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Noncovalent Interactions within a Synthetic Receptor Can Reinforce Guest Binding

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Abstract: Structural and thermodynamic data are presented on the binding properties of anion receptors containing two covalently linked cyclopeptide subunits that bind sulfate and iodide anions with micromolar affinity in aqueous solution. A synchrotron X-ray crystal structure of the sulfate complex of one receptor revealed that the anion is bound between the peptide rings of the biscyclopeptide. Intimate intramolecular contacts between the nonpolar surfaces of the proline rings of the individual receptor moieties in the complex suggest that hydrophobic interactions *within* the receptor that do not directly involve the guest contribute to complex stability. This finding is supported by a microcalorimetric analysis of the solvent dependence of complex stability, which showed that increasing the water content of the solvent has only a weak influence on the Gibbs energy of binding. Hence, the increasing amount of energy required for desolvating the binding partners in solutions containing more water is almost compensated by the increasingly favorable hydrophobic interactions. Further observations that suggest that guest-induced intra-receptor interactions contribute to guest binding are (i) anion binding of a monomeric cyclopeptide lacking the covalent linkage between the two rings leads to the formation of 2:1 complexes; (ii) in the crystal structure of the 2:1 iodide complex of this monotopic receptor, a similar arrangement of the two cyclopeptide rings has been found as in the sulfate complex of the biscyclopeptide; (iii) complex formation of the monomeric cyclopeptide in aqueous solution is highly cooperative with a large stability constant corresponding to the formation of the 2:1 complexes from relatively instable 1:1 complexes; (iv) the monomeric cyclopeptide forms only 1:1 anion complexes in DMSO where hydrophobic interactions do not take place; and (v) introducing polar hydroxy groups on the proline rings of the monomeric cyclopeptide disrupts cooperativity causing the formation of only 1:1 complexes even in aqueous solution. Taken together these observations demonstrate that, in addition to direct receptor−substrate interactions, noncovalent interactions between the two subunits of such biscyclopeptides contribute significantly to anion complex stability. Reinforcement of molecular recognition through intra-receptor interactions should be an attractive new strategy to boost host−guest affinities.

Introduction

Molecular recognition plays a key role in biology, providing the incentive to develop synthetic molecules that can interfere with biological systems through noncovalent interactions. While developing small synthetic molecules that bind with high affinities to biomacromolecules has met with considerable success, the development of synthetic receptors that bind to small biologically relevant molecules in water with similarly high affinities has proven to be more difficult. A recent survey reveals that water-soluble synthetic receptors are typically several orders of magnitude less efficient in binding their guests than their biomolecular counterparts.[1]

Thus far, the approach to the design of synthetic receptors has focused exclusively on the direct interactions between host and guest (i.e., on the development of host molecules presenting selected functional groups in the optimal arrangement for guest binding). However, recent insights into molecular recognition in proteins suggests that efficient guest binding may involve more that just the direct interactions of the guest with the functional groups in the binding pocket.[2] Proteins are molecules that fold into specific conformations as a result of an extensive network of noncovalent interactions *within* their structures. There are several lines of evidence that suggest that ligand−protein interactions and these intra-protein interactions can be highly interdependent. For a number of systems where ligands are held exceptionally strongly (including the streptavidin/biotin pair), ligand binding is accompanied by a dramatic reduction of the

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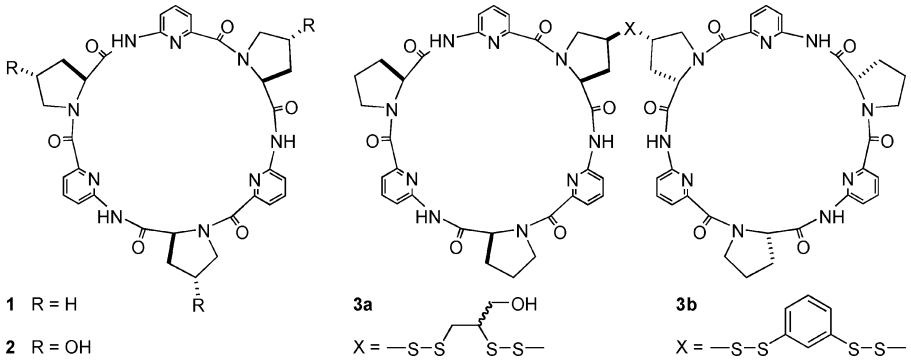
Chart 1



dynamics of the protein[3] and a rise in melting temperature (by a massive 37 °C for streptavidin/biotin).[4] These and other[5] results suggest that ligand binding and noncovalent interactions within proteins can be mutually reinforcing (i.e., noncovalent interactions within a biomolecular host can contribute to guest binding).

We now report the, as far as we are aware, first quantitative evidence that intra-receptor interactions can make an important contribution to guest binding by a synthetic host. Specifically, we show how hydrophobic interactions between two covalently linked peptide rings that do not directly involve the guest contribute to the complexation of iodide and sulfate by two synthetic biscyclopeptide-based anion receptors. Evidence comes from X-ray structure data, the solvent dependence of the anion affinity, and previous observations of the binding behavior of structurally related monomeric cyclopeptides.

Experimental Section

Materials. Cyclopeptides **1**,[6] **2**,[7] and **3**[8] were prepared as described previously. Analytical grade potassium sulfate (BDH Chemicals) and potassium iodide (Aldrich) were used without further purification.

Binding Experiments. Equilibrium constants, enthalpies, and entropies of binding were determined using isothermal titration calorimetry (MCS-ITC, Microcal LLC, Northampton, MA) at 298 K. Solutions of potassium sulfate (480−580 μM) or potassium iodide (2.5 mM) in aqueous acetonitrile (Milli-Q water and HPLC-grade acetonitrile [Fisher]) were titrated in 10 μL aliquots into solutions of the receptor (52−69 μM for sulfate binding; 250 μM for iodide binding) made up using the same batch of solvent. Binding constants and enthalpies of binding were obtained by curve fitting of the titration data using the one-site binding model available in the Origin 2.9 software.

X-ray Crystallography. Crystals of the sulfate complex of **3a** were grown in an open NMR tube by slow evaporation of a solution of 0.5 mg of **3a** and 0.6 μL of a 50 wt % aqueous solution of tetrabutylammonium sulfate (Fluka) in 0.5 mL of a 2:1 (v/v) mixture of acetonitrile and water. The crystals were extremely small and weakly diffracting, and a synchrotron radiation source was used to obtain diffraction data for this compound (at 150 K). Data were collected at Station 9.8, Daresbury SRS, UK,[9] using a Bruker SMART CCD diffractometer. The structures were solved by direct methods using the program SIR92.[10] The refinement and graphical calculations were performed using the CRYSTALS program suite.[11] The structures were refined by full-matrix least squares procedure on F. Chebychev weighting schemes,[12] and empirical absorption corrections (SADABS) were applied. All non-hydrogen atoms of the main receptor moiety were refined with anisotropic displacement parameters. Hydrogen atoms were located in Fourier maps and their positions adjusted geometrically after each cycle of refinement with isotropic thermal parameters. The sulfate anion, disordered over two positions, was modeled with refined occupancies: site (S100, O100, O200, O300, O400) occ 0.538 and site (S101, O101, O201, O301, O401) occ 0.462. Also the disulfide spacer X was disordered over two positions and was modeled with refined occupancies: site (S200, S300, C340, C350, C360, O500, S400, S500) occ 0.662 and site (S201, S301, C341, C351, C361, O501, S401, S501) occ 0.338. In view of severe shortage of data, the two (severely disordered) n-Bu_4N^+ counterions were refined with restrains and isotropic displacement parameters. A part of one of the butyl chains was modeled over two positions with refined occupancies: site (C871, C881, C891) occ 0.505 and site (C870, C880, C890) occ 0.495.

Crystal data: $C_{101}H_{144}N_{20}O_{18}S_5$, formula moiety: [$C_{69}H_{70}N_{18}O_{13}S_4$, $C_{16}H_{36}N$, $C_{16}H_{36}N$, O_4S, H_2O] $M = 2086.66$, $Z = 2$, monoclinic, space group $P21$, $a = 12.9731(10)$ Å, $b = 16.0397(10)$ Å, $c = 26.4260(10)$ Å, $\beta = 93.196(10)°$, $U = 5490.3(6)$ Å^3, $T = 150(2)$ K, $\mu = 0.178$ mm^{-1}, synchrotron radiation $\lambda = 0.6923$ Å. Of 35394 reflections measured, 8281 were independent ($R_{int} = 0.04$). Final $R = 0.1128$ (8231 reflections with $I > 2\sigma(I)$) and $wR = 0.1248$; CCDC 273684.

Results and Discussion

Cyclic hexapeptide **1** (Chart 1) has previously been found to bind with exceptional efficiency to iodide and sulfate anions in highly competitive aqueous solvents by forming a 2:1 complex in which the anion is sandwiched between two separate cyclopeptides.[6] We subsequently reported how linking the two cyclopeptides together covalently leads to an increase in binding affinity.[13] The best results were obtained when we used dynamic combinatorial chemistry to optimize the spacer between the cyclopeptides, resulting in **3a** and **3b**, which exhibited micromolar affinities for iodide and sub-micromolar affinities for sulfate in 2:1 acetonitrile−water mixtures.[8] As far as we are

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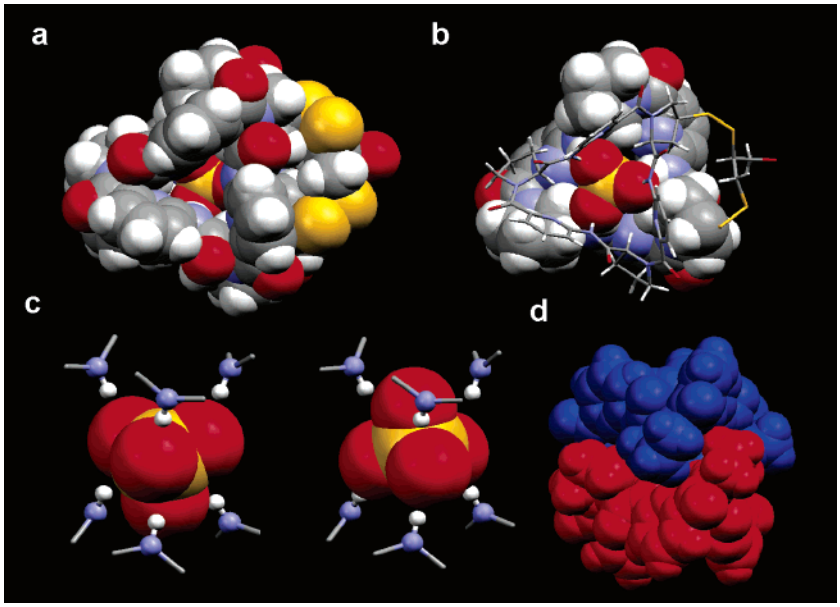

Figure 1. X-ray crystal structure of the complex of **3a** with $(NBu_4)_2SO_4$. (a) Space-filling representation showing how the receptor surrounds the central sulfate guest. (b) Same complex with one peptide ring and the disulfide spacer shown as sticks. (c) Detailed representation of the hydrogen-bonding arrangement around the sulfate anion, illustrating how the anion is disordered over two nearly identical positions. (d) Space-filling representation showing the two peptide rings in different colors, illustrating the close contacts between them. Counterions and solvent molecules are not shown for clarity.

aware, these compounds are currently the most efficient neutral synthetic receptors for anions in aqueous solution.[14]

We have now obtained detailed structural information on the binding mode of **3a** from a crystal structure of the sulfate complex of this biscyclopeptide. Small crystals that required a synchrotron radiation source to obtain diffraction data of sufficient quality were obtained by slow evaporation of an acetonitrile−water solution of **3a** and tetrabutylammonium sulfate. The resulting structure is depicted in Figure 1.[15] It shows that the anion is completely desolvated in the complex and sandwiched between the two peptide rings of **3a**. The six NH groups of both rings donate hydrogen bonds to the sulfate guest, which is disordered over two essentially equivalent positions (Figure 1c). In each of these positions, the three NH groups of one ring form essentially linear two-center hydrogen bonds with the three sulfate oxygen atoms facing this ring, while the other ring, which faces the remaining single oxygen atom, is involved in bifurcated hydrogen bonds (i.e., the hydrogen atom is shared between two oxygens). The disordered arrangement of the sulfate ion in the crystal structure of the complex suggests that the guest retains a considerable mobility inside the host cavity, thus reducing the entropic cost of complex formation. Consequently, a rapid interchange between various energetically similar arrangements of the sulfate ion in the host cavity can be expected in solution as evidenced, for example, by the very similar shifts all α protons of **3b** experience in the ^{1}H NMR upon binding of the guest (Supporting Information of ref 8).

We have compared the two hydrogen bonding arrangements found in the sulfate complex of **3a** with those occurring in the benchmark from nature, namely, the sulfate binding protein[16] and with the average values obtained from a statistical analysis of hydrogen bonding to nitrogen donors in sulfate containing crystal structures (Table 1).[17] For the first peptide ring, the observed ranges of N−H···O bond angles, N−O distances and S−O···H bond angles for the three hydrogen bonds compare favorably with the average values obtained from the statistical analysis. Moreover, the N−H···O bond angles are substantially closer to the ideal 180° than for the sulfate binding protein. For the second peptide ring the N−H···O bond angles, N−O distances, and S−O···H bond angles vary substantially as a result of the bifurcated nature of the hydrogen bonds. While such bifurcated hydrogen bonds are frequent in synthetic sulfate

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Table 1. Bond Angles and Distances in the Crystal Structure of **3a** with $(NBu_4)_2SO_4$ as Compared to CSD Statistical Averages[a] and the Corresponding Values for the Sulfate Binding Protein[16a]

	3a·$(NBu_4)_2SO_4$	CSD average[a]	sulfate binding protein
N−H···O bond angle (°)	179.6−179.9[b] 119.8−166.5[c]	158 ± 16	151−170
H···O−S bond angle (°)	126.7−137.8[b] 86.3−107.8[c]	117 ± 16	
N−O distance (Å)	2.74−2.83[b] 2.68−3.49[c]	2.89 ± 0.12	2.67−2.84

[a] Analysis of hydrogen bonding to nitrogen donors in sulfate containing crystal structures from the Cambridge Structural Database (CSD).[17] [b] Data for the peptide ring facing the three sulfate oxygen atoms. [c] Data for the peptide ring facing the single remaining sulfate oxygen atom involved in bifurcated hydrogen bonds.

complexes,[17] they do not appear to play a role in the sulfate binding protein.[16a]

Figure 1, panels a and d, shows how the receptor completely surrounds the guest in the complex, providing a binding site that is well-shielded from the solvent. Most importantly, the two peptide rings form extensive contacts with each other. A similar arrangement in which the proline rings of two cyclopeptides also approach each other almost within van der Waals contact has previously been observed in the crystal structure of the iodide sandwich complex of the monotopic cyclopeptide **1**.[6] Such close contacts between nonpolar parts of the peptide rings upon binding to the guest are likely to contribute to binding affinity in aqueous solution as a result of hydrophobic interactions.

Evidence for the involvement of hydrophobic intra-receptor interactions upon anion complexation comes from an analysis of the solvent dependence of sulfate and iodide affinity of receptor **3b** using isothermal titration microcalorimetry (ITC). Unfortunately, the limited solubility of **3b** prevents measurements in pure water. We have, however, been able to study binding of sulfate and iodide to **3b** in different acetonitrile/water mixtures. Figure 2a shows the Gibbs energies, enthalpies, and entropies of binding of sulfate as a function of the mole fraction of water $x(H_2O)$. Evidently, the Gibbs energy of binding depends linearly on the mole fraction of water in the range of the solvent mixtures used. It becomes less negative in more aqueous solvents, but the affinity for sulfate decreases only slowly with increasing water content, and an appreciable binding constant of 2.8×10^5 M^{-1} could still be observed in 87 mol % H_2O. This behavior is in sharp contrast to observations on other neutral anion receptors whose binding constants decrease dramatically with comparatively small increases in water content (as much as an order of magnitude for an increase in water content from 10 to 15%).[14y] In contrast to the Gibbs energy, the enthalpy and entropy changes with change in solvent composition are much more pronounced.

Figure 2b shows a similar analysis using iodide as substrate. In this case, the Gibbs energy of binding is even less sensitive to the solvent composition. A similar trend has previously been observed for the interaction of peptide **1** with iodide and sulfate in different solvent mixtures. While the stability of the $(\mathbf{1})_2$·sulfate complex decreases from 3.2×10^6 to 1.2×10^5 M^{-2} upon changing the solvent from 50% water−methanol to 80% water−methanol, the decrease is much less pronounced in iodide binding ($K_a = 2.3 \times 10^5$ M^{-2} in 50% water−methanol, 1.6×10^5 M^{-2} in 80% water−methanol).[7] The enthalpy and entropy changes upon complexation of iodide by **3b** follow the same trends as observed for sulfate only with a much smaller slope of the regression lines. Clearly the nature of the anion has a profound effect on the thermodynamics of binding. As the mode of binding of sulfate and iodide by **3** is likely to be similar with respect to the mutual arrangement of host and guest in the complex, receptor conformation, and intra-receptor interactions, we speculate that the difference in the binding thermodynamics between the two anions reflects the difference in their (de)-solvation. The large changes in the enthalpy and entropy of binding of sulfate as a function of the solvent composition could be a result of the tendency of sulfate to be preferentially solvated by water molecules. As the amount of water decreases the water−sulfate interactions should become enthalpically more favorable (less polar medium) and entropically more costly (less water available). As a consequence, desolvation of the guest upon complex formation will be entropically more favorable as the amount of water decreases but increasingly endothermic if the binding enthalpy cannot fully compensate for the enthalpy required for the release of solvent molecules from the anion.

Literature data are available on the thermodynamics of transfer of iodide from water to water−acetonitrile mixtures.[18] At mole fractions of water between 0.4 and 0.9, desolvating iodide becomes 1.9 kJ·mol^{-1} more costly in free energy for every 10% increase in $x(H_2O)$ (see Supporting Information). Thus, solely on the basis of the increased cost of desolvating the anion one would expect an 11-fold drop in affinity upon increasing the mole fraction of water from 49 to 80%. Yet, our experiments show that the binding constant for iodide only drops 2.2-fold from 9.9×10^4 to 4.5×10^4 M^{-1} over the same range. We attribute this effect to hydrophobic interactions in the complex between the two cyclopeptide rings that partially compensate the increasingly unfavorable desolvation process in mixtures containing more water.[19] Unfortunately, thermodynamic data for the transfer of sulfate from water to water−acetonitrile mixtures data are not available preventing a similar analysis for binding of this anion.

Additional arguments for intra-receptor interactions contributing to the anion affinity of biscyclopeptides **3a** and **3b** in aqueous solution come from previous investigations. The stepwise analysis of the binding of sulfate by cyclopeptide **1** in 1:1 D_2O-CD_3OD has shown, for example, that the first peptide ring binds the guest relatively weakly ($K_1 = 3.6 \times 10^2$ M^{-1}), whereas binding of the second peptide to the 1:1 complex is much stronger ($K_2 = 8.8 \times 10^3$ M^{-1}) (Scheme 1).[13] If binding would be completely independent, K_2 should be 4 times smaller than K_1.[20] However, the data show that K_2 is 24 times larger than K_1. Thus, binding is 2 orders of magnitude more efficient than statistically expected, corresponding to an increase in affinity of ca. 11 kJ·mol^{-1} resulting from the interactions between the two peptide rings.

In less polar solvents, the contribution of these intra-receptor interactions is greatly reduced, confirming that they are largely

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(19) This analysis ignores desolvation of the polar amide groups of the receptor which should make binding even more unfavorable at higher mol fractions of water.
(20) Statistically the rate constant of formation of the 1:1 complex is double that of the 2:1 complex, while the rate constant of dissociation of the 2:1 complex is double that of the 1:1 complex. Since the equilibrium constants equal the ratio between the rate constant of formation and the rate constant of dissociation, it follows that $K_2 = K_1/4$. See also: Ercolani, G. *J. Am. Chem. Soc.* **2003**, *125*, 16097.

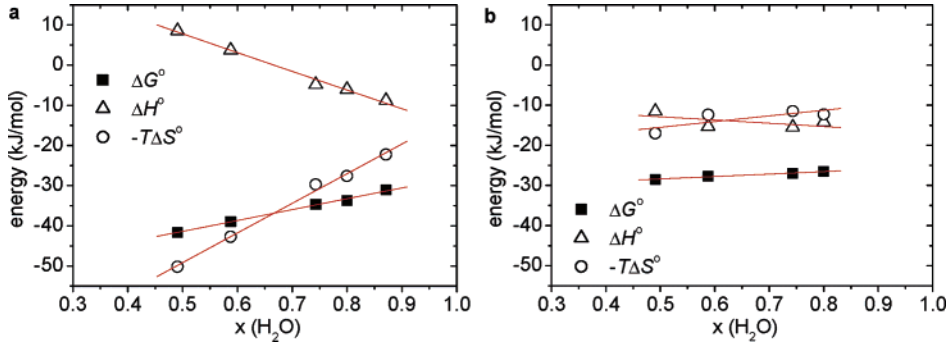
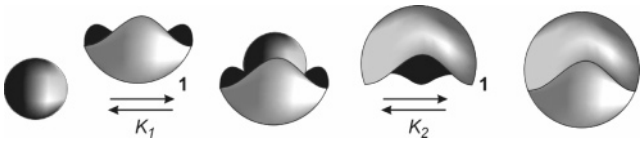


Figure 2. Gibbs energy ($\Delta G°$, ■), enthalpy ($\Delta H°$, △), and entropy ($-T\Delta S°$, ○) of binding of (a) K_2SO_4 and (b) KI to receptor **3b** as a function of the mole fraction of water $x(H_2O)$ in acetonitrile−water mixtures at 298 K.

Scheme 1. Schematical Representation of the Stepwise Formation of the 2:1 Sandwich Complex between Sulfate and **1**



hydrophobic in origin. Thus, while **1** forms termolecular sandwich complexes with sulfate and other anions in aqueous methanol solution, which structurally resemble the bimolecular sulfate complex of **3a**,[6] only moderately stable 1:1 anion complexes are formed in DMSO, a solvent in which hydrophobic interactions do not occur. The stability of the iodide complex of **1** in DMSO-d_6 amounts to only 150 M^{-1}, for example.[21]

Further evidence for the involvement of hydrophobic interactions comes from studies on the hydroxyproline containing peptide **2**.[7] This monotopic cyclopeptide only forms 1:1 complexes even in highly aqueous solvents presumably because the hydrophobic interactions between the proline rings are disrupted by the presence of the polar hydroxy groups.

Conclusions

Detailed studies on the binding of a series of cyclopeptide based receptors to sulfate or iodide anions show that binding affinity is not only due to the direct interactions between receptor and guest but also due to interactions within the receptor that do not directly involve the guest. While such reinforcement of molecular recognition through intra-receptor interactions is known to occur in some proteins, as far as we are aware this is the first example of a synthetic receptor for which this mechanism is demonstrated. Evidence for the involvement of intra-receptor interactions is derived from (i) the X-ray crystal structure of the sulfate complex of **3a** showing intimate contacts between hydrophobic surfaces of the two peptide rings of the receptor; (ii) the analysis of the solvent dependence of the anion affinity of biscyclopeptide **3b**, revealing that binding affinity falls off much less rapidly upon increasing the water fraction in the solvent mixture than expected on the basis of the solvation energy of iodide; (iii) the stepwise analysis of binding of cyclopeptide **1** to sulfate in 1:1 methanol−water to form a 2:1 sandwich complex, showing that the second peptide binds 100 times stronger that statistically expected, thus indicating that the intra-receptor interactions reinforce guest binding by ca. 11 kJ·mol^{-1} in this solvent mixture; (iv) the observation that the formation of a 2:1 complex is not favorable in DMSO where hydrophobic interactions can no longer take place; and (v) the fact that peptide **2** carrying hydrophilic hydroxy groups shows no propensity to form 2:1 complexes even in pure water.

These results demonstrate that the affinities of synthetic receptors can be increased substantially through the contribution of intra-receptor interactions. This effect is reminiscent of what has been observed for some noncovalent multicomponent capsular assemblies, where the stability of the capsules is critically dependent on the presence of the guest.[22] However, in the present cyclic peptide system the interactions between the receptor subunits are *intra*molecular and therefore better able to contribute to guest binding affinity (intermolecular interactions are generally entropically more unfavorable than intramolecular interactions). Still, the anion complexes of our biscyclopeptides are probably not the only host−guest systems in which *intra*molecular intra-receptor interactions contribute to complex stability. Other examples could be the folding oligomers described by Nishinaga et al.[23a] or the ATP binding β-hairpin developed in the Waters group.[23b] However, the data currently available for these systems do not allow an assessment of the extend of possible contributions from intra-receptor interactions to guest binding. While the reinforcement of host−guest interactions in our cyclic peptide system was largely serendipitous, we anticipate that engineering intra-receptor interactions into synthetic hosts may well become an attractive and powerful strategy to boost host−guest affinities.

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Supporting Information Available: X-ray structure data (CIF) for **3a**·$(NBu_4)_2SO_4$ (CCDC 273684); analysis of thermodynamic data for the transfer of iodide from water to water−acetonitrile mixtures. This material is available free of charge via the Internet at http://pubs.acs.org.

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