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Role of Pyridine Hydrogen-Bonding Sites in Recognition of Basic Amino Acid Side Chains

Thomas W. Bell,*,† Alisher B. Khasanov,† and Michael G. B. Drew‡

Contribution from the Department of Chemistry/216, University of Nevada, Reno, Nevada 89557-0020, and Department of Chemistry, The University, Whiteknights, Reading, RG6 6AD, U.K.

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Abstract: A series of three, new artificial receptors for guanidinium and ammonium guests has been synthesized. All three receptors have highly preorganized clefts bearing two carboxylate groups. They differ in the number of nitrogen atoms contained in their clefts, as follows: four N atoms in receptor **3**, three N atoms in **4**, and two nitrogens in **5**. Crystallographic studies have produced the solid-state structures of the following guanidinium complexes of each receptor: $\mathbf{3{\cdot}2CH_3CH_2NHC(NH_2)_2^+}$, $\mathbf{4{\cdot}2CH_3NHC(NH_2)_2^+}$, and $\mathbf{5{\cdot}2C(NH_2)_3^+}$. The conformations of the receptor molecules in all three complexes are very similar. *N*-Alkylguanidinium guests are bound in the clefts of **3** and **4** in similar manners, despite the loss of one hydrogen-bond acceptor nitrogen in **4** and the possible hindrance of the cavity by a CH group. In the guanidinium complex of **5**, neither guest enters the cavity containing two CH groups. Complexation studies were conducted in methanol by ^{1}H NMR titration for several guanidinium and ammonium guests, including derivatives of the amino acids arginine and lysine. Receptor **5** binds all such guests weakly (K_s < 4000), while **3** binds most guests very strongly (K_s > 100 000). Receptor **3** is selective for arginine versus lysine, while **4** binds lysine better than does **3**. The results generally underscore the importance of receptor preorganization and hydrogen-bonding complementarity in the design of receptors that can serve as probes for biomolecules.

Introduction

Events of noncovalent bonding between biological molecules are processes of major significance that regulate various functions in living systems. In many biological processes, basic amino acid residues such as arginine and lysine are critical in recognition of functionally important proteins. The recognition of specific plasma lipoproteins by the low-density lipoprotein (LDL) cell surface receptors on human fibroblasts, which initiates a series of events regulating intracellular cholesterol metabolism, depends on the presence of lysine and arginine residues.[1] The same residues in apoliprotein B, an essential structural protein required for the assembly of triglyceride-rich low-density lipoproteins by the liver, are found to be critical for its binding to microsomal triglyceride transfer protein.[2] RNA recognition processes have become important targets for the development of antiviral and antibiotic drugs.[3] The transcriptional activator tat[4−6] and the Rev protein[4,7] are arginine-rich RNA-binding proteins that regulate important steps in replication of the human immunodeficiency virus type I (HIV-1). The structure of the HIV-1 nucleocapsid protein bound to the genomic Ψ-RNA recognition element[8] displays a specific arginine−base interaction (Arg[32]-A[8]). Synthetic receptors capable of selective binding to arginine and lysine residues could lead to novel pharmaceuticals, as well as molecular probes that could facilitate the understanding and characterization of vital biological processes.

Previously designed receptors for guanidinium and alkylammonium ions are mostly crown ether derivatives.[9] More recent approaches involve polyaza-aromatic hexagonal lattice receptors for unsubstituted guanidinium ion,[10] tweezer-type receptors that can bind alkylguanidinium guests including arginine,[11] and

* To whom correspondence should be addressed. E-mail: twb@unr.edu. Fax: 775/784-6804.
† University of Nevada.
‡ The University, Reading.

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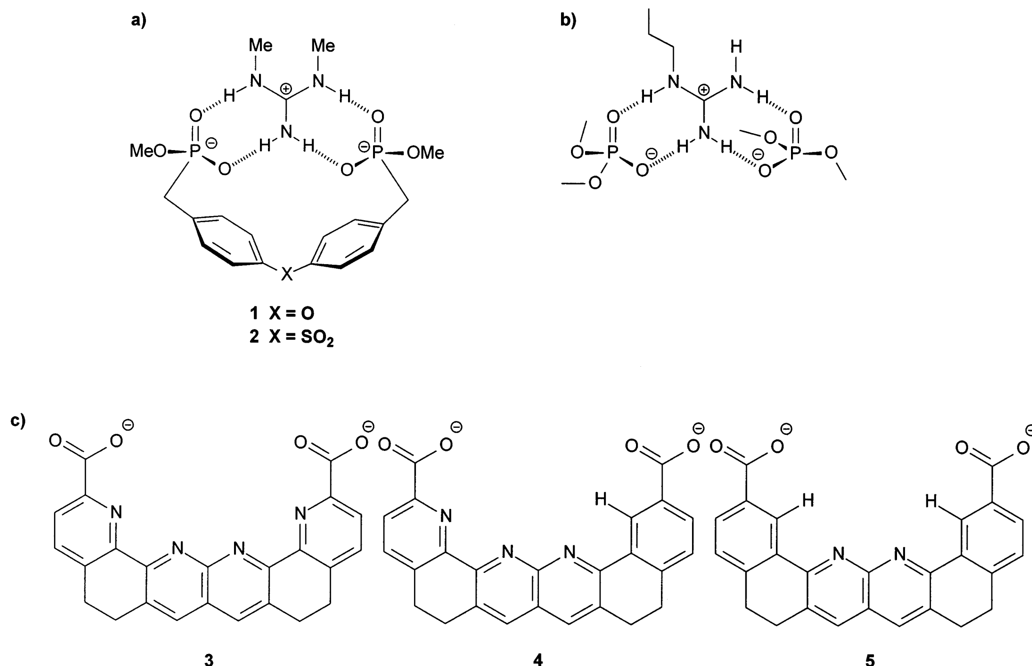

Figure 1. Structures of guanidinium receptors: (a) "molecular tweezers";[15] (b) the "arginine fork" of the HIV-1 tat-TAR complex;[6] (c) hexagonal lattice receptors.

polyanionic cyclophanes.[12] Receptors derived from cucurbituril,[13] calixarenes,[14] and xylylene bisphosphonate[15] have also been designed to bind alkylammonium ions.

To be useful for biological applications, a receptor for arginine or lysine must be reasonably soluble in water, it must have high affinity for *N*-alkylguanidinium or *N*-alkylammonium side chains, and it must display selectivity relative to other cations. One can develop such a receptor by imitating natural patterns of recognition or by employing a new, improved design. Following the biomimetic approach, Schrader[15b] synthesized "molecular tweezers" containing two phosphonate groups capable of binding guanidinium cations by four hydrogen bonds (Figure 1a). The structure of the resulting complex resembles that of the critical arginine residue of tat (Arg[52]) bound to phosphodiesters P22 and P23 of HIV-1 TAR RNA, a feature dubbed the "arginine fork" (Figure 1b) by Frankel et al.[6] However, molecular tweezer **2** shows only modest binding of *N*-alkylguanidinium guests even in methanol ($K_d \approx 2$ mM). This can be attributed to incomplete preorganization of hydrogen-bond acceptor sites in **2**, which allows free rotation about several single bonds, requiring that part of the binding energy be used to conformationally organize the receptor.

Several research groups have reported the formation of complexes with host molecules having hydrogen-bonding sites that are partially preorganized by attachment to or embedding within cyclic structures.[16] Our approach to recognition of small, planar molecules is through hexagonal lattice design[10,17] of relatively rigid receptor molecules with defined planar arrays of hydrogen-bonding groups. Following this approach, we designed and synthesized a water-soluble receptor for the *N*-alkylguanidinium cation (**3**, Figure 1c). This artificial receptor binds arginine in water ($K_a \approx 10^3$ M^{-1}) with about 7-fold selectivity over lysine, and it binds the dipeptide diarginine (K_a

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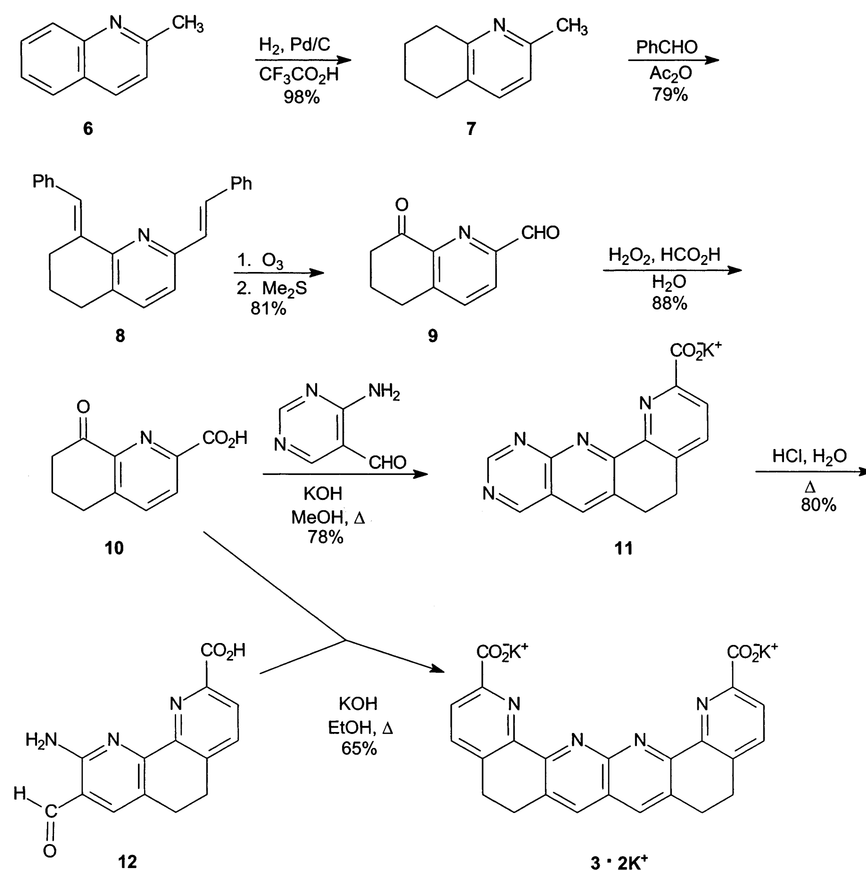
Scheme 1



$\approx 2 \times 10^4\ M^{-1}$) with 5-fold selectivity over dilysine.[17j] Receptor **3** has two negatively charged carboxylate groups, which make it soluble in water. The nitrogen and oxygen hydrogen-bond acceptor sites in **3** are preorganized for the formation of a robust hydrogen-bonding network with guanidinium ion. Conjugation between the carboxylate groups and the neighboring pyridine rings should stabilize the coplanar conformation. Rotation about the pyridine−carboxylate bond is expected, but the symmetry of the carboxylate group produces identical structures via 180° rotation about this bond.

The combination of receptor preorganization and attractive electrostatic interactions between the dianionic host and cationic guest makes complexes of **3** with *N*-alkylguanidinium stable even in aqueous media. To investigate how modification of binding sites in the cavity of this type of receptor affects binding motifs and affinities toward various guests, we designed molecules **4** and **5** (Figure 1c). In the structure of **4**, one of the pyridine rings of **3** is replaced with a benzene ring, introducing one hydrogen atom directly into the cavity. In complexes of **3**, this pyridine ring acts as a spacer between the 1,8-naphthyridine and carboxylate hydrogen-bond acceptors and also provides a stabilizing secondary electrostatic N···H interaction.[17i] The absence of this attractive interaction and steric interference between the benzene hydrogen atom and the planar *N*-alkylguanidinium guest is expected to decrease the stabilities of the complexes of **4**. Because of the smaller size of *N*-alkylammonium relative to *N*-alkylguanidinium, this modification may not decrease the affinity of **4** toward the former guest. Replacement of both pyridine rings in **3** with benzene rings makes the cavity of **5** even more congested for binding both *N*-alkylguanidinium and *N*-alkylammonium cations. Thus, the affinity of **5** for these cations should be much less than those of receptors **3** and **4**.

Results and Discussion

Our general strategy for the synthesis of target compounds **3**, **4**, and **5** involves a sequence of two Friedländer condensations of ketones with appropriate aminoaldehydes (Scheme 1). In the case of compound **3**, the key ketoacid (**10**) was obtained from quinaldine (**6**). The benzene ring of **6** was selectively reduced by catalytic hydrogenation with palladium on carbon in trifluoroacetic acid, using the procedure of Vierhapper and Eliel.[18] Condensation of **7** with benzaldehyde in acetic anhydride gave dibenzylidene derivative **8**. Ozonolysis[19] of **8** with reductive workup gave keto aldehyde **9**, which was oxidized to ketoacid **10** with Jones' reagent or hydrogen peroxide and formic acid. Friedländer condensation of **10** with 4-aminopyrimidine-5-carboxaldehyde[20] gave **11**, which was hydrolyzed to aminoaldehyde **12** with dilute hydrochloric acid. A second condensation of **10** with **12** in refluxing ethanol gave receptor **3** as the dipotassium salt (**3·2K**$^+$), which was isolated in 22% overall yield from quinaldine after purification by recrystallization.

To obtain compound **5**, 1-tetralone-7-carboxylic acid (**13**)[21] was used as a main building block (Scheme 2). As in the synthesis of **3**, 4-aminopyrimidine-5-carboxaldehyde[20] was used

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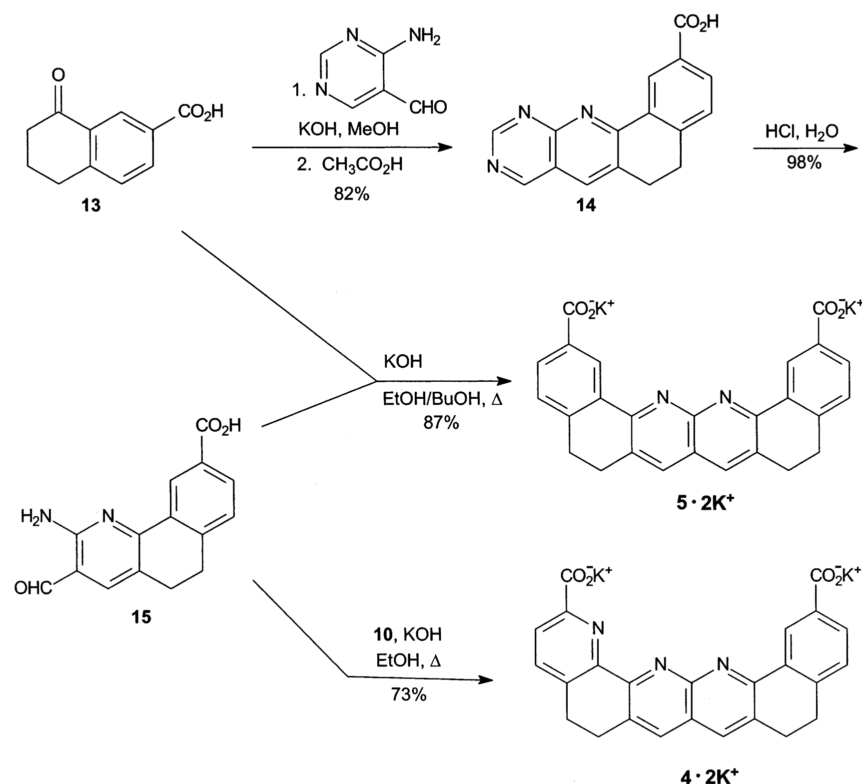
Scheme 2



in the first Friedländer condensation with tetralone derivative **13**. The product of this condensation was obtained in the carboxylic acid form (**14**), which was hydrolyzed to aminoaldehyde **15**. The condensation of **13** with **15** required a higher reaction temperature than that used in the synthesis of **3**; therefore, butanol was added as a higher boiling cosolvent to facilitate the formation of **5**. After recrystallization from an ethanol/water mixture, microanalytically pure **5·2K**$^+$ was obtained in 23% overall yield from **13**. The use of the same ketone (**10** or **13**) in both Friedländer condensations provided the molecular symmetry of **3** and **5**. However, two different ketones could be used to make unsymmetrical molecules of this type. This was successfully demonstrated by condensation of aminoaldehyde **15**, obtained from tetralone **13**, with ketoacid **10**. The resulting unsymmetrical compound **4** was obtained in 73% yield as its dipotassium salt (Scheme 2).

The dipotassium salts of diacids **3**, **4**, and **5** have good aqueous solubilities (ca. 0.3 M for **3·2 K**$^+$), which makes them very promising compounds for biological and medicinal studies. They are also slightly soluble in methanol, but are insoluble in dimethyl sulfoxide, dichloromethane, and chloroform. However, the solubilities of the corresponding neutral diacids are reversed. They have very low solubility in water ($<4\ \mu$M), but are moderately soluble in dimethyl sulfoxide.

Addition of guanidine hydrochloride, *N*-methylguanidine hydrochloride, or *N*-ethylguanidine sulfate to aqueous solutions of dipotassium salts of **3**, **4**, or **5** results in the formation of yellow precipitates. According to results of combustion microanalysis and ^{1}H NMR spectroscopy, dried samples of these precipitates consisted of the receptor dianion and the corresponding guanidinium cation in an exactly 1:2 ratio. The predicted binding motif of receptor **3** to guanidinium ion was proven by X-ray crystallographic analysis of a single crystal of the hydrated complex with *N*-ethylguanidinium, **3·2CH$_3$CH$_2$NHC(NH$_2$)$_2$$^+$**.[17j] Two molecules of the complex **3·2CH$_3$CH$_2$NHC(NH$_2$)$_2$$^+$** form a centrosymmetric, hydrogen-bonded dimer with eight water molecules in the solid state (Figure 2). As described previously,[17j] one ethylguanidinium guest (**A**) in each complex resides in the receptor cavity, and the other cation (**B**) binds externally to one carboxylate group of the same receptor. In the dimer structure (Figure 2), this second ethylguanidinium guest (**B**) and a water molecule (O53) bridge to the carboxylate at the opposite end of the other receptor. The other six water

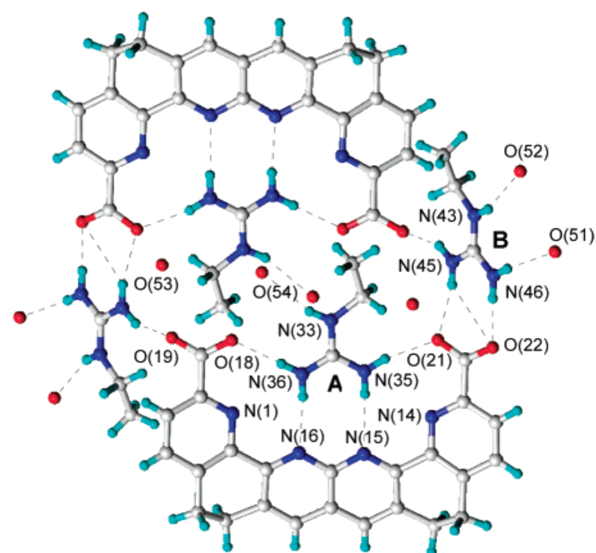


Figure 2. Crystal structure of complex **3·2CH$_3$CH$_2$NHC(NH$_2$)$_2$$^+$** showing two centrosymmetrically related *N*-ethylguanidinium cations positioned with respect to the two host receptors and four pairs of hydrogen-bonded water molecules (red spheres). Hydrogen bonds shown as dotted lines. Atom colors: gray, carbon; blue, nitrogen; red, oxygen; cyan, hydrogen.

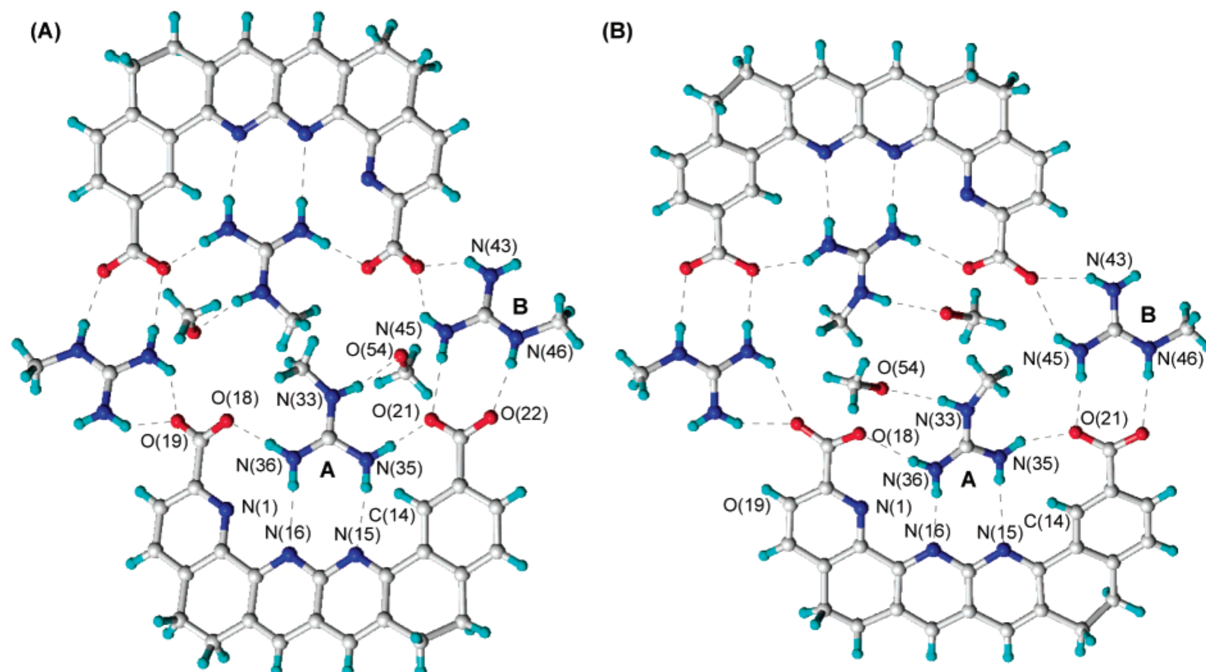


Figure 3. Crystal structure of complex $\mathbf{4{\cdot}2CH_3NHC(NH_2)_2^+}$ showing two centrosymmetrically related *N*-methylguanidinium cations positioned with respect to the two host receptors. The *N*-methyl group and hydrogen-bonded methanol solvent molecule are disordered over two possible pairs of centrosymmetric sites with 64% occupancy in A, and 36% occupancy in B. Atom colors: gray, carbon; blue, nitrogen; red, oxygen; cyan, hydrogen.

molecules in the dimer form hydrogen bonds to the remaining ethylguanidinium NH groups, and one also bridges to a carboxylate oxygen.

The complex $\mathbf{4{\cdot}2CH_3NHC(NH_2)_2^+}$ packs in a very similar centrosymmetric dimer pattern, with one guest (**A**) in the receptor cleft and the second bound externally to a carboxylate (Figure 3). The crystal contains one methanol molecule per 1:2 complex. This solvent molecule and the position of the internally bound guest (**A**) methyl group are disordered between two alternate trigonal positions. These disordered positions are apparently correlated as shown in Figure 3, because the best refinement was obtained with 64% weighting of the structure shown in Figure 3A and 36% weighting of the structure shown in Figure 3B. In both cases, the methanol molecule forms a hydrogen-bonding bridge between the CH_3NH donor of the internal guest and the nicotinic carboxylate of the host. Alternate positions of the guest methyl group were correlated with switching of the methanol bridge from the carboxylate of the receptor in the same 1:2 complex to the same carboxylate oxygen atom of the other receptor in the dimer.

In contrast, $\mathbf{5{\cdot}2C(NH_2)_3^+}$ crystallizes without a guest in the cleft and also lacks the eight-membered carboxylate-guanidinium chelate ring observed in the complexes of receptors **4** and **5**. The two guanidinium guests form six-membered chelate rings, one (**A**) with a water molecule O(501) occupying the central cavity of the receptor and the second (**B**) with a carboxylate oxygen (Figure 4). This water molecule is hydrogen-bonded to a second bridging water O(502), and two other water molecules are disordered between four positions, each with 50% occupancy.

All three receptors adopt relatively planar conformations, as judged from the deviations of the heteroatoms lining the cleft (2−4 nitrogens and 2 oxygens) from the least squares planes defined by these atoms (Table 1). In the series **3**−**4**−**5**, the angle between this plane and the more coplanar guest increases from

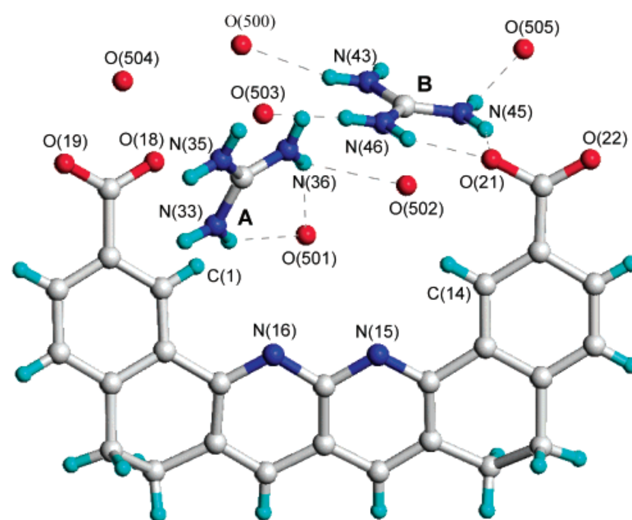


Figure 4. Crystal structure of complex $\mathbf{5{\cdot}2C(NH_2)_3^+}$ showing positions of the two independent guanidinium cations with respect to the receptor and six hydrogen-bonded water molecules (red spheres). Atom colors: gray, carbon; blue, nitrogen; red, oxygen; cyan, hydrogen.

7 to 58°, showing that sequential replacement of pyridine by benzene increasingly excludes the guest from the receptor cavity. In the guanidinium complex of receptor **5**, the central water molecule lies only 0.07 Å from the plane of the 1,8-naphthyridine unit of this receptor.

The conformations of receptors **3**−**5** in their complexes are also described by key torsion angles given in Table 2. The angles for X−C−C−N and X−C−C−O fragments within the cleft (X = N or C) fall in the range of 0−27°, while the torsion angles of the external $C{-}CH_2{-}CH_2{-}C$ bridges range from 25 to 53°. The signs of the ethylene bridge torsions are opposite in the cases of **3** and **5**, but they are the same for receptor **4**. These relationships indicate approximate mirror and C_2 sym-

Table 1. Deviations (Å) of Oxygen and Nitrogen Atoms from Receptor Planes and Angles (deg) between Least Squares Planes in Complexes of Receptors **3**, **4**, and **5** (Errors Given in Parentheses)

Plane 1: The Host Molecule

3 O(21) −0.18, N(14) 0.19, N(15) 0.03, N(16) −0.07, N(1) −0.13, O(18) 0.16
4 O(21) 0.03, N(15) −0.02, N(16) −0.05, N(1) 0.11, O(18) −0.07
5 O(21) 0.00, N(15) −0.01, N(16) 0.00, O(18) 0.00

	angles		
	3	4	5
between planes 1 and 2[a]	6.9(3)	25.6(2)	58.0(6)
between planes 1 and 3a[b]	29.6(2), 14.6(2)	5.7(2), 17.8(2)	4.8(6), 24.9(6)
between planes 3b[b] and 4[c]	22.6(1)	25.2(2)	
between planes 1 and 4	51.8(1)	20.6(2)	73.2(4)

[a] Plane 2: guest **A** (in cavity in complexes of **3** and **4**). [b] Planes 3a and 3b: carboxylates containing O(18), O(19) and O(21), O(22), respectively. [c] Plane 4: guest **B** (hydrogen bonded to carboxylate a in complexes of **3** and **4**).

Table 2. Torsion Angles (deg) in Receptor Molecules in the Crystal Structures of $\mathbf{3 \cdot 2CH_3CH_2NHC(NH_2)_2^+}$, $\mathbf{4 \cdot 2CH_3NHC(NH_2)_2^+}$, and $\mathbf{5 \cdot 2C(NH_2)_3^+}$ (Errors Given in Parentheses)

	receptor		
	3[a]	4[b]	5[c]
inner fragments:			
X(14)−C(13)−C(20)−O(21)	11.9(5)	15.0(7)	0.7(13)
X(14)−C(14A)−C(14B)−N(15)	19.0(5)	−16.9(6)	−13.9(10)
Y(1)−C(16B)−C(16A)−N(16)	−17.9(5)	−0.0(6)	16.6(10)
Y(1)−C(2)−C(17)−O(18)	26.5(6)	15.4(8)	11.5(15)
outer CCH_2CH_2C bridges:			
C(8A)−C(9)−C(10)−C(10A)	51.0(4)	−50.2(6)	−50.4(11)
C(4A)−C(5)−C(6)−C(6A)	−52.7(5)	−24.8(14)	53.2(11)

[a] X = Y= N. [b] X = C, Y = N. [c] X = Y = C.

metry, respectively. These variations suggest that such complexes are relatively conformationally mobile in solution.

Head-to-tail stacking interactions appear to be important in the crystallization of the complexes of all three receptors (Figure 5). In the *N*-ethylguanidinium complex of **3** (Figure 5A), the receptor molecules stack in pairs such that each naphthyridine nitrogen atom lies about 3.5 Å from an electronically complementary carbon atom of its neighbor. Receptor **4** forms similar dimers, which produce linear chains by stacking with other dimers with a larger offset between ring atoms (Figure 5B). In the complex of receptor **5**, a herringbone pattern is produced by continuously offset chains of head-to-tail stacked receptor molecules (Figure 5C).

All ^{1}H NMR signals of the dipotassium salts of **3**, **4**, and **5** in D_2O solutions exhibit downfield shifts upon dilution from 0.1 M to 0.1 mM, with chemical shift differences up to 1.6 ppm. This effect can be attributed to the aggregation of these molecules in aqueous solutions through hydrophobic interactions between nonpolar regions of the molecules that are remote from the charged carboxylate groups. Particularly, the aggregation is enhanced by the molecular planarity, which provides a large hydrophobic contact surface between stacked molecules. Similar head-to-tail stacking is observed in the crystal structures of the complexes (cf. Figure 5), but the proposed tail-to-tail stacking in water would not interfere with solvation of the polar, carboxylate headgroups. Addition of guanidinium or *N*-alkylguanidinium salts to aqueous solutions of **3**, **4**, or **5** significantly increases aggregation and upfield shifts, probably because the closer proximity of opposite charges in a complex makes the complex less polar than the dianionic receptor.

Table 3. Stability Constants (K_s, 10^3 M^{-1}) of 1:1 Complexes As Determined by ^{1}H NMR Titration in CD_3OD (25 °C)

	receptor		
guest	3	4	5
methylguanidine HCl	>100	3.9 ± 0.4	1.0 ± 0.1
creatine	14.5 ± 1.0	<1.0	<1.0
*N*α-acetyl-L-arginine	>100	1.2 ± 0.2	3.9 ± 2
L-arginine	>100	>100	1.1 + 0.2
L-lysine	29 ± 3	>100	1.1 ± 0.1
*N*α-acetyl-L-lysine	>100	>100	<1.0
6-aminocaproic acid	27 ± 10	>100	<1.0
1-propylamine HCl	>100	>100	1.6 ± 0.2
*N*ε-acetyl-L-lysine	1.2 ± 0.1	1.4 ± 0.3	1.4 ± 0.3

In less polar solvents, aggregation of these compounds should decrease, and determination of stability constants is expected to be easier than it was in water. Therefore, methanol-d_4 was chosen as a solvent for measurements of host−guest interactions. Dilute solutions of tetramethylammonium salts of receptors **3**, **4**, and **5** (0.1 mM) in CD_3OD were used for titrations. Various guests containing alkylguanidinium and alkylammonium groups were investigated for binding ability toward receptors **3**, **4**, and **5**. Downfield shift of the 1,8-naphthyridine protons of the host was observed, suggesting that the cationic guest binds first to the cavity. The calculated stability constants for 1:1 complexes are shown in Table 3. As expected, receptor **3** binds guanidinium-containing guests with very high affinity, and complexation shifts were not observed beyond 1:1 guest/host ratio. The 1:1 stability constants (K_s) of its complexes with *N*-methylguanidinium, arginine, and *N*α-acetyl-L-arginine in CD_3OD exceed 10^5 M^{-1} and cannot be measured accurately. However, the complex of **3** with lysine is less stable ($K_s = 29\,000$ M^{-1}). Thus, receptor **3** has a more than 3-fold selectivity toward binding arginine relative to lysine. Surprisingly, the affinity for *N*α-acetyl-L-lysine and propylammonium chloride was also found to be very high ($K_s \geq 10^5$ M^{-1}).

Receptor **4** displays a preference for binding primary alkylammonium guests, including L-lysine, *N*α-acetyl-L-lysine, 6-aminocaproic acid, and 1-propylamine ($K_s \geq 10^5$ M^{-1}). Among guanidinium guests, only arginine binds with very high affinity to **4**. The complex of **4** with *N*-methylguanidinium has a significantly smaller stability constant ($K_s = 3900$ M^{-1}). Apparently, the smaller cavity of **4** cannot comfortably accommodate the large guanidinium ion, but it accepts the smaller ammonium side chain of lysine. Creatine and *N*α-acetyl-L-arginine form moderate to weak complexes with **4**. The arginine complex of **4** is apparently further stabilized by electrostatic interaction between the α-ammonium group and one of the host carboxylates.

In the case of receptor **5**, the binding to almost all guests is weak. The cavity size of **5** is apparently too small to fit either ammonium or guanidinium ions. In general, receptor **5** does not discriminate between the cationic guests that were investigated. Apparently, its binding properties are dictated almost purely by attraction of opposite charges without stabilization from multiple hydrogen bonding.[22] Receptors **3** and **4** can form arrays of hydrogen bonds as well as electrostatic interactions with the investigated guests. These hydrogen bonds significantly

(22) As pointed out by a referee, the binding constants exhibitied by **5** are in line with results of studies done on the binding of carboxylate guests with guanidinium hosts: (a) Schiessl, P.; Schmidtchen, F. P. *Tetrahedron Lett.* **1993**, *34*, 2449−2452. (b) Schmuck, C. *Chem.-Eur. J.* **2000**, *6*, 709−718.

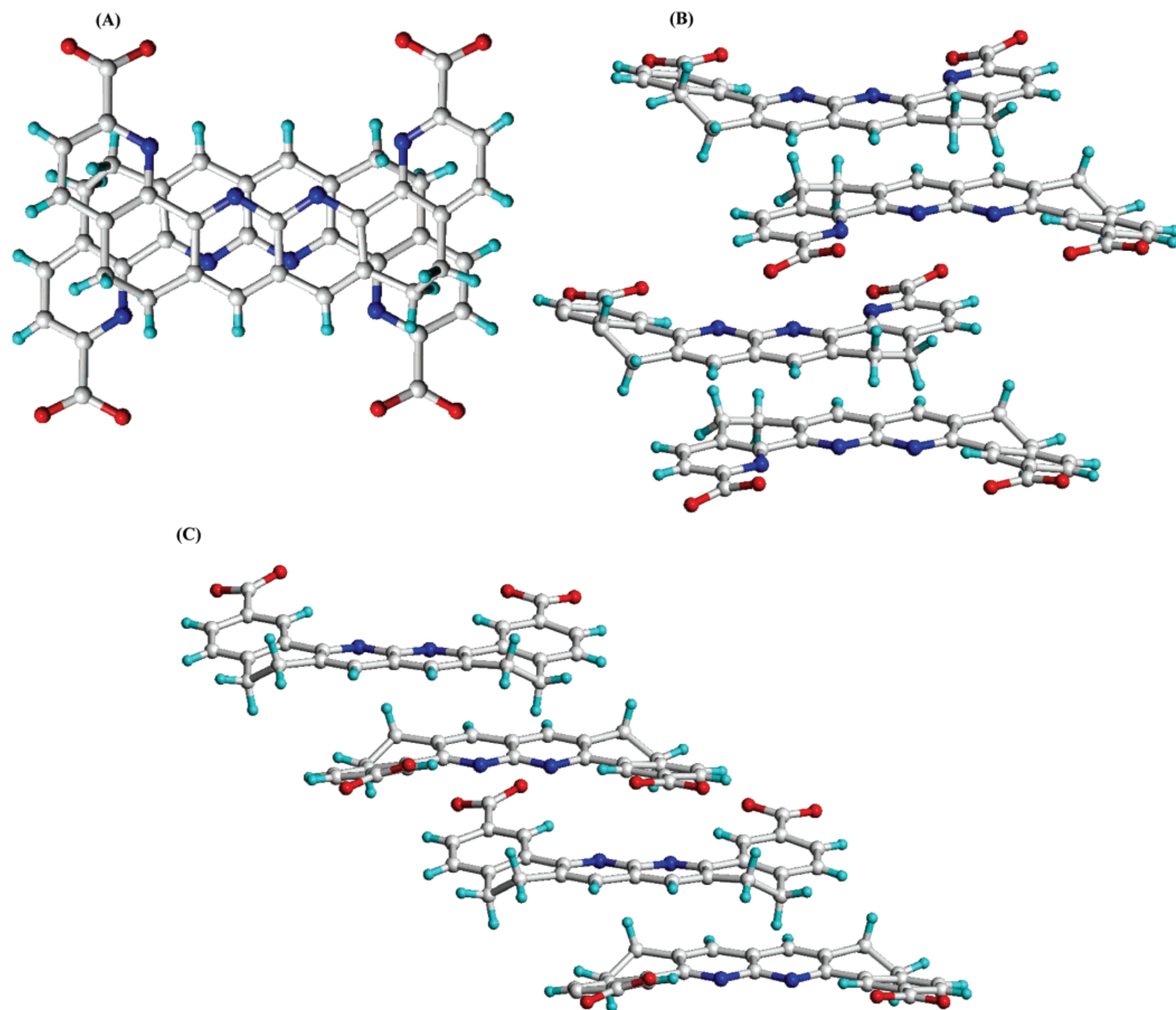


Figure 5. Stacking interactions between receptor molecules in the crystal structures of (A) **3·2CH$_3$CH$_2$NHC(NH$_2$)$_2$$^+$**; (B) **4·2CH$_3$NHC(NH$_2$)$_2$$^+$**; and (C) **5·2C(NH$_2$)$_3$$^+$**.

increase the stabilities of the complexes; thus it is clear that the composition of the cavity critically determines the molecular recognition properties of these receptors.

Receptor **4** tends to have higher affinity toward alkylammonium guests than alkylguanidinium, while receptor **3** binds alkylguanidinium molecules more strongly. This selectivity might be explained in terms of the energies of cavity solvation. The compact ammonium ion has a higher charge density than the planar guanidinium cation. Therefore, the alkylammonium ion is expected to form stronger attractive electrostatic interactions. The alkylguanidinium ion, on the other hand, is able to form more hydrogen bonds with the planar receptors **3** and **4**. The larger cavity of **3** is more highly solvated prior to binding than the smaller cavity of **4**. Upon replacement of multiple solvent molecules in the cavity of **3** with guests, the host desolvation energy is better compensated with multiple hydrogen bonds to the guanidinium ion than to the compact ammonium ion. In receptor **4**, the cavity is smaller and less solvated as compared to **3**. The guanidinium ion cannot form as energetically favorable hydrogen bonds with **4** as with **3**, because the steric interference of the hydrogen atom in the cavity of **4** causes the guanidinium ion to twist away from the plane of the cavity. This twist is shown in the crystal structure of the complex of **4** with the methylguanidinium ion (Figure 3). This increases the hydrogen-bond lengths and distorts their linear orientations and, as a result, decreases their stabilities. The binding motif of a smaller ammonium ion is not affected by the decrease in the cavity size of **4**, and its binding energy resulting from electrostatic attraction and some hydrogen bonding may be similar to that of **3**. Yet replacement of fewer solvent molecules from the cavity of **4** costs less energy than for **3**, which makes complexes of **4** with alkylammonium guests more stable.

The stabilities of complexes of **3** with zwitterionic guests also depend on the proximity of the negatively charged carboxylate groups of **3** and these guests. The comparison of the binding strengths of **3** with arginine and creatine demonstrates this dependence. Assuming the same binding motif of the guanidinium group with **3**, the carboxylate group of creatine should be less remote from the carboxylate of receptor **3** than that of arginine. Therefore, the repulsive interaction between carboxy-

late groups of creatine and **3** is enhanced, which results in a weaker complex with creatine (K_s = 14 500 M^{-1}) than with arginine or other guanidinium-containing guests ($K_s \geq 10^5$ M^{-1}). The close proximity between the α-ammonium and carboxylate groups of *N*ε-acetyl-L-lysine also leads to consistently weak complexes for all three receptors. A more detailed interpretation of the binding results presented in Table 3 would require additional thermodynamic data, such as enthalpic and entropic contributions to binding free energies.

Conclusions

The results presented here show that simple charge stabilization is not sufficient for strong binding of arginine and lysine side chains. High preorganization of hydrogen-bonding sites on a receptor and size complementarity between a receptor's cavity and a guest make a great contribution to the stabilization of a complex. Receptors **3** and **4** could lead to molecular probes that are specific for arginine and lysine residues in biomolecules. Simple modifications of these receptors, such as introducing groups for attachment to other host molecules, should produce useful building blocks for construction of receptors capable of recognizing particular peptides and proteins.

Experimental Section

Water used as a solvent for spectroscopic measurements was deionized and degassed by double distillation, followed by boiling immediately prior to use. Other solvents were purchased from Fisher Scientific Co. and were used as received. UV−visible spectra were recorded at 2 nm wavelength resolution using a cell of 1 cm path length. Chemical shifts in ^{1}H and ^{13}C NMR spectra were referenced to solvent resonances. Melting points are uncorrected. Elemental analyses were performed by Numega Resonance or Desert Analytics.

2-Methyl-5,6,7,8-tetrahydroquinoline (7).[18] A Morton flask equipped with a stirring bar was charged with 71.6 g (0.5 mol) of quinaldine (**6**) (Acros, 97%) and cooled in an ice bath. Next 200 mL of trifluoroacetic acid (Acros, 99%) and 7 g of palladium on activated carbon (Acros, 10% Pd) were added, and the flask was attached to a low-pressure hydrogenation apparatus. The entire system was evacuated at water aspirator pressure (20−30 mm) and filled with hydrogen gas. The evacuation/filling procedure was repeated three more times. The contents were stirred vigorously at room temperature over a period of 60 h, resulting in the consumption of 30.2 L (1.07 mol) of hydrogen. The catalyst was removed by vacuum filtration through Whatman number 2 filter paper and washed with 50 mL of water. The acidic solution was diluted with 250 mL of water, cooled by means of an ice bath, and basified to pH 10 with NaOH pellets. The product was extracted with hexanes (3 × 100 mL). The combined hexane extracts were washed with water (2 × 50 mL) and dried over Na_2SO_4. Solvent was evaporated under water aspirator pressure, and the remaining oil was dried overnight under vacuum (0.3 mm) to give 72.2 g (98%) of product as a colorless oil, pure by gas chromatography (column HP-1, injection temp 250 °C, column temp 80−250 °C, 10 °C/min, helium carrier gas). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.22 (d, *J* = 7.8 Hz, 1H), 6.86 (d, *J* = 7.8 Hz, 1H), 2.87 (t, *J* = 6.4 Hz, 2H), 2.70 (t, *J* = 6.4 Hz, 2H), 2.47 (s, 3H), 1.85 (m, 2H), 1.79 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.4, 155.0, 137.0, 128.8, 120.3, 32.5, 28.3, 24.0, 23.2, 22.8. EI-MS (70 eV) *m/z* (rel intensity): 147 (M^+, 100), 146 (100), 132 (36), 119 (48), 77 (19).

8-Benzylidene-2-styryl-5,6,7,8-tetrahydroquinoline (8). A 1 L round-bottomed flask equipped with a stirring bar, condenser, and gas inlet was charged with 72.2 g (0.49 mol) of 2-methyl-5,6,7,8-tetrahydroquinoline (**7**), 208 g (1.96 mol) of benzaldehyde (Acros, 98+%), and 201 g (1.97 mol) of acetic anhydride (Acros, 99.4%). The mixture was heated under reflux at 150−160 °C under nitrogen for 5 days. All volatile materials were then removed by rotary evaporation at 80 °C, and the remaining dark oil was fractionally distilled under vacuum. The fraction boiling at 140−160 °C (0.3 mm) was collected, mixed with 200 mL of hot ethanol, and stirred at 70 °C to form an emulsion. The stirred mixture was cooled to room temperature and seeded with crystals of the product to facilitate crystallization. The precipitate was collected by vacuum filtration, washed with 40 mL of ethanol, and dried under vacuum to give 126 g (79%) of pale yellow crystals of **8**, mp 76−80 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (s, 1H), 7.1−7.8 (m, 14H), 2.8−3.0 (m, 4H), 1.86 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.1, 152.4, 138.2, 137.3, 137.1, 135.7, 131.9, 131.3, 129.7 (2C), 128.7, 128.6 (2C), 128.1 (2C), 128.0, 127.3, 127.0 (2C), 126.7, 120.4, 29.7, 28.0, 22.9. EI-MS (70 eV) *m/z* (rel intensity): 324 (M + 1, 11), 323 (M, 59), 322 (100), 231 (19), 91 (20), 77 (12). Anal. Calcd for $C_{24}H_{21}N$: C, 89.13; H, 6.54; N, 4.33. Found: C, 88.92; H, 6.76; N, 4.64.

6,7-Dihydro-8(5*H*)-quinolinone-2-carboxaldehyde (9). To a 1 L round-bottomed flask were added 20.0 g (61.8 mmol) of 8-benzylidene-2-styryl-5,6,7,8-tetrahydroquinoline (**8**), 300 mL of CH_2Cl_2, and 100 mL of methanol. The solution was cooled to −77 °C by means of a dry ice/acetone bath, and a stream of O_3/O_2 was bubbled through the solution until it turned distinctly blue. The solution was purged by bubbling nitrogen gas for 1 h, and then 10 mL of dimethyl sulfide was added via syringe. The solution was allowed to warm to room temperature overnight, and then the solvents were removed by rotary evaporation below 25 °C, and the remaining oil was dried under vacuum (0.3−1.0 mm) for 11 h at room temperature. Next 400 mL of ether was added to the oily residue, and the mixture was cooled in a freezer (−15 to −20 °C) for 10 h. The white precipitate was collected by vacuum filtration, washed with 30 mL of ether, and dried under vacuum to give 7.06 g of **9**. Ether was removed from the mother liquor by rotary evaporation, and the remaining oil was dissolved in 300 mL of CH_2Cl_2. This solution was washed with 40 mL of water, dried over Na_2SO_4, and the solvent was evaporated. The remaining oil was dried under vacuum for 4 h, and then 300 mL of ether was added, and the mixture was cooled in a freezer for 10 h. The crystals were collected by vacuum filtration, washed with 20 mL of ether, and dried under vacuum to give 2.60 g of the second crop. Total yield: 9.7 g (89%), mp 164−168 °C. For microanalysis, a sample was recrystallized from ethyl acetate and dried under vacuum (0.1 mm, 25 °C, 2 d). ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.20 (s, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 7.8 Hz, 1H), 3.16 (t, *J* = 5.9 Hz, 2H), 2.88 (t, *J* = 6.4 Hz, 2H), 2.25 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.5, 192.7, 152.0, 148.4, 145.2, 139.0, 123.8, 39.6, 29.4, 22.2. EI-MS (70 eV) *m/z* (rel intensity): 175 (M^+, 28), 147 (79), 119 (100), 91 (53), 64 (45), 63 (32), 62 (26), 55 (50), 51 (27), 40 (29), 39 (45). Anal. Calcd for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.82; H, 5.02; N, 8.31.

6,7-Dihydro-8(5*H*)-quinolinone-2-carboxylic Acid (10). Method A. To a 500 mL Erlenmeyer flask were added 7.0 g (40 mmol) of 5,6-dihydro-8(7*H*)-quinolinone-2-carboxaldehyde (**9**) and 500 mL of acetone. The solution was vigorously stirred, and 13.0 mL of Jones' reagent (CrO_3 (Acros, 99.7%) 26.7 g (0.27 mol) dissolved in 23 mL of concentrated H_2SO_4 and 50 mL of water, and then diluted to 100 mL with water) was added dropwise over a period of 2 h at room temperature. The mixture was stirred for another 30 min, and 0.5 mL of 2-propanol was added. The acetone solution was decanted from the green precipitate, filtered through a sintered glass filter, and evaporated to dryness to give a solid organic residue. A solution of the green inorganic precipitate in 100 mL of 1 N aqueous sulfuric acid was extracted with CH_2Cl_2 (6 × 30 mL). The combined CH_2Cl_2 extracts were washed with 20 mL of water, dried over Na_2SO_4, and evaporated to dryness to give a second solid organic residue. Both organic residues were combined and dissolved in 15 mL of hot ethanol. This solution was cooled in a freezer (−15 to −20 °C) for 3 h, and white crystals

were collected by vacuum filtration, washed with 5 mL of ice cold ethanol, and dried under vacuum to give 5.4 g (70%) of **10**, mp 167−170 °C.

Method B. To a 500 mL Erlenmeyer flask equipped with a stirring bar were added 30.0 g (0.17 mol) of 6,7-dihydro-8(5*H*)-quinolinone-2-carboxaldehyde (**9**) and 34 g (0.74 mol) of formic acid (Acros, 98%). The mixture was stirred until a clear solution was formed. The mixture was then cooled by means of an ice bath, and 56 g of 30% H_2O_2 solution in water (Aldrich) was added dropwise over a period of 30 min (*Caution:* The reaction is highly exothermic in the beginning and may cause splashes). The resulting suspension was swirled every 3−5 min for a period of 1 h and left at room temperature for 2 h. Next 100 mL of water was added, and the mixture was swirled one more time and refrigerated for 24 h. The white precipitate was collected by vacuum filtration, washed with ice cold water (3 × 10 mL), and dried under vacuum (0.1 mm) over P_2O_5 over a period of 2 days to give 28.8 g (88%) of product **10**, mp 167−170 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.30 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 7.8 Hz, 1H), 3.15 (t, J = 6.1 Hz, 2H), 2.86 (t, J = 6.6 Hz, 2H), 2.26 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.9, 163.8, 146.4, 145.4, 144.9, 140.2, 126.4, 39.4, 29.1, 22.1. EI-MS (70 eV) *m/z* (rel intensity): 147 (M − 44, 80), 119 (25), 118 (43), 93 (48), 91 (100), 64 (23), 55 (29), 39 (28). Anal. Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.74; N, 7.33. Found: C, 64.66; H, 4.97; N, 7.87.

5,6-Dihydropyrimido[4,5-*b*][1,10]phenanthroline-2-carboxylic Acid, Potassium Salt (11). A 250 mL round-bottomed flask equipped with a stirring bar, condenser, and nitrogen gas inlet was charged with 2.00 g (16.2 mmol) of 4-amino-5-pyrimidinecarboxaldehyde,[20] 3.10 g (16.2 mmol) of 5,6-dihydro-8(7*H*)-quinolinone-2-carboxylic acid (**10**), and 100 mL of methanol. The mixture was heated to boiling, and then a solution of 15% KOH in methanol (w/v) was added dropwise over a period of 10 min to achieve pH 9, as measured by adding a drop of the solution to wet pH paper. The resulting mixture was heated at reflux under nitrogen for 24 h, resulting in the formation of a gray colored precipitate. The mixture was cooled to room temperature and refrigerated overnight. The precipitate was collected by vacuum filtration, washed with 10 mL of ethanol, and dried under vacuum (0.1 mm) to give 4.00 g (78%) of potassium salt **11**. For microanalysis, crude **11** was recrystallized from water and dried under vacuum (0.1 mm, 65 °C, 3 d), dec > 340 °C. ^{1}H NMR (300 MHz, D_2O): δ 9.02 (s, 1H), 8.96 (s, 1H), 7.84 (s, 1H), 7.43 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 2.94 (t, J = 7.1 Hz, 2H), 2.75 (t, J = 7.1 Hz, 2H). ^{13}C NMR (125.7 MHz, D_2O): δ 171.1, 160.5, 157.8, 156.3, 154.3, 151.5, 146.0, 138.3, 136.7, 135.3 (2C), 125.6, 118.4, 25.7, 25.0. FAB-MS *m/z* (rel intensity): 317.3 (M + 1, 100). Anal. Calcd for $C_{15}H_9N_4O_2K{\cdot}2H_2O$: C, 51.13; H, 3.71; N, 15.90. Found: C, 51.00; H, 3.40; N, 15.73.

5,6-Dihydro-9-amino-8-formyl[1,10]phenanthrolinecarboxaldehyde-2-carboxylic Acid (12). A 500 mL round-bottomed flask equipped with a stirring bar was charged with 4.00 g (12.6 mmol) of 5,6-dihydropyrimido[4,5-*b*][1,10]phenanthroline-2-carboxylic acid potassium salt (**11**) and 200 mL of water. The solution was stirred, and 15% aqueous HCl solution was added dropwise until the pH of the mixture reached 3−4 (approximately 2.5 mL of acid was required). A yellow precipitate formed, and the resulting suspension was heated under reflux for 10 h. The mixture was cooled to room temperature, saturated with NaCl (56 g), and transferred into a liquid−liquid continuous extractor attached to a 1 L round-bottomed flask containing 500 mL of $EtOH/CH_2Cl_2$, 1:7 (v/v). The suspension was extracted over a period of 4 days. The organic extract was then dried over Na_2SO_4, filtered through filter paper, and the solvent was removed by rotary evaporation. The remaining yellow solid was dried under vacuum (0.1 mm) to give 2.72 g (80%) of aminoaldehyde **12**, which was pure by ^{1}H NMR spectroscopy and could be used for the next step. For microanalysis, **12** was recrystallized from water and dried overnight under vacuum (0.1 mm) to give yellow, rod-shaped crystals, mp 364−366 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.91 (s, 1H), 8.01 (d, J = 7.8 Hz, 1H), 8.00 (s, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.68 (s, 2H), 3.02 (t, J = 6.8 Hz, 2H), 2.87 (t, J = 6.8 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 192.9, 166.2, 157.5, 154.5, 150.5, 147.5, 143.9, 139.4, 137.1, 125.0, 122.5, 113.0, 27.4, 25.2. FAB-MS *m/z* (rel intensity): 270.3 (M + 1, 100). Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.28; H, 4.15; N, 15.67.

5,6,9,10-Tetrahydro[1,10]phenanthrolino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic Acid, Potassium Salt ($3{\cdot}2K^+$). A 500 mL round-bottomed flask equipped with a stirring bar, condenser, and nitrogen gas inlet was charged with 1.00 g (3.71 mmol) of 5,6-dihydro-9-amino-8-formyl[1,10]phenanthrolinecarboxaldehyde-2-carboxylic acid (**12**), 0.71 g (3.71 mmol) of 5,6-dihydro-8(7*H*)-quinolinone-2-carboxylic acid (**10**), and 200 mL of ethanol. The mixture was heated to boiling, and then a solution of KOH in methanol was added dropwise over a period of 10 min to achieve pH 9 (approximately 7.0 mL of 1.06 N KOH solution was required). The resulting mixture was heated at reflux under nitrogen for 3 days, resulting in the formation of a gray colored precipitate. One-half of the solvent was removed by rotary evaporation, and 200 mL of ether was added to the mixture. The resulting gellike precipitate was collected by vacuum filtration, washed with 10 mL of ethanol, and dried under vacuum (0.1 mm) to give 1.64 g (88%) of potassium salt $3{\cdot}2K^+$, which was pure by ^{1}H NMR spectroscopy. For microanalysis, a sample of $3{\cdot}2K^+$ was dissolved in hot water, and hot ethanol was added until the solution remained cloudy. After storage in a refrigerator, the precipitate was collected by vacuum filtration and dried under vacuum (0.1 mm, 60 °C, 3 d), dec > 340 °C. ^{1}H NMR (300 MHz, D_2O): δ 7.85 (d, J = 7.8 Hz, 2H), 7.66 (d, J = 7.8 Hz, 2H), 7.54 (s, 2H), 2.90 (s, 8H). ^{13}C NMR (75 MHz, D_2O): δ 172.1 (2C), 153.8 (2C), 153.1, 152.4 (2C), 148.5 (2C), 137.9 (2C), 137.8 (2C), 135.1 (2C), 133.3 (2C), 125.2 (2C), 122.5, 26.3 (2C), 26.2 (2C). IR (KBr): 3382 (br), 2929 (w), 2842 (w), 1612 (s), 1559 (s), 1383 (s), 1253 (w), 1216 (w), 1159 (w), 1097 (w), 1072 (w), 1025 (w), 926 (w), 869 (w), 829 (w), 803 (w), 778 (m), 745 (w), 708 (w), 698 (w), 631 (w) cm^{-1}. FAB-MS *m/z* (rel intensity): 501 (M + 1, 38), 499 (41), 498 (100). Anal. Calcd for $C_{24}H_{14}K_2N_4O_4{\cdot}2H_2O$: C, 53.71; H, 3.38; N, 10.44. Found: C, 53.30; H, 3.78; N, 10.19.

5,6,9,10-Tetrahydro[1,10]phenanthrolino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic Acid ($3{\cdot}2H^+$). To a 50 mL Erlenmeyer flask equipped with a stirring bar were added 1.64 g (3.27 mmol) of potassium salt $3{\cdot}2K^+$ and 20 mL of water. The solution was stirred, and 15% aqueous HCl solution was added dropwise until the pH of the mixture reached 3−4. The resulting yellow precipitate was collected by vacuum filtration, washed with 10 mL of water, and dried under vacuum (0.1 mm) to give 1.34 g (97%) of crude diacid $3{\cdot}2H^+$. The crude product was dissolved in 2.5 L of $CHCl_3$/MeOH (3:1, v/v), which was heated under reflux over a period of 2 days. The resulting mixture was filtered through Whatman No. 5 filter paper, and the filtrate was slowly concentrated to a volume of 60 mL by evaporation. The precipitated solid was collected by vacuum filtration, washed with 10 mL of methanol, and dried under vacuum (0.1 mm) to give 0.70 g of a yellow product ($3{\cdot}2H^+$), mp 240−242 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.32 (s, 2H), 8.06 (d, J = 8.3 Hz, 2H), 7.98 (d, J = 8.3 Hz, 2H), 3.18 (m, 4H), 3.16 (m, 4H). FAB-MS *m/z* (rel intensity): 425.4 (M + 1, 100), 381.3 (46), 337.4 (36). Anal. Calcd for $C_{24}H_{16}N_4O_4{\cdot}H_2O{\cdot}CH_3OH$: C, 63.29; H, 4.67; N, 11.81. Found: C, 63.58; H, 4.36; N, 12.05.

Complex of 5,6,9,10-Tetrahydro[1,10]phenanthrolino[2,3-*b*][1,10]-phenanthroline-2,13-dicarboxylate with Ethylguanidinium ($3{\cdot}2CH_3CH_2NHC(NH_2)_2{}^+$). A 50 mL round-bottomed flask was charged with 100 mg (0.186 mmol) of 5,6,9,10-tetrahydro[1,10]phenanthrolino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic acid, dipotassium salt ($3{\cdot}2K^+$), and 3 mL of distilled water. The mixture was heated slightly to form a clear solution, and then a solution of 50.7 mg (0.276 mmol) of ethylguanidine sulfate (Aldrich, 98%) in 3 mL of water was added into the flask. A pale yellow precipitate formed after 30−60 s. Next 15 mL of distilled water was added, and the mixture was heated to

boiling until all of the solid dissolved. The hot solution was let to cool to room temperature and stored undisturbed for 2 days. Some of the resulting single crystals were removed with some mother liquor to prevent the loss of solvent from the crystal lattice and were used for X-ray analysis. The rest of the crystals were collected by vacuum filtration, washed with 5 mL of cold water, and dried under vacuum (0.1 mm) at 76 °C for 3 days. Anal. Calcd for $C_{30}H_{34}N_{10}O_4$·1.5 H_2O: C, 57.58; H, 5.96; N, 22.38. Found: C, 57.47; H, 6.10; N, 22.54.

5,6-Dihydrobenzo[*h*]pyrimido[4,5-*b*]quinoline-2-carboxylic Acid (14). A 500 mL round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and nitrogen gas inlet was charged with 6.5 g (52.6 mmol) of 4-amino-5-pyrimidinecarboxaldehyde,[20] 10.0 g (52.6 mmol) of 7-carboxy-1-tetralone (**13**),[21] and 300 mL of HPLC grade methanol. The contents were heated to boiling, and a solution of KOH in methanol was added dropwise until the pH reached 10; about 5.7 mL of 1.05 N KOH solution in methanol was required. The reaction mixture was stirred under nitrogen at reflux temperature for 3 days. The solution was then concentrated to 150 mL by boiling and acidified to pH 6 with acetic acid. The resulting mixture was refrigerated overnight. A yellow precipitate formed, which was collected by vacuum filtration and dried under vacuum (0.1 mm) to give 10.3 g of 5,6-dihydrobenzo[*h*]pyrimido[4,5-*b*]quinoline-2-carboxylic acid (**14**). The mother liquor was concentrated by rotary evaporation to a volume of 50 mL, and then 100 mL of water was added, and the mixture was refrigerated overnight. The deposited precipitate was collected by vacuum filtration and dried under vacuum (0.1 mm) to give the second crop of yellow solid (1.6 g). The combined crops of **14** (11.9 g, 82%) were pure by ^{1}H NMR and were used without further purification in the next step. For microanalysis, crude **14** was recrystallized from a MeOH/AcOH (3:5, v/v) mixture and dried under vacuum (0.1 mm, 90 °C) for 5 days, mp 356−360 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.2−13.0 (br s, 1H), 9.63 (s, 1H), 9.43 (s, 1H), 9.07 (d, J = 1.5 Hz, 1H), 8.49 (s, 1H), 8.05 (dd, ^3J = 7.8 Hz, ^4J = 2.0 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 3.09−3.22 (m, 4H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.0, 161.4, 159.1, 157.9, 156.6, 145.2, 135.1, 133.2 (2C), 131.8, 130.1, 128.9, 127.5, 119.2, 27.1, 27.0. FAB-MS m/z (rel intensity): 278.3 (M + 1, 100). Anal. Calcd for $C_{16}H_{11}N_3O_2$·0.2H_2O: C, 68.42; H, 4.09; N, 14.96. Found: C, 68.30; H, 4.05; N, 14.86.

2-Amino-5,6-dihydro-3-formylbenzo[*h*]quinoline-9-carboxylic Acid (15). A 500 mL round-bottomed flask, equipped with a magnetic stirring bar and reflux condenser, was charged with 9.24 g (33.3 mmol) of 5,6-dihydrobenzo[*h*]pyrimido[4,5-*b*]quinoline-2-carboxylic acid (**14**), 250 mL of water, and 2 mL of concentrated aqueous HCl solution. The suspension was heated to boiling and heated at reflux with stirring over a period of 24 h. The mixture was then refrigerated overnight. The resulting yellow solid was collected by vacuum filtration, washed with water (2 × 20 mL), and dried under vacuum (0.1 mm) to give 8.84 g (98%) of 2-amino-5,6-dihydro-3-formylbenzo[*h*]quinoline-9-carboxylic acid (**15**). For microanalysis, crude **15** was recrystallized from a H_2O/AcOH (3:5 v/v) mixture and dried under vacuum (0.1 mm, 95 °C, 5 d), dec > 230 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.0 (br, 1H), 9.85 (s, 1H), 8.81 (d, J = 1.5 Hz, 1H), 7.94 (dd, ^3J = 7.8 Hz, ^4J = 2.0 Hz, 1H), 7.93 (s, 1H), 7.5−7.7 (br, 2H), 7.44 (d, J = 7.8 Hz, 1H), 2.97 (t, J = 6.6 Hz, 2H), 2.85 (t, J = 6.8 Hz, 2H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 192.7, 167.2, 157.3, 154.8, 144.6, 143.9, 133.4, 130.8, 129.5, 128.5, 126.6, 119.9, 112.5, 27.8, 25.5. FAB-MS m/z (rel intensity): 269.3 (M + 1, 100). Anal. Calcd for $C_{15}H_{12}N_2O_3$·0.2H_2O: C, 66.27; H, 4.60; N, 10.30. Found: C, 66.28; H, 4.41; N, 10.38.

5,6,9,10-Tetrahydrodinaphtho[1,2-*b*:2′,1′-*g*][1,8]naphthyridine-2,13-dicarboxylic Acid, Dipotassium Salt (5·2K$^+$). A 250 mL round-bottomed flask, equipped with a magnetic stirring bar, reflux condenser, and nitrogen gas inlet, was charged with 0.55 g (2.87 mmol) of 7-carboxy-1-tetralone (**13**),[21] 0.77 g (2.87 mmol) of 2-amino-5,6-dihydro-3-formylbenzo[*h*]quinoline-9-carboxylic acid (**15**), and 50 mL of ethanol. The mixture was stirred and heated to boiling under nitrogen. A solution of KOH in methanol was then added dropwise until the pH reached 10; about 6 mL of 1.05 N methanolic KOH was required. Next 100 mL of butanol was added, and the mixture was heated at reflux under nitrogen for 1 week. The solution was cooled to room temperature and refrigerated overnight. The resulting yellow-green precipitate was collected by vacuum filtration, washed with 50 mL of cold anhydrous ethanol, and dried under vacuum (0.1 mm) to give 1.24 g (87%) of crude dipotassium salt **5·2K$^+$**. Of this material, 0.81 g was dissolved in 7 mL of water, and this solution was filtered through filter paper. The solution was heated to 80 °C, and ethanol was added dropwise until the solution became cloudy. Two drops of water were added to dissolve the cloudy precipitate, and the solution was refrigerated overnight. The resulting yellow-greenish solid was collected by vacuum filtration and dried at 80 °C under vacuum (0.1 mm) for 2.5 days to give 0.27 g (33% recovery) of **5·2K$^+$**. ^{1}H NMR (300 MHz, D_2O, 30 mM): δ 8.61 (s, 2H), 7.84 (d, J = 7.8 Hz, 2H), 7.48 (s, 2H), 7.22 (d, J = 7.8 Hz, 2H), 2.70 (m, 8H). IR (KBr): 3160 (bw), 2937 (m), 1712 (s), 1676 (m), 1631 (w), 1604 (m), 1573 (w), 1560 (w), 1541 (w), 1479 (m), 1466 (m), 1439 (w), 1388 (m), 1370 (m), 1314 (w), 287 (w), 1217 (m), 1175 (w), 1119 (w), 1106 (w), 1069 (w), 1025 (m), 772 (m), 735 (w), 706 (w), 624 (w) cm^{-1}. Anal. Calcd for $C_{26}H_{16}K_2N_2O_4$·2H_2O: C, 58.41; H, 3.77; N, 5.24. Found: C, 58.34; H, 3.42; N, 5.11.

5,6,9,10-Tetrahydrodinaphtho[1,2-*b*:2′,1′-*g*][1,8]naphthyridine-2,13-dicarboxylic Acid (5·2H$^+$). To a 25 mL Erlenmeyer flask equipped with a stirring bar were added 1.64 g (3.27 mmol) of potassium salt **5·2K$^+$** and 10 mL of water. The solution was stirred, and 15% aqueous HCl solution was added dropwise until the pH of the mixture reached 4−3. The yellow precipitate was then collected by vacuum filtration, washed with 5 mL of water, and dried under vacuum (0.1 mm) over P_2O_5 for 2 days to give 0.58 g of crude diacid **5·2H$^+$**. The crude product was dissolved in 10 mL of boiling DMSO, and 40 mL of water was added to the hot solution. The mixture was cooled to room temperature, and the precipitated solid was collected by vacuum filtration, washed with water (5 × 10 mL), then with methanol (5 × 10 mL), and dried under vacuum (0.1 mm) at 90 °C for 3 days to give 0.47 g (73%) of yellow product **5·2H$^+$**, dec > 400 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.1 (br s, 2H), 9.10 (s, 2H), 8.22 (s, 2H), 7.97 (d, J = 7.8 Hz, 2H), 7.49 (d, J = 7.8 Hz, 2H), 3.1 (m, 8H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.2, 154.1, 154.0, 144.3, 134.6, 133.9, 131.0, 130.6, 129.7, 128.6, 126.9, 122.1, 27.4, 27.1. FAB-MS m/z (rel intensity): 423.4 (M + 1, 100), 278.3 (15). Anal. Calcd for $C_{26}H_{18}N_2O_4$·H_2O: C, 70.90; H, 4.58; N, 6.36. Found: C, 71.04; H, 4.47; N, 6.50.

Complex of 5,6,9,10-Tetrahydrodinaphtho[1,2-*b*:2′,1′-*g*][1,8]-naphthyridine-2,13-dicarboxylate with Guanidinium (5·2C(NH$_2$)$_3$$^+$). A 12 mL centifuge tube was charged with 50 mg (0.10 mmol) of 5,6,9,10-tetrahydrodinaphtho[1,2-*b*:2′,1′-*g*][1,8]naphthyridine-2,13-dicarboxylic acid, dipotassium salt (**5·2K$^+$**), and 2 mL of distilled water. The mixture was heated slightly to form a clear solution. A solution of 38 mg (0.40 mmol) of guanidine hydrochloride (Aldrich, 98%) in 2 mL of water was then added. The solutions were thoroughly mixed and allowed to stand at 25 °C for 5 h. A pale yellow suspension formed, which was separated by centrifugation, followed by decantation of the clear supernatant solution. A solution of the precipitate in 10 mL of boiling water was slowly cooled to room temperature over a period of 12 h, and was then allowed to stand at 25 °C. Small crystals formed over a period of 3 d. For microanalysis, some of the crystals were withdrawn and dried under vacuum (0.1 mm, 76 °C, 3 d). Anal. Calcd for $C_{28}H_{28}N_8O_4$·0.5H_2O: C, 61.19; H, 5.32; N, 20.39. Found: C, 61.10; H, 5.21; N, 20.26. For X-ray diffraction studies, several small seed crystals were withdrawn and immediately (to prevent desolvation) placed into the crystallization chamber, which was filled with water, of an apparatus for growing crystals by slow diffusion of the reacting solutions.[23] One arm of the apparatus was charged with a solution of

(23) Khasanov, A. B. *Aldrichimica Acta* **1999**, *32*, 74−90.

Table 4. Crystal Data and Structure Refinement for Complexes of Receptors **3**, **4**, and **5**

complex of:	3	4	5
empirical formula	$C_{30}H_{42}N_{10}O_8$	$C_{30}H_{35}N_9O_5$	$C_{28}H_{36}N_8O_8$
formula weight	670.74	601.67	612.65
temperature/K	293(2)	293(2)	293(2)
wavelength/Å	0.71073	0.71073	0.71073
crystal system, space group	triclinic, *P*-1	monoclinic, $P2_1/n$	orthorhombic, *C2cb*
unit cell dimensions			
a/Å	10.606(12)	13.370(15)	21.20(2)
b/Å	11.412(10)	7.690(10)	27.35(3)
c/Å	14.128(15)	29.78(4)	10.808(12)
α/°	70.56(1)	(90)	(90)
β/°	79.36(1)	99.50(1)	(90)
γ/°	88.51(1)	(90)	(90)
volume/Å^3	1639(3)	3020(6)	6267(12)
Z, calculated density/ Mgm^{-3}	2, 1.359	4, 1.323	8, 1.299
reflections collected/ unique	5828	6823/4027	10 648/2944
data/restraints/ parameters	5828/0/436	4027/0/399	2944/0/416
final *R* indices [$I > 2\sigma(I)$]	R1 0.0900,	0.0987	0.1058
	wR2 0.2582	0.2712	0.2794
R indices (all data)	R1 0.1563	0.1668	0.1318
	wR2 0.3002	0.3179	0.3017
largest diff. peak and hole/e Å^{-3}	0.587, −0.367	0.464, −0.336	0.549, −0.268

50 mg (0.10 mmol) of 5,6,9,10-tetrahydrodinaphtho-[1,2-*b*:2′,1′-*g*][1,8]-naphthyridine-2,13-dicarboxylic acid and dipotassium salt (**5·2K**$^+$) in 3 mL of distilled water. The other arm was charged with a solution of 28 mg (0.30 mmol) of guanidine hydrochloride (Aldrich, 98%) in 3 mL of water. The apparatus was left undisturbed over 2 weeks to get X-ray quality crystals, which were withdrawn and stored with some of the mother liquor to prevent desolvation.

5,6,9,10-Tetrahydrobenzo[7,8]quino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic Acid, Dipotassium Salt (4·2K$^+$). A 250 mL round-bottomed flask equipped with a magnetic stirring bar, reflux condenser, and gas inlet was charged with 0.55 g (2.9 mmol) of 6,7-dihydro-8(5*H*)-quinolinone-2-carboxylic acid (**10**), 0.77 g (2.9 mmol) of 2-amino-5,6-dihydro-3-formylbenzo[*h*]quinoline-9-carboxylic acid (**15**), and 130 mL of ethanol. The mixture was stirred and heated to boiling under nitrogen. A solution of KOH in methanol was then added dropwise until the pH reached 10; about 2.5 mL of 1.05 N methanolic KOH was required. The resulting clear solution was stirred at reflux temperature under nitrogen for 5 days. The solution was then cooled to room temperature, and 100 mL of ether was added to cause precipitation. The mixture was refrigerated overnight. The precipitate was collected by vacuum filtration and dried under vacuum (0.1 mm) to give 1.5 g of crude dipotassium salt **4·2K**$^+$. A solution of the crude product in 10 mL of hot water was heated to 80 °C, and 20 mL of ethanol was added dropwise. The mixture was then refrigerated overnight. The resulting yellow-greenish solid was collected by vacuum filtration and dried at 80 °C under vacuum (0.1 mm) for 2 days to give 1.05 g (73%) of **4·2K**$^+$. ^{1}H NMR (300 MHz, D_2O, 30 mM): δ 8.65 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.17 (s, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.12 (s, 1H), 2.76 (s, 8H). IR (KBr): 3422 (bw), 2938 (m), 1709 (s), 1676 (s), 1608 (s), 1573 (w), 1478 (m), 1411 (m), 1374 (w), 1352 (w), 1312 (m), 1267 (m), 1223 (m), 1175 (w), 1131 (w), 912 (w), 809 (w), 770 (m), 736 (w), 707 (w), 618 (w), 577 (w) cm^{-1}. Anal. Calcd for $C_{25}H_{15}N_3O_4K_2\cdot H_2O$: C, 58.01; H, 3.31; N, 8.12. Found: C, 58.31; H, 3.13; N, 8.17.

5,6,9,10-Tetrahydrobenzo[7,8]quino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic Acid (4·2H$^+$). To a 25 mL Erlenmeyer flask equipped with a stirring bar were added 0.54 g (1.06 mmol) of potassium salt **4·2K**$^+$ and 10 mL of water. The solution was stirred, and 15% aqueous HCl solution was added dropwise until the pH of the mixture reached 4−3. The yellow precipitate was then collected by vacuum filtration, washed with 5 mL of water, and dried under vacuum (0.1 mm) over P_2O_5 for 2 days to give 0.41 g of crude diacid **4·2H**$^+$. The crude product was stirred and heated at reflux temperature in 280 mL of $CHCl_3$/MeOH (3:1, v/v) for a period of 2 days. The mixture was then concentrated by rotary evaporation to a volume of 100 mL and refrigerated overnight. The precipitate was collected by vacuum filtration, washed with 10 mL of methanol, and dried at 90 °C under vacuum (0.1 mm) for 3 days to give 0.33 g (73%) of yellow product **4·2H**$^+$, mp 264−266 °C (dec). ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.1 (br s, 2H), 9.16 (s, 1H), 8.32 (s, 1H), 8.29 (s, 1H), 8.09 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 3.2 (m, 8H). ^{13}C NMR (125.7 MHz, DMSO-d_6): δ 167.1, 166.4, 154.4, 154.2, 153.7, 150.8, 147.8, 144.4, 139.3, 137.5, 134.8, 134.7, 134.0, 133.0, 131.8, 130.8, 129.9, 128.7, 127.0, 125.1, 122.5, 27.4, 27.2, 27.0, 26.8. FAB-MS *m/z* (rel intensity): 424.3 (M + 1, 100), 380.3 (33). Anal. Calcd for $C_{25}H_{17}N_3O_4\cdot CH_3OH\cdot 0.5H_2O$: C, 67.24; H, 4.77; N, 9.05. Found: C, 67.54; H, 4.70; N, 9.25.

Complex of 5,6,9,10-Tetrahydro[7,8]quino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylate with Methylguanidinium (4·2$CH_3NHC(NH_2)_2^+$). A 25 mL round-bottom flask was charged with 30.7 mg (0.066 mmol) of 5,6,9,10-tetrahydro[7,8]quino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic acid (**4·2H**$^+$) and 5 mL of methanol. Next 56 μL of a 25.4% solution of tetramethylammonium hydroxide in methanol (Aldrich) was added, and the mixture was stirred to form a clear solution. A solution of 30 mg (0.27 mmol) of methylguanidine hydrochloride (Aldrich, 98%) in 1 mL of methanol was then added, and the mixture was stirred briefly and then allowed to stand at 25 °C. Small crystals formed over a period of 3 d. Several small crystals were withdrawn and immediately (to prevent desolvation) placed into the crystallization chamber of an apparatus for growing crystals by slow diffusion of the reacting solutions,[23] which was then filled with methanol. One arm of the apparatus was charged with a solution of 31 mg (0.067 mmol) of 5,6,9,10-tetrahydro[7,8]quino[2,3-*b*][1,10]phenanthroline-2,13-dicarboxylic acid (**4·2H**$^+$) and 58 μL of a 25.4% methanolic solution of tetramethylammonium hydroxide (Aldrich) in 3 mL of methanol. The other arm was charged with a solution of 32 mg (0.29 mmol) of methylguanidine hydrochloride (Aldrich, 98%) in 3 mL of methanol. The apparatus was left undisturbed over 2 weeks to obtain X-ray quality crystals, which were withdrawn and stored with some of the mother liquor (to prevent desolvation). For microanalysis, some of the crystals were dried under vacuum (0.1 mm, 90 °C, 5 d). Anal. Calcd for $C_{29}H_{31}N_9O_4\cdot 1.5H_2O$: C, 58.38; H, 5.74; N, 21.13. Found: C, 58.66; H, 5.43; N, 21.16.

Crystallographic Structure Determination. Crystal data were collected with Mo Kα radiation using the MARresearch Image Plate System. The crystals were positioned at 70 mm from the Image Plate. A total of 95 frames were measured at 2° intervals with a counting time of 2 min. Data analysis was carried out with the XDS program.[24] The structures were solved using direct methods with the Shelx86 program.[25] The complex of receptor **3** contained four solvent water molecules, each refined with full occupancy. In the complex of **4**, one guest molecule was disordered with the methyl group occupying two possible sites. Associated with this disorder were two superimposed methanol molecules, each compatible with one position of the methyl group. The two possible sets of disordered atoms were refined with occupancy factors of x and 1 − x, respectively. The value of x in the refined structure was 0.64(1). In the complex of **5**, six solvent water molecules were found, of which two (O(500) and O(501)) were refined with full occupancy and the others with 50% occupancy. In all three structures, all non-hydrogen atoms were refined anistropically (apart from disordered atoms). Hydrogen atoms bonded to carbon and nitrogen

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Table 5. Hydrogen-Bond Distances (Å) in Complexes of Receptors **3**, **4**, and **5**

	3	4
O(18)···N(36)	2.829(6)	2.791(8)
O(18)···O(54)	2.710(7)	
O(18)···O(100)		2.995(11)
O(19)···O(100)		3.046(12)
O(18)···O(200)		2.563(15)
N(33)···O(200)		3.380(14)
O(21)···N(35)	2.871(5)	2.926(7)
O(21)···N(45)	2.912(5)	2.844(7)
O(22)···N(46)	2.848(6)	2.837(6)
N(35)···N(15)	3.060(5)	3.155(8)
N(36)···N(16)	2.999(4)	3.050(8)
In **3**:		
N(33)···O(54)	3.037(7)	
N(43)···O(52)	2.886(7)	
N(46)···O(51)	2.890(7)	
O(51)···O(52)	3.167(10)	
O(53)···O(52)	2.790(8)	
O(18)···O(52) \$1	2.882(8)	
O(19)···O(53) \$2	2.753(6)	
O(19)···O(51) \$3	2.936(7)	
O(10)···N(45) \$4	2.932(7)	
symmetry elements:	\$1 $2 - x, -y, 2 - z$; \$2 $x, y, 1 + z$; \$3 $x - 1, y + 1, z$; \$4 $1 - x, -y, 2 - z$	
In **4**:		
O(22···N(43) \$1	2.782(6)	
O(19)···N(43) \$2	2.771(6)	
O(19)···N(45) \$2	2.931(6)	
N(33)···O(54) \$2	2.998(14)	
symmetry elements:	\$1 $1.5 - x, 0.5 + y, 0.5 - z$; \$2 $1 - x, -1 - y, -z$	

	5
O(21)···N(46)	2.924(12)
O(21)···N(45)	3.245(19)
O(21)···O(502)	2.79(2)
O(21)···O(500) \$9	2.795(10)
O(22)···O(500) \$1	2.825(11)
O(22)···N(35) \$7	2.912(15)
O(22)···N(33) \$7	2.974(16)
O(19)···N(43) \$8	2.823(13)
O(19)···O(505) \$8	2.90(3)
O(19)···N(35) \$4	3.037(16)
O(19)···O(505) \$5	2.90(3)
O(18)···O(503)	2.749(18)
O(18)···O(504)	2.95(2)
O(18)···N(36) \$4	2.994(19)
N(16)···O(501)	2.951(12)
N(15)···O(501)	3.283(12)
O(500)···N(43) \$2	2.925(13)
O(500)···O(504) \$2	3.06(3)
O(501)···O(502)	2.78(3)
O(501)···N(36)	3.084(18)
O(501)···N(33)	3.081(17)
O(501)···O(503)	3.25(4)
O(502)···N(36)	2.95(3)
O(502)···O(504) \$6	3.12(3)
O(503)···N(46)	2.81(2)
O(504)···O(504) \$3	2.53(4)
O(505)···O(505) \$4	2.72(7)
O(505)···N(45)	2.86(3)
O(505)···N(36) \$7	3.02(4)
symmetry elements:	\$1 $x - 1/2, y, -x + 3/2$;
\$2 $x + 1/2, y, -z + 1/2$;	\$3 $x, -y, -z - 1$;
\$4 $x, -y, -z$;	\$5 $x + 1/2, -y, z - 1/2$;
\$6 $x, y, 1 + z$;	\$7 $x - 1/2, y, -z + 1/2$;
\$8 $x + 1/2, y, -z - 1/2$;	\$9 $x - 1/2, -y, z - 1/2$

were included in calculated positions with thermal parameters set at 1.2 times those of the hydrogen atoms to which they were attached. Hydrogen atoms bonded to the water molecules could not be located and were not included. The structures were refined on F^2 using Shelx1.[26] Crystal data, data collection parameters, and refinement details are given in Table 4. Distances between hydrogen-bonded atoms and symmetry transformations are given in Table 5.

Binding Studies. ^{1}H NMR spectra were measured on a VARIAN UNITY spectrometer operating at 500 MHz. Chemical shifts were referenced to the solvent resonances. Stability constants (K_s) were calculated by fitting the equation described by Wilcox[27] to the experimental data by means of the program "Sigma Plot".

(26) Shelx1. Sheldrick, G. M. Program for crystal structure refinement; University of Gottingen, 1993.
(27) Wilcox, C. S. In *Frontiers of Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds; VCH: New York, 1991; pp 123−144.

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Supporting Information Available: Tables of crystal data, structure solution and refinement, atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for complexes of receptors **3**, **4**, and **5**, as well as tables of ^{1}H NMR data used for calculation of binding constants in methanol (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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