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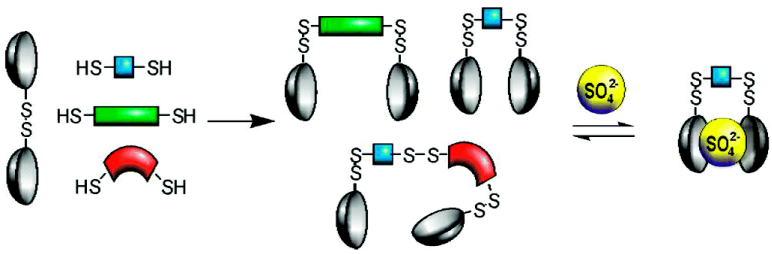

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Dynamic Combinatorial Optimization of a Neutral Receptor That Binds Inorganic Anions in Aqueous Solution

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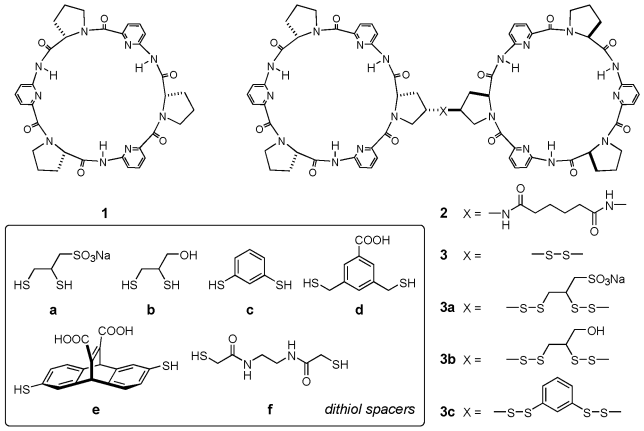
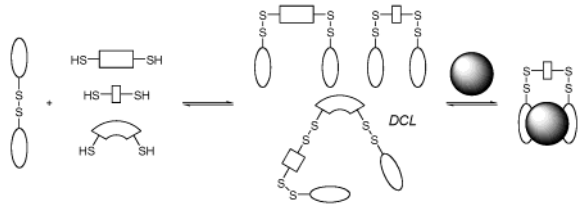
The development of synthetic receptors for small molecules or ions rivaling the binding efficiencies of proteins is a long-standing challenge. Traditionally, the approach to such receptors has involved an iterative process of design, synthesis, and assessment of affinity. With the advent of dynamic combinatorial chemistry,[1] an additional tool has become available that merges synthesis and affinity screening into a single process. In brief, dynamic combinatorial libraries (DCLs) are formed by linking building blocks together using reversible bonds causing all members of a DCL to interconvert continuously, forming an equilibrium mixture. Addition of a guest to a DCL of potential receptors shifts the equilibrium toward the receptor that binds the guest most efficiently. Since this concept was first articulated in 1996,[2] several examples of the successful use of dynamic combinatorial chemistry for the identification and synthesis of covalent receptors have been reported.[3]



The work described herein is based on the observation by one of us that the neutral cyclic hexapeptide **1** binds anions such as halides and sulfate in highly competitive aqueous solvent mixtures.[4] Structural investigations revealed that sandwich-type 2:1 complexes are formed, in which the anions are bound by six hydrogen bonds between two interdigitating cyclopeptide rings. In subsequent work, these aggregates could be stabilized by covalently linking two peptide units together via adipic acid to give receptor **2**.[5] Design of the linker was based on the crystal structure of the iodide complex of **1**.[4] Here, we report how dynamic combinatorial chemistry can be used to optimize the linking unit, resulting in two new receptors that bind iodide and sulfate anions with, for neutral species, unprecedented affinities.

Our approach is based on reversible disulfide chemistry[6] and is summarized in Scheme 1. Mixing disulfide **3** with dithiols **a**−**f** in

Scheme 1



the presence of oxygen from the air results in the formation of a DCL that contains potential receptors in which two peptide rings are separated by different dithiol-derived spacers. Note that, using this approach, compounds containing a combination of two or more spacers will also be formed. Addition of an anionic guest to this DCL should then lead to the amplification of good binders.

We prepared disulfide **3** from a derivative of **1** containing one tosylated 4*R* hydroxyproline subunit[5] by reaction with potassium thioacetate followed by basic hydrolysis of the acetyl group in the presence of oxygen. Preliminary binding studies confirmed that **3** is not a good receptor for iodide or sulfate anions ($K_a < 10^3$ M^{-1}). Because this compound is poorly soluble in highly aqueous solutions, DCLs were made by dissolving **3** and **a**−**f** in a 2:1 (v/v) mixture of acetonitrile and water. Oxidation was performed at pH 8−9 and in the presence of air over a period of 7 days. During this time, the residual thiols mediate reversible disulfide exchange, ensuring that the final library composition is under thermodynamic control.[6b] Figure 1a shows the HPLC trace of a disulfide library resulting from mixing 1 equiv of **3** with 0.33 equiv of each of the six dithiols **a**−**f**. The product distribution is dominated by the starting disulfide **3** as expected from the thermodynamic preference to form dimeric rather than higher-order structures.[7] Exposure of the DCL to KI or K_2SO_4 resulted in the marked amplification of three different receptors (Figure 1b and c). Similar studies using KBr resulted in a reduced amplification of the same set of receptors,[8] whereas NaCl and KF had no effect on library composition. The amplified compounds were isolated using HPLC, and, by using electrospray ionization mass spectrometry, structurally assigned to disulfides **3a**, **3b**, and **3c**, incorporating the smaller dithiols spacers **a**, **b**, and **c**, respectively. We have focused on **3b** and **3c** as these are more strongly amplified than **3a**. Both compounds were isolated efficiently from a second-generation biased dynamic library made from equimolar amounts of **3** and **b** or **c** in the presence of an excess of an anionic template.[9] Figure 2 shows how the introduction of guest shifts the composition of these biased libraries to produce **3b** and **3c** in 63% and 77% yield. The only significant side product is starting material **3**, which can of course be recycled.

Next, we compared iodide and sulfate complexation of receptors **3b** and **3c** obtained using dynamic combinatorial chemistry with

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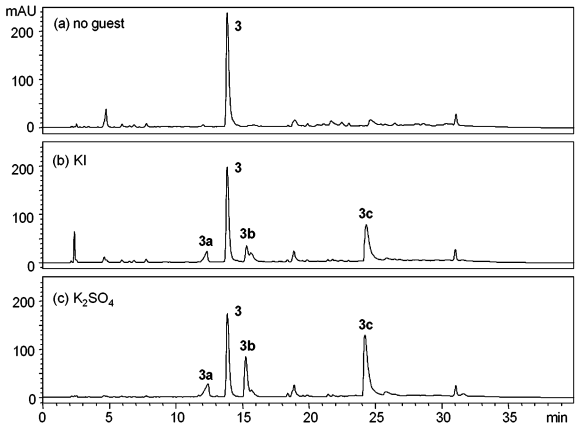

Figure 1. HPLC analysis of a DCL made by mixing **3** (1.33 mM) with **a**−**f** (0.44 mM each) in the absence of guest (a) and in the presence of 10 mM KI (b) or 10 mM K_2SO_4 (c) showing amplification of receptors **3a**−**3c**.

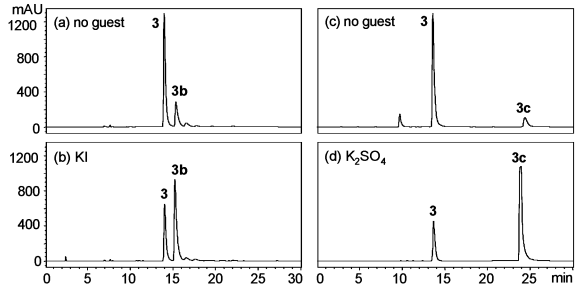

Figure 2. HPLC analysis of biased DCLs made by mixing **3** (1.33 mM) with **b** (1.33 mM) in the absence of guest (a) and in the presence of 10 mM KI (b). HPLC trace of the DCL made from **3** (1.33 mM) and **c** (1.33 mM) in the absence (c) and in the presence (d) of 10 mM K_2SO_4. Libraries in the absence of guest showed signs of precipitation after 2−3 days.

Table 1. Association Constants, Gibbs Energies, Enthalpies, and Entropies of Binding of KI and K_2SO_4 to Receptors **2**, **3b**, and **3c**[a]

		K_a	$\Delta G°$	$\Delta H°$	$T\Delta S°$
KI	**2**	3.3×10^3	−20.0	−4.3	15.7
	3b	2.9×10^4	−25.5	−20.7	4.8
	3c	5.6×10^4	−27.1	−13.4	13.7
K_2SO_4	**2**	2.0×10^5	−30.2	10.7	41.0
	3b	5.4×10^6	−38.4	1.8	40.1
	3c	6.7×10^6	−39.0	3.7	42.7

[a] Recorded in 2:1 (v/v) acetonitrile/water at 298 K; binding constants in M^{-1} and energies in kJ mol^{-1}.

that of the previously designed receptor **2**. In the ^{1}H NMR spectrum, the characteristic downfield shift of the H(α) signals of **3c** is visible in the presence of sulfate, indicating that the mode of interaction of this receptor with the anion is similar to that of **2**.[5] Quantitative binding studies were carried out in 2:1 (v/v) acetonitrile/water mixtures using isothermal titration microcalorimetry (ITC). This technique provides the complex stoichiometry, which was invariably 1:1 for all host−guest pairs described here, as well as the binding constants and enthalpies from which the entropies of binding can be calculated. The results show that **3b** and **3c** bind sulfate and iodide anions an order of magnitude more efficiently than does **2** (Table 1). As far as we are aware, these binding constants are the highest obtained thus far for the complexation of anions by a neutral receptor in aqueous solution.[10]

Thermodynamic analysis of the binding shows some interesting trends. For all receptors, sulfate binding is strongly entropy driven, suggesting that liberation of solvent from around the sulfate anion is the major driving force. For the less strongly solvated iodide ion,[10a] this effect is much reduced, and the enthalpy change upon binding becomes significant. When comparing receptors **3b** and **3c** with **2**, the improved affinity of the former results predominantly from a more favorable enthalpy change upon binding. One possible explanation could be that the new receptors form stronger or better-aligned hydrogen bonds with the anion, although we cannot exclude that the more favorable enthalpy of complexation arises from, for instance, different conformations of the unbound states of **3b** or **3c** in comparison to **2**.

In summary, we have shown that dynamic combinatorial chemistry can be used to optimize the binding properties of bis-(cyclopeptide) **2** toward anions in aqueous solution. Two new receptors were obtained with, for this class of neutral compounds, unprecedented binding efficiencies. Our results demonstrate that dynamic combinatorial optimization of designed receptors can be a powerful strategy, bringing synthetic receptors with efficiencies approaching those of proteins one step closer.

Acknowledgment. We are grateful to Jeremy K. M. Sanders for stimulating discussions. S.O. thanks the Royal Society for a University Research Fellowship. S.K. thanks the Deutsche Forschungsgemeinschaft for funding and H. Ritter for his support.

Supporting Information Available: Procedures for the preparation and analysis of DCLs, the ITC experiments, the syntheses of **3**, **3b**, and **3c**, and ^{1}H NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For reviews, see: (a) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Curr. Opin. Chem. Biol.* **2002**, *6*, 321−327. (b) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Drug Discovery Today* **2002**, *7*, 117−125. (c) Lehn, J.-M.; Eliseev, A. V. *Science* **2001**, *291*, 2331−2332. (d) Karan, C.; Miller, B. L. *Drug Discovery Today* **2000**, *5*, 67−75.

(2) Brady, P. A.; Bonar-Law, R. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K. M. *Chem. Commun.* **1996**, 319−320.

(3) (a) Eliseev, A. V.; Nelen, M. I. *Chem.—Eur. J.* **1998**, *4*, 825−834. (b) Berl, V.; Huc, I.; Lehn, J.-M.; DeCian, A.; Fischer, J. *Eur. J. Org. Chem.* **1999**, 3089−3094. (c) Storm, O.; Lüning, U. *Chem.—Eur. J.* **2002**, *8*, 793−798. (d) Furlan, R. L. E.; Ng, Y.-F.; Cousins, G. R. L.; Redman, J. E.; Sanders, J. K. M. *Tetrahedron* **2002**, *58*, 771−778. (e) Roberts, S. L.; Furlan, R. L. E.; Cousins, G. R. L.; Sanders, J. K. M. *Chem. Commun.* **2002**, 938−939. (f) Furlan, R. L. E.; Ng, Y.-F.; Otto, S.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2001**, *123*, 8876−8877. (g) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590−593. (h) Brisig, B.; Sanders, J. K. M.; Otto, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1270−1273.

(4) Kubik, S.; Goddard, R.; Kirchner, R.; Nolting, D.; Seidel, J. *Angew. Chem., Int. Ed.* **2001**, *40*, 2648−2651.

(5) Kubik, S.; Kirchner, R.; Nolting, D.; Seidel, J. *J. Am. Chem. Soc.* **2002**, *124*, 12752−12760.

(6) (a) Hioki, H.; Still, W. C. *J. Org. Chem.* **1998**, *63*, 904−905. (b) Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *J. Am. Chem. Soc.* **2000**, *122*, 12063−12064. (c) Ramström, O.; Lehn, J.-M. *ChemBioChem* **2000**, *1*, 41−48.

(7) Ercolani, G.; Mandolini, L.; Mencarelli, P.; Roelens, S. *J. Am. Chem. Soc.* **1993**, *115*, 3901−3908.

(8) When using KBr instead of K_2SO_4 as a template, the areas of the peaks in the HPLC traces due to **3a**, **3b**, and **3c** decreased by approximately 70%, 50%, and 30%, respectively.

(9) Starting from racemic **b**, we obtained receptor **3b** as a mixture of diastereomers. The receptor can be isolated using either Na_2SO_4 or KI as a template. In our hands, templating by Na_2SO_4 is rather erratic, however, and more reproducible results are obtained with KI.

(10) For reviews on anion receptors, see: (a) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609−1646. (b) Antonisse, M. M. G.; Reinhoudt, D. N. *Chem. Commun.* **1998**, 443−448. (c) Snowden, T. S.; Anslyn, E. V. *Curr. Opin. Chem. Biol.* **1999**, *3*, 740−746. (d) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486−516. For studies on neutral synthetic anion receptors in aqueous mixtures, see: (e) Fan, E.; Van Arman, S. A.; Kincaid, S.; Hamilton, A. D. *J. Am. Chem. Soc.* **1993**, *115*, 369−370. (f) Jagessar, R. C.; Shang, M.; Scheidt, W. R.; Burns, D. H. *J. Am. Chem. Soc.* **1998**, *120*, 11684−11692. (g) Anzenbacher, P., Jr.; Jursíková, K.; Sessler, J. L. *J. Am. Chem. Soc.* **2000**, *122*, 9350−9351.

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