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Anion Binding by Fluorescent Biimidazole Diamides

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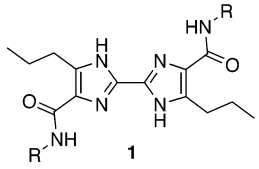
Six 2,2′-biimidazoles with various amide groups at the 4- and 4′-positions were prepared from 5-propyl-1*H*-imidazole-4-carboxylic acid ethyl ester. In the final step of the synthesis, biimidazole C2−C2′ bond formation was accomplished in 33−45% yield by palladium(0)-catalyzed homocoupling of the corresponding 2-iodoimidazoles. Four of the biimidazoles were studied by X-ray diffraction. In the solid state, all display coplanar imidazole rings, an anti relationship of amide groups, and intramolecular ($NH_{amide}\cdots N_{imid}$) and intermolecular ($NH_{imid}\cdots O_{amide}$) hydrogen bonding. In CH_2Cl_2, the emission intensity of the biimidazoles is quenched by the presence of dihydrogenphosphate and chloride anions, but no shifts in λ_{emiss} are observed. Binding constants for 1:1 biimidazole−anion complexation (K_{assoc}) are on the order of 10^4 M^{-1} for $H_2PO_4^-$ and Cl^-. One of the receptors (bearing 3,5-difluorobenzylamides) is selective for chloride. The participation of the amide N*H* atoms in anion binding was established by ^{1}H NMR.

Introduction

Artificial receptors that are capable of selectively binding anionic species show promise in the diagnosis and treatment of diseases, and in environmental remediation.[1] Sensors for inorganic phosphate, for example, could be used to monitor ATP synthesis/hydrolysis or kinase-dependent cell signaling. "Carrier" molecules can enhance through-membrane transport of chloride ion, which is a goal of cystic fibrosis research. Extraction of nitrate from rivers and lakes is expected to inhibit eutrophication, and the associated oxygen depletion and fish kills. Several anion receptors have been constructed from five-membered heterocycles,[2] (thio)amides,[3] or both,[4] because these groups form relatively strong NH···anion hydrogen bonds. Previous work has also established that coupling luminescent moieties to H-bond donors can yield sensors that operate at low anion concentrations.[5] We describe here a series of anion receptors that incorporate all of these features into a single molecular unit.[6] Specifically, electrically neutral biimidazole diamides **1** are shown to simultaneously serve as multiple H-bond donors[7] and as anion-sensitive fluorophores.



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Results and Discussion

Synthesis of the biimidazoles is shown in Scheme 1. In the first step, the known ester **2**[8] was hydrolyzed to a carboxylic acid in quantitative yield with concentrated aqueous HCl at reflux. Elemental analysis confirmed that the product is isolated as a hydrochloride salt. Repeated attempts to couple acid **3** to amines (using DCC, BOP, or HBTU)[9] failed to produce amides, so this compound was instead converted to the corresponding acid chloride **4** by reaction with excess oxalyl chloride in CH_3CN at reflux.[10] Once dried, solid **4** could be stored in a tightly sealed container at room temperature for several months

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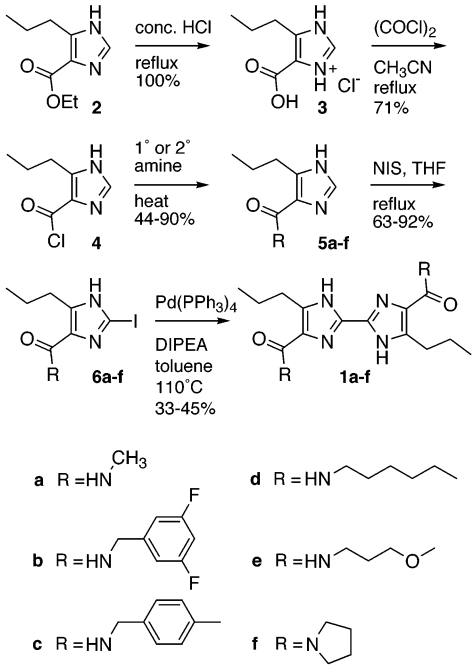
SCHEME 1



without decomposition. In addition, **4** is stable toward TLC analysis on silica gel plates with EtOAc as the eluent. Amides **5a**−**f** were prepared by treating **4** with a series of neat liquid amines. Removal of excess amine followed by recrystallization or column chromatography afforded the desired amides as solids that are freely soluble in CH_3OH and moderately soluble in CH_2Cl_2 or EtOAc. Installation of a halogen at the imidazole 2-position of **5a**−**f**, required for subsequent biimidazole bond formation,[11] was accomplished by treating the amides with *N*-iodosuccinimide (NIS) in THF at reflux. In the final step of the synthesis, a palladium(0)-promoted homocoupling reaction, originally developed for preparation of biimidazole di*esters*,[11] was employed. Here, iodides **6a**−**f** were treated with *N*,*N*-diisopropylethylamine and a catalytic amount of $Pd(PPh_3)_4$ in toluene at 110 °C. The six biimidazole products **1a**−**f** display a characteristic blue fluorescence under short-wave UV irradiation on silica gel TLC plates.

Four of the final products (**1a**−**d**) were analyzed by single-crystal X-ray diffraction. In the solid state, the imidazole rings and amide CONH atoms of these four derivatives are essentially congruent, with no deviations in bond lengths or angles greater than 2%. Significant differences across the series appear only in the conformations of the 5,5′-dipropyl side chains and in the alkyl/benzyl groups attached to the amide NH units. Therefore, only a representative biimidazole (**1a**) is described here in detail. This molecule adopts a conformation such that the amide groups are anti to one another, and the heterocyclic rings are coplanar (Figure 1). These structural features have also been observed in bipyrrole-containing compounds.[12] Although imidazoles typically

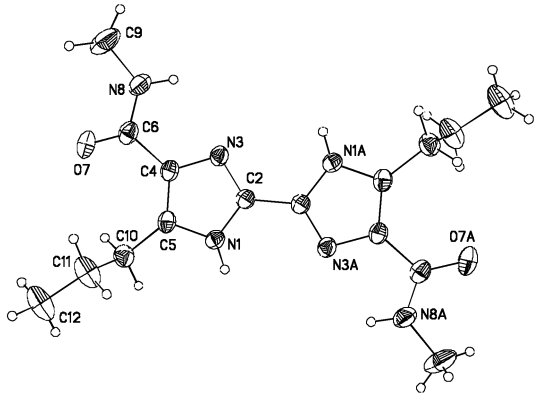


FIGURE 1. Structure of **1a** in the crystal. The molecule sits on a crystallographic inversion center. Thermal ellipsoids are scaled to the 50% probability level.

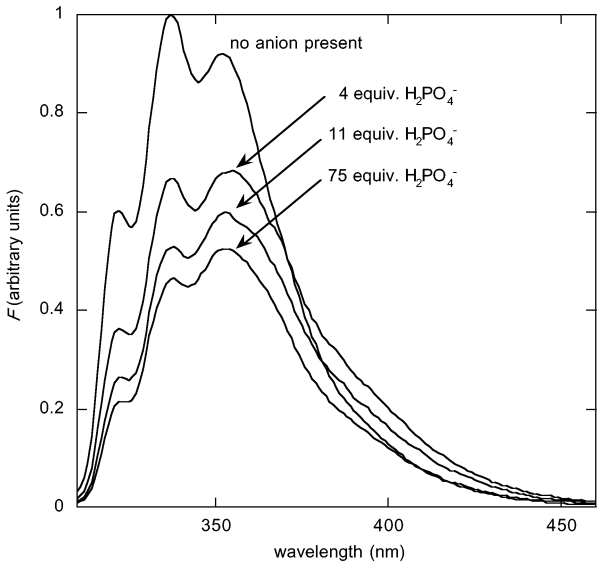


FIGURE 2. Representative emission spectra of **1c** (2.5×10^{-6} M) in aerated CH_2Cl_2 during titration with $Bu_4N^+ H_2PO_4^-$. λ_{excit} = 300 nm.

display NH tautomerism, only one tautomer is readily apparent in crystals of **1a**. This form allows for intramolecular hydrogen bonding between the amide N8−H8 atoms and the lone pair electrons of imidazole nitrogens N3 ($H_{amide}\cdots N_{imid} \approx 2.3$ Å). Furthermore, each molecule of **1a** H-bonds to four adjacent molecules by donating two $NH_{imid}\cdots O_{amide}$ bonds and accepting two $NH_{imid}\cdots O_{amide}$ bonds (see the Supporting Information).

With their ability to donate hydrogen bonds established, the fluorescence behavior of **1b**−**f**[13] was examined in the presence of anionic species. As an example, Figure 2 shows the evolution of the emission bands of **1c** (2.5×10^{-6} M in CH_2Cl_2)[14] as tetrabutylammonium dihydrogenphosphate ($Bu_4N^+ H_2PO_4^-$) was introduced. The first addition of anion (to give a solution with a 4-fold excess

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(13) Quantum yields Φ_F for **1c**, **1d**, and **1e** were measured to be 0.29, 0.32, and 0.32 (±10%), respectively, with excitation at 300 nm in N_2-purged absolute ethanol at room temperature (relative to 7-amino-4-methylcoumarin). These determinations were carried out according to: Fery-Forgues, S.; Lavabre, D. *J. Chem. Educ.* **1999**, *76*, 1260. For examples of 2,2′-bithiazoles that display intense fluorescence, see: Wagner, M.; Engrand, P.; Regnouf-de-Vains, J.-B.; Marsura, A. *Tetrahedron Lett.* **2001**, *42*, 5207.

TABLE 1. Binding Constants, K_{assoc} (M^{-1}), for 1b−f with Dihydrogenphosphate and Chloride in CH_2Cl_2 at 23 °C

biimidazole	$H_2PO_4^-$	Cl^-
1b	6.8×10^4	1.4×10^5
1c	4.6×10^4	3.7×10^4
1d	4.7×10^4	2.7×10^4
1e	4.1×10^4	2.5×10^4
1f	2.0×10^4	4.0×10^3

of $H_2PO_4^-$ relative to **1c**) caused the integrated emission intensity F to decrease by about 15%. At the conclusion of the titration, the presence of 75 equiv of $H_2PO_4^-$ quenched slightly more than 40% of the initial fluorescence. Chloride ion was found to be a less effective quencher, with 75 equiv of this anion effecting a decrease of just 15% in the total emission of **1c** in CH_2Cl_2.[15] No shifts in the wavelengths of the emission peaks were observed.

For each of **1b**−**f**, plots of F/F_0 versus [$H_2PO_4^-$] or [Cl^-] are hyperbolic, demonstrating saturation behavior. Table 1 summarizes the 1:1 biimidazole−anion binding constants (K_{assoc}) for dihydrogenphosphate and chloride that were derived from fluorescence titrations.[16] For all but **1b**, the K_{assoc} values for $H_2PO_4^-$ are higher than those for Cl^-, a result that has been observed with other nonmacrocyclic receptors.[5d,6] On the basis of its larger size alone, binding of dihydrogenphosphate ion was expected to be sensitive to the identity of the substituents attached to the amide N atoms. Instead, chloride binding showed a higher degree of "tunability".[17] Affinities for $H_2PO_4^-$ are clustered in the range $(2-7) \times 10^4$ M^{-1}, while Cl^- is bound nearly 2 orders of magnitude more strongly by **1b** than **1f**. The apparently anomolous behavior of **1b**[18] prompted further titrations with nitrate and bromide; however, neither anion formed an unusually robust complex with this receptor. K_{assoc} for **1b** + NO_3^- and **1b** + Br^- were both determined to be $<2 \times 10^4$ M^{-1}. Diether **1e** was designed to exploit additional $OH_{phosphate}\cdots O_{ether}$ interactions with $H_2PO_4^-$, but the presence of such H-bonds cannot be established from the K_{assoc} value. Biimidazole **1f**, which lacks amide N*H* atoms, interacts with anions most weakly of all the receptors tested.

Two control titrations were performed to confirm that the decrease in fluorescence arises from actual anion *binding*, and not from nonspecific collisional quenching, changes in ionic strength, or protonation of imidazole nitrogen atoms by the $H_2PO_4^-$ ion (a potential source of H^+). For the first titration, the solvent system was changed to CH_2Cl_2−CH_3OH (9:1 v/v). The presence of an anion-solvating, hydrogen-bond donor solvent is expected to render anion binding less favorable.[19] Indeed, no quenching of the fluorescence of biimidazole **1d** was observed when Cl^- was added, even in 300-fold excess. In the second control experiment, the emission intensity of **1c** (in CH_2Cl_2) remained constant during treatment with trifluoroacetic acid.

Proton NMR experiments performed upon biimidazole **1c** in a mixture of CD_2Cl_2 and acetone-d_6 (2:1 v/v) emphasize the role of the amide N*H* atoms in anion coordination. (The modest solubility of **1c** in neat dichloromethane prevented this solvent from being used in the NMR studies, as it was in the fluorescence experiments.) As the amount of added Bu_4N^+ $H_2PO_4^-$ was gradually raised from 0 to 0.9 equiv, the amide protons of sensor **1c** shifted downfield by about 0.55 ppm. Further addition of dihydrogenphosphate induced small *upfield* shifts in δ_{NH}. No C*H* protons of **1c** were found to shift dramatically during these titrations. For example, the 4-methylbenzyl CH_2 nuclei, which are constrained to lie in proximity to a bound anion, moved upfield by 0.04 ppm. The data were best fit[20] to a binding model in which the predominant complex has 2:1 receptor−anion stoichiometry when [**1c**] > [$H_2PO_4^-$]. The overall binding constant β for **1c**$_2$·$H_2PO_4^-$ was calculated to be 1.8×10^5 M^{-2}. A Job plot also indicated 2:1 binding in CD_2Cl_2−acetone-d_6 (2:1 v/v). However, the calculated concentration profiles[20] for the **1c** + $H_2PO_4^-$ titration show the binding stoichiometry to be largely 1:1 when [biimidazole] ≪ [anion]. Although the use of different solvents for the fluorescence and NMR binding titrations prevents the results from being directly compared, the observation of 1:1 binding in the presence of excess anion is in accord with the fluorescence data.

As expected, more polar solvents lead to smaller anion-induced downfield shifts of the amide N*H* protons, and to weaker binding. When a titration of **1c** with Bu_4N^+ $H_2PO_4^-$ was performed in CD_2Cl_2−DMSO-d_6 (2:1 v/v), the amide N*H* resonance shifted by 0.31 ppm. Simple 1:1 binding stoichiometry was operative in this case, with $K_{assoc} = 2.7 \times 10^3$ M^{-1}. Introduction of a 15-fold excess of Bu_4N^+ Cl^- to a solution of **1e** in DMSO-d_6 caused a change in δ_{NH} of just 0.08 ppm; K_{assoc} for the 1:1 interaction was calculated to be ≈5 M^{-1}. A single set of biimidazole peaks appeared throughout these experiments, indicative of fast exchange. Severe broadening of the imidazole N*H* peaks precluded their use in K_{assoc} determinations.

Among several possible anion binding modes for the biimidazole unit, two are shown in Figure 3. Assuming that the anti configuration observed in the solid state is the predominant biimidazole conformation in solution, *anti-2* complexation requires minimal reorganization of receptors **1**. In principle this mode could accommodate 1:2 biimidazole−anion binding stoichiometry, although complexes of this type were not observed even under conditions of excess anion. Maximal H-bond donation to

(14) The emission spectra of **1b**−**f** are sensitive to concentration. When excited at 300 nm, aerated CH_2Cl_2 solutions of **1b**−**f** of concentrations greater than approximately 5×10^{-6} M emit light of wavelengths 310−460 nm in a single, relatively featureless emission band ($\lambda_{max} \approx 365$ nm). Below about 3×10^{-6} M, however, the fluorescence spectra display three maxima near 322, 337, and 353 nm. These results are consistent with biimidazole−biimidazole self-association occurring. Aggregation was particularly problematic in the case of dimethyl derivative **1a**, for which the emission spectrum did not become resolved into three peaks even at a concentration as low as 9×10^{-7} M.

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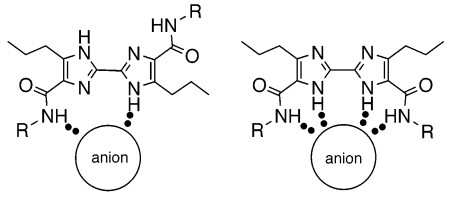

anti-2 *syn-4*

FIGURE 3. Possible structures of biimidazole−anion complexes.

an anionic guest is only possible if both imidazole NH tautomerism and rotation about the central C2−C2′ bond occur, as in *syn-4*. Each heterocycle-amide "half" of the biimidazole would provide two convergent NH groups,[4] allowing **1** to define a crescent-shaped binding pocket.

Conclusions

Biimidazole diamides serve as electrically neutral, relatively unselective sensors for anions in organic solution. The intrinsic fluorescence of the biimidazole unit obviates the need for luminescent metal ions or covalently appended, dedicated chromophores. Incorporation of biimidazole units into macrocycles and/or spherical receptors, thereby constraining the biimidazoles to adopt only the syn conformation, is expected to improve the anion affinities and selectivities of this receptor class.

Experimental Section

5-Propyl-1*H*-imidazole-4-carboxylic Acid Hydrochloride (3). 5-Propyl-1*H*-imidazole-4-carboxylic acid ethyl ester **2** (2.00 g; 11.0 mmol) and concentrated aqueous HCl (40 mL) were combined and heated to reflux with stirring for 24 h. After cooling to room temperature, the light tan solution was diluted with water (100 mL) and extracted with EtOAc. The aqueous layer was then evaporated under reduced pressure. The residue was dissolved in 2-propanol (20 mL) and cooled to −78 °C. Upon addition of Et_2O, a white precipitate formed. This material was collected by filtration and dried under vacuum to afford 2.10 g (100%) of **3**. ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H), 1.69 (m, 2H), 2.92 (t, 2H), 9.17 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 13.9, 22.4, 26.4, 120.9, 135.7, 140.0, 160.7. Anal. Calcd for $C_7H_{11}ClN_2O_2 \cdot 0.66(C_3H_9O) \cdot 0.33(H_2O)$: C 45.67, H 7.24, N 11.84, Cl 14.98. Found: C 45.27, H 7.17, N 11.86, Cl 14.68.

5-Propyl-1*H*-imidazole-4-carbonyl Chloride (4). 5-Propyl-1*H*-imidazole-4-carboxylic acid hydrochloride **3** (1.0 g; 5.3 mmol) was added to nitrogen-purged CH_3CN (10 mL). Oxalyl chloride (2.5 mL; 29 mmol) was then added, and the mixture was heated to reflux under N_2 for 1 h. The brown solution was then allowed to cool, and the product began to precipitate. Ice-cold dry Et_2O (30 mL) was added to the reaction flask to complete the precipitation. The green-gold solid was collected by suction filtration, washed with Et_2O, and dried under vacuum to afford 0.65 g (71%) of **4**. ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H), 1.70 (m, 2H), 2.90 (t, 2H), 8.70 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 13.6, 21.3, 29.4, 118.1, 137.9, 150.46, 155.8.

General Procedure for Preparation of Amides 5a−f. Under N_2, a round-bottomed flask containing a stir bar was charged with 5-propyl-1*H*-imidazole-4-carbonyl chloride **4** (0.40 g, 2.3 mmol) and 3−4 mL of a neat liquid amine. The mixture was stirred with gentle warming from a heat gun for 5 min, during which time a cloud of white vapor appeared in the flask. The mixture was partitioned between 100 mL of water and 100 mL of an organic solvent (CH_2Cl_2 or EtOAc−THF (1:1 v/v), as required for solubility). The organic phase was then washed with saturated aqueous NaCl and was dried over Na_2SO_4 or $MgSO_4$, as appropriate. Filtration, rotary evaporation of the filtrate, and drying for 24 h under high vacuum afforded the crude amide as a yellow-orange oil or solid. Further purification was performed as described below.

5-Propyl-1*H*-imidazole-4-carboxylic Acid Methylamide (5a). By using a solution of 40% aqueous methylamine, 0.31 g (80%) of amide **5a** was obtained as colorless prisms after recrystallization from CH_3CN-CH_3OH. Mp 173−175 °C; ^{1}H NMR (CD_3OD) δ 0.97 (t, 3H), 1.65 (m, 2H), 2.55 and 2.90 (both s, 3H total), 2.95 (t, 2H), 7.50 (s, 1H), 7.85 (br t, 1H); ^{13}C NMR (CD_3OD) δ 14.0, 23.8, 25.8, 27.8, 135.0, 137.7, 166.7; LRMS (CI$^+$) *m*/*z* (%) 168 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_8H_{14}N_3O$ 168.1137, found 168.1141.

5-Propyl-1*H*-imidazole-4-carboxylic Acid 3,5-Difluorobenzylamide (5b). By using 3,5-difluorobenzylamine, crude amide **5b** was obtained as a viscous yellow oil after flash column chromatography on silica gel with EtOAc as the eluent. Recrystallization from Et_2O−hexanes afforded 0.32 g (50%) of pure **5b** as colorless needles. Mp 104−105 °C; TLC (EtOAc) $R_f = 0.33$; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.63 (m, 2H), 2.97 (t, 2H), 4.59 (d, 2H), 6.60 (t, 1H), 6.80 (d, 2H), 7.32 (s, 1H), 8.00 (br t, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 22.8, 27.2, 42.2, 110.0, 129.5, 133.4, 137.2, 143.1, 161.8, 164.7; LRMS (CI+) *m*/*z* (%) 280 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_{14}H_{16}F_2N_3O$ 280.1261, found 280.1263. Anal. Calcd for $C_{14}H_{15}F_2N_3O$: C 60.21, H 5.41, N 15.05. Found: C 60.21, H 5.43, N 14.98.

5-Propyl-1*H*-imidazole-4-carboxylic Acid 4-Methylbenzylamide (5c). By using 4-methylbenzylamine, 0.53 g (90%) of amide **5c** was obtained as an off-white solid after flash column chromatography on silica gel with EtOAc as the eluent. Mp 131−133 °C; TLC (EtOAc): $R_f = 0.28$; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.65 (m, 2H), 2.30 (s, 3H), 3.00 (t, 2H), 4.57 (d, 2H), 7.10 (dd, 4H), 7.25 (s, 1H), 7.62 (br t, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 21.7, 22.5, 27.1, 43.0, 133.6, 135.8, 137.7, 164.8; LRMS (CI+) *m*/*z* (%) 258 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_{15}H_{20}N_3O$ 258.1606, found 258.1600.

5-Propyl-1*H*-imidazole-4-carboxylic Acid Hexylamide (5d). By using 1-hexylamine, 0.49 g (90%) of amide **5d** was obtained as light yellow plates after recrystallization from CH_3CN. Mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (br t, 6H), 1.30 (m, 6H), 1.61 (m, 2H), 1.65 (m, 2H), 3.00 (t, 2H), 3.37 (q, 2H), 7.22 (br t, 1H), 7.48 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.2, 22.8, 22.9, 27.2, 30.0, 31.3, 31.8, 39.1, 132.8, 136.0, 164.2; LRMS (CI$^+$) *m*/*z* (%) 238 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_{13}H_{24}N_3O$ 238.1919, found 238.1912. Anal. Calcd for $C_{13}H_{23}N_3O$: C 65.79, H 9.77, N 17.70. Found: C 65.89, H 9.38, N 17.72.

5-Propyl-1*H*-imidazole-4-carboxylic Acid (3-Methoxypropyl)amide (5e). By using 3-methoxypropylamine, 0.44 g (85%) of amide **5e** was obtained as yellow crystals after flash column chromatography with EtOAc−CH_3OH (9:1 v/v) as the eluent. Mp 61−63 °C; TLC (EtOAc−CH_3OH, 9:1) $R_f = 0.30$; ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H), 1.55 (m, 2H), 1.75 (m, 2H), 2.90 (t, 2H), 3.21 (s, 3H), 3.40 (m, 4H), 7.35 (s, 1H), 7.44 (br t, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 23.7, 27.7, 30.5, 37.3, 58.9, 71.5, 134.1, 138.3, 164.6; LRMS (CI$^+$) *m*/*z* (%) 226 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_{11}H_{20}N_3O_2$ 226.1556, found 226.1553.

(5-Propyl-1*H*-imidazol-4-yl)pyrrolidin-1-ylmethanone (5f). By using pyrrolidine, 0.21 g (44%) of amide **5f** was obtained as yellow needles after recrystallization from CH_3CN. Mp 121−122 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H), 1.61 (m, 2H), 1.90 (br m, 4H), 2.84 (t, 2H), 3.60 (br s, 2H), 3.90 (br s, 2H), 7.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.5, 22.5, 23.8, 44.9, 46.3, 46.7, 48.8, 131.0, 136.5, 164.5; LRMS (CI$^+$) *m*/*z* (%) 208 (100) [M + H]$^+$; HRMS (CI$^+$) calcd for $C_{11}H_{18}N_3O$ 208.1450, found 208.1445. Anal. Calcd for $C_{11}H_{17}N_3O$: C 63.74, H 8.27, N 20.27. Found: C 63.57, H 8.30, N 20.26.

General Procedure for Preparation of 2-Iodo Amides 6a−f. An imidazole amide **5** (1−2 mmol) was dissolved in dry THF (25 mL) in a round-bottomed flask containing a stir bar. *N*-Iodosuccinimide (NIS) (2.0 equiv) was added in one portion,

and the flask was covered with foil to exclude light. The mixture was heated to reflux for 24 h under N_2, then was allowed to cool to room temperature. Saturated aqueous $NaHSO_3$ (7 mL) was added dropwise to destroy the excess iodine reagent. The solvents were removed under reduced pressure, and the residue was partitioned between water and an organic solvent ($CHCl_3$ or EtOAc, as determined by solubility). The organic phase was dried over Na_2SO_4 and filtered, and the filtrate was evaporated to yield the 2-iodo product as a solid. In most cases, this crude material was used in the subsequent Pd(0)-coupling step without additional purification.

2-Iodo-5-propyl-1*H*-imidazole-4-carboxylic Acid Methylamide (6a). Starting from **5a** (0.30 g; 1.8 mmol), 0.45 g (87%) of **6a** was obtained as a yellow solid. ^{1}H NMR (CD_3OD) δ 0.89 (t, 3H), 1.61 (m, 2H), 2.63 and 2.82 (both s, 3H total), 2.91 (t, 2H), 7.85 (s, 1H); ^{13}C NMR (CD_3OD) δ 13.9, 23.7, 25.9, 27.7, 134.5, 142.0, 165.3; LRMS (CI^+) *m*/*z* (%) 294 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_8H_{13}IN_3O$ 294.0103, found 294.0010.

2-Iodo-5-propyl-1*H*-imidazole-4-carboxylic Acid 3,5-Difluorobenzylamide (6b). Starting from **5b** (0.32 g; 1.1 mmol), 0.39 g (84%) of **6b** was obtained as yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3H), 1.62 (m, 2H), 2.99 (t, 2H), 4.58 (d, 2H), 6.68 (t, 1H), 6.84 (d, 2H), 7.64 (br t, 1H), 9.14 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.9, 22.8, 27.1, 42.3, 110.5, 133.8, 141.7, 143.0, 161.6, 163.0, 165.1; LRMS (CI^+) *m*/*z* (%) 406 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{14}H_{15}F_2IN_3O$ 406.0228, found 406.0219.

2-Iodo-5-propyl-1*H*-imidazole-4-carboxylic Acid 4-Methylbenzylamide (6c). Starting from **5c** (0.30 g; 1.2 mmol), 0.38 g (85%) of **6c** was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3H), 1.64 (m, 2H), 2.33 (s, 3H), 3.01 (t, 2H), 4.56 (d, 2H), 7.17 (dd, 4H), 7.40 (br t, 1H); ^{13}C NMR ($CDCl_3$) δ 14.0, 21.3, 22.8, 29.8, 42.9, 128.1, 129.5, 135.6, 137.1, 141.0, 162.5; LRMS (CI^+) *m*/*z* (%) 290 (100), 384 (10) $[M + H]^+$; HRMS (CI^+) calcd for $C_{15}H_{19}IN_3O$ 384.0573, found 384.0585.

2-Iodo-5-propyl-1*H*-imidazole-4-carboxylic Acid Hexylamide (6d). Starting from **5d** (0.30 g; 1.3 mmol), 0.42 g (92%) of **6d** was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.81 (br t, 6H), 1.23 (m, 6H), 1.57 (m, 4H), 2.96 (t, 2H), 3.35 (q, 2H), 7.16 (br t, 1H), 11.39 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 14.0, 22.6, 26.7, 26.9, 29.6, 31.5, 39.0, 134.0, 140.8, 162.5; LRMS (CI^+) *m*/*z* (%) 364 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{13}H_{23}IN_3O$ 364.0886, found 364.0877.

2-Iodo-5-propyl-1*H*-imidazole-4-carboxylic Acid (3-Methoxypropyl)amide (6e). Starting from **5e** (0.56 g; 2.5 mmol), the crude product obtained from the General Procedure was further purified by flash column chromatography on silica gel with EtOAc as the eluent. Fractions containing the fast spot were combined and evaporated to afford 0.55 g (63%) of **6e** as a white solid. TLC (EtOAc) R_f = 0.48; ^{1}H NMR (CD_3OD) δ 0.80 (t, 3H), 1.52 (m, 2H), 1.76 (m, 2H), 2.85 (t, 2H), 3.23 (s, 3H), 3.29 (m, 2H), 3.37 (t, 2H), 7.70 (br t, 1H); ^{13}C NMR (CD_3OD) δ 13.9, 23.7, 27.7, 30.5, 58.9, 71.5, 134.5, 142.1, 164.6; LRMS (CI^+) *m*/*z* (%) 352 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{11}H_{19}IN_3O_2$ 352.0522, found 352.0528.

(2-Iodo-5-propyl-1*H*-imidazol-4-yl)pyrrolidin-1-ylmethanone (6f). Starting from **5f** (0.21 g; 1.0 mmol), 0.30 g (89%) of **6f** was obtained as a yellow solid. ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H), 1.60 (m, 2H), 1.90 (br m, 4H), 2.80 (t, 2H), 3.61 (br s, 2H), 3.85 (br s, 2H), 9.30 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 21.0, 22.6, 23.9, 26.4, 46.6, 49.0, 136.5, 141.9, 163.0; LRMS (CI^+) *m*/*z* (%) 334 (100) $[M + H]^+$; HRMS (CI^+): calcd for $C_{11}H_{17}IN_3O$ 334.0416, found 334.0419.

General Procedure for Preparation of Biimidazole Diamides 1a−f. An iodide **6** (1.0−1.6 mmol) and dry toluene (30 mL) were placed in a thick-walled pressure tube containing a stir bar. The solution was purged with N_2 for 5 min, then *N*,*N*-diisopropylethylamine (2.0 equiv) and tetrakis(triphenylphosphine)palladium(0) (0.040 equiv) were added to the reaction mixture. The tube was sealed, covered in foil, and heated at 110 °C for 48 h, during which time the reaction turned dark red-brown. Subsequent isolation and purification of products **1a**−**f** varied, as described below.

5,5′-Dipropyl-1*H*,1′*H*-[2,2′]biimidazolyl-4,4′-dicarboxylic Acid Bis(methylamide) (1a). Iodide **6a** (0.35 g; 1.2 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3OH and filtered. The filtrate was evaporated under vacuum, and the residue was dissolved in CH_2Cl_2. Upon standing, a white solid precipitated. This material was collected by filtration and was washed with ice-cold CH_2Cl_2 to afford 0.080 g (43%) of **1a**. Mp >260 °C; TLC (EtOAc) R_f = 0.32; ^{1}H NMR (DMSO-d_6) δ 0.83 (t, 6H), 1.61 (m, 4H), 2.73 (d, 6H), 2.89 (t, 4H), 7.50 (br t, 2H); ^{13}C NMR (DMSO-d_6) δ 13.1, 21.9, 24.7, 30.2, 130.3, 135.6, 163.0; UV/vis (CH_3OH) λ_{max} (ϵ $M^{-1}cm^{-1}$) 286 (18500), 294 (18200), 309 (9860). Anal. Calcd for $C_{16}H_{24}N_6O_2 \cdot 0.25(CH_2Cl_2)$: C 55.19, H 6.98, N 23.76. Found: C 54.93, H 6.71, N 23.74.

5,5′-Dipropyl-1*H*,1′*H*-[2,2′]biimidazolyl-4,4′-dicarboxylic Acid Bis(3,5-difluorobenzylamide) (1b). Iodide **6b** (0.44 g; 1.1 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3OH and filtered. The filtrate was evaporated under vacuum, and the residue was dissolved in $CHCl_3$. Slow addition of CH_3CN caused a brown solid to precipitate. This material was collected by filtration and washed with CH_3CN to afford 0.12 g (40%) of **1b** as an off-white solid. Mp 173−174 °C; TLC (CH_2Cl_2−EtOAc, 1:1) R_f = 0.51; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 6H), 1.60 (m, 4H), 2.90 (t, 4H), 4.42 (d, 4H), 6.99 (d, 4H), 7.05 (t, 2H), 8.20 (br t, 2H); ^{13}C NMR (DMSO-d_6) δ 14.1, 23.1, 30.2, 41.8, 110.9, 131.0, 141.7, 145.5, 161.3, 163.8, 164.7; UV/vis (CH_3OH) λ_{max} (ϵ M^{-1} cm^{-1}) 287 (21800), 294 (20700), 309 (10700); LRMS (CI^+) *m*/*z* (%) 557 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{28}H_{29}F_4N_6O_2$ 557.2288, found 557.2292.

5,5′-Dipropyl-1*H*,1′*H*-[2,2′]biimidazolyl-4,4′-dicarboxylic Acid Bis(4-methylbenzylamide) (1c). Iodide **6c** (0.45 g; 1.2 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3OH and filtered. The filtrate was evaporated under vacuum, and the residue was triturated with CH_3CN. The solid that remained undissolved was collected by filtration and washed with CH_3CN to afford 0.30 g (38%) of **1c** as an off-white microcrystalline solid. An analytical sample was recrystallized from CH_3OH. Mp 226−227 °C; TLC (CH_2Cl_2−EtOAc, 1:1) R_f = 0.60; ^{1}H NMR (CD_2Cl_2) δ 0.80 (t, 6H), 1.59 (m, 4H), 2.21 (s, 6H), 2.86 (t, 4H), 4.39 (d, 4H), 7.05 (dd, 8H), 7.22 (br t, 2H); ^{13}C NMR (DMSO-d_6) δ 14.2, 21.4, 23.3, 27.0, 49.6, 128.3, 129.9, 131.0, 137.0, 138.7, 164.2; UV/vis (CH_3OH) λ_{max} (ϵ M^{-1} cm^{-1}) 287 (25100), 295 (25100), 310 (13700); LRMS (CI^+) *m*/*z* (%) 513 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{30}H_{37}N_6O_2$ 513.2978, found 513.2981. Anal. Calcd for $C_{30}H_{36}N_6O_2 \cdot 0.33(H_2O)$: C 68.68, H 7.17, N 16.02. Found: C 68.58, H 7.17, N 16.11.

5,5′-Dipropyl-1*H*,1′*H*-[2,2′]biimidazolyl-4,4′-dicarboxylic Acid Bis(hexylamide) (1d). Iodide **6d** (0.45 g; 1.1 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3OH and filtered. The filtrate was evaporated under vacuum, and the residue was recrystallized from CH_3OH to afford 0.12 g (45%) of **1d** as a white solid. Mp >260 °C; TLC (CH_2Cl_2−EtOAc, 1:1) R_f = 0.51; ^{1}H NMR ($CDCl_3$) δ 0.88 (br t, 6H), 0.97 (t, 6H), 1.31 (br m, 12H), 1.60 (m, 4H), 1.72 (m, 4H), 3.06 (t, 4H), 3.38 (q, 4H), 7.00 (br t, 2H); ^{13}C NMR (DMSO-d_6) δ 14.2, 14.6, 22.8, 23.1, 26.9, 30.2, 31.7, 38.7, 131.3, 137.2, 163.5; UV/vis (CH_3OH) λ_{max} (ϵ M^{-1} cm^{-1}) 287 (26000), 295 (25900), 309 (14100); LRMS (CI^+) *m*/*z* (%) 473 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{26}H_{45}N_6O_2$ 473.3604, found 473.3601. Anal. Calcd for $C_{26}H_{44}N_6O_2$: C 66.07, H 9.38, N 17.78. Found: C 66.26, H 9.54, N 17.83.

5,5′-Dipropyl-1*H*,1′*H*-[2,2′]biimidazolyl-4,4′-dicarboxylic Acid Bis[(3-methoxypropyl)amide] (1e). Iodide **6e** (0.55 g; 1.6 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3-

OH and filtered. The filtrate was evaporated under vacuum, and the residue was recrystallized from EtOAc to afford 0.13 g (33%) of **1e** as colorless prisms. Mp >260 °C; TLC (EtOAc) $R_f = 0.31$; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 6H), 1.65 (m, 4H), 1.82 (m, 4H), 3.00 (t, 4H), 3.34 (s, 6H), 3.48 (m, 8H), 7.29 (br t, 2H); ^{13}C NMR (DMSO-d_6) δ 14.3, 24.1, 26.7, 32.3, 61.9, 72.3, 137.5, 145.1, 167.3; UV/vis (CH_3OH) λ_{max} (ϵ M^{-1} cm^{-1}) 287 (25600), 295 (25400), 309 (13800); LRMS (CI^+) *m*/*z* (%) 449 (100) $[M + H]^+$; HRMS (CI^+) calcd for $C_{22}H_{37}N_6O_4$ 449.2876, found 449.2875. Anal. Calcd for $C_{22}H_{36}N_6O_4$: C 58.91, H 8.09, N 18.74. Found: C 59.00, H 8.09, N 18.78.

[5,5′-Dipropyl-4′-(pyrrolidine-1-carbonyl)-1*H*,1′*H*-[2,2′]-biimidazolyl-4-yl]pyrrolidin-1-ylmethanone (1f). Iodide **6f** (0.34 g; 1.0 mmol) was used as starting material. After cooling to room temperature, the reaction mixture was diluted with CH_3OH and filtered. The filtrate was evaporated under vacuum, and the residue was recrystallized from CH_3OH to afford 0.080 g (38%) of **1f** as yellow prisms. Mp >260 °C; TLC (EtOAc) $R_f = 0.14$; ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 6H), 1.59 (m, 4H), 1.80 (br m, 8H), 2.80 (t, 4H), 3.55 (br m, 4H), 3.90 (br m, 4H); ^{13}C NMR (DMSO-d_6) δ 14.3, 23.4, 24.2, 26.9, 31.4, 46.8, 49.0, 132.9, 138.0, 163.7; UV/vis (CH_3OH) λ_{max} (ϵ M^{-1} cm^{-1}) 287 (21200), 294 (20900), 309 (sh); LRMS (CI^+) *m*/*z* (%) 208 (100), 413 (70) $[M + H]^+$; HRMS (CI^+) calcd for $C_{22}H_{33}N_6O_2$ 413.2665, found 413.2662. Anal. Calcd for $C_{22}H_{32}N_6O_2 \cdot 0.33(H_2O)$: C 63.13, H 7.87, N 20.08. Found: C 63.21, H 7.82, N 20.08.

Fluorescence Titrations. Biimidazole diamides **1b**−**f** were dissolved in spectrophotometric grade dichloromethane such that the concentration of each solution was between 1×10^{-6} and 3×10^{-6} M (sonication was required to effect complete dissolution in some cases). An electronic absorption spectrum was acquired for each sample to ensure that the optical density was less than 0.1. A 3.0-mL sample of biimidazole diamide solution was transferred to a quartz cuvette and placed into the fluorometer. The sample was excited at 300 nm, and an emission spectrum from 310 to 460 nm was recorded. Upon completion of the scan, the area contained under the emission band (*F*) was computed. Aliquots of an approximately 0.03 M solution of anion (as tetrabutylammonium (Bu_4N^+) salt) in CH_2Cl_2 were then injected into the sample solution through a small hole in the cap. The sample solution was magnetically stirred for 1 min after each addition, then was scanned again. This process was repeated until the change in fluorescence intensity became insignificant. The total volume of the sample solution changed by less than 2% over the course of the experiment.

Binding constants (K_{assoc}) were derived from plots of F/F_0 vs [anion].[16] During iterative fitting to the equation $F/F_0 = (1 + (k_{complex}/k_{biimid})K_{assoc}[anion])/(1 + K_{assoc}[anion])$, the values of $k_{complex}$ and K_{assoc} were allowed to vary freely. The constant k_{biimid} is equal to $F_0/[biimid]_0$. Results reported in Table 1 are averages of at least two replicate titrations.

^{1}H NMR Binding Studies. A 0.0132 M solution of **1c** in CD_2Cl_2−acetone-d_6 (2:1 v/v) was prepared. A portion (0.75 mL) of this solution was transferred to an NMR tube; to the remainder of the solution was added Bu_4N^+ $H_2PO_4^-$ such that its concentration was 0.189 M. A known volume of the anion-containing solution was added to the NMR tube, the tube was inverted several times to mix the contents, and the chemical shift of the amide N*H* protons of **1c** was determined with use of a 500-MHz instrument. This process was repeated until these protons ceased to move downfield. Procedures similar to the one above were also used to assess the binding of **1c** to $H_2PO_4^-$ in CD_2Cl_2−DMSO-d_6 (2:1 v/v), and the binding of **1e** to Cl^- in DMSO-d_6. Binding constants were calculated with the nonlinear curve fitting program WinEQNMR.[20]

The stoichiometry of **1c** + $H_2PO_4^-$ complexation in the NMR concentration regime was determined by the method of continuous variations (i.e., a Job analysis). Thus, equimolar solutions of **1c** and Bu_4N^+ $H_2PO_4^-$ were prepared (in CD_2Cl_2−acetone-d_6 (2:1 v/v)) and were mixed in varying ratios in a series of NMR tubes such that the total number of moles of **1c** + $H_2PO_4^-$ was the same in each tube. The chemical shift of the amide N*H* protons of **1c** was recorded for each sample. A plot of $\{(\delta_{obs} - \delta_0)/\delta_{max}\}$[**1c**] vs mole fraction of **1c** was then generated; it displayed a maximum at a mole fraction **1c** of ≈0.65.

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Supporting Information Available: A crystal packing diagram for **1a**, and ORTEP views of single molecules of **1b**−**d**; tables of crystal data, bond lengths and angles, atomic coordinates, and thermal parameters for **1a**−**d**; and binding plots from fluorescence and ^{1}H NMR titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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