Porphyrinatoerbium–crown ether conjugate for synergistic binding and chirality sensing of zwitterionic amino acids

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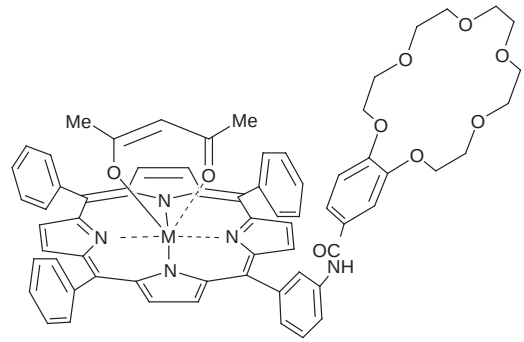
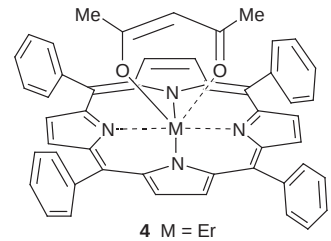
Received (in Cambridge, UK) 15th April 1999, Accepted 26th April 1999

A porphyrinatoerbium–benzo-18-crown-6 conjugate offers efficient extraction and chirality sensing of amino acids under neutral conditions *via* synergistic binding of zwitterions.

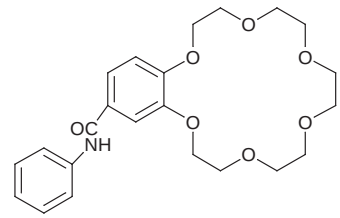
Lanthanide complexes are widely employed as shift reagents in NMR spectroscopy,[1] catalysts in organic synthesis and biotechnology,[2] and probes for fluorescence/MRI sensing.[3] We recently demonstrated that lanthanide tris(β-diketonate)s and tetraphenylporphyrin complexes offered efficient extraction of unprotected amino acids under neutral pH conditions and also acted as circular dichroism (CD) probes capable of chirality sensing of the bound guests.[4,5] In these systems, the central lanthanide(III) ions were electrically neutralized by coordination from the diketonate and/or porphyrinate anions, with the additional CO_2^- group of the guest amino acids forming highly coordinated complexes.[4] Since zwitterionic amino acids are hydrophilic and bifunctional guests, more effective receptors should have multiple binding sites highly complementary to the NH_3^+ and CO_2^- sites of the guest. Thus, we designed a novel type of conjugate receptor for synergistic binding of zwitterionic amino acids in which a benzo-18-crown-6 moiety is covalently connected to the lanthanide porphyrin complex **1**–**3**. Because benzo-18-crown-6 coordinates to the NH_3^+ moiety and since lanthanide porphyrinate binds the CO_2^- moiety, these conjugates with two different kinds of binding sites within a molecule are expected to offer multiple binding of zwitterionic, chiral amino acids and sensitive CD probing of their chirality.[6]



1 M = Er
2 M = Gd
3 M = Yb



4 M = Er
5 M = Gd
6 M = Yb



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Conjugates **1**–**3** were prepared from lanthanide tris(acetylacetonate)s and the corresponding crowned porphyrin ligand,[7] which was derived from amino-substituted tetraphenylporphyrin and 4-chlorocarbonylbenzo-18-crown-6. Their receptor–probe abilities were characterized by liquid–liquid extraction of amino acids and subsequent CD measurements. The concentrations of the amino acids in the aqueous phases were determined, based on UV spectroscopy and/or amino acid analysis (ninhydrin colorimetry). Mixtures of lanthanide porphyrin complexes and benzo-18-crown-6 derivatives,[7,8] *i.e.* **4** + **7**, **5** + **7** or **6** + **7**, were also examined to compare the 'intermolecular' cooperativity of two different binding sites with the 'intramolecular' one.

Porphyrinatoerbium–crown ether conjugate **1** offered efficient extraction of several amino acids from neutral aqueous solution into CH_2Cl_2 solution (Fig. 1). Its extraction ability of tryptophan (Trp) (40%) is twice as high as those of **4** alone (18%) and the mixture **4** + **7** (18%). Although **4** and **7** have binding sites for CO_2^- and NH_3^+ groups respectively, the covalent connection between the two binding sites increased the extraction ability. In other words, bifunctional amino acids are believed to be cooperatively bound to the erbium center and 18-crown-6 ring of conjugate **1**. The extracted amount of Trp by conjugate **1** strongly depended on the pH of the aqueous solution, suggesting that the guest Trp was extracted in zwitterionic form: 13% (pH = 3.5) < 40% (pH = 5.7) ≃ 38% (pH = 8.1) > 28% (pH = 9.4) > 8% (pH = 10.1). When the concentration of Trp in the aqueous phase increased, the mole ratio of the extracted Trp to conjugate **1** increased and reached 0.9, suggesting 1 : 1 complexation. The organic phase was characterized using NMR spectroscopy after extraction experiments. The signals for the aliphatic protons of the extracted Trp disappeared in the presence of conjugate **1**, while those for the aromatic protons broadened but still appeared around 5.5–6.0 ppm. These observations support the suggestion that the extracted Trp locates near the erbium center and also above the porphyrin plane of conjugate **1**. This conjugate extracted a dipeptide as well as other amino acids more effectively than the mixture **4** + **7**: [extraction percentage by **1**]/[extraction percentage by **4** + **7**] = 40%/20% for tryptophanyl-glycine (Trp-Gly);

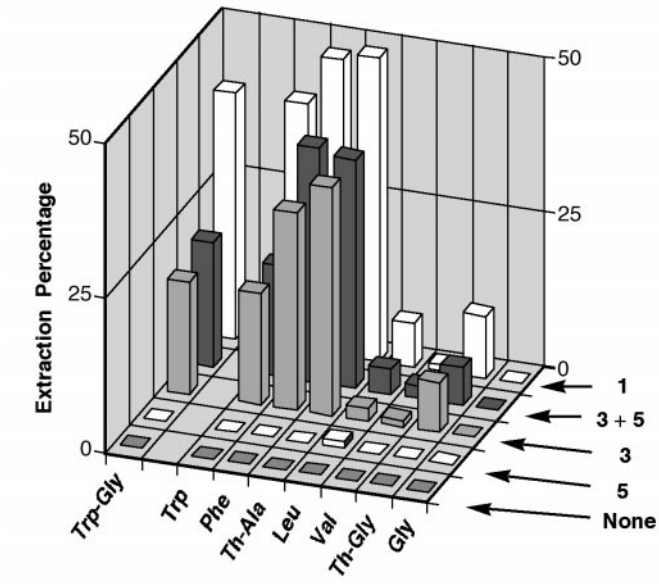

Fig. 1 Extraction of zwitterionic amino acids. Conditions: amino acid = 125 nmol, H_2O = 2.5 ml (pH = 5.9–6.2); receptor = 125 nmol, CH_2Cl_2 = 10 ml.

48%/38% for phenylalanine (Phe); 49%/37% for 3-(2-thienyl)-alanine (Th-Ala); 7%/4% for leucine (Leu); 10%/6% for (2-thienyl)glycine (Th-Gly).[9] In contrast, conjugates **2** and **3** containing gadolinium and ytterbium porphyrinates extracted Trp with comparable efficiencies to those with corresponding mixtures under the employed conditions: 39% for **2** and 34% for **5** + **7**; 14% for **3** and 10% for **6** + **7**. Thus, the nature of the lanthanide center is an important factor in designing effective conjugate receptors.

Conjugate **1** exhibited chirality-specific CD signals *via* complexation with chiral amino acids (Fig. 2). After extraction experiments with L-Trp, L-Phe, L-Th-Ala, L-Th-Gly and L-Leu guests, the resulting CH_2Cl_2 solutions gave the'reversed' S-shaped CD bands around the Soret band, while their D-isomers offered the S-shaped CD bands (Fig. 2). Their CD amplitudes ($[\theta$ at 1st $\lambda] - [\theta$ at 2nd $\lambda]/10^5$ deg cm^2 dmol^{-1}) were larger

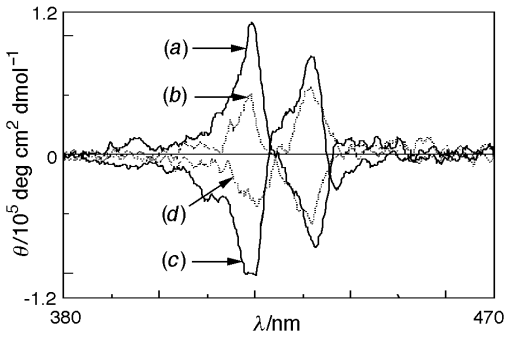

Fig. 2 CD spectra of conjugate **1** or mixture **4** + **7** in CH_2Cl_2 after shaking with an aqueous solution of L- or D-Trp. Conditions: see Fig. 1. (*a*) L-Trp·**1**, (*b*) L-Trp·**4** + **7**, (*c*) D-Trp·**1**, (*d*) D-Trp·**4** + **7**.

than those with **4** + **7**: **1** (1.9) > **4** + **7** (1.0) for L-Trp; **1** (1.8) > **4** + **7** (0.8) for L-Phe; **1** (2.3) > **4** + **7** (1.1) for L-Th-Ala; **1** (0.6) > **4** + **7** (0.5) for L-Leu; **1** (0.9) > **4** + **7** (0.4) for L-Th-Gly. Conjugate **1** was not only an effective extracting reagent of unprotected amino acids but also a sensitive CD probe for their chirality determination.

We have demonstrated that the effective cooperativity of porphyrinatoerbrium and 18-crown-6 ring promoted improved extraction of amino acids and sensitive CD probing of their chirality. A further hybridization of lanthanide complexes with receptor molecules provides promising possibilities in the development of effective sensing, transport and separation of multi-functional substrates of biological interest.

The authors are grateful to Ms M. Doe of the Analytical Center, Graduate School of Science, Osaka City University for determination of amino acid concentrations. This work was supported by a Grant-in-Aid for Scientific Research (No. 10440198) from the Ministry of Education, Science, Culture and Sports, Japan.

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7 *Selected data* for crowned porphyrin ligand: mp 176–180 °C; *m/z* [FABMS (*m*-nitrobenzyl alcohol)] 990 (M + Na^+) (Calc. for $C_{61}H_{53}N_5O_7{\cdot}0.5H_2O$; C, 74.98; H, 5.57; N, 7.17. Found: C, 74.97; H, 5.45; N, 7.15%). For **1**: mp 249 °C (decomp.); *m/z* [FABMS (*m*-nitrobenzyl alcohol)] 1230 (M^+) (Calc. for $C_{66}H_{58}N_5O_9Er{\cdot}H_2O$; C, 63.39; H, 4.84; N, 560. Found: C, 63.33; H, 4.69; N, 5.80%). For **4**: mp > 300 °C; *m/z* (EIMS) 877 (M^+) (Calc. for $C_{49}H_{35}N_4O_2Er{\cdot}H_2O$; C, 65.60; H, 4.16; N, 6.25. Found: C, 65.69; H, 4.06; N, 6.52%). For **7**: mp 139–141 °C (Calc. for $C_{23}H_{29}NO_7{\cdot}H_2O$; C, 61.46; H, 6.95; N, 3.12. Found: C, 61.21; H, 6.98; N, 3.13%).
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9 The observed extraction efficiency was mostly dependent on the hydrophobicity of the guest amino acid. Octan-1-ol–water distribution co-efficients (log *D*) were determined at pH 5: Trp, −1.15; Phe, −1.44; Th-Ala, −1.50; Leu, −1.72; Val, −2.29; Th-Gly, −2.64; Gly, −3.02. See N. E. Tayar, R.-S. Tsai, P.-A. Carrupt and B. Testa, *J. Chem. Soc., Perkin Trans. 2*, 1992, 79 and ref. 4.

Communication 9/03024G