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Luminescent Crown Ether Amino Acids: Selective Binding to *N*-terminal Lysine in Peptides

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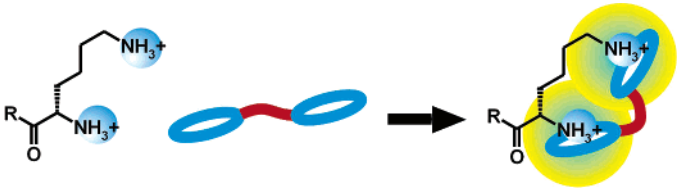

Crown ether amino acids (CEAAs) with a luminescent phthalic ester or phthalimide moiety have been prepared. Simple peptide chemistry covalently tethers the macrocycles to give ditopic ammonium-ion binders. The binding events of both crown ether groups are monitored independently by changes of their specific emission properties. The affinity of the bis-CEAA to bis-ammonium ions is distance dependent, which allows distinguishing between isomeric small peptides containing a lysine residue in different positions.

Introduction

Selective recognition of short peptides plays an essential role in nature. Many cell−cell interactions for example depend on the recognition of the Arg-Gly-Asp sequence.[1] The efforts made to mimic nature's ability to selectively bind peptide sequences have led to diverse receptor structures.[2] Principally, two different strategies have been followed. Recognition of the secondary structure has attracted serious attention, the main target being the β-strand structure. It can be recognized by β-strand mimics as has been shown by Nowick,[3−7] Schrader,[8−10] and others.[11] Binding to α-helices by recognition of carboxylate side chain functional groups, as Hamilton has shown,[12,13] utilizes the second principle of peptide recognition, which relates to the binding of side and end-of-chain functionalities. Hossain and Schneider[14] designed complementary molecules to the zwitterionic form of natural unprotected peptides.[15] An 18-crown-6 moiety served as a binding unit for the ammonium end and a peralkylated ammonium molecule as a means of binding carboxylates through coulomb interactions. Schmuck[16] recently reported heterocyclic peptide binders, which use the strong guanidinium carboxylate interaction and work in water.[17] Breslow[18] and coworkers used covalently linked cyclodextrins to bind phenyl residues within the peptide chain. Hamachi[19]

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(1) Kessler, H. *Angew. Chem.* **1982**, *94*, 509−520.
(2) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479−2494.
(3) Nowick, J. S.; Lam, K. S.; Khasanova, T. V.; Kemnitzer, W. E.; Maitra, S.; Mee, H. T.; Lui, R. *J. Am. Chem. Soc.* **2002**, *124*, 4972−4973.
(4) Nowick, J. S.; Chung, D. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 1765−1768.
(5) Chung, D. M.; Nowick, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 3062−3063.
(6) Nowick, J. S.; Chung, D. M.; Maitra, K.; Maitra, S.; Stigers, K. D.; Sun, Y. *J. Am. Chem. Soc.* **2000**, *122*, 7654−7661.
(7) Nowick, J. S. *Acc. Chem. Res.* **1999**, *32*, 287−296.
(8) Kirsten, C. N.; Schrader, T. H. *J. Am. Chem. Soc.* **1997**, *119*, 12061−12068.
(9) Cernovska, K.; Kemter, M.; Gallmeier, H.-C.; Rzepecki, P.; Schrader, T.; Koenig, B. *Org. Biomol. Chem.* **2004**, *2*, 1603−1611.
(10) Wehner, M.; Schrader, T. *Angew. Chem.* **2002**, *114*, 1827−1831.
(11) Eblinger, F.; Schneider, H.-J. *Chem. Commun.* **1998**, 2297−2298.
(12) Peczuh, M. W.; Hamilton, A. D.; Sanchez-Quesada, J.; de Mendoza, J.; Haack, T.; Giralt, E. *J. Am. Chem. Soc.* **1997**, *119*, 9327−9328.
(13) Haack, T.; Peczuh, M. W.; Salvatella, X.; Sánchez-Quesada, J.; de Mendoza, J.; Hamilton, A. D.; Giralt, E. *J. Am. Chem. Soc.* **1999**, *121*, 11813−11820.
(14) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−11209.
(15) For the use of porphyrines, see: Sirish, M.; Schneider, H.-J. *Chem. Commun.* **1999**, 907−908. Sirish, M.; Chertkov, V. A.; Schneider, H.-J. *Chem. Eur. J.* **2002**, *8*.
(16) Schmuck, C.; Geiger, L. *J. Am. Chem. Soc.* **2004**, *126*, 8898−8899.
(17) Binding to the carboxy-terminus of a peptide: Braxmeier, T.; Demarcus, M.; Fessmann, T.; McAteer, S.; Kilburn, J. D. *Chem. Eur. J.* **2001**, *7*, 1889−1898.
(18) Breslow, R.; Yang, Z.; Ching, R.; Trojandt, G.; Odobel, F. *J. Am. Chem. Soc.* **1998**, *120*, 3536−3537.

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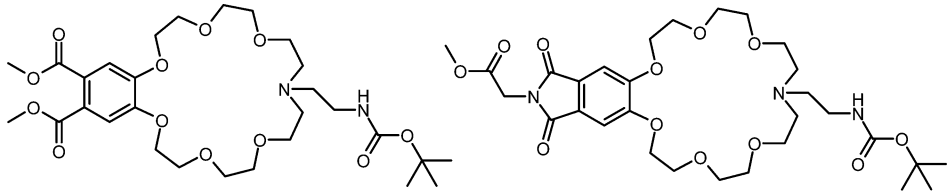
SCHEME 1. Structures of Crown Ether Amino Acids (CEAA) 1 and 2 Used in This Work



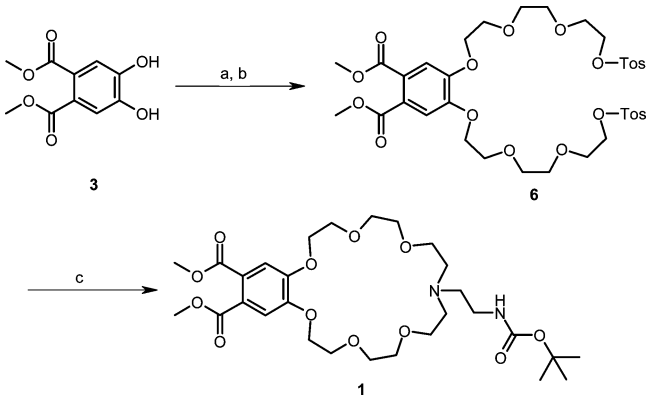
1 2

were able to bind monophosphorylated peptides with bis-[zinc(II)-dipicolamine]-based receptors. Another approach based on metal ion coordination was achieved by Anslyn.[20] They designed a tweezer-like receptor where a copper terpyridine in the center binds to histidine. Additional carboxylate groups stabilize the tweezer complex by formation of salt bridges. Shea[21] used polymer imprinted complexes for the binding of His-containing small peptides.[22−,27]

Although these approaches all show good binding and recognition abilities, they are rather restricted as they bear no functional groups which make an oligomerization or combination with other recognition moieties simple and straightforward. This report illustrates an approach for receptor creation based on a building block system. Our aim is to address ammonium ions within peptide chains, which can be either *N*-terminal or in lysine side chains. We chose crown ethers as recognition moiety.

The use of covalently linked crown ethers[28] to create function goes back to the seminal work of Gokel,[29−31] who employed such systems as artificial ion channels. As simple crowns such as the 18-crown-6 are not connectable, our investigation centered on crown ether amino acids.[32] These should be easy to oligomerize or combine with other recognition moieties with use of standard peptide synthesis methods. Furthermore, a fluorescent phthalic ester or phthalimide moiety was incorporated as a luminescent probe for binding events.[33] The combination of a crown ether ammonium ion binding site with phenolphthalein to create a sensory system for diamines has been previously reported by Fuji et al.[34] The hybrid compound allows distinguishing between aliphatic diamines of various lengths, but the chemosensor cannot be easily extended, modified, or combined with other groups. To overcome such drawbacks, we synthesized several different crown ether amino acids (CEAA) of which compounds **1** and **2** (Scheme 1) proved to be the most suitable for our purposes.

SCHEME 2. Synthesis of a Luminescent Crown Ether Amino Acid[a]



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[a] Reagents and conditions: (a) $Tos(CH_2O)_3H$, K_2CO_3, DMF, 60 °C, 79%. (b) TosCl, KOH, THF, H_2O, 0 °C → RT, 95%. (c) $H_2N(CH_2)_2NHBoc$, K_2CO_3, KI, MeCN, H_2O, 81 °C, 79%.

Results and Discussion

Syntheses. The synthesis of **1** and **2** was straightforward starting with the phthalic diester (**3**) [or the phthalimide (**4**) for the synthesis of **2**, respectively; see the Supporting Information for the structure]. Dialkylation with triethyleneglycol-monotosylate led to **5** followed by treatment with tosyl chloride to give the ditosylated **6**. Cyclization with *N*-Boc-ethyldiamine gave the desired building block **1** in good yields (79%) (Scheme 2). CEAA **1** and **2** show different absorption and emission properties due to their different chromophors. Both absorption and emission are shifted bathochromically in phthalimide CEAA **2** with respect to **1**. The phthalic ester CEAA **1** emits at about 390 nm, the phthalimide CEAA **2** at about 490 nm (Figure 1). Both CEAA **1** and **2** have been tested for ammonium binding ability with *n*-butylammonium chloride in methanol. Both show a mediocre binding affinity but good enhancement of fluorescence intensity. Although structurally quite similar, the two crown ethers

(19) Ojida, A.; Mito-oka, Y.; Sada, K.; Hamachi, I. *J. Am. Chem. Soc.* **2004**, *126*, 2454−2463.
(20) Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6*, 1341−1344.
(21) Hart, B. R.; Shea, K. J. *J. Am. Chem. Soc.* **2001**, *123*, 2072−2073.
(22) For other types of artificial peptide-binding molecules, see: Allott, C.; Adams, H.; Bernad, P. L.; Hunter, C. A.; Rotger, C.; Thomas, J. A. *Chem. Commun.* **1998**, 2449−2450.
(23) Borchardt, A.; Still, W. C. *J. Am. Chem. Soc.* **1994**, *116*, 7467−7468.
(24) Use of diketopiperazine binding sites: Conza, M.; Wennemers, H. *J. Org. Chem.* **2002**, *67*, 2696−2698.
(25) Dowden, J.; Edwards, P. D.; Flack, S. S.; Kilburn, J. D. *Chem. Eur. J.* **1999**, *5*, 79−89.
(26) Yoon, S. S.; Still, W. C. *J. Am. Chem. Soc.* **1993**, *115*, 823−824.
(27) Sun, S.; Fazal, Md. A.; Roy, B. C.; Mallik, S. *Org. Lett.* **2000**, *2*, 911−914.
(28) Gunning, P.; Benniston, A. C.; Peacock, R. D. *Chem. Commun.* **2004**, 2226−2227.
(29) Gokel, G. W.; Murrilo, O. *Acc. Chem. Res.* **1996**, *29*, 425−432.
(30) Shabany, H.; Pajewski, R.; Abel, E.; Mukhopadhyay, A.; Gokel, G. W. *J. Heterocycl. Chem.* **2001**, *38*, 1393−1400.
(31) Leevy, W. M.; Donato, G. M.; Ferdani, R.; Goldman, W. E.; Schlesinger, P. H.; Gokel, G. W. *J. Am. Chem. Soc.* **2002**, *124*, 9022−9023.
(32) Artificial amino acids with crown ether side chain functionality have been previously described. Kotha, S.; Brahmachary, E. *Ind. J. Chem., B* **2001**, *40B*, 1−4. Meillon, J.-C.; Voyer, N. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 967−969. Mazaleyrat, J.-P.; Gaucher, A.; Goubard, Y.; Savrda, J.; Wakselman, M. *Tetrahedron Lett.* **1997**, *38*, 2091−2094.
(33) For other emitting crown ether derivatives, see: Collins, G. E.; Choi, L.-S. *Chem. Commun.* **1997**, 1135−1136.
(34) Sensory system for the visualization of the distance of amino groups in α,ω-diamines: Fuji, K.; Tsubaki, K.; Tanaka, N.; Hayashi, T.; Otsubo, T.; Kinoshita, T. *J. Am. Chem. Soc.* **1999**, *121*, 3807−3808.

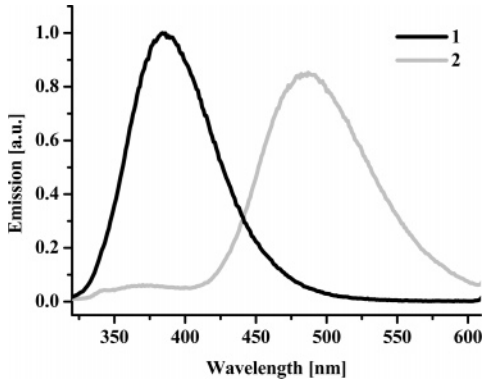


FIGURE 1. Emission spectra of compounds **1** and **2**.

TABLE 1. Binding Affinity and Emission Enhancement of Compounds 1 and 2 in the Presence of *n*-butylammonium Chloride in Methanol

	log K	I_∞/I_0
1	2.25	3.7
2	2.16	4.4

differ in their binding and signaling abilities. CEAA **1** binds slightly better but shows less enhancement of fluorescence intensity than **2** upon binding of ammonium ions (Table 1). Combination of two molecules of **1** led to bis-CEAA **7** (Scheme 3). This was achieved with standard peptide chemistry and **7** could be obtained in only 3 steps

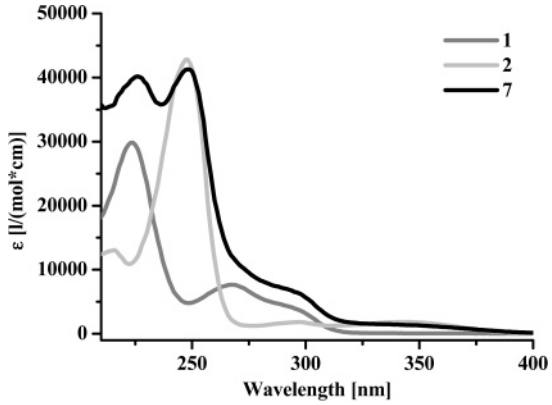
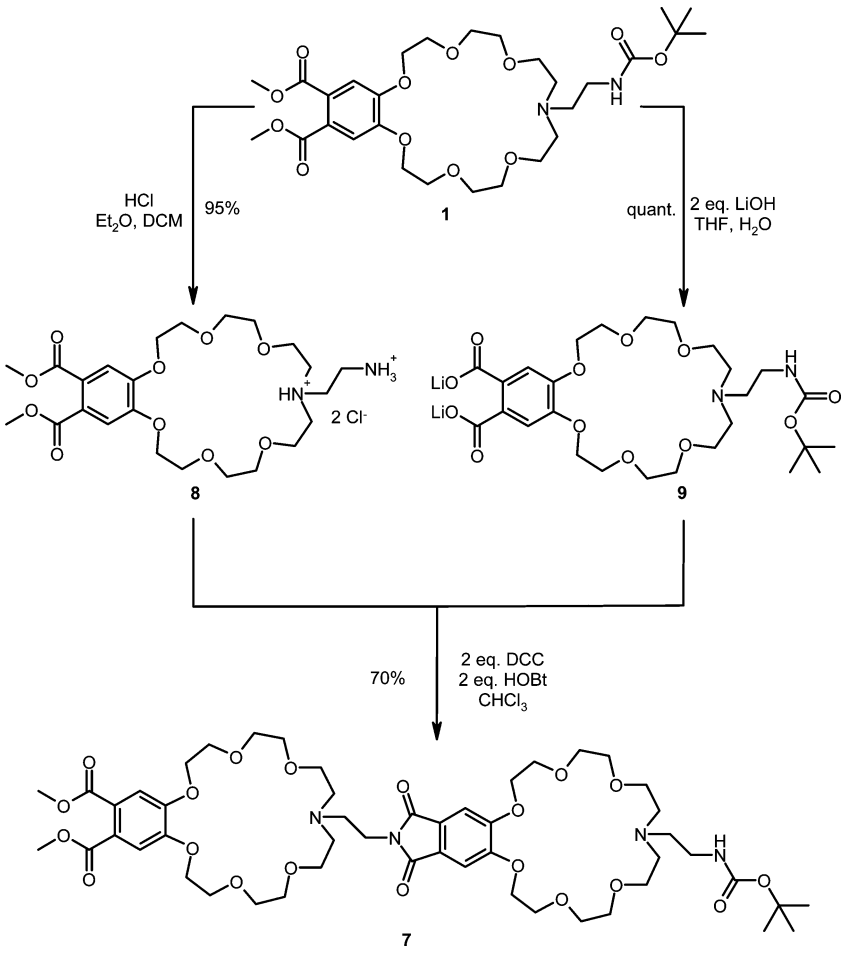


FIGURE 2. Absorption spectra of compounds **1**, **2**, and **7**.

of which 2 were deprotection steps. Bis-CEAA **7** comprises two different chromophors, which can be seen as parts of the corresponding CEAA **1** and **2**. Therefore the UV spectrum of **7** resembles a combination of the two spectra of **1** and **2** (Figure 2). As both the phthalic diester part of **7** and CEAA **1** do not absorb light at wavelengths greater than 320 nm, the fluorescence spectrum of **7** is dependent on the excitation wavelength. The spectrum only comprises both emission peaks when the molecule is excited at wavelengths shorter than 320 nm. This feature can be used to selectively monitor both crown ether unities within the bis-CEAA **7**. By exciting the molecule at wavelengths greater than 320 nm only the

SCHEME 3. Synthesis of Bis-CEAA 7 from Compound 1



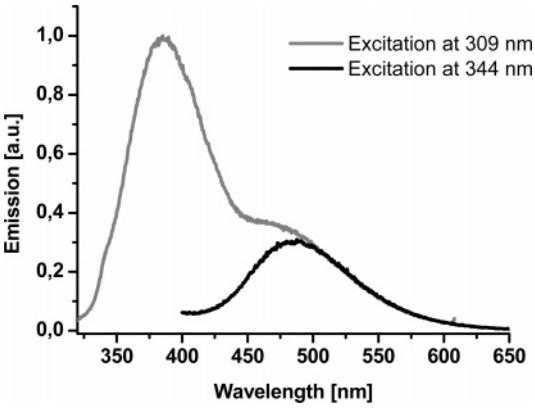


FIGURE 3. Emission spectra of compound **7** upon excitation at different wavelengths.

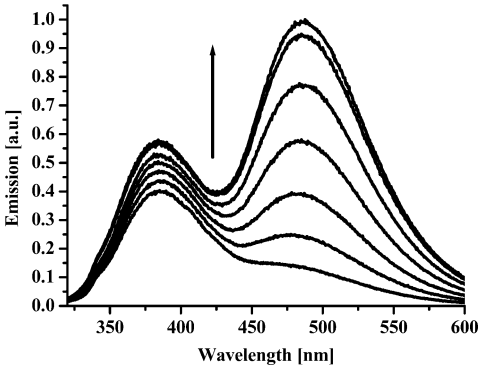


FIGURE 4. Changes in the emission spectrum of **7** upon addition of *n*-butylammonium chloride.

phthalimide part emits and therefore binding events at this part of **7** can be followed. To observe what happens at the phthalic ester part of **7**, one can integrate that part of the fluorescence spectrum of **7** at which the phthalimid part does not emit (i.e. 320−390 nm). This concept of selective monitoring was first tested with *n*-butylammonium chloride to see the changes in binding properties of the two parts of **7** with respect to the structurally similar CEAA **1** and **2**. The obtained binding curves reflected a one-to-one stoichiometry and for both parts a separate binding constant could be obtained. Interestingly, the phthalimide part of **7** shows a slightly increased fluorescence enhancement ($I_\infty/I_0 = 5.0$) with respect to **2**, whereas the fluorescence of the isophthalic diester part shows much less of an increase ($I_\infty/I_0 = 1.3$) than **1**. This effect can clearly be seen in the emission spectra (Figure 4). A comparison shows that the diester moieties in both **1** and **7** bind ammonium ions slightly better than the phthalimide moieties in **2** and **7**. Furthermore, a small decrease in binding affinity is observed on the crown ethers of **7** in comparison to those for **1** and **2**. However, as the binding constants do not change drastically and since a one-to-one stoichiometry can be found for both parts, it can be concluded that the binding properties of the mono-CEAA **1** and **2** persist when extended and incorporated into a bis-crown receptor.

TABLE 2. Affinity Constants of Lysine Methyl Ester Binding by 7 in Protic Solvents

solvent	crown ether part of **7**	log *K*	I_∞/I_0
methanol	phthalic diester	4.7	1.5
	phthalimide	4.3	8
H_2O (1.6% MeOH)	phthalic diester	4.5	1.4
	phthalimide	4.7	1.7
H_2O, HEPES (50 mM, pH 7.5, 1.6% MeOH)	phthalic diester	2.2	2.0
	phthalimide	1.5	1.4

As bis-CEAA **7** consists of two crown ether units, the next step was to test bisammonium guests. Lysine methyl ester dihydrochloride was chosen as a simple guest of biological origin. The titrations to determine binding affinities were conducted in three different solvent systems: methanol, water, and buffered aqueous solution. In comparison to *n*-butylammonium chloride, lysine methyl ester dihydrochloride is bound much more strongly. The binding affinity increases more than 100-fold. This indicates that cooperative binding takes place and each of the lysine ammonium ions is bound by one crown ether of **7**. The binding curve reflects a one-to-one stoichiometry. The formation of this complex stoichiometry is also supported by a Job's plot analysis with maximum at $X = 0.5$ (Figure 5). Additionally, the fluorescence increase in the phthalimide part of **7** is increased from 5- to 8-fold with respect to the binding of *n*-butylammonium chloride, which is beneficial for sensing purposes due to its better signal-to-noise ratio. As bis-CEAA **7** is able to recognize lysine, the ability of **7** to differentiate between short peptides which contain lysine in different positions was examined. Therefore three sequentially different peptides were synthesized. Each of them contains one lysine and two glycines. Peptide **10** (H-Lys-Gly-Gly-OMe) has an *N*-terminal lysine and is therefore structurally quite

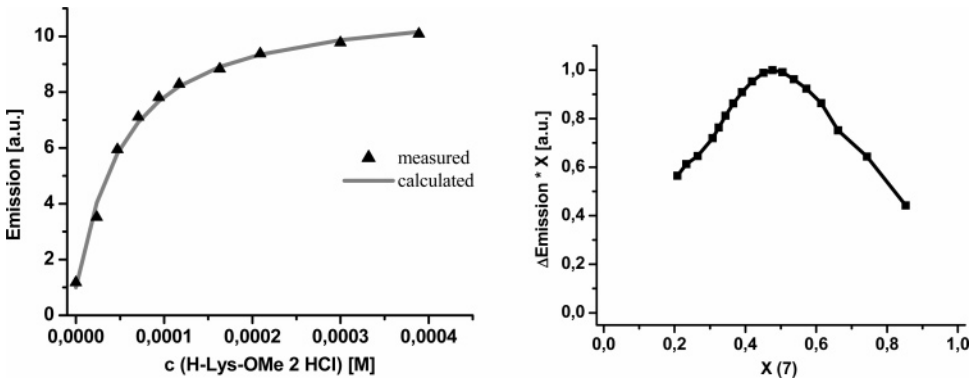


FIGURE 5. Emission titration curve (left, $c = 1.63 \times 10^{-5}$ mol/L) and Job's plot analysis (right) of bis-crown ether **7** with lysine methyl ester in methanol.

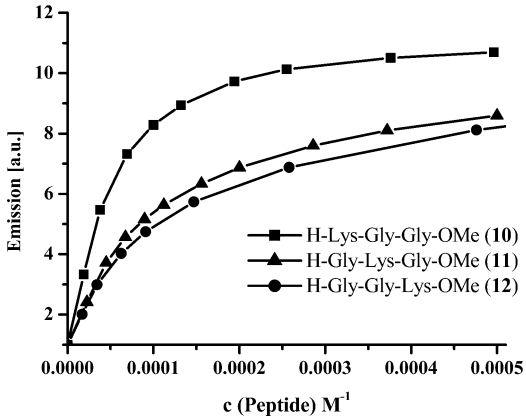


FIGURE 6. Titration curves of **7** ($c = 2.06 \times 10^{-5}$ M^{-1}) with tripeptide dihydrochlorides in methanol. The response of the phthalimide part is shown.

TABLE 3. Binding Affinities of Tripeptides to Compound 7 in Methanol

peptide	examined part of **7**	log *K*	I_∞/I_0
H-Lys-Gly-Gly-OMe (**10**)	diester	4.7	1.4
	imide	4.4	14
H-Gly-Lys-Gly-OMe (**11**)	diester	4.4	1.4
	imide	4.0	10
H-Gly-Gly-Lys-OMe (**12**)	diester	4.1	1.7
	imide	3.9	9

similar to lysine methyl ester (with respect to the distribution of ammonium ions within the molecule). In the second peptide **11** (H-Gly-Lys-Gly-OMe), lysine occupies the middle position. Thus one additional spacer compared to **10** is inserted between the two ammonium ions. Two glycine spacers compared to **10** are inserted in peptide **12** (H-Gly-Gly-Lys-OMe). The binding affinities of all three peptides as dihydrochlorides to compound **7** were determined by titration in methanol (Figure 6; Table 3). The error of the affinity constants of peptides and the phthalimide crown ether part is below 10% for the phthalic diester part at about 20%.

As shown above, the phthalic diester part of **7** binds better than the phthalimide part, whereas the phthalimide shows a better fluorescence response. This effect is even increased when binding to peptides and can lead to a large difference between the two parts of **7**. Here the binding of **7** to **10** gives a 1.4-fold increase at the diester part and a 14-fold increase at the imide part. Due to this strong enhancement of fluorescence the imide binding constants can be determined more precisely, as the diester part affinity constant. Therefore only the phthalimide part is used to evaluate the ability of **7** to differentiate between sequentially isomeric peptides (Figure 7). The binding affinities of lysine methyl ester and **10** to **7** are rather similar. Moving the Lys into the peptide chain and thus increasing the distance of the *N*-terminal and the side chain ammonium group leads to a significant decrease of the affinity and emission

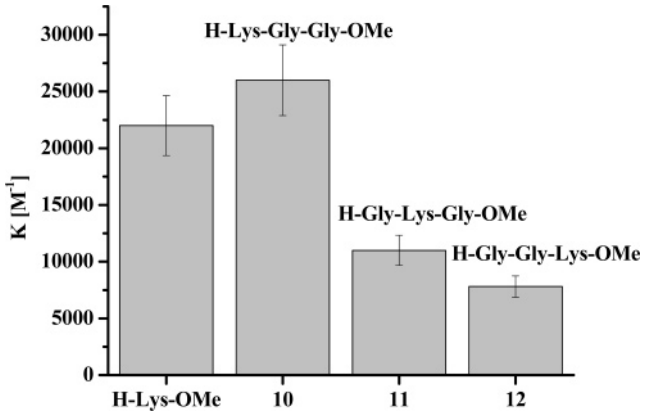


FIGURE 7. Comparison of the binding affinity of **7** to H-Lys-OMe and three isomeric tripeptides **10**, **11**, and **12**.

response. Bis-CEAA **7** obviously binds best to peptides with *N*-terminal lysine.

Conclusions

Luminescent crown ether amino acids (CEAA) have been synthesized, which have affinity to ammonium ions and signal the binding processes by an increase in emission. Selective deprotection and peptide bond formation led to bis-CEAA (**7**), a process that may be extended to the incorporation of luminescent crown ether moieties into peptide structures or the preparation of more extended crown ether oligoamide chains. In CEAA dipeptide **7** both crown ether parts bind independently to monoammonium guests with affinity similar to that of monomeric CEAAs. A bisammonium guest, such as lysine methyl ester, is cooperatively bound with a much higher affinity. The binding process to each of the two crown ether moieties of **7** can be monitored independently by its emission. Sequentially isomeric tripeptides containing one Lys show different emission response with **7** depending on the distance between ammonium ions within the peptides. Peptides with *N*-terminal lysine show the highest affinity to **7**. CEAA **2** allows the preparation of more extended and complex synthetic receptors with use of standard and even automated peptide chemistry. Compounds of this kind should show a specific affinity to complementary ammonium ion patterns in peptide structures or on protein surfaces.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft (GRK 760) and the Fonds der chemischen Industrie for support of this work.

Supporting Information Available: General experimental procedures, experimental procedures, and spectral data for the synthesis of compounds **1**, **2**, **4**, **5**, **6**, **7**, **8**, **9**, **14**, **15**, **16**, **17**, and **18**, as well as ^{1}H and/or ^{13}C NMR charts for all new compounds, and comparison of *n*-butylammonium binding to **1**, **2**, and both crown ether moieties of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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