

## Binding biomolecules with designed, hydrogen-bonding receptors

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### Abstract

An approach to the design of artificial receptors is described involving the fusion of several six-membered rings, preorganizing nitrogen and oxygen hydrogen bonding sites. A hexagonal lattice chemosensor extracts the blood metabolite creatinine into water and acts as a chromogenic reagent for this clinical analyte. Another receptor binds urea via six hydrogen bonds, forming a complex that is stable even in DMSO. A water-soluble receptor has now been synthesized that recognizes the guanidinium sidechain of arginine in aqueous media.

### INTRODUCTION

Artificial receptors can be tailored to bind small, biologically-relevant molecules, such as metabolites, amino acids and nucleotides. Our host compounds are not intended to serve as models of natural receptors, carriers or enzymes. Instead, we hope to probe fundamental aspects of molecular recognition through design and synthesis of completely artificial receptors, followed by examination of the structures and stabilities of their complexes. Targeting guest molecules of biological importance also invites opportunities for practical applications of the hosts, e.g. as chemosensors (ref. 1,2) or chemotherapeutic agents.

Our approach utilizes fused-ring structures to control the molecular architecture of the host and consequently to preorganize metal-ligating atoms or hydrogen bonding sites. The high affinities of hosts that are conformationally organized for complexation and have poorly solvated binding sites were previously recognized by Cram (ref. 3). Figure 1 shows key examples of two types of

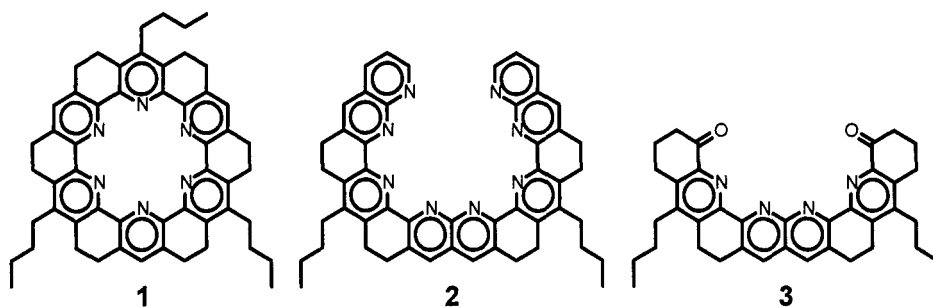


Fig. 1. A metal-binding torand (1) and hexagonal lattice receptors for guanidinium (2) and urea (3).

host compounds consisting of several pyridine and six-membered carbocyclic rings fused into a relatively rigid, planar molecular framework. Metal-binding host 1 (ref. 4) exemplifies the torands, in which the perimeter of the macrocycle is defined by smaller, fused rings (ref. 5-8). Pyridine heterocycles also form strong hydrogen bonds, and incorporation of 1,8-naphthyridine moieties into the host backbone produces "hexagonal lattice" receptors (ref. 9), such as 2 and 3.

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U-shaped receptor **2** binds the guanidinium cation via six hydrogen bonds (ref. 10) and diketone **3** forms a stable complex with urea containing only four hydrogen bonds (ref. 11). As pointed out previously (ref. 2), these complexes are stabilized by several attractive secondary electrostatic interactions between hydrogen bond donating NH groups of the guests and pre-organized hydrogen bond acceptor atoms (N and O) of the hosts.

We are now extending the hexagonal lattice approach to develop molecular sensors for small molecules of biological importance, including urea and creatinine. Artificial receptors for nucleotides and amino acids are also of interest as novel molecular probes and potentially as biopolymer-binding drugs.

### OPTICAL CHEMOSENSORS FOR CREATININE

Blood levels of the nitrogenous metabolites urea and creatinine are used to monitor human kidney function, making these clinical analytes excellent targets for developing novel chemosensors (ref. 1,2,12). Figure 2 shows our approach to a chromogenic reagent for creatinine (**5**) based conceptually on urea receptor **3** (Fig. 1). In the design of creatinine receptor **4** the right-hand portion of **3** was removed and the chromogenic dinitrophenol group was incorporated into the left-hand portion. The synthesis of **4** (ref. 12) was carried out in five steps from an intermediate used in the preparation of torand **1** and related fused-ring terpyridyl ligands (ref. 13). Creatinine is extracted from water into dichloromethane solutions of receptor **4**, forming the colored complex (**6**). As shown in Fig. 2, binding of creatinine by three hydrogen bonds causes a proton to be transferred from the OH group, apparently to the terminal nitrogen atom of the receptor. The receptor moiety in **6** has a delocalized, dipolar structure, producing a broad absorption band in the visible region (400-500 nm). This absorption spectrum is consistent with structure **6** found in the crystalline complex (ref. 12), but recent theoretical studies suggest an

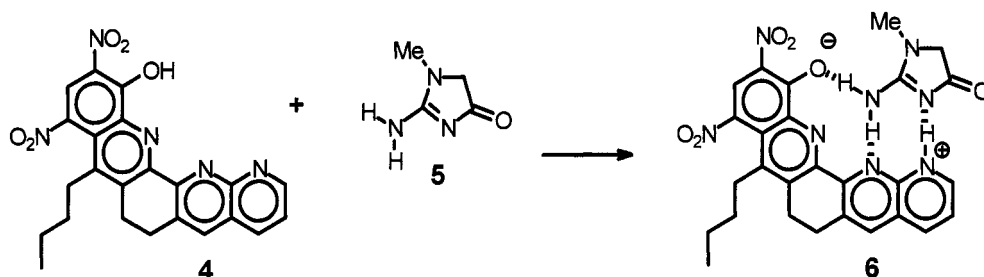


Fig. 2. A chromogenic receptor (**4**) for creatinine (**5**) and the solid-state structure of the resulting complex (**6**).

alternate solution structure in which the creatinine molecule is protonated and the host is anionic (ref. 14).

The chromogenic response of receptor **4** is sufficiently sensitive for measurement of creatinine at normal blood levels with correction for background levels of Na<sup>+</sup>, which produces an 80-fold weaker response. Current research efforts are directed at improving the creatinine/sodium selectivity and at creating fluorescent reagents for creatinine. Figure 3 shows synthetic routes for incorporating two different fluorophores into the receptor backbone. Both syntheses start with intermediate **7**, which was used previously in the synthesis of a torand analog of U-shaped receptor **2** (ref. 10). Friedländer condensation with homophthalimide introduces the benz-anthryridone fluorophore in **8**, which is converted to ketone **9** by ozonolysis. In the second synthesis, intermediate **7** is converted first to free base form **10** before condensation with acetic anhydride. Ozonolysis of **11** yields ketone **12** containing a 1,8-naphthyrindone fluorophore.

Ketones **9** and **12** could serve as intermediates in the synthesis of fluorescent reagents containing phenolic or other acidic groups, but they are also useful as model compounds for probing optical response resulting from the incorporation of such structures as intrinsic fluorophores in hexagonal lattice receptors (ref. 1).

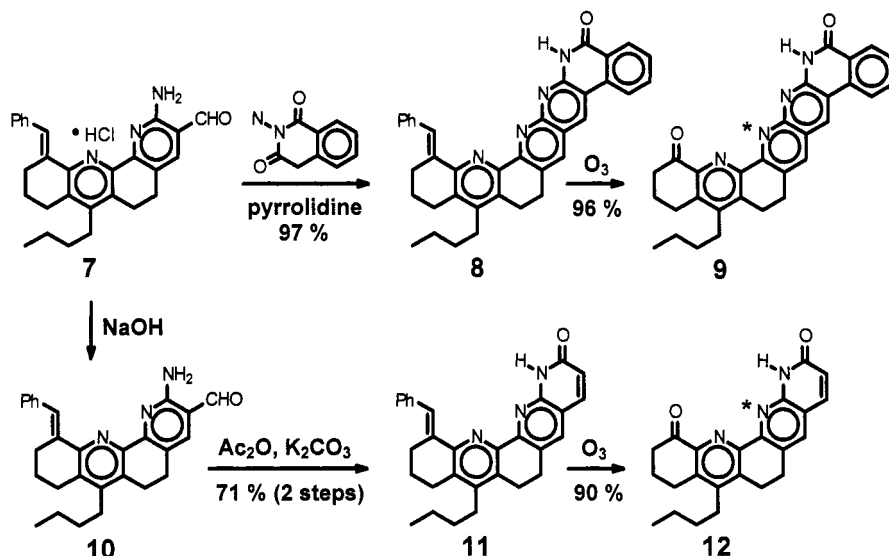


Fig. 3. Syntheses of two fluorescent intermediates (**9** and **12**; asterisk indicates site of protonation by  $\text{CF}_3\text{CO}_2\text{H}$ ).

We have examined the spectroscopic effects of protonation of ketones **9** and **12**, considering that proton transfer might be used to signal binding of creatinine or other guests by receptors containing similar intrinsic fluorophores. In ethanol the emission maximum of **9** shifts from 400 to 438 nm upon protonation, while that of **12** shifts from 378 to 406 nm. Addition of trifluoroacetic acid to solutions of **9** in  $\text{CDCl}_3/\text{methanol-}d_4$  causes the largest downfield shifts for the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals of the pyridine ring marked by an asterisk in Fig. 3 and protonation of **12** appears to occur at the corresponding position. The longer wavelength absorption and emission of **9** and the apparent formation of a favorable array of donor and acceptor sites upon protonation make the benzanthyridone moiety attractive for further development as an intrinsic fluorophore in receptors for creatinine and similar guest molecules.

#### A U-SHAPED RECEPTOR FOR UREA

Hexagonal lattice receptor design makes possible the complexation of urea via a maximum of six hydrogen bonds, as in the guanidinium complex of **2** (Fig. 1.), while diketone **3** only forms four hydrogen bonds with the NH donors of urea (ref. 11). Figure 4 shows a U-shaped artificial receptor (**13**) that fully satisfies the hydrogen-bonding capacity of urea (ref. 15). Complex **14** is very stable, even in competitive hydrogen-bonding solvents, such as DMSO. In fact, receptor **13** is best purified after synthesis by thermal recrystallization of **14** from DMSO, followed by extraction of urea from the complex by boiling water.

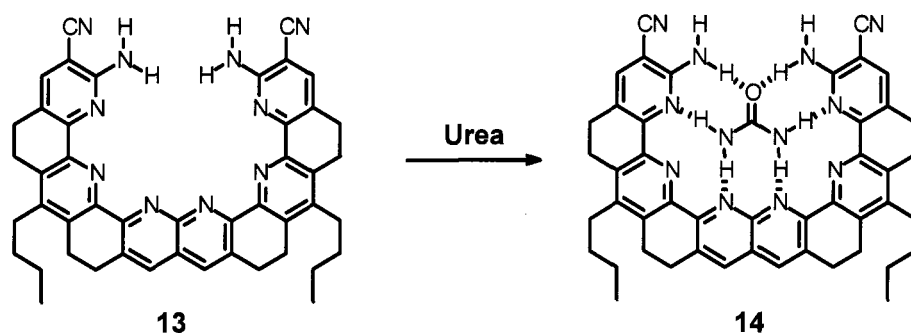


Fig. 4. A U-shaped receptor (**13**) and its urea complex (**14**).

Complex **14** was characterized by combustion microanalysis and by IR spectroscopy, showing the hydrogen bonded C=O absorption at  $1655\text{ cm}^{-1}$  (ref. 11). Binding of urea can be followed by  $^1\text{H}$  NMR spectroscopy using 1:1 (v/v)  $\text{CDCl}_3/\text{DMSO}-d_6$ , but calculation of a stability constant is complicated by the concentration dependence of the chemical shifts of free **13** in this solvent mixture. Complexation also causes a 16 nm bathochromic shift of the longest wavelength absorption maximum of **13** (394 nm) and  $K_s = 1.4 \times 10^4$  was obtained from titration data using 1:1  $\text{CHCl}_3/\text{DMSO}$  (ref. 11). This optical response is specific for urea; binding of *N*-butylurea causes small changes in the absorption spectrum of **13**, which shows negligible responses to *N,N'*-dimethylurea and *N,N*-dimethylurea. Among complexes in which urea is bound solely by neutral hydrogen bonds, **14** is uniquely stable.

#### A WATER-SOLUBLE GUANIDINIUM RECEPTOR

Most designed receptors for the guanidinium cation are flexible, crown ether derivatives. Previously reported hexagonal lattice receptors, including guanidinium receptor **2** (Fig. 1), are extractants and membrane transport agents, but their poor water solubilities limit homogeneous binding studies to organic solvents. Lacking alkyl sidechains, dicarboxylic acid **15** was expected to be soluble in water, particularly in dianionic form **16** (Fig. 5). The nitrogen and oxygen hydrogen-bond acceptor sites in **16** are preorganized for binding *N*-alkylguanidinium cations to form complex **17**. Electronic interactions (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*  $180^\circ$  rotation about this bond. The combination of receptor preorganization and attractive electrostatic interactions between the dianionic host and cationic guest led to our hypothesis that complexes of type **17** would be stable, even in aqueous media.

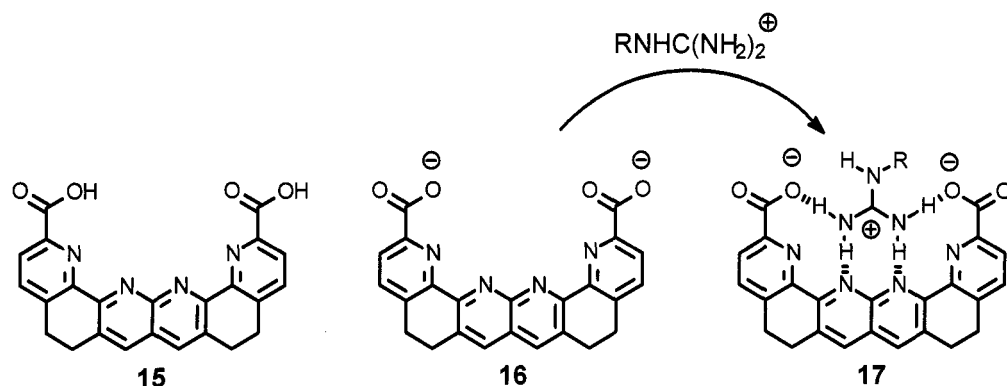


Fig. 5. A guanidinium receptor in the neutral form (**15**), water-soluble dianion (**16**) and *N*-alkylguanidinium complex (**17**).

The target arginine receptor was synthesized in seven steps as dipotassium salt **25** (Fig. 6). The benzene ring of quinaldine (**18**) was selectively reduced by catalytic hydrogenation in trifluoroacetic acid. Condensation of **19** with benzaldehyde in acetic anhydride gave dibenzylidene derivative **20**, which was conveniently purified by vacuum distillation. Ozonolysis gave ketoaldehyde **21**, which underwent Jones oxidation to ketoacid **22**. Friedländer condensation of **22** with 4-aminopyrimidine-5-carboxaldehyde gave **23**, which was hydrolyzed to aminoaldehyde **24**. A second condensation of **22** with **24** gave potassium salt **25**, which was purified by recrystallization and obtained in 18% overall yield from quinaldine. The molecular symmetry of **25** results from the use of the same ketone (**22**) in both Friedländer condensations (e and g), but two different ketones could be used to make unsymmetrical molecules of type **25** bearing functional groups other than carboxylate. This route could also be used for the preparation of arginine receptors that are substituted to allow immobilization, attachment of other recognition sites or conjugation to biomolecules.

As anticipated, dipotassium salt **25** has relatively high solubility in water (*ca.* 0.3 M). It is slightly soluble in methanol and insoluble in dimethyl sulfoxide (DMSO), dichloromethane and chloroform. The corresponding carboxylic acid (**15**) has very low water solubility (*ca.* 4  $\mu$ M), but is moderately soluble in DMSO. The ability of **25** to bind N-alkylguanidinium cations was tested by mixing an aqueous solution of **25** with a dilute solution of ethylguanidine sulfate in water, resulting in the formation of a yellow precipitate. Crystals of this complex formed when a hot aqueous solution was cooled to room temperature but crumbled upon drying under vacuum or in air. According to results of combustion microanalysis and  $^1\text{H-NMR}$  spectroscopy, dried samples of the complex consisted of the receptor dianion (**16**) and N-ethylguanidinium cation in

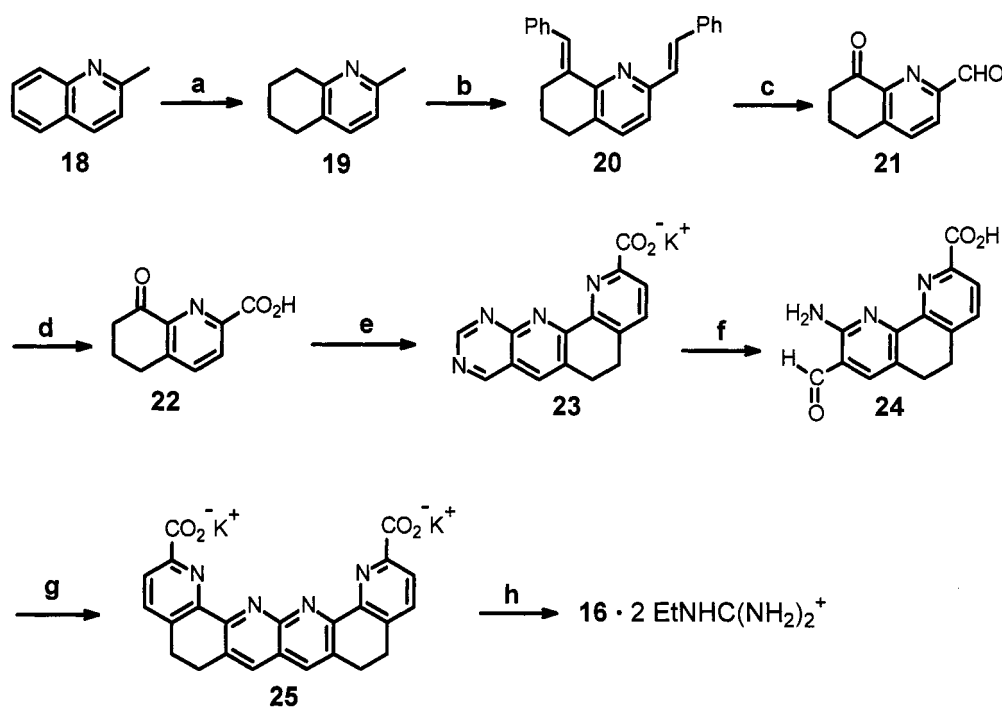


Fig. 6. Synthesis of a water-soluble guanidinium receptor as the dipotassium salt (**25**). Conditions a:  $\text{H}_2$  (1 atm), 10% Pd/C,  $\text{CF}_3\text{CO}_2\text{H}$ , 20 °C. b: benzaldehyde,  $(\text{CH}_3\text{CO})_2\text{O}$ , 150–160 °C. c: 1)  $\text{O}_3/\text{O}_2$ ,  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , -77 °C; 2)  $\text{Me}_2\text{S}$ ,  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , -77 – 20 °C. d:  $\text{CrO}_3/\text{H}_2\text{SO}_4$ ,  $(\text{CH}_3)_2\text{CO}$ , 20 °C. e: 4-amino-5-pyrimidinecarboxaldehyde, KOH,  $\text{CH}_3\text{OH}$ , reflux. f: HCl,  $\text{H}_2\text{O}$ , reflux. g: **22**, KOH, EtOH, reflux. h: ethylguanidine sulfate,  $\text{H}_2\text{O}$ , reflux.

exactly a 1:2 ratio. This stoichiometry is expected for a simple salt of a dicarboxylic acid with a base, but the predicted binding motif (**17**, Fig. 5) was proven by X-ray crystallographic analysis of a single crystal of hydrated complex  $16 \cdot 2\text{EtNHC}(\text{NH}_2)_2^+$ .

The ability of receptor **25** to bind guanidinium guests in water was examined by several methods, including  $^1\text{H-NMR}$  and UV-visible spectroscopy, as well as microcalorimetry (ref. 16). The  $^1\text{H-NMR}$  signals of **25** in  $\text{D}_2\text{O}$  solutions are significantly shifted upon addition of guanidinium chloride or N-ethylguanidinium sulfate. In the absence of guanidinium guests, the  $^1\text{H-NMR}$  chemical shifts of **25** are also concentration-dependent in the range of 0.1 mM to 0.1 M, complicating the calculation of dissociation constants. Receptor **25** and its complexes apparently aggregate in water through hydrophobic interactions between nonpolar regions of the molecule that are remote from the charged carboxylate groups. The UV-visible absorption spectra of aqueous solutions of **25** follow Beer's Law over the concentration range of 1-100  $\mu\text{M}$ , suggesting the absence of aggregation at low concentration. In order to understand the ionization states of **25** in water, we performed a series of pH titrations and followed changes in the UV-visible spectrum. Addition of HCl to aqueous solutions of **25** caused a bathochromic shift of the  $\pi\text{-}\pi^*$  band ( $\lambda_{\text{max}}$  384 nm) and a loss of fine structure. Line shape analysis of the pH titration curve measured at 414 nm gave the following ionization constants for dicarboxylic acid **15**:  $\text{pK}_1$  4.7;  $\text{pK}_2$  6.0.

The UV-visible absorption spectrum of receptor **25** in water or methanol is not significantly affected by complexation of guanidinium cations, so binding of various guests was evaluated by  $^1\text{H-NMR}$  spectroscopy. As mentioned previously, receptor **25** aggregates in water at concentrations needed for NMR spectroscopy, so methanol was chosen as the solvent for NMR titrations. The  $^1\text{H-NMR}$  signals of the receptor hydrogen atoms shift downfield upon complexation of cationic guests in  $\text{CD}_3\text{OD}$ . The experimental data points best fit the theoretical curve calculated from a dissociation constant ( $K_d$ ) equal to 34  $\mu\text{M}$  for the amino acid L-(+)-lysine. Similar titrations with methylguanidinium chloride or arginine gave  $K_d$  values for the 1:1 complexes that were too small to be measured accurately by this experimental method. Upper limits of 10  $\mu\text{M}$  were estimated for the  $K_d$  values of these guests. Thus, in methanol receptor **25** binds guanidinium cations at least 3 times more strongly than a primary ammonium guest, and **25** binds the methylguanidinium cation at least 200 times more strongly than does a recently reported molecular tweezer (ref. 17). Compound **25** is unique among artificial receptors in that it forms the strongest known complexes of N-alkylguanidinium cations in water.

## CONCLUSION

Highly preorganized hexagonal lattice receptors can be designed and synthesized for complexation of biologically important molecules, such as urea, creatinine, guanidines and amino acids. The resulting complexes are exceptionally stable, even in competitive hydrogen-bonding solvents, such as DMSO and water. Such artificial receptors can be used as extractants, chemosensors and probes for biological systems.

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