DOI: 10.1002/chem.200501062

One-Armed Artificial Receptors for the Binding of Polar Tetrapeptides in Water: Probing the Substrate Selectivity of a Combinatorial Receptor Library

Carsten Schmuck* and Martin Heil[a]

Dedicated to Professor Frank-Gerrit Klärner on the occasion of his 65th birthday

Abstract: We have recently developed a new class of one-armed artificial receptors **1** for the binding of the polar tetrapeptide *N*-Ac-D-Glu-L-Lys-D-Ala-D-Ala-OH (EKAA) **2** in water using a combined combinatorial and statistical approach. We have now further probed the substrate selectivity of this receptor library **1** by screening a second tetrapeptide substrate (**3**) with the inverse sequence *N*-Ac-D-Ala-D-Ala-L-Lys-D-Glu-OH (AAKE). This "inverse" substrate is also efficiently bound by our receptors, with $K_{ass} \approx 6000\,M^{-1}$ for the best receptors, as determined both by a quantitative on-bead binding assay and by UV and fluorescence titration studies in free solution. Hence, the inverse tetrapeptide **3** is in general bound two to three times less efficiently than the "normal" peptide **2** ($K_{ass} \approx 17000\,M^{-1}$), even though the complexation mainly involves long-range electrostatic interactions and both the receptor and substrate are rather flexible. Molecular modeling and ab initio calculations have been used to rationalize the observed substrate selectivity and to analyze the various binding interactions within the complex.

Keywords: combinatorial chemistry · guanidinium cations · molecular recognition · peptides · supramolecular chemistry

Introduction

Intermolecular interactions involving peptides or protein fragments are rather important supramolecular events and are central to a variety of biochemical and medicinal processes.[1] For example, signal transduction and enzymatic activities, as well as pathological processes such as amyloid formation responsible for Alzheimer's disease or bacterial cell wall maturation, critically depend on molecular recognition events involving peptides. Therefore, artificial receptors that are capable of selectively binding a given peptide under physiological conditions (in water) are not only interesting as model systems for studying the principles of the underlying supramolecular chemistry, but also as starting points for the development of sensors as diagnostic tools[2] or as molecular probes capable of interfering with an actual biological event.[3]

For such purposes, strong complexation of the given target peptide by the artificial host is necessary.[4,5] This, however, is quite challenging. On the one hand, in water, electrostatic interactions such as ion pairs or H-bonds,[6] which due to their specificity and in some cases directionality[7] are quite useful for imposing selectivity in supramolecular complex formation,[8] are weakened due to competitive solvation by the polar water molecules. On the other hand, hydrophobic interactions,[9] which are more important in water, are rather non-specific, making the design of selective host molecules difficult.[10] For Nature, this does not normally pose many problems. Molecular recognition events often take place in clefts or cavities within proteins, which provide a less polar microenvironment for the binding than the bulk solvent, allowing the effective exploitation of electrostatic interactions.[11,12] For artificial chemical receptors, however, which in general are much smaller and therefore structurally less well defined than proteins, this often represents a severe limitation in terms of both their design and development as well as for any potential application, which necessarily has to take place under physiological conditions. Therefore, most peptide receptors reported to date require additional hydrophobic,[13] aromatic,[14] and/or much stronger metal-ligand interactions[15] for efficient substrate binding in water.

[a] Prof. Dr. C. Schmuck, Dr. M. Heil
Institut für Organische Chemie
Universität Würzburg, Am Hubland
97074 Würzburg (Germany)
Fax: (+49) 931-888-4626
E-mail: schmuck@chemie.uni-wuerzburg.de

We have recently shown for the first time that binding of the non-hydrophobic tetrapeptide *N*-Ac-D-Glu-L-Lys-D-Ala-D-Ala-OH (EKAA) **2** in water is possible without the need for additional hydrophobic or metal–ligand interactions.[16] This tetrapeptide sequence is interesting in terms of its relevance to bacterial cell wall maturation.[17] During the synthesis of the bacterial cell wall, linear peptidoglycans are cross-linked through a transamidation reaction involving this tetrapeptide sequence, which is also the point of attack of the glycopeptide antibiotic vancomycin. This substrate is therefore not only challenging in terms of its highly flexible and polar character, but is also of value in view of its biological relevance. Receptors that selectively bind this peptide sequence could be of interest in improving our understanding of the molecular basis of vancomycin antibiotic activity and resistance.[4c,j] Through a quantitative on-bead screening of a medium-sized but focused combinatorial receptor library **1** with 512 members, we were able to identify efficient receptors that bind the tetrapeptide **2** with $K_{ass} \approx 10^4\,M^{-1}$. A statistical QSAR analysis of the experimental data underlined the fact that peptide binding is solely based on electrostatic interactions in this case.[16]

We now present herein the results of a more thorough investigation of the binding properties of receptors of type **1**. We have probed the substrate selectivity of this receptor class by using the inverse tetrapeptide *N*-Ac-D-Ala-D-Ala-L-Lys-D-Glu-OH (AAKE) **3** as a second substrate. Furthermore, we present the results of detailed binding studies for both substrates, on-bead and in free solution, by means of experimental NMR, UV, and fluorescence titrations as well as molecular mechanics and ab initio calculations. Comparison of the respective experimental binding data for the two substrates **2** and **3** reveals interesting insights into the structural requirements for efficient peptide complexation by this receptor class.

Results and Discussion

Design and synthesis of the library: The general design of the receptor library **1** is based on our guanidiniocarbonyl pyrrole binding motif, which we developed for efficient complexation of carboxylates in aqueous solvents.[18,19] To this carboxylate binding site (CBS) for the complexation of the C-termini of peptides, additional interaction sites in the form of a linear tripeptide unit have been attached. The peptidic nature of the receptor allows the formation of a hydrogen-bonded antiparallel β-sheet[20] with the backbone of the tetrapeptide substrate **2**, as shown schematically in Figure 1. Furthermore, multiple electrostatic interactions between the amino acid side chains on both the substrate and the receptor should further stabilize the complex and provide the necessary substrate selectivity. Based on this receptor design (abbreviated as CBS-AA^1-AA^2-AA^3), facile and fast solid-phase peptide synthesis can be performed, which also allows the introduction of structural diversity by using a combinatorial variation of the amino acids in the tripeptide

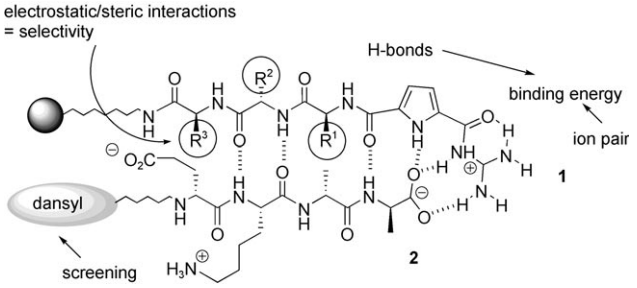
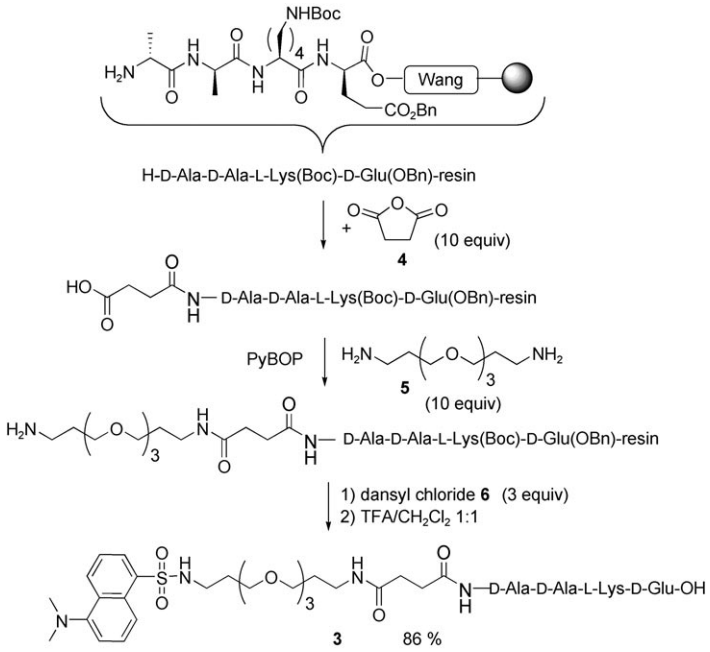


Figure 1. Schematic representation of complex formation between the receptor library **1** and the dansylated tetrapeptide substrate **2**.

unit.[4j,21] Therefore, a combinatorial receptor library[22] was synthesized on amino-TentaGel as the solid support according to a standard Fmoc protocol using the split-and-mix approach[23] in combination with IRORI radiofrequency tagging technology[24] as described previously.[4e] In each of the three coupling steps, the same eight different amino acids were used, namely Lys(Boc), Tyr(*t*Bu), Ser(*t*Bu), Glu(OBn), Phe, Val, Leu, and Trp, giving rise to a library with 512 different members. These specific amino acids used were chosen among the proteinogenic amino acids to provide a representative range of varying polar, charged, and hydrophobic residues within the final receptor library.[16] Deprotection of the side-chain functionalities was finally achieved by using 25% HBr in acetic acid.

In a first experiment, this library **1** was screened for its affinity for the polar tetrapeptide *N*-Ac-D-Glu-L-Lys-D-Ala-D-Ala-OH (EKAA) **2** in water.[16] Based on this initial library screening, receptors were found that bind **2** with an affinity of $K_{ass} \approx 10^4\,M^{-1}$. To probe the substrate selectivity of the receptors, we have now also prepared the inverse sequence, *N*-Ac-D-Ala-D-Ala-L-Lys-D-Glu-OH (AAKE), in the form of a dansylated derivative **3** on Wang resin as a solid support by means of a standard solid-phase peptide synthesis (SPPS) based on an Fmoc protocol (Scheme 1). To ensure water solubility, a hydrophilic triethyleneglycol spacer was introduced between the fluorescent dansyl label and the tetrapeptide. Thus, the free N-terminal amino group of the side-chain-protected tetrapeptide obtained after SPPS was reacted sequentially with succinic anhydride **4**, the triethyleneglycol diamine **5**, and dansyl chloride **6**. The labeled tetrapeptide **3** was cleaved from the resin with TFA in CH_2Cl_2 (1:1 mixture). Under these conditions, the protecting groups on the amino acid side chains are also cleaved, to afford analytically pure **3** in 83% yield.

Binding studies on-bead: To qualitatively probe the entire receptor library for its binding properties towards this inverse substrate **3**, aliquots of the 512 resin-bound deprotected receptors **1** were pooled and the combined mixture was incubated with a 5 μM solution of the tetrapeptide substrate **3** in 20 μM bis-tris-buffer at pH 6.0 in water. After the supernatant solution had been washed off, the beads were screened under UV light using a fluorescence microscope. A selective binding of the tetrapeptide substrate **3** by some,



Scheme 1. Synthesis of the dansylated tetrapeptide substrate **3**.

but not all, of the 512 receptors **1** could be observed, as indicated by the strong fluorescence activity of individual beads (Figure 2).

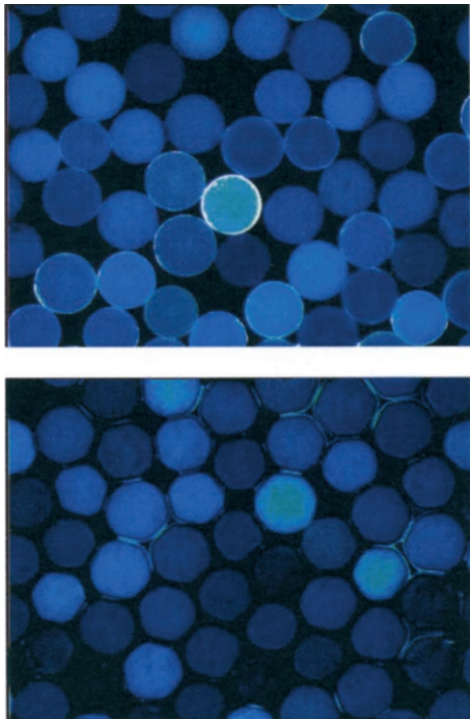


Figure 2. On-bead binding assay in buffered water ([substrate] = 5 μM, 20 μM bis-tris buffer, pH 6.0). Strong fluorescence activity indicates selective binding of tetrapeptide **2** (EKAA) to selected cationic receptors **1** (top), whereas the inverse tetrapeptide **3** (AAKE) is bound less efficiently at the same concentration (bottom).

Only those beads on which the attached receptor is capable of binding the peptide even in water show the characteristic fluorescence of the dansyl group. All of the other receptors that do not bind the peptide under these experimental conditions remain dark. The percentage of beads in the mixture taking up the fluorescence was found to be directly correlated with the concentration of the substrate solution used for incubation. This underlines the fact that the observed differences in fluorescence activity are indeed due to different binding affinities of the individual receptors and not to a selective fluorescence quenching within the complex (which would be independent of concentration). However, this qualitative screening gave a first indication that the binding of the inverse substrate **3** (AAKE) by receptors of type **1** is weaker compared with that of the "normal" substrate **2** (EKAA), as indicated by the higher concentrations needed to achieve a significant peptide binding, reflected by the intensity of the fluorescence staining (Figure 2).

The weaker binding of the inverse substrate **3** relative to **2** was confirmed by a quantitative on-bead fluorescence assay using a high-throughput microtiter plate reader.[25] The IRORI tagging technology provides the individual library members locally separated and in amounts of about 20–30 mg resin, which is enough material for a quantitative screening. For each receptor, a precisely measured sample (15–25 mg of resin with a 0.22 mmol g^{-1} loading, as determined by quantification of the Fmoc-piperidine adduct during receptor synthesis) was incubated with 2 mL of a 39 μM solution of the dansylated tetrapeptide **3** in 200 μM bis-tris-buffer at pH 6.0 for 20 h. After equilibration, 200 μL of the supernatant solution was removed and the fluorescence intensity of the solution was measured. From the fluorescence intensity before and after incubation and the loading of the resin, the association constants K_{ass} for each receptor could be calculated. The best receptors were found to have a $K_{ass} \approx 6000\,M^{-1}$ for **3** and in general K_{ass} is around two to three times smaller than for the "normal" substrate **2**, for which the best receptors had $K_{ass} = 17100\,M^{-1}$ (Table 1).

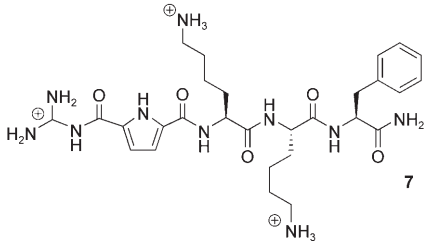
Table 1. Selected association constants K_{ass} (in M^{-1})[a] for the complexation of tetrapeptides **2** (EKAA) and **3** (AAKE) by receptors of type **1** (CBS-AA^1-AA^2-AA^3-resin) in water, as obtained from the library screening.

Entry	Receptor	AAKE **3**	EKAA **2**
1	CBS-KKF	4800	17100
2	CBS-KKL	5600	15400
3	CBS-KKE	5700	15300
4	CBS-KSK	5700	13200
5	CBS-KFK	6000	12900
6	CBS-KYY	3300	12300
7	CBS-KKK	4300	12000
8	CBS-KKV	5500	11500
9	CBS-LYK	2900	10000
10	CBS-KLF	2700	8800
11	CBS-FKK	2800	6200
12	CBS-FEK	500	1600
13	CBS-VYV	140	300
14	CBS-VEF	<100	200

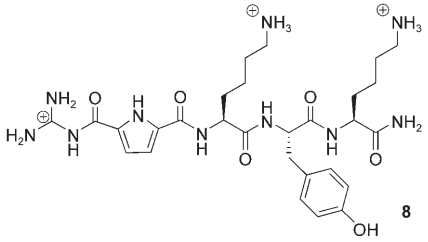
[a] Estimated error in K ±20%.

Interestingly, the best receptor for the inverse substrate **3** (CBS-KFK, entry 5) is not the same as that for substrate **2** (CBS-KKF, entry 1), even though the binding affinities for substrate **3** do not differ significantly. In general, any receptor that contains two lysine residues—irrespective of their position—seems to bind the inverse substrate **3** rather well. This again underlines the fact that the dominant binding forces are charge interactions. Due to the flexibility of the lysine side chain, no significant dependence on its position in the receptor is therefore expected.

Complex formation in solution: To validate the binding data obtained from the solid-phase screening, complexation studies in free solution were also performed.[26] Two members of the library, receptors **7** and **8**, were therefore synthesized on



7



8

Rink amide resin by using a standard Fmoc protocol and were obtained in analytically pure form after cleavage from the solid support. Receptor **7** (CBS-KKF) was the most efficient one for **2** in the on-bead screening, whereas receptor **8** (CBS-KYK) showed a medium affinity ($K_{ass} \approx 5800\,\text{M}^{-1}$ for **2** and $4500\,\text{M}^{-1}$ for **3**, respectively). The unlabeled N-acetylated tetrapeptide substrates **9** (EKAA) and **10** (AAKE) needed for the binding studies were synthesized by means of a standard Fmoc protocol on Wang resin. Due to their charged side chains, both tetrapeptides as well as the receptors are well soluble in water, even at millimolar concentrations.

We first tried ^{1}H NMR titrations to reveal complex formation in solution. In water/DMSO (90:10), complexation-induced shift changes could indeed be observed upon the addition of tetrapeptide **9** (NMe_4^+ salt) to a solution of the receptor **8** (CBS-KYK, chloride salt). For example, the signals of the amide NHs of the tripeptide backbone and of the tyrosine CHs of the receptor were shifted downfield, whereas the signals of the pyrrole CHs were shifted upfield, in accordance with observations for other guanidiniocarbonyl pyrrole receptor systems.[18] Unfortunately, due to the complexity of the spectra and extensive overlap between the signals of the receptor and those of the substrate, a quantitative analysis of the shift changes was not possible. Therefore, complex formation in water can only be qualitatively demonstrated by these NMR experiments. Nevertheless, as the shift changes of the signals of the tyrosine CHs and the various amide NHs suggest, the intermolecular interaction is not limited to ion-pair formation between the guanidiniocarbonyl pyrrole cation and the carboxylate of the tetrapeptide, but also extends well into the tripeptide unit of the receptor, in agreement with the model schematically depicted in Figure 1.

For a quantitative determination of the complex stabilities in free solution, we performed UV and fluorescence titration experiments in buffered water.[26] Stock solutions of the tetrapeptides ($c = 7.7\times10^{-4}\,\text{M}$) and of the receptors ($c = 5.4\times10^{-5}\,\text{M}$) were freshly prepared in buffered water (bis-tris-buffer, pH 6.15, $c = 1.6\times10^{-3}\,\text{M}$). Aliquots of the appropriate tetrapeptide solution were then added to a solution of the receptor and the changes in the UV and fluorescence spectra were recorded after each addition. In the UV spectra, the decrease of the absorbance A of the pyrrole band at 300 nm was followed. A nonlinear curve-fitting based on a 1:1 complexation model was used to calculate the binding constant K from the isotherms according to the following equation.[26c]

$$A = \varepsilon_R \cdot [R]_0 + \varepsilon_L \cdot [L]_0 + \frac{K \cdot \Delta\varepsilon \cdot [R]_0 \cdot [L]}{1 + K \cdot [L]}$$

with:

$$[L] = [L]_0 - \frac{K \cdot [R]_0 \cdot [L]_0}{(1 + K \cdot [L]_0)^2 + K \cdot [R]_0}$$

where $[L]$ = concentration of free ligand (tetrapeptide) in the solution, $[L]_0$ = total concentration of ligand (tetrapeptide) in the solution (free and bound), $[R]_0$ = total concentration of receptor in the solution (free and bound), and ε_{RL}, ε_R, ε_L = absorption coefficients of the complex, the receptor, and the ligand, respectively.

$$\Delta\varepsilon = \varepsilon_{RL} - \varepsilon_R - \varepsilon_L$$

Furthermore, the total concentrations of receptor $[R]_0$ and ligand $[L]_0$ in the actual sample can be calculated from the initial concentrations of the stock solutions used ($[R]_0$* and $[L]_0$*) by accounting for the change in volume caused by each substrate addition using a dilution factor x that relates the volume added in each titration step (V_{added}) to the initial volume of the sample at the beginning of the titration ($V_{initial}$):

$$[R]_0 = \frac{[R]_0{}^*}{1+x} \quad [L]_0 = \frac{[L]_0{}^* \cdot x}{1+x} \quad \text{with } x = \frac{V_{added}}{V_{initial}}$$

The absorption coefficients of the receptors ($\varepsilon_R = 25300\,\text{M}\,\text{cm}^{-1}$ for **7** and $\varepsilon_R = 22250\,\text{M}\,\text{cm}^{-1}$ for **8**) as well as of the tetrapeptide substrates ($\varepsilon_L = 200\,\text{M}\,\text{cm}^{-1}$ for both **9**

and **10**) were determined from independent measurements and were used as constants in the data analysis. The only parameters to be fitted were therefore the absorption coefficient of the complex ε_{RL} and the binding constant K itself. A representative binding isotherm is shown in Figure 3. These

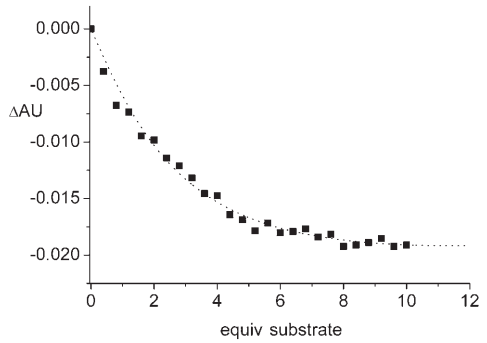


Figure 3. Binding isotherm at $\lambda = 300$ nm for the complexation of tetrapeptide **9** by receptor **7** as obtained from a UV titration experiment corrected for absorption changes due to dilution. The dotted line represents the curve-fitting for a 1:1 complexation.

titration experiments confirm that our receptors also form stable complexes with both tetrapeptides in free solution. For receptor **7** (CBS-KKF), which showed the highest affinity for substrate **2** in the solid-phase screening, the binding constant for tetrapeptide **9** ($K_{ass} = 15400\,M^{-1}$) is of the same order of magnitude but slightly lower than that obtained from the solid-phase screening ($K_{ass} = 17100\,M^{-1}$). In good agreement with the data derived from the solid-phase screening, the inverse peptide **10** is also bound less efficiently in free solution ($K_{ass} = 4500\,M^{-1}$) but again with similar affinity as on-bead ($K_{ass} = 4800\,M^{-1}$). For receptor **8** (CBS-KYK), the binding constants in free solution are $K_{ass} = 6200\,M^{-1}$ for **9** (EKAA) and $K_{ass} = 5300\,M^{-1}$ for **10** (AAKE), again in excellent agreement with the binding affinities on-bead ($K_{ass} = 5800$ and $K_{ass} = 4500\,M^{-1}$, respectively). The results show that at least for this specific receptor class the solid support itself does not have any significant influence on the complexation event.[27]

We also used fluorescence titration as a second independent method to determine the binding constant for receptor **8**. In this case, the decrease in the fluorescence emission of the pyrrole moiety at 329 nm upon complex formation was followed. To avoid a signal overlap with the Raman peak of water, an excitation wavelength > 300 nm had to be used (here we used 310 nm). In preliminary tests, we observed that in a certain concentration range the fluorescence intensity of the receptor actually increased upon dilution of the sample (Figure 4). The most probable explanation is that a non-specific fluorescence quenching occurs with increasing concentration. Another conceivable explanation, self-association of the receptor, seems rather unlikely as no similar effect was observed in the UV spectra. As Figure 4 shows, the starting concentration of the receptor has to be $c < 5 \times 10^{-5}$ M to ensure Lambert–Beer behavior. Therefore, the

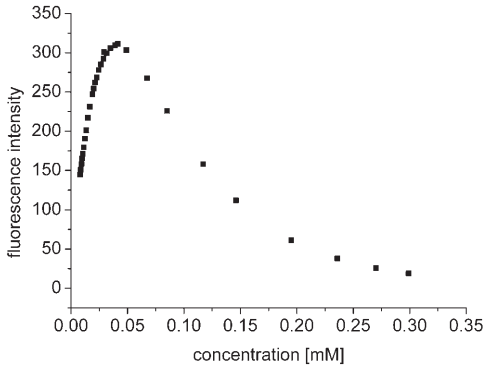


Figure 4. Dilution curve for the fluorescence intensity at $\lambda = 329$ nm of receptor **8** in buffered water. Above a concentration of 50 μM, fluorescence quenching is observed.

fluorescence titration was performed with an initial receptor concentration of $c = 1 \times 10^{-5}$ M.

Furthermore, the bis-tris buffer solution showed a significant deterioration with age, leading to an intense fluorescence emission at around 300 nm after a few weeks. All solutions were therefore freshly prepared. Even then, it took up to 45 min after each substrate addition until a constant and reproducible fluorescence intensity was observed. Whether this was due to mixing effects or unfavorable dynamics of complex formation is not known, but the fluorescence emission of pyrrole receptors of type **1** is generally rather sensitive to dissolved gases or solvent composition, pH or even temperature changes. These experimental problems made fluorescence titration less well suited for the determination of complex stabilities as a routine method, even though it is more sensitive due to the larger absorption changes. We therefore only investigated the complex formation between receptor **8** (CBS-KYK) and tetrapeptide **9** (EKAA) by fluorescence titration (Figure 5). As the tetrapeptide **9** also has significant emission bands in the same spectral range (due to the protonated ammonium group of the lysine residue), we first determined its emission coefficient by means of a dilution experiment. Perfect Lambert–Beer behavior was observed at 329 nm with an emission coefficient of $\varepsilon = 54740\,M\,cm^{-1}$, which together with that of receptor **8** ($\varepsilon = 7.7 \times 10^6\,M\,cm^{-1}$) was again used as a constant in the nonlinear curve-fitting of the binding isotherm (Figure 5). The calculated binding constant of $K_{ass} = 6100\,M^{-1}$ is in good agreement with that obtained from the UV titration ($K_{ass} = 6200\,M^{-1}$). These titration experiments show that fully flexible one-armed peptide receptors of type **1** are indeed capable of binding tetrapeptides such as **9** or **10** based on charge interactions and H-bonds even in free solution, confirming the results obtained from the screening of the solid-phase-bound library. However, the sequence of the tetrapeptide substrate does have a significant effect on the binding affinity, even though complexation is mainly dominated by rather long-range electrostatic interactions. As discussed previously, based on a statistical QSAR analysis, hydrophobic interactions are not important for substrate binding in this case.[16]

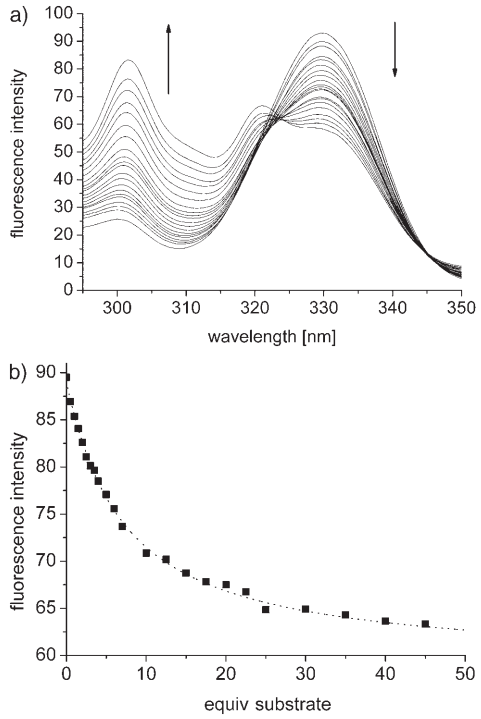


Figure 5. Changes in the fluorescence spectrum due to complex formation between receptor **8** and tetrapeptide **9** (a) and the resulting binding isotherm at $\lambda = 329$ nm (b).

Modeling studies: To further elucidate this difference in the complex stabilities of the two substrates **9** and **10**, we performed molecular mechanics calculations (Macromodel V. 8.0, Amber* force field, GB/SA water solvation).[28] Energy-minimized structures were obtained from a Monte Carlo conformational search (at least 50000 steps, until the minimum structure was found multiple times). The obtained energy-minimized structures were then further subjected to a MD simulation (10 ps at 300 K). According to these calculations, the weaker binding of the inverse tetrapeptide AAKE **3** is probably due to intramolecular ion-pair formation between the lysine and the two glutamate carboxylates (Figure 6). Both carboxylates of the inverse tetrapeptide AAKE, the one in the side chain and the one at the C-terminus, can interact simultaneously with the positively charged ammonium group of the lysine. These intramolecular ion pairs have to be broken before an efficient intermolecular interaction with the receptor can take place. In contrast, in tetrapeptide EKAA, the glutamate is located at the N-terminus and therefore the lysine can only interact with one of the two carboxylate groups of this tetrapeptide, the one on the glutamate side chain. The C-terminal alanine carboxylate is too far away and is not affected by this intramolecular ion-pair formation. It is therefore available for complexation by the guanidiniocarbonyl pyrrole receptor. This difference in intramolecular charge interactions within the substrate could explain the better binding of the tetrapeptide EKAA **2** compared to the inverse sequence AAKE **3**.

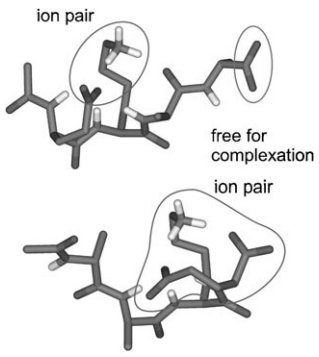


Figure 6. Calculated energy-minimized structures of the tetrapeptide substrates **9** (EKAA, top) and **10** (AAKE, bottom) in water.

The calculated energy-minimized structure of the complex between the most efficient receptor **7** (CBS-KKF) and the tetrapeptide **9** (EKAA) is shown in Figure 7. The C-terminal carboxylate of the tetrapeptide substrate is bound by the guanidiniocarbonyl pyrrole moiety and simultaneously by the lysine in position 1 of the receptor. This explains the significant effect of this position on complex stability, as also seen in the library screening (Figure 8). When the amino acid sequence of the tripeptide part of the receptor is systematically varied, every eighth member has a lysine in position 1 next to the guanidinium cation, corresponding to a general receptor sequence CBS-Lys-AA^2-AA^3. This causes a significant increase in the association constant determined in the quantitative on-bead screening compared to related

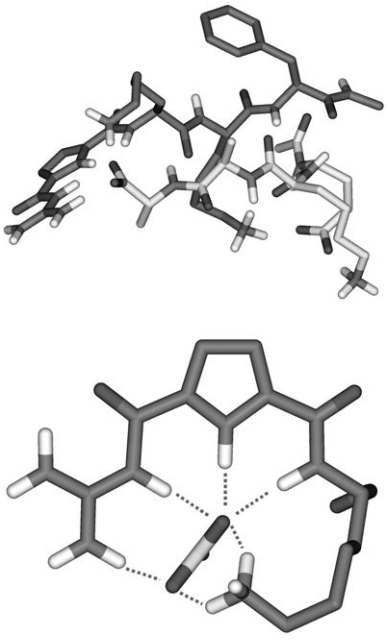


Figure 7. Energy-minimized structure of the complex between receptor **7** (CBS-KKF, dark grey) and tetrapeptide **9** (EKAA, light grey) (top) in water, and the binding scheme of the C-terminal carboxylate (bottom) showing the stabilizing effect of lysine in position 1 of the receptor [nonpolar hydrogens omitted for clarity].

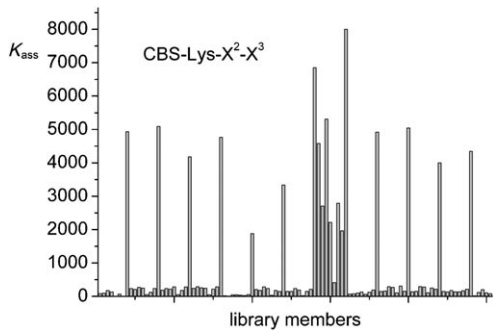


Figure 8. Selection of association constants K_{ass} [in M^{-1}] determined from quantitative on-bead screening by systematic sequence variation of the tripeptide part of the receptor. Every eighth column corresponds to a receptor of the general sequence CBS-Lys-AA^2-AA^3, each of which shows significantly increased affinity compared with the other receptors. This demonstrates the additional stabilization of the complex by a lysine in position 1 of the receptor.

sequences, as shown in Figure 8. This demonstrates the additional stabilization of the complex by a lysine in position 1 of the receptor. The second lysine of the receptor CBS-KKF ion pairs with the glutamate side chain of the substrate, which is also stabilized by the adjacent lysine of the substrate (reflecting the intramolecular ion pair that can be seen in Figure 6). Hence, complex formation can be summarized as follows: strong double ion-pair formation to the C-terminal carboxylate and a second, somewhat weaker, charge interaction with the N-terminal glutamate.

This efficient binding of the C-terminal carboxylate is further illustrated by the calculated electrostatic surface potential of the complex (Figure 9). The charge of the C-terminal carboxylate (left side, green circle) is efficiently neutralized by the cationic guanidiniocarbonyl pyrrole moiety and the ammonium ion of lysine 1 in the receptor. This is most evident by comparison with the N-terminal glutamate carboxylate (right side, red circle), which despite being ion paired with two ammonium cations still bears a significant negative charge density. Even the various carbonyl oxygens (visible, for example, at the lower rim of the complex) have a higher negative charge density than the C-terminal carboxylate bound by the guanidiniocarbonyl pyrrole moiety.

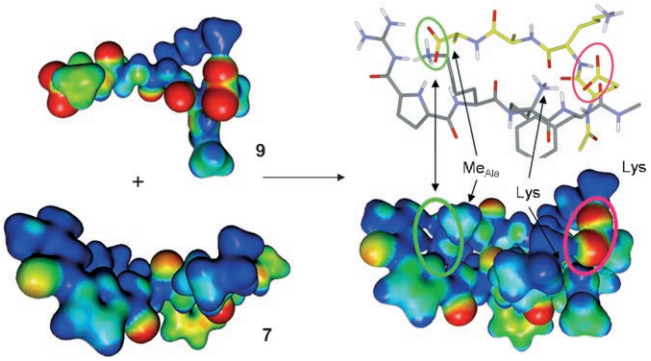


Figure 9. Calculated electrostatic surface potential (HF/6-31 G*) mapped onto the electron density, illustrating the charge complementarity between receptor **7** and tetrapeptide **9** (left), and the efficient charge neutralization especially for the C-terminal carboxylate upon complex formation (right). [Contour value = 0.02 e Å^{-3}; the geometries were taken from the force field calculations; color code for **7**: red < +0.15; yellow +0.2; green +0.25; light blue +0.3; blue +0.35; for **9**: red < −0.15; yellow −0.1; green −0.05; light blue 0.00; blue +0.05; for the complex: red < +0.05; yellow +0.1; green +0.15; light blue +0.2; blue +0.25].

Furthermore, the calculated dynamic stability of the complex is surprising in view of the flexibility of both the host and guest. According to an MD simulation in water at 300 K, no significant changes of the general complex structure and hence in the corresponding binding interactions are observed over a time period of 100 ps (Figure 10). The complex is therefore conformationally rather well defined.

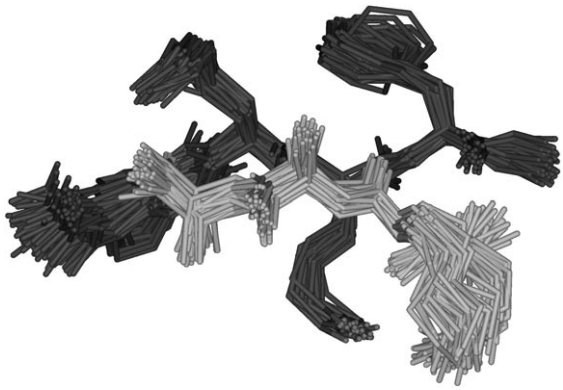


Figure 10. Superposition of complex structures as obtained from a molecular dynamics simulation over a time period of 100 ps (one structure taken every 2 ps) [grey: tetrapeptide **9**; black: receptor **7** CBS-KKF; hydrogens omitted for clarity].

Conclusion

Our experiments show that fully flexible one-armed receptors of the general type **1** form stable complexes based on electrostatic interactions with polar anionic tetrapeptides such as **9** or **10**, even in water. In spite of the long range of these charge interactions, the complex stability depends significantly on the amino acid sequence of the substrate. Moving the glutamate side chain from the N- to the C-terminus as in the inverse substrate **10** significantly affects the binding affinity. Hence, even rather flexible receptors such as **1** can exhibit substrate selectivity. We are currently exploring the usefulness of receptors such as CBS-KKF in the design of selective peptide sensors.

Experimental Section

General remarks: Reaction solvents were dried and distilled under argon before use. All other reagents were used as obtained from either Aldrich or Fluka. ^{1}H and ^{13}C NMR chemical shifts are reported relative to the signals of the deuterated solvents. Peak assignments are based on either DEPT, 2D NMR studies, and/or comparison with literature data. IR

spectra were recorded from samples prepared as KBr pellets. Melting points are not corrected.

Synthesis of the dansylated tetrapeptide substrate 3: The fluorophore-labeled tetrapeptide **3** was synthesized on Wang resin according to a standard protocol. Wang resin (300 mg, 1.11 mmol g^{-1}, 0.33 mmol) was swollen in CH_2Cl_2/DMF (8:2) for 1.5 h, and the first amino acid was coupled to the resin by the application of Fmoc-D-Glu(OBn)-OH (2.0 equiv), diisopropyl carbodiimide (DIC, 2.0 equiv), and dimethyl aminopyridine (DMAP, 0.1 equiv) in CH_2Cl_2/MeOH (8:2) for a reaction time of 20 h. The coupling step was repeated twice. The Fmoc group was then cleaved with piperidine in DMF (20%). The next three amino acids (L-Lys(Boc), D-Ala, and D-Ala, respectively) were coupled under the following conditions: amino acid (2.5 equiv) and (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP) (2.5 equiv) in DMF containing 3% *N*-methylmorpholine (NMM; 10 mL) for 4 h. The free amino function of the tetrapeptide was then coupled with succinic anhydride **4** (10.0 equiv) in CH_2Cl_2/DMF (8:2) for 3 h. The resulting carboxylic acid was coupled with 1,13-diamino-4,7,10-trioxaundecane **5** in the presence of PyBOP (10.0 equiv) in DMF (reaction time 3 h). The resulting amine was treated with dansyl chloride **6** (3.0 equiv) in DMF for 20 h. Cleavage from the resin and deprotection of the side chains was performed by exposure to CH_2Cl_2/TFA (1:1) for 2 h. The solvents were then evaporated, and the resulting red oil was treated with dry diethyl ether to obtain a light-green solid that was found to be analytically pure.

Tetrapeptide 3 (AAKE): Yield: 165 mg, 0.17 mmol, 52%; m.p. 106°C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.18 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3H; CH_3), 1.23 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3H; CH_3), 1.25–1.35 (m, 2H; CH_2), 1.49–1.60 (m, 6H; CH_2), 1.66 (m, 1H; CH_2), 1.77 (m, 1H; CH_2), 1.96 (m, 1H; CH_2), 2.22 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H; CH_2), 2.30–2.39 (m, 4H; CH_2), 2.70 (m, 2H; CH_2), 2.83 (q, $^3J_{H\text{-}H}$ = 5.9 Hz, 2H; CH_2), 3.03 (m, 2H; CH_2), 3.14 (s, 6H; CH_3), 3.21–3.24 (m, 4H; CH_2), 3.31–3.37 (m, 4H; CH_2), 3.38–3.42 (m, 4H; CH_2), 3.44–3.50 (m, 2H; CH_2), 4.14–4.23 (m, 4H; CH), 7.77 (m, 2H; ar-CH), 7.84 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 2H; ar-CH), 7.95 (t, 1H; NH), 7.99 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1H; NH), 8.00 (s, 3H; NH_3^+), 8.06 (t, $^3J_{H\text{-}H}$ = 5.2 Hz, 1H; NH), 8.13 (d, $^3J_{H\text{-}H}$ = 6.9 Hz, 1H; NH), 8.18 (m, 2H; ar-CH), 8.61 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H; NH), 8.86 (d, $^3J_{H\text{-}H}$ = 7.4 Hz, 1H; NH); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 17.7, 17.8 (CH_3), 22.3, 26.5, 26.6, 29.5, 29.6, 30.1, 31.0, 31.1, 31.4, 36.0, 38.6, 40.2 (CH_2), 46.2 (CH_3), 48.7, 49.1, 51.2, 52.6 (CH), 67.5, 68.2, 69.5, 69.7, 69.8, 69.9 (CH_2), 125.4, 127.3, 127.8, 128.4, 129.0, 129.1 (CH), 136.7, 171.7, 172.0, 172.2, 172.5, 172.7, 173.2, 173.9 (C_q); MS (ESI, DMSO/H_2O): *m*/*z*: 953 [M^+], 806 [$M^+ - C_5H_8NO_4$], 678 [$M^+ - C_{11}H_{20}N_3O_5$], 607 [$C_{29}H_{43}N_4O_8S^+$], 536 [$C_{26}H_{38}N_3O_7S^+$], 452 [$C_{22}H_{34}N_3O_5S^+$]; HR-MS (pos. ESI): *m*/*z*: calcd for $C_{43}H_{68}N_8O_{14}S^+$: 953.465; found: 953.466; FT-IR (KBr disk): $\tilde{\nu}$ = 3413 (s), 3268 (s), 2925 (m), 1732 (m), 1647 (s), 1542 (m), 1450 (w), 1321 (w), 1320 (w), 1214 (w), 1143 (w), 795 cm^{-1} (w).

Synthesis of receptors 7 and 8 (general protocol): The synthesis was performed on Rink amide resin following a standard protocol. Rink amide resin (300 mg, 0.74 mmol g^{-1}, 0.22 mmol) was swollen in DMF for 1.5 h. The Fmoc protecting group was then removed by agitation with piperidine in DMF (20%) for 20 min. The conditions for the coupling of amino acids were as follows: Fmoc amino acid (2.5 equiv) and PyBOP (2.5 equiv) in DMF containing 3% NMM (10 mL). The mixture was shaken for 3.5 h to ensure quantitative coupling. The yield was monitored indirectly by the UV absorption of the Fmoc-piperidine adduct at 300 nm. The attachment of the 5-guanidiniocarbonylpyrrole-2-carboxylic acid was performed under similar conditions, carboxylic acid (2.5 equiv) and PyBOP (2.5 equiv) in DMF containing 5% NMM, for extended reaction times of 24 h. To ensure a quantitative coupling, the last step was repeated. The product was cleaved from the solid support by shaking the resin with CH_2Cl_2/TFA (5:95). The solvents were evaporated and the remaining oil was treated with dry diethyl ether. To obtain the hydrochloride salt, the resulting white solid was dissolved in methanol (1 mL) and acidified with 0.1 N hydrochloric acid (4 mL) and the mixture was lyophilized. This was repeated three times to ensure complete exchange of trifluoroacetate for chloride.

Receptor 7 (CBS-KKF): Yield: 151 mg, 0.21 mmol, 98%; m.p. 247°C (decomp); ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.16–1.28 (m, 2H; CH_2), 1.29–1.42 (m, 2H; CH_2), 1.44–1.75 (m, 8H; CH_2), 2.66–2.71 (m, 2H; CH_2), 2.73–2.79 (m, 2H; CH_2), 2.80–2.86 (m, 1H; CH), 2.98–3.02 (m, 1H; CH_2), 4.12–4.17 (m, 1H; CH), 4.40–4.45 (m, 2H; CH), 6.91 (s, 1H; py-CH), 7.12 (s, 1H; NH_2), 7.18–7.25 (m, 5H; ar-CH), 7.45 (s, 1H; NH_2), 7.60 (m, 1H; py-CH), 7.88 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H; NH), 7.92 (brs, 6H; NH_3^+), 8.27 (d, $^3J_{H\text{-}H}$ = 7.8 Hz, 1H; NH), 8.51 (s, 2H; gua-NH_2), 8.67 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1H; NH), 8.71 (s, 2H; gua-NH_2), 12.17 (s, 1H; gua-NH), 12.52 (s, 1H; py-NH); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 22.3, 22.7, 26.6, 26.7, 31.2, 31.3, 37.8, 38.7, 40.2 (CH_2), 53.0, 53.0, 53.8 (CH), 113.8, 116.0 (py-CH), 125.8, 132.5 (py-C_q), 126.4, 128.2, 129.4, 155.7 (gua-C_q), 137.9, 159.1, 159.9, 171.3, 171.8, 173.0 (C_q); MS (ESI, DMSO/H_2O): *m*/*z*: 599 [M^+], 377 [$C_{21}H_{37}N_6O_3^+$], 300 [$(M+H)^{2+}$], 179 [$C_7H_7N_4O_2^+$]; FT-IR (KBr disk): $\tilde{\nu}$ = 3326 (s), 3061 (s), 2949 (m), 1702 (s), 1654 (s), 1541 (m), 1472 (w), 1276 (w), 1198 (w), 815 (w), 754 cm^{-1} (w).

Receptor 8 (CBS-KYK): Yield: 110 mg, 0.15 mmol, 69%; m.p. 209°C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 1.20–1.32 (m, 4H; CH_2), 1.47–1.69 (m, 6H; CH_2), 1.65–1.70 (m, 2H; CH_2), 2.69–2.77 (m, 5H; CH_2), 2.90–2.96 (m, 1H; CH), 4.33–4.43 (m, 2H; CH), 6.59 (d, $^3J_{H\text{-}H}$ = 8.6 Hz, 2H; ar-CH), 6.91 (s, 1H; py-CH), 7.01 (d, $^3J_{H\text{-}H}$ = 8.6 Hz, 2H; ar-CH), 7.06 (s, 1H; NH_2), 7.25 (s, 1H; NH_2), 7.61 (m, 1H; py-CH), 7.87 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H; NH), 7.97 (brs, 6H; NH_3^+), 8.20 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H; NH), 8.53 (s, 2H; gua-NH_2), 8.66 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1H; NH), 8.72 (s, 2H; gua-NH_2), 9.18 (brs, 1H; OH), 12.15 (s, 1H; gua-NH), 12.51 (s, 1H; py-NH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 22.3, 22.6, 26.7, 26.8, 31.3, 31.5, 36.3, 38.7 (CH_2), 52.4, 53.2, 54.6 (CH), 113.8, 116.0 (py-CH), 115.0, 130.2 (ar-CH), 125.8, 132.5 (py-C_q), 155.8 (gua-C_q), 127.9, 156.0, 159.9, 171.0, 171.6, 173.5 (C_q); MS (ESI, DMSO/H_2O): *m*/*z*: 615 [M+H^+], 437 [$M^+ - C_7H_9N_4O_2$], 308 [$(M+H)^{2+}$], 179 [$C_7H_7N_4O_2^+$]; FT-IR (KBr disk): $\tilde{\nu}$ = 3421 (s), 2938 (m), 1710 (s), 1654 (s), 1558 (m), 1477 (w), 1268 (w), 1194 (w), 821 (w), 758 cm^{-1} (w).

Synthesis of the acetylated tetrapeptides 9 and 10: The acetylated tetrapeptides **9** and **10** were synthesized on Wang resin according to a standard protocol. Wang resin (300 mg, 1.11 mmol g^{-1}, 0.33 mmol) was swollen in CH_2Cl_2/DMF (8:2) for 1.5 h, and the first amino acid was coupled to the resin by the application of Fmoc-D-Ala-OH (2.0 equiv) or Fmoc-D-Glu-OH (2.0 equiv), respectively, diisopropylcarbodiimide (DIC, 2.0 equiv), and dimethylaminopyridine (DMAP, 0.1 equiv) in CH_2Cl_2/MeOH (8:2) for a reaction time of 20 h. The coupling step was repeated twice. The Fmoc group was then cleaved with piperidine in DMF (20%). The other three amino acids (D-Ala, L-Lys, D-Glu and L-Lys, D-Ala, D-Ala, respectively) were coupled under the following conditions: amino acid (2.5 equiv) and (benzotriazol-1-yloxy)trispyrrolidinophosphonium hexafluorophosphate (PyBOP) (2.5 equiv) in DMF containing 3% *N*-methylmorpholine (NMM; 10 mL) for 4 h. The resulting free amino functions were acetylated using acetic anhydride (340 mg, 3.3 mmol, 10 equiv) and DMAP (0.1 equiv) in CH_2Cl_2/DMF (8:2) for 8 h. The resin was washed thoroughly with CH_2Cl_2, methanol, diethyl ether, and further CH_2Cl_2 to remove traces of DMF. Cleavage of the product from the resin was achieved by treatment with 50% TFA in CH_2Cl_2 and it was then precipitated by adding dry diethyl ether to the solution. The white solids were lyophilized twice with water containing 2 mL of 0.1 N HCl to ensure the presence of a hydrochloride salt. The white solids thus obtained were found to be analytically pure.

Tetrapeptide 9 (*N*-Ac-EKAA-OH): Yield: 82 mg, 0.17 mmol, 52%; m.p. 187°C; ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.18 (d, $^3J_{H\text{-}H}$ = 7.0 Hz, 3H; CH_3), 1.26 (d, $^3J_{H\text{-}H}$ = 7.3 Hz, 3H; CH_3), 1.21–1.35 (m, 4H; CH_2), 1.49–1.56 (m, 4H; CH_2), 1.63 (m, 1H; CH_2), 1.72 (m, 1H; CH_2), 1.83 (m, 1H; CH_2), 1.84 (s, 3H; CH_3), 2.23 (m, 2H; CH_2), 2.72 (m, 2H; CH_2), 4.15–4.22 (m, 3H; CH), 4.30 (m, 1H; CH), 7.93 (brs, 3H; NH_3^+), 8.04 (d, $^3J_{H\text{-}H}$ = 7.9 Hz, 1H; NH), 8.04 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1H; NH), 8.09 (d, $^3J_{H\text{-}H}$ = 7.6 Hz, 1H; NH), 8.13 (d, $^3J_{H\text{-}H}$ = 7.3 Hz, 1H; NH); ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 16.9, 18.2, 22.4 (CH_3), 22.1, 26.4, 27.1, 30.1, 31.0, 38.4 (CH_2), 47.4, 47.6, 52.1, 52.4 (CH), 169.8, 171.2, 171.6, 171.9, 174.0, 174.1 (C_q); MS (ESI, DMSO/H_2O): *m*/*z*: 460 [M^++H], 396 [M^+ +Na$-C_3H_6NO_2$], 389 [$C_{18}H_{33}N_2O_7^+$], 290 [$C_{13}H_{24}NO_6^+$], 203 [$C_8H_{15}O_5Na^+$]; FT-IR (KBr disk): $\tilde{\nu}$ = 3422 (s), 3074 (s), 2922 (s), 1728

(m), 1654 (s), 1542 (m), 1459 (w), 1378 (w), 1213 (m), 1170 (m), 1046 (w), 826 cm^{-1} (w).

Tetrapeptide 10 (*N*-Ac-AAKE-OH): Yield: 220 mg, 0.21 mmol, 98% (as trifluoroacetate salt); m.p. 154°C; ^1H NMR (600 MHz, [D$_6$]DMSO): δ = 1.19 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 3H; CH_3), 1.23 (d, $^3J_{H\text{-}H}$ = 7.3 Hz, 3H; CH_3), 1.25–1.34 (m, 2H; CH_2), 1.47–1.56 (m, 3H; CH_2), 1.69 (m, 1H; CH_2), 1.81 (m, 1H; CH_2), 1.84 (s, 3H; CH_3), 1.98 (m, 1H; CH_2), 2.29 (t, $^3J_{H\text{-}H}$ = 7.9 Hz, 2H; CH_2), 2.75 (m, 2H; CH_2), 4.16–4.29 (m, 4H; CH), 7.72 (brs, 3H; NH_3^+), 7.85 (d, $^3J_{H\text{-}H}$ = 8.2 Hz, 1H; NH), 8.04 (m, 2H; NH), 8.11 (d, $^3J_{H\text{-}H}$ = 7.7 Hz, 1H; NH), 12.44 (brs, 1H; COOH); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 18.1, 18.2, 22.6 (CH_3), 22.1, 26.3, 26.7, 30.2, 31.5, 38.9 (CH_2), 48.5, 48.7, 51.4, 52.0 (CH), 169.5, 171.6, 172.2, 172.5, 173.2, 173.9 (C_q); MS (ESI, DMSO/H_2O): *m*/*z*: 460 [M^++H], 346 [M^+ −$C_5H_7NO_2$], 274 [M^+−$C_8H_{13}N_2O_3$]; FT-IR (KBr disk): $\tilde{\nu}$ = 3423 (s), 3071 (s), 2927 (s), 1729 (m), 1655 (s), 1542 (m), 1450 (w), 1374 (w), 1210 (m), 1172 cm^{-1} (m).

Acknowledgements

Financial support from the Fonds der Chemischen Industrie and the Deutsche Forschungsgemeinschaft (SFB 630) is gratefully acknowledged. We thank Sebastian Schlund and Prof. Dr. Bernd Engels (University of Wuerzburg) for performing the ESP calculation.

[1] a) H.-J. Böhm, G. Klebe, *Angew. Chem.* **1996**, *108*, 2750–2778; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2588–2614; b) E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 1244–1287; *Angew. Chem. Int. Ed.* **2003**, *42*, 1210–1250.

[2] For some recent examples of the use of artificial receptors as sensors, see: a) M. Maue, T. Schrader, *Angew. Chem.* **2005**, *117*, 2305–2310; *Angew. Chem. Int. Ed.* **2005**, *44*, 2265–2270; b) W. L. Wong, K.-H. Huang, P.-F. Tei, C.-S. Lee, H.-L. Kwong, *Chem. Commun.* **2005**, 384–385; c) Z. Zhong, E. V. Anslyn, *J. Am. Chem. Soc.* **2002**, *124*, 9014–9015; d) S. E. Schneider, S. N. O'Neil, E. V. Anslyn, *J. Am. Chem. Soc.* **2000**, *122*, 542–543; e) K. Niikura, A. Metzger, E. V. Anslyn, *J. Am. Chem. Soc.* **1998**, *120*, 8533–8534.

[3] A recent example can be found in: a) H. Yin, A. D. Hamilton, *Angew. Chem.* **2005**, *117*, 4200–4235; *Angew. Chem. Int. Ed.* **2005**, *44*, 4130–4163; b) for another interesting example also involving a combinatorial library approach, see: D. L. Dong, R. Liu, R. Sherlock, M. H. Wigler, H. P. Nestler, *Chem. Biol.* **1999**, *6*, 133–141.

[4] For selected examples of artificial peptide receptors, see: a) P. Krattiger, H. Wennemers, *Synlett* **2005**, 706–708; b) C. Schmuck, L. Geiger, *J. Am. Chem. Soc.* **2004**, *126*, 8898–8899; c) C. Chamorro, J.-W. Hofman, R. M. J. Liskamp, *Tetrahedron* **2004**, *60*, 8691–8697; d) H. Wennemers, M. C. Nold, M. M. Conza, K. J. Kulicke, M. Neuburger, *Chem. Eur. J.* **2003**, *9*, 442–448; e) C. Schmuck, M. Heil, *ChemBioChem* **2003**, *4*, 1232–1238; f) S. Rensing, T. Schrader, *Org. Lett.* **2002**, *4*, 2161–2164; g) K. Jensen, T. M. Braxmeier, M. Demarcus, J. G. Frey, J. D. Kilburn, *Chem. Eur. J.* **2002**, *8*, 1300–1309; h) M. Conza, H. Wennemers, *J. Org. Chem.* **2002**, *67*, 2696–2698; i) H. Wennemers, M. Conza, M. Nold, P. Krattiger, *Chem. Eur. J.* **2001**, *7*, 3342–3347; j) R. Xuo, G. Greiveldinger, L. E. Marenus, A. Cooper, J. A. Ellman, *J. Am. Chem. Soc.* **1999**, *121*, 4898–4899; k) T. Fessmann, J. D. Kilburn, *Angew. Chem.* **1999**, *111*, 2170–2174; *Angew. Chem. Int. Ed.* **1999**, *38*, 1993–1996; l) M. Sirish, H.-J. Schneider, *Chem. Commun.* **1999**, *10*, 907–908; m) M. Davies, M. Bonnat, F. Guillier, J. D. Kilburn, M. Bradley, *J. Org. Chem.* **1998**, *63*, 8696–8703; n) A. Hossain, H. J. Schneider, *J. Am. Chem. Soc.* **1998**, *120*, 11208–11209; o) R. Breslow, Z. Yang, R. Ching, G. Trojandt, F. Odobel, *J. Am. Chem. Soc.* **1998**, *120*, 3536–3537; p) J. S. Albert, M. W. Peczuh, A. D. Hamilton, *Bioorg. Med. Chem.* **1997**, *5*, 1455–1467.

[5] For review articles on artificial peptide receptors, see: a) M. W. Peczuh, A. D. Hamilton, *Chem. Rev.* **2000**, *100*, 2479–2494; b) H.-J. Schneider, *Adv. Supramol. Chem.* **2000**, *7*, 185–216; c) H.-J. Schneider, *Angew. Chem.* **1993**, *105*, 890–892; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 848–850; d) T. H. Webb, C. S. Wilcox, *Chem. Soc. Rev.* **1993**, *22*, 383–395.

[6] a) M. Meot-Ner, *Chem. Rev.* **2005**, *105*, 213–284; b) G. A. Jeffrey, *An Introduction to Hydrogen Bonding*, Oxford University Press, New York, **1997**; c) J. Israelachvili, *Intermolecular and Surface Forces*, 2nd ed., Academic Press, London, **1992**.

[7] In contrast to coulombic interactions between two point charges, which are of course non-directional, charge interactions with organic molecules such as guanidinium cations are directional. This may be due to the formation of H-bond-enforced ion pairs and/or the anisotropic solvation of such ions: P. E. Mason, G. W. Neilson, J. E. Enderby, M.-L. Saboungi, C. E. Dempsey, A. D. MacKerell Jr., J. W. Brady, *J. Am. Chem. Soc.* **2004**, *126*, 11462–11471. For a general discussion of the directionality of non-covalent interactions, see: J. P. Glusker, *Top. Curr. Chem.* **1998**, *198*, 1–56.

[8] L.-J. Prins, D. N. Reinhoudt, P. Timmerman, *Angew. Chem.* **2001**, *113*, 2446–2492; *Angew. Chem. Int. Ed.* **2001**, *40*, 2383–2426.

[9] For reviews on hydrophobic interactions, see: a) B. Widom, P. Bhimalapuram, K. Koga, *Phys. Chem. Chem. Phys.* **2003**, *5*, 3085–3093; b) L. R. Pratt, A. Pohorille, *Chem. Rev.* **2002**, *102*, 2671–2691; c) N. T. Southall, K. A. Dill, A. D. J. Haymet, *J. Phys. Chem. B* **2002**, *106*, 521–533.

[10] A. M. Davis, S. J. Teague, *Angew. Chem.* **1999**, *111*, 778–792; *Angew. Chem. Int. Ed.* **1999**, *38*, 736–749.

[11] S. Kumar, R. Nussinov, *ChemBioChem* **2002**, *3*, 604–617.

[12] For example, Fersht has estimated that charge interactions within proteins are much stronger than those in free solution: a) A. R. Fersht, *Trends Biochem. Sci.* **1987**, *12*, 301–304; b) A. R. Fersht, J. P. Shi, J. Knill-Jones, D. M. Lowe, A. J. Wilkinson, D. M. Blow, P. Brick, P. Carter, M. M. Waye, G. Winter, *Nature* **1985**, *314*, 235–238.

[13] The importance of hydrophobic interactions for peptide-peptide interactions within model systems ("hydrophobic cluster formation") was, for example, demonstrated by Kelly in his work on β-sheet nucleators: a) P. Chitnumsub, W. R. Fiori, H. A. Lashuel, H. Diaz, J. W. Kelly, *Bioorg. Med. Chem.* **1999**, *7*, 39–59; b) K. Y. Tsang, H. Diaz, N. Graciana, J. W. Kelly, *J. Am. Chem. Soc.* **1994**, *116*, 3988–4005; c) J. P. Schneider, J. W. Kelly, *Chem. Rev.* **1995**, *95*, 2169–2187.

[14] For reviews on aromatic interactions within peptides and in supramolecular model systems, see: a) M. L. Waters, *Biopolymers* **2004**, *76*, 435–445; b) M. L. Waters, *Curr. Opin. Chem. Biol.* **2002**, *6*, 736–741; c) C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc. Perkin Trans. 2* **2001**, 651–669; d) S. K. Burley, G. A. Petsko, *Science* **1985**, *229*, 23–28; e) for a critical comment, see: D. M. Chung, Y. Dou, P. Baldi, J. S. Nowick, *J. Am. Chem. Soc.* **2005**, *127*, 9998–9999.

[15] Some recent examples of metal-based peptide binding: a) A. Buryak, K. Severin, *Angew. Chem.* **2004**, *116*, 4875–4878; *Angew. Chem. Int. Ed.* **2004**, *43*, 4771–4774; b) H. Imai, H. Munakata, Y. Uemori, N. Sakura, *Inorg. Chem.* **2004**, *43*, 1211–1213; c) A. T. Wright, E. V. Anslyn, *Org. Lett.* **2004**, *6*, 1341–1344; d) M. Sirish, V. Chertkov, H.-J. Schneider, *Chem. Eur. J.* **2002**, *8*, 1181–1188; e) H. Ogoshi, T. Mizutani, *Acc. Chem. Res.* **1998**, *31*, 81–89.

[16] C. Schmuck, M. Heil, K. Baumann, J. Scheiber, *Angew. Chem.* **2005**, *117*, DOI: 10.1002/ange. 200501812; *Angew. Chem. Int. Ed.* **2005**, *44*, DOI: 10.1002/anie.200501812.

[17] a) R. D. Süssmuth, *ChemBioChem* **2002**, *3*, 295–298; b) K. C. Nicolaou, C. N. Boddy, S. Bräse, N. Wissinger, *Angew. Chem.* **1999**, *111*, 2230–2287; *Angew. Chem. Int. Ed.* **1999**, *38*, 2096–2152; c) D. H. Williams, B. Bardsley, *Angew. Chem.* **1999**, *111*, 1264–1286; *Angew. Chem. Int. Ed.* **1999**, *38*, 1173–1193; d) C. T. Walsh, S. L. Fisher, I.-S. Park, M. Prahalad, Z. Wu, *Chem. Biol.* **1996**, *3*, 21–28.

[18] a) C. Schmuck, V. Bickert, *Org. Lett.* **2003**, *5*, 4579–4581; b) C. Schmuck, L. Geiger, *Curr. Org. Chem.* **2003**, *7*, 1485–1502; c) C. Schmuck, *Chem. Eur. J.* **2000**, *6*, 709–718.

[19] For comprehensive reviews of anion recognition, see the following: a) K. A. Schug, W. Lindner, *Chem. Rev.* **2005**, *105*, 67–113; b) M. D. Best, S. L. Tobey, E. V. Anslyn, *Coord. Chem. Rev.* **2003**, *240*, 3–15;

c) P. A. Gale, *Coord. Chem. Rev.* **2003**, *240*, 191–221; d) R. J. Fitzmaurice, G. M. Kyne, D. Douheret, J. D. Kilburn, *J. Chem. Soc. Perkin Trans. 1* **2002**, 841–864; e) T. S. Snowden, E. V. Anslyn, *Curr. Opin. Chem. Biol.* **1999**, *3*, 740–746; f) P. D. Beer, P. Schmitt, *Curr. Opin. Chem. Biol.* **1997**, *1*, 475–482; g) A. Bianchi, K. Bowman-James, E. Garcia-España, *Supramolecular Chemistry of Anions*, Wiley-VCH, New York, **1997**; h) F. P. Schmidtchen, M. Berger, *Chem. Rev.* **1997**, *97*, 1609–1646; i) C. Seel, A. Galán, J. de Mendoza, *Top. Curr. Chem.* **1995**, *175*, 101–132.

[20] For examples of supramolecular interactions within artificial β-sheets, see: a) M. Kruppa, C. Bonauer, V. Michlova, B. Koenig, *J. Org. Chem.* **2005**, *70*, 5305–5308; b) D. M. Chung, J. S. Nowick, *J. Am. Chem. Soc.* **2004**, *126*, 3062–3063; c) H. Zeng, X. Yang, A. L. Brown, S. Martinovic, R. D. Smith, B. Gong, *Chem. Commun.* **2003**, 1556–1557; d) H. Zeng, X. Yang, R. A. Flowers, B. Gong, *J. Am. Chem. Soc.* **2002**, *124*, 2903–2910; e) J. S. Nowick, *Acc. Chem. Res.* **1999**, *32*, 287–296; f) J. S. Nowick, E. M. Smith, M. Pairish, *Chem. Soc. Rev.* **1996**, *25* 401–415.

[21] Examples of combinatorial libraries based on peptidic structures: a) R. Boyce, G. Li, H. P. Nestler, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1994**, *116*, 7955–7956; b) E. J. Iorio, Y. Shao, C.-T. Chen, H. Wagner, W. C. Still, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1635–1638; c) R. Arienzo, J. D. Kilburn, *Tetrahedron* **2002**, *58*, 711–719; d) M. R. Carrasco, W. C. Still, *Chem. Biol.* **1995**, *2*, 205–212.

[22] For review articles on the use of combinatorial receptor libraries, see: a) N. Srinivasan, J. D. Kilburn, *Curr. Opin. Chem. Biol.* **2004**, *8*, 305–310; b) B. Linton, A. D. Hamilton, *Curr. Opin. Chem. Biol.* **1999**, *3*, 307–312; c) Y. R. DeMiguel, J. M. K. Sanders, *Curr. Opin. Chem. Biol.* **1998**, *2*, 417–421; d) W. C. Still, *Acc. Chem. Res.* **1996**, *29*, 155–163.

[23] K. S. Lam, M. Lebl, V. Krchnak, *Chem. Rev.* **1997**, *97*, 411–448.

[24] A. W. Czarnik, *Curr. Opin. Chem. Biol.* **1997**, *1*, 60–66.

[25] a) S. S. Yoon, W. C. Still, *Tetrahedron* **1995**, *51*, 567–578; b) Y. Cheng, T. Suenaga, W. C. Still, *J. Am. Chem. Soc.* **1996**, *118*, 1813–1814; c) P. W. Smith, G. Chang, W. C. Still, *J. Org. Chem.* **1988**, *53*, 1587–1590.

[26] a) C. S. Wilcox, in *Frontiers in Supramolecular Chemistry and Photochemistry* (Eds.: H.-J. Schneider, H. Dürr), VCH, Weinheim, **1990**, pp. 123–144; b) K. Hirose, *J. Inclusion Phenom. Macrocyclic Chem.* **2001**, *39*, 193–209; c) K. A. Connors, *Binding Constants*, Wiley, New York, **1987**; d) L. Fielding, *Tetrahedron* **2000**, *56*, 6151–6170.

[27] For the influence of the solid support and the microenvironment on weak interactions, see, for example: a) M. Conza, H. Wennemers, *Chem. Commun.* **2003**, 866–867; b) K. Ariga, T. Kunitake, *Acc. Chem. Res.* **1998**, *31*, 371–378.

[28] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem.* **1990**, *11*, 440–467.

Received: August 30, 2005
Published online: November 28, 2005

