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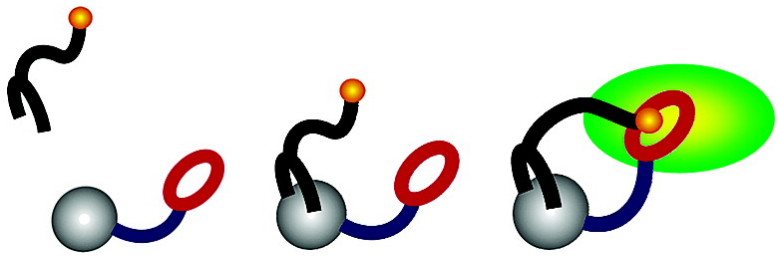
J. Am. Chem. Soc., **2005**, 127 (10), 3362-3365• DOI: 10.1021/ja043930h • Publication Date (Web): 18 February 2005



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Published on Web 02/18/2005

A Luminescent Receptor with Affinity for N-Terminal Histidine in Peptides in Aqueous Solution

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Abstract: Crown ethers of suitable size are the perfect artificial host compounds for ammonium ion binding, but the rather low affinity in aqueous solution prevents their use at physiological conditions. We report here the synthesis and properties of a luminescent benzo crown ether with a pendant copper imidodiacetic acid complex, which coordinates with high affinity to histidine. The emission intensity of the benzo crown ether increases significantly in the presence of ammonium ions in methanol. At physiological conditions in buffered water at pH 7.5 these interactions are too weak to be detected. If an ammonium ion *and* an imidazole moiety are present in the analyte, such as in His-Lys-OMe or His-OMe, high binding affinity in aqueous solution is restored. The binding event is signaled by an increase in emission intensity, which can even be observed with the naked eye. This allows the selective detection of small peptides containing N-terminal histidine or histidine among all other amino acids at physiological conditions.

Introduction

Ammonium ions are present in many compounds of biological or pharmaceutical interest, ranging from simple amino acids, neurotransmitters, and amino sugars to peptides and proteins. The classic hosts for ammonium ion binding[1] are crown ethers of appropriate size, but high binding affinities are only observed in organic solvents, such as methanol.[2] At physiological conditions in water or in DMSO as solvent, the binding constants are usually smaller than $\log K = 2$. A more favorable intramolecular hydrogen bond formation between crown ether and ammonium ion[3] is achieved by combining it with a stronger ionic or reversible covalent intermolecular interaction, creating di- or polytopic receptors.[4] Recent examples used the combination of crown ethers with boronic acids[5] to bind glucosamine, and guanidinium[6,7] and ammonium and nonpolar groups[8] to bind amino acids.[9−11] Reversible coordination to metal complexes can provide high affinity and selectivity in aqueous solution, but it has not been used to amplify crown ether−ammonium ion binding.[12] We report here the preparation and peptide binding properties of the luminescent crown ether[13] **5** (see the Supporting Information for details of its synthesis), which contains a pendant copper iminodiacetic acid (Cu−IDA) complex.[14,15]

Results and Discussion

Synthesis. The Cu^{II}−IDA motif is well-known for its ability to bind to imidazole and histidine.[16−21] To combine this property with the ammonium ion affinity of a crown ether, amine **2** was allowed to react with bistosylate **3** followed by deprotection to give the aza crown **4** (Scheme 1). The phthalic ester moiety of **4** allows one to monitor the binding of an ammonium ion to the crown ether by an increase in its emission intensity (excitation, $\lambda = 305$ nm; detection, $\lambda \approx 377$ nm). Upon addition of copper(II) ions, a stable complex (**5**) is formed containing the IDA ligand (see the Supporting Information for experimental details). Compound **6** was prepared for comparison from **3** by reaction with mono-Boc-protected 1,2-ethylenediamine to investigate the response of the crown ether emission to ammonium ion binding.

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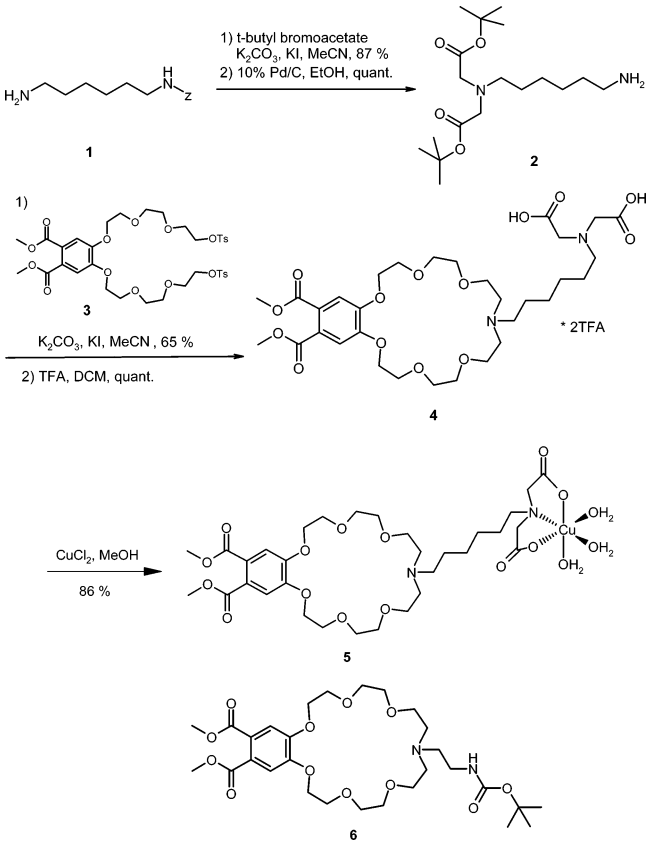
(3) Examples of intramolecular crown ether−ammonium ion interactions: (a) Al-Sayah, M. H.; Branda, N. R. *Org. Lett.* **2002**, *4*, 881−884. (b) Shinkai, S.; Ishihara, M.; Ueda, K.; Manabe, O. *J. Chem. Soc., Perkin Trans. 2* **1985**, 511−518.
(4) Examples of ditopic peptide receptors: (a) Cheng, Y.; Suenaga, T.; Still, W. C. *J. Am. Chem. Soc.* **1996**, *118*, 1813−1814. (b) Wennemers, H.; Conza, M.; Nold, M.; Krattiger, P. *Chem.—Eur. J.* **2001**, *7*, 3342−3347.
(5) Cooper, C. R.; James, T. D. *Chem. Commun.* **1997**, *15*, 1419−1420.
(6) Metzger, A.; Gloe, K.; Stephan, H.; Schmidtchen, F. P. *J. Org. Chem.* **1996**, *61*, 2051−2055.
(7) de Silva, A. P.; Gunaratne, H. Q. N.; McVeigh, C.; Maguire, G. E. M.; Maxwell, P. R. S.; O'Hanlon, E. *Chem. Commun.* **1996**, 2191−2192.
(8) Hossain, M. A.; Schneider, H.-J. *J. Am. Chem. Soc.* **1998**, *120*, 11208−11209.
(9) Receptor for binding of *N*-acylated dipeptides in water: Schmuck, C.; Geiger, L. *J. Am. Chem. Soc.* **2004**, *126*, 8898−8899.
(10) For a recent example of cooperative metal coordination and ion pairing in peptide recognition, see: Wright, A. T.; Anslyn, E. V. *Org. Lett.* **2004**, *6*, 1341−1344.
(11) Aspartic acid receptor using reversible coordination: Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296−11297.
(12) For lanthanide complex−crown ether conjugates which show emission response to potassium or sodium, see: Gunnlaugson, T.; Leonard, J. P. *Chem. Commun.* **2003**, 2424−2425.
(13) Otsuki, J.; Yamagata, T.; Ohmuro, K.; Araki, K.; Takido, T.; Seno, M. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 333−337.
(14) For a recent example of pyridine-substituted crown ether and its coordination to a metal complex, see: Chi, K.-W.; Addicott, C.; Stang, P. J. *J. Org. Chem.* **2004**, *69*, 2910−2912.
(15) For a recent report of a crown ether based luminescent metal ion sensor, see: Kim, H. M.; Jeong, M.-J.; Ahn, H. C.; Jeon, S.-J.; Cho, B. R. *J. Org. Chem.* **2004**, *69*, 5749−5751.

10.1021/ja043930h CCC: $30.25

Scheme 1. Synthesis of Luminescent Benzo Crown Ethers **4**−**6**



Binding Studies. To investigate the photophysical properties of the crown ether part, absorption and emission spectra of compound **6** were recorded in methanol, and their response to the presence of ammonium ions was tested (see the Supporting Information for the spectra). Upon excitation at the absorption maximum around $\lambda_{max} = 268$ nm an emission with a maximum at 385 nm is observed. The quantum yield of $\Phi = 0.09$ is rather low.[22] Upon addition of KSCN or *n*-$BuNH_3Cl$ the emission intensity increases significantly by a factor of 2.2 or 3.7, respectively. Table 1 summarizes the binding constants derived from the titration data (see the Supporting Information for the titration curves).

As expected, no response of the emission properties is observed for **5**[23] or **6** if treated with KSCN or *n*$BuNH_3Cl$ in buffered aqueous solution (50 mM HEPES, pH 7.5, $c = 10^{-5}$ mol/L, up to 1000 equiv of ammonium ion), even with a large excess of the salts. The aqueous solvent competes with the crown ether for cation binding, and the ammonium−crown ether interaction is intercepted. The situation changes if the dipeptide His-Lys-OMe is added to a solution of **5**.

Table 1. Binding Affinities and Emission Response of Compound **6** in Methanol to the Addition of Potassium or Ammonium Ions

guest	K (M^{-1})	log K	I_{max}/I_0
KSCN	4790	3.68	2.22
n$BuNH_3Cl$	178	2.25	3.68

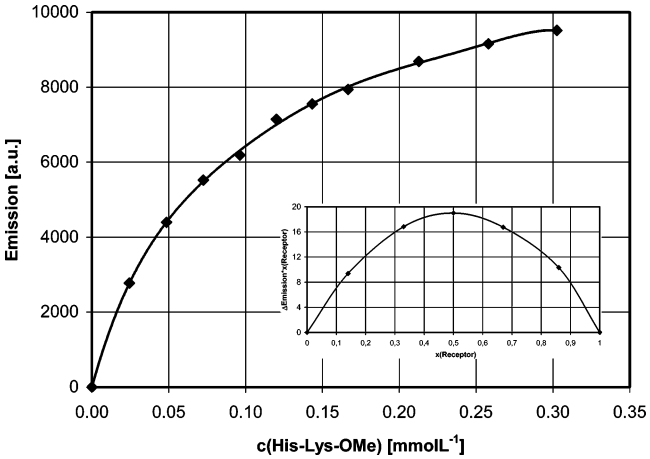
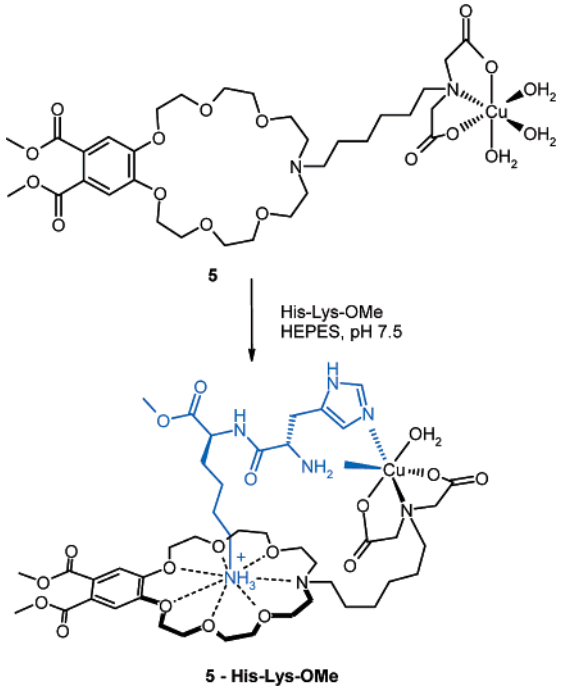


Figure 1. Emission titration of **5** ($c = 10^{-5}$ mol/L) with His-Lys-OMe in buffered aqueous solution (50 mM HEPES, pH 7.5). Inset: Job's plot analysis.

Scheme 2. Proposed Mode of Binding of Luminescent Crown Ether **5** and His-Lys-OMe



(16) A major application of this binding process is found in the purification of His-tagged proteins by affinity chromatography techniques.
(17) Examples for the use of metal−IDA complexes in synthetic receptors: (a) Sun, S.; Fazal, M. A.; Roy, B. C.; Mallik, S. *Org. Lett.* **2000**, *2*, 911. (b) Sun, S.; Fazal, M. A.; Roy, B. C.; Chandra, B.; Mallik, S. *Inorg. Chem.* **2002**, *41*, 1584. (c) Roy, B. C.; Fazal, M. A.; Sun, S.; Mallik, S. *J. Chem. Soc., Chem. Commun.* **2000**, 547. (d) Fazal, M. A.; Roy, B. C.; Sun, S.; Mallik, S.; Rodgers, K. R. *J. Am. Chem. Soc.* **2001**, *123*, 6283. (e) Banerjee, A. L.; Swanson, M.; Roy, B. C.; Jia, X.; Haldar, M. K.; Mallik, S.; Srivatava, D. K. *J. Am. Chem. Soc.* **2004**, *126*, 10875−10883.
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(20) Copper(II)-based receptor for imidazole and histidine: Fabbrizzi, L.; Francese, G.; Licchelli, M.; Perotti, A.; Tagliett, A. *Chem. Commun.* **1997**, 581−582.
(21) Fluorescent sensor for histidine based on a Co(II)−free base porphyrin dimer: Yang, R.; Wang, K.; Long, L.; Xiao, D.; Yang, X.; Tan, W. *Anal. Chem.* **2002**, *74*, 1088−1096.
(22) All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound ($\Phi = 0.546$).
(23) Upon incorporation of copper ions the quantum yield of the emission of **5** decreases to $\Phi = 0.0045$ compared to that of **4** ($\Phi = 0.62$, all quantum yields relative to quinine sulfate in 1 N H_2SO_4). The presence of the paramagnetic transition-metal ion may quench the emission of the crown ether. Binding of His-containing peptides restores parts of the emission intensity, presumably by part suppression of the quenching mechanism.

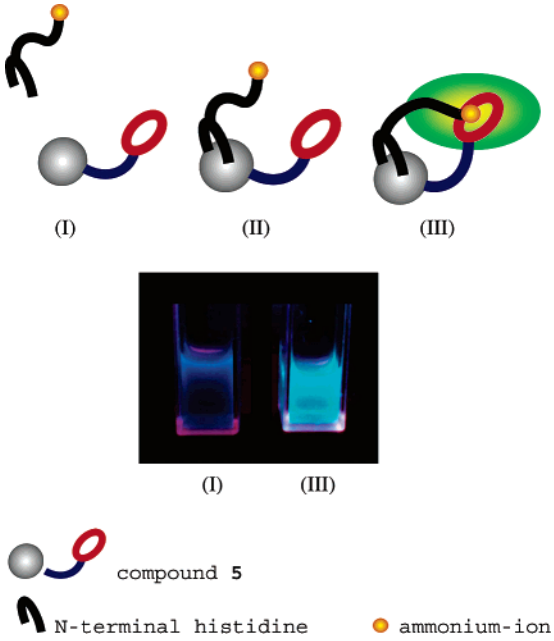

Figure 2. Schematic binding process and emission intensity changes of solutions of **5** in buffered water in the presence of His-Lys-OMe.

Coordination of the N-terminal His to the Cu^{II}−IDA complex[24] of **5** makes the crown ether−ammonium ion binding intramolecularly much more favorable. Titration of **5** with His-Lys-OMe in HEPES buffer (50 mM, pH 7.5)[25] resulted in a 1:1 complex (Scheme 2) as shown by a Job's plot analysis (see Figure 1). With a binding constant of log $K = 4.22 \pm 0.05$ compound **5** binds the ammonium group of His-Lys-OMe with high affinity in buffered water. Calorimetric titrations confirmed the strong interaction of **5** and His-Lys-OMe. The emission intensity change of **5** in the presence of His-Lys-OMe can even be observed with the naked eye (see Figure 2). No emission response is detected under the same conditions with the N-terminal acylated dipeptide Ac-His-Lys-OMe, which proves the importance of an N-terminal His for the overall binding process.

Surprisingly, using His-OMe as an added guest molecule, nearly the same enhancement of emission intensity is observed as with His-Lys-OMe. Job's plot analysis shows a stoichiometry of 2:1 for the His-OMe−**5** aggregate (Figures 3 and 4). The coordination chemistry of the Cu^{II}−IDA complex[24] provides a likely rationale for this observation: After binding of one His-OMe to Cu^{II}−IDA, one coordination site remains, which can accommodate the imidazole moiety of a second His-OMe while its ammonium group is bound by the crown ether, leading to an increased emission intensity.[26] The overall binding constant of His-OMe to **5** was determined to be log $K = 3.8 \pm 0.1$. The binding motif allows the selective detection of N-terminal His groups, which is illustrated by the binding of tripeptide His-Gly-Gly. This peptide binds to **5** with the same 2:1 stoichiometry as observed for histidine and an overall affinity of log $K = 3.71 \pm 0.05$.

(24) Hopgood, D.; Angelici, R. J. *J. Am. Chem. Soc.* **1968**, *90*, 2508−2513.
(25) At this pH the IDA−metal complex is stable, imidazole is not protonated and coordinates to the metal complex, and the lysine side chain bears an ammonium ion, which can bind to the crown ether.
(26) A rapid exchange of bound histidines within the dynamic assembly is expected.

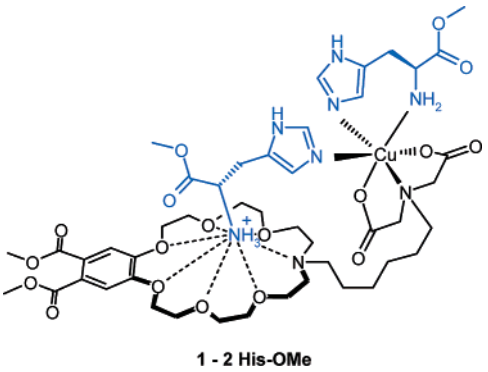

Figure 3. Proposed structure of the assembly of two His-OMe peptides with **5**.

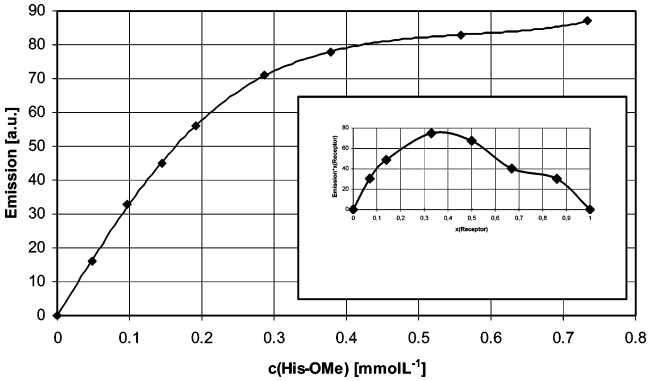

Figure 4. Emission titration curve of **5** with His-OMe in buffered aqueous solution at pH 7.5. Inset: Job's plot analysis.

The binding studies show that compound **5** has the ability to detect the simultaneous presence of an imidazole *and* an ammonium ion in one molecule. The stoichiometry of the resulting assembly with **5** depends on their geometrical arrangement. A simple illustration of an application of such a property is the selective detection of the amino acid His among 20 natural α-amino acids (Figure 5).[27] Only His with imidazole *and* an ammonium group leads to emission enhancement, while the presence of all other amino acids does not induce significant emission changes. Side chain functionalities of amino acids, such as Lys, Cys, Ser, or Met, are known to coordinate to the IDA−Cu^{II} complex, but only this interaction alone does not trigger the emission response. The interaction of the ammonium group with the benzo crown, which only becomes possible intramolecularly,[28] is the necessary second process to signal the binding event.

Conclusions

The combination of an imidazole-coordinating metal complex with a luminescent ammonium-binding crown ether results in a simple synthetic receptor with high affinity in buffered water for molecules bearing *both* functional groups. The emission response is triggered by the weak ammonium ion binding, which only becomes possible intramolecularly within the assembly.

(27) Only Tyr and Trp could not be used in this assay. Tyr is not soluble in HEPES buffer, and the indole emission of Trp interferes with the emission of **5**. However, calorimetric titration showed no binding of the amino acids to **5**.
(28) All amino acids bear an ammonium group under the experimental conditions. But in aqueous solution the affinity of the ammonium group to the benzo crown ether is too weak to give any emission response.

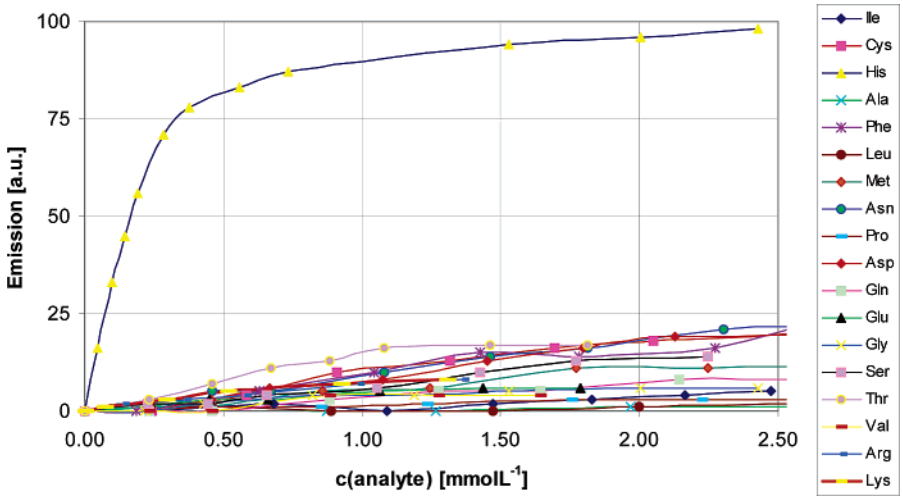

Figure 5. Response of the emission intensity of **5** to the presence of 20 natural α-amino acids in aqueous buffered solution (50 mM HEPES, pH 7.5).

Only the presence of an ammonium group, even in large excess, does not trigger an optical output. Binding selectivities, which are different from the present one, may be accomplished by other combinations of strongly coordinating metal complexes and binding sites of weak affinity.

Acknowledgment. We thank the Fonds der Chemischen Industrie and the Volkswagen Foundation for support of this work.

Supporting Information Available: Synthesis and characterization of compounds **4**−**6**, UV and emission spectra of compound **6** in methanol, titration of compound **6** with *n*$BuNH_3Cl$ in methanol, titration of **5** with His-Lsy-OMe and His-OMe in buffered aqueous solution, and Job's plot analyses (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA043930H