DOI: 10.1002/ejic.201000239

Luminescent Hybrids Combining a Metal Complex and a Crown Ether – Receptors for Peptidic Ammonium Phosphates

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Keywords: Crown compounds / Zinc / N ligands / Peptides / Luminescence / Amines / Cations

Hybrid compounds combining a luminescent aza crown ether and 1,4,7,10-tetraazacyclododecane (cyclene) or bis-(pyridin-2-ylmethylamine) (bpa) metal complexes were prepared. These synthetic receptors signal the presence of peptidic ammonium phosphates in buffered aqueous solution by changes in their emission intensity. Reversible phosphate ion coordination to the metal complex binding sites decreases the emission intensity of the phthalic ester fluorophore. Structurally suitable ammonium phosphates, such as the C-terminal domain of RNA polymerase II or the protein kinase G target sequence, bind to the synthetic receptors by an additional intramolecular interaction between the ammonium ion and the aza crown ether. The binding of the ammonium ion to the aza crown ether increases the emission intensity of the fluorophore. In aqueous solution, this weak interaction can only occur within aggregates of phosphates and metal complex receptors of matching geometry;therefore, it allows the distinction of structurally related phosphorylated peptides in buffered aqueous solution.

Introduction

Molecular recognition of molecules or ions of biological relevance in aqueous media by synthetic receptors is still a challenge.[1] Ammonium ions are found in all amino acids[2] and in many compounds of biological interest, such as the neurotransmitters dopamine[3] and γ-aminobutyric acid.[4] Of equal importance are phosphate esters,[5] which are found in nucleoside phosphates (nucleotides) as components of RNA and DNA,[6] in ATP[7] or phosphorylated amino acids in proteins involved in intracellular signalling and regulation.[8] Phosphorylation is one of the most biologically relevant and ubiquitous post-translational modifications of proteins;[9] this reversible process is used by all organisms to regulate many cellular activities. Phosphorylation occurs at the hydroxy residues of serine, tyrosine and threonine,[10] having a key role in cell growth regulation systems such as apoptosis or cell cycle progression and various cellular expression processes,[11] proliferation and energy metabolism,[12] enzyme activity control[13] or in many signal transduction pathways as well as in gene expression and regulation.[14] Misregulated phosphorylations are directly connected to severe diseases including cancer and neuropathogenesis.

Two important enzymes in the context of phosphorylation are cyclic guanosine monophosphate (GMP)-dependent protein kinase G (PKG) and RNA polymerase II (Pol II). PKG catalyzes the transfer of phosphate from ATP to specific serine or threonine residues in target proteins. It phosphorylates a number of biologically important targets involved in the regulation of smooth muscle relaxation, cell division and nucleic acid synthesis.[15] Studies have revealed highly specific substrate sequences like Arg-Lys-Arg-pSer-Arg-Ala-Glu (**2**), which show a strong preference for PKG I_α (K_m = 59 μM).[16]

Pol II unwinds the DNA double helix, synthesizes the messenger RNA and proofreads the nascent transcript in the eukaryotic transcription cycle. It is precisely regulated by phosphorylation in all transcription steps: initiation, elongation and termination.[17] Its C-terminal domain (CTD) seems to be key to the complex reaction sequence of gene expression.[18] The CTD sequence consists of the heptapeptide repeats Tyr-Ser-Pro-Thr-Ser-Pro-Ser (**1**). The binding of regulatory factors in the transcription elongation process depends on a specific CTD phosphorylation pattern, which changes during the transcription cycle.[19] Mainly, serine residues in positions 2 and 5 of the heptapeptide repeat are phosphorylated and dephosphorylated during the transcription cycle. On average, only one phosphate is present per repeat.[20] Both phosphorylations trigger different functions,[21] for example, phosphorylation at the serine residue in position 5 takes place during transcription initiation, whereas transcription elongation is connected with CTD phosphorylation at the serine residue in position 2.[22] The CTD is dephosphorylated during transcription termination.

The phosphorylated target peptide sequences of PKG and Pol II are shown in Figure 1. An element common to both structures is the close proximity of the phosphate ester

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejic.201000239.

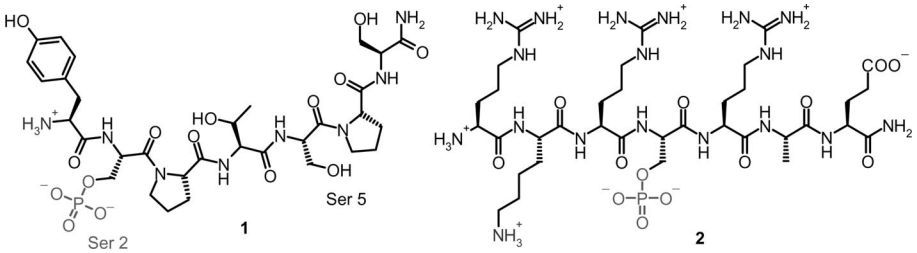


Figure 1. Phosphorylated target peptides **1** and **2**.

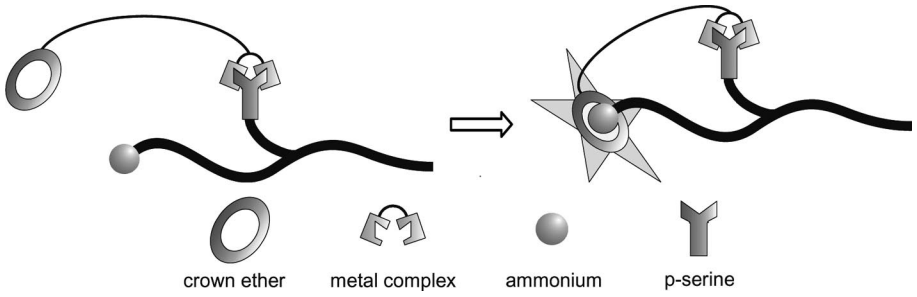


Figure 2. Schematic two-step binding of a receptor consisting of a metal complex and an aza crown ether to a peptide bearing ammonium and phosphate ions: (1) reversible coordination of the metal complex to the phosphate ion and (2) intramolecular interaction of the ammonium ion and crown ether, which leads to an increase in emission intensity.

and an ammonium ion. A luminescent synthetic receptor binding simultaneously to the ammonium and phosphate ions, which are close together, should be useful to monitor the phosphorylation state of the proteins as a chemosensor. We selected the reversible coordination of a Lewis acid metal complex to the phosphate anion as the interaction is strong. This places an aza crown ether moiety in close proximity to the ammonium group. Although the interaction of an ammonium ion with a crown ether is weak in aqueous solution, the intramolecular arrangement facilitates the binding. The binding of an ammonium ion is signalled by changes in the emission properties of the crown ether unit (Figure 2).

Zinc–dipicolylamine [Zn^{II}-dpa],[23,24] dinuclear Zn^{II}-dpa[25] and zinc–1,4,7,10-tetraazacyclododecane [Zn^{II}-cyclene] complexes[26] are strong phosphate ion binders in aqueous solution.[27–30] A comprehensive review on the molecular interactions of Zn^{II}-cyclene and its derivatives has recently been published.[31] Zn^{II}-dipicolylamine-stilbazoles have been shown to bind to a natural phosphoprotein recognition domain.[32] They were used in conjugates of peptide nucleic acids (PNA) and metal binding ligands recognizing phosphates in DNA.[33] Bis(picolylamine)–metal complexes have found application as artificial phosphoprotein sensors in protein separation and analysis by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), which retains phosphorylated proteins more strongly than corresponding nonphosphorylated proteins,[34–36] in fluorescent chemosensing arrays for phosphate derivatives[23,37] and for chemosensing phosphatidylserine-containing membranes and membranes of bacterial cells.[38] Several dpa-based fluorescent chemosensors for ATP have been reported.[39]

We have combined such metal complexes with luminescent crown ether amino acids (CEAAs) that respond to the presence of ammonium ions by changes in their emission properties.[40,41] The units are connected by peptidic linkers to allow variations in separation. Figure 3 shows the general structure of the receptors that can bind to ammonium and phosphate simultaneously.

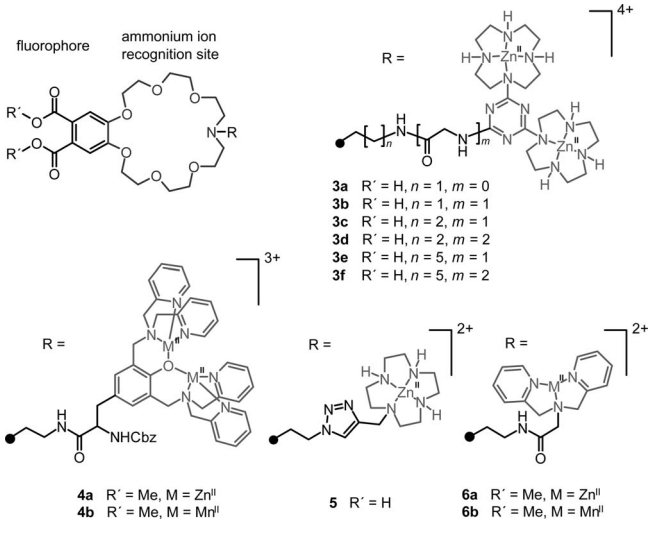
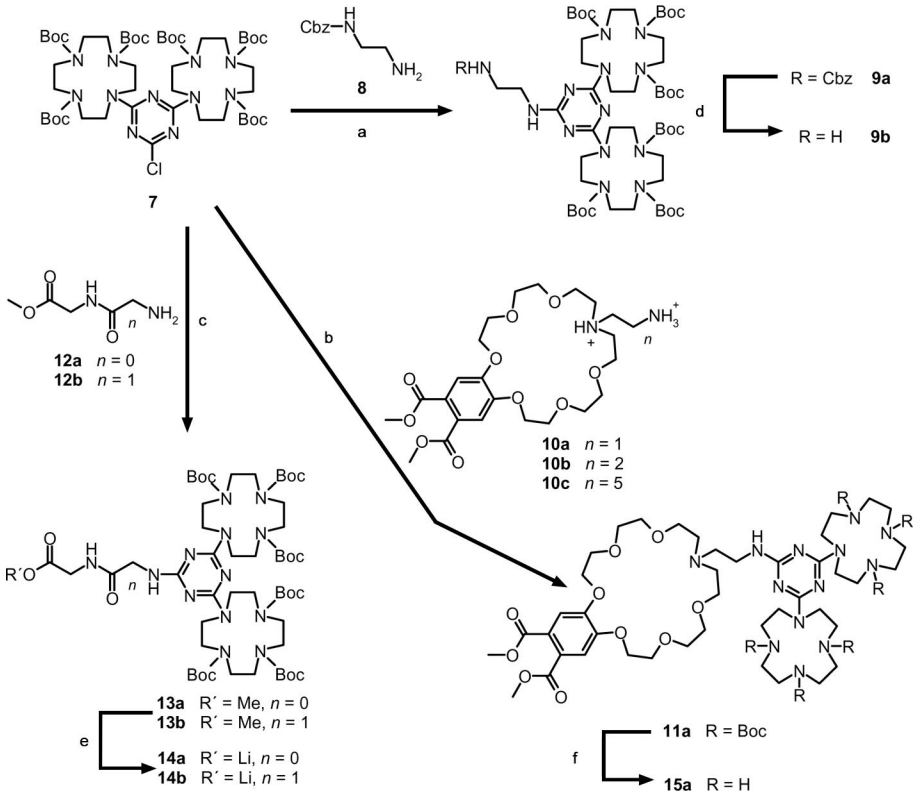


Figure 3. General structure of synthetic receptors with ammonium and phosphate ion affinity. The luminescent crown ether is connected to bis(zinc–cyclene) complex **3a**–**f**, Tyr–bis(dpa) complex **4**, zinc–cyclene complex **5** and dipicolylamine complex **6**. Counterions are not shown.

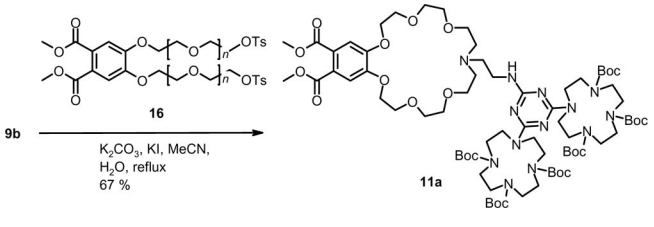


Scheme 1. Synthetic routes to compounds **9**, **14** and **15**: (a) K_2CO_3, dioxane, 2 d, 120 °C; 86%; (b) K_2CO_3, dioxane, 2 d, 140 °C; 41%; (c) K_2CO_3, dioxane, 2 d, 140 °C; 76–79%; (d) EtOH Pd/C 10%, 20 bar H_2, 4 d; 97%; (e) LiOH, acetone, 2 d, r.t.; quant.; (f) TFA, DCM, 4 h, r.t., ion exchange with strongly basic resin in H_2O; 91%.

Results and Discussion

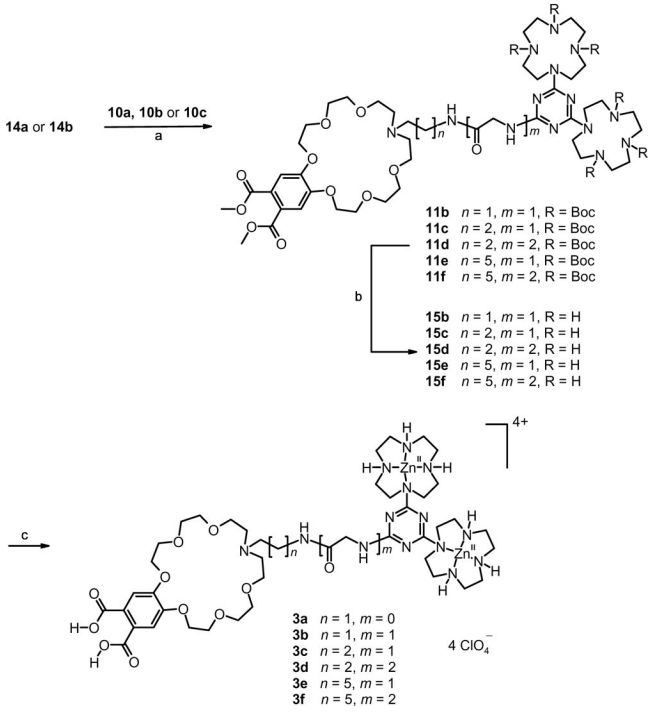
Synthesis

The synthesis started with protected bis(cyclene) **7**. The chloride group was substituted by orthogonal monoprotected 1-*N*-benzyloxycarbonyl ethylenediamine (**8**), methyl glycinate (**12a**) or methyl glycylglycinate (**12b**). Subsequent deprotection steps proceeded in excellent yields (Scheme 1). Starting materials and crown ether amino acid esters **10** were prepared and deprotected as described previously.[42,43] Crown ether bis(cyclene) **11a** can also be prepared by the route depicted in Scheme 1; however, a better method is the reaction of **9b** with tosylate **16** (Scheme 2).



Scheme 2. Synthesis of ligand **11a** by a ring closing S_N reaction.

Peptide coupling of building blocks **10** and **14** gave the protected ditopic crown ether bis(cyclene) receptor ligands **11b**–**11f**.[44] All compounds were deprotected with TFA[45]



Scheme 3. Synthesis of complexes **3** by peptide coupling, deprotection and complexation. (a) EDC, HOBt, DIPEA, $CHCl_3$, 0 °C, r.t.→ 40 °C, overnight; 51–65%; (b) TFA, DCM, 4 h, r.t., ion exchange with strongly basic resin in H_2O; 86–93%; (c) $Zn(ClO_4)_2$, $NaHCO_3$, MeOH, H_2O, 60 °C → 80 °C, 6 h; 79–95%.

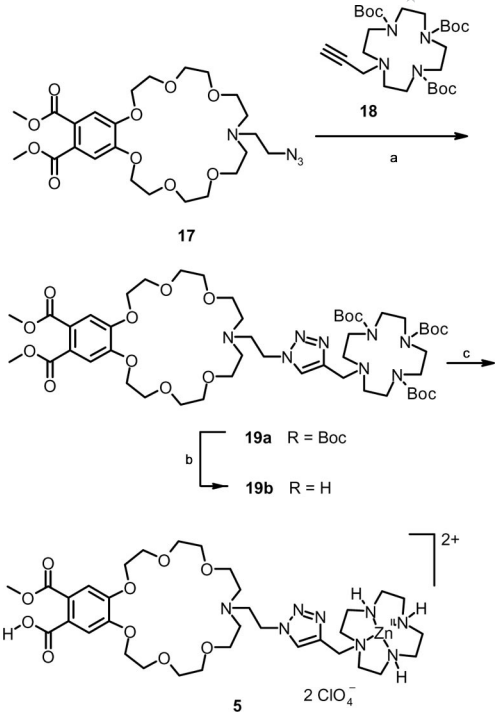
and, after neutralization with the aid of a strong basic ion exchange resin, complexed with zinc ions under reflux in water/methanol solution (Scheme 3).

Dinuclear Zn^{II} complexes **3a**–**3f** were isolated in analytical purity in good yields (79–95%) from the reaction of the ligand with zinc perchlorate in aqueous solution followed by precipitation with ethanol. Characterization confirmed a metal-to-ligand ratio of 2:1. The methyl esters are partially cleaved to yield the free acids under the conditions of the complexation reaction or through storage of the complexes in buffer. Therefore, conditions that resulted in complete ester hydrolysis were chosen for the complexation reactions in order to avoid mixtures.[46]

Monocyclene crown **5** was prepared for comparison (Scheme 4). The compound was obtained by cycloaddition of Huisgen azide **17** with alkyne **18**. Acidic cleavage of the Boc protecting groups and subsequent treatment of the TFA salt with a basic anion exchange resin yielded the free amine ligand. Complex formation with one equivalent of $Zn(ClO_4)_2$ gave the mononuclear Zn^{II}-cyclene derivative **5**.[47]

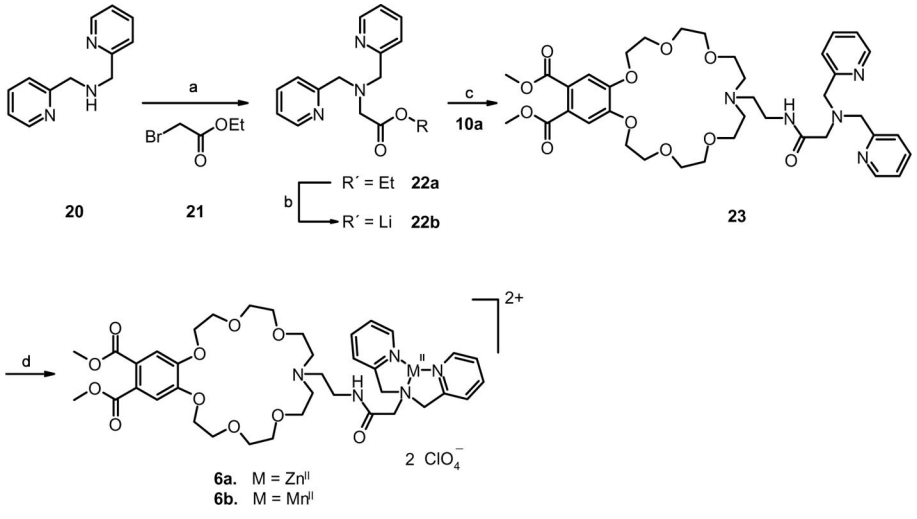
Zinc and manganese complexes (**6a** and **6b**) of dipicolylamine (dpa) with crown ether ligand **23** were prepared from 2,2-dipicolylamine (**20**). Carboxylic acid **22b** was coupled with crown ether amino acid TFA salt **10a** by peptide bond formation. Manganese and zinc complexes **6** were obtained in methanol at ambient temperature (Scheme 5). The complexes are fluorescent, as zinc and manganese ions do not quench the emission.

Analogously, the lithium salt of Cbz-protected tyrosine–bis(dpa) **24** was treated with crown ether **10a** yielding the desired crown-appended bis(dpa) ligand **25**. The ligand was converted in aqueous methanol solution into the dizinc(II) or dimanganese(II) complex (**4a** or **4b**) by treatment with zinc perchlorate or manganese chloride, respectively (Scheme 6). Overall, the complexes were prepared in good yield.

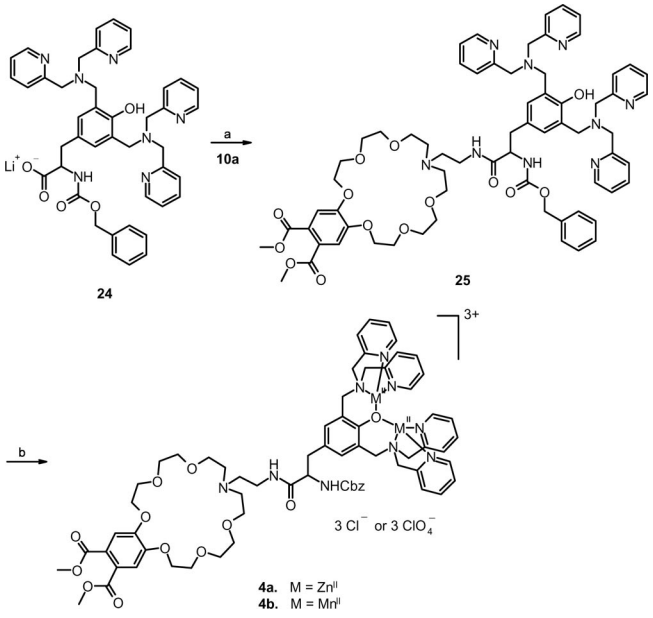


Scheme 4. Synthesis of complex **5**. (a) $CuSO_4$, sodium ascorbate, MeOH, H_2O, r.t. then reflux, 5 h; 93%; (b) TFA, DCM, 4 h, r.t., ion exchange with strongly basic resin in H_2O; 97%; (c) $Zn(ClO_4)_2$, $NaHCO_3$, MeOH, H_2O, 60 °C → 80 °C, 6 h; quant.

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



Scheme 5. Synthesis of complexes **6**. (a) MeCN, K_2CO_3, 0 °C then r.t.; 81%; (b) NaOH, MeOH, H_2O, 6 h, r.t.; quant.; (c) EDC, HOBt, DIPEA, $CHCl_3$, DMF, 0 °C, r.t.→ 40 °C, overnight; 61%; (d) $Zn(ClO_4)_2$, MeOH, H_2O, 40 °C, 2 h; quant.; or $MnCl_2$, MeOH, H_2O, 40 °C, 2 h; quant.



Scheme 6. Preparation of complexes **4**. (a) EDC, HOBt, DIPEA, DMF (dry), $CHCl_3$ (dry), 0 °C, r.t.→ 60 °C, overnight; 47%; (b) $Zn(ClO_4)_2$, MeOH, H_2O, 40 °C, 2 h; quant.; or $MnCl_2$, MeOH, H_2O, 40 °C, 2 h; quant.

Recognition Properties of the Crown Ether Metal Complex Receptors

Photophysical Properties

Compounds **3a**–**3f** and **5** show absorption maxima in water at 220 nm and 270 nm and emit upon excitation at 380 nm with a quantum yield of about $\phi = 0.05$.[48] The absorption properties are only marginally affected by the nature of the tether.[49] Compounds **4a**,**b** and **6a**,**b** show a similar behaviour, with absorption bands at 220 nm and 270 nm. The emission maximum is observed at 390 nm, and the quantum yield was determined to be $\phi = 0.1$ (Figure 4).

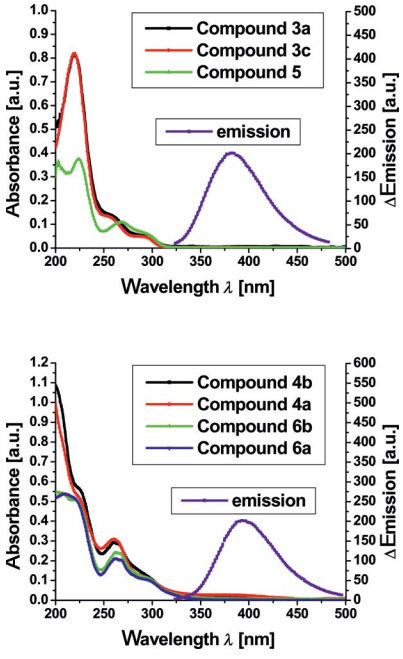


Figure 4. Absorption and emission spectra of selected metal complexes in water; top: compounds **3a**, **3c** and **5** ($c = 2.1 \times 10^{-5}$ mol/L), bottom: compounds **4a**, **4b**, **6a** and **6b** ($c = 2.2 \times 10^{-5}$ mol/L).

Binding Properties of the Individual Binding Sites

To evaluate the ammonium ion affinity of the crown ether binding site, protected ligands **11a**–**f** were titrated with *n*-butylammonium chloride and potassium isothiocyanate in methanol, giving $\log K$ values of 2.5 and 3.5 for *n*-butylammonium chloride and potassium isothiocyanate, respectively, which matches literature data.[42] In HEPES buffered aqueous solution, no interaction of the cations with the crown ether was detectable, even when 2000 equiv. of the salts were added.

Compound **3e** was titrated in aqueous HEPES buffer (50 mM, pH 7.4) with adenosine monophosphate (AMP, **26**) yielding a binding constant of $\log K = 4.1$. The binding of the phosphate ion to the metal complex leads to a decrease in luminescence (Figure 5).

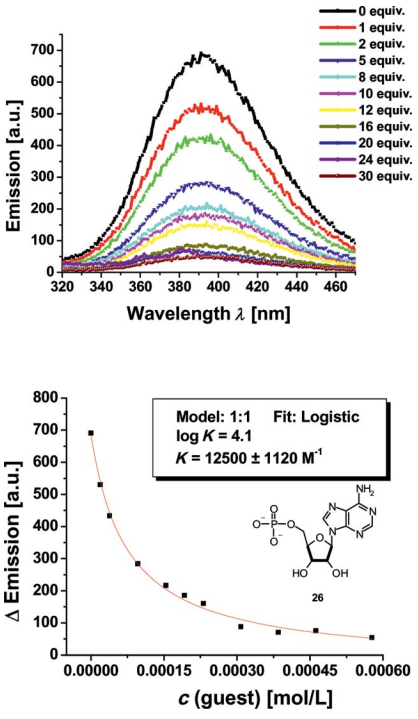


Figure 5. Titration of compound **3e** with AMP (**26**); top: observed emission changes; bottom: nonlinear fit corresponds to a 1:1 binding model.

Binding of Ammonium Phosphate

The simultaneous binding ability of receptors **3** to **6** to phosphate and ammonium ions was investigated with phosphoserine (**27**), PKG substrate **2** and monophosphorylated CTD heptapeptide **1** (Figure 6). The distance between the phosphate and ammonium ions increases from **27** to **1**, but the structure of the peptides is very flexible in all cases. To quickly identify the best matches between synthetic recep-

tors and target peptides, a well plate screening of the library of complexes **3**–**6** against **1**, **2** and **27** was performed in buffered aqueous solution (HEPES 50 mM, pH 7.4, 25 °C).

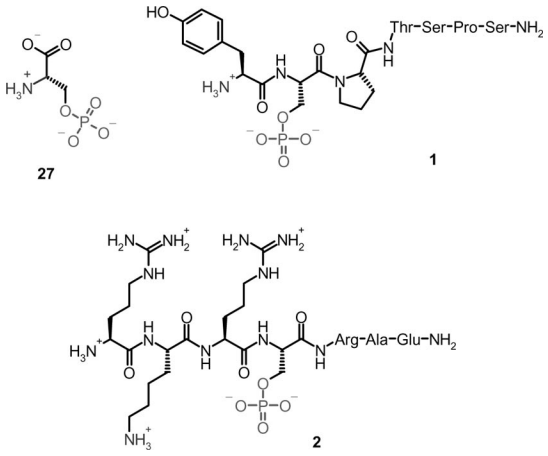


Figure 6. Ammonium phosphates used to evaluate the binding properties of compounds **3** to **6**: phosphoserine (**27**), CTD sequence repeat (pSer 2) **1** and PKG-substrate (pSer 4) **2**.

Twenty equivalents of peptide **1** or **2**, or a hundred equivalents of **27**, were added to a solution of the synthetic receptor (1×10^{-5} M), and the fluorescence emission intensity of **3a**, **3b** and **5** at 380 nm, and that of **4a**, **4b**, **6a** and **6b** at 390 nm (λ_{ex} = 300 nm), was recorded. The fluorescence enhancement factors with volume correction were calculated by Equation (1).

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

A value of 1.00 indicates no significant interaction, a value smaller than 1.00 means coordination of the metal complex to a phosphoserine residue, and a value larger than 1.00 points to additional intramolecular interaction of ammonium ions and crown ethers. Figure 7 summarizes the results.

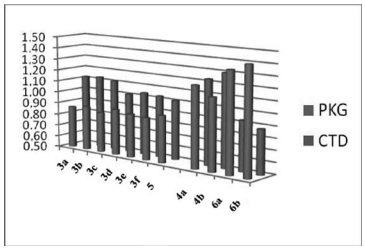


Figure 7. Ammonium phosphate induced fluorescence enhancement factors of receptors **3** to **6**: 1×10^{-5} M receptor in 50 mM HEPES buffer at pH = 7.4; excitation wavelength 300 nm; emission wavelength 390 nm; the errors are estimated to be approximately 10%; [guest] = 2×10^{-4} M.

The presence of phosphoserine (**27**) decreases the luminescence intensity of all of the investigated complexes. Phosphate coordination to the metal complex occurs, but the distance or geometry of the ammonium ion group is not favourable for intramolecular inclusion into the crown ether. A significant fluorescence enhancement was observed for **6a** and **6b** with both peptides **1** and **2**, and for

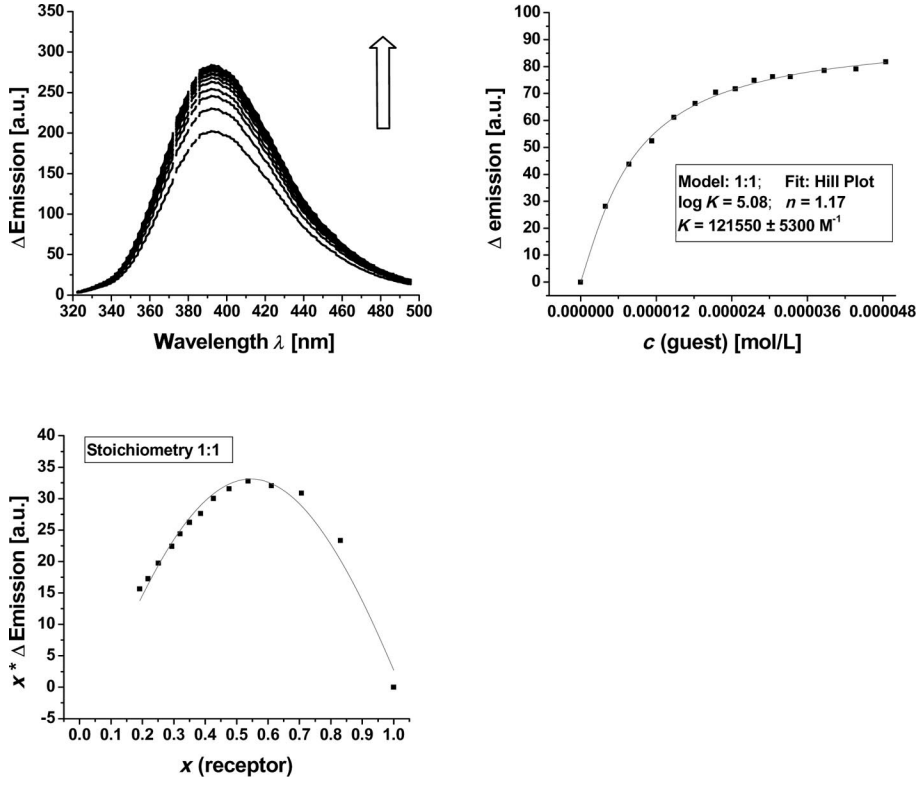


Figure 8. Top: Emission intensity changes of **4b** (2×10^{-5} M, λ_{ex} = 300 nm) upon addition of **2**. Bottom: Job's plot analysis.

4a and **4b** upon binding to **2**. Synthetic receptors **3a**, **3b** and **3c** show weak increases of the fluorescence intensity ($F/F_0 < 1.2$) in the presence of CTD motif **1**. To confirm that the phosphorylated site is essential in the target peptides in order to trigger emission changes, complexes **3**, **4** and **6** were treated under identical conditions with the amino acids cysteine, serine, arginine, histidine, glutaminic acid, glutamine and tetrabutylammonium acetate. The presence of the amino acids or the ammonium salt resulted in only negligible changes in the emission intensities of the complexes.

The peptide–receptor combinations with the largest fluorescence enhancement response in the screening assay were investigated by emission titrations in HEPES-buffered aqueous solution (50 mM, pH 7.4). The stoichiometry was determined by Job plot analyses extracted from titration data. Fluorescence intensities were volume-corrected, plotted against the concentration of the peptide and evaluated

Table 1. Binding constants (K) and fluorescence enhancement (F/F_0) values for selected combinations of complexes **3**, **4** and **6** with peptides **1** and **2** and phosphoserine.

Receptor[a]	PKG-substrate **2**[b]		CTD-heptapeptide **1**[b]		Phosphoserine (**27**)[b]	
	K [$\times 10^3$ M^{-1}]	F/F_0	K [$\times 10^3$ M^{-1}]	F/F_0	K [$\times 10^3$ M^{-1}]	F/F_0
4a	81 ± 6 log K = *4.91*	1.39	–[c]	–[c]	13 ± 2 log K = *4.12*	0.92
4b	121 ± 5 log K = *5.08*	1.40	–[c]	–[c]	10 ± 1 Log K = *4.02*	0.84
6a	33 ± 3 log K = *4.52*	1.17	64 ± 2 log K = *4.80*	1.23	7 ± 1 log K = *3.86*	0.82
6b	78 ± 6 log K = *4.89*	1.11	70 ± 3 log K = *4.84*	1.31	8 ± 1 log K = *3.92*	0.79
3a	–[c]	–[c]	72 ± 4 log K = *4.86*	1.16	5 ± 1 log K = *3.7*	0.69
3b	–[c]	–[c]	82 ± 5 log K = *4.91*	1.14	5 ± 1 log K = *3.7*	0.72
3c	–[c]	–[c]	92 ± 4 log K = *4.96*	1.12	5 ± 1 log K = *3.7*	0.71

[a] 2×10^{-5} M receptor in HEPES buffer (50 mM, pH 7.4, no salt added, adjusted with Et_4NOH and HCl); the counterion is Cl^-. [b] Concentration [guest] = 0.0002 M or 0.001 M. [c] Not determined.

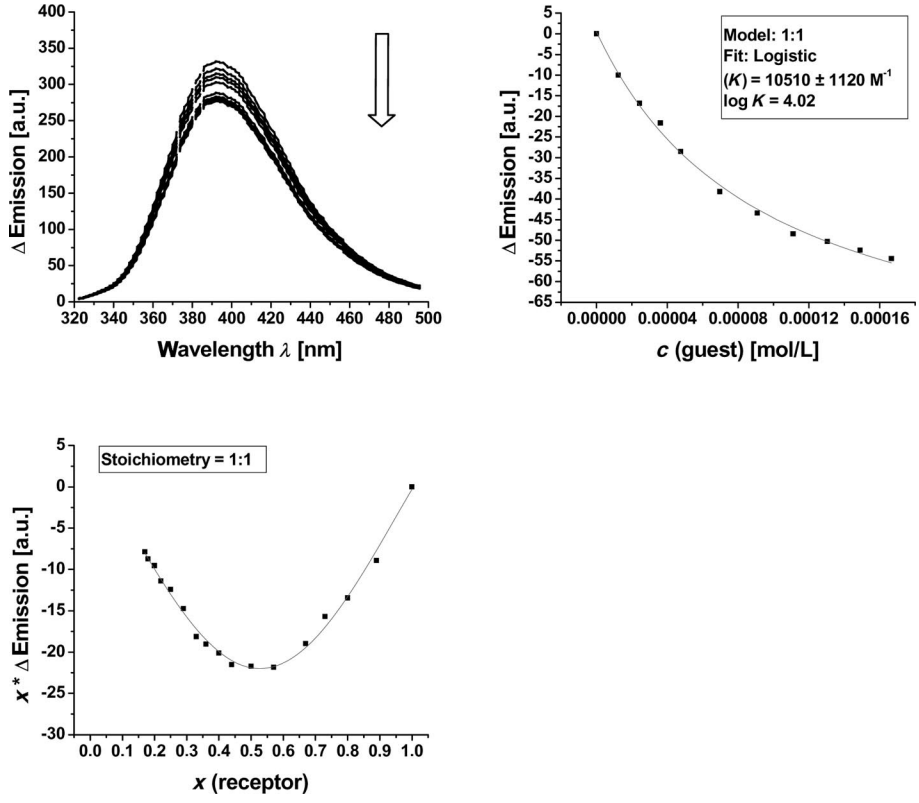


Figure 9. Top: Emission titration of **4b** with phosphoserine (**27**). Bottom: Job's plot analysis.

by nonlinear fitting methods to determine the binding constants. Figure 8 shows as a representative example of the emission titration curve and Job plot analysis for the titration of **4b** with peptide **2**.

Table 1 summarizes all binding data derived from the titrations. The affinities of **4a** and **4b** for peptide **2** and of **6a**, **6b**, **3a**, **3b** and **3c** for peptide **1** are in the micromolar range and identical within the error of experiment. Job plot analyses[49] confirmed stoichiometric ratios of the receptor–peptide aggregates of 1:1 in all cases. All receptors show a similar affinity to phosphoserine (**27**), which is 1–2 orders of magnitude smaller than that to **1** or **2**. Emission intensities decrease during titration (Figure 9). The difference in emission response indicates the mode of binding: phosphate-to-metal coordination for **27** and ditopic ammonium phosphate binding in the case of **1** and **2**. Hill plot analysis[50] shows a weak cooperativity of the supramolecular aggregate formation for the binding of the metal complexes with CTD heptapeptide **1** (Hill coefficient n = 1.4–1.8) and PKG substrate **2** (Hill coefficient n = 1.1–1.4).

Conclusions

We have prepared luminescent synthetic receptors **3**, **4** and **6** for the binding of peptidic ammonium phosphates **1**, **2** and **27** in aqueous buffer. The compounds consist of a metal complex, which reversibly coordinates phosphate anions, and a luminescent aza crown ether with a weak affinity for ammonium ions. The binding of ammonium ions to the crown ether in aqueous solution is only possible in an intramolecular fashion subsequent to phosphate ion coordination. The coordination of phosphate ions to the metal complex decreases the emission intensity of the fluorophore, whereas ammonium ion binding to the aza crown ether increases the emission intensity. This allows a distinction between phosphate coordination and ditopic ammonium phosphate binding. Phosphoserine coordinates to all synthetic receptors with millimolar affinity, but its geometry does not favour the simultaneous interaction of the phosphate and ammonium ions with the receptor, as indicated by a decreased emission intensity. Phosphorylated peptides **1** and **2** bind to the synthetic receptors in ditopic fashion with micromolar affinity. The increase in emission intensity is a clear indication of the interaction of the aza crown ether with the ammonium ion. The conformationally flexible structures of the short peptides **1** and **2** and the receptors does not lead to large differences in selectivity upon structural variation. However, from the screening experiment, best matches were identified for the combinations of receptor **4** with peptide **2**, and receptors **3a**–**c** or **6** with peptide **1**. Our examples show that by combining weak and strong reversible interactions, the selective detection of phosphorylated peptide sequences in aqueous buffered solution becomes possible. Nonphosphorylated amino acids do not interfere, and phosphate coordination without ammonium ion binding is indicated by a decrease in emission intensity. The synthetic receptors presented here may not currently fit the requirements for practical chemosensor applications in bioanalytics, such as excitation and emission wavelength, membrane permeability or tolerance to other species present, but they prove that combinations of strong and weak binding sites allow a distinction between phosphorylated peptides in aqueous buffers.

Experimental Section

Syntheses: Compounds **7**,[29,51] **8**,[52] **10a**,[53] **10c**,[43] **16**,[53] **17**,[42] **18**,[54] **20**[55] and **24**[56] were prepared by following literature procedures. The PKG substrate (pSer 4) was purchased from Genscript (> 95%) and used as received. The synthesis and characterization of compounds **3**, **4** and **6** is described in the Supporting Information to this article.

Fluorescence Screening, Absorption and Emission Titrations: The fluorescence output was recorded in the wavelength range of 330–500 nm, above and below this range no signal is detected. The excitation of the fluorophore is possible between 280 to 310 nm, below this range the nucleotide adenine and the amino acid tyrosine interfere.

Screening of Amino Acid and Peptide Binding Affinities: The screenings were performed in black UV star well plates with 384 cells (130 μL volume per cell) in HEPES buffer (50 mM) at pH 7.4. To a 1×10^{-5} M solution of a particular receptor compound were added twenty equivalents of peptide (2×10^{-4} M) or one hundred equivalents of amino acid (1×10^{-3} M) (1:1 v/v). The mixtures were pipetted row by row, mixed with the aid of the pipette and allowed to equilibrate for 10 min. The fluorescence spectrum was recorded (λ_{ex} = 300 nm) and compared to diluted blank receptor solutions. All measurements were repeated twice.

Titration with Nucleotides and Phosphoserine in a Well Plate: The solutions of the hosts (5×10^{-5} mol/L) and guests (5×10^{-5} mol/L)/(1×10^{-3} mol/L) were prepared in aqueous HEPES buffer (50 mM; pH = 7.4). The titrations were performed in black UV star well plates with 384 cells. Different small aliquots (0, 1, 2, 5, 8, 10, 12, 16, 20, 24 and 30 equiv.) of the guest were added to the receptor. The final concentration of the receptor was 1.92×10^{-5} mol/L in each well. The luminescence output was plotted against the concentration of the guest, and the binding constant was evaluated by non-linear fitting methods.

Binding Affinity Titration with Peptides in a Cuvette: All binding studies were conducted in buffered aqueous solution (50 mM HEPES, pH 7.4, adjusted with Et_4NOH, no salt added) at a constant temperature of 298 K. The receptor (1.0 mL, 2×10^{-5} mol/L) in the cuvette was titrated stepwise with small amounts (10–50 μL, 0.1–1.0 equiv.) of the peptide solution (2×10^{-4} mol/L). After each addition, the solution was allowed to equilibrate for 10 min before the fluorescence intensity (λ_{ex} = 300 nm) was recorded. The stoichiometry was determined by Job plot analysis extracted from titration data.[49] To determine the binding constant, fluorescence intensities were volume-corrected, plotted against the concentration of peptide and evaluated by nonlinear fitting methods.

Supporting Information (see footnote on the first page of this article): (1) experimental section, (2) NMR spectra of selected molecules, (3) details of the fluorescence studies, fluorescence titration curves and Job plot analyses, (4) additional studies on phosphate recognition.

Acknowledgments

We thank the Deutsche Forschungsgemeinschaft (GRK 760) and the University of Regensburg for support of this work. We thank Dr. A. Grauer for preparation of compound **13b**, Dr. S. Stadlbauer for supplying 2,2-*N*,*N*-dipicolylamine (**20**), F. Schmidt for preparation of CTD heptapeptide **1** and A. Riechers for discussions.

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[45] If HCl in ethyl ether is used for Boc cleavage, the product may precipitate so quickly that a repetition of the deprotection step is necessary to remove all protecting groups. If traces of water are present, the crown ether methyl esters are quantitatively hydrolyzed.
[46] It is known that zinc–cyclene complexes promote the hydrolysis of activated phosphate and carboxy esters, and even of certain peptide bonds. Typically, the hydrolysis rate of carboxy esters at neutral pH is low. We suggest that the structure of the compounds described here promotes intramolecular ester hydrolysis. Peptide bond hydrolysis: C. Bazzicalupi, A. Bencini, E. Berni, A. Bianchi, P. Fornasari, C. Giorgi, B. Valtancoli, *Eur. J. Inorg. Chem.* **2003**, 1974–1983. Carboxyester hydrolysis: M. Subat, K. Woinaroschy, S. Anthofer, B. Malterer, B. König, *Inorg. Chem.* **2007**, *46*, 4336–4356.
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[48] All quantum yields were determined with quinine disulfate in 1 N H_2SO_4 as the reference compound (Φ = 0.546).
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Received: February 28, 2010
Published Online: May 19, 2010

