Pergamon

Tetrahedron Letters 42 (2001) 7583–7585

TETRAHEDRON LETTERS

Synergistic binding and chirality sensing of unprotected amino acids with ferrocenecarboxylic acid–crown ether conjugate

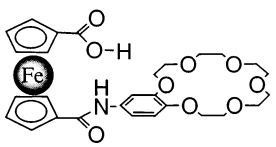
Hiroshi Tsukube,* Hiroshi Fukui and Satoshi Shinoda

Department of Chemistry, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan

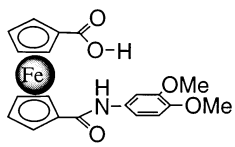
Received 22 June 2001; revised 15 August 2001; accepted 17 August 2001

Abstract—A novel conjugate receptor was designed to bind unprotected amino acids under acidic conditions and sense their chirality, in which 18-crown-6 ring was connected with carboxylic acid function via ferrocene spacer.

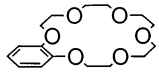
Amino acids are one of the most important substrates in both biological and artificial processes. Although various types of receptors have been reported to interact with ammonium or carboxylate moieties of the amino acid derivatives, the number of effective receptors for unprotected amino acids is still limited.[1] Since amino acids are hydrophilic and bifunctional species, effective receptors should have multiple binding sites complementary to $-NH_2$ (or $-NH_3^+$) and $-CO_2H$ (or $-CO_2^-$) sites of amino acids.[2] Here, we present a novel conjugate receptor **1** for synergistic binding of protonated amino acids, in which benzo-18-crown-6 ring is connected with carboxylic acid function through a ferrocene spacer. In this conjugate, the crown ether ring can bind $-NH_3^+$ cation of the guest,[3] while the carboxylic acid can form two-point hydrogen bondings with $-CO_2H$ moiety of the guest.[4] Since some ferrocene derivatives acted as molecular bearing and chromophore functions,[5] conjugate **1** is expected to offer effective extraction and chirality sensing of protonated amino acids, NH_3^+-CH(R)-CO_2H.



1



2



3

Conjugate **1** and reference **2** were prepared by the reaction of ferrocene dicarboxylic acid chloride with aminobenzo-18-crown-6 and dimethoxyaminobenzene, respectively.[6] Since ferrocenecarboxylic acid has a pK_a value of 4.2 in H_2O,[7] this conjugate can provide a highly polar microenvironment complementary to the protonated form of α-amino acid. The extraction experiments were carried out by adding a CH_2Cl_2 solution of conjugate **1** (0.0090 mmol, 1.8 mL) to a D_2O solution (1.8 mL, pD was adjusted with HCl) of amino acid (0.0090 mmol) and $LiClO_4$ (0.90 mmol). After the mixture had been stirred for 2 h, the D_2O phase was separated and the extracted amount of the amino acid was determined based on integration of ^1H NMR signals for the amino acid protons before and after extraction. We confirmed that the leakage of conjugate **1** into the D_2O phase was negligible (<3%). The pH-titration curves obtained for the employed amino acids in D_2O revealed that the protonated form of each substrate predominantly existed below pD = 1.0.

Ferrocenecarboxylic acid–crown ether conjugate **1** extracted several amino acids from the acidic solutions (pD = 0.4) and its extraction profiles were apparently determined by hydrophobicity of the guest amino acids (Table 1):[8] 55% for tryptophan (Trp); 55% for phenylalanine (Phe); 29% for *tert*-leucine (*t*-Leu); 25% for valine (Val); 7% for alanine (Ala). Since the mixed receptor **2**+**3** and benzo-18-crown-6 **3** exhibited lower extraction abilities for all the examined amino acids, the

Keywords: crown ethers; ferrocenes; amino acids; extraction; chirality sensing.

* Corresponding author. Tel./fax: +81-6-6605-2560; e-mail: tsukube@sci.osaka-cu.ac.jp

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01549-0

Table 1. Extraction of unprotected amino acids by conjugate **1** and related systems[a]

Amino acid	Extraction percentage (%)[b]		
	Receptor: **1**	**2**+**3**	**3**
L-Trp	55	23	14
L-Phe	55	22	20
L-*t*-Leu	29	8	6
L-Val	25	7	4
L-Ala	7	2	0

[a] pD values slightly changed after extraction.
[b] The averaged values of two or three independent experiments were shown. Reproducibility <±5%.

conjugation of 18-crown-6 ring and carboxylic acid via ferrocene spacer operated in the extraction of the unprotected amino acids. The extracted amount of Trp also depended on pD value of the aqueous solution: 55% (pD = 0.4); 49% (pD = 1.6); 36% (pD = 2.8); and 11% (pD = 5.5). Since the pK_a value of Trp was estimated to be 2.0 in D_2O, the percentage of the protonated form was estimated as 97% (pD = 0.4), 71% (pD = 1.6), 13% (pD = 2.8) and 0% (pD = 5.5). Therefore, the guest Trp was thought to be extracted as the protonated form, NH_3^+-CH(R)-CO_2H. When the concentration of conjugate **1** in the CH_2Cl_2 phase was fixed at 0.005 mol/L, the extracted amount of L-Trp increased with its concentration in the aqueous phase (0–0.025 mol/L), and the saturated amount of the extracted L-Trp indicated 1:1 stoichiometry (conjugate **1**: protonated amino acid). Similar 1:1 complexes were also detected by FAB-MS analysis, while very weak signals derived from 2:2 complex were observed.

Conjugate **1** gave induced CD signals via complexation with chiral amino acids, the signs of which were significantly specific to the chirality of the bound amino acids. After the extraction experiment with protonated L-*t*-Leu, the CH_2Cl_2 solution gave W-shaped CD bands around the absorption of the ferrocene function (250–350 nm), while D-*t*-Leu offered the symmetrical M-shaped CD bands (Fig. 1). L-Trp, L-Phe and L-Val exhibited similar W-shaped CD bands, indicating that the stereochemistry of the bound amino acids could be sensed using this CD method.[9] Although the CD spectra of chiral ferrocene derivatives have not been studied in detail,[10] the CD spectra observed here probably suggest that the cooperative binding of the protonated amino acids with 18-crown-6 ring and carboxylic acid function fixes the orientation of two cyclopentadienyl rings in an asymmetrical fashion. Since the mixed receptor **2**+**3** modestly extracted amino acids and gave no detectable CD signal, conjugate **1** was confirmed to act as not only an effective extracting reagent of unprotected amino acids but also a sensitive CD probe for their chirality determination.

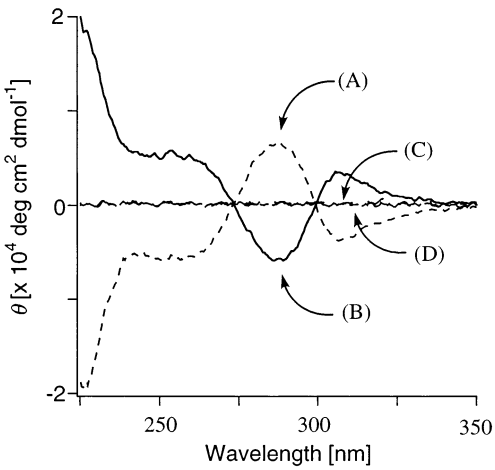


Figure 1. CD spectra of conjugate **1** and mixture **2**+**3** in CH_2Cl_2 after extraction of L- or D-*t*-Leu. (A) L-*t*-Leu **1**, (B) D-*t*-Leu **1**, (C) L-*t*-Leu **2**+**3**, (D) D-*t*-Leu **2**+**3**.

We have demonstrated that a new type of conjugate **1** offered efficient extraction of protonated amino acids and effective CD sensing of their chirality. Further conjugations of characteristic binding units with ferrocene spacers can provide promising possibilities in the development of effective sensing, transport and separation systems for multi-functional substrates of biological interest. The authors are grateful to Professors Kiyoshi Isobe and Isamu Kinoshita of Osaka City University for valuable comments on CD measurements.

References

1. Reetz, M. T. In *Comprehensive Supramolecular Chemistry*; Voegtle, F., Ed.; Oxford, 1996; Vol. 2, p. 553.
2. Ditopic receptors for unprotected amino acids: (a) Sessler, J. L.; Andrieusky, A. *Chem. Eur. J.* **1998**, *4*, 159; (b) Rosa, D. T.; Coucouvanis, D. *Inorg. Chem.* **1998**, *37*, 2328; (c) Mizutani, T.; Wada, K.; Kitagawa, S. *J. Am. Chem. Soc.* **1999**, *121*, 11425; (d) Sansone, F.; Barboso, S.; Casnati, A.; Sciotto, D.; Ungaro, R. *Tetrahedron Lett.* **1999**, *40*, 4741; (e) Suzuki, I.; Obata, K.; Anzai, J.; Ikeda, H.; Ueno, A. *J. Chem. Soc., Perkin Trans. 2* **2000**, 1705.
3. Crown ether based conjugate receptors: (a) Santos, O.; Lajmi, A. R.; Canary, J. W. *Tetrahedron Lett.* **1997**, *38*, 4383; (b) Antipin, I. S.; Stoikov, I. I.; Pinkhassik, E. M.; Fitseva, N. A.; Stibor, I.; Konovalov, A. I. *Tetrahedron Lett.* **1998**, *38*, 5865; (c) Tsukube, H.; Wada, M.; Shinoda, S.; Tamiaki, H. *Chem. Commun.* **1999**, 1007; (d) Chrisstoffels, L. A. J.; de Jong, F.; Reinhoudt, D. N.; Sivelli, S.; Gazzola, L.; Casnati, A.; Ungaro, R. *J. Am. Chem. Soc.* **1999**, *121*, 10142; (e) Deetz, M. J.; Shang, M.; Smith, B. D. *J. Am. Chem. Soc.* **2000**, *122*, 6201.
4. Carboxylic acid-based conjugate receptors: (a) Andreu, C.; Galan, A.; Kobiro, K.; Mendoza, J.; Park, T. K.; Rebek, J. *J. Am. Chem. Soc.* **1994**, *116*, 5501; (b) Wang, Z.-H.; Hirose, T.; Baldwin, B. W.; Yang, Y. *Chem. Commun.* **1997**, 297; (c) Zhang, X.-X.; Lippard, S. J. *J. Org. Chem.* **2000**, *65*, 5298.
5. Ferrocene-based conjugate receptors: (a) Li, C.; Medina, J. G. C.; Maguire, G. E. M.; Abel, E.; Atwood, J. L.; Gokel, G. W. *J. Am. Chem. Soc.* **1997**, *119*, 1609; (b) Beer, P. D.; Gale, P. A.; Chen, G. Z. *J. Chem. Soc., Dalton* **1999**, 1897; (c) Inouye, M.; Takase, M. *Angew. Chem., Int. Ed.* **2001**, *113*, 1796.

6. Selected data for conjugate **1**: mp 63–64°C; Anal. calcd for $C_{28}H_{33}FeNO_9$ $0.3H_2O$: C, 57.11; H, 5.78; N, 2.45. Found: C, 57.12; H, 5.75; N, 2.38. Reference **2**: mp 178–179°C; Anal. calcd for $C_{20}H_{19}FeNO_5$: C, 58.70; H, 4.68; N, 3.42. Found: C, 58.9; H, 4.64; N, 3.57.
7. Matsue, T.; Evans, D. H.; Osa, T.; Kobayashi, N. *J. Am. Chem. Soc*. **1985**, *107*, 3411.
8. Octan-1-ol/water distribution coefficients were determined at pH 5: Trp, −1.61; Phe, −1.75; *t*-Leu, −1.97; Val, −2.18; Ala, −2.71. See: Tayar, N. E.; Tsai, R.-S.; Carrupt, P.-A.; Testa, B. *J. Chem. Soc., Perkin Trans. 2* **1992**, 79.
9. CD sensing with ferrocene chromophores: (a) Ueno, A.; Chen, Q.; Suzuki. I.; Osa, T. *Anal. Chem*. **1992**, *64*, 1650; (b) Takeuchi, M.; Mizuno, T.; Shinkai, S.; Shirakami, S.; Itoh, T. *Tetrahedron*: *Asymmetry* **2000**, *11*, 3311.
10. Recent examples of chiral ferrocenes: (a) Cui, X. L.; Wa, Y. J.; Du, C. X.; Yang, L. R.; Zhu, Y. *Tetrahedron*: *Asymmetry* **1999**, *10*, 1255; (b) Shirakami, S.; Itoh, T. *Tetrahedron*: *Asymmetry* **2000**, *11*, 2823; (c) Moriuchi, T.; Nomoto, A.; Yoshida, K.; Ogawa, A.; Hirao, T. *J. Am. Chem. Soc*. **2001**, *123*, 68.