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Anion-Binding Properties of a Cyclic Pseudohexapeptide Containing 1,5-Disubstituted 1,2,3-Triazole Subunits

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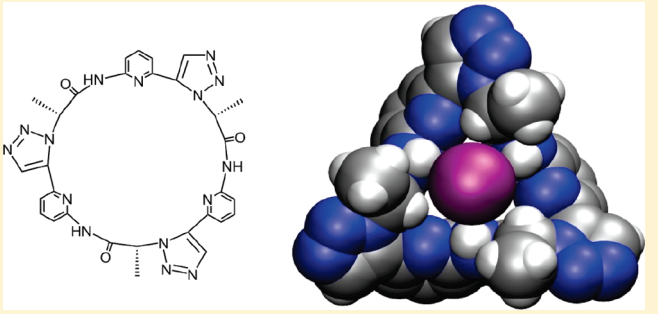
Supporting Information

ABSTRACT: A C_3 symmetric cyclic pseudohexapeptide containing 2-aminopicoline-derived subunits and 1,5-disubstituted 1,2,3-triazole rings is introduced as a potent anion receptor. This macrocycle was designed to mimic both the conformation and the receptor properties of a previously described cyclic hexapeptide containing alternating L-proline and 6-aminopicolinic acid subunits. Conformational analyses demonstrate that the cyclic peptide and the cyclic pseudopeptide are structurally closely related. Most importantly, both exhibit a converging arrangement of the NH groups, hence a good preorganization for anion binding. As a consequence, the pseudopeptide also very efficiently interacts with halide and sulfate ions, and this is the case even in competitive aqueous solvent mixtures. However, there are clear differences in the structures of both compounds, which translate into characteristic differences in receptor properties. Specifically, (i) the pseudopeptide possesses an anion affinity intrinsically higher than that of the cyclopeptide, (ii) the pseudopeptide is well preorganized for anion binding in a wider range of solvents from aprotic to protic, (iii) anion affinity in aprotic solvents is very high and associated with complexation equilibria that are slow on the NMR time-scale, (iv) the propensity of the pseudopeptide to form sandwich-type 2:1 complexes with two receptor molecules surrounding one anion is significantly lower than that of the cyclopeptide. A solvent-dependent calorimetric characterization of the binding equilibria of both compounds provided clear evidence for the stabilizing effect of hydrophobic interactions between the receptor subunits in such 2:1 complexes. The pseudopeptide thus represents the first member of a new family of anion receptors whose properties may be fine-tuned by varying the side chains in the periphery of the cavity.



INTRODUCTION

Different aspects govern the overall affinity of a synthetic receptor to its substrate. The most important of these are structural complementarity, conformational flexibility, and solvation effects. To include all these facets in receptor design is challenging and new receptors are therefore rarely designed from scratch but rather based on well-established building blocks with known binding properties.[1] Appropriate structural modifications, preferentially in the periphery of the receptor cavity, should then produce a predictable outcome in terms of binding affinity or selectivity.[2] Alternatively, combinatorial methods are also useful for receptor identification or optimization.[3]

While being conceptually straightforward, the deliberate control over the properties of a synthetic receptor by structural modification of its basic molecular framework can sometimes yield unexpected outcomes, such as a significantly lower than expected substrate affinity. Possible causes are unpredicted large effects of structural changes on receptor conformation or solvation. An example of a receptor in which a relative subtle structural modification caused profound changes in the binding model is cyclopeptide **1a**, which was introduced by our group as a receptor for inorganic anions such as sulfate or halides in aqueous solution.[4a] Complex formation involves interdigitation of two cyclopeptide rings to form a cavity into which the six peptide NH groups from both rings are projected, thus allowing hydrogen-bonding interactions with an included anion.[5] Binding affinity is high even in 80% water/methanol partly because the apolar proline rings of the two cyclopeptide moieties induce hydrophobic interactions when they approach one another to almost van der Waals contact in the sandwich-type complex.[4b] Unexpectedly, the ability of the cyclopeptide to form such sandwich complexes is completely lost if the proline subunits in **1a** are replaced with 4*R*-hydroxyproline subunits.[4c] The corresponding peptide **1b** also binds anions in aqueous media, but only in the form of 1:1 complexes. Besides steric effects of the hydroxy groups in the hydroxyproline residues, solvation effects could account for the different behavior of **1b**: hydroxyproline is presumably more strongly hydrated in aqueous solution and, as a consequence, desolvation is energetically more costly than that of proline.

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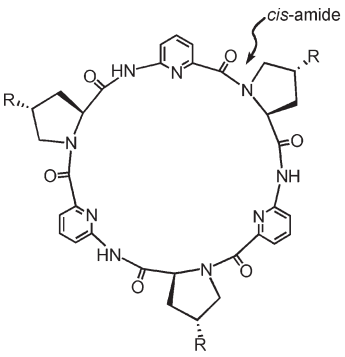
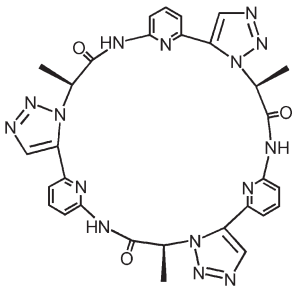
Assembling two cyclopeptide rings in a sandwich-type complex is therefore thermodynamically less favorable.



1a R = H
1b R = OH



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Evaluation of the receptor properties of other cyclopeptide derivatives would undoubtedly provide further insight into causes for the, in particular for a neutral compound, unusually high anion affinity of **1a** in aqueous solution.[6−9] Unfortunately, structural modification of **1a** beyond the introduction of hydroxyproline and substituted hydroxyproline derivatives is synthetically very challenging. We were therefore interested in the development of a structural analogue of **1a** with similar conformational preferences in protic solvents that, in turn, would produce similar receptor properties. In addition, this analogue should allow a straightforward structural variation in a relatively wide range.

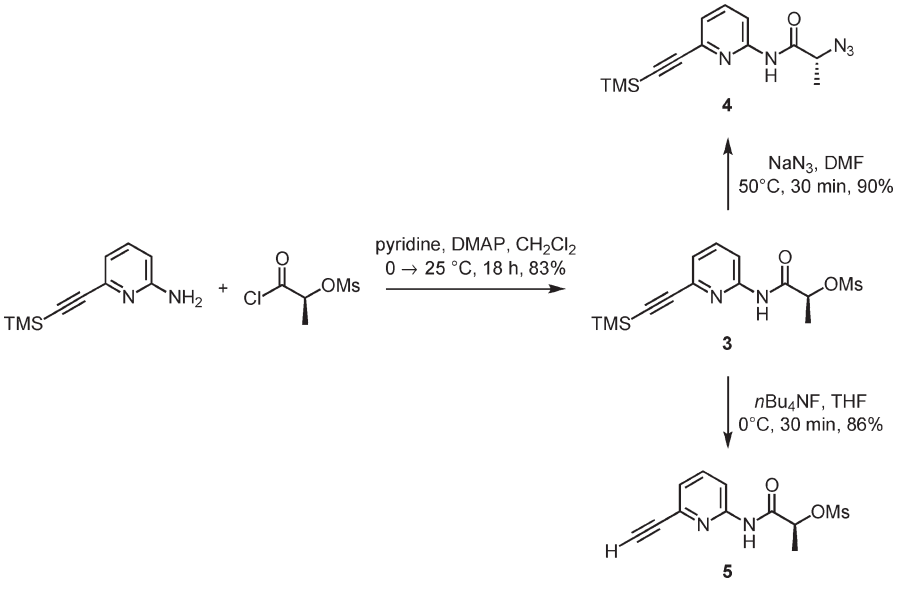
The most important aspects of the preferred conformations of **1a** in aqueous solution are three converging NH groups and cis-conformations at the proline amide groups.[4a] Since a cyclopeptide with alternating proline and 3-aminobenzoic acid residues is much less well preorganized for anion-binding and has only *trans*-amides, it seems as if the 6-aminopicolinic acid subunits in **1a** are essential.[4d] This leaves the proline subunits for structural modification, which should ideally be replaced with a nonlinear amino acid to gain flexibility in the type of substituent arranged along the perimeter of the cyclopeptide cavity, while retaining the cis-conformations of the corresponding amide bonds. Recent investigations in the field of peptidomimetics[10] have demonstrated that stabilization of *cis*-amides in a peptide backbone can efficiently be achieved by replacement of the amide group with a 1,5-disubstituted 1,2,3-triazole ring[11] or related heterocyclic subunits.[12] Accordingly, pseudopeptide **2** should represent a very promising structural analogue of **1a**, an assumption that was confirmed by force-field calculations. Compound **2** could provide information on whether the unusual anion-binding properties of **1a** are unique or a general phenomenon for a certain class of anion receptors. In addition, evaluation of the properties of derivatives differing in the substituents at the stereogenic centers of **2** should also give insight into the interplay between structural parameters and the thermodynamics of complex formation.

First indications for the potential feasibility of this concept were obtained from the structural characterization of smaller homologues of **1a** and **2**, namely a cyclic tetrapeptide and a cyclic pseudotetrapeptide, which indeed adopt structurally very similar conformations.[4e] Here, we show that there are also close structural analogies between **2** and **1a**. Subtle differences in the structures of both macrocycles translate into clear differences in binding behavior, however, thus providing information about the critical parameters responsible for the anion affinity of **1a**.

RESULTS AND DISCUSSION

Synthesis. Our synthetic strategy to prepare the cyclic pseudohexapeptide involved the use of **3**, obtained by condensing TMS-protected 2-amino-6-ethynylpyridine with the mesylate of (*S*)-lactic acid chloride, as the central building block (Scheme 1). Chain elongation and cyclization was achieved by repeated ruthenium(II)-catalyzed azide−alkyne-cycloadditions yielding the desired 1,5-disubstitued 1,2,3-triazole rings.[13] To introduce the functional groups necessary for azide−alkyne cycloaddition, **3** was converted into azide **4** by nucleophilic substitution of the mesyl group. Since **3** contains the (*S*)-enantiomer of lactic acid, this transformation produced a configuration at the stereogenic center opposite to the one in **2** (this procedure will therefore

Scheme 1



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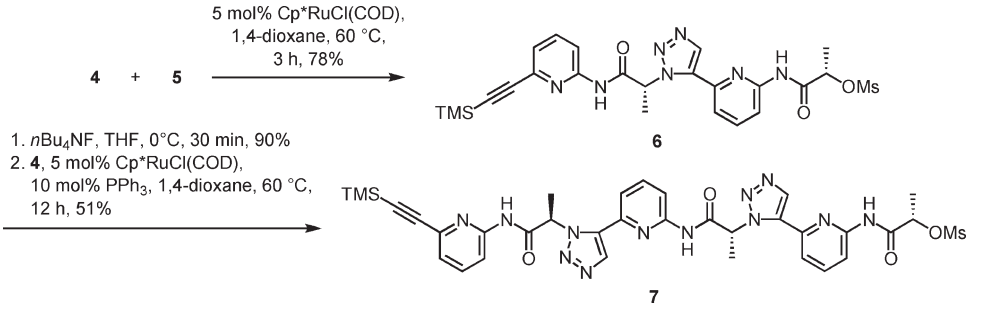
Scheme 2

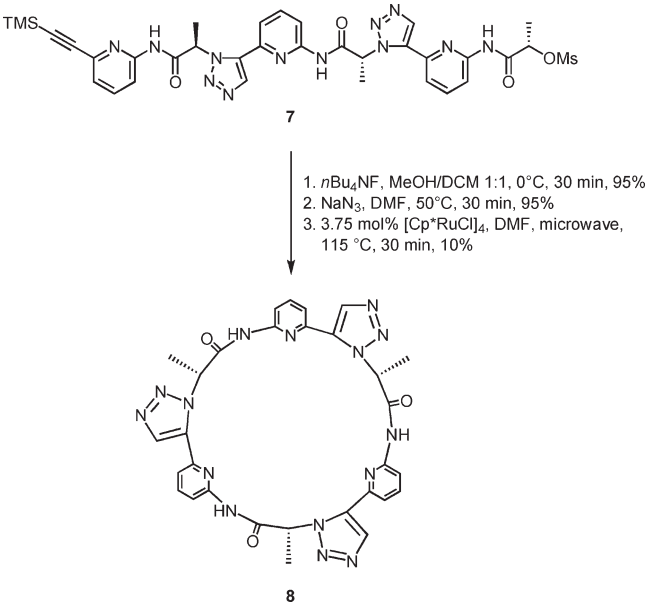


eventually furnish the enantiomer of **2**, compound **8**). In an independent reaction, cleavage of the TMS-group in **3** led to alkyne **5**. At this stage, compounds **4** and **5** possessed the appropriate functional groups to allow for the first chain elongation reaction. This reaction was performed by treating equimolar amounts of both compounds with 5 mol % of Cp*RuCl(COD) (Scheme 2). It should be noted that it is important to use a freshly prepared sample of **4** for this reaction because this compound is relatively prone to cyclodimerization.[4e]

Tetramer **6** thus obtained was deprotected at the alkyne moiety. The resulting product afforded crystals whose X-ray crystallographic characterization unequivocally confirmed the regioselective formation of the desired 1,5-disubstituted 1,2,3-triazole ring in the previous step (see Supporting Information). Coupling of the TMS-deprotected derivative of **6** to **4**, using the same ruthenium(II) catalyst as in the first chain elongation step, resulted in hexamer **7** (Scheme 3). Again, a crystal structure confirmed the correct substitution patterns at the triazole moieties in this compound and also showed the folding of this oligomer in the crystal (see Supporting Information). Unsatisfactory yields in this step (ca. 35%) could be somewhat improved to 51% by adding 10 mol % of triphenylphosphine to the reaction mixture, which presumably increases stability of the active catalyst. Compound **7** was then converted into the immediate precursor of **8** by initial cleavage of the TMS-group and subsequent conversion of the mesylate at the far end of the chain into the azide. Cyclization of this precursor was performed under microwave irradiation in the presence of 3.75 mol % of $[Cp^*RuCl]_4$.

Chromatographic analysis of the reaction mixtures indicated almost complete disappearance of the starting material after ca. 30 min under the chosen conditions and the formation of two main products in a ratio of ca. 2.5:1, both of which have the mass of macrocycle **8** according to MALDI-TOF mass spectrometric analysis. These compounds were separated by chromatography on silica gel. Whereas the major product has a simple ^{1}H NMR spectrum correlating with a C_3 symmetric averaged conformation, the spectrum of the other product is significantly more complex (see Supporting Information). NMR spectroscopy thus indicated that the major fraction corresponds to the symmetric macrocycle **8** while the other product is most probably also a macrocycle but an unsymmetrical one possibly with the triazole ring formed during the macrocyclization step having the 1,4-disubstitution pattern. Formation of this side product can be rationalized by assuming that the final azide−alkyne cycloaddition proceeds to a significant extent thermally and, hence, with a reduced regioselectivity under the microwave conditions. A control experiment indeed showed that performing the reaction

Scheme 3



in the absence of catalyst has a strong influence on the product ratio. Under these conditions, the unsymmetrical product is formed in 2-fold excess relative to the symmetrical one.

Product **8** was obtained in analytically pure form after recrystallization with a yield of 10% in the cyclization step. It should be noted that there are only very few other examples of macrocyclizations involving ruthenium-catalyzed azide−alkyne cycloadditions and to the best of our knowledge ours is the first to afford a cyclic pseudopeptide derivative.[14,15]

Conformational Analysis. Initial information about the structure of **8** was obtained by X-ray crystallographic analysis of crystals of **8** grown from acetone with trace water. Each molecule of **8** is associated with one acetone molecule and one molecule of water in the crystal structure obtained (Figure 1). The molecular structure of **8** is almost C_3 symmetrical, the aromatic moieties are arranged in a tilted fashion pointing into one direction, and the methyl groups at the stereogenic centers point into the opposite direction. The vectors defining the NH bonds converge at the narrow opening of the macrocycle. These groups are therefore well preorganized for the interaction with an anionic guest. The three protons at the stereogenic centers are in relative close proximity of the NH protons with distances ranging

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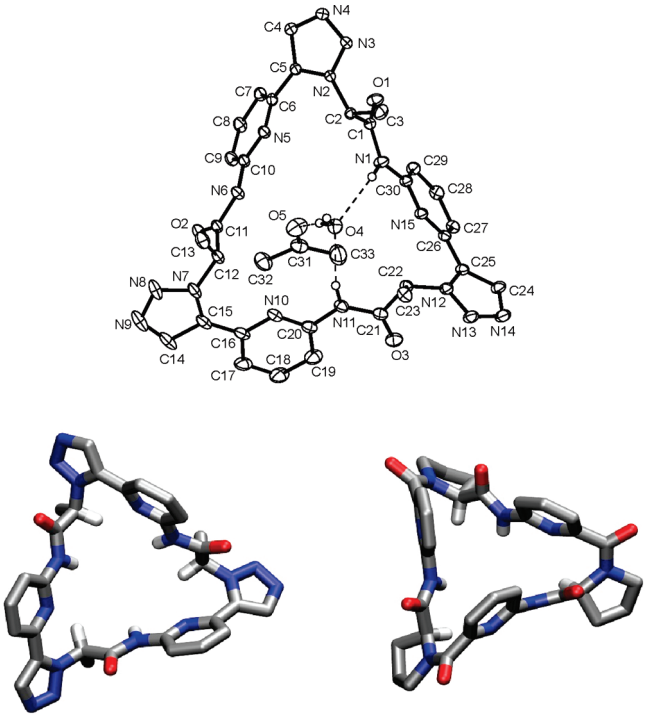


Figure 1. The top representation shows the molecular structure of $8 \cdot C_3H_6O \cdot H_2O$. Beneath, the molecular structures of $8 \cdot C_3H_6O \cdot H_2O$ (left) and $\mathbf{1a} \cdot (H_2O)_3$[4a] (right) are compared. Solvent molecules and hydrogen atoms in the bottom structures are omitted for clarity except for acidic hydrogen atoms and hydrogen atoms at the stereogenic centers of both compounds. Note that **8** and **1a** have opposite configurations at the stereogenic centers.

between 2.23 and 2.53 Å. The acetone molecule is included into the bowl-shaped cavity formed by the aromatic moieties. The water molecule, which is located near the center of the narrow opening, is hydrogen-bonded to the acetone carbonyl group. The other hydrogen atom of the water molecule interacts with one ring nitrogen atom of a triazole moiety of another macrocycle in the crystal.

Overall, the conformation of **8** in the crystal very well reproduces the characteristic elements of the preferred conformation of **1a**.[4a] Most importantly, the converging arrangement of the NH groups on the side of the macrocycle where the amino acid residues are located, in this case the proline rings, and the arrangement of the aromatic subunits in the opposite direction can also be seen in the crystal structure of $\mathbf{1a} \cdot (H_2O)_3$ (Figure 1). In contrast to **8**, where the mean planes of the aromatic moieties make an average angle of about 20° to the approximate C_3 axis, the mean planes of the aromatic rings in **1a** are arranged almost parallel to the C_3 axis. The cavity defined by the aromatic rings is therefore larger in the case of **8**, allowing the inclusion of an acetone molecule. Despite the stronger tilting of the aromatic rings in **8**, the diameter of the narrower opening does not differ significantly between **1a** and **8**, however.

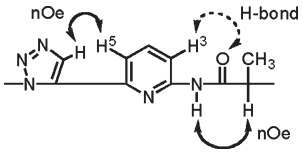
The different arrangements of the aromatic rings in the crystal structures of **1a** and **8** do not necessarily indicate intrinsic differences in the conformational preferences of both compounds since the wider cavity of **8** could also be a consequence of the included acetone molecule. However, in another crystal modification of **8**, crystallized from acetone and water and containing one acetone and *two* water molecules, the acetone occupies a different position, but the arrangement of the aromatic subunits is very similar to the one shown in Figure 1. In addition, we have a crystal structure of **1a** which contains an acetone molecule just outside but close to the cavity composed of the aromatic moieties, and the conformation of the cyclopeptide in this structure is practically the same as in $\mathbf{1a} \cdot (H_2O)_3$.[4a] In combination, these results support the assumption that the crystal structures shown in Figure 1 reflect the intrinsically preferred conformations of both **8** and **1a**. Both structures are obviously similar in many aspects although characteristic differences can be noted.

NMR spectroscopic studies were performed to obtain information about the conformational behavior of **8** in solution. The ^{1}H NMR spectra of **8** turned out to be strongly solvent dependent. In all solvents used, namely $CDCl_3$, acetone-d_6, DMSO-d_6, and D_2O/CD_3OD 1:2, simple spectra were obtained, albeit of significantly different quality. The quality of the spectrum is best in the protic solvent mixture (Figure 3), whereas in the other solvents, line broadening of some (mainly of signals corresponding to the protons arranged around the narrow opening of **8**) or all signals is observed (see Supporting Information). This effect is most pronounced for DMSO-d_6. Since the spectrum at 100 °C in DMSO or the spectrum at 25 °C in the presence of anions that bind to **8** (vide infra) exhibit sharper signals, it seems as if the free macrocycle is involved in a slow equilibrium in DMSO at room temperature involving different conformations of **8** and/or tight interactions of the macrocycle with solvent molecules (e.g., via N−H···O═S interactions). An argument in favor of the strong solvation of **8** in DMSO is the downfield shift of the NH signal when changing the solvent from $CDCl_3$ to acetone-d_6 and DMSO-d_6. Irrespective of the individual effects of the different solvents, ^{1}H NMR spectroscopy indicates that the conformation of **8** remains C_3 symmetric on average in all tested solvents.

A pronounced solvent dependency of the ^{1}H NMR spectra was also observed for **1a**, which distinctly differs from that of **8**, however. The spectra of **1a** in DMSO-d_6 and in aqueous solvent mixtures exhibit sharp signals and are simple, consistent with averaged C_3 symmetric conformations.[4a] In acetone-d_6 or $CDCl_3$, on the other hand, complex spectra were observed, indicating the presence of an asymmetric conformation. A detailed analysis showed this conformation to result from the rearrangement of one *cis*-amide bond along the macrocycle into a *trans*-amide bond, which brings an amide NH in close proximity to a picolinic acid ring nitrogen.[4f] The resulting intramolecular hydrogen bond causes stabilization of a conformation lacking a C_3 axis. This occurs, however, only in aprotic solvents. Since a similar conformational rearrangement is prevented by the triazole moieties in **8**, an analogous asymmetric conformation is most likely not accessible, and this compound should therefore be well preorganized for anion binding also in apolar solvents.

Information about the relationship between the molecular structure of **8** observed in the crystal and the solution structure was obtained from NOESY NMR spectroscopy. This spectrum was recorded in acetone-d_6, in which the signals are relatively sharp and the NH signals are still visible (see Supporting Information). There are two important cross-peaks in this spectrum that provide structural information. The first involves the signal of the triazole protons and the signal of the protons in the 5-position of the aromatic subunits. The second cross-peak is visible between the NH signals of **8** and the signal of the protons at the stereogenic centers (Scheme 4). Both cross-peaks are consistent with the conformation of **8** in the crystal, in which the spatial proximity of the corresponding pairs of protons is clearly

Scheme 4



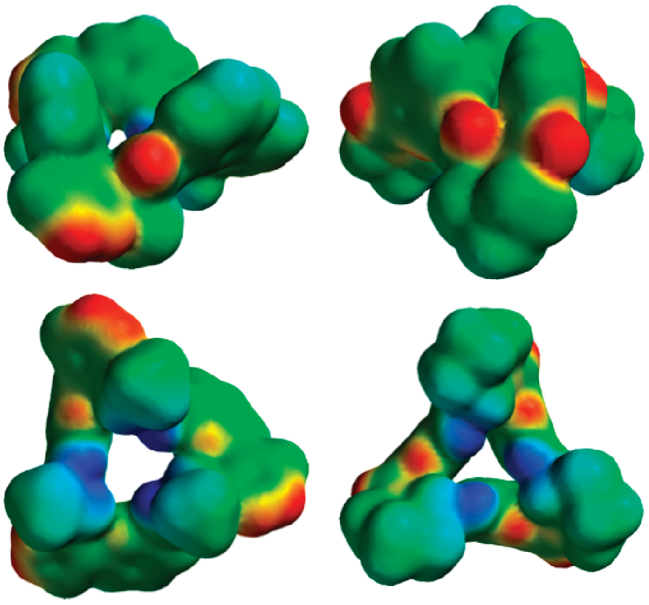


Figure 2. Electrostatic potential surfaces of cyclic pseudopeptide **8** (left) and cyclic peptide **1a** (right). These surfaces were generated with MacSpartan 04 (Wavefunction, Inc.) by mapping AM1 electrostatic potentials onto surfaces of molecular electron density (0.002 electron/Å) followed by color-coding. In all surfaces, the potential energy values range from +50 kcal/mol to −50 kcal/mol, with red signifying a value greater or equal to the maximum in negative potential and blue signifying a value smaller or equal to the maximum in positive potential.

visible. No cross-peak is observed between the NH signals and the signal of the protons in the 3-position of the pyridinyl rings, showing that the rotation of the NH groups is restricted and that the NH protons are preferentially projected toward the narrower opening of the macrocycle. This arrangement allows hydrogen-bonding interactions between H^3 and the oxygen atoms of the amide groups, which account for the relatively high resonance of these aromatic protons. Similar conformational preferences of amide groups in the 2-position of pyridine rings have been detected in certain oligoamides.[16] Taken together, the NMR spectroscopic investigations provide strong evidence that the preferred solution conformations of **8** closely resemble the conformation found in the crystal.

Figure 2 shows the electrostatic potential surfaces calculated on the basis of the crystal structures for **1a** and **8**. The different overall shape of both compounds is clearly visible. In addition, the high positive potential in the periphery of the narrow opening is also evident, which is even slightly more positive in the case of **8**, indicating that complex formation should take place in similar regions of the molecules in both cases. It is worth noting, however, that there are profound steric differences in these regions. The proline rings in **1a** cause a relatively strong steric shielding of

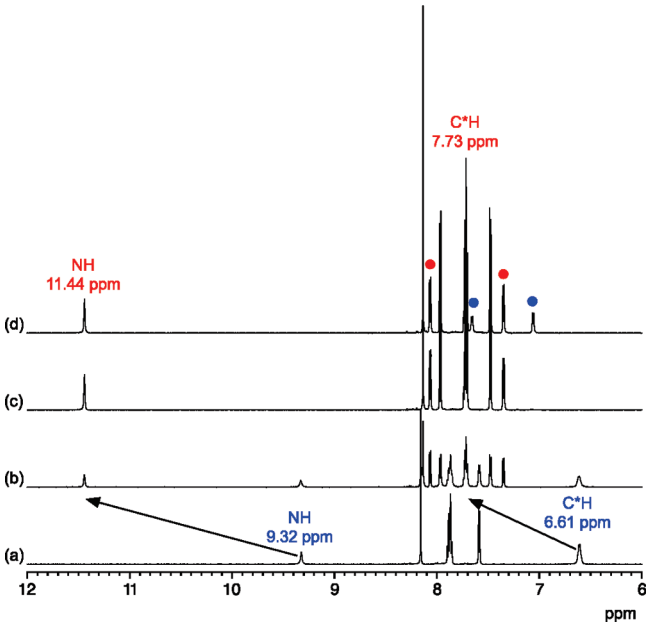


Figure 3. ^{1}H NMR spectrum of **8** in acetone-d_6 (1 mM) in the region 6−12 ppm (a) and the corresponding spectra in the presence of 0.5 equiv (b), 1.0 equiv (c), 1.5 equiv (d) of *n*-butyltrimethylammonium tosylate at 25 °C. The blue assignments correspond to the signals of the free receptor and the red ones to those of the complex. The dots mark the aromatic tosylate signals with red indicating complexed and blue indicating free anion.

the binding site, albeit at a larger distance to the NH groups than the methyl groups do in **8**. As a consequence, the entrance of the binding site in **8** seems to be somewhat restricted.

Qualitative Binding Properties. Anion-binding is associated with characteristic changes in the NMR spectrum of **1a**. In the polar aprotic solvent DMSO-d_6, a downfield shift of the NH signal is observed, which typically accounts for hydrogen bond formation between the NH protons and the anionic guests. In addition, the signal of the proline H(α) protons also exhibits a characteristic downfield shift because the electron density on the corresponding protons is strongly affected by the anions bound in close proximity. Minor shifts of the aromatic signals and of the signals of the other proline protons are due to electronic effects in combination with conformational changes in the cyclopeptide upon complex formation.[4a,c,f] In protic solvents, where the NH signals are not visible, the shifts of the other signals, particularly the strong downfield shift of the H(α) signals, are retained.

Anion effects on the ^{1}H NMR spectrum of **8** are strikingly similar. As an example, the effects of the incremental addition of *n*-butyltrimethylammonium tosylate on the ^{1}H NMR spectrum of **8** in acetone-d_6 are shown in Figure 3. Pronounced signal shifts caused by the presence of the salt, in particular the strong downfield shifts of the NH signal and of the signal corresponding to the protons at the stereogenic centers of **8**, denoted C*H, are clearly visible. Interestingly, complex formation is slow on the NMR time-scale, as the incremental addition of the salt causes the signals of the free receptor to progressively decrease and new signals corresponding to the tosylate complex to appear. Full complexation of **8** is observed after addition of 1 equiv of the salt. Further salt addition does not affect the spectrum of the complex and only causes signals of uncomplexed tosylate anions to appear. This behavior accounts for a 1:1 stoichiometry and a high stability

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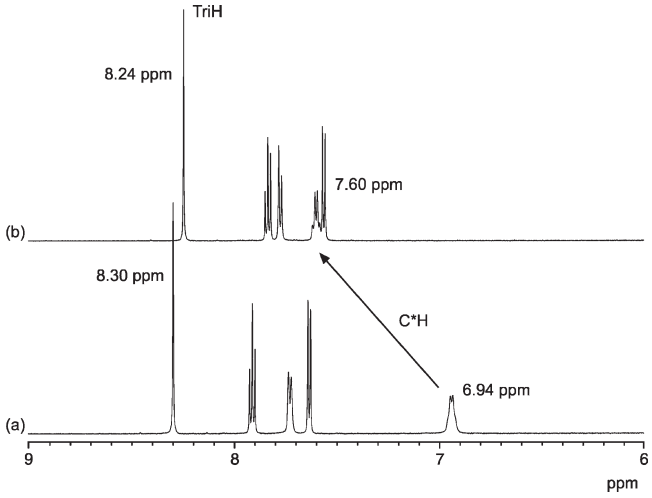

Figure 4. ^{1}H NMR spectrum of **8** in 1:2 (*v*/*v*) D_2O/CD_3OD (2 mM) in the region 6−9 ppm in the absence (a) and in the presence (b) of 2 equiv of Na_2SO_4 at 25 °C.

of the tosylate complex of **8** in acetone. The corresponding stability constant K_a can be estimated on the basis of the spectra depicted in Figure 3 to exceed 10^5 M^{-1}.

Anion-binding in DMSO-d_6 causes sharpening of the signals in the ^{1}H NMR spectrum, indicating a conformational stabilization of **8** upon complex formation. Complex formation is again slow on the NMR time-scale, and the signal shifts are similar to the ones observed in acetone (see Supporting Information). Importantly, the strong deshielding of the C*H protons of **8** is even retained when appropriate salts are added to a solution of the pseudopeptide in 1:2 (*v*/*v*) D_2O/CD_3OD (Figure 4), clearly demonstrating that anion-binding is not restricted to aprotic solvents but, in accordance with the envisaged close relationship between **1a** and **8** both in terms of structure and anion-binding properties, also takes place in much more competitive media. Complex formation is fast on the NMR time-scale in this solvent mixture albeit associated with significant line broadening of the shifting signals (see Supporting Information). The remarkable downfield shift of the C*H signal of 0.66 ppm caused by the addition of 2 equiv of sodium sulfate to a 2 mM solution of **8** in 1:2 (*v*/*v*) D_2O/CD_3OD relative to the corresponding signal of the uncomplexed macrocycle attests for tight and strong interactions with the anion.

In none of the solvents tested does the signal of the triazole proton shift to a large extent upon complex formation. The presence of sulfate ions even causes an upfield shift in the protic solvent mixture. This indicates that the triazole protons are not engaged in direct interactions with the anion as in other recently described triazole-containing anion receptors.[5d,17] Considering the diverging arrangement of this proton from the binding site of **8** (Figure 1), its participation in binding is indeed very unlikely.

Information about complex stoichiometry was derived from electrospray ionization mass spectrometry (ESI-MS). Negative mode mass spectra of **8** in 1:1 (*v*/*v*) H_2O/CH_3OH in the presence of the three different halides chloride, bromide, and iodide showed four signals whose *m*/*z* ratios correspond to those of the deprotonated macrocycle and the three halide 1:1 complexes (see Supporting Information). In contrast to corresponding spectra obtained for **1a**, no 2:1 receptor/anion complexes are visible.[4a] Thus, these spectra provide evidence that, although **8** is clearly able to interact with anions in aqueous media, the propensity of this compound to form sandwich-type complexes is smaller than that of **1a**. Complexes of **8** with a bound sulfate ion could not be observed mass spectrometrically.

Quantitative Binding Properties. Anion affinity of **8** was evaluated quantitatively by using isothermal titration calorimetry (ITC). This method has the advantage that it provides information about complex stoichiometry, as well as the full thermodynamic signature of complex formation in a single measurement.[18] Figure 5 shows two representative binding isotherms obtained for titrating tetramethylammonium bromide into methanolic solutions of **1a** or **8**. The different shapes of the curves indicate 1:1 binding for pseudopeptide **8**, as evidenced by an inflection point of the binding isotherm close to 1, and 2:1 binding for cyclopeptide **1a**. Similar results were obtained in other titrations (see Supporting Information). Thus, different methods had to be used to derive the binding constants from the titration data. In the case of 1:1 complexes, the one-site binding model was used whereas higher complexes were evaluated on the basis of the sequential binding model to obtain information about the stepwise binding constants K_{11} and K_{21}. The results obtained for the iodide complex of **8** were confirmed by performing an inverse titration during which a receptor solution was titrated into a salt solution. Normal and inverse titrations furnished the same results within the error limits.

Although we were most interested in the anion affinity of **8** in aqueous solvent mixtures, the heat of complex formation in water/methanol mixtures, unfortunately, turned out to be too small to obtain reliable binding data, at least for halide complexation. Therefore, titrations were mainly performed in methanol. For comparison, complex formation of **1a** was studied calorimetrically in the same solvent. Only in the case of sulfate binding was it possible to investigate the effect of solvent composition on binding properties of **8**. The results of these ITC measurements are summarized in Tables 1 and 2.

Stability constants of the 1:1 complexes between the three halides and **8** are in the submillimolar range, showing that the pseudopeptide possesses high anion affinity even in the competitive protic solvent methanol. Pseudopeptide **8** also binds nitrate anions, but in accordance with the poor coordinating nature of this anion, the binding is relatively weak. Halide affinity decreases in the order $Br^- > I^- > Cl^-$, thus deviating from selectivity of cyclopeptide **1a** whose anion affinity monotonously decreases with decreasing size of the anion.[4c] A correlation between ionic radius and halide selectivity has also been observed for a cyclic triamide developed in the Hamilton group.[5c] In both this triamide and **1a**, binding selectivity was rationalized by the geometric match between receptor cavity and substrate that causes the anion which is able to bind to all three hydrogen bond donors of the receptor simultaneously to be bound best. The deviating anion selectivity of **8** has subtler reasons, which can best be derived by considering the individual enthalpic and entropic components of complex formation.

Complex formation between halides and **8** is associated with a relatively large negative binding enthalpy, which becomes more favorable in the order $Cl^- < Br^- < I^-$. Thus, the iodide complex of **8** is enthalpically the most stable complex in methanol. This trend is opposite to the intrinsic strength of the halide···H−N hydrogen bond, which is the stronger the higher the charge density of the anion. The increase in the absolute binding enthalpy from complexation of chloride over bromide to iodide is

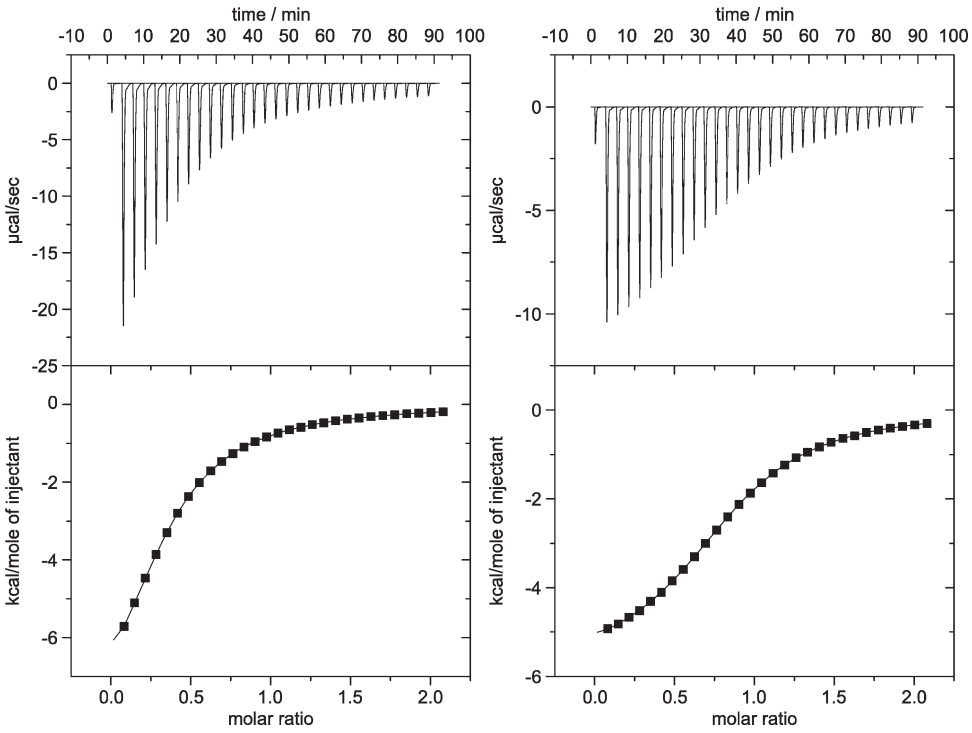

Figure 5. ITC traces and binding isotherms for the titrations of receptors **8** (0.5 mM) with $N(CH_3)_4Br$ (6 mM) (right) and **1a** (1.0 mM) (left) with $N(CH_3)_4Br$ (12 mM) in CH_3OH at 25 °C.

Table 1. Stoichiometry Factor *n*, Association Constants K_a, Gibbs Energies ΔG, Enthalpies ΔH, and Entropies $T\Delta S$ Associated with the Complexation of Chloride, Bromide, Iodide, or Nitrate by Receptors 1a and 8 in CH_3OH at 25 °C

anion[a]	receptor	n[b]	K_a[c]	ΔG[d]	ΔH[d]	$T\Delta S$[d]
chloride	**1a**		$K_{11} = 1060 \pm 70$ $K_{21} = 1070 \pm 180$ $K_T = 1.14 \pm 0.24 \times 10^6$	-34.6 ± 0.5	-17.8 ± 2.0	16.8 ± 2.5
	8	0.80	7380 ± 540	-22.0 ± 0.2	-20.8 ± 0.4	1.2 ± 0.5
bromide	**1a**		$K_{11} = 980 \pm 90$ $K_{21} = 3640 \pm 460$ $K_T = 3.54 \pm 0.39 \times 10^6$	-37.4 ± 0.3	-37.4 ± 1.4	0.0 ± 1.6
	8	0.88	16170 ± 1110	-24.0 ± 0.2	-24.5 ± 0.3	-0.5 ± 0.2
iodide	**1a**		$K_{11} = 1480 \pm 270$ $K_{21} = 4550 \pm 740$ $K_T = 6.57 \pm 0.30 \times 10^6$	-38.9 ± 0.1	-40.0 ± 0.6	-1.1 ± 0.7
	8	0.84	10970 ± 230	-23.1 ± 0.1	-25.9 ± 1.0	-2.8 ± 0.9
nitrate	**1a**		$K_{11} = 690 \pm 250$ $K_{21} = 1050 \pm 150$ $K_T = 6.98 \pm 1.81 \times 10^5$	-33.4 ± 0.7	-15.4 ± 5.3	18.0 ± 6.0
	8	1.0	2540 ± 260	-19.5 ± 0.3	-18.1 ± 1.4	1.4 ± 1.7

[a] All anions as tetramethylammonium salts. [b] Error ≤ 15%. [c] K_{11}, K_{21} in M^{-1}, K_T in M^{-2}. [d] ΔG, ΔH, $T\Delta S$ in kJ mol^{-1}; all results are averages over at least three measurements.

therefore either a result of the cavity size of **8** which renders interactions between the largest halide and all three NH groups of the receptor to be most efficient and/or of solvent effects which cause the overall enthalpic gain of complex formation to be largest for the most weakly solvated anion. This clear correlation between binding enthalpy and size of the halide does not directly translate into binding selectivity, however, because of the small but decisive entropic terms. Entropy strengthens chloride binding presumably because chloride anions are more strongly solvated in methanol and the release of solvent molecules upon binding is therefore entropically favored. In contrast, iodide complexation is associated with an adverse effect of entropy, which causes the overall stability of this complex to fall behind that of the bromide complex of **8** whose binding entropy is zero.

Since cyclopeptide **1a** forms 2:1 complexes with all halides even in methanol, direct comparison of the anion affinity of the

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Table 2. Association Constants K_a, Gibbs Energies ΔG, Enthalpies ΔH, and Entropies $T\Delta S$ Associated with the Complexation of Tetramethylammonium Sulfate by Receptors 1a and 8 in CH_3OH/H_2O Mixtures of Varying Composition at 25 °C (pH 6.7)

CH_3OH/H_2O (*v*/*v*)	receptor	K_a[a]	ΔG[b]	ΔH[b]	$T\Delta S$[b]
1: 0	**1a**	K_{11} = n.d.[c]	−58.5 ± 0.1	−34.5 ± 0.2	24.0 ± 0.3
		K_{21} = n.d.[c]			
		$K_T = 1.72 \pm 0.03 \times 10^{10}$			
	8	K_{11} = 31200 ± 4730	−50.8 ± 0.2	−34.6 ± 1.0	16.2 ± 0.7
		K_{21} = 25230 ± 1600			
		$K_T = 7.86 \pm 1.14 \times 10^8$			
4: 1	**1a**	K_{11} = 9060 ± 990	−46.7 ± 0.1	−21.5 ± 0.1	25.2 ± 0.1
		K_{21} = 18900 ± 1250			
		$K_T = 1.70 \pm 0.09 \times 10^8$			
	8	K_{11} = 5130 ± 810	−42.7 ± 0.7	−30.5 ± 0.9	12.2 ± 0.6
		K_{21} = 6190 ± 430			
		$K_T = 3.18 \pm 0.6 \times 10^7$			
2: 1	**1a**	K_{11} = 360 ± 120	−39.6 ± 0.2	−25.4 ± 0.1	14.2 ± 0.1
		K_{21} = 26000 ± 8400			
		$K_T = 8.81 \pm 0.13 \times 10^6$			
	8	K_{11} = 1010 ± 470	−39.5 ± 0.2	−37.0 ± 2.2	2.5 ± 2.2
		K_{21} = 9650 ± 4080			
		$K_T = 8.51 \pm 0.60 \times 10^6$			

[a] K_{11}, K_{21} in M^{-1}, K_T in M^{-2}. [b] ΔG, ΔH, $T\Delta S$ in kJ mol^{-1}; all results are averages over at least three measurements. [c] The steep shape of the binding isotherm prevented calculation of reliable stepwise binding constants in methanol.

cyclopeptide and the pseudopeptide is not straightforward. However, the fact that all stability constants associated with the formation of the 1:1 complexes of **1a**, K_{11}, are smaller than the stability constants of the complexes of **8** is an indication for an intrinsically higher anion affinity of the pseudopeptide, in accordance with the results of the EPS calculations (Figure 2). To support this assumption, we also determined the stability of the bromide complex of cyclopeptide **1b** in methanol, which forms only 1:1 complexes. Binding turned out to be too weak to be reliably quantified by an ITC titration. An NMR titration was therefore performed which gave a K_a of 580 M^{-1}, providing strong evidence for a more than 1 order of magnitude higher anion affinity of **8** in comparison to **1a**.

Table 2 shows that in all solvent mixtures studied, **1a** and **8** bind sulfate in exothermic reactions with favorable entropic components. In contrast to halide binding, sulfate binding consistently leads to the formation of 2:1 complexes, however. Thus, the higher charge and tetrahedral geometry in combination with the four strongly coordinating oxygen atoms enable the sulfate anion to recruit a second receptor unit for complex formation. Overall, binding of **8** is very strong but almost noncooperative in methanol as evidenced by the smaller binding constant K_{21} with respect to K_{11}. Interestingly, increasing the water content of the solvent mixture has a pronounced effect on this ratio. In solvent mixtures containing more water, K_{21} increases relative to K_{11} so that in 2:1 CH_3OH/H_2O complex formation becomes a strongly cooperative process. A similar trend is observed for **1a**. This result is a clear indication for the stabilizing effects of hydrophobic interactions on the 2:1 sandwich-type complexes of such receptors.[4b] Additional stabilizing forces such as dipole−dipole interactions as in the triazolophanes developed in the Flood group,[5d] which cause aggregation of the receptors even in the absence of the guests, seem to be absent. Formation of the 2:1 complexes is more favorable in the case of the cyclopeptide, but because of the intrinsically higher anion affinity of **8**, signified by the consistently larger binding constants K_{11}, the overall stability of the sulfate complexes of both macrocycles is comparable in 2:1 CH_3OH/H_2O.

Our results thus provide qualitative and quantitative information about the influence of hydrophobic effects within a receptor or between receptor subunits on noncovalent interactions in aqueous media.[19] Clearly, macrocyclic receptors such as **8** or cyclopeptide **1a** are very interesting systems to study these effects since they are among the few systems that combine high anion affinity in competitive solvents and sufficient water solubility to perform binding studies in the aqueous environment. Major advantages of **8** are the intrinsically high anion affinity of this receptor, the well-defined predictable conformation, and the possibility of being able to systematically vary the structure of the side chains. These factors render **8** an ideal basis for the further development of synthetic anion receptors which are active in aqueous solution.

CONCLUSIONS

The features responsible for the unique anion-binding properties of cyclopeptide **1a** were transferred to a macrocyclic analogue by replacing the tertiary amide groups with 1,5-disubstituted 1,2,3-triazole rings and the proline subunits with noncyclic amino acid derivatives. These modifications leave the overall conformation of **1a**, viz., the converging arrangement of the NH groups, the shielding of the binding site by hydrophobic substituents, and the structural complementarity of two rings, intact. As a consequence, the corresponding pseudopeptide **8** also very efficiently interacts with anions in competitive solvent mixtures. However, there are clear differences in the structures of **1a** and **8**, which translate into characteristic differences in receptor properties. Specifically, the pseudopeptide possesses an anion affinity intrinsically higher than that of the cyclopeptide, but its propensity to form sandwich-type 2:1 complexes with two receptor molecules surrounding one anion is significantly lower.

In the case of **1a**, the main driving force for the formation of these sandwich-type complexes comes from hydrophobic effects between proline rings of the two cyclopeptide moieties making formation of the 2:1 complexes a highly cooperative process. Because the hydrophobic regions around the binding site of pseudopeptide **8** are smaller than in **1a**, contributions of hydrophobic effects to complex formation are presumably reduced. As a result, halides are bound in the form of 1:1 complexes in methanol. A 2:1 complex is formed with sulfate, most likely due to the different shape, higher charge, and stronger hydrogen-bond acceptor properties of this anion, indicating that there are no structural reasons that prevent **8** from forming sandwich-type complexes, but complex formation is not cooperative in methanol. However, increasing the water content of the solution has a strongly stabilizing influence on the second binding step, the formation of the 2:1 complex from the 1:1 complex, clearly demonstrating the stabilizing effects of hydrophobic interactions on such systems.

This work thus provides detailed information about the effects that influence the anion affinity of **1a** and **8**. It shows that the arrangement of the NH groups in **8** is well suited for strong interactions with anionic substrates and that the environment of the binding site has pronounced effects on binding kinetics, complex stability, and stoichiometry. Fortunately, structural variation of **8**, involving introduction of functional groups around the cavity, is relatively straightforward. Consequently, there is room for further optimization of the receptor properties of **8**. Work in this respect can be expected to furnish new potent receptors for anions active in apolar *and* polar media in addition to improving our understanding of the structural and thermodynamic parameters that mediate anion binding in water.

EXPERIMENTAL SECTION

General Details. Peak assignments in the ^{1}H NMR spectra were confirmed by using H,H-COSY spectra. The only signals that could not be assigned unambiguously with this method are those of the H^3 and H^5 protons on the aromatic moieties. Due to hydrogen bond formation to the amide C═O group, the signal of the H^3 proton is generally the more deshielded one, an assignment that was confirmed for selected compounds by HMBC spectra and for cyclic pseudopeptide **8** by NOESY NMR spectroscopy (see Supporting Information). HSQC spectra were used to interpret the ^{13}C NMR spectra on the basis of the previously assigned proton spectra. NMR spectra become increasingly complex for the longer oligomers; however, signals of equivalent protons in the individual subunits of the oligomers tend to cluster in relatively narrow spectral regions and can therefore be assigned in a straightforward manner. This method allows assignment of the signals to the type of absorbing nuclei but not to their position along the chain. No attempts were made to determine this position because the spectra are simplified substantially upon cyclization of the hexamer to the C_3 symmetrical product. The following abbreviations are used: Epa, 2-amino-6-ethynyl-2-pyridine; Lac, lactic acid; Tri, 1,2,3-triazole.

ITC Titrations. The ITC titrations were carried out in methanol or in mixtures of methanol and water. The different salts and receptors were weighed using an analytical precision balance and dissolved in a known volume of solvent or mixture of solvents and loaded into the system for immediate analysis. Solutions involved in the same titration experiment were made up from the same batch of solvent. The pH of the aqueous solvent mixtures amounted to 6.7. No substantial change in pH was observed during the course of the titration. The standard ITC experiment involved the titration of a solution of the salt into a solution of the receptor at 25 °C. For this, the salt solution was added in 30 injections of 8 μL, separated by an interval of 180 s between injections, with the exception of the first addition, which was 2 μL. In the titration of **1a** with tetramethylammonium sulfate in methanol, 30 injections of 6 μL of the salt solutions were used. Binding constants and enthalpies of binding were obtained by curve fitting of the titration data using the one-site binding model for 1:1 complexes and the sequential binding model with the option "ligand in cell" for 2:1 complexes both of which are implemented in the Origin 7.0 software provided by the manufacturer. The peak produced by the first injection was discarded during data processing.

6-[(Trimethylsilyl)ethynyl]pyridin-2-amine. 2-Amino-6-bromopyridine (1.5 g, 8.7 mmol), $(Ph_3P)_2PdCl_2$ (240 mg, 345 μmol, 4 mol %), bis(2-diphenylphosphinophenyl) ether (DPEphos) (185 mg, 345 μmol, 4 mol %), and CuI (165 mg, 870 μmol, 10 mol %) were stirred in freshly distilled NEt_3 (30 mL) for 30 min at room temperature. To this yellow solution was added ethynyltrimethylsilane (1.3 mL, 9.6 mmol, 1.2 equiv) dropwise. The resulting black solution was stirred for an additional 12 h. After removal of the solvent, the residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, *v*/*v*) to give the product as an off-white solid, which was pure enough for the next step. Analytically pure material was obtained by sublimation (100 °C, 5×10^{-2} mbar). Yield 1.6 g (97%); mp 126 °C; ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 0.21 (s, 9H, $Si(CH_3)_3$), 6.11 (s, 2H, NH_2), 6.43 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(3)), 6.63 (d, 1H, ^3J(H,H) = 7.9 Hz, EpaH(5)), 7.33 (t, 1H, ^3J(H,H) = 8.2, EpaH(4)); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = −0.2 ($Si(CH_3)_3$), 91.4 (Si-C≡C), 105.5 (Si−C≡C), 108.9 (EpaC(3)), 115.6 (EpaC(5)), 137.3 (EpaC(4)), 139.7 (EpaC(6)), 159.7 (EpaC(2)); EI-MS (70 eV): *m*/*z* (%): 175.06 (100%) [$M^+ - CH_3$], 190.09 (83%) [M^+]; elemental analysis calcd (%) for $C_{10}H_{14}N_2Si$: C 63.11, H 7.41, N 14.72; found C 63.32, H 7.39, N 14.80.

TMS-Epa-(*S*)-Lac-OMs (3). (*S*)-1-Chloro-1-oxopropan-2-yl methanesulfonate (4.0 g, 19.8 mmol) (obtained in three steps from (*S*)-methyl lactate by mesylation,[20a] ester hydrolysis,[20b] and conversion of the free acid into the acid chloride[20c] by following the described procedures) in dry dichloromethane (3 mL) was added dropwise to a solution of 6-[(trimethylsilyl)ethynyl]pyridine-2-amine (2.5 g, 13.2 mmol), pyridine (1.5 mL, 18.5 mmol), and DMAP (8 mg, 0.5 mol %) in dry dichloromethane (15 mL) at 0 °C. The solution was stirred overnight while it was allowed to reach room temperature. Afterward, it was washed with 10% aqueous K_2CO_3 (20 mL) and water (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, *v*/*v*) to give the crude product. Recrystallization from hexane/ethyl acetate afforded it in analytically pure form as white needles. Yield 3.7 g (83%); mp 117−118 °C; $[\alpha]^{20}_D$ = −46.7 (*c* = 1, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 0.24 (s, 9H, $Si(CH_3)_3$), 1.52 (d, 3H, ^3J(H,H) = 6.7 Hz, $LacCH_3$), 3.25 (s, 3H, SO_2CH_3), 5.23 (q, 1H, ^3J(H,H) = 6.7 Hz, LacCH), 7.30 (d, 1H, ^3J(H,H) = 7.7 Hz, EpaH(5)), 7.83 (t, 1H, ^3J(H,H) = 7.9, EpaH(4)), 8.07 (d, 1H, ^3J(H,H) = 7.9 Hz, EpaH(3)), 11.00 (s, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = −0.4 ($Si(CH_3)_3$), 18.6 ($LacCH_3$), 38.1 (SO_2CH_3), 75.2 (LacCH), 94.1 (Si-C≡C), 103.5 (Si−C≡C), 114.1 (EpaC(3)), 123.1 (EpaC(5)), 139.3 (EpaC(4)), 140.4 (EpaC(6)), 151.4 (EpaC(2)), 168.2 (CO); EI-MS (70 eV): *m*/*z* (%): 217.08 (52%) [$M^+ - CH(CH_3)OSO_2CH_3$], 261.11 (100%) [$M^+ - SO_2CH_3$], 340.09 (11%) [M^+]; elemental analysis calcd (%) for $C_{14}H_{20}N_2O_4SSi$: C 49.39, H 5.92, N 8.23, S 9.42; found C 49.39, H 5.95, N 8.16, S 9.27.

TMS-Epa-(*R*)-Lac-N_3 (4). Compound **3** (500 mg, 1.47 mmol) and NaN_3 (115 mg, 1.77 mmol, 1.2 equiv) were stirred in DMF (3 mL) at 50 °C for 30 min. Ethyl acetate (10 mL) was added, and the mixture was washed with water (10 mL). The solvent was evaporated and the crude product subjected to column chromatography (silica gel; hexane/ethyl acetate, 3:1, *v*/*v*) to give **4** as a slightly yellow oil. This compound is unstable and was therefore immediately used for the next step. Yield 380

mg (90%); ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 0.23 (s, 9H, Si(CH_3)$_3$), 1.43 (d, 3H, ^3J(H,H) = 6.9 Hz, LacCH_3), 4.09 (q, 1H, ^3J(H,H) = 6.9 Hz, LacCH), 7.28 (d, 1H, ^3J(H,H) = 7.3 Hz, EpaH(5)), 7.82 (t, 1H, ^3J(H,H) = 7.9, EpaH(4)), 8.09 (d, 1H, ^3J(H,H) = 8.4 Hz, EpaH(3)), 11.03 (s, br, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = −0.4 (Si(CH_3)$_3$), 16.6 (LacCH_3), 57.1 (LacCH), 94.0 (Si-C≡C), 103.5 (Si−C≡C), 114.0 (EpaC(3)), 123.0 (EpaC(5)), 139.2 (EpaC(4)), 140.1 (EpaC(6)), 151.6 (EpaC(2)), 170.1 (CO); IR (KBr): $\tilde{\nu}$ = 2123 cm^{-1} (s, azide).

H-Epa-(*S*)-Lac-OMs (5). To a solution of **3** (1.24 g, 3.65 mmol) in THF (10 mL) was added a solution of nBu$_4$NF·3 H_2O (1.73 g, 7.29 mmol, 1.5 equiv) in THF (10 mL) dropwise at 0 °C. The reaction mixture was stirred at this temperature for 30 min and then diluted with ethyl acetate (20 mL). The resulting mixture was washed with water (40 mL). The water phase was extracted twice with ethyl acetate (20 mL), and the combined organic layers were concentrated in vacuo. The residue was subjected to column chromatography (silica gel; hexane/ethyl acetate, 2:1, *v*/*v*) to give **5** as a white solid. Recrystallization from hexane/ethyl acetate afforded the pure compound as colorless needles. Yield 840 mg (86%); $[\alpha]_D^{20}$ = −48.0 (*c* = 1, $CHCl_3$); mp 104 °C; ^{1}H NMR (600 MHz, acetone-d_6, 25 °C) δ = 1.68 (d, 3H, ^3J(H,H) = 6.8 Hz, LacCH_3), 3.28 (s, 3H, SO_2CH_3), 3.78 (s, 1H, HC≡C), 5.34 (q, 1H, ^3J(H,H) = 6.8 Hz, LacCH), 7.33 (d, 1H, ^3J(H,H) = 7.5 Hz, EpaH(5)), 7.84 (t, 1H, ^3J(H,H) = 8.0, EpaH(4)), 8.29 (d, 1H, ^3J(H,H) = 8.4 Hz, EpaH(3)), 9.48 (s, br, 1H, NH); ^{13}C NMR (151 MHz, acetone-d_6, 25 °C) δ = 19.1 (LacCH_3), 38.7 (SO_2CH_3), 76.7 (HC≡C), 78.8 (LacCH), 83.2 (HC≡C), 114.9 (EpaC(3)), 124.6 (EpaC(5)), 139.7 (EpaC(4)), 141.5 (EpaC(6)), 152.3 (EpaC(2)), 169.0 (CO); EI-MS (70 eV): *m*/*z* (%): 145.04 (100%) [M$^+$ − CH(CH$_3$)OSO$_2$CH$_3$], 189.07 (45%) [M$^+$ − SO$_2$CH$_3$], 268.05 (11%) [M$^+$]; IR (KBr) $\tilde{\nu}$ = 2160 (w, ν(CH) alkyne) cm^{-1}; elemental analysis calcd (%) for $C_{11}H_{12}N_2O_4S$: C 49.24, H 4.51, N 10.44, S 11.95; found C 49.53, H 4.59, N 10.32, S 11.75.

TMS-Epa-(*R*)-Lac-Tri-Epa-(*S*)-Lac-OMs (6). Freshly prepared **4** (1.52 g, 5.67 mmol) and **5** (1.72 g, 5.95 mmol) were dissolved in dry 1,4-dioxane (10 mL) under an atmosphere of nitrogen. Cp*RuCl(COD) (107.7 mg, 0.26 mmol, 5 mol %) was added, and the reaction mixture was stirred for 3 h at 60 °C. The solvent was then removed in vacuo and the crude product subjected to column chromatography on silica (hexane/ethyl acetate, 2:3, *v*/*v*) to give **6** as a colorless solid. Yield: 2.47 g (78%); mp 126 °C; $[\alpha]_D^{20}$ = +70 (*c* = 0.5, $CHCl_3$/CH_3OH 1:1, *v*/*v*); ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 0.24 (s, 9H, Si(CH_3)$_3$), 1.54 (d, 3H, ^3J(H,H) = 6.8 Hz, LacCH_3), 2.00 (d, 3H, ^3J(H,H) = 7.1 Hz, LacCH_3), 3.28 (s, 3H, SO_2CH_3), 5.27 (q, 1H, ^3J(H,H) = 6.8 Hz, LacCH), 6.55 (q, 1H, ^3J(H,H) = 7.1 Hz, LacCH), 7.24 (d, 1H, ^3J(H,H) = 7.5 Hz, EpaH(5)), 7.73 (t, 1H, ^3J(H,H) = 8.6 Hz, EpaH(4)), 7.74 (d, 1H, ^3J(H,H) = 7.7 Hz, EpaH(5)), 7.88 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(3)), 7.95 (t, 1H, ^3J(H,H) = 8.1 Hz, EpaH(4)), 8.01 (d, 1H, ^3J(H,H) = 8.2 Hz, EpaH(3)), 8.49 (s, 1H, TriH), 9.13 (s, 1H, NH), 9.35 (s, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = −0.2 (Si(CH_3)$_3$), 17.4 + 19.2 (LacCH_3), 38.6 (SO_2CH_3), 60.0 + 75.2 (LacCH), 94.2 (Si-C≡C), 103.7 (Si−C≡C), 113.3 + 113.7 (EpaC(3)), 118.8 + 123.2 (EpaC(5)), 134.2 (TriC(4)), 134.7 (TriC(5)), 139.4 (EpaC(4)), 140.2 (EpaC(6)), 140.4 (EpaC(4)), 145.2 (EpaC(6)), 150.4 + 151.8 (EpaC(2)), 168.1 + 169.6 (CO); MS (ESI-TOF, positive mode): *m*/*z* (%): 556.2 (100%) [M + H$^+$], 578.2 (91%) [M + Na$^+$]; elemental analysis calcd (%) for $C_{24}H_{29}N_7O_5SSi$ · 0.5 H_2O: C 51.05, H 5.35, N 17.36, S 5.68; found C 51.33, H 5.38, N 17.36, S 5.83.

TMS-Epa-[(*R*)-Lac-Tri-Epa]$_2$-(*S*)-Lac-OMs (7). To a solution of **6** (1.50 g, 2.70 mmol) in THF (15 mL) was added a solution nBu$_4$NF·3 H_2O (1.27 g, 4.00 mmol) in THF (5 mL) dropwise at 0 °C. The reaction mixture was stirred at this temperature for 30 min and then diluted with diethyl ether (50 mL). The resulting mixture was washed twice with water (50 mL). The aqueous layer was extracted with diethyl ether (50 mL), and the combined organic layers were concentrated in vacuo. The crude product was subjected to column chromatography on silica (hexane/ethyl acetate, 1:3, *v*/*v*) to give the alkyne as a colorless solid. The product crystallized as colorless needles from a 1:1 mixture of methanol and dichloromethane upon slow evaporation. Yield: 1.17 g (90%); mp > 180 °C (dec); $[\alpha]_D^{20}$ = +92.7 (*c* = 0.5, $CHCl_3$/CH_3OH 1:1, *v*/*v*); ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 1.54 (d, 3H, ^3J(H,H) = 6.8 Hz, LacCH_3), 2.01 (d, 3H, ^3J(H,H) = 7.2 Hz, LacCH_3), 3.29 (s, 3H, SO_2CH_3), 4.35 (s, 1H, HC≡C), 5.24 (q, 1H, ^3J(H,H) = 6.8 Hz, LacCH), 6.54 (q, 1H, ^3J(H,H) = 7.2 Hz, LacCH), 7.27 (d, 1H, ^3J(H,H) = 7.4 Hz, EpaH(5)), 7.74 (d, 1H, ^3J(H,H) = 7.4 Hz, EpaH(5)), 7.75 (t, 1H, ^3J(H,H) = 7.8 Hz, EpaH(4)), 7.89 (d, 1H, ^3J(H,H) = 8.5 Hz, EpaH(3)), 7.96 (t, 1H, ^3J(H,H) = 7.9 Hz, EpaH(4)), 8.02 (d, 1H, ^3J(H,H) = 8.2 Hz, EpaH(3)), 8.48 (s, 1H, TriH), 10.34 (s, 1H, NH), 10.86 (s, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = 17.4 + 19.1 (LacCH_3), 38.7 (SO_2CH_3), 59.9 + 75.3 (LacCH), 80.6 (HC≡C), 82.5 (HC≡C), 113.4 + 113.8 (EpaC(3)), 118.9 + 123.5 (EpaC(5)), 134.3 (TriC(4)), 134.9 (TriC(5)), 139.4 (EpaC(4)), 140.1 (EpaC(6)), 140.4 (EpaC(4)), 145.2 (EpaC(6)), 150.4 + 151.8 (EpaC(2)), 168.1 + 169.6 (CO); IR (KBr) $\tilde{\nu}$ = 2117 (w, ν(CH) alkyne) cm^{-1}; MS (ESI-TOF, positive mode): *m*/*z* (%): 484.2 (65%) [M + H$^+$], 506.2 (100%) [M + Na$^+$]; elemental analysis calcd (%) for $C_{21}H_{21}N_7O_5S$: C 52.17, H 4.38, N 20.28, S 6.63; found C 52.08, H 4.60, N 20.22, S 6.57. The free alkyne thus obtained (1.05 g, 2.17 mmol) and **4** (0.69 g, 2.39 mmol) were dissolved in dry 1,4-dioxane (15 mL) under an atmosphere of nitrogen. Cp*RuCl(COD) (41 mg, 0.11 mmol, 5 mol %) and PPh_3 (57.0 mg, 0.22 mmol, 10 mol %) were added, and the reaction mixture was stirred for 12 h at 60 °C. The solvent was then removed in vacuo and the crude product subjected to column chromatography on silica (hexane/ethyl acetate, 1:3, *v*/*v*) to give **7** as a colorless solid. The product crystallized from methanol/dichloromethane 1:1 upon slow evaporation. Yield: 0.85 g (51%); mp > 220 °C (dec); $[\alpha]_D^{20}$ = +60.0 (*c* = 0.5, $CHCl_3$/CH_3OH 1:1, *v*/*v*); ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ = 0.21 (s, 9H, Si(CH_3)$_3$), 1.67 (s, br, 6H, LacCH_3), 2.02 (d, 3H, ^3J(H,H) = 7.0 Hz, LacCH_3), 3.36 (s, 3H, SO_2CH_3), 5.20 (q, 1H, ^3J(H,H) = 6.8 Hz, LacCH), 5.37 (s, br, 1H, LacCH), 5.96 (s, br, 1H, LacCH), 7.21 (d, 1H, ^3J(H,H) = 7.3 Hz, EpaH(5)), 7.38 (d, 1H, ^3J(H,H) = 7.6 Hz, EpaH(5)), 7.49 (d, 1H, ^3J(H,H) = 7.6 Hz, EpaH(5)), 7.72 (s, br, 1H, EpaH(4)), 7.81 (t, 1H, ^3J(H,H) = 8.0 Hz, EpaH(4)), 7.85 (t, 1H, ^3J(H,H) = 8.0 Hz, EpaH(4)), 8.01 (s, 1H, TriH), 8.05 (d, 1H, ^3J(H,H) = 8.4 Hz, EpaH(3)), 8.14 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(3)), 8.15 (s, 1H, TriH), 8.22 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(3)), 8.90 (s, br, 1H, NH), 9.02 (s, br, 1H, NH), 9.26 (s, br, 1H, NH); ^{13}C NMR (151 MHz, $CDCl_3$, 25 °C) δ = −0.4 (Si(CH_3)$_3$), 16.4 + 16.9 + 19.2 (LacCH_3), 38.7 (SO_2CH_3), 60.6 + 62.6 + 74.6 (LacCH), 95.7 (Si-C≡C), 102.8 (Si−C≡C), 113.5 + 115.0 (EpaC(3)), 118.9 + 119.0 + 124.1 (EpaC(5)), 134.0 + 134.2 (TriC(4)), 135.1 + 135.3 (TriC(5)), 139.9 + 140.3 (EpaC(4)), 143.9 + 144.3 (EpaC(6)), 150.4 + 150.9 (EpaC(2)), 167.7 + 170.7 (CO); MS (ESI-TOF, negative mode): *m*/*z* (%): 673.3 (32%) [M − (C≡CTMS)$^+$], 769.3 (100%) [M − H$^+$], 805.3 (6%) [M + Cl$^-$]; elemental analysis calcd (%) for $C_{34}H_{38}N_2O_6SSi$: C 52.97, H 4.97, N 21.80, S 4.16; found C 52.74, H 4.77, N 21.56, S 3.96.

Cyclo[(*R*)-Lac-Tri-Epa]$_3$ (8). To a solution of **7** (0.20 g, 0.26 mmol) in 1:1 (*v*/*v*) dichloromethane/methanol (20 mL) was added a solution nBu$_4$NF·3H_2O (0.12 g, 0.39 mmol) in THF (5 mL) dropwise at 0 °C. The reaction mixture was stirred at this temperature for 60 min and then diluted with diethyl ether (50 mL). The resulting mixture was washed twice with water (50 mL). The aqueous layer was extracted with diethyl ether (50 mL), and the combined organic layers were concentrated in vacuo. The crude product was subjected to column chromatography on silica (ethyl acetate) to give the free alkyne of **7** as a colorless solid. The product crystallized from 1:1 (*v*/*v*) methanol/dichloromethane upon slow evaporation. Yield: 0.17 g (95%); mp > 160 °C (dec.); $[\alpha]_D^{20}$ = +139.0 (*c* = 0.5, $CHCl_3$/CH_3OH 1:1, *v*/*v*); ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ = 1.64 (d, 3H, ^3J(H,H) = 6.9 Hz, LacCH_3),

1.72 (d, 3H, ^3J(H,H) = 6.0 Hz, LacCH$_3$), 2.03 (d, 3H, ^3J(H,H) = 7.2 Hz, LacCH$_3$), 3.04 (s, 1H, HC≡C), 3.26 (s, 3H, SO$_2$CH$_3$), 5.14 (q, 1H, ^3J(H,H) = 6.9 Hz, LacCH), 5.37 (s, br, 1H, LacCH), 6.05 (q, 1H, ^3J(H,H) = 7.2 Hz, LacCH), 7.23 (d, 1H, ^3J(H,H) = 7.4 Hz, EpaH(5)), 7.39 (d, 1H, ^3J(H,H) = 7.6 Hz, EpaH(5)), 7.49 (d, 1H, ^3J(H,H) = 7.6 Hz, EpaH(5)), 7.74 (t, 1H, ^3J(H,H) = 8.0 Hz, EpaH(4)), 7.81 (t, 1H, ^3J(H,H) = 8.0 Hz, EpaH(4)), 7.78 (t, 1H, ^3J(H,H) = 8.0 Hz, EpaH(4)), 7.98 (s, 1H, TriH), 8.11 (s, 1H, TriH), 8.13 (d, 1H, ^3J(H,H) = 8.5 Hz, EpaH(3)), 8.13 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(3)), 8.21 (d, 1H, ^3J(H,H) = 8.4 Hz, EpaH(3)), 8.92 (s, 1H, NH), 9.23 (s, 1H, NH), 10.29 (s, 1H, NH); ^{13}C NMR (151 MHz, CDCl$_3$, 25 °C) δ = 16.2 + 16.8 + 19.1 (LacCH$_3$), 38.6 (SO$_2$CH$_3$), 59.8 + 62.3 + 74.7 (LacCH), 78.0 (HC≡C), 81.9 (HC≡C), 113.6 + 114.5 + 115.7 (EpaC(3)), 118.2 + 119.5 + 124.1 (EpaC(5)), 134.0 + 134.1 (TriC(4)), 135.1 + 135.3 (TriC(5)), 139.3 + 139.9 + 140.5 (EpaC(4)), 143.9 + 144.5 (EpaC(6)), 150.3 + 150.9 + 151.2 (EpaC(2)), 167.4 + 167.7 + 170.6 (CO); IR (KBr) $\tilde{\nu}$ = 2108 (vw, ν(CH) alkyne) cm^{-1}; MS (ESI-TOF, negative mode): m/z (%): 601.3 (64%) [M − CH$_3$SO$_3$H − H$^+$], 697.2 (48%) [M − H$^+$], 733.2 (4%) [M + Cl$^-$]; elemental analysis calcd (%) for C$_{31}$H$_{30}$N $_{12}$O$_6$S · H$_2$O: C 51.95, H 4.50, N 23.45, S 4.47; found C 52.03, H 4.68, N 23.29, S 4.78. The free alkyne thus obtained (60 mg, 86 μmol) and sodium azide (9 mg, 129 μmol) in DMF (1 mL) were stirred at 50 °C for 45 min. The reaction mixture was diluted with diethyl ether (5 mL) and washed with water (5 mL). The solvent was removed in vacuo and the crude product subjected to column chromatography on silica (ethyl acetate) to give the linear precursor of **8** as a colorless solid. Yield: 52.7 mg (95%); ^{1}H NMR (600 MHz, DMSO-d_6, 25 °C) δ = 1.13 (d, 3H, ^3J(H,H) = 6.9 Hz, LacCH$_3$), 1.82 (d, 3H, ^3J(H,H) = 7.0 Hz, LacCH$_3$), 1.97 (d, 3H, ^3J(H,H) = 7.2 Hz, LacCH$_3$), 3.88 (q, 1H, ^3J(H,H) = 6.9 Hz, LacCH), 4.26 (s, 3H, SO$_2$CH$_3$), 6.06 (q, 1H, ^3J(H,H) = 7.0 Hz, LacCH), 6.28 (q, 1H, ^3J(H,H) = 7.2 Hz, LacCH), 7.21 (d, 1H, ^3J(H,H) = 7.3 Hz, EpaH(5)), 7.58 (d, 1H, ^3J(H,H) = 7.6 Hz, EpaH(5)), 7.67 (t, 1H, ^3J(H,H) = 4.2 Hz, EpaH(4)), 7.77 (t, 1H, ^3J(H,H) = 8.2 Hz, EpaH(4)), 7.78 (d, 1H, ^3J(H,H) = 8.3 Hz, EpaH(5)), 7.91−7.94 (m, 3H, EpaH(4)+EpaH(3)), 7.98 (d, 1H, ^3J(H,H) = 8.4 Hz, EpaH(3)), 8.27 (s, 1H, TriH), 8.40 (s, 1H, TriH), 9.87 (s, 1H, NH), 10.24 (s, 1H, NH), 10.94 (s, 1H, NH); ^{13}C NMR (151 MHz, DMSO-d_6, 25 °C) δ = 16.3 + 17.4 + 17.8 (LacCH$_3$), 57.4 + 58.8 + 59.8 (LacCH), 80.5 (HC≡C), 82.4 (HC≡C), 113.6 + 113.9 + 114.1 (EpaC(3)), 119.2 + 119.5 + 124.5 (EpaC(5)), 133.8 + 134.1 (TriC(4)), 135.4 + 135.8 (TriC(5)), 139.3 + 140.0 + 140.1 (EpaC(4)), 140.2 + 145.1 + 145.2 (EpaC(6)), 150.5 + 151.1 + 151.9 (EpaC(2)), 168.6 + 169.0 + 169.5 (CO); IR (KBr) $\tilde{\nu}$ = 2106 (s, azide) cm^{-1}. The obtained product (75 mg, 116 μmol) was dissolved in DMF (60 mL), and [Cp*RuCl]$_4$ (4.4 mg, 4.00 μmol, 3.75 mol %) was added. The reaction mixture was then rapidly transferred to a microwave reactor and stirred for 30 min at 115 °C [Discover (CEM), 200 W, standard mode, stirring device, cooling fan, and temperature measurement by an IR sensor]. In total, 12 reactions were carried out this way to convert altogether 900 mg (1.39 mmol) of the starting material. All reaction mixtures were combined, and the solvent was removed in vacuo. The residue was dissolved in dichloromethane (20 mL) and washed with water (20 mL). The organic layer was concentrated and subjected to column chromatography on silica. A solvent gradient was used to elute the product starting with ethyl acetate, which was changed to 1:1 (v/v) acetone/dichloromethane and finally to acetone. The isolated product was further purified on a RP-8 column. For this, it was dissolved in a small amount of DMF and applied to a column conditioned with 1:10 (v/v) acetone/H$_2$O. The eluent composition was gradually changed until the pure product eluted (acetone/water, 1:1, v/v). Pure product crystallized as colorless needles from the fractions containing **8** upon slow evaporation. Yield: 90 mg (10%); mp 229−232 °C; $[\alpha]^{20}_D$ = −10.0 (c = 0.5, CHCl$_3$/CH$_3$OH 1:1, v/v); ^{1}H NMR (600 MHz, 10% DMSO-d_6/acetone-d_6, 25 °C) δ = 2.11 (d, 9H, ^3J(H,H) = 7.1 Hz, LacCH$_3$), 6.76 (q, 3H, ^3J(H,H) = 7.1 Hz, LacCH), 7.22 (d, 3H, ^3J(H,H) = 7.4 Hz, EpaH(5)), 7.80−7.85 (m, 6H, EpaH(3)+EpaH(4)), 8.18 (s, 3H, TriH), 9.67 (s, 3H, NH); ^{13}C NMR (151 MHz, 10% DMSO-d_6/acetone-d_6, 25 °C) δ = 17.3 (LacCH$_3$), 59.2 (LacCH), 114.1 (EpaC(3)), 119.1 (EpaC(5)), 133.9 (TriC(4)), 135.9 (TriC(5)), 139.9 (EpaC(4)), 145.8 (EpaC(2)), 151.3 (EpaC(6)), 169.0 (CO); IR (KBr) $\tilde{\nu}$ = 3138 (w), 1707 (s), 1577 (s), 1522 (s), 1457 (s), 1283 (m), 1165 (m), 806 (s); MS (ESI-TOF, negative mode): m/z (%): 644.3 (12%) [M − H$^+$], 680.3 (100%) [M + Cl$^-$], 707.2 (23%) [M + NO$_3^-$]; elemental analysis calcd (%) for C$_{30}$H$_{27}$N$_{15}$O$_3$·2 H$_2$O·1.5 C$_3$H$_6$O: C 53.90, H 5.24, N 27.33; found C 53.86, H 5.20, N 27.46.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectroscopic characterization of compounds **3**−**8**, NOESY NMR spectra of **8**, X-ray crystallographic analyses of the TMS-deprotected derivative of **6**, **7**, **8**·C$_3$H$_6$O·H$_2$O, and **8**·C$_3$H$_6$O·2H$_2$O, ^{1}H NMR spectrum of the side product obtained during the cyclization, and results of the ESI mass spectrometric, ^{1}H NMR spectroscopic, and ITC binding studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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