor $\mathbf{R_{dxd}}$ in fact exhibits only a small selectivity for **T1** over **T2** ($\alpha_{T1/T2}$ = 2.0). Bis-imidazole binding by this receptor is not particularly favored: the association constants for both **T1** and **T2** are much smaller than those observed for binding to receptors $\mathbf{R_{cxc}}$ and $\mathbf{R_{cnc}}$ (Table 2). This unfavorable two-point binding is consistent with receptor $\mathbf{R_{dxd}}$'s relatively small affinity for a second 1-benzylimidazole (K_2 = 140 M^{-1}, Table 1).

Addition of the four methyl groups to the phenyl ring in $\mathbf{R_{crc}}$ reduces the affinity for 1-benzylimidazole by approximately a factor of 2, relative to receptor $\mathbf{R_{cxc}}$ without the methyl groups (Table 1). Although $\mathbf{R_{crc}}$ was designed with the idea that restricting the free rotations about the benzylic C—C bonds would increase selectivity for **T2** over **T1**, the net effect of the methyl groups is to decrease this selectivity, from 11.5 to 2.7 (Table 3). The source of this decrease is a 2-fold increase in affinity for the nontarget **T1** (relative to binding two 1-benzylimidazoles) (K_{T1}/K_1K_2 = 24/470 versus 10/420 for $\mathbf{R_{cxc}}$) as well as a reduction in affinity for the target **T2** (K_{T2}/K_1K_2 = 89/470 versus 140/420 for $\mathbf{R_{cxc}}$) (Table 2). This shift toward binding the smaller bis-imidazole is not apparent from the distances predicted using simple computer modeling of the individual receptors and targets. It appears that the second conformationally restricted receptor $\mathbf{R_{cc}}$ is unable to bind either its target bis-imidazole **T1** or **T2** by two-point binding: bis-imidazole binding is only marginally favored over 1-benzylimidazole (Table 2). Consequently the receptor's selectivity for the bis-imidazole pair ($\alpha_{T2/T1}$) is negligible (very close to 1).

The results reported here are relevant to the design of synthetic receptors for proteins. These studies demonstrate that significant selectivity can be achieved by matching the distance between two metals in a receptor to two metal-coordinating ligands on a target molecule. When extended to protein recognition, this approach relies on the advantage of a two-point metal-to-ligand interaction with a pair of surface-exposed histidines over (one-point) interactions with other surface histidines or nonmatching histidine pairs. Because the steric bulk of the protein is expected to prevent it from forming 2:1 complexes with a receptor, selectivities for protein binding will be equivalent to the maximum selectivities obtained here at low concentrations of imidazole and should not depend strongly on concentration. Assuming the recognition behaves comparably in an aqueous medium, binding a specific histidine pair could be favored by a factors of 10^2–10^3 over binding to independent surface histidines. This model system also demonstrates that it should be possible to distinguish among histidine pairs differing in distance by as little as 4 Å with relatively high selectivity (>10). These selectivities and binding affinities (>10^6 M^{-1}) are not unlike the selectivities and affinities exhibited by antibodies. To further improve specificity and broaden the potential applicability, metal coordination could be used in conjunction with other interactions.

Experimental Section

General Method. Anhydrous solvents were purchased from Aldrich and used as received. All reactions were carried out under a slow stream of dry nitrogen. Cyclam was purchased from Strem, and all other chemicals were obtained from Aldrich. Chemicals were used as received from the suppliers.

^{1}H and ^{13}C NMR spectra were recorded using a General Electric QE-300 (300 MHz) spectrometer. Chemical shifts are reported in ppm. High resolution FAB mass spectra were obtained from Southern California Regional Mass Spectrometry Center, Department of Chemistry, University of California, Riverside, CA. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

4,4″-Bis(imidazol-1-ylmethyl)-*p*-terphenyl (T3).

To a suspension of NaH (126 mg, 5.25 mmol) in THF (50 mL) was added imidazole (360 mg, 5.25 mmol). After the evolution of hydrogen gas ceased, the resulting suspension was heated to reflux for 30 min. It was cooled to room temperature and 420 mg (1.28 mmol) of solid 4,4″-bis(chloromethyl)-*p*-terphenyl[27] was added in one portion. The reaction mixture was heated to reflux for 48 h, cooled to room temperature and water (1 mL) was added to quench the reaction. THF was evaporated under reduced pressure to give a final volume of 10 mL and was poured into 250 mL of cold water. The resulting fine white precipitate was separated from the supernatant by centrifugation, washed with cold water, and then lyophilized. The crude product was purified by recrystallization from $CHCl_3$/hexane to afford a white solid (375 mg, 75%): mp 214–215 °C; ^{1}H NMR (DMSO-d_6) δ 7.78 (s, 2H), 7.73 (s, 4H), 7.69 (d, 4H, J = 8.2 Hz), 7.34 (d, 2H, J = 8.2 Hz), 7.22 (s, 2H), 6.91 (s, 2H), 5.23 (s, 4H); ^{13}C NMR (DMSO-d_6) δ 139.4, 139.2, 137.9, 137.7, 129.2, 128.6, 127.7, 127.4, 120.1, 49.6; HRMS calcd for $C_{26}H_{22}N_4$: 390.1844, found 390.1832.

2,6-Bis[4′,8′,11′-tris(*p*-toluenesulfonyl)-1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl]naphthalene (1). 1,4,8-Tris(*p*-toluenesulfonyl)-1,4,8,11-tetraazacyclotetradecane (cyclam tritosylate) was prepared by literature procedure.[28] To a solution of this compound (1.9 g, 2.87 mmol) in 50 mL of hot acetonitrile were added 2,6-bis(bromomethyl)naphthalene[29] (450 mg, 1.43 mmol) and 2 g of anhydrous K_2CO_3 (14.4 mmol). The resulting reaction mixture was heated to reflux the 40 h and cooled to room temperature, and the solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 (150 mL) and filtered to remove insoluble inorganic materials. The volume of the filtrate was reduced to 40 mL and methanol was added to induce crystallization. The white crystals were filtered, washed with methanol, and then vacuum-dried (1.7 g, 81%): mp 206–208 °C; ^{1}H NMR ($CDCl_3$) δ 7.75–7.69, 7.45–7.27, 7.13–7.10 (m, 30H), 3.74 (s, 4H), 3.25–2.98 (m, 24H), 2.76 (t, br, 4H, J = 6.0 Hz), 2.57 (t, br, 4H, J = 6.0 Hz), 2.46 (s), 2.44 (s), 2.36 (s, 18H), 1.97 (t, br, 4H, J = 4.0 Hz), 1.76 (t, br, 4H, J = 4.0 Hz). Anal. Calcd for $C_{74}H_{92}N_8O_{12}S_6$: C, 60.14; H, 6.27; N, 7.58. Found: C, 59.91; H, 6.37; N, 7.57.

2,6-Bis(1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl)naphthalene (2). To a solution of phenol (3 g, 32 mmol) in 60 mL of HBr–AcOH (30 wt %) was added 1.4 g of **1** (0.95 mmol) and the reaction mixture was heated at 100 °C for 15 h. The hexatosylate **1** dissolved within 15 min and slowly the reaction mixture turned dark brown. It was cooled to room temperature and ether (100 mL) was added. The deep brown precipitate was filtered, washed with ether, and then vacuum-dried. This was taken up in 10 mL of water and passed through Dowex-1 ion-exchange column (hydroxide form, generated from the commercially available chloride form with 10% aqueous KOH) and was eluted with water until the pH of the eluant was 7. Water was removed mostly under reduced pressure and then lyophilized to afford the pure product as a white solid (553 mg, 86%): mp 127–129 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 2H, J = 8.6 Hz), 7.71 (s, 2H), 7.51 (d, 2H, J = 8.6 Hz), 3.72 (s, 4H), 2.91–2.56 (m, 38H), 1.92–1.90 (m, 4H), 1.70–1.68 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 136.3, 132.8, 127.9, 127.6, 105.0, 58.3, 54.9, 53.6, 50.9, 49.5, 49.4, 49.2, 48.3, 47.8, 28.9, 26.6; HRMS calcd for $C_{32}H_{56}N_8$ 552.4627, found 552.4639.

Diaqua-2,6-bis(1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl)naphthalene Bis(mercury(II)) Tetraperchlorate ($\mathbf{R_{cnc}}$). $Hg(ClO_4)_2 \cdot 3H_2O$ (450 mg, 1 mmol) was dissolved in 10 mL of methanol and to this solution was added dropwise a solution of the free base **2** (233 mg, 0.42 mmol) in 10 mL of methanol. A white precipitate appeared immediately. The reaction was stirred at room temperature for 3 h, and then 20 mL of ether was added. The precipitate was filtered, washed with ether, and vacuum-dried to afford the bis-Hg^{2+} complex as a white solid: 523 mg, 87%; mp 183–184 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.97 (d, 2H, J = 8.4 Hz), 7.84 (s, 2H), 7.43 (d, 2H, J = 8.4 Hz), 5.44–5.29 (m, br, 4H), 4.49–4.44 (m, br, 2H), 4.28 (d, 2H, J = 4.3 Hz), 3.95 (d, 2H, J = 4.3 Hz); rest of the

(27) Campbell, T. W. *J. Am. Chem. Soc.* **1960**, *82*, 3126–3128.
(28) Helps, I. M.; Parker, D.; Morphy, J. R.; Chapman, J. *Tetrahedron* **1989**, *45*, 219–226.
(29) Diekmann, J.; Hertler, W. R.; Benson, R. E. *J. Org. Chem.* **1963**, *28*, 2719–2724.

hydrogens appear as multiplets between 3.17–2.47, 2.30–2.25, 1.90–1.75, 1.62–1.60. Anal. Cacld for $C_{32}H_{60}N_8Cl_4Hg_2O_{18}$: C, 27.69; H, 4.35; N, 8.07. Found: C, 27.96; H, 4.51; N, 7.89.

Diaqua-1,4-bis(1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl)benzene Bis(mercury(II)) Tetraperchlorate (R_{cxc}). A solution of the bis-cyclam free base[20] (120 mg, 0.24 mmol) in 5 mL of methanol was added dropwise to a stirred solution of $Hg(ClO_4)_2 \cdot 3H_2O$ (250 mg, 0.55 mmol) in 5 mL of methanol. A white precipitate was observed immediately. The reaction was stirred at room temperature for 3 h and ether (10 mL) was added. The precipitate was filtered, washed with ether, and then vacuum-dried to afford the pure complex as a white powder (255 mg, 79%): mp 175–176 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.28 (m, 4H), 5.38 (s, br, 2H), 5.27 (s, br, 2H), 4.51 (s, br, 2H), 4.16 (d, 2H, J = 8.0 Hz), 3.74 (d, 2H, J = 8.0 Hz), 3.19–2.99 (m), 2.79–2.71 (m), 2.59–2.48 (m), 2.28–2.14 (m), 1.96–1.54 (m). Anal. Calcd for $C_{28}H_{58}N_8Cl_4Hg_2O_{18}$: C, 25.14; H, 4.37; N, 8.38. Found: C, 25.52; H, 4.27; N, 8.66.

α,α′-Bis[*N*,*N*-bis[(phthalimidoethyl)amino]-*p*-xylene (4). α,α′-Dibromo-*p*-xylene (870 mg, 3.3 mmol), phthalimide protected diethylenetriamine **3**[21] (2.4 g, 6.6 mmol), and anhydrous K_2CO_3 (4 g, 29 mmol) were heated to reflux in 100 mL of acetonitrile for 48 h and then cooled to room temperature, and acetonitrile was evaporated under reduced pressure to a final volume of 20 mL. This was poured into 250 mL of ice-cold water, and the resultant white precipitate was filtered, washed with water, and lyophilized. The product was obtained as a white solid (2.56 g, 94%) and was found to be quite pure by NMR: mp 218–220 °C; ^{1}H NMR ($CDCl_3$) δ 7.73 (s, br, 18H), 6.67 (s, 4H), 3.75–3.71 (m, 8H), 3.51 (s, 4H), 2.77–2.74 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 168.7, 137.6, 134.2, 132.9, 129.3, 123.6, 58.4, 52.1, 36.4. As this compound is reported in the literature,[21] it was not characterized further.

1,4-Bis[[bis(2-aminoethyl)amino]methyl]benzene (5). The protected derivative **4** (1.64 g, 1.98 mmol) and hydrazine hydrate (3 mL, 62 mmol) were heated to reflux in 100 mL of absolute ethanol for 5 days. Ethanol and hydrazine were removed under reduced pressure (this is important since any remaining hydrazine was found to complicate the isolation procedure). The residual white solid was dissolved in 100 mL of water and was heated to reflux with 10 mL of concd HCl for 10 h. The reaction mixture was cooled to room temperature, the precipitate was filtered, and the filtrate was concentrated. Additional precipitate that appeared during evaporation was also filtered. The filtrate was concentrated to a final volume of 10 mL and poured into 100 mL of absolute ethanol. The fine white precipitate was filtered, washed with ethanol, and vacuum-dried. The free base was generated from this hexahydrochloride salt, as described for compound **2**, to afford the amine as a pale yellow syrup: 565 mg, 92%; ^{1}H NMR ($CDCl_3$) δ 7.21 (s, 4H), 3.52 (s, 4H), 2.71 (t, 8H, J = 6.0 Hz), 2.47 (t, 8H, J = 6.0 Hz), 1.31 (s, br, 8H N*H*); ^{13}C NMR ($CDCl_3$) δ 138.4, 128.8, 58.9, 57.4, 39.8. As this compound is reported in the literature,[21] it was not characterized further.

Hexaaqua-1,4-bis[[bis(2-aminoethyl)amino]methyl]benzene Bis(mercury(II)) Tetraperchlorate (R_{dxd}). To a solution of $Hg(ClO_4)_2 \cdot 3H_2O$ (450 mg, 1 mmol) in 10 mL of methanol was added dropwise a solution of the base **5** in 5 mL of methanol. A white precipitate appeared immediately. After stirring the reaction mixture at room temperature for 2 h, the precipitate was filtered, washed with methanol, and vacuum-dried: yield 359 mg, 70%; mp 146–148 °C dec; ^{1}H NMR (DMSO-d_6) δ 7.29 (s, 4H), 3.74 (s, 4H), 3.12–3.02 (m), 2.83–2.96 (m), 2.32–2.23 (m). Anal. Calcd for $C_{16}H_{32}N_6Hg_2Cl_4O_{16}$: C, 17.35; H, 2.91; N, 7.59. Found: C, 17.61; H, 3.31; N, 7.35.

1,4-Bis[4′,8′,11′-tris(*p*-toluenesulfonyl)-1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl]-2,3,5,6-tetramethylbenzene (7). This was prepared by a procedure analogous to **1**. Thus 275 mg (0.86 mmol) of the bromide **6**,[30] 1.14 g (1.72 mmol) of cyclam tritosylate,[29] and 1.2 g (8.7 mmol) of anhydrous K_2CO_3 afforded 1.08 g (85%) of the hexatosylate after recrystallization from $CHCl_3$/hexane: mp 140–142 °C; ^{1}H NMR (DMSO-d_6) δ 7.78–7.48 (m, 24H), 3.78 (s, 4H), the hydrogens from the cyclam rings appear as multiplets between 3.20–3.08, 2.97–2.73, 2.66–2.46; 1.88 (s, br), 1.71 (s, br); 2.27 (s), 2.29 (s) and 2.32 (s) for the methyl groups, 2.15 (s, 12H); this compound was found to be associated with one molecule of $CHCl_3$ (8.54 ppm, s) which was very difficult to remove. Anal. Calcd for $C_{74}H_{98}N_8O_{12}S_6 \cdot CHCl_3$: C, 56.18; H, 6.22; N, 6.99. Found: C, 56.62; H, 6.21; N, 7.02.

1,4-Bis(1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl)-2,3,5,6-tetramethylbenzene (8). This was prepared by a procedure similar to **2**. Hexatosylate **7** (900 mg, 0.61 mmol), 2 g of phenol (21 mmol), and 30 mL of 30 wt % HBr–AcOH afforded 210 mg (62%) of the free base as a white solid: mp 135–137 °C; ^{1}H NMR ($CDCl_3$) δ 3.59 (s, 4H), 2.71–2.43 (m, 38H), 2.26 (s, 12H), 1.76–1.63 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 134.4, 133.8, 54.4, 53.2, 53.0, 50.8, 49.4, 48.6, 48.4, 47.7, 29.4, 28.4, 26.7, 17.3; HRMS calcd for $C_{32}H_{62}N_8$ 558.5094, found 558.5082.

Diaqua-1,4-bis(1′,4′,8′,11′-tetraazacyclotetradecan-1′-ylmethyl)-2,3,5,6-tetramethylbenzene Bis(mercury(II) Tetraperchlorate (R_{crc}). This was prepared by a procedure similar to R_{cxc}. Thus 210 mg (0.46 mmol) of $Hg(ClO_4)_2 \cdot 3H_2O$ and 100 mg (0.18 mmol) of **8** gave 193 mg (78%) of the complex as a white solid: mp 165–167 °C dec; ^{1}H NMR (DMSO-d_6) δ 5.58 (s, br, 3H), 4.15 (s, br superposed with a broad singlet, 7H), 2.28 (s, 12H); rest of the hydrogens appear as multiplets between 3.32–2.67, 1.90–1.84. Anal. Calcd for $C_{32}H_{66}N_8Hg_2Cl_4O_{18}$: C, 27.57; H, 4.77; N, 8.04. Found: C, 27.91; H, 5.01; N, 8.49.

Diaqua-6,6′-bis(1,4,8,11-tetraazacyclotetradecane) Bis(mercury(II)) Tetraperchlorate (R_{cc}). To a solution of $Hg(ClO_4)_2 \cdot 3H_2O$ (450 mg, 1 mmol) in 10 mL of methanol was added dropwise a solution of the free base[19] (150 mg, 0.38 mmol) in 5 mL of methanol. A transient white precipitate was observed. The resulting clear reaction mixture was stirred at room temperature for 2 h and 20 mL ether was added. The white precipitate was filtered, washed with ether, and vacuum-dried to give the complex as a white powder: 282 mg, 60%; mp 188–190 °C dec; ^{1}H NMR (DMSO-d_6) δ 4.78 (s, br, 4H), 4.76 (s, br, 4H); rest of the hydrogens appear as multiplets between 3.18–3.07, 2.98–2.64, 1.89–1.84. Anal. Calcd for $C_{20}H_{46}N_8Hg_2Cl_4O_{16} \cdot 2H_2O$: C, 19.47; H, 4.08; N, 9.08. Found: C, 19.32; H, 4.32; N, 9.11.

Appendix

Titrations of Bis-Imidazoles. The analysis the bis-imidazole titration experiments assumes that the bis-imidazole forms only 1:1 complexes the receptor and that coordinated imidazoles are in fast-exchange with free imidazoles. If the concentration of free bis-imidazole [T] is known, then the concentration of bound species can be determined by a mass balance on the total amount of receptor (R_t)

$$[R \cdot T] = \frac{K_T R_t [T]}{1 + K_T [T]} \tag{8}$$

where the association constant (K_T) is as defined in eq 5. In the fast-exchange limit, the change in observed imidazole chemical shift (δ(T)) of the bis-imidazole is

$$\delta(T) = \delta_T \frac{[R \cdot T]}{T_t} \tag{9}$$

where δ_T is the imidazole chemical shift change of T when bound to receptor. If this quantity is known, then the concentration of free bis-imidazole can be calculated directly from the observed chemical shift change

$$[T] = T_t \left(1 - \frac{\delta(T)}{\delta_T}\right) \tag{10}$$

This set of equations is suitable for nonlinear regression (SigmaPlot v. 6.0, Jandel Scientific). For initial estimates of δ_T and K_T, eqs. 8–10 calculate a predicted chemical shift change. The optimum values of K_T and δ_T minimize the error of the predicted chemical shift change from the observed one (δ(T)).

Competition Among Bis-Imidazoles. In addition to the above assumptions, the analysis of the competition experiments between bis-imidazoles assumes that no mixed complexes are formed. If the concentration of free bis-imidazoles [T1] and [T2] are known, then the concentrations of bound species are given by

$$[R \cdot T1] = \frac{K_{T1} R_t [T1]}{1 + K_{T1}[T1] + K_{T2}[T2]} \tag{11}$$

$$[R \cdot T2] = \frac{K_{T2} R_t [T2]}{1 + K_{T1}[T1] + K_{T2}[T2]} \tag{12}$$

The association constants (K_{T1} and K_{T2}) are defined in eq 5. This set of equations is also suitable for nonlinear regression. The free

(30) Made, A. W. V.; Made, R. H. V. *J. Org. Chem.* **1993**, *58*, 1262–1263.

concentration of each bis-imidazole is calculated from the observed chemical shifts by eq 10 using the bound chemical shift changes (δ_{T1} and δ_{T2}) determined from the equilibrium titration experiments. For initial estimates of the association constants K_{T1} and K_{T2}, eqs 9–12 calculate predicted chemical shift changes (δ(T1) and δ(T2)). The optimum values of K_{T1} and K_{T2} simultaneously minimize the error of both predicted chemical shift changes from the observed ones.

This apparent selectivity (eq 7) is measured directly from the observed chemical shift changes with the chemical shift parameters (δ_{T1}, δ_{T2}) determined from the equilibrium titration experiments. In the case of the bis-imidazoles, for the assumptions outlined above, the apparent selectivity is predicted to be independent of concentration

$$\alpha_{T1/T2} = \frac{\delta_{T2}/\delta(T2) - 1}{\delta_{T1}/\delta(T1) - 1} = \frac{K_{T1}}{K_{T2}} \tag{13}$$

Titrations of 1-Benzylimidazole. The analysis of the 1-benzylimidazole titration experiments assumes that the imidazole forms 1:1 and 2:1 complexes with the receptor and that coordinated imidazoles are in fast-exchange with free imidazoles. These titration experiments were analyzed using a variation of the method of Lenkinski[22] for molecular complexes of 2:1 stoichiometry. If the concentration of free imidazole [I] is known, then the concentrations of bound species are given by

$$[\mathbf{R}\cdot\mathbf{I}] = \frac{K_1\mathbf{R}_t[\mathbf{I}]}{1 + K_1[\mathbf{I}] + K_1K_2[\mathbf{I}]^2} \tag{14}$$

$$[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}] = \frac{K_1K_2\mathbf{R}_t[\mathbf{I}]^2}{1 + K_1[\mathbf{I}] + K_1K_2[\mathbf{I}]^2} \tag{15}$$

Assuming fast-exchange, the observed chemical shift change (δ(I)) is the sum of the contributions from the two bound species

$$\delta(\mathbf{I}) = \delta_1\frac{[\mathbf{R}\cdot\mathbf{I}]}{\mathbf{I}_t} + 2\delta_2\frac{[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}]}{\mathbf{I}_t} \tag{16}$$

If the chemical shift changes δ_1 and δ_2 are assumed equal, then the concentration of free imidazole can be calculated directly from the observed chemical shift change by analogy to eq 10. If δ_1 and δ_2 are different, then this analogy no longer holds. However, if we take them to be *approximately* equal, then a weighted average leads to the following expression for [I]

$$[\mathbf{I}] = \mathbf{I}_t\left(1 - \frac{\delta(\mathbf{I})}{\delta_{avg}}\right) + \frac{\Delta}{2\delta_{avg}}(\beta([\mathbf{R}\cdot\mathbf{I}] + [\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}]) - 2[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}]) \tag{19}$$

where the average is given by ($1 < \beta < 0$)

$$\delta_{avg} = (1 - \beta)\delta_1 + \beta\delta_2 \tag{17}$$

$$\Delta = \delta_1 - \delta_2 \tag{18}$$

Thus this case can be taken as a perturbation of the case $\delta_1 = \delta_2$. For the correct value of β, the second term of this equation will vanish, and the free concentration can be determined from the observed chemical shift change

$$\beta = \frac{2[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}]}{2[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}] + [\mathbf{R}\cdot\mathbf{I}]} = \frac{2K_2[\mathbf{I}]}{2K_2[\mathbf{I}] + 1} \tag{20}$$

This system of equations is now suitable for nonlinear regression. For initial estimates of δ_1, δ_2, K_1, and K_2, initial estimates of δ_{avg} and of [I] are calculated assuming $\beta = 1/2$. From these estimates, a more accurate β is calculated by eq 20, and thus more accurate estimates of δ_{avg} and the concentration of free **I**. The optimum values of the four parameters minimize the error in the chemical shift change predicted by eq 16.

Competition of Bis-Imidazole with 1-Benzylimidazole. The assumptions used to analyze the bis-imidazole competition experiments are the same as above. In this experiment we consider the formation of three species

$$[\mathbf{R}\cdot\mathbf{T}] = \frac{K_T\mathbf{R}_t[\mathbf{T}]}{1 + K_1[\mathbf{I}] + K_1K_2[\mathbf{I}]^2 + K_T[\mathbf{T}]} \tag{21}$$

$$[\mathbf{R}\cdot\mathbf{I}] = \frac{K_1\mathbf{R}_t[\mathbf{I}]}{1 + K_1[\mathbf{I}] + K_1K_2[\mathbf{I}]^2 + K_T[\mathbf{T}]} \tag{22}$$

$$[\mathbf{I}\cdot\mathbf{R}\cdot\mathbf{I}] = \frac{K_1K_2\mathbf{R}_t[\mathbf{I}]^2}{1 + K_1[\mathbf{I}] + K_1K_2[\mathbf{I}]^2 + K_T[\mathbf{T}]} \tag{23}$$

The analysis of the equilibrium competition experiments proceeds from the parameters determined in the equilibrium titration experiments. In the case of bis-imidazole (T) competition with 1-benzylimidazole (**I**), the parameters δ_1, δ_2, δ_T, K_1, and K_2 are taken from the equilibrium titration experiments of both species. The concentrations of free bis-imidazole (T) and free 1-benzylimidazole (**I**) are determined by the procedures outlined above. The ratio of the association constants of bis-imidazole and 1-benzylimidazole (K_T/K_1) can then be calculated by minimizing the square error in the predicted chemical shift changes of both species.

In contrast with the previous case, the selectivity that a receptor shows for T over **I** (eq 6) cannot be calculated directly from the observed chemical shift changes, because the average chemical shift of 1-benzylimidazole depends on the parameter β. We are most interested in predicting the behavior of this system at high dilution. We can calculate an apparent selectivity using $\beta = 0$ and $\delta_{avg} = \delta_1$, and the error will be minimized for that concentration regime

$$\alpha_{T/I} = \frac{(\delta_1/\delta(\mathbf{I}) - 1)}{(\delta_T/\delta(\mathbf{T}) - 1)} = \frac{K_T}{K_1}\frac{1 + (\Delta/\delta_1)K_1K_2[\mathbf{R}][\mathbf{I}]}{1 + K_2[\mathbf{I}](2 - \Delta/\delta_1)} \tag{24}$$

The apparent selectivity is predicted to reach a maximum of K_T/K_1 at low concentrations of competitor **I**.

Acknowledgment. This research was supported by Whitaker Foundation, the Office of Naval Research (N000014-02-5-1178) and the National Science Foundation (BCS-9108502). F.H.A acknowledges a National Science Foundation PYI award and a fellowship from the David and Lucile Packard Foundation. S.M. is grateful to Ipsita Mallik for help in typing the manuscript.

Supplementary Material Available: Table containing the change in chemical shift of imidazole C2-H between free and receptor bound forms for **T1**, **T2**, **T3**, and **I**; titration and competition curves for the receptors $\mathbf{R}_{cnc}$, $\mathbf{R}_{crc}$, $\mathbf{R}_{dxd}$, and $\mathbf{R}_{cc}$ (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.