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Optically Active Cyclic Hexapeptides with Covalently Attached Pyrene Probes: Selective Alkaline Earth Metal Ion Recognition Using Excimer Emission

Jörg Strauss and Jörg Daub*

Institut für Organische Chemie, Universität Regensburg, Universitätsstrasse 31, D-93053 Regensburg

joerg.daub@chemie.uni-regensburg.de

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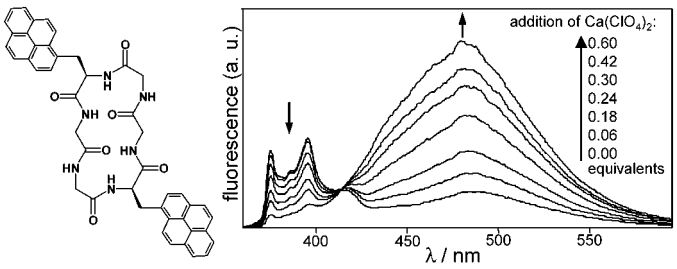
ABSTRACT



The synthesis of two optically active pyrene-modified cyclic hexapeptides and their selectivity toward the complexation of alkaline earth metal ions are reported. Complexation was studied by optical and chiroptical methods. The cyclic peptides are forming 2:1 sandwich complexes with the metal ions.

Cyclic peptides are important building blocks in life science and molecular material chemistry, operating in diverse areas such as physiological processes, supramolecular chemistry, or nanoscale materials. They are useful for the study of cell-adhesion processes,[1] the construction of transmembrane nanotube channels in order to transport molecules and ions,[2] and for molecular recognition of amino acids,[3] anions,[4] and cations.[5] The labeling of biomolecules such as peptides with dye units enables the probing of intrinsic properties using emission and absorption spectroscopy. In the past, pyrenyl-derivatized peptides[6] and enzymes[7] were successfully used to elucidate peptidic structures and functions with optical spectroscopy. Here we report a new protocol for the

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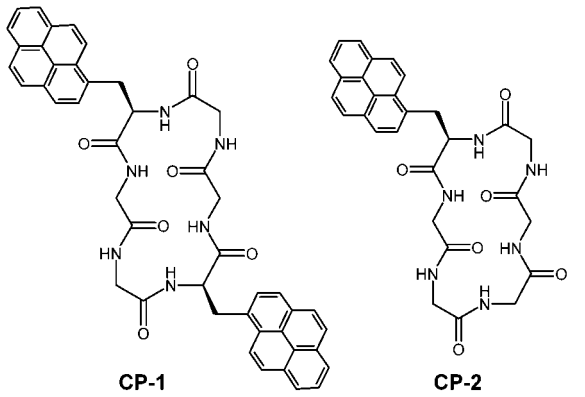
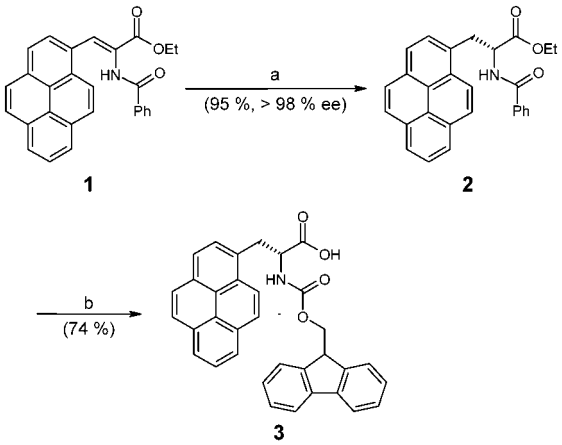
enantioselective synthesis of pyrenyl-substituted D-alanine and its incorporation into cyclic peptides. As a first recognition study it is shown by UV/vis absorption and emission spectroscopy that the resulting compounds **CP-1** and **CP-2** are forming complexes with divalent alkaline earth metal ions. Comparable metal ion complexes of cyclic peptides, which are suitable for the transportation of ions through membranes,[8] have so far been established by NMR, circular dichroism, or Raman spectroscopy.[9] Compared to fluorescence, these methods are less selective in in vivo studies.



Preparation of the nonnatural pyrenyl amino acid starts with the transformation of 1-pyrenecarboxyaldehyde to 2-phenyl-4-(1-pyrenylmethylene)-5-(4*H*)-oxazolone. Subsequent ring opening of the heterocyclic unit results in dehydroamino acid derivative **1**.[10] Asymmetric hydrogenation employing (*R*,*R*)-Et-DUPHOS-Rh(I) as a catalyst yields **2** in high enantiomeric purity (ee > 98%, Scheme 1).[11] The reaction sequence given in Scheme 1 leads to the Fmoc-protected amino acid **3**.[12] This amino acid (**3**) and glycine are subjected to a Merrifield solid-phase peptide synthesis using *o*-chlorotrityl chloride (cTrt) resin and applying the Fmoc strategy.[13] The Fmoc-protected amino acids are coupled using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU), 1-hydroxybenzotriazole (HOBt), and diisopropylamine (DIEA) as the base.[13] The linear hexapeptides H_2N-Gly-D-Pya-Gly_2-D-Pya-Gly-OH·HOTf[14] and H_2N-Gly_3-D-Pya-Gly_2-OH·HOTf are obtained in more than 80% yield.

Scheme 1. Enantioselective Preparation of Fmoc-Protected Pyrenyl Amino Acid[a]



[a] Key: (a)(i) (*R*,*R*)-Et-DUPHOS, $[Rh(COD)Cl]_2$, CH_2Cl_2; (ii) H_2 (725 psi), MeOH, 60 °C. (b)(i) HCl, HOAc, 100 °C; (ii) Fmoc-ONSu, $NaHCO_3$, dioxane, H_2O.

Peptide cyclization is carried out at high dilution (7×10^{-4} M) in DMF using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and collidine.[15] Subsequent purification by reversed-phase HPLC delivers the cyclic peptides **CP-1** and **CP-2** with a yield of over 70%.[16] These new optically active cyclic peptides are showing typical pyrene absorption and emission behavior.[17] The UV/vis spectra display vibrationally splitted transitions at 339 nm (**CP-1**, $\epsilon = 44340$ L mol^{-1} cm^{-1}; **CP-2**, $\epsilon = 24610$ L mol^{-1} cm^{-1}), 273 nm (**CP-1**, $\epsilon = 46680$ L mol^{-1} cm^{-1}; **CP-2**, $\epsilon = 27680$ L mol^{-1} cm^{-1}) and 240 nm (**CP-1**, $\epsilon = 77240$ L mol^{-1} cm^{-1}; **CP-2**, $\epsilon = 42120$ L mol^{-1} cm^{-1}).[18] The fluorescence spectra exhibit intensive pyrene monomer emission in the region of 375 to 418 nm. An additional pyrene excimer band appears at 487 and 481 nm in **CP-1** and **CP-2**, respectively. The excimer emission of **CP-2** is concentration dependent, and its intensity is much lower than that of **CP-1**. On the contrary, the excimer emission of **CP-1** is almost independent of concentration. This proves an intramolecular excimer formation.

Addition of calcium perchlorate to a solution of cyclic peptide **CP-1** causes a considerable increase of the excimer to monomer emission ratio (I_E/I_M). After the cation concentration has reached half of the cyclic peptide concentration, further addition of calcium(II) ions is ineffective (Figure 1).

The titration of **CP-1** with barium(II) yields an analogous emission response. However, it is found that the I_E/I_M ratio is lower on barium(II) addition than on calcium(II) addition.

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(18) Only the longest wavelength bands and their molar extinction coefficients of the vibrationally split transitions are given.

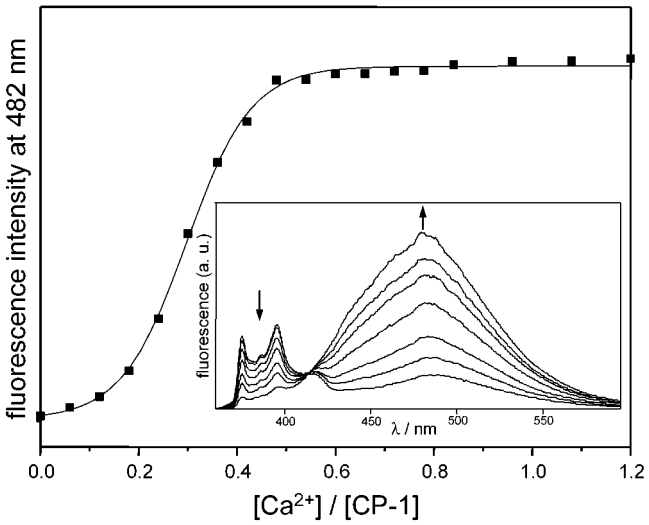

Figure 1. Change of emission intensity at 482 nm during titration of **CP-1** with $Ca(ClO_4)_2$. Inset: fluorescence of **CP-1** after addition of 0, 0.06, 0.18, 0.24, 0.3, 0.42, and 0.6 equiv of $Ca(ClO_4)_2$.[19]

Also the (**CP-1**)−Ba(II) complex is found to be more stable than the (**CP-1**)−Ca(II) complex (Table 1).

Table 1. I_E/I_M Ratio of Cyclic Peptides **CP-1** and **CP-2** as well as Their Alkaline Earth Metal Ion Complexes and the Corresponding Complexation Constants

	I_E/I_M	log K
CP-1	0.6	
CP-1 + Ca^{2+}	14.9	5
CP-1 + Ba^{2+}	3.4	8
CP-2	0.1	
CP-2 + Ca^{2+}	8.5	6
CP-2 + Ba^{2+}	0.7	5

Unlike Ca(II) and Ba(II) titration, the titration with magnesium perchlorate does not affect the emission of cyclic peptide **CP-1**. This indicates the high selectivity of **CP-1** for Ca(II) and Ba(II) cations versus Mg(II) ions. Peptide **CP-2** shows analogous fluorescence characteristics on the addition of alkaline earth metal ions. However, the I_E/I_M ratios of these complexes are much lower in comparison to the **CP-1** complexes. It is also found that the two **CP-2** complexes have the same stability within the scope of error (Table 1).

The addition of Ca(II) ions as well as Ba(II) ions to **CP-1** and **CP-2** causes a decrease in intensity and a bathochromic shift of the absorption bands in the UV/vis spectra (Figure 2). This is in agreement with the formation of pyrene dimers in the ground state.[6a,20] The excitation spectra of the free

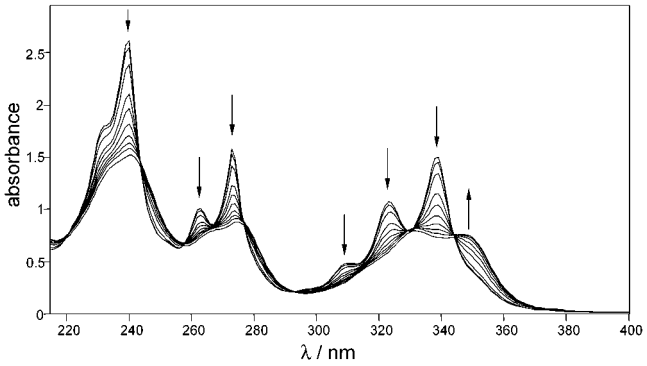

Figure 2. UV/vis spectra of **CP-1** after addition of 0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.45, and 0.8 equiv of $Ca(ClO_4)_2$.[19]

peptides and their complexes coincide with their corresponding UV/vis spectra.

Characteristic absorption bands in UV spectra appear at λ_{max} of 348 nm (ϵ = 22460 L mol^{-1} cm^{-1}), 275 nm (ϵ = 26690 L mol^{-1} cm^{-1}) and 241 nm (ϵ = 46220 L mol^{-1} cm^{-1}). As also found by fluorescence spectroscopy, the UV/vis spectra do not change beyond a molar ratio of **CP-1** to metal cation higher than 2:1.

The complexation of alkaline earth metal ions with the cyclic peptides **CP-1** and **CP-2** is also detectable by circular dichroism (CD). Free **CP-1**, for instance, displays weak CD intensity. On calcium(II) complexation, an intense CD band at 244 nm occurs.

Because of their spectral properties, the pyrene probes allow a more detailed stoichiometry and structure description of the complexes. In contrast to other known pyrene-modified metal sensors,[22] the I_E/I_M ratio of **CP-1** and **CP-2** fluorescence increases after metal ion addition. This leads to almost complete quenching of pyrene monomer emission (Table 1, Figure 1). This finding and the proof of pyrene ground-state dimers verifies a stacked and parallel arrangement of pyrene subunits in the complexes, formed both in the excited state and in the ground state. Job plots of UV/vis titrations result in a 2:1 stoichiometry of the cyclic peptide metal ion complexes. Accordingly, the cyclic peptides **CP-1** and **CP-2** are forming 2:1 sandwich complexes with Ba(II) and Ca(II) cations. The pyrene subunits of the upper and the lower cyclic peptide units are supposed to stack together in an almost parallel way. This explains the insensitivity of the UV/vis absorption bands as well as the emission bands at cyclic peptide to metal ion ratios higher than 2:1.

(19) All fluorescence and UV/vis spectra were carried out in 1,1,1,3,3,3-hexafluoro-2-propanol/methanol (4/1) at concentrations of 10^{-4}−10^{-5} M.

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(21) Logarithmic complexation constants (log K) are estimated numerically by the computer program HypNMR. Errors are valued with 15%. For details on HypNMR, see: Alderighi, L.; Bianchi, A.; Biondi, L.; Calabi, L.; De Miranda, M.: Gans, P.; Ghelli, S.; Losi, P.; Paleari, L.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2741−2745.

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As known from literature, normally three carbonyl oxygens of each peptide molecule in the complex are coordinating the metal ion in an octahedral manner.[23] It is also well established that the complex stability critically depends on the cation size.[9a] Due to the formation of a 2-fold pyrene/pyrene interaction[6g] in the **CP-1** complexes, the peptide conformation should be less flexible than in the corresponding **CP-2** complexes. This may lead to a more stable (**CP-1**)$_2$Ba(II) complex (ionic radius of Ba(II) at coordination number 6 = 1.35 Å) in comparison to (**CP-1**)$_2$Ca(II) (ionic radius of Ca(II) at coordination number 6 = 1.00 Å). Due to the single pyrene−pyrene interaction,[6g] the **CP-2** complexes of Ba(II) and Ca(II) are of almost equal stability (Table 1). With its ionic radius of 0.72 Å at coordination number 6, Mg(II) seems to be too small to form sandwich complexes with peptide **CP-1** or peptide **CP-2**.[9a]

Assuming an octahedral complex structure in which the distance between the Ca(II) cation and oxygen amounts to 2.2−2.6 Å,[9a,23] the (**CP-1**)$_2$Ca(II) complex was calculated with Spartan.[24] After geometry optimization using force field methods, the calculations were carried out on a semiempirical level using PM3 (Figure 3). On the basis of these calculations, the pyrene−pyrene distance in the (**CP-1**)$_2$Ca(II) complex amounts to 6−7 Å. This is larger than the expected distance of 4 Å for dynamic pyrene excimers. According to that and due to the proven pyrene ground-state dimers, the excimer emission is determined to be a static one, which occurs at distances between 3 and 10 Å.[25]

The reported pyrenyl-modified cyclic hexapeptides enable detection of cyclic hexapeptide−metal cation complexes using the intensity ratio of the pyrene monomer and excimer emission for the first time. The reported route of synthesis allows modification of the ligands structurally and stereochemically. Thus, potential applications in different fields

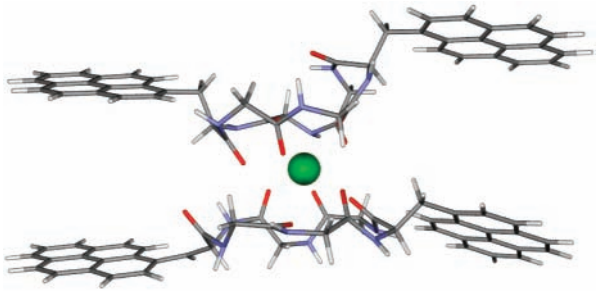

Figure 3. Supposed structure of the (**CP-1**)$_2$Ca(II) complex (colors: gray = C, blue = N, red = O, white = H, and green = Ca^{2+}).

arise such as (i) metal ion sensing, (ii) specific interaction targeting natural receptor systems, (iii) analyzing ion transportation by fluorescence spectroscopy, (iv) the determination of structure−activity relationships using customized cyclic peptides, and (v) molecular switches operating under chiroptical signal expression.[26]

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Supporting Information Available: Experimental procedure and full characterization for compound **2**, CD spectra of compound **CP-1** and its calcium complex, and a Job plot for (**CP-1**)$_2$(Ca^{2+}) complex formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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