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Ditopic crown ether–guanidinium ion receptors for the molecular recognition of amino acids and small peptides

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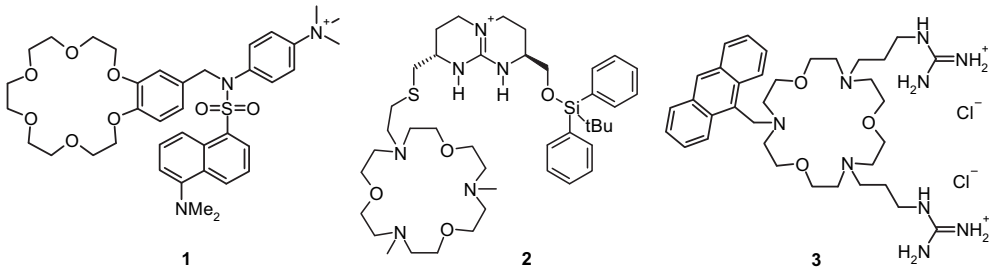
ABSTRACT

A series of ditopic synthetic receptors based on a crown ether–guanidinium ion recognition motif is reported. The compounds show binding affinity to selected amino acids, including important neurotransmitters. The effect of the distance of the ammonium and the carboxylate ion, the rigidity of the spacer, and the use of pre-organized pyrrole– and pyrene–guanidinium groups on binding affinity and selectivity are discussed.

1. Introduction

The selective recognition of amino acids and small peptides by synthetic receptors is still a challenge, whereby the detection of analytes, which are directly related to a biological function is of particular interest. Neurotransmitters are one group of such analytes. They play a critical role in the living organisms.[1,2] A typical example is γ-aminobutyric acid (GABA), a non-proteinogenic γ-amino acid, which binds specifically to the GABA receptors[3] located in nerve cells.[4]

The combination of crown ethers with ammonium or guanidinium ion binding sites has been successfully demonstrated in the design of synthetic amino acids receptors[5–9] by several examples. Crown ether **1** with a peralkylated ammonium group was tested for peptide binding: Triglycine showed the highest binding affinity in water (K=200 M^{-1}) and methanol (K=13,000 M^{-1}). Tripeptides bearing aromatic side chain functionalities showed a significantly increased affinity (K=2150 M^{-1} for Gly-Trp-Gly), which was explained by additional π-stacking and hydrophobic interactions with the luminescent dansyl group.[5] Compound **2** combines a chiral bicyclic guanidinium salt for carboxylate ion binding, a triaza-crown ether as an ammonium binding moiety and a hydrophobic silyl ether. The compound was used to bind amino acid zwitterions and transfers them into an organic phase. Small hydrophilic (Ser, Gly) and aromatic (Phe, Trp), but no charged amino acids were extracted.[6] Receptor **3** binds preferably glycine, lysine, and 4-amino butyric acid. The binding is indicated by an emission intensity increase (Fig. 1).[7]



1 2 3

Figure 1. Reported ditopic amino acid receptors containing crown ether and guanidinium or quaternary ammonium ion binding sites.

We have recently developed a luminescent crown ether amino acid, which represents a particular suitable building block for the construction of amino acid receptors when combined with guanidinium ions groups. The 21-azacrown-7 structure was optimized for ammonium on binding.[10] Figure 2 shows the general structure of the envisaged amino acid receptors. We discuss in the following the synthesis of a series of such compounds with systematically altered structure and their binding ability toward selected amino acids.

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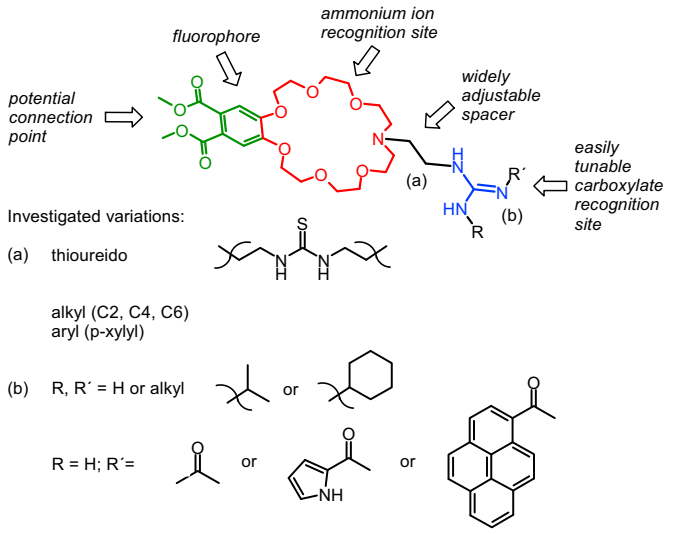


Figure 2. General structure and variations of the investigated crown ether guanidine conjugates.

2. Results and discussion

2.1. Synthesis

The synthesis of the amino acid receptors starts from crown ether amino esters **4a**, **4b**, **4c** and **4d** with ethyl, butyl, hexyl, and *p*-xylyl spacer, respectively, connecting the crown ether dimethyl phthalate to the amino moiety. A general synthetic route for their preparation[11] and several examples[11] have been published previously. A crown ether amino ester with thioureido **4e** was prepared from **4a** using the isothiocyanate of *N*-Boc-ethylenediamine (Scheme 1).[12]

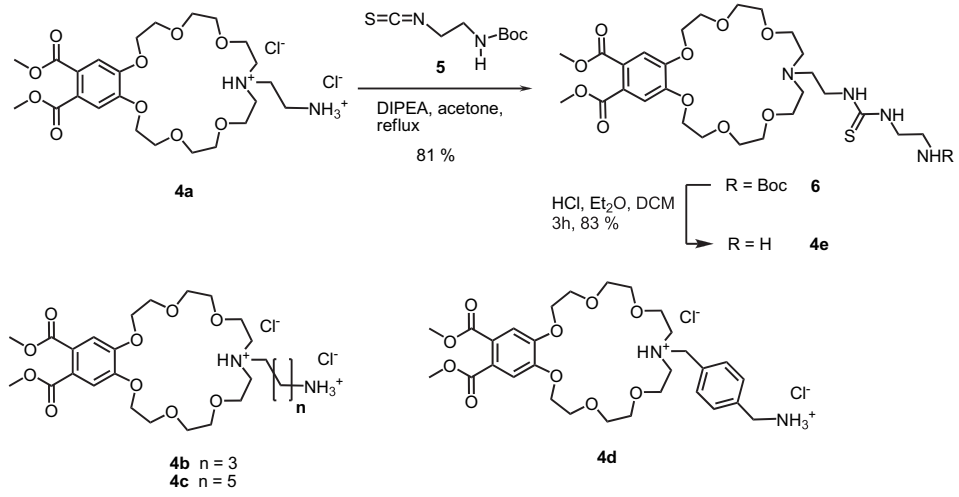
dry conditions. Guanidines **14** and **16** were obtained from reaction with methyl-*iso*-thiourea and subsequent deprotection.[23,24] The routes allow the introduction of a broad variety of substituents in good overall yield. The reactions are shown exemplarily for the conversion of **4a** into **11a**, **12a**, **14a**,[25] and **16a** in Scheme 2. Compound **4b** was converted analogously into **11b**, **12b**, **14b**, and **16b**, compound **4c** into **11c**, **12c**, **14c**, and **16c**, compound **4d** into **11d**, **12d**, **14d**, and **16d** and compound **4e** into **11e**, **12e**, **14e**, and **16e**. Table 1 summarizes the yields of the transformations.

Acetylation as in compounds **16** increases the acidity of the guanidinium NHs and pre-organizes the host by intramolecular hydrogen bonds, resulting in more directional H-bonds aiding the guest binding.[32] In competitive solvents the binding strength depends mainly on the energy penalty necessary to remove the solvation shell around the host.[26] Large alkyl and aryl substitution patterns can serve as an unpolar shielding at the guanidinium site, excluding the more destabilizing influences of the surrounding polar solvent.[27]

For the synthesis of compound **16e** a different route (Scheme 3) was used: **4a** was converted into thioisocyanate **17**, reacted with the Boc-protected amine **18** to give **13e**, which was deprotected and acylated to yield **16e**.

For comparison with the effect of the guanidinium moieties, quaternary amines were introduced as carboxylate binding sites.[5,28] Two such receptors **21** and **24**, and one guanidinium substituted compound **27** were prepared by the Huisgen-cycloaddition reaction. The starting alkyne **19** and azide **22** have been published recently.[11] 4-Azidoanilin[29] and propargylamine were converted into their corresponding iodide salts **20** and **23** in high yields. The protected guanidine **25** resulted from the reaction of propargylbromide with bis-Boc-protected guanidine under phase transfer conditions in excellent yield (Scheme 4).[30]

The Schmuck group developed a binding site for carboxylates by connecting a guanidinium group with pyrrole building blocks.[31,32]



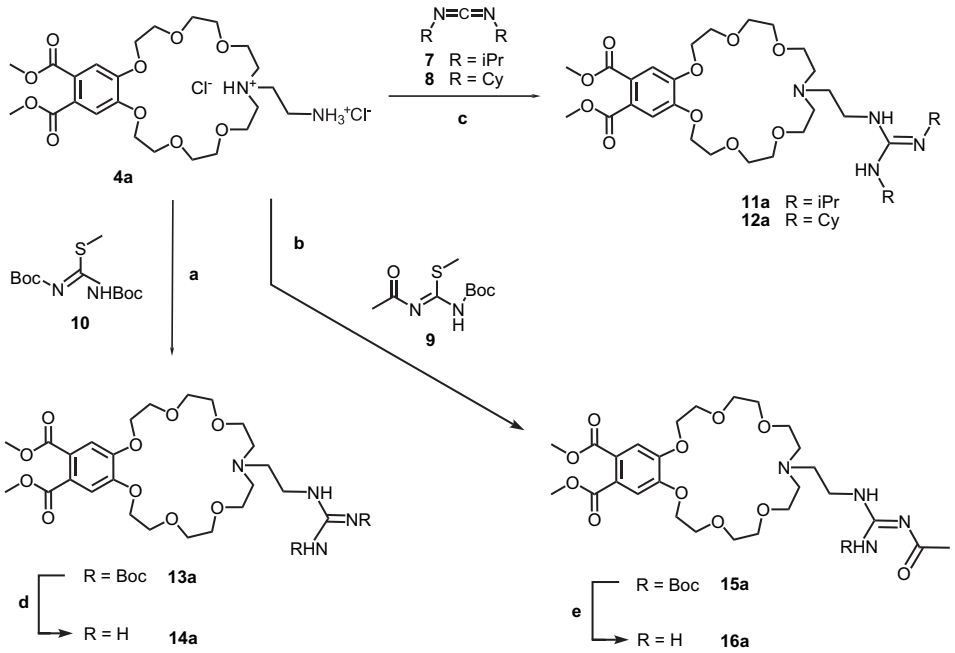
Scheme 1. Structures of crown ether amino esters **4a**–**e** and the synthesis of **4e** and **4a**.

The synthesis of guanidines involves treatment of an amine with an electrophilic amidine species. Several reviews have been published on this topic.[13] The most commonly used reagents include derivatives of pyrazole-1-carboxyamidine,[14] triflylguanidines,[15] *S*-alkyl-*iso*-thioureas,[16] and protected thiourea[17] derivatives, the latter often activated by mercury salts[18] or EDC.[19] Alternatively phase transfer catalytic substitution[20] or the Mitsonubo reaction on carbamate-protected guanidines[21] can be employed. For the conversion of an tableamine to a symmetrically substituted guanidine carbodiimides[22] are often used.

Amines **4** were converted into the corresponding guanidines **11** and **12** using symmetrically substituted carbodiimides **7** or **8** under

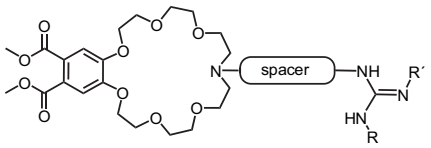
It has been demonstrated, that additional H-bonds, hydrophobic effects, and π-interactions enhance the binding strength, but mainly lead to a better selectivity of recognition. Pyrene features π-stacking abilities and can supply an unpolar shielding area. The fluorophore has been extensively used, e.g., developing fluorescent sensors for phosphates or DNA.[33,34] As binding site, the anticipated complexation with carboxylate anions will alter the redox potential of the guanidinium ion, which may change the emission properties of the fluorophore.

The guanidinium–pyrrol and guanidinium–pyrene compounds were prepared starting from amines **4a** and **4d**. In Scheme 5 the reactions are shown for **4d**; the synthesis using **4a** is analogous



Scheme 2. Routes to the crown ether guanidine receptors; conditions: a) NEt_3, $CHCl_3$ (dry), 2d, rt to 40 °C; 78%; b) NEt_3, $HgCl_2$, DMF (dry), $CHCl_3$ (dry), rt 2–4 h, over night 40 °C; 71%; c) 5 equiv DIC or DCC, DIPEA, dry MeCN or THF, 4d, reflux under nitrogen; 52–57%; ^{i}Pr=isopropyl; Cy=cyclohexyl; d) CH_2Cl_2, HCl in Et_2O, 5 h; 95%; e) CH_2Cl_2, HCl in Et_2O, 4–6 h, rt; quant.

Table 1
Structures and yields of prepared crown ether–guanidinium compounds with different spacer units



Amino ester	Spacer	Yields of corresponding guanidines [%]			
		11	**12**	**14**	**16**
		(R, R′=^{i}Pr)	(R, R′=Cy)	(R, R′=H)	(R=H, R′=Ac)
4a	Ethyl	**11a**: 69	**12a**: 76	**14a**: 78 (96)[a]	**16a**: 69 (91)[a]
4b	Butyl	**11b**: 58	**12b**: 62	**14b**: 75 (94)[a]	**16b**: 74 (90)[a]
4c	Hexyl	**11c**: 29	**12c**: 34	**14c**: 51 (78)[a]	**16c**: 43 (86)[a]
4d	*p*-Xylyl	**11d**: 61	**12d**: 68	**14d**: 82 (95)[a]	**16d**: 73 (93)[a]
4e	Bis-ethyl-thioureido	**11e**: 36	**12e**: 39	**14e**: 42 (76)[a]	—[b]

[a] The yield of the deprotection reaction is given in brackets.
[b] Not prepared.

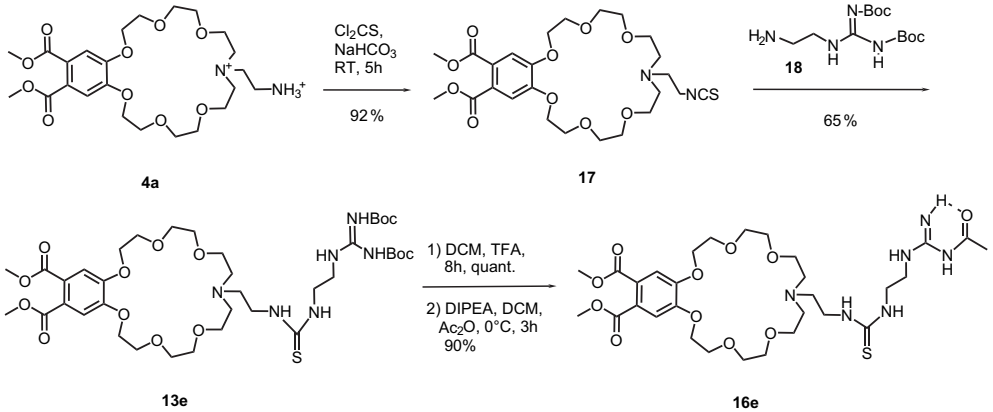
leading to compounds **37**–**40**. The methyl-*iso*-thioureas building blocks were prepared in good yields by peptide coupling of 1-Boc-2-methyl-*iso*-thiourea with pyrrole-2-carboxylic acid and pyrene-1-carboxylic acid, respectively. Compound **36** was prepared for comparison to probe the effect of the crown ether moiety on the binding.

Peptides for the determination of binding selectivity were prepared in solution and by standard solid phase methods.

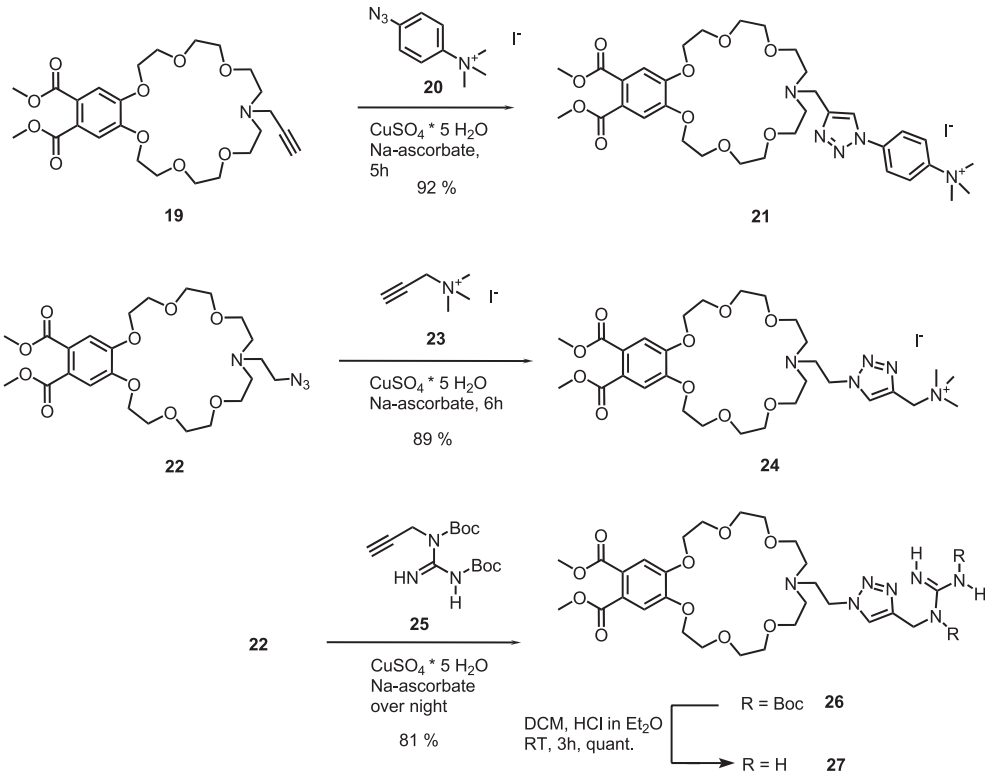
2.2. Recognition properties of ditopic amino acid receptors

2.2.1. Photophysical properties. Compounds **11**–**16** show absorption maxima in methanol at 220 nm and 270 nm (Fig. 3), and emit upon excitation at 390 nm (Fig. 4) with a quantum yield of about ϕ=0.1.[35] The absorption and emission properties are only marginally affected by the nature of the substituent *R*.[36] Compounds **21**, **24**, and **27** behave similar, only **21** with its triazolyl-anilinium system in the side chain shows a stronger absorption at 270 nm. The emission maximum and quantum yield of these compounds match with the values observed for the receptors **11**–**16**.

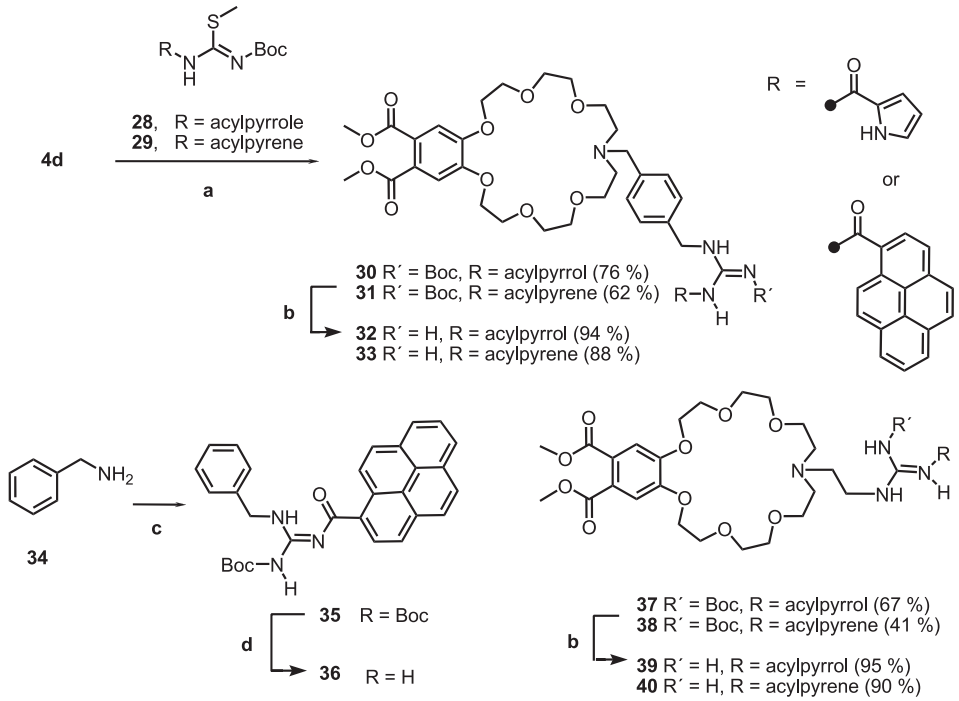
The pyrrole compounds **32** and **39** absorb at 220 nm and 300 nm in methanol, and emit upon excitation at 390 nm with a quantum



Scheme 3. Alternative synthesis for crown ether–guanidinium combinations (**13e** and **16e**) with thioureido spacer.



Scheme 4. Receptors **21**, **24**, and **27** for ammonium and carboxylate ion binding obtained by 'click reaction'.



Scheme 5. Synthesis of crown ether receptors **32**, **33**, **39**, and **40** with pyrrolecarbonyl–guanidinium and pyrenecarbonyl–guandinium groups; conditions: a) NEt_3, Hg^{2+}, DMF, $CHCl_3$, over night, rt; 69%; b) CH_2Cl_2, HCl in Et_2O, 4–6 h, rt; quant.; c) DMF, Hg^{2+}, rt, then 40 °C, 6 h, 91%; d) MeOH, $HCl_{(aq)}$, 3 h, rt; quant. Synthesis of model compound **36**.

yield of about ϕ=0.1.[35] The emission spectrum of the parent molecule **14a** is given for comparison with **39**. The receptors **32** and **39** consist of two electronically not coupled parts, therefore the UV spectrum is the sum of the spectra of the pyrrole–guanidinium-(λ_{max}=300 nm) and the crown ether unit (λ_{max}=220 and 300 nm).

Compounds **33** and **40** show absorption maxima in methanol at 240 nm, 280 nm, and 350 nm, and emit upon excitation at 390 nm with a quantum yield of about ϕ=0.1.[35] Emission maxima and quantum yield are pH dependent. The emission intensity increases upon addition of acid and decreases upon base addition (Fig. 5, right).

2.2.2. Influence of the protonation on the emission—pH range for the measurements. Receptor protonation increases the luminescent output of the receptor compounds[37] and can therefore interfere

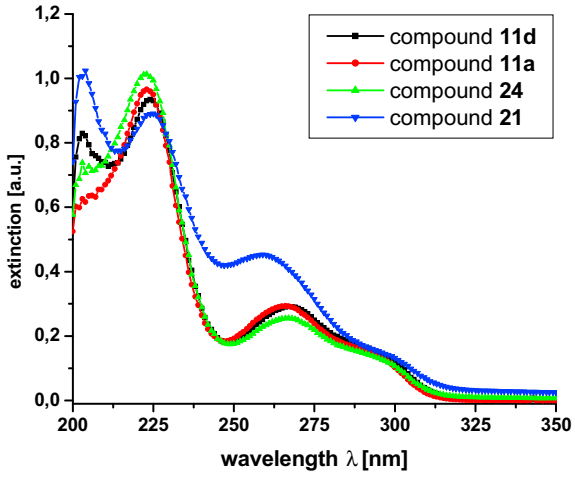


Figure 3. Absorption spectra of compounds **11a**, **11d** (c=3.4×10^{-5} mol/L) and **21**, **24** (c=3.5×10^{-5} mol/L) in methanol solution.

with the amino acid binding measurements. To minimize this effect, the optimum pH range for the investigations was evaluated by recording the emission intensity of selected receptor compounds depending on the pH value. A receptor solution in methanol (5×10^{-5} mol/L) was mixed with aqueous buffer solutions from pH 3 to pH 10 in the ratio 9/1.[38] After short equilibration time the fluorescence spectrum was recorded (Fig. 6).

2.2.3. The emission of most receptors increases from neutral pH values with increasing acidity. Therefore a weakly basic pH (7.5–8.5) is suitable for binding measurements. Ethylene and thioureido bridged systems can be used for emission binding titrations down to a pH=6.5 without interference. The pH range of the receptors for binding studies depends on the guanidine moiety, which has to be protonated for carboxylate ion binding.[39]

2.2.4. Binding of guanidinium and ammonium ions. The crown ether binding affinity for ammonium ions in aqueous solution at physiological pH is rather weak.[40] The ammonium binding properties were

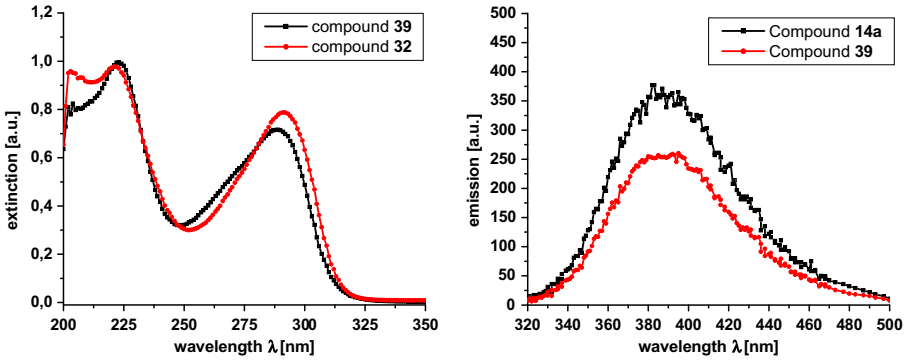


Figure 4. Absorption spectra of compounds **32** and **39** (3.6×10^{-5} mol/L) and the emission of compounds **14a** and **39** in comparison (c=2.0×10^{-5} mol/L) in methanol solution (λ_{ex}=300 nm).

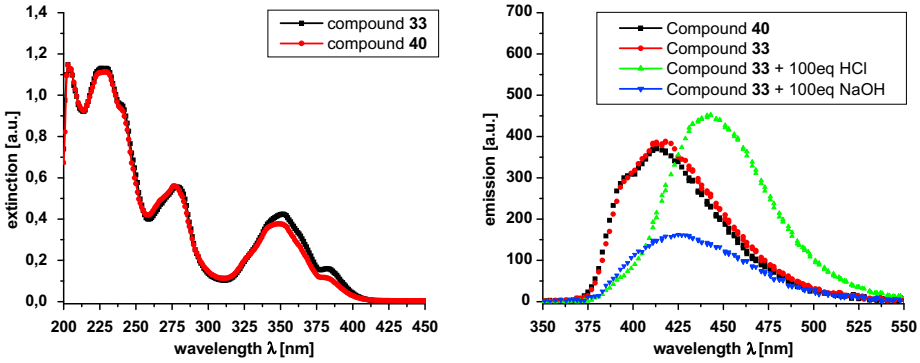


Figure 5. Absorption- (left) and emission (right) spectra of compounds **33** and **40** in methanol solution (c=1.6×10^{-5} mol/L); excitation wavelength (λ_{ex}) 390 nm.

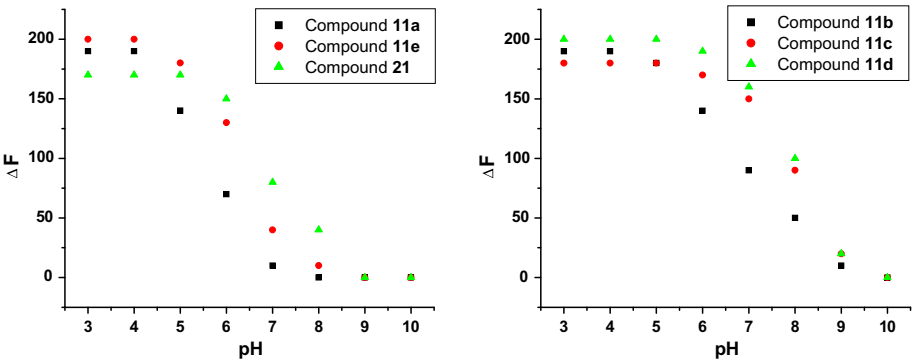


Figure 6. pH dependence of the emission intensity of selected receptors in methanol/water 9/1; λ_{max}=300 nm.

investigated in pH adjusted water/methanol systems using *n*-butyl ammonium chloride and acetyl lysine methyl ester hydrochloride with free side chain[41] by emission titration. Both ammonium species bind with comparable strength ($K \sim 200$–$300\ M^{-1}$ in methanol, $<100\ M^{-1}$ in methanol/water 9/1) matching the expected value for the crown ether alone determined in previous studies.[11,10] Addition of guanidine and acetyl-guanidinium hydrochloride (up to 2000 equiv) gave a negligible response in the fluorescence study.

As guanidinium ions show affinity to crown ether moieties,[42] we investigated a potential inter- or intramolecular self-aggregation of the guanidinium-crown ether receptors **11a**/**c**, **12a**/**c**, **14a**/**c** and **16a**/**c** under the experimental conditions. Self-aggregation would lead to competing equilibria and altered stoichiometries, which may complicate the determination of affinity constants. NMR dilution experiments revealed that unsubstituted moieties (**11a** and **11c**) showed minimal chemically induced shifts and weak aggregation in chloroform, but in aqueous methanol no aggregation is observed.

2.2.5. Screenings of the receptor library with amino acids and small peptides. Selectivities and relative response depending on the spacer length and the substitution pattern of the carboxylate binding sites were screened with compounds **11a**–**e**, **12a**–**e**, **14a**–**e**, **16a**–**e**, **21**, **24**, and **27**.

Different amino acids were selected as guests representing every mode of distance dependent binding, all kinds of polarity, charge, basicity, and acidity.[43] To study the influence of rigidity of the guest on binding, differently long glycine sequences and β-, γ-, and ε-amino acids, such as β-alanine, GABA, and AHX, were chosen. Upon binding the fluorescence increased. Taking into account the error of the spectrometer and the well plate reading system, only events with more than 10% increase were registered as binding and the fluorescence enhancement factors (z) were calculated as:

$$Z = \frac{F}{F_0}\left(\frac{v_0 + v}{v_0}\right) \tag{1}$$

Eq. 1 is used to calculate the fluorescence enhancement factors with volume corrections. F: observed fluorescence, F_0: fluorescence blanc sample, v_0: volume before addition, v: volume addition.

The increase of emission increase is a relative indicator for the binding strength (Fig. 7).

Asparagine, glutamine, leucine, phenylalanine, serine, cysteine, methionine, guanidine hydrochloride, and tetrabutylammonium acetate were also tested in the microtiter array and gave no response. In addition, the fluorescence response upon strong increase of the polarity[44] in the solution was investigated with 0.1 M sodium perchlorate in aqueous methanol and no change in emission intensity or shift of the fluorescence was observed.[45]

The receptors display distance dependent recognition ability. Only compounds with the short ethylene (**11a**, **12a**, **14a**, and **16a**), the rigid xylylene (**11d**, **12d**, **14d**, and **16d**) spacer or the triazole system with an appended aromatic quaternary amine (**21**) show moderate to good binding strengths and selectivities. Especially the xylylene spacered molecules respond very well and a significant increase of the emission intensity is observed with GABA (n=4). It is especially noteworthy, that glycine and glutaminic acid, the physiological precursor of GABA, induce a negligible fluorescence response, even if added in large excess with respect to the sensor. With receptors **24** and **27** good selectivities, but weak binding is observed. Receptors bridged by longer alkyl chains were rather

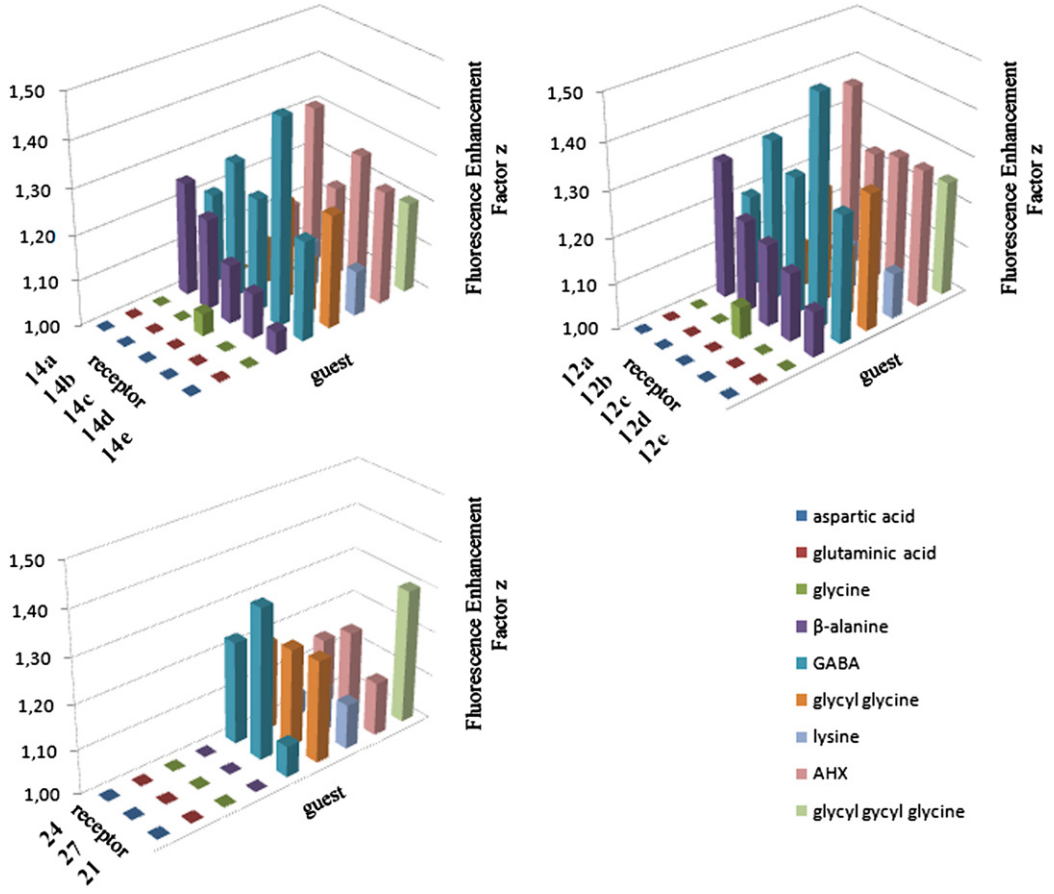


Figure 7. Selectivities and fluorescence enhancement factors of representative molecules; conditions: 2×10^{-5} M receptor in MeOH/water 9/1 at pH 7.5–8.5 adjusted with Et_4NOH or HCl, excitation wavelength 300 nm, emission wavelength 390 nm, the errors are estimated as ~10%; [guest]=0.02 M.

unselective and weak binders. The guanidines carrying cyclohexyl substituents showed no enhanced binding. All acetyl-guanidines are stable under the experimental conditions.[46]

2.2.6. Selectivities of crown-guanidino-pyrroles and -pyrenes. Upon the addition of a carboxylate guest, the UV bands of **33**, **36**, and **40** became broader and red shifted. In the fluorescence spectra a change in emission from 410 nm to 440 nm accompanied by quenching is observed. No excimer formation can be found in the presence of the charged guanidinium moiety. These receptors bind with the same selectivities as their isopropyl-substituted analogs. Guest addition results in a gradual decrease of luminescence intensity, which allows monitoring of the binding event by optical spectroscopy (Fig. 8).

tri- and tetrapeptide with one or, respectively, two inserted glycines in the sequence. The N-terminus of the peptides is acylated and the C-terminus functionalized as amide to allow only the interaction of the peptides side chains with the receptor. Additionally, four isomeric tetrapeptides containing glutaminic acid and three glycines were used to investigate the influence of the ammonium-to-carboxylate ion distance on the binding. To quickly select the peptide sequences with highest affinity a screening was performed in aqueous methanol solution (pH adjusted to 8–8.5) using a microtiter array as before. Figure 12 summarizes the results.

Glu-Lys peptides induced a significant increase in emission, revealing a preference for the shortest example (Fig. 10, right). The four isomeric glutaminic acid tetrapeptides show a luminescence enhancement depending on their carboxyl to N-terminus distance

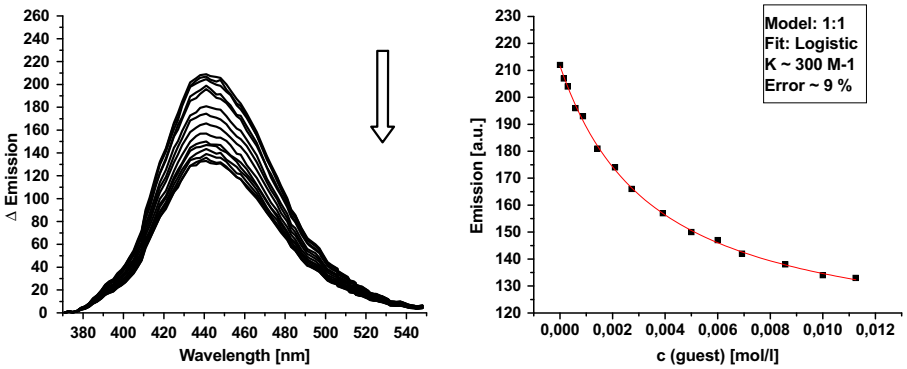


Figure 8. Fluorescent spectrum of **33** (8×10^{-6} M, λ_{ex}=350 nm, pH 6.5) at different ratios of γ-aminobutyric acid in aqueous methanol (see binding studies).

The binding of the receptors with pyrrole substituents **32** and **39** cannot be screened in the microtiter array due to the strong absorbance of the pyrrole system at 300 nm overlapping with the crown ether luminophore. No interpretable change in fluorescence intensity arises when a guest is added. The receptors were therefore investigated by UV spectroscopy in a cuvette. Following the results of the first screening only β-alanine, γ-aminobutyric acid, and ε-aminohexanoic acid were compared to glycine, glycyl glycine, lysine, aspartic, and glutaminic acid. In cases, where a ditopic binding is possible due to the right distance, the strongest change is observed.

2.2.7. Binding of short peptide sequences. The peptide binding selectivity of the crown ether with xylylene bis-isopropyl-guanidinium-binder (**11d**) was evaluated with small peptide sequences different in rigidity, distance of the ammonium, and carboxylate group and substituents. A lysine–glutaminic acid dipeptide was chosen due its biological relevance[47] and compared to the related (Fig. 10, left). Corresponding sequences containing glutamine instead of glutaminic acid did not show an increase in emission (data not shown), supporting the need of the additional coordination to the guanidine for recognition.

The peptide sequences with significant responses in the screening assay were investigated by emission titrations with **11d** (pH 8–8.5) or UV measurement with **32** (pH 6.5) in aqueous methanol solution. Their binding constants (log *K*) were derived from the titration data by non-linear fitting methods. The stoichiometry of the binding events was determined by Job's plot[48] analyses. Table 2 summarizes representative values for the isomeric Glu tetrapeptides.

Only peptide sequences, which bind with their carboxyl donor site to the guanidine, and, at the same time, have an ammonium ion in the right distance available, show strong and specific response. EGGG shows the highest affinity, as it provides an optimal geometry and distance to undergo bidentate coordination. Larger distances between both binding sites lead to a decrease in affinity.

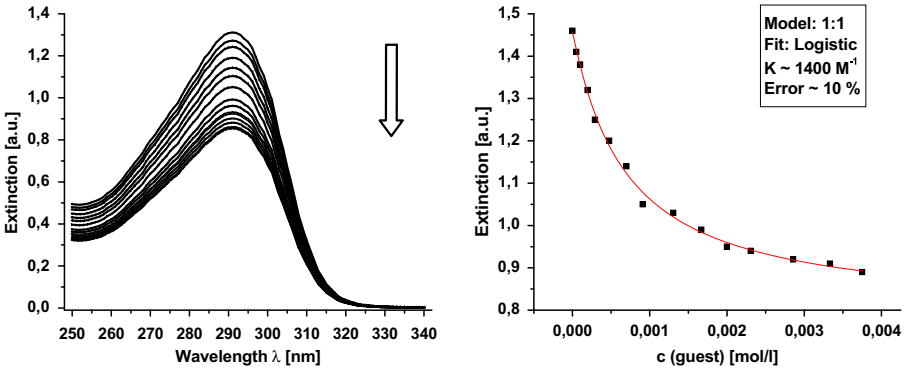


Figure 9. UV spectrum of the pyrrole part of **32** (2×10^{-5} M, pH 6.4) at different ratios of γ-aminobutyric acid in aqueous methanol (see binding studies).

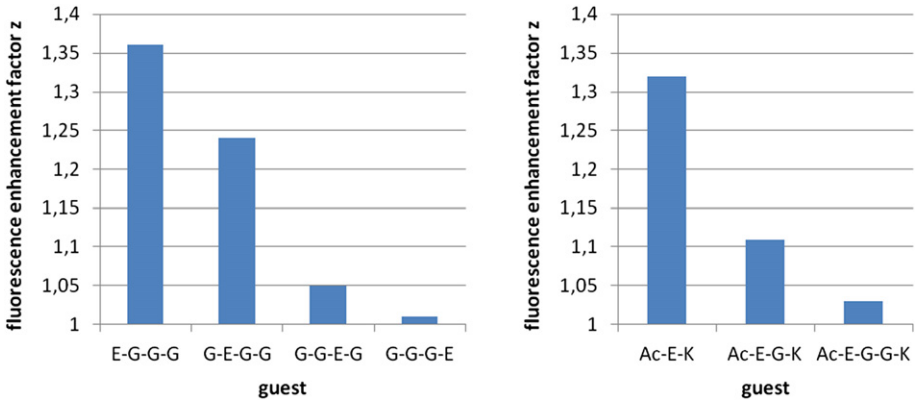


Figure 10. Fluorescence enhancement factors for **11d** with isomeric glutaminic acid (E)—glycine (G)—tetrapeptides (left) and Ac-E-K-NH_2, Ac-E-G-K-NH_2, Ac-E-G-G-K-NH_2 sequences; the error is assumed as ~10%.

Table 2
Binding strengths and selectivities of selected receptors versus short peptide sequences. The errors are assumed to be ~10%, conditions: T=25 °C, counter ion is chloride, pH values for the measurements were adjusted with HCl or Et_4NOH

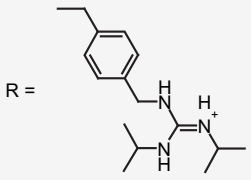
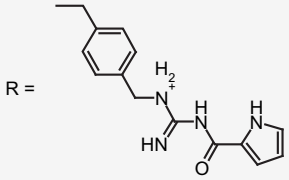
	Binding constant K [M^{-1}] in methanol (10% water)		
	H-E-G-G-G-OH	H-G-E-G-G-OH	H-G-G-E-G-H
R =  **11d**	400	200	<100
R =  **32**	1300	1100	1000

Figure 11 shows the emission titration curve for Ac-Glu-Lys-amide and **11d**. Peptide Ac-Glu-Lys-amide and other Glu-Lys sequences are binding to **11d** with a stoichiometry differing from 1/1 (the lined curve indicates the theoretical 1/1 binding curve). Therefore, no binding affinity could be derived, but saturation in the range of a 10^{-2} M guest concentration indicates low millimolar affinities (log K~2–3).

2.2.8. Fluorescence titrations with amino acids. The binding affinities of the best performing receptors using the same conditions against selected amino acids were investigated. Depending on the receptor, β-alanine, γ-aminobutyric acid, ε-aminohexanoic acid, lysine, and glycyl glycine were added. Lysine did not show any detectable binding event with one of the receptors. Table 3 summarizes the results. The best binding value for a particular receptor/guest-couple is indicated in bold numbers.

Although the binding affinities and differences are small, a few general trends can be noted: The guanidines bind slightly better under the experimental conditions than the quaternized ammonium ions (receptors **21** and **24**). The flexibility of the binding site bridge is of importance. For the hexyl spaced receptors (**11c**, **12c**, **14c**, and **16c**) all responses were too small to derive extract binding values (log K<2). For the xylyl spaced materials (**11d**, **12d**, **14d** and **16d**) higher binding constants are always achieved with GABA as the preferred guest. This finds its reason in a better pre-organization of the two binding sites by the aromatic platform. The binding constants of the isopropyl-guanidines (**11a**/**d**) and the pyrene substituted moieties (**33** and **40**) are comparable. Figure 12 shows exemplarily the rise of luminescence in the fluorescence spectrum and the according titration curve for **11d** with GABA. Pyrrole-substituted receptors **32** and **39** (for a titration curve see Fig. 9) show a twofold higher affinity for a specific guest compared to crown guanidinium receptors with isopropyl residues (**11a**/**d**). The pyrrole group stabilizes the aggregate by an additional H-bond and a less polar microenvironment.

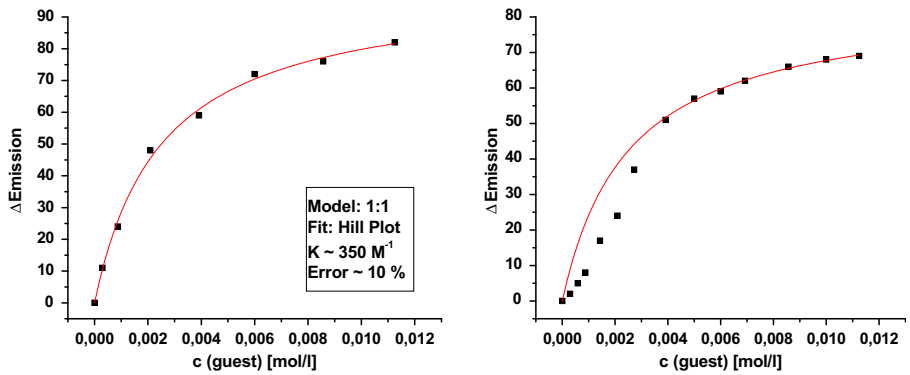
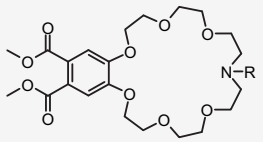
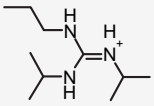
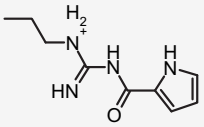
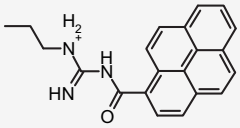
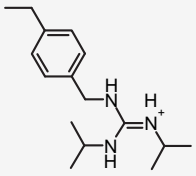
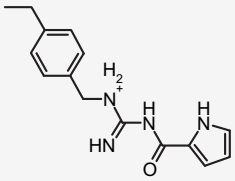
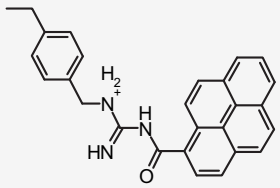
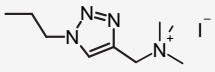


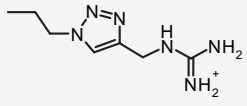
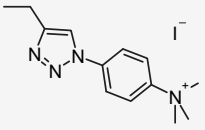
Figure 11. Emission titration curve of H-E-G-G-G-amide (left) and Ac-Glu-Lys-amide (right) with **11d** in aqueous methanol.

Table 3
Binding constants of the receptors against various guests; — no binding detected, n.d. means not determined. The errors are assumed to be around 10%, conditions: T=25 °C, counter ion is chloride, pH values for the measurements were adjusted with HCl or Et_4NOH to a) pH=7.6; b) values determined by UV titration in methanol/water 9/1; c) pH=6.4; d) pH=8.5

		Binding constant K [M^{-1}] in methanol/water 9/1				
		β-Alanine	GABA	Glycyl glycine	AHX	Glycyl glycyl glycin
R =  **11a**	a)	300	200	—	<100	—
R =  **39**	b)	**1300**	1000	—	1000	—
R =  **40**	c)	300	200	—	200	—
R =  **11d**	d)	100	**500**	—	400	—
R =  **32**	b)	n.d.	**1300**	—	1100	—
R =  **33**	c)	200	300	—	300	—
R =  **24**	a)	n.d.	100	200	<100	—

(*continued on next page*)

Table 3 (*continued*)

		Binding constant K [M^{-1}] in methanol/water 9/1				
		β-Alanine	GABA	Glycyl glycine	AHX	Glycyl glycyl glycin
R =  **27**	a)	n.d.	200	<100	200	—
R =  **21**	a)	—	<100	200	200	**600**

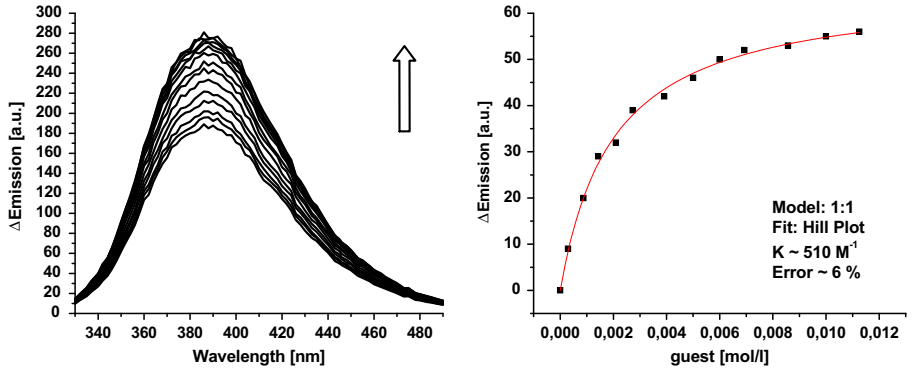


Figure 12. Fluorescent spectrum of **11d** (2×10^{-5} M, λ_{ex}=300 nm) at different ratios of γ-aminobutyric acid in aqueous methanol.

3. Conclusions

We have reported receptors for zwitterionic amino acids and for the recognition of the neurotransmitter γ-aminobutyric acid (GABA) in polar protic solvents. Compound **11d** and **32** show selectivity toward GABA compared to similar amino acids and distinguish GABA from its biological precursor glutaminic acid. In addition, molecule **21** was found to be suitable for recognition of tripeptides. The distance dependence of the binding affinity was demonstrated with different amino acids and small peptide sequences. Figure 13 summarizes typical titration curves and Job's plot analyses[48] (small insertions) for the isopropyl guanidinium receptor **11d** with GABA and AHX, as well as **21** and $(Gly)_3$.

Based on these results and taking into account comparable literature examples[6,8] and energy minimization studies with the aid of the program package Spartan, the following structure for the receptor–peptide aggregates are proposed (Fig. 14):

In the course of the studies, we were able to lower the pH value for investigations with such systems by more than one order of magnitude in comparison to literature known examples.[5–8] Aza-crown ether–guanidinium systems, which are stable, applicable and insensitive to fluctuations over a broad pH range should carry

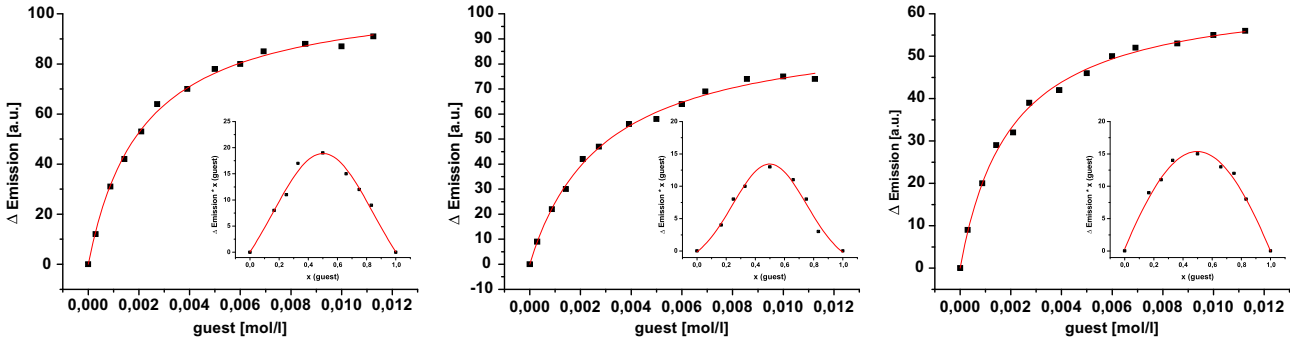


Figure 13. Comparison of the emission titrations of **11d** with GABA (left), **11d** with the similar guest AHX (middle) and **21** with Gly-Gly-Gly (right).

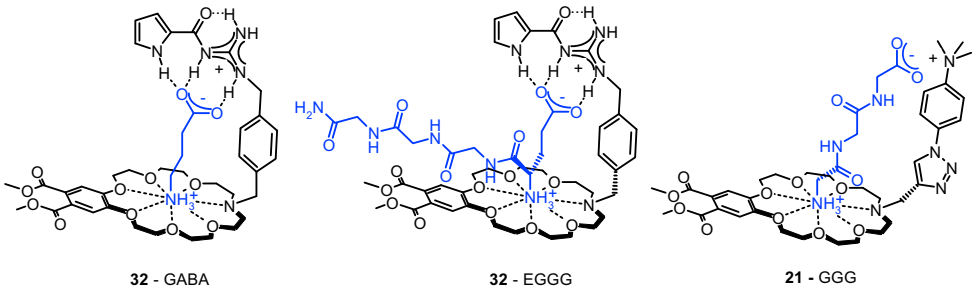


Figure 14. Illustrations of proposed structures[49] of stable peptide aggregates of GABA, Gly-Gly-Gly, and EGGG.

alkyl chains with a maximum length of C2 appended to a carbamide, thioureido, ureido or triazole substituent[50] at the azacrown ether nitrogen atom. They allow the determination of amino acid binding at pH values close to 6.

The selectivity and affinity of the receptors is currently not sufficient for practical use in sensing of unprotected amino acids or peptide, but the investigations clearly show that the interaction of such ditopic compounds with amino acids and peptides can be rationalized on the basis of established binding motifs. This contributes to the development of a more rational design of sequence selective peptide chemosensors.

4. Experimental section

4.1. General method for the preparation of aza-benzo-21-crown-7-ethers with appended bis-Boc-protected guanidines (GP III)

The crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) or TFA salt (0.3 mmol, approx. 300 mg) was dissolved in 5.0 mL of dried chloroform, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was dropped in and the mixture was stirred for 20 min. at room temperature. After addition of 290 mg of 1,3-bis(Boc)-2-methyl-2-*iso*-thiourea (1.0 mmol), the well stirred solution was to heated to reflux under nitrogen atmosphere and held at this temperature for 2 days. The solution was cooled to room temperature, diluted with 30.0 mL of ethyl acetate, and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate, and the solvent was evaporated. After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate/ethanol or chloroform/methanol as eluent. (R_f ca. 0.2–0.3 in EE/EtOH 3/1).[51]

*4.1.1. 14-[2-[2,3-Di-(tert-butyloxycarbonyl)-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13a**).* Compound **4a** (176 mg, 0.30 mmol) was converted to the product by *GP III*. The substance is a colorless glass (195 mg, 0.260 mmol, 78%) (R_f [$CHCl_3$/MeOH 12/1]=0.27; R_f [EE/EtOH 3/1]=0.16). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm]=1.42 (s, 9H), 1.43 (s, 9H), 2.63 (t, 2H, J=5.4 Hz), 2.71–2.74 (t, 4H, J=5.4 Hz), 3.37–3.40 (q, 2H), 3.51–3.54 (t, 4H), 3.56–3.58 (m, 4H), 3.67–3.70 (m, 4H), 3.80 (s, 6H), 3.83–3.86 (t, 4H), 4.12–4.15 (t, 4H), 7.12 (s, 2H), 8.56 (br s, 1H), 11.38 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm]=28.0 (+, 3C), 28.3 (+, 3C), 38.8 (−, 1C), 52.5 (+, 2C), 53.8 (−, 1C), 54.1 (−, 2C), 69.3 (−, 2C), 69.5 (−, 2C), 70.1 (−, 2C), 70.7 (−, 2C), 71.1 (−, 2C), 79.0 (C_{quat}, 1C), 82.6 (C_{quat}, 1C), 113.7 (+, 2C), 125.3 (C_{quat}, 2C), 150.5 (C_{quat}, 2C), 152.8 (C_{quat}, 1C), 156.0 (C_{quat}, 1C), 163.5 (C_{quat}, 1C), 167.7 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3312 (br m), 2938 (m), 2866 (m), 1792 (m), 1720 (m), 1633 (m), 1521 (m), 1434 (m), 1347 (m), 1285 (s), 1251 (s), 1125 (s), 1058 (s), 982 (m), 949 (m), 875 (m), 773 (m), 657 (m); MS (ESI–MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): m/z (%)=757.4 (100, MH+); UV (MeOH): λ (ε)=268 (7400), 224 (30,200); HRMS (EIMS 70 eV): calcd for $C_{35}H_{56}N_4O_{14}$+: 757.3871, found: 757.3856; MF: $C_{35}H_{56}N_4O_{14}$, FW: 756.85 g/mol.

*4.1.2. 14-[4-[2,3-Di-(tert-butyloxycarbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**13d**).* Compound **4d** (203 mg, 0.30 mmol) was reacted after *GP III*. The product appears as a yellow glass (205 mg, 0.247 mmol, 82%) (R_f [$CHCl_3$/MeOH 12/1]=0.3; R_f [EE/EtOH 3/1]=0.18). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm]=1.46 (s, 9H), 1.50 (s, 9H), 2.76 (m, 4H), 3.52–3.60 (m, 4H), 3.62–3.66 (m, 6H), 3.73–3.77 (m, 4H), 3.86 (s, 6H), 3.89–3.93 (m, 4H), 4.13–4.21 (m, 4H), 4.56 (d, 2H, J=5.3 Hz), 7.18 (s, 2H), 7.20 (d, 2H, J=4.6 Hz), 7.27 (d, 2H, J=4.6 Hz), 8.53 (br s, 1H), 11.51 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm]=28.1 (+, 3C), 28.3 (+, 3C), 44.8 (−, 1C), 52.6 (+, 2C), 53.7 (−, 2C), 59.3 (−, 1C), 69.3 (−, 2C), 69.5 (−, 2C), 69.8 (−, 2C), 70.7 (−, 2C), 71.2 (−, 2C), 79.4 (C_{quat}, 1C), 83.1 (C_{quat}, 1C), 113.6 (+, 2C), 125.3 (C_{quat}, 3C), 127.7 (C_{quat}, 2C), 129.3 (C_{quat}, 2C), 135.8 (C_{quat}, 1C), 150.5 (C_{quat}, 2C), 153.2 (C_{quat}, 1C), 156.1 (C_{quat}, 1C), 163.6 (C_{quat}, 1C), 167.8 (C_{quat}, 2C); -IR (KBr): ν (cm^{-1})=3327 (br m), 2933 (m), 2877 (m), 2053 (w), 1970 (w), 1721 (m), 1612 (m), 1518 (m), 1433 (m), 1410 (m), 1353 (m), 1325 (m), 1286 (s), 1125 (s), 1058 (m), 981 (m), 914 (m), 803 (m), 778 (m), 731 (m), 649 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): m/z (%)=833.4 (22%, MH^+), 417.2 (100%, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=268 (7700), 224 (32,100); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{41}H_{61}N_4O_{14}^+$: 833.4184, found: 833.4191; MF: $C_{41}H_{66}N_4O_{14}$, FW: 832.95 g/mol.

4.2. General procedure for the Boc deprotection of the crown ether guanidines (GP V)

The according starting material (0.1 mmol) was dissolved in 0.5 mL of dry dichloromethane and a saturated solution of hydrochloric acid in diethylether (0.3 mL) was added. After 4 h of stirring at room temperature the HCl gas was removed by bubbling N_2 through the solution. All volatiles were evaporated at reduced pressure. The residue was taken up in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethylether. The solution was decanted off the precipitate, it was washed once with diethylether, decanted off again, and the product was dried in the vacuum to furnish a hygroscopic powder in nearly quantitative yield.

*4.2.1. 14-[2-Guanidino-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**14a**).* Mp=72–74 °C (uncorrected); ^{1}H NMR (300 MHz, MeOD): δ [ppm]=2.68–2.73 (m, 6H), 3.24 (m, 2H), 3.52–3.59 (m, 4H), 3.61–3.66 (m, 4H), 3.74–3.79 (m, 4H), 3.85 (s, 6H), 3.89–3.93 (m, 4H), 4.25–4.28 (m, 4H), 7.32 (s, 2H); ^{13}C NMR (75 MHz, MeOD): δ [ppm]=42.3 (−, 1C),

53.2 (+, 2C), 56.5 (−, 2C), 58.4 (−, 1C), 70.2 (−, 2C), 70.5 (−, 2C), 70.6 (−, 2C), 71.8 (−, 2C), 72.3 (−, 2C), 114.6 (+, 2C), 126.9 (C_{quat}, 2C), 151.7 (C_{quat}, 2C), 159.3 (C_{quat}, 1C), 166.2 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3340 (br m), 2947 (m), 2882 (m), 1719 (m), 1659 (m), 1520 (m), 1439 (m), 1352 (m), 1292 (m), 1198 (s), 1130 (s), 1052 (m), 946 (m), 812 (m), 729 (m), 650 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=557.1 (100, MH^+), 298.4 (23, $(M+2Na^+)^{2+}$), 278.9 (23, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=268 (7200), 223 (29,100); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{25}H_{41}N_4O_{10}^+$: 557.2823, found: 557.2825; MF: $C_{25}H_{40}N_4O_{10}Cl_2$, FW: 625.51 g/mol.

*4.2.2. 14-[4-(Guanidino-methyl)-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**14d**).* Mp=81–83 °C (uncorrected); ^{1}H NMR (300 MHz, MeOD): δ [ppm]=3.21–3.28 (m, 4H), 3.69–3.80 (m, 8H), 3.86 (s, 6H), 3.88–4.23 (m, 12H), 4.29 (m, 2H), 4.69 (m, app. s, 2H), 7.02 (s, 2H), 7.11 (d, 2H, *J*=4.6 Hz), 7.34 (d, 2H, *J*=4.6 Hz); ^{13}C NMR (75 MHz, MeOD): δ [ppm]=45.3 (−, 1C), 53.3 (+, 2C), 53.6 (−, 2C), 57.0 (−, 1C), 65.5 (−, 2C), 69.5 (−, 2C), 70.6 (−, 2C), 71.0 (−, 2C), 71.2 (−, 2C), 113.4 (+, 2C), 126.1 (C_{quat}, 2C), 128.3 (+, 2C), 130.8 (C_{quat}, 1C), 133.2 (+, 2C), 139.2 (C_{quat}, 1C), 151.5 (C_{quat}, 2C), 158.9 (C_{quat}, 1C), 169.8 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3411 (br m), 2924 (m), 2071 (w), 1672 (m), 1600 (m), 1521 (m), 1436 (m), 1351 (m), 1288 (s), 1181 (s), 1126 (s), 1061 (m), 974 (m), 916 (m), 882 (m), 800 (m), 720 (m), 653 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=633.3 (19, MH^+), 317.0 (100, $(M+2H^+)^{2+}$); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{31}H_{45}N_4O_{10}^+$: 633.3136, found: 633.3147; MF: $C_{31}H_{50}N_4O_{10}$, FW: 701.61 g/mol.

4.3. Synthesis of aza-benzo-21-crown-7-ethers with *N,N*-dialkyl-substituted guanidine motifs (GP VI)

The crown ether amino acid hydrochloride (0.3 mmol, approx. 200 mg) was dissolved in 10.0 mL of dry acetonitrile in a protective nitrogen atmosphere, freshly distilled DIPEA (1.0 mmol, 129 mg, 0.17 mL) was dropped in and the mixture was stirred 30 min. While cooling to 2–5 °C DIC (190 mg) or DCC (310 mg) (1.5 mmol) was added in one portion and the solution was, after 2 h of stirring in the ice bath, carefully heated to reflux temperature and held under dry conditions at this temperature for 4 days. After cooling to room temperature, the reaction mixture was filtered over Celite and the filter cake was washed several times with acetonitrile and dichloromethane. The solvent was evaporated; the crude product was thoroughly dried and purified by column chromatography with silica gel and chloroform/methanol as eluent (R_f ca. 0.2–0.3 in chloroform/methanol 4/1).

*4.3.1. 14-[2-[2,3-Di-isopropyl-guanidino]-ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11a**).* Converting compound **4a** (176 mg, 0.30 mmol) after *GP VI* to the product gives a colorless glass (133 mg, 0.207 mmol, 69%) (R_f [EE/MeOH 5/2]=0.39; R_f [EE/EtOH 3/1]=0.1). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm]=1.18–1.22 (d, 12H, *J*=5.4 Hz), 2.79 (m, 6H), 3.32 (m, 2H), 3.49–3.59 (m, 8H), 3.61–3.69 (m, 4H), 3.78–3.87 (m, 4H), 3.86 (s, 6H), 4.01 (heptett, 2H, *J*=5.4 Hz), 4.12–4.22 (m, 4H), 7.86 (br s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm]=21.9 (+, 2C), 22.1 (+, 2C), 43.8 (+, 2C), 51.6 (+, 2C), 53.2 (−, 1C), 54.6 (−, 1C), 68.1 (−, 4C), 68.5 (−, 2C), 68.6 (−, 2C), 69.4 (−, 2C), 70.1 (−, 2C), 112.7 (+, 2C), 124.4 (C_{quat}, 2C), 149.4 (C_{quat}, 2C), 153.9 (C_{quat}, 1C), 167.7 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3402 (br m), 2940 (m), 2874 (m), 1719 (m), 1615 (m), 1519 (m), 1435 (m), 1348 (m), 1285 (s), 1183 (s), 1124 (s), 1061 (m), 976 (m), 945 (m), 878 (m), 782 (m), 767 (m), 730 (m), 698 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=641.4 (100, MH^+); UV (MeOH): λ (ε)=268 (8500), 224 (28,800); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{31}H_{53}N_4O_{10}^+$: 641.3762, found: 641.3745; MF: $C_{31}H_{52}N_4O_{10}$, FW: 640.78 g/mol.

*4.3.2. 14-[4-[2,3-Di-isopropyl-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11d**).* Compound **4d** (203 mg, 0.30 mmol) was submitted to *GP VI* to give the according product as a yellow glass (131 mg, 0.183 mmol, 61%) (R_f [$CHCl_3$/MeOH 6/1]=0.2). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm]=1.12 (d, 12H, *J*=6.4 Hz), 2.77 (m, 4H), 3.56–3.61 (m, 8H), 3.68–3.71 (m, 4H), 3.66–3.73 (m, 2H), 3.81 (s, 6H), 3.83–3.87 (m, 4H), 3.96–4.03 (heptett, 2H, *J*=6.4 Hz), 4.10–4.15 (m, 4H), 4.46 (d, 2H, *J*=5.3 Hz), 6.51 (d, 2H, *J*=4.6 Hz), 7.09 (s, 2H), 7.24 (dd, 4H, *J*=4.6 Hz, 2.1 Hz), 8.41 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm]=22.8 (+, 4C), 45.3 (−, 1C), 45.4 (+, 2C), 52.7 (+, 2C), 53.5 (−, 2C), 59.0 (−, 1C), 69.1 (−, 2C), 69.2 (−, 2C), 69.4 (−, 2C), 70.5 (−, 2C), 71.0 (−, 2C), 113.3 (+, 2C), 125.3 (C_{quat}, 2C), 125.3 (C_{quat}, 1C), 127.4 (+, 2C), 129.9 (+, 2C), 150.4 (C_{quat}, 2C), 154.3 (C_{quat}, 1C), 167.9 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3180 (br m), 2931 (m), 2872 (m), 2194 (w), 1721 (m), 1612 (m), 1517 (m), 1437 (m), 1350 (m), 1288 (s), 1197 (s), 1125 (s), 1049 (m), 979 (m), 913 (m), 796 (m), 728 (m), 643 (m); **MS** (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=717.5 (13%, MH^+), 359.2 (100%, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=268 (8600), 224 (29,000); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{37}H_{56}N_4O_{10}^+$: 716.3996, found: 716.3986; MF: $C_{37}H_{56}N_4O_{10}$, FW: 716.88 g/mol.

*4.3.3. 14-[2-(3-(2-(2,3-Di-cyclohexyl-guanidino)ethyl)thioureido)ethyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**11e**).* Reacting **4e** (210 mg, 0.30 mmol) after GPVI gives a yellow glass as the product (96 mg, 0.13 mmol, 39%) (R_f [$CHCl_3$/MeOH 4/1]=0.31). ^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm]=1.13–1.23 (m, 2H), 1.32–1.43 (m, 8H), 1.54–1.66 (m, 2H), 1.69–1.80 (m, 4H), 1.89–2.03 (m, 4H), 2.75 (m, 6H), 3.48–3.63 (m, 10H), 3.61–3.66 (m, 4H), 3.73–3.79 (m, 4H), 3.87 (s, 6H), 3.88–3.93 (m, 4H), 4.19–4.24 (m, 4H), 6.41 (m, 1H), 7.20 (s, 2H), 7.63 (br s, 2H), 8.70 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm]=24.8 (−, 4C), 25.0 (−, 2C), 33.0 (−, 4C), 40.0 (−, 1C), 41.8 (−, 1C), 51.9 (+, 2C), 52.6 (+, 2C), 54.1 (−, 1C), 54.4 (−, 2C), 54.9 (−, 1C), 68.8 (−, 2C), 69.4 (−, 2C), 69.5 (−, 2C), 70.4 (−, 2C), 70.9 (−, 2C), 113.5 (+, 2C), 125.4 (C_{quat}, 2C), 150.3 (C_{quat}, 2C), 153.3 (C_{quat}, 1C), 155.1 (C_{quat}, 1C), 166.7 (C_{quat}, 2C); IR (KBr): ν (cm^{-1})=3220 (br m), 3196 (br m), 3086 (m), 2931 (m), 2859 (m), 2202 (w), 2028 (w), 1722 (m), 1614 (m), 1556 (m), 1521 (m), 1438 (m), 1350 (m), 1287 (s), 1197 (s), 1123 (s), 1052 (m), 981 (m), 915 (m), 799 (m), 729 (m), 659 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=823.5 (11, MH^+), 412.1 (100, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=268 (7900), 224 (28,700); HRMS (LSI-MS FAB glycerine): calcd for $C_{40}H_{67}N_6O_{10}S^+$: 823.4639, found: 823.4649; MF: $C_{40}H_{66}N_6O_{10}S$, FW: 823.07 g/mol.

4.4. Synthesis of protected aza-benzo-21-crown-7-ethers with pyrrole- and pyrene substituted guanidine motifs (GP VII)

The crown ether amino acid hydrochloride or TFA salt (0.2 mmol, approx. 200 mg as the TFA salt) was dissolved in 3.0 mL of dry DMF, freshly distilled triethylamine (1.0 mmol, 101 mg, 0.14 mL) was dropped in and the mixture was stirred for 20 min at room temperature. After addition of the appropriate 2-methyl-2-isothiourea (0.3 mmol), the well stirred solution was heated to 40 °C under nitrogen atmosphere and held at this temperature over night. The solution was cooled to room temperature, diluted with 30.0 mL of DCM, and filtered. The filtrate was washed with saturated ammonium chloride solution (10 mL) and water (10 mL), dried over magnesium sulfate, and the solvent was evaporated.

After vacuum drying the crude product was purified by column chromatography on silica gel with ethyl acetate/ethanol as eluent (R_f ca. 0.3 in EE/EtOH 6/1).

*4.4.1. 14-[4-[2-(tert-Butyloxycarbonyl)-3-(pyrrole-2-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**30**).* Reacting **4d** (136 mg, 0.20 mmol) after *GP VII* a yellow glass is obtained (125 mg, 0.151 mmol, 76%). Ethanol/ethyl acetate 1/6 was used for purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm]=149–1.51 (s, 9H), 2.62–2.81 (m, 4H), 3.49–3.70 (m, 10H), 3.71–3.80 (m, 4H), 3.83–3.96 (m, 4H), 3.82 (s, 6H), 4.11–4.21 (m, 4H), 4.61 (m, 2H), 6.21 (m, 1H), 6.85 (m, 1H), 6.95 (m, 1H), 7.17 (s, 2H), 7.21–7.33 (m, 4H), 8.62 (br s, 1H), 9.39 (br s, 1H), 12.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm]=28.0 (+, 2C), 28.2 (+, 1C), 44.7 (−, 1C), 52.5 (+, 2C), 53.8 (−, 2C), 59.2 (−, 1C), 69.3 (−, 2C), 69.4 (−, 2C), 69.8 (−, 1C), 70.6 (−, 2C), 71.2 (−, 2C), 83.1 (C_{quat}, 1C), 110.4 (+, 1C), 113.6 (+, 1C), 114.1 (+, 2C), 121.8 (+, 1C), 125.3 (C_{quat}, 2C), 127.4 (+, 2C), 129.3 (+, 2C), 131.1 (C_{quat}, 1C), 139.0 (C_{quat}, 1C), 150.5 (C_{quat}, 2C), 153.3 (C_{quat}, 1C), 155.8 (C_{quat}, 1C), 167.8 (C_{quat}, 2C), 171.0 (C_{quat}, 1C); IR (KBr): ν (cm^{-1})=3319 (br m), 2941 (m), 2873 (m), 1718 (m), 1614 (m), 1577 (m), 1407 (m), 1352 (s), 1287 (s), 1195 (m), 1125 (s), 1061 (m), 1027 (s), 980 (m), 911 (m), 846 (m), 780 (m), 731 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=826.3 (81, MH^+), 413.6 (100, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=303 (22,900), 222 (27,500); HRMS (PI-LSI-MS FAB Glycerine): calcd for $C_{41}H_{56}N_5O_{13}^+$: 826.6870, found: 826.3866; MF: $C_{41}H_{55}N_5O_{13}$, FW: 825.92 g/mol.

*4.4.2. 14-[4-[2-(tert-Butyloxycarbonyl)-3-(pyrene-1-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester (**31**).* Conversion of **4d** (136 mg, 0.20 mmol) by *GP VII* yields a yellow glass (119 mg, 0.124 mmol, 62%). Ethanol/ethyl acetate 1/9 was used for purification. ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm]=1.48 (s, 3H), 1.52 (s, 6H), 1.86 (m, 2H), 2.71 (m, 4H), 3.59–3.61 (m, 8H), 3.62–3.71 (m, 4H), 3.72–3.93 (m, 4H), 3.82 (s, 6H), 4.09–4.18 (m, 4H), 4.71 (d, 2H, *J*=5.3 Hz), 7.09–7.18 (m, 2H), 7.25–7.32 (m, 4H), 8.68 (d, 1H, *J*=4.6 Hz), 8.91 (br s, 1H), 9.30 (d, 1H, *J*=4.6 Hz), 9.56 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm]=28.1 (+, 2C), 28.3 (+, 1C), 44.8 (−, 1C), 52.5 (−, 1C), 52.6 (+, 2C), 53.6 (−, 2C), 69.3–71.2 (−, 10C), 83.4 (C_{quat}, 1C), 114.0 (+, 2C), 124.1 (+, 1C), 124.6 (C_{quat}, 1C), 124.8 (+, 1C), 125.0 (C_{quat}, 1C), 125.2 (+, 1C), 125.2 (C_{quat}, 2C), 125.6 (+, 1C), 125.7 (C_{quat}, 1C), 126.0 (+, 1C), 126.4 (C_{quat}, 1C), 126.7 (C_{quat}, 1C), 127.1 (C_{quat}, 1C), 127.4 (+, 1C), 127.7 (+, 2C), 127.9 (+, 2C), 128.3 (+, 1C), 128.4 (+, 1C), 128.7 (+, 1C), 130.3 (C_{quat}, 1C), 150.2 (C_{quat}, 2C), 153.3 (C_{quat}, 1C), 156.0 (C_{quat}, 1C), 163.9 (C_{quat}, 1C), 167.7 (C_{quat}, 2C), 180.9 (C_{quat}, 1C); IR (KBr): ν (cm^{-1})=3312 (br m), 2943 (m), 2873 (m), 1719 (m), 1598 (m), 1575 (m), 1514 (m), 1434 (m), 1398 (m), 1346 (m), 1287 (s), 1193 (m), 1125 (s), 1050 (s), 982 (m), 911 (m), 847 (m), 808 (m), 730 (a), 646 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=960.1 (100, MH+), 480.6 (100, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=348 (35,600), 281 (42,100), 223 (74,600); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{53}H_{61}N_4O_{13}^+$: 961.4235, found: 961.4263; MF: $C_{53}H_{60}N_4O_{13}$, FW: 961.09 g/mol.

4.5. Deprotection of aza-benzo-21-crown-7-ethers with pyrrole- and pyrene substituted guanidine motifs (GP VIII)

The starting material (0.1 mmol) was dissolved in 2.0 mL of dry, cold dichloromethane and a cold solution of HCl in diethylether (1.0 mL) was added. After 4–6 h of stirring at room temperature under moisture protection, all volatiles were removed at reduced pressure. The residue was dissolved in a minimum amount of dichloromethane and the product was precipitated carefully by slow addition of diethylether. The solution was centrifuged, the diethylether was decanted off, and the process was repeated. The product was dried in the vacuum to give a fine hygroscopic, faintly yellow powder. The yield is nearly quantitative.

*4.5.1. 14-[4-[2-(Pyrrole-2-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**32**).* Deprotection of **30** (83 mg, 0.10 mmol) by *GP VII* gives a yellow, hygroscopic, glassy solid (68 mg, 0.094 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm]=3.12–3.30 (m, 4H), 3.49–3.69 (m, 8H), 3.71–3.96 (m, 8H), 3.84 (s, 6H), 4.06–4.25 (m, 4H), 4.41 (m, 2H), 4.52 (m, 2H), 6.21 (m, 1H), 6.90–7.11 (m, 5H), 7.28–7.32 (m, 3H), 9.71 (br s, 1H), 10.30 (br s, 1H), 11.12 (br s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm]=44.1 (−, 1C), 52.5 (−, 2C), 52.8 (+, 2C), 57.1 (−, 1C), 65.0 (−, 2C), 68.1 (−, 2C), 69.1 (−, 2C), 69.9 (−, 2C), 70.1 (−, 2C), 111.3 (+, 1C), 112.3 (+, 2C), 118.4 (+, 1C), 123.4 (C_{quat}, 1C), 125.1 (C_{quat}, 2C), 126.2 (+, 2C), 127.2 (+, 2C), 128.9 (C_{quat}, 1C), 132.0 (+, 1C), 136.2 (C_{quat}, 1C), 149.7 (C_{quat}, 2C), 155.4 (C_{quat}, 1C), 168.0 (C_{quat}, 2C); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=726.3 (23, MH^+), 363.7 (100, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=298 (22,000), 220 (26,700); HRMS (FABMS glycerine): calcd for $C_{36}H_{48}N_5O_{11}^+$: 726.3350, found: 726.3346; MF: $C_{36}H_{51}N_5O_{11}Cl_2$, FW: 800.74 g/mol.

*4.5.2. 14-[4-[2-(Pyrene-1-carbonyl)-guanidino-methyl]-benzyl]-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocycloheneicosene-2,3-dicarboxylic acid dimethyl ester hydrochloride (**33**).* Compound **31** (96 mg, 0.10 mmol) was deprotected according to *GP VII* to give the product **33** as a yellow glass (76 mg, 0.088 mmol, 88%). ^{1}H NMR (600 MHz, $CDCl_3$): δ [ppm]=3.21 (m, 4H), 3.61–3.68 (m, 8H), 3.81–3.96 (m, 8H), 3.86 (s, 6H), 4.09–4.14 (m, 4H), 4.51 (m, 2H), 4.63 (m, 2H), 6.93 (s, 2H), 7.11 (d, 2H, *J*=4.6 Hz), 7.41 (d, 2H, *J*=4.6 Hz), 7.97–8.11 (m, 3H), 8.13–8.21 (m, 2H), 8.22–8.28 (m, 2H), 8.36 (m, 1H), 8.71 (m, 1H), 10.23 (br s, 1H), 10.96 (br s, 1H), 13.51 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ [ppm]=44.3 (−, 1C), 52.2 (−, 2C), 52.9 (+, 2C), 56.8 (−, 1C), 65.6 (−, 2C), 68.2 (−, 2C), 69.2 (−, 2C), 70.0 (−, 2C), 70.2 (−, 2C), 112.3 (+, 2C), 123.9 (+, 1C), 124.2 (C_{quat}, 1C), 124.3 (+, 1C), 124.9 (C_{quat}, 2C), 125.2 (C_{quat}, 1C), 126.4 (+, 1C), 126.5 (+, 1C), 126.6 (C_{quat}, 1C), 126.9 (C_{quat}, 1C), 127.0 (+, 2C), 127.4 (+, 1C), 129.9 (+, 1C), 130.2 (+, 1C), 130.6 (C_{quat}, 1C), 130.7 (C_{quat}, 1C), 132.2 (+, 5C), 134.7 (+, 1C), 135.8 (+, 1C), 131.3 (C_{quat}, 1C), 150.0 (C_{quat}, 2C), 155.8 (C_{quat}, 1C), 168.2 (C_{quat}, 2C), 172.2 (C_{quat}, 1C); IR (KBr): ν (cm^{-1})=3302 (br m), 2916 (m), 2878 (m), 1675 (s), 1596 (m), 1515 (m), 1436 (m), 1351 (m), 1267 (m), 1197 (s), 1129 (s), 1066 (m), 977 (m), 913 (m), 832 (m), 725 (m); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): *m/z* (%)=861.4 (21, MH+), 431.8 (100, $(M+2H^+)^{2+}$); UV (MeOH): λ (ε)=346 (22,500), 279 (35,000), 227 (68,700); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{48}H_{53}N_4O_{11}^+$: 861.3711, found: 861.3686; MF: $C_{48}H_{56}N_4O_{11}Cl_2$, FW: 931.88 g/mol;

4.6. Example for the synthesis of receptors with the quaternary ammonium motif via Huisgen-cycloaddition reaction

*4.6.1. {4-[4-(2,3-Bis-methoxycarbonyl-6,7,9,10,12,13,15,16,18,19,21,22-dodecahydro-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosen-14-ylmethyl)-2,3-dihydro-[1,2,3]triazol-1-yl]-phenyl}-trimethyl-ammonium; iodide (**21**).* Compound **19** (122 mg, 0.24 mmol) was dissolved together with compound **20** (61 mg, 0.2 mmol) in 1.0 mL of methanol. A solution of copper(II)sulfate pentahydrate (10 mg, 0.02 mmol) and sodium ascorbate (16 mg, 0.1 mmol) in water (0.5 mL) was added drop wise. After stirring for 1 h at room temperature, this solution was added again and the vigorously stirred reaction mixture was heated 4 h to 60 °C under nitrogen. After cooling to room temperature, 9.0 mL dichloromethane were added, the aqueous layer was separated off and the organic phase was washed with 3.0 mL of brine. After drying the solution over $MgSO_4$ the solvent was distilled off and the solid residue was purified on

a small pellet of silica gel. All impurities were washed from the column with chloroform/methanol 9/1 (TLC control); the product was eluated with chloroform/methanol 3/1. The solvent was evaporated, the residue was sonicated in 5.0 mL of chloroform and the silica gel was filtered off. The filtrate was evaporated to give the pure product as orange, sticky oil (150 mg, 0.184 mmol, 92%).

^{1}H NMR (300 MHz, $CDCl_3$): δ [ppm]=3.08–3.15 (m, 4H), 3.71 (s, 9H), 3.66–3.87 (m, 8H), 3.84 (s, 6H), 3.91–3.98 (m, 4H), 4.01–4.09 (m, 4H), 4.12–4.19 (m, 4H), 4.76 (m, 2H), 7.02 (s, 2H), 7.86 (d, 2H, J=4.6 Hz), 8.07 (d, 2H, J=4.6 Hz), 8.52 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm]=47.0 (−, 1C), 53.2 (+, 2C), 54.7 (−, 2C), 58.0 (+, 3C), 67.8 (−, 2C), 69.8 (−, 2C), 70.7 (−, 2C), 71.1 (−, 2C), 71.7 (−, 2C), 113.5 (+, 2C), 121.9 (+, 1C), 122.3 (+, 2C), 123.2 (+, 2C), 125.8 (C_{quat}, 1C), 126.2 (C_{quat}, 2C), 138.7 (C_{quat}, 1C), 147.8 (C_{quat}, 1C), 151.4 (C_{quat}, 2C), 169.4 (C_{quat}, 2C); MS (ESI-MS, CH_2Cl_2/MeOH+10 mmol NH_4OAc): m/z (%)=686.3 (12, M^+), 343.6 (100, $(M^+ + H^+)^{2+}$); UV (MeOH): λ (ε)=260 (13,400), 227 (25,100), 204 (31,100); HRMS (PI-LSI-MS FAB glycerine): calcd for $C_{34}H_{48}N_5O_{10}^+$: 686.3401, found: 686.3391; MF: $C_{34}H_{48}N_5O_{10}$, FW: 813.69 g/mol.

The molecular structures, syntheses, and characterization of all further compounds are described in the Supporting data.

4.7. Investigation of the binding properties

4.7.1. Screening of peptide binding affinities. The estimation of binding affinities were performed in half area UV star well-plates with 96 cells using a methanol/water mixture 9/1.[52] Depending on the expected binding strength 100–500 equiv of peptide or amino acid (5×10^{-2} M to 1×10^{-3} M) were added to a 2×10^{-5} M solution of a particular receptor compound. Every solution-crown-guanidine receptors or amino acids was, if necessary, adjusted[53] in it's pH to weakly basic (pH 7.5–8.5) with Et_4NOH or HCl according to Table 2.[54] The mixtures were quickly pipetted row by row, mixed with the aid of the pipette, and allowed to equilibrate for 5 min. The fluorescence spectrum was recorded (λ_{ex}=300 nm) and compared to a blanc sample of the receptor with the same concentration. All measurements were repeated twice.

4.7.2. Binding affinity titrations. In every titration 1.0 mL of a 2×10^{-5} M solution of receptor was used and titrated according to the following procedure in a cuvette. To the pH adjusted solutions[53] of the crown ether receptors in 9/1 methanol/water rising equivalents of the amino acids or peptides in the according solvent at the same pH were successively added in 10 μL (≙5–25 equiv) aliquots (5×10^{-2} M to 1×10^{-2} M). After each addition the solution was allowed to equilibrate for 6 min and the emission intensity was recorded at 25 °C (λ_{ex}=300 nm). To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non-linear fitting methods.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2010.01.028.

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36. All compounds show due to their close structural relationship very similar photophysical properties: ε (nm)=220 (27,000–30,000), 270 (7000–8500).
37. a) The increase in emission by guest binding is most likely caused by the intercepted photoinduced electron transfer from the nitrogen atom of the crown ether to the fluorophore. b) The pK_a values of the tertiary amine in the crown ether in dependency on the spacer were evaluated by titration with perchloric acid (see Supplementary data). The aliphatic amines possess about the same basicity and are all between 5.6 and 6; only the aromatic substituent differs slightly with a value of 6.6.
38. To prevent a potential cleavage of the methyl esters and decomposition of the acetyl-guanidine moiety, the measurements are restricted to the range pH>5 to max pH=10.
39. The pK_a value of the guanidinium moiety may vary depending on the adjacent group effects, in general it will be lowered by aromatic acyl>aliphatic acyl>phenyl>alkyl Schug, K. A.; Lindner, W. *Chem. Rev.* **2005**, *105*, 67–113; Storey, B. T.; Sullivan, W. W.; Moyer, C. L. *J. Organomet. Chem.* **1964**, *29*, 3118–3120 Briefly summarized, the pK_a of aromatic acyl-guanidines is between 6 and 7 [see also Ref. 35], of aliphatic examples it is between 7 and 8. The alkylated or unsubstituted guanidines with about five orders of magnitude higher values can be assumed as always protonated under the given conditions.
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43. Tryptophan was excluded, as it absorbs in the same range as the crown ether fluorophore. The binding of tyrosine was not investigated; it is not sufficiently soluble under the experimental conditions.
44. Solvatochromatic effects may lead to additional changes in the emission.
45. The crown ether guanidinium combinations with thioureido chain or with triazole rings coordinate mercury ions weakly. Stable mercury compounds of triazoles are known Müller, E.; Meier, H. *Liebigs Ann. Chem.* **1968**, *716*, 11–18.
46. Most acetyl-guanidines are comparable in their stability with esters. Acyl-guanidines are in general isolable compounds and can be stored as salts in non-nucleophilic solvents over a long time without any decomposition. A possible intramolecular nucleophilic attack can accelerate the decomposition reaction enormously Brennauer, A.; Keller, M.; Freund, M.; Bernhard, G.; Buschauer, A. *Tetrahedron Lett.* **2007**, *48*, 6996–6999. The acyl-guanidines proved to be stable for at least several days in solution. No different behaviour was observed if the screenings are repeated after 2–3 days. No strong nucleophile is present and the pH of the measurement never exceeded the range between 6 and 8.
47. The combination Glu-Lys as in the dipeptide is found in functional regions of many important peptides as ubiquitin Pickart, C. M.; Eddins, M. J. *Biochim. Biophys. Acta* **2004**, *1695*, 55–72; It is highly conserved in nearly all human endorphins, for examples see Ling, N.; Burgus, R.; Guillemin, R. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 3942–3946; Ramabadran, K. *Singapore Med. J.* **1983**, *24*, 235–240.
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52. Amino acids in their zwitterionic form are not sufficiently soluble in pure methanol, therefore the measurements have to be conducted in aqueous mixtures.
53. A pH electrode for methanolic solutions was used and calibrated once a day.
54. Salt addition is avoided; it increases the polarity of the solvent systems and interferes with the guanidinium binding.