Water-soluble doubly N-confused hexaphyrin : a near-IR fluorescent Zn(II) ion sensor in water

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バージョン：

Water-soluble doubly N-confused hexaphyrin: A near-IR fluorescent Zn(II) ion sensor in water

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A water-soluble doubly N-confused hexaphyrin (N_2CH) having two octa-arginine peptide arms displays an enhanced near-infrared (NIR) emission around 1050 nm in the presence of Zn^{2+} in aqueous solution.

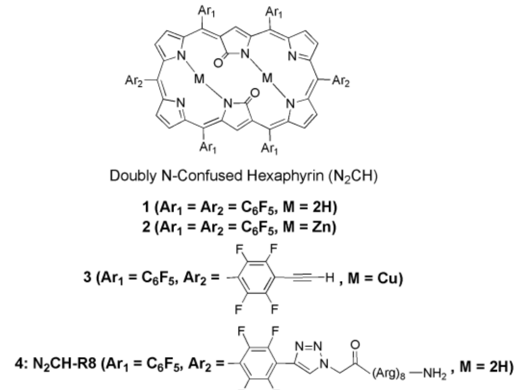
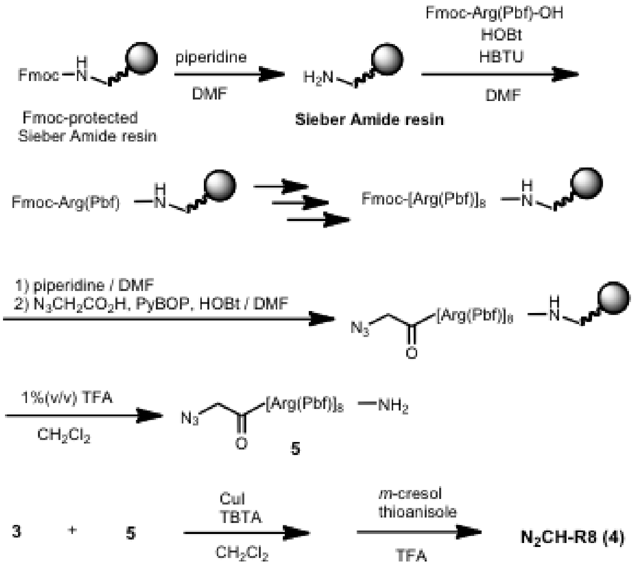


Chart 1 Structures of doubly N-confused hexaphyrins.

In the biomedical/bioanalytical fields, NIR-light technology has been of growing importance since the interference by biomolecules could be minimized in the NIR region.[1] Among a variety of targets for NIR-biosensing, Zn^{2+} ion gathers wide attention because of its importance as one of the most essential metal ions in the human body and the interest for the neurochemical functions.[2] Previously, in a series of studies on N-confused porphyrinoids,[3] we have shown doubly N-confused hexaphyrin (N_2CH, **1**, Chart 1), a kind of expanded porphyrin, forms bis-metal complexes with various divalent and trivalent metal ions such as Cu^{2+}, Ni^{2+}, Zn^{2+}, Mn^{3+}, and Fe^{3+}, and displays an intensified near-infrared (NIR) emission with $\lambda_{em} > 1000$ nm in CH_2Cl_2 when Zn^{2+} ions are coordinated.[4] Since then, we have been interested in synthesizing a water-soluble derivative, which serves as a NIR fluorescent sensor molecule, especially, for Zn^{2+} in aqueous media. Herein, we report the synthesis of a water-soluble derivative of N_2CH possessing two highly hydrophilic octa-arginine peptides (**N_2CH-R8**, **4**) and its emission behaviour in the presence of various metal ions in aqueous media. A largely enhanced NIR emission around 1050 nm in water by Zn^{2+} coordination was demonstrated for the first time.

Synthesis of **N_2CH-R8** was performed by conjugation of two peptides and N_2CH via a Cu(I)-catalyzed "click reaction" between azide groups and terminal alkynes (Scheme 1),[5] which are installed in the peptide and N_2CH, respectively.[6] At first, N_2CH derivative possessing two ethynylaryl groups (**3**), which was derived from *meso*-aryl N_2CH having two 2,3,5,6-tetrafluoro-4-iodophenyl groups, was synthesized through Sonogashira coupling reactions with $Pd(PPh_3)_2Cl_2$ and CuI in 49% yield.[7] The counterpart, octa-arginine (R8) peptide derivative (**5**), in which the arginine side-chains were protected with 2,2,4,6,7-pentamethyldihydro-benzofuran-5-sulfonyl (Pbf) groups and its N-terminus bears an azide moiety, was prepared by the solid-phase synthesis, which started from fluoren-9-ylmethyloxycarbonyl (Fmoc) protected aminoxanthen-3-yloxy-polystyrene resin (Sieber Amide resin).[6,7] Then, N_2CH derivative **3** was conjugated with peptide **5** by Cu(I)-catalyzed Huisgen 1,3-dipolar cycloadditions between the terminal alkynes in **3** and the azide moiety at the N-terminus of **5** (Scheme 1).[7] The reaction mixture was treated with trifluoroacetic acid (TFA) to remove Pbf groups and Cu^{2+} ions from the arginine side-chains and the macrocycle, affording the desired freebase N_2CH-peptide



Scheme 1 Synthesis of **N_2CH-R8** via conjugation of N_2CH (**3**) and protected octa-arginine peptide (**5**) by a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition.

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† Electronic Supplementary Information (ESI) available: Details of the experiments, theoretical calculations and spectroscopic measurements.

conjugate, **N_2CH-R8**. The resulting conjugate was purified by a reverse phase HPLC with CH_3CN/H_2O and obtained as a TFA salt (46% yield based on **3**). By partition experiments between ultrapure water and CH_2Cl_2, **N_2CH-R8** was selectively extracted to the aqueous phase.[8]

At first, we examined Zn^{2+} complexation of N_2CH **1** in organic solvent by spectroscopic methods. In CH_2Cl_2, UV-vis-NIR spectrum of **1** with excess $Zn(OAc)_2$ (ca. 500 equiv) was nearly identical to that of isolated **2**.[6] For 4.0 μM of **1**, completion of the spectral changes required 200 μM (50 equiv) of $Zn(OAc)_2$. The observation of isosbestic points at 591, 652, 764 and 830 nm suggests the coordination of second Zn^{2+} is rather fast to afford bis-Zn^{2+} complex, spontaneously.[6] NIR-fluorescence spectra of **1**, **2**, and **1** treated *in situ* with excess $Zn(OAc)_2$ were compared at ambient temperature. Upon adding $Zn(OAc)_2$, fluorescence of **1** was enhanced approximately 10-fold,[6] exhibiting a sharp emission band around 1047 nm, which was nearly identical to that of **2**. This result indicates that *in situ* formation of bis-Zn^{2+} complex of **1** proceeds almost quantitatively in CH_2Cl_2 at ambient temperature.

The structure of bis-Zn^{2+} complex **2** was revealed by X-ray crystallography (Fig. 1).[‡] The complex shows a slightly distorted structure with a mean plane deviation of 0.0798 Å defined by 36 heavy atoms without oxygen in the macrocycle. The displacement of Zn^{2+} ion is 0.0572 Å and the Zn-Zn distance is 4.806 Å. One H_2O molecule is coordinated at the axial position of each Zn atom with a Zn-O distance of 2.038 Å. The water molecule might come from the adventitious moisture in the solvent and such coordination would be usual for a water-soluble derivative of **2** in aqueous media.

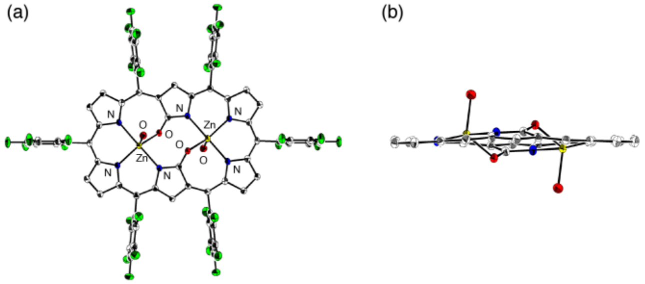

Fig. 1 ORTEP structure of **2**: a) top view, b) side view. Pentafluorophenyl groups are omitted for clarity in (b). Thermal ellipsoids are shown at the 30% probability level.

Then, **N_2CH-R8** was subjected to the spectroscopic measurements in aqueous solution (Fig. 2). The absorption λ_{max} values of the Soret-like (566 nm) and Q-like bands (730, 807, 902, 1040 nm) were similar to the spectrum of **1** in DMF, indicating that neither attachment of octa-arginine peptides via triazole linkers to N_2CH nor aqueous media intrinsically perturbs the absorption property of N_2CH macrocycle. Furthermore, in the fluorescence spectrum of **N_2CH-R8** in aqueous solution, an emission band at 1047 nm was observed similar to **1** in CH_2Cl_2 (1053 nm) as well as **1** and **N_2CH-R8** in DMF (1053 nm). The fluorescence efficiency of **N_2CH-R8** in H_2O appears similar or even better than **1** in CH_2Cl_2,[6] indicating that the aqueous media do not affect the fluorescence property of N_2CH macrocycle adversely.

With addition of an excess $Zn(OAc)_2$ to the aqueous solution of **N_2CH-R8**, the absorption λ_{max} of the Soret-like band shifted to 600 nm, which is identical to that of **1** with $Zn(OAc)_2$ in CH_2Cl_2. Relative fluorescence quantum yield increased 14-fold without shifting the emission maximum (λ_{em}). Therefore, both the changes in the absorption and fluorescence spectra indicate that the N_2CH macrocycle spontaneously captures Zn^{2+} ions in aqueous solution and its coordination mode is very similar to that of **1** in organic solvent (Fig. 2b, see also Supporting Information). The fluorescence spectra also indicate that **N_2CH-R8** is significantly responsive to Zn^{2+} in a pH region under neutral to weakly alkaline conditions (Fig. 2c). An excess amount of $Zn(OAc)_2$ (800 μM, 200 equiv) was insufficient for rapid completion of the spectral changes with **N_2CH-R8** (4.0 μM) (Fig. 2d), suggesting that the sensitivity of Zn^{2+} complexation of **N_2CH-R8** in H_2O is several fold lower than that of **1** in CH_2Cl_2.

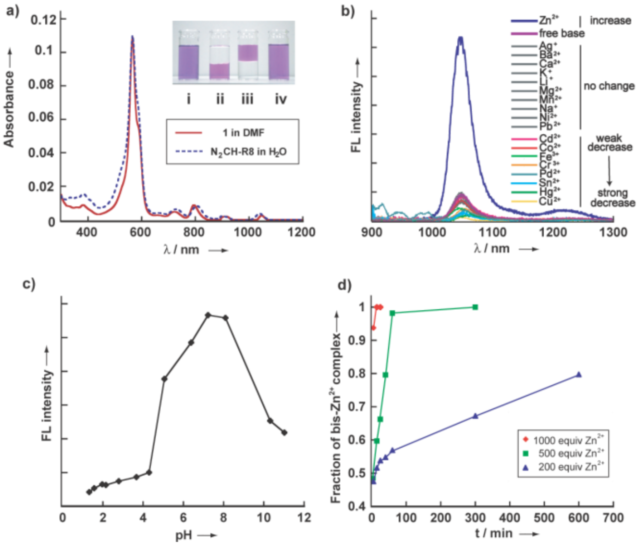

Fig. 2 (a) UV-vis-NIR spectra of N_2CH (**1**) in DMF and **N_2CH-R8** in H_2O. [**1**] = [**N_2CH-R8**] = 0.27 μM. The inset shows partition experiments of **1** and **N_2CH-R8** (6.5 μM). i: **1** in DMF, ii: **1** in H_2O-CH_2Cl_2, iii: **N_2CH-R8** in H_2O-CH_2Cl_2, iv: **N_2CH-R8** in DMF. (b) NIR fluorescence spectra of **N_2CH-R8** (0.53 μM, λ_{ex} = 588 nm) in H_2O in the absence or presence of 1000 equiv of metal ions. (c) pH dependent fluorescence intensity changes of **N_2CH-R8** (0.53 μM, λ_{ex} = 600 nm) with 1000 equiv Zn^{2+}. pH values were adjusted by aqueous HCl or NaOH. **N_2CH-R8** became insoluble when pH exceeded 11. (d) Time dependent Zn^{2+} coordination of **N_2CH-R8** (4.0 μM) in H_2O determined from absorption spectra.

Next, to gain insights into the complexation of other metals, absorption spectra of **N_2CH-R8** were investigated in aqueous solution upon addition of 19 metal ions. Besides Zn^{2+}, eight metal ions [Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, Pd^{2+}, Sn^{2+}] were coordinated by the macrocycle judging from the changes of the absorption spectra.[6] Among coordinated metals, only Zn^{2+} showed a marked enhancement in emission. On the other hand, the fluorescence spectra of **N_2CH-R8** in the presence of Cd^{2+}, Co^{2+}, and Fe^{3+} were similar to that of freebase. In contrast, remaining five metal ions, Cu^{2+}, Cr^{3+}, Hg^{2+}, Pd^{2+}, and Sn^{2+} quenched the emission (Figs. 2b and 3a).[6]

Then, the effect of metal ions on the fluorescence property of Zn^{2+} **N_2CH-R8** complex was examined. Metal ions (1000 equiv) that cause no effect on the absorption spectra of **N_2CH-R8** also did not show any change in the fluorescence spectra of **N_2CH-R8** with 1000 equiv Zn^{2+}.[6] The fluorescence was not affected by Cd^{2+} or Co^{2+}, whereas it was partially quenched by Cr^{3+}, Fe^{3+}, or Sn^{2+} and strongly quenched by 1000 equiv Cu^{2+}, Hg^{2+}, or Pd^{2+},[6] suggesting that the affinity of the last three metal ions to **N_2CH-R8** is comparable to or higher than that of Zn^{2+}. The strong inhibitory effect of Cu^{2+} ion is demonstrated by the experiment, in which 0.53 μM Cu^{2+} quenched the fluorescence of same molar concentration of **N_2CH-R8** complex nearly completely within 30 min (Fig. 3b, Fig. S13). This observation suggests that the Zn^{2+} complex of **N_2CH-R8** is also capable of serving as a "switch-off" fluorescent sensor to detect Cu^{2+} ions in aqueous environment.[9]

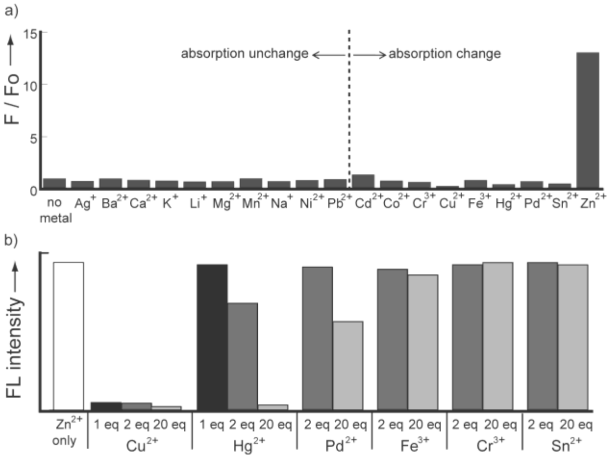

Fig. 3 (a) Relative fluorescence intensity of **N_2CH-R8** (0.53 μM, λ_{em} = 600 nm) in H_2O with 1000 equiv of metal ions of interest. (b) Fluorescence quenching of **N_2CH-R8** (0.53 μM, λ_{ex} = 600 nm) with 1000 equiv Zn^{2+} ions by 1.0, 2.0 or 20 equiv of metal ions.

In summary, we have synthesized a water-soluble derivative of doubly N-confused hexaphyrin (**N_2CH-R8**), which exhibits enhanced NIR fluorescence around 1050 nm only by Zn^{2+} ion coordination in aqueous solution. Thus, **N_2CH-R8** is a promising platform to develop a "switch-on" NIR fluorescent sensor for Zn^{2+} in aqueous solution albeit further improvement of coordination affinity and specificity, as well as emission efficiency would be necessary.[10] Furthermore, Zn^{2+} complex of **N_2CH-R8** can also serve as a promising platform for a "switch-off" NIR fluorescent sensor for Cu^{2+} ion, a similarly important metal ion involved in a number of biological processes in living cells.[11] As octa-arginine (R8) is known as a member of peptides showing a cell penetrating property that enables various molecules to be introduced into mammalian cells,[12] *in vivo* application of **N_2CH-R8** would be of interest. Because **N_2CH-R8** would be applicable directly to *in vivo* analysis, investigation of its photophysical and coordination properties in cultured cells is now underway.

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Notes and references

‡ Crystal data for **2**: $C_{66}H_{10}F_{30}N_6O_2$•6(C_4H_8O)•4(O), M_r = 2116.20, monoclinic, space group $P2_1/n$ (no. 14), a = 20.43(3), b = 8.939(11), c = 25.15(3) Å, β = 97.89(6)°, V = 4550(10) Å^3, Z = 2, ρ_{calcd} = 1.545 gcm^{-3}, $\mu(Mo_{K\alpha})$ = 0.650 cm^{-1}, T = 123.1 K; Rigaku RAXIS-RAPID; 55° > 2θ > 6°, 23026 measured reflections, 10446 unique reflections, 5121 with $I > 2\sigma(I)$ used in F^2 refinement, 632 parameters, R = 0.0666, wR = 0.1920 (all data), GOF = 0.955. CCDC 759874 contains the supplementary crystallographic data for **2**.

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