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Ferrocenyl-Bearing Cyclopseudopeptides as Redox-Switchable Cation Receptors

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A family of ferrocenyl-bearing cyclopseudopeptides (**1**−**10**) designed for redox-switchable receptors of cations was synthesized. Circular dichroism (CD) and cyclic valtammetry (CV) studies of cation binding properties in both the reduced (K_1) and oxidized (K_2) forms revealed that the binding preference is mainly governed by the charges and radius of the guest cation as well as by the suitability of the host to accommodate the guest. Particularly worth mentioning is the fact that some synthesized cyclopseudopeptides showed high binding affinity and selectivity toward alkaline-earth ions. For example, the K_1 of compound **2** binding with Ca^{2+} is 4.37×10^6 mol^{-1}·L and its Ca^{2+}/K^+ selectivity is 3.1×10^5:1, both values are much greater than those of an excellent natural ionophore, valinomycin (1×10^5 mol^{-1}·L and 0.33:1, respectively). The linear relationship between the shifts of half-wave potentials ($\Delta E_{1/2}$) and the radius/charge [$r/(+)$] ratios suggests that the sensitivity of electrochemical responses to cation complexation be dominated by repulsion factors between the redox center and the incoming cation guest.

Introduction

Redox behavior as influenced by molecular recognition in host−guest systems has received considerable attention in the past two decades with a view toward advancement of chemical sensor technology.[1] Since binding usually affects electrochemical response, introduction of a redox-active moiety to the vicinity of a host binding site has been widely practiced in the literature as a prototype in the search of electrochemical sensors for particular target guest species. In this regard, many recently accomplished works demonstrate the possibility of such designed macrocycles to electrochemically recognize cationic or anionic species in polar solvents and in some cases water.[2−4] However, one important host system, the cyclic peptides, or the pseudopeptide-type metallocenycryptands/metallocenycavitands, has not yet drawn much attention in the related works.

Cyclopeptides are natural macrocycles well-known for their biological activities as antibiotics and ion-transport regulators in membranes.[5] These properties are attributed to their capability to capture and release metal ions.[6] However, it is recognized that to achieve good artificial cyclopeptide ionophores, one should first depress their conformational freedom by introducing some constraints to the cyclic skeleton in order to reduce the structural complication.[7−10] Herein, we report a design of cyclopeptide series **1**−**10** where both ferrocenyl and

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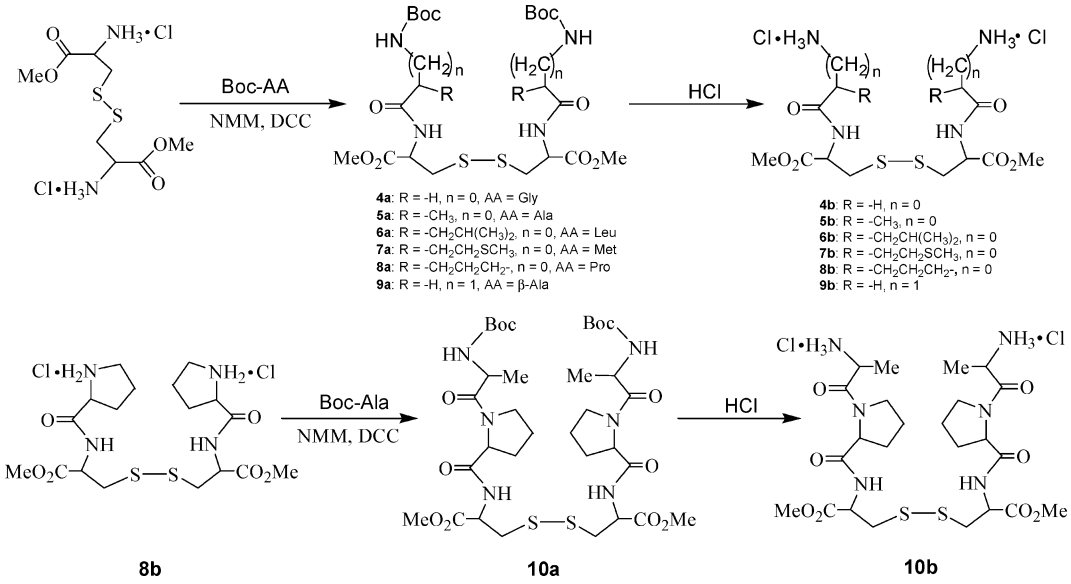
SCHEME 1. Synthesis of Linear Peptides 4a−10a and Their Deprotection



disulfide linkage are used as conformation constraints in improving their ion-binding property. Since the ferrocenyl unit is an electroactive group of reversible response, the potential of such designed cyclopeptides for cation sensors has also been examined. Ion binding thermodynamics of both the parent cyclopeptides and their oxidized forms, derived respectively from CD spectra and reversible electrochemical responses, are presented and discussed to reveal their cation binding preference.

Results and Discussion

Syntheses and Conformation Aspects. Condensation of 1,1′-bis(chlorocarbonyl)ferrocene with L-cystine dimethyl ester in highly diluted CH_2Cl_2 solution in the presence of *N*-methylmorphine (NMM), after column separation, produced the desired cyclopseudopeptides **1**−**3** through 1+1, 2+2, and 3+3 cyclization, respectively (Scheme 2).

The synthesis of compounds **4**−**10** was accomplished by cyclization of the corresponding linear peptides. The *N*-protected bis-peptides **4a**−**10a** were prepared via the conventional dicyclohexylcarbodiimide (DCC) method (Scheme 1). Deprotection of the *tert*-butoxycarbonyl (Boc) group followed by reactions of the linear peptides with 1,1′-bis(chlorocarbonyl)ferrocene through 1+1 cyclization then provided the corresponding compounds **4**−**10** (Scheme 2), which were isolated by column chromatography. All the synthetic cyclopseudopeptides **1**−**10** were identified by spectral analyses (see Supporting Information).

It is worth noting that in addition to the formation of the 1+1 macrocycle (**1**) as the major product, some 2+2 and 3+3 products (**2**−**3**) were also isolated. On the other hand, larger macrocyles **4**−**10** were observed to be the only products in the cyclizations of the corresponding linear bis-peptides **4b**−**10b**. It is thus believed that the complication of product formation in the reaction of 1,1′-bis(chlorocarbonyl)ferrocene with cystine dimethyl ester was probably caused by the internal constrain of the macrocycle.

Inspection of the ^{1}H NMR spectra (see Supporting Information) of peptides **1**−**10** (except **8**) shows that there is only one set of proton resonances for each type of proton. This suggests that these compounds may be either quite symmetrical or in a rapid exchange among configurations in solution. It is noted, however, that compound **8** has two double-peaks at 9.03 and 7.28 ppm (corresponding to amide protons in cystine) and two single-peaks at 3.86 and 3.76 ppm (corresponding to $-OCH_3$), implying that it may adopt two stable conformations. A similar phenomenon was also observed in structurally analogous systems in our previous study[11] and in precursor peptide **8a** (see Supporting Information). The cis−trans isomerism at a single peptide bond between the proline unit and the preceding residue might be the origin of the conformation multiplicity.[12]

The solubility difference observed within subgroup **1**−**3** is also interesting. While peptides **1** and **3** dissolve very well in low-polarized solvents ($CHCl_3$, CH_2Cl_2, etc.), **2** is highly soluble only in high-polarized solvents such as

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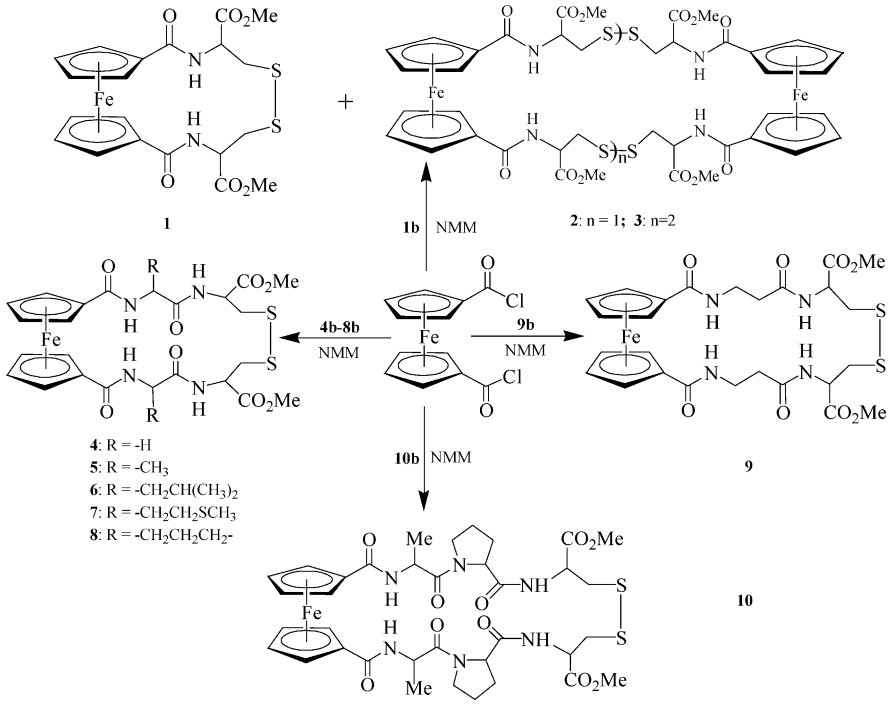
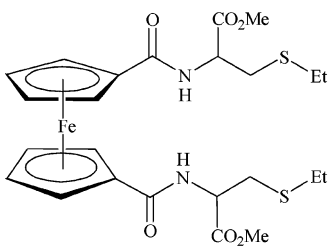
SCHEME 2. Synthesis of Cyclopseudopeptides 1−10

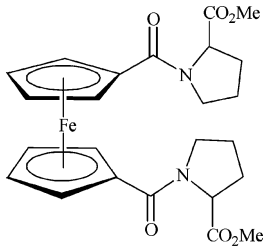


DMSO and MeOH. Since this may well be related to the difference in their configuration in solution, conformational analysis of **1** and **2** was conducted. The 2D-NMR spectra (NOESY, see Supporting Information) demonstrate that there are NOE cross-links between the amide protons and the α-protons of the cystine units or the protons of the ferrocene units. This is actually what has been expected since the amide protons are adjacently placed to these two types of protons in space. Besides, an NOE interaction between the amide protons and cystine *β*-protons was also observed but only for peptide **2**. This again suggests that these two sets of protons have been organized adjacently in this compound. The NOEs of **2** immediately imply that the hydrophobic protons are oriented mostly toward the "inside", leaving the hydrophilic carbonyl oxygens exposed to the "outside" of the macrocycle, thus allowing them to associate with polar solvent molecules. Obviously, the absence of such internal binding with cystine *β*-protons makes peptide **1** more hydrophobic compared to **2**, therefore it should be better solvated by nonpolar solvent molecules.

Binding Constants. Binding properties of peptides **1**−**10** and two open-chain analogues (**11** and **12**[13]) with alkali and alkaline-earth metal ions were examined with use of circular dichroism (CD) titration.[14] Substantial CD spectrum changes were recorded (Figure 1, taking the interaction of **1** with Ca^{2+} as an example) and used to evaluate the equilibrium constants on the basis of the changes of Cotton effects.[15] The derived binding constants are listed in Table 1.



11



12

Table 1 shows that the binding constants of peptides **1**−**12** with alkaline-earth ions (generally $>10^3$ mol^{-1}·L) are much greater than those with alkali ions (generally $<10^2$ mol^{-1}·L). This is in support of the previous report of Blout et al. that cyclopeptides often show higher binding affinity to cations of higher charge.[16] The best alkali binding was observed with Li^+. This is understandable since the charge density at Li^+ is the highest within the series.[17] It is worth noting that compared with the stability constants of a good natural ionophore, valinomycin, binding with K^+ and Ca^{2+} (3×10^5 and 1×10^5 mol^{-1}·L in acetonitrile, respectively[18]), the artificial cyclopseudopeptides **2**, **9**, and **10** synthesized in this work are shown to be better receptors for alkaline-earth ions (see Table 1). In addition, the Ca^{2+}/K^+ selectivity of the peptides of the present work ($\sim 1 \times 10^3$ to 3×10^5) was all observed to be much better than that of the natural

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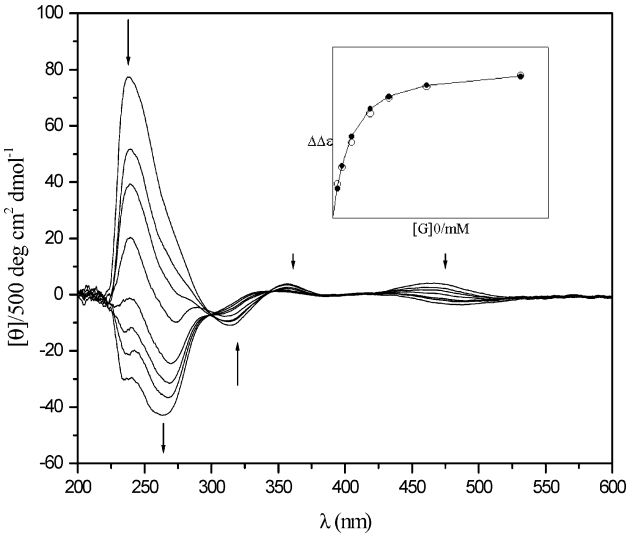

FIGURE 1. Circular dichroism spectrum changes of compound **1** upon addition of $Ca(ClO_4)_2$ in CH_3CN and nonlinear recursive based on the Cotton effect changes at 233 nm. (The concentration of **1** is 0.2 mmol·L^{-1}, and the concentrations of Ca^{2+} are 0, 0.1, 0.2, 0.4, 0.8, 1.2, 2.0, and 4.0 mmol·L^{-1}, respectively).

TABLE 1. Binding Constants of Ferrocenyl Peptides 1−12 with Alkali and Alkaline-Earth Ions[a]

	binding constant K_1 (mol^{-1}·L)[b]					
compd	Li^+	Na^+	K^+[c]	Mg^{2+}	Ca^{2+}	Ba^{2+}
1	75.4	27.3	40.0	1.01×10^3	4.12×10^3	5.85×10^3
2	102	<10	13.9	3.82×10^5	4.37×10^6	6.59×10^3
3	66.5	91.0	10.8	2.40×10^4	1.58×10^5	8.47×10^4
4	52.3	10.9	24.3	1.08×10^4	6.19×10^3	3.89×10^3
5	31.3	17.5	43.7	2.57×10^4	6.89×10^3	9.10×10^2
6	100.1	11.6	40.0	5.25×10^3	6.98×10^3	2.93×10^3
7	15.6	13.9	42.0	4.50×10^3	2.62×10^3	3.21×10^3
9[d]	958	31.3	18.9	1.06×10^6	7.88×10^5	
10	5.58×10^3	<10	<10	1.51×10^5	7.21×10^5	8.76×10^5
11	107	52.6	20.8	4.91×10^3	3.39×10^3	1.26×10^3
12	1.46×10^3	<10	<10	1.65×10^4	1.82×10^4	2.69×10^3

[a] Measured in CH_3CN at 25 °C except for compound **8** due to its conformational complication. Unless otherwise specified, ClO_4^- is the counterion. [b] Data evaluated from the following equation: $\Delta[\theta]^2 - (\alpha[H]_0 + \alpha[G]_0 + 1/K_1)\Delta[\theta] + \alpha^2[H]_0[G]_0 = 0$.[15] [c] PF_6^- is the counterion. [d] The binding constant of Ba^{2+} was not evaluated because the complex formed was observed to be in a 2:1 ratio.

valinomycin, with **2** (Ca^{2+}/K^+ ratio of 3.1×10^5) and **10** (Ca^{2+}/K^+ higher than 7.2×10^4) best demonstrating their superior potentials in relevant applications.

The binding orders of the synthesized peptides with alkaline-earth ions appear to be rather complicated. In sub-series **1**−**3**, the best affinity and selectivity were found with compound **2**. This is presumably because its ring size is most suitable for Ca^{2+}. The ring size of **1** may be too small to host any alkaline-earth ions, as implicated from its worst binding constants and lowest selectivity within the sub-family. These observations indicate that the binding affinity should be related to the suitability of the cavity size in accommodating the guest. Conceivably, compound **3** having the largest ring opening in this sub-series binds best with Ba^{2+} but not as well with the smaller Ca^{2+} and Mg^{2+}. Similarly, the best Ba^{2+} binding in all the synthesized peptides was found with compound **10** (8.76×10^5 mol^{-1}·L), and is understandable because its ring size is the largest of all. The best Mg^{2+} binding of compound **9** (1.06×10^6 mol^{-1}·L) may also be understood because it is smaller than **10** (and **2**) but larger than **4**−**7**. The moderate bindings of the latter sub-series may also be explained on the basis of the ring size argument.

A closer inspection of the data for sub-family **4**−**7** (Table 1) reveals a general trend of their binding preference of $Mg^{2+} > Ca^{2+} > Ba^{2+}$. This again reflects the effect of ring size. It also shows that the side chains can bring a small but notable change in binding constant, as exhibited by the larger values of **4** and **5** (with shorter arms) relative to those of **6** and **7** (with longer arms). This suggests that the hydrophobic effect of the longer side chains may weaken the interaction between cyclopeptide and the charged guest, especially for the small cation Mg^{2+}.[19]

It may also be interesting to compare the binding properties of the cyclic molecules with those of the open chain analogues. Compounds **11** and **12** were synthesized for this purpose and their binding properties examined. Not surprisingly, both the binding affinity and selectivity of peptides **11** and **12** toward alkaline-earth ions were observed to be rather poor (Table 1) compared to the cyclic compounds. This is logical because the ring structure combined with conformation constraints (e.g., the disulfide linkage) provides a better cavity for hosting cations of a suitable size. The relatively better binding of **12** (compared to **11**) is also understandable by considering a possible "pocket effect" that results from the two rigid pyrrolidine units for the incoming cations. The flexibility of the "pocket" opening explains its poor selectivity.

Electrochemistry. The cyclopseudopeptides designed in this work are composed of one or more ferrocene units and various amino acids. Subsequently, they should show good electrochemical response, and therefore may be used as probes for molecular recognition. The redox behavior of compounds **1**−**10** in the presence or in the absence of alkali and alkaline-earth metal cations was indeed observed to be very good. The reversible electrochemical data are summarized in Table 2.

Inspection of the electro-oxidation data of compounds **1**−**7** reveals that in comparing with their quite substantial changes of the ring size or the side chain, the changes of their redox potentials are small, with a span of only 40 mV. This suggests that there is no substantial molecular folding (or bending) toward the redox center; therefore the half-wave potential ($E_{1/2}$) should mainly be affected by the most adjacent structure. The small increase (i.e., more positive) of $E_{1/2}$ for sub-series **1**−**3**, as the number of ferrocenyl group goes up, obviously reflects the electrostatic repulsion between the ferrocene units in the same molecule.

Compound **8** is the only peptide in sub-series **4**−**8** that bears tertiary nitrogen atoms in the vicinity of the oxidizing ferrocene. Table 2 shows that this structure causes quite a substantial effect (by an average margin of 72 mV) on downshifting the ferrocene half-wave potential, due to the stronger electron-releasing ability of the tertiary nitrogen. When the proline unit is moved further apart from the oxidizing center by inserting an alanine unit in between, as in the case of compound **10**,

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TABLE 2. Half-Wave Potentials of Cyclopseudopeptides 1−10 with or without Alkali and Alkaline-Earth Ions[a]

compd	$E_{1/2}$ (mV)	$\Delta E_{1/2}$ (mV)[b]					
		Li^+	Na^+	K^+[c]	Mg^{2+}	Ca^{2+}	Ba^{2+}
1	385	48	<5	<5	18	104	51
2	413	31	<5	<5	53	117	53
3	417	37	<5	<5	39	134	44
4	384	47	<5	<5	98	97	45
5	377	42	<5	<5	43	98	34
6	389	38	<5	<5	63	91	29
7	396	35	<5	<5	30	70	31
8	315	87	<5	18	64	108	69
9	316	103	24	<5	161	144	99
10	342	69	11	9	96	146	91

[a] Reversible oxidation potentials recorded on BAS100B with a Pt working electrode in 0.1 M $^nBu_4N^+PF_6^-$/MeCN solution at 25 °C by cyclic voltammetry (CV) (scan rate: 100 mV/s) versus ferrocenium/ferrocene (Fc^+/Fc) redox couple. [b] The shift of half-wave potential recorded upon addition of 10 molar equiv of cation salt (unless otherwise specified, ClO_4^- is the counterion). $E_{1/2}$ data were reproducible up to 3 mV, and $\Delta E_{1/2}$ values were reproducible up to 5 mV. [c] PF_6^- is the counterion.

TABLE 3. Redox Potential Shifts of Ferrocene-Containing Cyclopseudopeptides 4 and 9 upon Addition of Cations[a]

cation	r (pm)	r/(+) (pm)	$\Delta E_{1/2}$ (mV)[b]	
			4	**9**
Li^+	68	68	47	103
Na^+	95	95	<5	24
Mg^{2+}	65	32.5	98	161
Ca^{2+}	99	49.5	97	144
Ba^{2+}	135	67.5	45	99
La^{3+}	106	35.3	132	197
Eu^{3+}	95	31.7	153	217
Dy^{3+}	91	30.3	147	215
Ho^{3+}	89	29.7	168	217
Er^{3+}	95	31.7	155	213
Yb^{3+}	86	28.7	151	210

[a] Recorded on BAS100B with a Pt working electrode in 0.1 M $^nBu_4N^+PF_6^-$/MeCN solution at 25 °C by cyclic voltammetry (scan rate: 100 mV/s) versus ferrocenium/ferrocene (Fc^+/Fc) redox couple. [b] The shift of half-wave potential upon addition of 10 molar equiv of cation (ClO_4^- is the counterion). The uncertainty is less than 5 mV.

a more positive $E_{1/2}$ was observed because the through-bond donor effect is chain-length sensitive. On the other hand, for peptide **9** whose electron-withdrawing carbonyl moiety is better insulated by the two β-alanine methylene carbons ($-CH_2CH_2-$), a less positive half-wave potential should be expected, and this is indeed observed to be the case with $E_{1/2} = 316$ mV.

The redox property of these cyclopseudopeptides in the presence of alkali or alkaline-earth ions was also investigated. Upon addition of excess metal ions, an anodic shift (see $\Delta E_{1/2}$ values in Table 2) was observed (see also Supporting Information).[20] This suggests that there exists an electrostatic repulsion between the redox center (Fc^+/Fc) and the guest cation. A general inspection of the $\Delta E_{1/2}$ data reveals some features worth mentioning. First of all, the anodic shifts by Li^+ are all substantially greater than those by Na^+ and K^+. This should be understandable because the positive charge on the associated Li^+ is more concentrated than that on Na^+ or K^+, and should have a stronger electrostatic effect on the ferrocene iron, making it more difficult to release an electron. The second feature that the anodic shifts by Ca^{2+} are almost always found to be the largest among the three alkaline-earth cations cannot be similarly explained, because the charge density of Ca^{2+} is not the largest among the three alkaline-earth cations. In searching the possible cause of this unexpected phenomenon, the relationship of anodic shift (i.e., $\Delta E_{1/2}$) vs charge density was examined, taking the bindings of compounds **4** and **9** with lanthanides as two examples. The corresponding data are presented in Table 3, where the charge density is expressed as the radius vs charge ratio r/(+). The linear regression lines are shown in Figure 2.

Figure 2 demonstrates a good linear correspondence between the $\Delta E_{1/2}$ and r/(+) values for the cations under study (except for Mg^{2+}). This indicates that the repulsion effect between the redox center and the guest cation is the dominant factor in perturbing electrochemical responses. This also implies that the superficial "superior" sensitivity of Ca^{2+} vs Mg^{2+} observed for most cyclopseudopeptides is actually not due to an extraordinarily high response to Ca^{2+}, but simply results from an unexpected abnormally low sensitivity for Mg^{2+}, as seen clearly from the two off liners of Mg^{2+}. The cause of this abnormal behavior of Mg^{2+} is not yet understood and needs further investigation.[21]

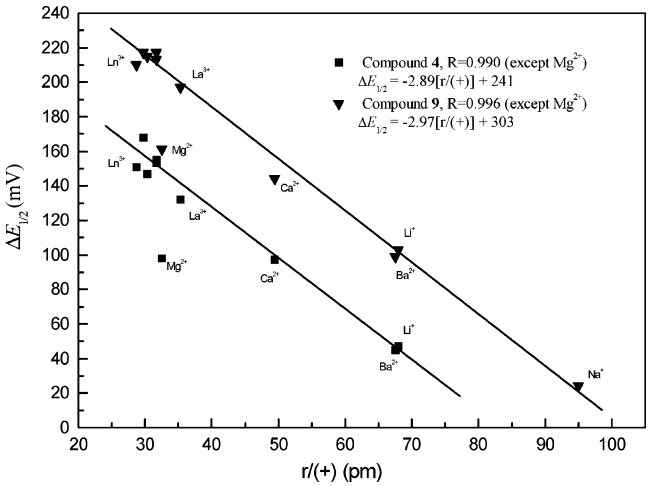


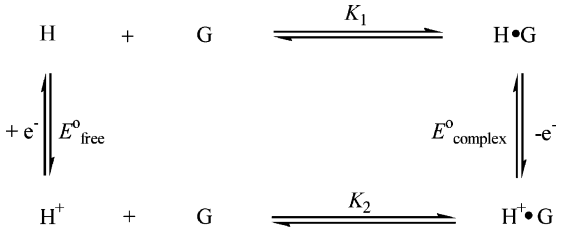
FIGURE 2. Plot of the potential shift ($\Delta E_{1/2}$) vs radius/ionic charge [r/(+)] for complexation of cyclopseudopeptides **4** and **9** with cations (the point corresponding to $\Delta E_{1/2}$ lower than 5 mV is not shown.)

Binding Constants of Oxidized Substrates (1^+−10^+). Though the $\Delta E_{1/2}$ data in Tables 2 and 3 seem to have no direct connection to the corresponding binding affinities (i.e., K_1), according to the relationship shown in Scheme 3 and in eq 1, these two sets of values are

(20) The oxidation potentials of the pseudopeptides upon association with cations were still reversible. However, two separated oxidation waves were not observed in excess of peptides. Though this phenomenon is not unusual, the reasons may be complicated and may be referred to some previous works for more detailed discussions (see ref 22 and publications listed below). (a) Beer, P. D.; Chen, Z.; Ogden, M. I. *J. Chem. Soc., Faraday Trans.* **1995**, *91*, 295. (b) Medina, J. C.; Goodnow, T. T.; Rojas, M. T.; Atwood, J. L.; Lynn, B. C.; Kaifer, A. E.; Gokel, G. W. *J. Am. Chem. Soc.* **1992**, *114*, 10583.

(21) The $\Delta E_{1/2}$ for Mg^{2+} also showed a notable negative departure from the correlation line for other systems reported in the literature with no intended explanation. See: Hall, C. D.; Sharpe, N. W.; Danks, I. P.; Sang, Y. P. *J. Chem. Soc., Chem. Commun.* **1989**, 419.

SCHEME 3. Redox and Complex Equilibria for a Redox-Sensitive Ionophore, Where H and G Represent Ionophore and Guest Molecules[a]



[a] $E°_{free}$ and $E°_{complex}$ are the oxidation potentials of the free and complexed species of the host molecules; K_1 and K_2 represent the binding constants of the free and oxidized species of ionophore, respectively.

TABLE 4. Binding Constants (K_2) of Oxidized Cyclopseudopeptides 1^+−10^+ with Alkaline-Earth Ions[a,b]

	K_2 (mol^{-1}·L)		
compd	Mg^{2+}	Ca^{2+}	Ba^{2+}
1	*2.24 × 10³*	*4.5 × 10*	*4.36 × 10²*
2	4.90 × 10⁴	4.57 × 10⁴	*8.32 × 10²*
3	5.25 × 10³	8.51 × 10²	1.51 × 10⁴
4	2.18 × 10²	*1.41 × 10²*	*6.76 × 10²*
5	4.79 × 10³	*1.51 × 10²*	*2.40 × 10²*
6	*4.57 × 10²*	*2.04 × 10²*	*9.55 × 10²*
7	*1.41 × 10³*	*1.73 × 10²*	*9.55 × 10²*
9[c]	2.04 × 10³	2.95 × 10³	
10	3.63 × 10³	2.45 × 10³	2.51 × 10⁴

[a] Calculated according to eq 1 based on the data in Tables 1 and 2. [b] Data in italics were estimated by using K_1 values of less than 10^4 mol^{-1}·L (but $>10^3$ mol^{-1}·L) (see text). No evaluation was attempted if K_1 was smaller than 10^3 mol^{-1}·L. [c] K_2 for Ba^{2+} was not estimated due to the low K_1 ($<10^3$ mol^{-1}·L).

$$\Delta E_{1/2} = E°_{complex} - E°_{free} = \frac{RT}{nF}\ln\left(\frac{K_1}{K_2}\right) \qquad (1)$$

actually indirectly related to each other. This relationship (eq 1) was then used to evaluate the binding constants (K_2) of the peptides in a higher oxidation state (H^+). The derived data are listed in Table 4.

It needs to be pointed out that eq 1 is reported to be suitable only for strongly interacting systems with $K_1 > 10^4$ mol^{-1}·L.[22] Because there is currently no method in the literature that provides an accurate description for the cases in which $1 < K_1 < 10^4$ mol^{-1}·L, the K_2 values with use of K_1 values that are greater than 10^3 but a bit lower than the limit of 10^4 mol^{-1}·L are not so precise and may be used only as a reference.

A comparison of the data in Table 4 with those in Table 1 indicates that all the K_2 values are smaller than the corresponding K_1 data; and this is expected, since the ferrocene iron in a high oxidation state (i.e., Fe^{3+}) is electrostatically more repulsive to guest cations than the ferrous Fe^{2+}, therefore making the complex of the oxidized species H^+·G less stable than H·G. Further inspection of Table 4 shows that the K_2 values of the oxidized complexes of Ba^{2+} are generally greater than those of Ca^{2+} (except for **2**), thus suggesting that the stability of these complexes should be dominated by an electrostatic repulsion force between Fe^{3+} and the guest cation. This is deduced from the fact that the Fe^{3+}/Ba^{2+} repulsion is less serious (causing a stability increase) as compared to that of the Ca^{2+} counterpart, because the charge density of Ca^{2+} is greater than that of Ba^{2+}. The exceptional observation for compound **2** may be simply the result of its extraordinary affinity to Ca^{2+} (due to its best suitability for hosting Ca^{2+}, vide supra) that is strong enough to override the repulsion effect.

As implied in the above case (i.e., **2**/Ca^{2+}), there exists a competition between the driving force of cation-carbonyl binding and the electrostatic repulsion for the overall cation association. It is conceivable that the small cation is in favor of the cation−carbonyl binding because of its more condensed positive charge, but meanwhile, it also exhibits a stronger repulsion to the redox center, and vice versa. The observation that almost all the K_2 values with Mg^{2+} are larger than those with Ca^{2+} clearly indicates that the association factor outruns the corresponding repulsion factor for the cases of H^+·Mg^{2+} complex formation (vs H^+·Ca^{2+}), a situation just opposite to that of H^+·Ba^{2+} complex formation (vs H^+·Ca^{2+}).

Conclusions

A series of ferrocene-bridged cystine cyclopeptides **1**−**10** have been designed and synthesized as redox-switchable receptors for cations. Binding constants derived from CD spectra revealed that the binding ability of these compounds with various cations is associated mainly with the radius and charge of the cation as well as with the cavity size of the host. Investigation on the binding of the compounds with alkali and alkaline-earth metal ions showed that all the synthesized cyclopeptides have higher affinity for the latter over that of the former. In particular, the best binding affinity and selectivity for Ca^{2+} was found with peptide **2**, which showed an excellent binding constant of 4.37 × 10^6 mol^{-1}·L and a remarkably high $K_1(Ca^{2+})/K_1(K^+)$ ratio of 3.1 × 10^5. Both of the values are much greater than those of the good natural ionophore valinomycin [$K(Ca^{2+})$ of 1 × 10^5 mol^{-1}·L and $K(Ca^{2+})/K(K^+)$ of 0.3].[18]

The perturbation of the guest cations to the redox potentials of the cyclopseudopeptides was investigated. The half-wave shifts ($\Delta E_{1/2}$) were found to correspond linearly with the radius-per-charge ratios $r/(+)$ (i.e., polarizing power, or reciprocally, charge density) of the guest cations (except for Mg^{2+}). The stability constants of the ferri-peptide−cation complexes (K_2) were also evaluated (or estimated) and they indicated a lower binding attraction relative to that of the ferro-counterparts as a reflection of the stronger repulsion to positive charge.

Experimental Section

Preparation of 1,1′-Bis(chlorocarbonyl)ferrocene. 1,1′-Ferrocendicarboxylic acid was commercially made and used without further purification. Preparation of 1,1′-bis(chlorocarbonyl)ferrocene was accomplished by treatment of 1,1′-ferrocendicarboxylic acid with PCl_5 in anhydrous benzene. After removing the solvent, extraction of the residue by anhydrous cyclohexane provided the expected product.

General Procedure for the Preparation of Linear Bispeptides 4a−10a. To a well-stirred and ice−salt cooled solution (about −5 °C) of 5 mmol of L-cystine dimethyl ester (or bis-peptide **8b**), 10 mmol of Boc protected amino acid (Boc-

(22) Miller, S. R.; Gustowski, D. A.; Chen, Z.-H.; Gokel, G. W.; Echegoyen, L.; Kaifer, A. E. *Anal. Chem.* **1988**, *60*, 2021.

AA, AA = Gly, Ala, Pro, Leu, Met, β-Ala), and 12 mmol of *N*-methylmorphine (NMM) in 80 mL of anhydrous $CH_2Cl_2$was added a solution of 11 mmol of dicyclohexylcarbodiimide (DCC) in 20 mL of dry CH_2Cl_2 dropwise over a period of 0.5 h. The resulting solution was stirred at −5 °C for 2 h, and at room temperature for 12 h. After filtration of the precipitate, the solution was then washed sequentially with 20 mL of H_2O, 1 N HCl, H_2O, 5% aqueous $NaHCO_3$, and H_2O twice, and the organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified on a short column of silica gel with use of a mixture of ethyl acetate and petroleum ether (60−90 °C) as eluent to afford the linear peptides **4a**−**10a** in high yields.

General Procedure for the Deprotection of Bis-peptides 4a−10a. A 10-mL 4 N HCl/AcOEt solution was added to the solution of 3.5 mmol of bis-peptides **4a**−**10a** in 15 mL of CH_2Cl_2. The solution was stirred at room temperature for 2 h, then evaporated in vacuo. The residue was then dissolved in trifle dry methanol, to which dry ether was added to afford the deprotected bis-peptides **4b**−**10b**.

General Procedure for the Coupling of 1,1′-Bis(chlorocarbonyl)ferrocene with Cystine Dimethyl Ester or Deprotected Bis-peptides 4b−10b. A solution of 1.5 mmol of freshly prepared 1,1′-bis(chlorocarbonyl)ferrocene in 50 mL of dry CH_2Cl_2 was added dropwise over a period of 2 h to a well-stirred solution of 3 mmol of cystine dimethyl ester (or deprotected bis-peptides **4b**−**10b**) and 15 mmol of NMM in 800 mL of dry CH_2Cl_2. The reaction mixture was then stirred at room temperature for 24 h. After the solution was concentrated to about 100 mL and filtered to remove the precipitate, the solution was washed sequentially with 20 mL of H_2O, 1 N HCl, H_2O, 5% aqueous $NaHCO_3$, and H_2O. The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was purified on a short column of silica gel with use of a mixture of ethyl acetate, petroleum ether (60−90 °C), and methanol as eluent to provide ferrocene-containing cyclopseudopeptides **1**−**10** in good yields.

Binding Constants. The binding equilibrium constants were measured in acetonitrile by using circular dichroism (CD) titration. The CD spectra were recorded in a quartz cell of 1 cm path length at 25 ± 1.0 °C. Data are represented as molar ellipticities. The concentration of the peptide was 1×10^{-4} mol·L^{-1} and the concentration of the cation was increased stepwise in a range of 10^{-5} to 10^{-3} mol·L^{-1}. Values for the equilibrium constants are reported as K_1, which were determined by using the methods previously reported by Liu et al.[15] The error from two measurements is less than 20%.

Electrochemistry. Tetrabutylammonium hexafluorophosphate was prepared from the reaction of $^nBu_4NHSO_4$ with HPF_6 in water. It was recrystallized three times from CH_2Cl_2 and Et_2O, and was vacuum-dried at 80 °C before usage. Cyclic voltammetric studies were performed on a BAS100B electrochemical analyzer in 0.1 M $^nBu_4N^+PF_6^-$/MeCN solution with a Pt electrode (φ 0.5 mm) as the working electrode at 25 ± 1.0 °C (scan rate: 100 mV/s). A 0.1 M Ag/$AgNO_3$ electrode (in 0.1 M $^nBu_4N^+PF_6^-$/MeCN) was employed as reference electrode and a Pt electrode (φ 0.3 mm) was used as the supporting electrode. Pt electrode surfaces were polished with 0.05-μm alumina, sonicated in water, and air-dried immediately before usage. Redox potentials are reported vs the ferrocenium/ferrocene (Fc^+/Fc) redox couple. Redox potential shifts were obtained after an excess of ions (10 equiv) was added to the solution of the cyclopseudopeptides (5×10^{-3} M) in 0.1 M $^nBu_4N^+PF_6^-$/MeCN solution (5 mL).

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Supporting Information Available: General experimental information, spectroscopic data (specific optical rotation, IR, ^{1}H NMR, MS, and elemental analysis) together with the yields of compounds **4a**−**10a** and **1**−**10**, ^{1}H NMR spectra of compounds **1**−**10**, NOESY spectra of compounds **1** and **2**, CD spectra of compounds **1**−**12**, some examples of CD changes of compounds **1**−**10** upon addition of a cation, and some CV figures with or without a cation. This material is available free of charge via the Internet at http://pubs.acs.org.

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