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COMMUNICATION

Dynamic combinatorial development of a neutral synthetic receptor that binds sulfate with nanomolar affinity in aqueous solution†

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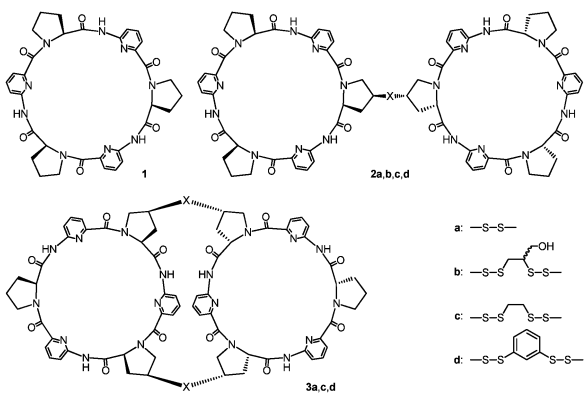
Using dynamic combinatorial disulfide chemistry we have developed a new generation of neutral synthetic receptors for anions, based on a macrobicyclic peptide structure. These receptors show an exceptional affinity and selectivity for sulfate ions in aqueous solution [log K_a = 8.67 in 41 mol% (67 volume%) acetonitrile in water]. The high affinity depends on a delicate balance between rigidity and flexibility in the structure of the receptor.

Molecular recognition plays a central role in biology, spurring attempts to develop synthetic host–guest systems that operate in aqueous solution.[1] However, obtaining synthetic receptors that bind their guests with good affinity and selectivity in water is a considerable challenge, as water molecules are strong competitors for most noncovalent interactions. Where nature has solved this problem long ago, chemists still struggle; synthetic receptors that operate in water are, on average, six orders of magnitude less efficient in binding guests than biomolecules.[1a] Yet, high affinities are essential for use of synthetic receptors in biological contexts.[2] We have already made significant progress in developing receptors that strongly bind hydrophobic cations in water.[3] We now report a new generation of hosts for hydrophilic anions, which are much more challenging targets as anions are usually more strongly solvated in aqueous solution than cations.[4]

One of the hallmarks in the recognition of anions in biology is the sulfate-binding protein (SBP) that presents a neutral binding pocket for the anionic guest.[5] Several neutral synthetic anion receptors have been developed, many of which are inspired by the binding motif of SBP.[6,7] While good binding affinities have been obtained in organic solvents, only few systems retain detectable anion binding in aqueous solution.[8] Often, the addition of even small amounts of water eliminates binding.[4] Cyclopeptide **1**, developed by one of us, is a notable exception: this receptor binds sulfate and halides in highly aqueous media.[9] The 2 : 1 sandwich complexes formed by two units of **1** with the anion can be further stabilized by covalently linking the two cyclopeptide rings.[10] Selection of the optimal linkers using dynamic combinatorial chemistry has yielded neutral anion receptors, **2b** and **2d**, which exhibit micromolar affinities for sulfate in mixtures of water and acetonitrile.[10d] To the best of our knowledge, these are the highest affinities reported for neutral anion receptors in aqueous solution prior to the present study. The extraordinary affinity is partly due to reinforced molecular recognition.[11] In the conformation in which the bis(cyclopeptide) binds the anion, hydrophobic interactions between the two cyclopeptide units contribute to the overall complex stability. We have previously estimated that these interactions enhance binding affinity by two orders of magnitude.[12]



We have since extended our studies to target receptors, in which the two cyclopeptide rings are connected *via* two linkers. Such macrobicyclic receptors should be significantly better preorganized for complex formation. Access to these complex architectures is difficult when using traditional synthesis, but relatively straightforward when using a dynamic combinatorial strategy (Scheme 1). Dynamic combinatorial chemistry[13] relies on equilibrium mixtures that respond to the addition of template molecules (*i.e.* anionic guests) by amplifying strongly binding library members at the expense of the other

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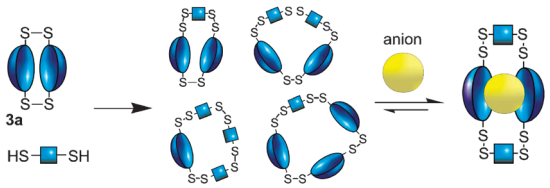
† Electronic supplementary information (ESI) available: Synthesis of **3a**, preparation of the DCLs, isolation and characterization of receptors **2c**, **3c** and **3d** and ITC (displacement) binding assays. See DOI: 10.1039/c1cc13451e



Scheme 1 Anion-induced amplification of a macrobicyclic receptor from a dynamic combinatorial library of disulfides.

compounds in the mixture. We have used dynamic combinatorial libraries (DCLs) based on disulfide exchange.[14] Such libraries can be generated from thiols which readily oxidize when exposed to oxygen from the air at neutral pH. Disulfide exchange is mediated by residual thiolate anions and can be halted by addition of an acid.

Bis(cyclopeptide) **3a** was prepared from a monocyclic peptide with alternating L-proline and 6-aminopicolinic acid subunits in which two proline rings contain 4*S*-thiobenzoate substituents. The monocyclic peptide was synthesized by assembling the linear precursor in solution from appropriately functionalized dipeptides followed by cyclization under pseudo high-dilution conditions.†

DCLs were prepared by mixing bis(cyclopeptide) **3a** with various dithiol spacers in acetonitrile–water mixtures‡, using sulfate salts or salts of other anions as templates. After allowing the libraries to equilibrate for six days the composition of the DCLs in the presence and absence of different anions was analyzed by HPLC-MS. Fig. 1 shows the results for the two most successful spacers **c**§ and **d** with the anions that produced the biggest amplifications: sulfate, iodide and selenate. Significant amplifications of macrobicycles **3c** and **3d** were observed in all cases, most notably with sulfate. Receptors **3c** and **3d** were isolated by preparative HPLC from sulfate-templated libraries. Unlike the previous generation of receptors, it was necessary to remove most of the sulfate prior to injection by precipitation with $BaCl_2$ and filtration, otherwise the receptors retained the sulfate anion during chromatography. This already suggested that these compounds exhibit a strong affinity for sulfate, which was confirmed using isothermal titration calorimetry (ITC).

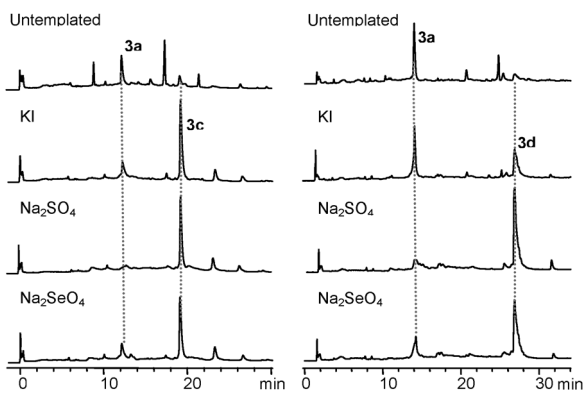


Fig. 1 HPLC analysis (260 nm) for DCLs containing bis(cyclopeptide) **3a** (0.67 mM), spacer **c** (1.33 mM, left) or **d** (1.33 mM, right) and various salts (10 mM) in CH_3CN/H_2O 2 : 1 (v/v) at pH 9 after 6 days of equilibration.

Determining the binding constants of sulfate and selenate with receptors **3c** and **3d** required the use of displacement techniques[15] as binding of these anions was too strong to be accurately measured by direct titration. We first performed titrations of the relatively weakly binding iodide anion. After binding was saturated we titrated in salts containing the more strongly binding anions (sulfate or selenate) and recorded the heat associated with displacing the iodide anion from the binding site of the receptor. The results of the ITC binding studies are summarized in Table 1. For comparison we also include data obtained for the corresponding receptors linked by only one spacer **2c** (prepared for the present study) and **2d** (reported previously).[10d]

Table 1 clearly shows that our new anion receptors possess exceptionally high anion affinities; the highest reported so far in aqueous media for neutral synthetic receptors. They are also the strongest binders of all synthetic receptors developed through dynamic combinatorial chemistry reported to date. In addition, our results provide valuable information about effects that control binding affinities and selectivities in these receptors. The singly linked bis(cyclopeptide) receptors **2c** and **2d** have comparable anion affinities, almost independent of whether the spacer is flexible (as in **2c**) or rigid (as in **2d**). In contrast, for the doubly linked receptors **3c** and **3d** the nature of the spacer has a pronounced effect on anion binding. While the expectation is that by increasing the preorganization of the receptor through the introduction of a second linker the binding affinity should increase, this effect is only modest (4.3 kJ mol^{-1} in the case of sulfate binding) for aromatic linker **d**. The corresponding effect for the more flexible aliphatic linker **c** is much larger: affinity for sulfate increases by 11 kJ mol^{-1}, highlighting the importance of flexibility in achieving high affinities. Note that for both sulfate and iodide binding, the enhanced binding affinity upon introduction of a second spacer is solely an entropic effect and accompanied by an unfavorable change in the enthalpy of binding. It is unlikely that the entropic gain upon introducing a second spacer is associated with *anion* desolvation. We have shown previously that, even for the singly linked receptors, the anion binding pocket of the receptor is well shielded from the solvent and that the bound anion is completely desolvated.[12] Assuming that also in the doubly-linked bis(cyclopeptides) **3c** and **3d** the bound anion is completely desolvated, this suggests that

Table 1 Association constants, Gibbs binding energies, enthalpies and entropies of various anions to receptors **2c**, **2d**, **3c**, and **3d**[a]

Salt	Receptor	log K_a	$\Delta G^{\circ b}$	$\Delta H^{\circ b}$	$T\Delta S^{\circ b}$
KI	**2c**	4.89	−27.9	−18.7	9.2
	3c	6.04	−34.4	−12.7	21.7
	2d	4.75	−27.1	−13.4	13.7
	3d	5.08	−28.9	−7.2	21.7
Na_2SO_4	**2c**	6.78	−38.6	2.8	41.4
	3c	8.67	−49.5	9.6	59.1
	2d	6.83	−39.0	3.7	42.7
	3d	7.59	−43.3	6.0	49.3
Na_2SeO_4	**3c**	8.04	−46.0	16.0	62.0
	3d	6.60	−37.7	[c]	[c]

[a] Recorded in CH_3CN/H_2O 2 : 1 (v/v) at 298 K. [b] Energies in kJ mol^{-1}. [c] The relatively weak affinity of SeO_4^{2-} relative to I^- did not allow obtaining accurate values for the binding enthalpy and entropy.

introducing a second spacer gives rise to more favorable *receptor* desolvation. A well ordered array of water molecules has recently been observed in the solid state inside the cavity of a triply-linked bis(cyclopeptide).[10e] If the cavities of bis(cyclopeptides) **3c** and **3d** contain similarly well-ordered water molecules in the absence of anions, complex formation should indeed benefit significantly from the release of these solvent molecules. In addition, the more favorable entropic terms observed for the doubly-linked bis(cyclopeptides) with respect to the singly-linked analogs could also reflect a smaller loss of conformational flexibility when **3c** and **3d** interact with anions, *i.e.* the doubly linked receptors are better preorganized. The more favorable entropic contribution to anion binding resulting from introduction of the second linker is partly compensated by a reduced binding enthalpy. This reduction could result from the second linker preventing the two cyclopeptide rings from adopting the optimal mutual arrangement for anion binding and/or from a larger enthalpy required for desolvation of **3c** and **3d**.

The properties of receptors **3c** and **3d** thus depend on a delicate balance between rigidity and flexibility.[16] Rigidification of the receptor into a macrobicyclic structure by incorporating two spacers enhances affinity and selectivity, when compared to the corresponding receptors with zero or one spacer. The best results were obtained when using two flexible linkers **c** as compared to the more rigid linker **d**. These results illustrate the challenge posed by rigidification: the positioning of the atoms involved in binding needs to be exactly right for optimal interactions, which is extremely difficult to achieve when this positioning is dictated by covalent bonds. Nature's solution to this problem is probably to shape binding sites using noncovalent interactions (*i.e.* through protein folding), which allow small conformational adjustments to be made more easily. In our covalently assembled system, the more flexible linker that is more tolerant toward conformational adjustments (*i.e.* induced fit) during complex formation gives rise to the better receptor. The importance of the right degree of flexibility[16] in the covalent framework is further underlined by the recent work in which two cyclopeptide subunits were covalently linked using click chemistry.[10e] In this system, the increase in the number of linkers between the two cyclopeptides from one to three has only a moderate impact on sulfate binding since the linkers adopt energetically unfavorable conformations in the complexes. We are currently investigating whether even better receptors can be obtained through a dynamic combinatorial approach targeting triply-linked bis(cyclopeptides).

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Notes and references

‡ Addition of organic cosolvent was required to ensure sufficient solubility of cyclopeptides **2** and **3**.
§ Symmetric spacer **c** was used instead of **b** as the latter gave rise to complex mixtures of stereoisomeric products.

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