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A cystine-bearing pseudo-cyclopeptide as a new amphi-receptor

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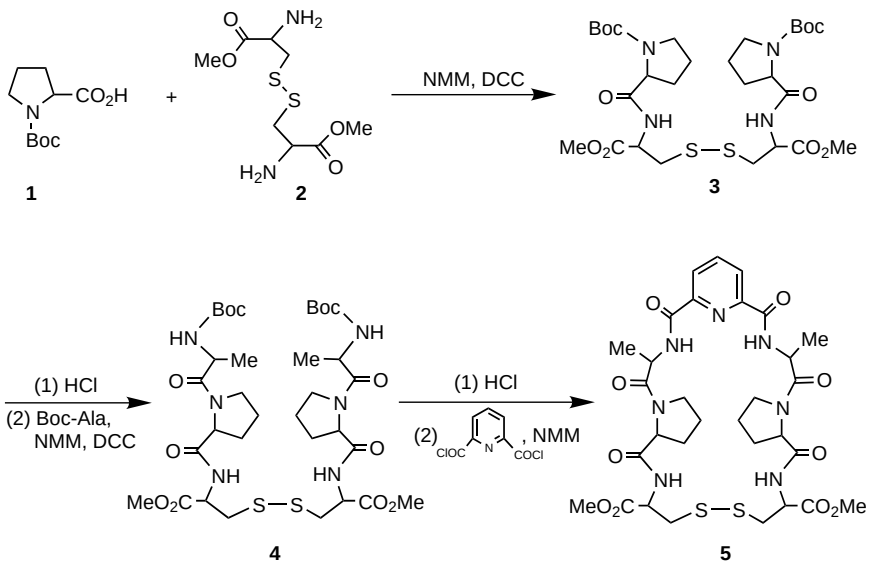
Abstract—A conformationally constrained cyclic peptide was designed and synthesized as a novel amphi-receptor. It binds to cations or anions through the carbonyl or amino groups, respectively. The stability of the cation complexes relies largely on the polarizability of the cation, whereas the stability of the anion complexes relies on the strength of the H-bond as well as the polarizability of the anion.

Host–guest complexation is widely observed in many biological processes such as ion transfer, enzyme catalysis, and enzyme inhibition.[1] Despite the remarkable progress achieved in finding host molecules which respectively serve as receptors for cations, anions, or small molecules,[2,3] only in very few cases have the so-called amphi-receptors been reported which show binding properties to both cations and anions.[4–9] Generally, amphi-ionophores are found to associate with cations or anions through their separate binding sites (e.g. amide group for anions, crown ether unit for cations[8]). Based on ab initio calculations, Kim and co-workers proposed that cyclic peptides may be able to serve as good amphi-ionophores because of the availability of both amide and crown-like structures within the molecule.[10] In fact, Pons and co-workers by chance observed amphi-recognition for a disulfide-bonded cyclic peptide, though no special attention has been paid to this interesting phenomenon.[11] However, together with the recent findings on the binding properties of conformationally constrained cyclopeptides,[12–14] we were stimulated to design and synthesize a cystine-containing cyclopseudopeptide **5** in order to define a new range of amphi-ionophores. Preliminary NMR, IR



Scheme 1. Synthesis of disulfide-bonded and pyridine-containing cyclopseudopeptide **5** (NMM = *N*-methylmorphine).

Keywords: pseudo-cyclopeptide; amphi-receptor; cation; anion.

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and circular dichroism (CD) studies showed that it does indeed possess the predicted amphi-binding property.

The synthesis of cyclopseudopeptide **5** was carried out by conventional solution methods with a Boc (*tert*-butyloxycarbonyl) group to protect the amino group, a methyl ester to protect the carboxyl group, and DCC as coupling reagent. Reaction of *C*-protected cystine **2** with Boc-proline **1** provided bis-proline-cystine peptide **3**. After removal of the Boc group with HCl/AcOEt, the diamine was reacted with Boc-alanine to yield bis(alanineproline)-cystine peptide **4**. Finally, deprotection of **4**, then condensation with pyridine-2,6-dicarbonyl dichloride in very dilute CH_2Cl_2 solution afforded cyclopseudopeptide **5** in a good yield through [1+1] cyclization (Scheme 1).[15] Elemental and ^1H NMR analyses showed that there are four H_2O molecules associated with the peptide product.

^1H NMR, FT-IR, and 2D NMR (NOESY, Fig. 1, NOE interactions are shown as crosslinks) spectra were used to analyze the hydrogen-bonding in the molecule. The ^1H NMR spectrum (CD_3CN) indicated that compound **5** adopts a stable C_2-symmetry conformation. The high chemical shift of the Ala amide protons (8.87 ppm in CD_3CN) suggests that these hydrogen atoms may be involved in intramolecular hydrogen bonding. This is supported by the FT-IR spectrum in acetonitrile, which shows that the N–H stretches of the alanine and cystine units are located at 3368 cm^{-1}, which is lower than the wavenumbers commonly observed in non-H-bonded situations. The NOESY spectrum in Fig. 1 shows that the Ala amide protons have no NOE interaction with the pyridine β and γ protons, suggesting that the two amide protons orient towards the center of the macrocycle, and are located adjacent to the pyridine nitrogen as shown in **5**. As reported by Ranganathan et al. for a similar situation,[12] the intramolecular hydrogen bond should form a three-H···N···H center. The proton shift of 7.41 ppm and N–H stretching of 3368 cm^{-1} further suggest that the Cys amide protons may be involved in relatively weak intermolecular hydrogen bonds. The NOE interaction between the Ala α protons and the Cys-OCH_3 indicates that an intermolecular hydrogen bond must exist between the Cys amide protons and the Ala carbonyl groups. The observation of both the intramolecular and intermolecular H-bonds suggest that introduction of a semi-rigid pyridine group to the backbone indeed decreases the rotational freedom of the peptide skeleton, and therefore may play a role in making the cyclic peptide into a better ionophore.

Circular dichroism (CD) is known to be very useful in conformational analysis and binding constant determinations for cation-cyclopeptide complexes,[11] and was applied in the present work. Addition of a cation (ClO_4^- as counter-ion) to the solution of **5** in acetonitrile (1×10^{-4} mol L^{-1}, 25°C) resulted in a decrease in the peaks at 275 and 215 nm, and the trough at 235 nm, for most cations tested. This suggests that both the carbonyl groups and the pyridine are involved in cation complexation. A similar spectroscopic change was also observed upon addition of anions. The spectroscopic

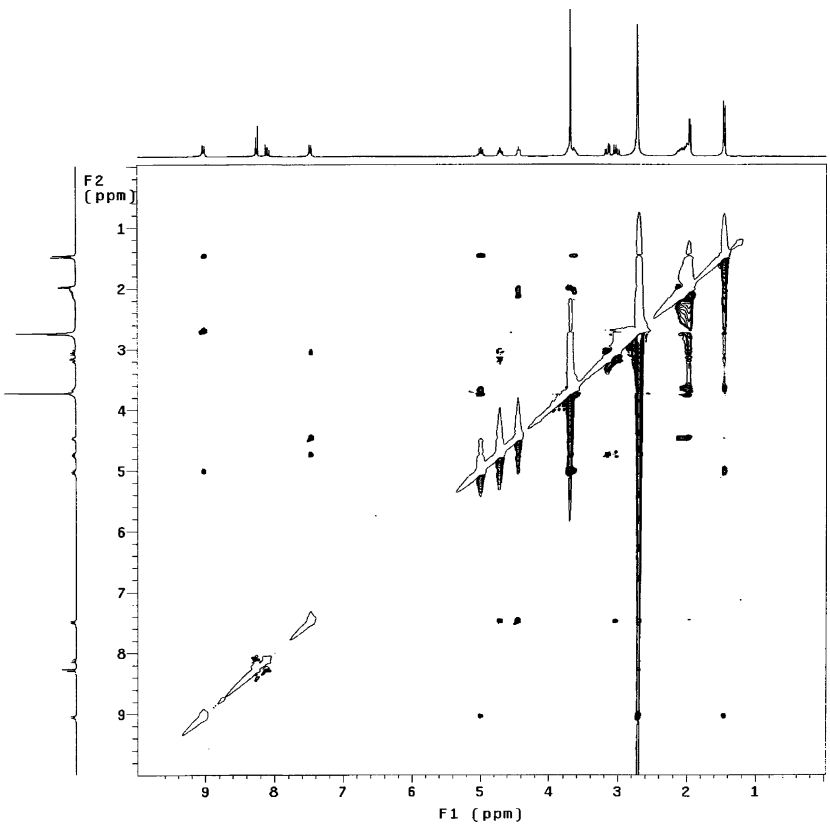

Figure 1. NOESY spectrum of cyclopseudopeptide **5** in CD_3CN.

changes of the Cotton effects at 275 and 235 nm allowed the equilibrium constants of the 1:1 complexes (Table 1) to be evaluated by linear or non-linear recursive methods according to standard procedures.[16]

Inspection of the binding constants (K) in Table 1 reveals that cyclopseudopeptide **5** can associate well with cations in CH_3CN with equilibrium constants ranging from about 100 to 45,000 with an interesting selectivity for Ca^{2+}. Previous work in the literature pointed out that the strength of interaction of an artificial cyclic peptide with cations should depend not only on the radius but also on the charge of the cation. It was reported that artificial cyclic peptides often showed high binding affinity for cations of high charge.[17,18] In the present work, it seems that the high Ca^{2+} selectivity can be rationalized in terms of the normalized polarizability (i.e. the radius/charge ratio). This can be seen clearly by the close match of the binding constants of Li^+ and Ba^{2+}, which share a similar radius/charge ratio [$r/(+)$ for Li^+ and Ba^{2+} are 0.68 and 0.675, respectively]. The relationship between $\log K$ and $r/(+)$ is shown in Fig. 2.

Table 1. Binding constants of cyclopeptide **5** with cations and anions[a]

Cation[b]	K[d] (mol^{-1} L)	Anion[c]	K[f] (mol^{-1} L)
Li^+	1.65×10^3	F^-	4.18×10^2
Na^+	1.55×10^2	Cl^-	30
K^+[c]	1.11×10^2	Br^-	1.44×10^2
Mg^{2+}	4.62×10^2	$H_2PO_4^-$	1.55×10^2
Ca^{2+}	4.43×10^4	NO_3^-	46
Ba^{2+}	1.87×10^3	OAc^-	1.12×10^2

[a] Determined in CH_3CN at 25°C.[19]
[b] ClO_4^- as counter-ion unless otherwise noted.
[c] PF_6^- as counter-ion due to the poor solubility of $KClO_4$ in CH_3CN.
[d] Derived from the Cotton effect at 275 nm.
[e] Bu_4N^+ as counter-ion.
[f] Derived from the Cotton effect at 235 nm.

One may note from Table 1 that the bindings of compound **5** to anions are only moderate in scale. The best binding was found with F^- ($K=4.18\times10^2$), indicating that H-binding may also play a central role in anion association. However, as seen from the data of other anions, it is obvious that H-bonding is definitely not the only factor to influence the binding. As the polarizability grows larger along the series of Cl^-, NO_3^-, OAc^-, Br^-, and $H_2PO_4^-$, a similar trend in the binding constant change was observed. Beer et al.[19] noted that the binding strength for anions depends largely on the polarity of the solvent, so it is expected that if CH_3CN was replaced by a high-polarity solvent such as DMSO, the binding affinity of the same peptide **5** to anions would be improved. However, an attempt to test this failed to yield reliable data, due to conformational complications of **5** in DMSO.

Investigation of binding sites was also attempted by means of ^1H, ^{13}C NMR and FT-IR studies. Addition of one equivalent of $^nBu_4N^+H_2PO_4^-$ to a CD_3CN solution of **5** resulted in a significant downfield shift of the Cys amide protons from 7.41 to 7.75 ppm. This implies that the Cys protons must have been involved in anion complexation. The small upfield shift of the Ala amide protons (from 8.87 to 8.78 ppm) can be understood by considering a possible replacement of the previously mentioned three-centered intramolecular H-bond between the Ala amide protons and the pyridine nitrogen by the newly formed H-bond between the Ala amide protons and $H_2PO_4^-$. The latter is probably not as strong as the former. Similarly, addition of one equivalent of $Ca(ClO_4)_2$ to a CD_3CN solution of **5** resulted in significant shifts in the ^{13}C NMR spectrum for the amide carbons from 172.8, 172.7, and 163.5 ppm to 174.9, 174.5, and 166.0 ppm, respectively. On the other hand, no shift was observed for the ester carbonyl groups (at 172.2 ppm). This suggests that the amide carbonyl groups rather than the ester C=O groups are involved in the cation binding. This was further supported by the FT-IR spectra in CH_3CN

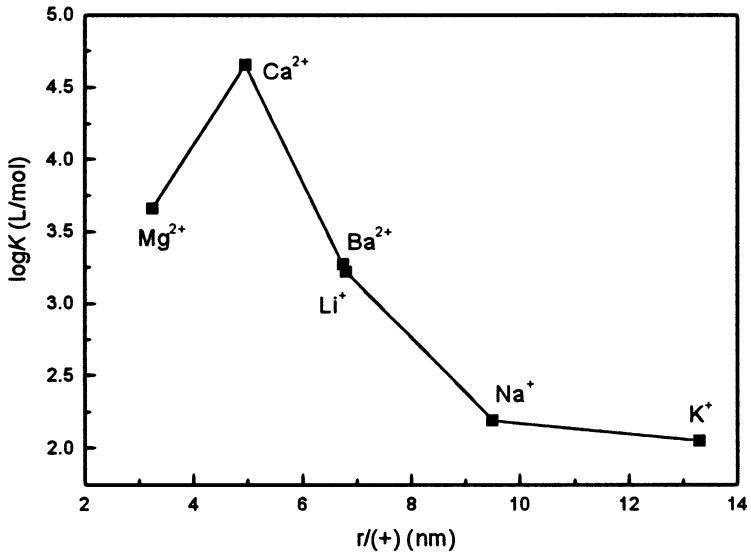

Figure 2. Relationship between the logarithm of the binding constants ($\log K$) and the radius/charge ratio [$r/(+)$].

which showed that the stretch of the amide carbonyl groups moved from 1682 to 1670 cm^{-1}, while the C=O stretch of the ester group at 1748 cm^{-1} remained unchanged upon addition of Ca^{2+}. From the above discussion, one may conclude that the C=O groups of the cyclic peptide should orientate towards the center when it complexes with cations, whereas the N–H groups should instead orientate towards the center when it complexes with anions.

In summary, we have designed and synthesized a novel cyclopseudopeptide and found that it is an efficient amphi-receptor. The cation binding was observed to rely largely on the polarizability of the cation, whereas the anion binding depended on the strength of the H-bond as well as the polarizability of the anion.

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References

1. Dugas, H. *Bioorganic Chemistry*, 3rd ed.; New York: Springer-Verlag, 1996.
2. Vögtle, F. *Supramolecular Chemistry*; John Wiley & Sons: New York, 1993.
3. Izatt, R. M.; Pawlak, K.; Bradshaw, J. S. *Chem. Rev.* **1995**, *95*, 2529–2586.
4. Arafa, E. A.; Kinnear, K. I.; Lockhart, J. C. *J. Chem. Soc., Chem. Commun.* **1992**, 61–64.
5. Flack, S. S.; Chaumette, J.-L.; Kilburn, J. D.; Langley, G. J.; Webster, M. *J. Chem. Soc., Chem. Commun.* **1993**, 399–401.
6. Savage, P. B.; Holmgren, S. K.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 4069–4070.
7. Rudkevich, D. M.; Brzozka, Z.; Palys, M.; Visser, H. C.; Verboom, W.; Reinhoudt, D. N. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 467–468.
8. Beer, P. D.; Chen, Z.; Ogden, M. I. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 295–302.
9. Kubik, S.; Goddard, R. *J. Org. Chem.* **1999**, *64*, 9476–9486.
10. Kim, K. S.; Cui, C.; Cho, S. J. *J. Phys. Chem. B* **1998**, *102*, 461–463.
11. García-Echeverría, C.; Albericio, F.; Giralt, E.; Pons, M. *J. Am. Chem. Soc.* **1993**, *115*, 11663–11670.
12. Ranganathan, D.; Haridas, V.; Karle, I. L. *J. Am. Chem. Soc.* **1998**, *120*, 2695–2702.
13. Mu, L.; Cheng, J.-P.; Huang, H.; Lu, J.; Hu, X. *Synth. Commun.* **1998**, *28*, 3091–3096.
14. Hu, X.; Chan, A. S. C.; Han, X.; He, J.; Cheng, J.-P. *Tetrahedron Lett.* **1999**, *40*, 7115–7118.
15. Yield: 38%. Mp 137–138°C. ^{1}H NMR, δ (200 MHz, $CDCl_3$), 1.52 (6H, d, $J=7.05$ Hz, Ala H^{β}), 1.87–2.04 (4H, m, Pro H^{γ}), 2.13 (8H, br, $4H_2O$), 2.32 (4H, m, Pro H^{β}), 3.10–3.23 (4H, m, Cys H^{β}), 3.62 (4H, m, Pro H^{δ}), 3.75 (6H, s, Cys OCH_3), 4.60 (2H, m, Pro H^{α}), 4.76 (2H, m, Cys H^{α}), 5.10 (2H, m, Ala H^{α}), 7.75 (2H, d, $J=7.31$ Hz, Cys NH), 8.00 (1H, t, Pyr H^{γ}), 8.28 (2H, d, $J=7.54$ Hz, Pyr H^{β}), 9.37 (2H, d, $J=8.84$ Hz, Ala NH). FT-IR (KBr, cm^{-1}): 3475, 3390, 3305, 1748, 1673, 1632, 1528, 1461, 1438, 1210; elemental analysis ($C_{31}H_{41}N_7O_{10}S_2+4H_2O$), calcd C, 46.09; H, 6.11; N, 12.14; found: C, 46.00; H, 6.32; N, 12.25. FAB-MS (m/z): 735[$M^{+\bullet}$].
16. For a 1:1 complexation, equilibrium constant $K=[HG]_e/([H]_0-[HG]_e)([G]_0-[HG]_e)$, where $[H]_0$ and $[G]_0$ represent the total concentrations of host and guest, respectively. If both free and complexed species have single conformations in solution, a CD change for a certain wavelength upon addition of guest can be demonstrated as $\Delta\Delta\varepsilon=\Delta\varepsilon_f-\Delta\varepsilon_e$, where $\Delta\varepsilon_e=[H]_e\Delta\varepsilon_f/[H]_0+[HG]_e\Delta\varepsilon_c/[H]_0$ ($\Delta\varepsilon_f$ and $\Delta\varepsilon_c$ represent the CD values for the free and complexed host, respectively). If $\alpha=\Delta\varepsilon_f-\Delta\varepsilon_c$, then $\Delta\Delta\varepsilon^2-\alpha([H]_0+[G]_0+1/K)\Delta\Delta\varepsilon/[G]_0+\alpha^2[H]_0/[G]_0=0$ (Eq. (1)). If $[G]_0 \gg [H]_0$, Eq. (1) can be simplified as $1/\Delta\Delta\varepsilon=1/\alpha K[G]_0+1/\alpha$ (Eq. (2)). In this work, cyclic peptide **5** was found to have a stable conformation in CH_3CN, and Ks are calculated according to Eq. (1) ($K>10^3$ mol^{-1} L) and Eq. (2) ($K<10^3$ mol^{-1} L). The errors are less than 20%.
17. Degelaen, J. P.; Pham, P.; Blout, E. R. *J. Am. Chem. Soc.* **1984**, *106*, 4882–4890.
18. Baron, D.; Pease, L. G.; Blout, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 8299–8306.
19. Beer, P. D.; Shade, M. *Chem. Commun.* **1997**, 2377–2378.