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A Molecular Oyster: A Neutral Anion Receptor Containing Two Cyclopeptide Subunits with a Remarkable Sulfate Affinity in Aqueous Solution

Stefan Kubik,*,† R. Kirchner,‡ D. Nolting,§ and J. Seidel‡

Contribution from the Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine-Universität, Universitätsstrasse 1, D-40225 Düsseldorf, Germany, Institut für Physikalische Chemie, Technische Universität Bergakademie Freiberg, Leipziger Strasse 29, D-09596 Freiberg, Germany, and Institut für Physikalische Chemie und Elektrochemie, Heinrich-Heine-Universität, Universitätsstrasse 1, D-40225 Düsseldorf, Germany

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Abstract: An artificial anion receptor is presented, in which two cyclohexapeptide subunits containing L-proline and 6-aminopicolinic acid subunits in an alternating sequence are connected via an adipinic acid spacer. This compound was devised to stabilize the 2:1 sandwich-type anion complexes that are observed when the two cyclopeptide moieties are not covalently connected and to obtain a 1:1 stoichiometry for these aggregates. Electrospray ionization mass spectrometry and NMR spectroscopic investigations showed that the bridged bis(cyclopeptide) does indeed form defined 1:1 complexes with halides, sulfate, and nitrate. ROESY NMR spectroscopy and molecular modeling allowed a structural assignment of the sulfate complex in solution. The stabilities of various anion complexes were determined by means of NMR titrations and isothermal titration microcalorimetry in 50% water/methanol. Both methods gave essentially the same quantitative results, namely stability constants that varied in the range 10^5-10^2 M^{-1} and decreased in the order $SO_4^{2-} > I^- > Br^- > Cl^- > NO_3^-$. This order was rationalized in terms of the size of the anions with the larger anions forming the more stable complexes because they better fit into the cavity of the host. The ability of sulfate to form stronger hydrogen bonds to the NH groups of the receptor, in addition to its slightly larger ionic radius with respect to iodide, causes the higher stability of the sulfate complex. No significant effect of the countercation on complex stability was observed. Furthermore, complex stability is enthalpically as well as entropically favored. A comparison of the iodide and sulfate complex stabilities of the ditopic receptor with those of a cyclopeptide that forms 1:1 anion complexes in solution showed that the presence of a second binding site increases complex stability by a factor of 100−350.

Introduction

Many vitally important biochemical processes rest upon the specific interaction between proteins and anionic substrates such as carbonate, sulfate, or phosphate.[1] To achieve the high substrate affinity and selectivity necessary in these interactions, Nature has devised a number of efficient binding motifs. In the phosphate binding protein (PBP), for example, a protonated guanidinium group suitably placed inside the active center is an important element of substrate recognition.[2] Other natural systems, such as the sulfate binding protein (SBP), do not require strong electrostatic interactions for substrate binding. In these systems, affinity and selectivity is mainly controlled by a defined array of hydrogen bonds between the anion and NH groups of the protein backbone, serine OH, or tryptophane NH groups.[3]

These basic principles of anion complexation have been used extensively in the context of supramolecular chemistry for the design of artificial anion receptors.[4] Host molecules have been described, for example, that bind anions efficiently in polar, even aqueous, solution by means of strong electrostatic (or coordinative) interactions. Other receptors only use hydrogen bonds for substrate recognition.[5] Although these neutral systems often form stable anion complexes in competitive solvents such as DMSO or acetonitrile, complex formation in water generally proved to be weak if present at all, one reason for this being the high hydration energies of many anions. An exception in this respect is the cyclic hexapeptide with alternating L-proline

* To whom correspondence should be addressed. E-mail: kubik@uni-duesseldorf.de. Fax: +49-211-81-14788.

† Institut für Organische Chemie und Makromolekulare Chemie, Heinrich-Heine-Universität.

‡ Institut für Physikalische Chemie, Technische Universität Bergakademie Freiberg.

§ Institut für Physikalische Chemie und Elektrochemie, Heinrich-Heine-Universität.

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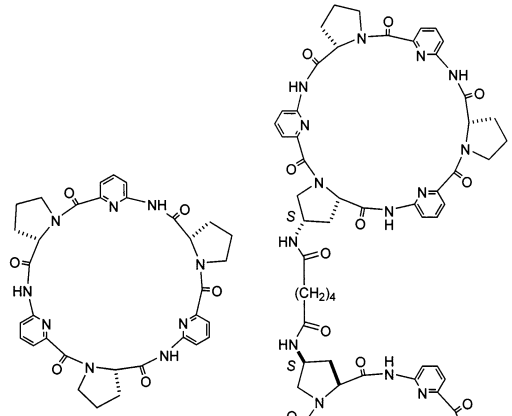
and 6-aminopicolinic acid subunits (**1**) that has recently been described by us.[6] This compound interacts with anions such as halides or sulfate by hydrogen bond formation even in water/methanol mixtures and thus represents a promising candidate for the development of new biomimetic anion hosts. On the basis of **1**, we have now developed a receptor of the next generation, whose synthesis and properties we describe here. Our new host **2** binds sulfate with a high affinity and selectivity in 50% water/methanol and can thus be regarded as a model for the sulfate binding protein.



1

2

Results and Discussion

Design and Synthesis. The unusual receptor properties of **1** are mainly a consequence of the special geometry of the anion complexes formed. Our structural assignment has shown that **1** preferentially forms sandwich-type 2:1 complexes with anions,[7] in which the guests reside in a cavity formed by the aggregation of two almost perfectly shape-complementary molecules of **1**.[6] In this cavity, the anions can interact with all six NH groups of the peptide moieties simultaneously and are effectively shielded from the surrounding solvent, a combination of effects that could provide a possible explanation for the stability of such complexes in aqueous solution.[8]

Recently, we succeeded in a quantitative determination of the stability of some anion complexes of **1**.[9] We found high overall stability constants for halide and sulfate complexes ranging from 10^4 to 10^5 M^{-2} in 80% D_2O/CD_3OD. Moreover, complex formation is cooperative; i.e., K_1, the stability constant of the 1:1 complex, is in every case significantly smaller than K_2, the stability constant that describes the equilibrium between the 1:1 and the 1:2 complex. Although higher aggregates are obviously preferred over 1:1 complexes, the composition of a 2:1 anion complex of **1** still depends markedly on the ratio and the concentration of receptor and substrate in solution. In general, a 2:1 complex predominates when an excess of receptor is present, whereas, in an excess of guest, a 1:1 complex is preferentially formed. Since the high anion affinity of **1** is mainly caused by the ability of the peptide to form sandwich-type complexes, we looked for ways to stabilize these aggregates. A possible approach is based upon a strategy that has been successfully applied in supramolecular chemistry to a number of artificial receptors, more recently, e.g., to cyclodextrins,[10] calixarenes,[11] or, in the context of anion receptors, Schmidtchen's macrotricyclic host[12] or sapphyrines,[13] namely the covalent linkage of two receptor subunits. Increasing the number of binding sites usually causes an improved affinity or selectivity of a host toward a substrate of interest, but in the case of **1**, we expected that connecting two cyclopeptide rings also provides a means to convert the observed 2:1 anion complexes into 1:1 complexes, whose binding equilibria are easier to describe. Success of this approach requires that the two cyclopeptide moieties are connected via a spacer that does not prevent their cooperative action in anion binding. Chosen appropriately, the spacer should allow a certain control over the binding properties of the corresponding host, however.

The proline rings in **1** are the most suitable positions for the introduction of a linker unit since they come in close contact in the anion complexes, and by replacement of one proline subunit of the peptide with a hydroxyproline residue, a functionalization in the form of a hydroxyl group can easily be introduced into the ring. Hydroxyl groups have the disadvantage, however, that they usually possess a low reactivity in hydroxyproline residues. Furthermore, we have shown that *4R*-configured hydroxyl groups in the proline residues of **1** prevent the formation of 2:1 complexes possibly because of steric effects.[9] We therefore decided to use a cyclopeptide as a building block for a ditopic receptor, in which one proline subunit of **1** carries an *S*-configured amino group in the 4 position (**3**). This compound was prepared from a derivative of **1** containing one tosylated *4R*-configured hydroxyproline by nucleophilic substitution of the tosyl group with azide followed by hydrogenation of the resulting product (Scheme 1).

We then used molecular modeling to identify a dicarboxylic acid able to connect two molecules of **3** while still enabling them to bind anions in a cooperative manner.[14] These calcula-

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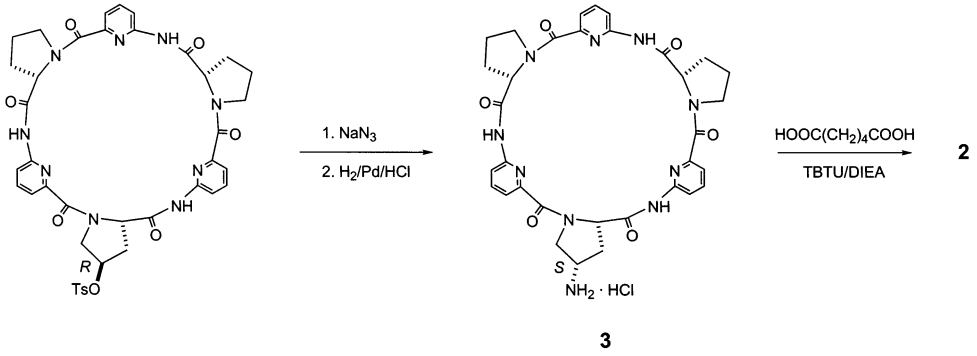
Scheme 1. Synthesis of Compounds **3** and **2**



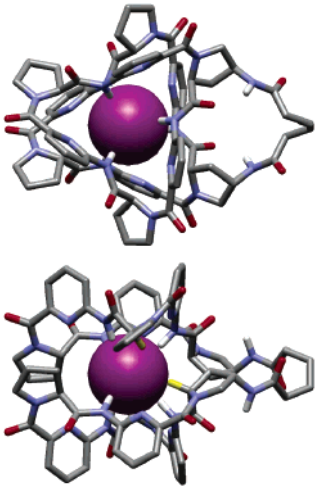


Figure 1. Top (above) and side (below) view of the calculated structure of the iodide (purple) complex of **2**. All hydrogen atoms except the ones at nitrogens have been omitted for reasons of clarity. In the side view, H(α) protons of a substituted and an unsubstituted proline ring are marked in yellow. The distance of these atoms amounts to 2.5 Å.

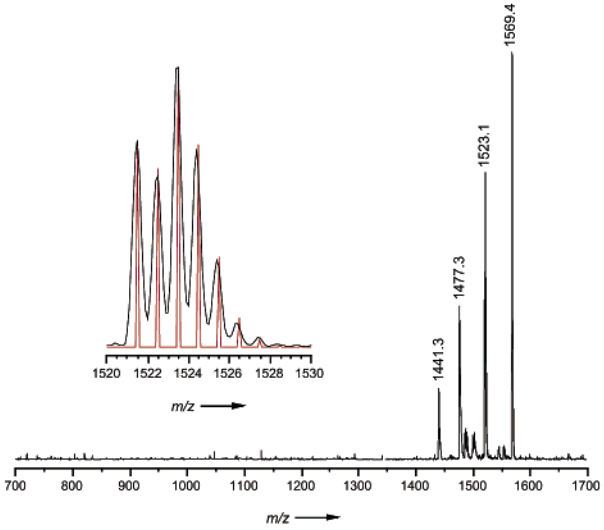


Figure 2. ESI mass spectrum of **2** in 80% H_2O/CH_3OH (0.1 mM) after addition of 0.33 equiv of each NaI, NaBr, and NaBr. The inset shows the fine structure of the signal of the 1:1 bromide complex of **2** with the black line representing the observed isotope distribution and the red line the calculated one.

tions were based on the crystal structure of the iodide complex of **1**. They indicated that adipinic acid should have the appropriate length and flexibility, making **2** a promising receptor candidate. The optimized structure of the iodide complex of **2** is depicted in Figure 1. Its structural relationship with the corresponding complex of **1** is high.[6] In both aggregates, the anion binds to all six peptide NH groups via hydrogen bonds, the two cyclopeptide subunits interlock perfectly like gears, and pairs of proline rings from different receptor moieties come in close contact. The overall symmetry of the iodide complex of **2** is C_2, however, because of the presence of the adipinic acid spacer.

Receptor **2** was obtained by reaction of **3** with adipinic acid under standard peptide coupling conditions in good yields and analytically pure after recrystallization (Scheme 1). Compound **2** is somewhat less soluble than **1**. Still, for electrospray mass spectrometric investigations, the same solvent mixture could be used as for **1** (80% H_2O/CH_3OH). NMR spectroscopic and microcalorimetric experiments had to be carried out in 50% D_2O/CD_3OD or 50% H_2O/CH_3OH, respectively.

Complex Stoichiometry. First indications of the anion affinity of **2** were obtained by using electrospray ionization mass spectrometry (ESI MS). This method is well suited for investigations on the complex formation between anions and macrocyclic host molecules as recently demonstrated by us and others.[6,15] In the negative ion mode, ESI MS not only allows a direct determination of the *m*/*z* ratio of the anion complexes formed but can also give information on their relative stabilities.[16]

The mass spectrum of a 0.1 mM solution of **2** in 80% H_2O/CH_3OH, to which 0.33 equiv of each NaCl, NaBr, and NaI was added, is depicted in Figure 2.

In addition to the signal of the free receptor [**2** − H^+] (*m*/*z* 1441.6), three other prominent signals are visible in this spectrum, whose *m*/*z* ratios correspond to complexes of the composition [**2** + Cl^-] (*m*/*z* 1477.5), [**2** + Br^-] (*m*/*z* 1523.5), and [**2** + I^-] (*m*/*z* 1569.5).[17] Thus, no significant amounts of complexes were detected, in which **2** binds two anions. Higher complexes with, e.g., two anions bound by two molecules of **2** have the same *m*/*z* ratio as simple 1:1 complexes. Such complexes are distinguishable from 1:1 complexes in the MS,

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(17) The smaller signals visible in the mass spectrum can be assigned to complexes of the composition [**2** + NO_2^-] and [**2** + NO_3^-]. Small amounts of these anions were obviously present in the solutions investigated. They originate either from the salts or the solvents used.

however, by analyzing the fine structures of the signals, which reflect the isotope distribution of an ion and thus differ distinctly for ions of different composition. Since the fine structures of the signals in Figure 2 are only consistent with 1:1 complexes between **2** and the different halides, a formation of higher aggregates can be ruled out (inset in Figure 2 and Supporting Information).[18] This result is further corroborated by the lack of signals in the spectrum that can be attributed to possible complexes, in which two or more molecules of **2** bind *different* anions.

The stoichiometry of the anion complexes of **2** could also be determined by NMR spectroscopy. For **1** we have shown that anion binding results in a downfield shift of the H(α) signal of the peptide in the ^{1}H NMR spectrum because of the spatial proximity of the corresponding protons to the negative charge density of the guest in the complex.[6,9] The reduced symmetry of **2** causes the three H(α) protons in a cyclopeptide subunit to be nonequivalent, and the ^{1}H NMR spectrum thus contains three H(α) signals, with the signal of the proton located in the substituted proline unit clearly separated from the other two that possess very similar chemical shifts. Upon addition of anions such as halides, sulfate, or nitrate to solutions of **2** in 50% D_2O/CD_3OD, all three H(α) signals shift downfield in the ^{1}H NMR spectrum, which clearly indicates that complex formation occurs. In addition, signals corresponding to aromatic protons at the aminopicolinic acid subunits of **2** are also shifted, an effect that has analogously been observed for **1**.[6,9] All of these signal shifts conveniently allow an evaluation of complex stoichiometry and stability by means of Job's method of continuous variation[19,20] and host−guest titrations,[19,21] respectively.

The Job plot of the iodide complex of **2** possesses a sharp maximum at an equimolar ratio of host and guest, which, taking the results of the mass spectrometric experiments also into consideration, indicates a 1:1 complex stoichiometry (Figure 3a).[19,20] In the case of the sulfate complex of **2**, a similar straightforward analysis is more difficult because an addition of less than 1 equiv of Na_2SO_4 to a solution of **2** in 50% D_2O/CD_3OD results in a significant line broadening of the H(α) signals in the ^{1}H NMR spectrum that prevents a quantitative evaluation of their shift. This effect is less pronounced for signals of the aromatic protons of **2**, which can be followed over the whole concentration range of a Job plot. All receptor signals become sharp again when the solution contains more than 1 equiv of Na_2SO_4, and the resonances of the H(α) signals in the corresponding NMR spectra are located downfield by ca. 1.1 ppm with respect to their positions in the spectrum of the free receptor. The observed line broadening in the NMR spectrum indicates a strong interaction between **2** and sulfate, and the fact that complex formation is almost complete after addition of only 1 equiv of guest is consistent with a 1:1 complex stoichiometry. This interpretation is corroborated by

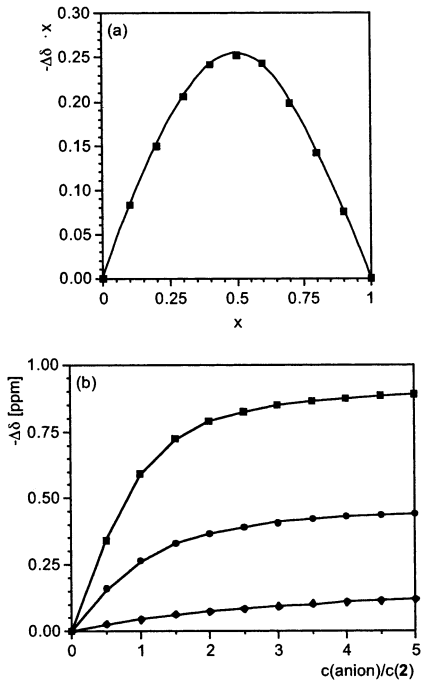

Figure 3. Job plot of the iodide complex of **2** (a) and ^{1}H NMR titration curves (b) of the iodide (squares), bromide (circles), and chloride (diamonds) complexes of **2** in 50% D_2O/CD_3OD. Experimental results are depicted as symbols, and the lines represent the calculated saturation curves.

the Job plots that have been obtained from analyzing the shifts of the aromatic protons of **2**. Taken together, both the ESI MS and the NMR investigations strongly indicate the formation of defined 1:1 complexes between anions and **2**, a stoichiometry that is in fact entropically the most favorable one.

Complex Structure. The high affinity of **2** toward sulfate allowed a structural investigation of the corresponding complex in solution by using ROESY NMR spectroscopy. In the ROESY NMR spectrum of uncomplexed **2** in 50% D_2O/CD_3OD (1 mM), pronounced cross-peaks are visible between the H(α) and H(γ) signals of the substituted prolines and between H(α) and H(δ) signals of the unsubstituted prolines, all of which indicate a spatial proximity of the corresponding pairs of protons within the five membered rings. Interactions between the two cyclopeptide moieties of **2** cannot be observed. Such effects are clearly visible in the spectrum of a solution of **2** (1 mM), however, that contains 5 equiv of Na_2SO_4. In particular, characteristic cross-peaks between the H(α) signals of a substituted and an unsubstituted proline unit and ones between the H(α) of a substituted and the H(β) of an unsubstituted proline unit (and vice versa) occur that account for a close contact of a substituted and an unsubstituted proline ring in the sulfate complex of **2**. The corresponding arrangement of the two cyclopeptide subunits of **2** is consistent with the one we calculated for the iodide complex (Figure 1). In this optimized structure, the distance between H(α) protons of substituted and unsubstituted proline rings (marked yellow in Figure 1) amounts to 2.5 Å, and that between H(α) and H(β) protons, to 3.3 Å. Assuming that on average **2** preferentially adopts a similar solution conformation in the sulfate complex, both distances are small enough to allow for cross-peaks in ROESY or NOESY NMR spectra. The NMR spectroscopic results thus indicate that the calculated structure in Figure 1 reflects characteristic aspects of the anion complexes of **2**. In the absence of suitable anions

(18) Besides the expected [**2** + SO_4^{2-}] complex, a much more intensive signal is observed in our electrospray mass spectrometric investigations of the sulfate complex of **2**, whose *m*/*z* ratio can be assigned to a complex of the composition [$\mathbf{2}_2$ + SO_4^{2-}]. Upon collision-induced dissociation (CID) of this ion the three fragments [**2** + SO_4^{2-}], [**2** + HSO_4^-], and [**2** − H^+] are obtained in almost equal amounts. A similar 2:1 complex could not be detected in solution. Its presence in the mass spectrometric investigations thus has to be attributed to the solvent-free conditions of the method.
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Table 1. Stability of Various Anion Complexes of **2** in 50% D_2O/CD_3OD Determined by NMR Titrations at $T = 298$ K[a]

	K_a	$\Delta\delta_{max}$	radius[23]
Na_2SO_4	3.5×10^5	0.20	230
NaI	8900	0.94	220
KI	11000	0.93	220
$(CH_3)_4NI$	11300	0.93	220
NaBr	5300	0.48	196
NaCl	710	0.19	181
$NaNO_3$	130	0.61	179

[a] K_a stability constants in M^{-1}. Errors in K_a <15%. $\Delta\delta_{max}$ = maximum chemical shifts of the receptor signal followed during the titration in ppm: In the case of the sulfate complex, the signal of protons in the 3 position of an aromatic subunit were used, in all other cases, the signal of the protons in α position of the substituted proline rings. Ionic radii of the anions are in pm.

in solution, **2** seems to prefer more open conformations as indicated by the lack of cross-peaks between the cyclopeptide moieties in the ROESY NMR spectrum.

Complex Stability. We first used NMR titrations to determine the stability of some anion complexes of **2** (Figure 3b).[19,21] Only salts of strong acids were used as guests in these measurements to avoid protonation equilibria during the titrations and the need to use buffers. The stability constant of the sulfate complex of **2** could only be determined by using a dilution titration and by analyzing the signal shifts of the aromatic receptor protons because of the high stability of this complex and the line broadening of the H(α) signals in the NMR spectrum. The resulting K_a approaches the upper limit that can be determined reliably by NMR titrations, and its experimental error is therefore higher than that in the other stability constants determined.[22] The order of magnitude of the stability of the sulfate complex should be correctly reflected in the K_a obtained, however. All stability constants were independent of the proton that was followed during the titration, which is a further indication that the model of a 1:1 complex stoichiometry is correct and that no higher equilibria have to be considered. The results of our investigations are summarized in Table 1.

Table 1 shows that the stability constants of the anion complexes investigated cover a range of 3 orders of magnitude between 10^2 and 10^5 M^{-1}, and as a consequence, the binding selectivity of **2** is quite good. The only stability constants that differ by a relatively small factor of 1.7 are the ones of the iodide and the bromide complex of **2**. For all other pairs of anion complexes, a ratio of at least 5 is observed in their K_a.[24] The observed trend in complex stability seems to depend mainly on the size of the bound anion with larger anions forming more stable complexes most probably because they better fit into available space between the two cyclopeptide moieties of **2**. Thus, despite the fact that, e.g., the smaller halides can form stronger hydrogen bonds to the NH groups of **2**, the better size complementarity between iodide and the receptor cavity, which is evident in Figure 1, is responsible for the higher complex stability. A similar trend has also been observed for one of Schmidtchen's macrotricyclic anion hosts in methanol.[25] The slightly larger size of the sulfate ion with respect to iodide most probably cannot account alone for the high stability of the sulfate complex. Important additional factors that favorably contribute to complex stability are the higher charge of sulfate and its ability to form strong hydrogen bonds to NH groups via the oxygens.[26] Table 1 also shows that, within the error limits, the stability of the iodide complex is not significantly affected by the countercation. To the best of our knowledge, no other neutral anion receptor that forms similar stable complexes in protic solvents has been described so far.

To support these results, we also investigated the anion affinity of **2** by using isothermal titration calorimetry (ITC).[27] This method directly measures the heat evolved or absorbed during complex formation in dependence on the molar ratio of receptor to ion. It thus provides a means to not only determine the enthalpy of complex formation (ΔH) but also to evaluate the stability constant (K_a) and the free energy of complex formation (ΔG) as well as the entropic contribution (ΔS). A number of different anion receptors were characterized microcalorimetrically, and it was shown that, in accordance with the simple electrostatic model of ion pairing,[28] the interactions between anions and charged receptors in polar solvents are often endothermic making entropic factors, i.e., the release of solvent molecules from the solvation spheres of host and guest, the major driving force of complex formation.[29] In less polar solvents or with neutral hosts also exothermic anion complexation has been observed with either a favorable[30] or unfavorable[31] contribution of entropy. In a preliminary microcalorimetric investigation, we observed a small endothermic effect when iodide interacted with **1** in 80% H_2O/CH_3OH, indicating that complex formation is mainly driven by entropy.[6] By using receptor **2** instead of **1** and changing the solvent mixture to 50% H_2O/CH_3OH (as well as using a more sensitive microcalorimeter), we were now able to investigate complex formation much more accurately (Figure 4). The results of our measurements are summarized in Table 2.

For every anion complex, the stability constant determined by ITC is slightly smaller than the corresponding one determined by NMR titration. Nevertheless, the agreement between the spectroscopically and calorimetrically determined log K_a values is quite good. The only case in which a larger difference between the two stability constants is observed is the sulfate complex, most probably because of the experimental error that has to be considered for the spectroscopically determined K_a. The general trend of anion complex stabilities of **2**, namely $SO_4^{2-} > I^- > Br^- > Cl^-$, is clearly confirmed by ITC, however.

Table 2 shows that complex formation is in every case enthalpically as well as entropically favored. The fact that all

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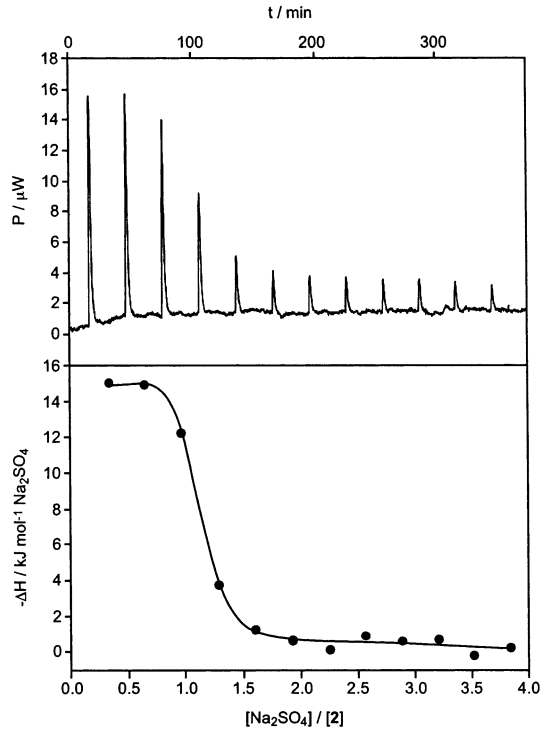


Figure 4. Isothermal calorimetric titration of **2** (0.45 mM) with Na_2SO_4 (14 mM) in 50% H_2O/CH_3OH at 298 K. The upper graph shows the measured heat pulses. The molar heats/pulse are depicted along with the curve fit (solid line) in the lower diagram.

Table 2. Stability of Various Anion Complexes of **2** in 50% H_2O/CH_3OH Determined by Isothermal Microcalorimetry at T = 298 K

	NMR	ITC			
	log K_a	log K_a	ΔG (kJ·mol^{-1})	ΔH (kJ·mol^{-1})	$T\Delta S$ (kJ·mol^{-1})
Na_2SO_4	5.54	4.55 ± 0.23	−26.0 ± 1.3	−15.0 ± 0.9	11.0 ± 2.2
NaI	3.95	3.79 ± 0.26	−21.6 ± 1.5	−13.2 ± 1.0	8.4 ± 2.5
KI	4.04	3.51 ± 0.34	−20.0 ± 1.9	−14.7 ± 1.0	5.3 ± 2.8
NaBr	3.72	3.45 ± 0.39	−19.7 ± 2.2	−11.2 ± 0.9	8.5 ± 3.1
NaCl	2.85	2.51 ± 0.86	−14.3 ± 4.9	−10.5 ± 1.5	3.8 ± 6.4

reactions are exothermic demonstrates that the inclusion of an anion into the cavity of **2** represents an energetically more stable situation for the system than the separated hydrated components even in the protic solvent mixture used. The dependence of complex stability on the type of anion is mainly determined by ΔH, and the more negative ΔH observed for the complexation of the larger anions again reflects the importance of an optimal fit of the guests in the available cavity. The difference in ΔH upon iodide and sulfate complexation is consistent with the fact that sulfate can form stronger hydrogen bonds to the NH groups of **2**. Entropic factors are also important for anion binding and constitute ca. 40% of the observed free enthalpy of complex formation in every case. The dependence of ΔS on the type of anion is less obvious, however, possibly because of superimposing effects of desolvation, not necessarily complete desolvation, of both host and guest. Our ITC measurements thus nicely complement the NMR experiments as well as provide valuable information on the thermodynamics of anion complexation by **2**. They support the view that, by the right choice of receptor structure, artificial receptors can be devised that strongly bind to suitable substrates by weak interactions even in highly competitive solvents.

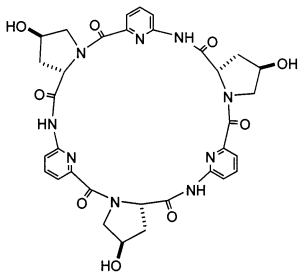
Table 3. Comparison of the Stabilities of the Sulfate and Iodide Complexes of **1** and **2** at T = 298 K[a]

		50% D_2O/ CD_3OD					80% D_2O/CD_3OD	
			1		**4**		**1**	
		K_a for **2**	K_a	$\Delta\delta_{max}$	K_a	$\Delta\delta_{max}$	K_a[9]	$\Delta\delta_{max}$
Na_2SO_4	K_1	35500	360	0.59	260	0.59	96	0.44
	K_2		8760	0.96			1270	0.77
	K_T		3.15×10^6				1.22×10^5	
	ΔG	−26.0	−37.1		−13.8		−29.0	
NaI	K_1	8900	30	0.64	26	0.69	22	0.55
	K_2		7670	0.82			7380	0.84
	K_T		2.30×10^5				1.62×10^5	
	ΔG	−22.6	−30.6		−8.1		−29.7	

[a] K_1 and K_2 in M^{-1}. K_T in M^{-2}. Errors in stability constants for the complexes of **1** < 40%. ΔG in kJ·mol^{-1}. $\Delta\delta_{max}$ = maximum chemical shifts of a H(α) signal of **1** or **4** in ppm.

Monotopic Receptor vs Ditopic Receptor. So far, we have shown that a receptor composed of two covalently linked molecules of **1** can bind anions in protic solvents with a defined 1:1 stoichiometry and high affinity. How does the anion affinity of this compound compare to that of the parent peptide **1**, however?

Complex stability of **1** is more difficult to determine because of the higher stoichiometry of the anion complexes of this peptide. Saturation curves obtained from NMR titrations, e.g., have to be fitted to four parameters, namely K_1, K_2, $\Delta\delta_{max1}$, and $\Delta\delta_{max2}$, to describe the underlying equilibrium correctly. The required mathematical treatment very often leads to more than one solution, which complicates a straightforward analysis. We recently solved this problem by comparing the anion affinity of **1** with the one of a structurally related cyclopeptide **4**, in which all three proline subunits are replaced by 4*R*-configured hydroxyprolines.[9]



4

This peptide adopts a similar conformation in solution as **1**. Because of the additional hydroxyl groups, **4** can only form 1:1 complexes with anions, however, whose stabilities are of the same order of magnitude as those of the 1:1 complexes of **1**. The stability constants of the anion complexes of **4** thus provide a good quantitative estimation for the first binding step in the interaction of **1** with anions and as a consequence allow a reliable evaluation of the whole complexation equilibrium by using NMR titrations. We used this strategy to determine the stability constants of a number of anion complexes of **1** in 80% D_2O/CD_3OD.[9] To be able to compare the anion affinities of **1** and **2**, we now repeated these measurements in 50% D_2O/CD_3OD for the iodide and the sulfate complex. The results obtained are summarized in Table 3, which also contains the complex stabilities in 80% D_2O/CD_3OD for comparison.

Table 3 shows that the change of the solvent has only a minor effect on the stability of the iodide complex of **1**. The sulfate

complex, however, is significantly more stable in 50% D_2O/CD_3OD than in 80% D_2O/CD_3OD. This result is consistent with our assumption that hydrogen bonds are an important factor in the stabilization of the sulfate complexes of **1** or **2**. These interactions become considerably stronger when the polarity of the solvent decreases, thus causing the observed increase in complex stability. The strong dependence of sulfate complex stability on the solvent composition also suggests, however, that the binding selectivity of **2** for sulfate over iodide in 50% D_2O/CD_3OD could be an effect of the solvent and not an intrinsic property of the receptor. This assumption is supported by the fact that the selectivity observed for **1** in 50% D_2O/CD_3OD is not only reduced but even reversed in favor of iodide in 80% D_2O/CD_3OD. To be able to study whether the same trend is observed for **2** we will now synthesize a better water-soluble derivative of this receptor.

A comparison of the anion complex stabilities of receptors **1** and **2** in 50% D_2O/CD_3OD on the basis of ΔG shows that the anion affinity of the ditopic receptor is smaller, possibly because the adipinic acid spacer in **2** affects the optimal complex geometry of the 2:1 complexes of **1** unfavorably. To gain a deeper insight into which thermodynamic factor is responsible for the reduced anion affinity of **2**, we characterized the iodide and the sulfate complex of **1** also calorimetrically. For the sulfate complex, we obtained an enthalpy of complex formation ΔH of -19.3 ± 1.6 kJ·mol^{-1} and a complex stability of $\log K_T = 6.48 \pm 0.08$, which is in excellent agreement with the stability constant determined by NMR titration ($\log K_T = 6.50$). Entropy $T\Delta S$ thus amounts to 17.7 kJ·mol^{-1}. Moreover, the microcalorimetric titration clearly confirmed the 2:1 complex stoichiometry of the sulfate complex of **1**.

The more exothermic reaction observed in the interaction between sulfate and **1** (for **2,** $\Delta H = -15.0 \pm 0.9$ kJ·mol^{-1}) supports our assumption that the sulfate complex of **1** is more stable than that of **2**. The fact that also entropy favors the complex formation of the monotopic receptor was surprising because we expected that a chelate effect, which is usually discussed in terms of entropy, would act in favor of the bis-(cyclopeptide). A similar entropic disadvantage for complex formation has also been reported for cyclodextrin dimers, however, and this result was interpreted in terms of a reduced flexibility of the ditopic hosts in the complex.[32] The large increase in stability observed for the complexes of cyclodextrin dimers with respect to their monotopic counterparts has therefore been ascribed to an *enthalpic* chelate effect.[32] In comparing the sulfate affinity of receptors **1** and **2**, we could detect neither an entropic nor an enthalpic chelate effect, however.

For iodide complexation of **1,** the microcalorimetric titration gave a ΔH for complex formation in 50% H_2O/CH_3OH, which is of the same order of magnitude as that of the iodide complex of **2** (**1**, $\Delta H = -14.8 \pm 2.8$ kJ·mol^{-1}; **2**, $\Delta H = -13.2 \pm 1.0$ kJ·mol^{-1}). The smaller stability of the iodide complex of **2** must therefore also be caused by an unfavorable entropic effect. Unfortunately, we were not able to get a good fit for the calorimetric binding curve in this case so that the exact contribution of entropy could not be quantified.

That the covalent linkage of two subunits of **1** does have a pronounced effect on receptor properties is evident when the anion complex stabilities of **2** are compared with those of **4** (or with K_1 of the complexes of **1**). Doubling the number of receptor sites in **2** with respect to **4** causes a 2-fold increase in the free energy of complex formation, which translates into a 140-fold increase of complex stability (100-fold increase of K_1 of **1**) in the case of the sulfate complex. The receptor properties of **2** thus almost exactly reflect the individual contributions of the two cyclopeptide subunits. A larger cooperative effect is observed for iodide binding. In this case, ΔG is 2.7 times larger than that of the iodide complex of **4** (and 2.6 times larger than the ΔG corresponding to K_1 of **1**), which translates into a ca. 340 times increase in the stability constant. This comparison thus clearly demonstrates the cooperativity of the two cyclopeptide moieties of **2** in anion complexation. An additional stabilizing effect that seems to operate in the sandwich-type anion complexes of **1** is most probably compensated by an unfavorable influence of the adipinic acid spacer in **2**. Future investigations will show if cooperativity can be further improved by a structural optimization of the spacer between the two cyclopeptide subunits.

Conclusion

In conclusion, we have shown that by covalently linking two cyclopeptide molecules derived from **1** with a suitable spacer, an artificial receptor is accessible that strongly binds anions in 50% water/methanol. In this solvent, we observed a particularly high affinity and selectivity for sulfate. Microcalorimetric investigations showed that anion binding is enthalpically as well as entropically driven. We use the term *molecular oyster* for this type of receptor because the two perfectly interlocking receptor subunits that are connected via a flexible hinge resemble the two halves of a shell. In the presence of suitable anions, this shell closes and binds the guests in an inner cavity like pearls. We expect that receptor properties and solubility of such anion hosts can be controlled by varying the nature (and the number) of the spacer unit. Investigations in this context are currently underway.

Experimental Section

General Methods. Analyses were carried out as follows: melting points, Büchi 510 apparatus; optical rotation, Perkin-Elmer 241 MC digital polarimeter ($d = 10$ cm); NMR, Varian VXR 300, Bruker DRX 500 equipped with an automatic sampler; FT IR, Bruker Vector 22 FT-IR spectrometer; FAB MS, Finnigan MAT 8200; ESI MS, Bruker Esquire 3000; microcalorimetry, Thermal Activity Monitor with 4 mL high performance calorimetric unit and titration microreaction cell, Thermometric AB; elemental analysis, Pharmaceutical Institute of the Heinrich-Heine-University, Düsseldorf, Germany; RP chromatography, MERCK LiChroprep RP-8 (40−63 μm) prepacked column size B (310-25). The following abbreviations are used: Bn, benzyl; BOC, *tert*-butoxycarbonyl; DIEA, *N*-ethyldiisopropylamine; TFA, trifluoroacetic acid; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; TBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; Hyp, L-hydroxyproline; Pro, L-proline; APA, 6-aminopicolinic acid; Adi, adipinic acid.

Job Plot. Equimolar solutions of **2** (0.5 mM) and of the guest in 50% D_2O/CD_3OD were prepared and mixed in various ratios. ^{1}H NMR spectra of the solutions were recorded, and the change in chemical shifts of the proline H(α), APAH(3), and APAH(5) protons were analyzed.

NMR Titrations. Stock solutions of the guest (50 μmol/500 μL for titrations with **1** or **4**, 2.5 μmol/500 μL for titrations with **2**) in D_2O and a peptide (1 μmol/500μL for titrations with **1** or **4**, 0.5 μmol/500μL

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for titrations with **2**) in 0.002% TMS/CD_3OD were prepared. In total, 11 NMR tubes were set up for titrations with **2** or **4** and 21 for titrations with **1** by adding increasing amounts of the guest solution (0−500 μL) to 500 μL aliquots of the host solution. All samples were made up to a volume of 1 mL with D_2O, and the respective ^{1}H NMR spectra were recorded. The chemical shifts of the H(α), APAH(3), or APAH(5) protons of the peptide were referenced against the internal standard used, and plotted against the ratio of guest/host concentration. From the resulting saturation curves, K_a and $\Delta\delta_{max}$ were calculated by the suitable nonlinear least-squares fitting method described for 1:1[19,21] or 1:2[33] complexes using the SIGMA Plot 3.0 (Jandel Scientific) software package.

Microcalorimetry. In a typical experiment, solutions of the guest (15 mM) in 50% H_2O/CH_3OH were injected stepwise (8 μL per step) at a rate of 1 μL/s to a solution of **1** or **2** in the same solvent mixture (0.75 mL, 0.5 mM). The next titration step was always started after reaching chemical and thermal equilibrium. The measured heat flow was recorded as function of time and converted into enthalpies by integration of the appropriate reaction peaks. Dilution effects were corrected by subtracting the results of a blank experiment with a solution of 50% H_2O/CH_3OH in place of the receptor solution under identical experimental conditions. The association parameters were evaluated by means of a computer program generated using the MATLAB programming package (Mathworks Inc., Natick, MA).

Dipeptide BOC-(4*R*)-Hyp-APA-OBn. 6-Amino-2-picolinic acid benzyl ester (1.71 g, 7.50 mmol) (supplementary information of ref 6), BOC-*(4R)*-L-hydroxyproline (2.61 g, 11.3 mmol), and PyCloP (4.76 g, 11.3 mmol) were dissolved in CH_2Cl_2 (150 mL). At room temperature, DIEA (3.9 mL, 22.6 mmol) was added dropwise, and then the reaction mixture was stirred for 5 d. The solvent was subsequently evaporated in vacuo, and the residue was subjected to a silica gel column (hexane/ethyl acetate, 3:1; ethyl acetate). All fractions containing the product were collected and evaporated to dryness in vacuo. The remaining residue was dissolved in CH_2Cl_2 (100 mL), and this solution was poured into diethyl ether (800 mL) under stirring. After 10 min, the precipitate was filtered off, and the filtrate was again evaporated to dryness. From the residue, pure product was isolated by another chromatographic purification step on a silica gel column (hexane/ethyl acetate, 1:15): yield 2.41 g (73%); mp 78−82 °C; $[\alpha]^{25}_D = -39.0$ ($c = 2$, MeOH); ^{1}H NMR (300 MHz, [d_6]DMSO, 100 °C, TMS) δ 1.32 (s, 9H; *t*BuCH_3), 1.97 (m, 1H; HypC(β)H), 2.15 (m, 1H; HypC(β)H), 3.30 (d, ^2J = 11.0 Hz, 1H; HypC(δ)H), 3.49 (dd, ^2J = 11.0 Hz, ^3J = 4.8 Hz, 1H; HypC(δ)H), 4.30 (m, 1H; HypC(γ)H), 4.55 (t, ^3J = 7.6 Hz, 1H; HypC(α)H), 4.71 (d, ^3J = 3.8 Hz, 1H; OH), 5.38 (s, 2H; $PhCH_2$), 7.39 (m, b, 5H; PhH), 7.76 (dd, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H; APAH(3)), 7.94 (t, ^3J = 7.7 Hz, 1H; APAH(4)), 8.26 (dd, ^3J = 7.7 Hz, ^4J = 1.0 Hz, 1H; APAH(5)), 10.47 (s, 1H; APANH). Anal. Calcd for $C_{23}H_{27}N_3O_6 \cdot 2H_2O$ (M_r = 477.5): C, 57.85; H, 6.54; N, 8.80. Found: C, 58.16; H, 6.21; N, 8.64.

Tosylated Dipeptide BOC-(4*R*)-4TsHyp-APA-OBn. BOC-(4*R*)-Hyp-APA-OBn (2.21 g, 5.00 mmol) was dissolved in a mixture of methylene chloride/pyridine, 1:1 (10 mL). Toluenesulfonyl chloride (4.77 g, 25 mmol) was added in one portion, and the reaction mixture was stirred at room temperature overnight. Afterward, the solvent was evaporated in vacuo, and the residue was dissolved in ethyl acetate. The resulting solution was washed three times with 10% aqueous Na_2CO_3 and three times with water, and the solvent was again removed. The product was isolated from the residue chromatographically (hexane/ethyl acetate, 1:1). The product solidified upon drying in vacuo: yield 2.74 g (92%); mp 67−73 °C; $[\alpha]^{25}_D = -26.6$ ($c = 2$, MeOH); ^{1}H NMR (300 MHz, [d_6]DMSO, 25 °C, TMS) (some signals split in the spectrum due to a slow rotation around the tertiary amide bond; at elevated temperatures, decomposition of the product occurred in DMSO) δ 1. 24 + 1.34 (2 s, 9H; *t*BuCH_3), 2.14 (m, 1H; HypC(β)H), 2.37 (m, 1H; HypC(β)H), 2.44 (s, 3H; TsCH_3), 3.46 (dt, ^2J = 12.3 Hz, 1H; HypC(δ)H), 3.54 (dd, ^2J = 12.4 Hz, ^3J = 3.5 Hz, 1H; HypC(δ)H), 4.55 (m, 1H; HypC(α)H), 5.11 (m, 1H; HypC(γ)H), 5.39 (s, 2H; $PhCH_2$), 7.35−7.53 (m, b, 7H; PhH + TsH(3)), 7.83 (m, 3H; APAH(3) + TsH(4)), 7.99−8.02 (2 t, ^3J = 8.2 Hz, 1H; APAH(4)), 8.29 + 8.34 (2 d, ^3J = 8.2 Hz, 1H; APAH(5)), 11.04 + 11.11 (2 s, 1H; APANH). Anal. Calcd for $C_{30}H_{33}N_3O_8S$ (M_r = 595.7): C, 60.49; H, 5.58; N, 7.05. Found: C, 60.42; H, 5.60; N, 6.94.

Tosylated Cyclopeptide. For the synthesis of the linear hexapeptide precursor of a cyclopeptide moiety of **2**, the BOC deprotected dipeptide H-(4*R*)-4TsHyp-APA-OBn and a tetrapeptide with proline subunits BOC-[Pro-6APA]$_2$-OH was coupled as described previously by us for similar compounds (see for example, supplementary information of ref 6). For the cyclization, this hexapeptide (1.03 g, 1 mmol) was deprotected at both ends of the peptide chain and dissolved in a mixture of degassed DMF (40 mL) and DIEA (1.04 mL, 6 mmol). The resulting solution was added dropwise over the course of 4 h to a solution of TBTU (1.60 g, 5 mmol) and DIEA (0.42 mL, 2.4 mmol) in degassed DMF (200 mL) at 80 °C. If necessary, the pH of the reaction mixture was adjusted afterward to ca. 9 by adding more DIEA, and stirring was continued for 1 h at 80 °C. The solvent was then evaporated in vacuo, and the product was isolated from the residue by chromatographic workup. An initial purification step was carried out using a silica gel column (acetone). The material recovered was further purified on an RP-8 column. For this, it was dissolved in a small amount of DMF and applied to a column conditioned with 1,4-dioxane/H_2O, 1:10. The eluent composition was gradually changed until the pure product eluted (1,4-dioxane/H_2O, 1:1). The material thus obtained was dissolved in acetone (20 mL), and the resulting solution was poured slowly in diethyl ether (200 mL) under stirring. Stirring was continued for 15 min, and the precipitate was filtered off and dried in vacuo: yield 0.33 g (40%); mp 205 °C (dec); $[\alpha]^{25}_D = -516.5$ ($c = 2$, DMF); ^{1}H NMR (300 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.84 (m, 4H; ProC(γ)H_2), 2.06 (m, 2H; ProC(β)H), 2.23 (m, 1H; HypC(β)H), 2.45 (s, 3H; TsCH_3), 2.62 (m, 2H; ProC(β)CH), 2.96 (m, 1H; HypC(β)H), 3.55−3.75 (m, b, 6H; ProC(δ)H_2 + HypC(δ)H_2), 5.14 (m, 1H; HypC(γ)H), 5.54 (m, 2H; ProC(α)H), 5.66 (dd, $^3J(H_{ax},H_{ax})$ = 7.0 Hz, $^3J(H_{ax},H_{eq})$ = 6.2 Hz, 1H; HypC(α)H), 7.14 (d, ^3J = 8.2 Hz, 1H; APAH(3)), 7.22 (d, ^3J = 8.2 Hz, 1H; APAH(3)), 7.27 (d, ^3J = 8.2 Hz, 1H; APAH(3)), 7.45 (m, b, 3H; APAH(5)), 7.54 (d, ^3J = 7.9 Hz, 2H; TsH(3)), 7.69−7.78 (m, b, 3H; APAH(4)), 7.88 (d, ^3J = 8.2 Hz, 2H; TsH(2)), 9.70 + 9.72 + 9.83 (3 s, 3 × 1H; APANH); ^{13}C NMR (75 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.3 + 22.4 (ProC(γ)), 30.6 (TsCH_3), 32.3 + 32.5 (ProC(β)), 38.2 (HypC(β)), 47.9 + 48.1 (ProC(δ)), 54.0 (HypC(δ)), 59.9 (HypC(α)), 61.4 + 61.5 (ProC(α)), 79.0 (HypC(γ)), 115.4 + 115.6 + 116.2 (APAC(3)), 119.5 + 119.6 + 119.7 (APAC(5)), 127.5 (TsC(2)), 130.3 (TsC(3)), 132.6 (TsC(4)), 138.8 + 138.9 (APAC(4)), 145.3 (TsC(1)), 148.3 + 148.5 + 148.6 (APAC(2)), 151.3 + 151.7 + 152.0 (APAC(6)), 165.7 + 166.1 + 166.3 (APACO), 169.6 + 170.8 + 171.1 (HypCO/ProCO). Anal. Calcd for $C_{40}H_{39}N_9O_9S \cdot 2H_2O$ (M_r = 857.9): C, 56.00; H, 5.05; N, 14.69. Found: C, 55.87; H, 5.07; N, 14.42. FAB-MS [*m*/*z* (relative intensity)]: 822 (30) [M + H^+].

Cyclopeptide Azide. The tosylated cyclopeptide (0.45 g, 0.55 mmol) was dissolved in DMF (10 mL). After the addition of sodium azide (0.18 g, 2.75 mmol), the reaction mixture was heated to 80 °C for 6 h. The solvent was then removed in vacuo, and the product was isolated from the residue by chromatographic workup using a silica gel column (CH_2Cl_2/MeOH, 5:1). Trituration with diethyl ether of the material recovered afforded an off white solid: yield 0.33 g (87%); mp 184 °C (dec); $[\alpha]^{25}_D = -530.0$ ($c = 2$, DMF); ^{1}H NMR (500 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.84 (m, 4H; ProC(γ)H_2), 2.05 (m, 2H; ProC(β)H), 2.41 (td, ^2J = 13.6 Hz, 1H; HypC(β)H), 2.57 (m, 2H; ProC(β)H), 2.86 (m, 1H; HypC(β)H), 3.59 (m, 2H; ProC(δ)H), 3.71 (m, 3H; ProC(δ)H + HypC(δ)H), 3.83 (dd, ^3J = 6.0 Hz, ^2J = 12.9 Hz, 1H; HypC(δ)H), 4.48 (m, 1H; HypC(γ)H), 5.60 (t, ^3J = 7.0 Hz, 1H; ProC(α)H), 5.64

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(t, ^3J = 7.1 Hz, 1H; ProC(α)H), 5.85 (dd, $^3J(H_{ax},H_{ax})$ = 8.7 Hz, $^3J(H_{ax},H_{eq})$ = 3.6 Hz, 1H; HypC(α)H), 7.15 (d, ^3J = 8.2 Hz, 1H; APAH(3)), 7.34 (d, ^3J = 8.2 Hz, 1H; APAH(3)), 7.43 (m, 3H; APAH(3) + 2 APAH(5)), 7.49 (d, ^3J = 7.3 Hz, 1H; APAH(5)), 7.71 (t, ^3J = 7.9 Hz, 1H; APAH(4)), 7.75 (t, ^3J = 7.6 Hz, 2H; APAH(4)), 9.58 + 9.61 + 9.69 (3 s, 3 × 1H; APANH); ^{13}C NMR (125 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.2 + 22.3 (ProC(γ)), 32.3 + 32.4 (ProC(β)), 36.9 (HypC(β)), 48.0 (ProC(δ)), 52.2 (HypC(δ)), 56.4 (HypC(γ)), 60.1 (HypC(α)), 61.3 + 61.5 (ProC(α)), 114.9 + 115.6 + 116.1 (APAC(3)), 119.4 + 119.7 (APAC(5)), 138.8 + 139.0 + 139.1 (APAC(4)), 148.4 + 148.5 + 148.7 (APAC(2)), 151.4 + 151.7 + 151.9 (APAC(6)), 165.7 + 165.8 (APACO), 169.6 + 170.9 + 171.0 (HypCO/ProCO); IR (KBr) 2107 cm^{-1} (azide). Anal. Calcd for $C_{33}H_{32}N_{12}O_6 \cdot 2.5H_2O$ (M_r = 737.7): C, 53.73; H, 5.06; N, 22.78. Found: C, 54.02; H, 5.17; N, 22.51. FAB-MS [*m*/*z* (relative intensity)]: 693 (30) [M + H$^+$].

Bis(cyclopeptide) (2). The azide prepared in the previous step (310 mg, 0.45 mmol) was dissolved in CH_2Cl_2/MeOH, 1:1 (75 mL). After the addition of 1 N HCl (0.45 mL) and 10% Pd/C (100 mg), the resulting reaction mixture was subjected to hydrogenation at 1 atm for 18 h. The catalyst was then filtered off by passage through a layer of Celite and washed with methanol. The combined filtrate and washings were evaporated to dryness in vacuo, the residue was dissolved in CH_2Cl_2, and the solvent was evaporated again. The crude product (**3**) was dried in vacuo and was used for the coupling reaction without further purification.

Cyclopeptide **3** (270 mg, 0.4 mmol) and adipinic acid (30 mg, 0.2 mmol) were dissolved in DMF (20 mL). In succession, TBTU (140 mg, 0.44 mmol) and DIEA (220 μL, 1.28 mmol) were added, and the resulting mixture was stirred 3 h at room temperature. Afterward, the solvent was evaporated in vacuo, and the product was isolated by chromatographic workup. For this, the residue was dissolved in a small amount of DMF. The resulting solution was applied to a RP-8 column conditioned with 1,4-dioxane/H_2O, 1:10, and the eluent composition was gradually changed to 1,4-dioxane/H_2O, 1:2, with which pure product eluted. The material recovered was dissolved in boiling methanol (50 mL). Under reflux, water (200 mL) was slowly added, and the solution was kept at room-temperature overnight. The precipitated product was filtered off, washed with water, and dried: yield 0.21 g (73%); mp > 250°C; $[\alpha]^{25}_D = -538.3$ (*c* = 2, DMF); ^{1}H NMR (500 MHz, [d_6]DMSO, 25 °C, TMS) δ 1.27 (m, 4H; AdiC(β)H_2), 1.74−2.09 (m, b, 18H; AdiC(α)H_2 + ProC(γ)H_2 + ProC(β)H + HypC(β)H), 2.57 (m, 4H; ProC(β)H), 2.84 (m, 2H; HypC(β)H), 3.43 (m, 2H; HypC(δ)H), 3.59 (m, 4H; ProC(δ)H), 3.68 (m, 4H; ProC(δ)H), 3.87 (m, 2H; HypC(δ)H), 4.28 (m, 2H; HypC(γ)H), 5.54 (t, ^3J = 6.9 Hz, 2H; HypC(α)H), 5.60 (m, 4H; ProC(α)H), 7.20 (d, ^3J = 8.2 Hz, 2H; APAH(3)), 7.22 (d, ^3J = 8.2 Hz, 2H; APAH(3)), 7.27 (d, ^3J = 8.2 Hz, 2H; APAH(3)), 7.41 (d, ^3J = 7.6 Hz, 2H; APAH(5)), 7.42 (d, ^3J = 7.6 Hz, 2H; APAH(5)), 7.45 (d, ^3J = 7.6 Hz, 2H; APAH(5)), 7.74 (m, 6H; APAH(4)), 7.89 (d, ^3J = 6.3 Hz, 2H; AdiNH), 9.60 + 9.62 + 9.68 (3 s, 3 × 1H; APANH); ^{13}C NMR (125 MHz, [d_6]DMSO, 25 °C, TMS) δ 22.2 + 22.3 (ProC(γ)), 24.6 (AdiC(β)), 32.3 + 32.4 (ProC(β)), 34.9 (AdiC(α)), 37.2 (HypC(β)), 46.0 (HypC(γ)), 48.0 (ProC(δ)), 51.9 (HypC(δ)), 60.4 (HypC(α)), 61.3 + 61.4 (ProC(α)), 115.5 + 115.7 + 115.8 (APAC(3)), 119.6 (APAC(5)), 138.8 + 138.9 + 139.0 (APAC(4)), 148.4 + 148.5 (APAC(2)), 151.4 + 151.8 + 151.9 (APAC(6)), 165.8 + 165.9 + 166.0 (APACO), 170.5 + 170.8 + 170.9 (HypCO/ProCO/AdiCO). Anal. Calcd for $C_{72}H_{74}N_{20}O_{14} \cdot 6H_2O$ (M_r = 1551.6): C, 55.74; H, 5.59; N, 18.05. Found: C, 55.47; H, 5.55; N, 17.95. FAB-MS: [*m*/*z* (relative intensity)]: 1443 (3) [M + H$^+$].

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Supporting Information Available: ^{1}H and ROESY NMR spectra of **2** and its sulfate complex, observed and calculated isotope distribution of the complexes between **2** and iodide, bromide, and chloride, and Job plots and NMR titration curves of anion complexes of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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