Development of New Double-Stranded Phenylalanyl Chelators Using $\eta$-$\chi$ Diagrams and Binding Constants for Chelators and Lanthanide Ions

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We examined the interaction between several ligands and alkaline (M$^+$) or lanthanide (Ln$^{3+}$) ions using $\eta$-$\chi$ diagrams. $\eta$ and $\chi$ represent absolute hardness and absolute electronegativity, respectively. Based on the diagrams, new double-stranded amino acid chelators (1) conjugated to Cat (catechol) were synthesized. They had two cavities coordinating hard metal ions, such as Li$^+$ and K$^+$, and soft metal ions, such as Lu$^{3+}$ and Eu$^{3+}$. I formed a solvated molecular complex, 1–Ln$^{3+}$, with a molar ratio of 1 : 1 at a binding constant ($K_b$) of $10^6$–$10^8$ with Y$^{3+}$, La$^{3+}$, Eu$^{3+}$, and Lu$^{3+}$, respectively. With a chemical hard ion, Li$^+$, we found that the $K_b$ of the 1–Eu$^{3+}$ complex increased. The optimized geometries of 1–Lu$^{3+}$ and 1–La$^{3+}$ complexes were obtained from B3LYP correlation functions using Stuttgart-Dresden-Bonn (SDD) Effective Core Potential (ECP) basis sets for Lu$^{3+}$ and La$^{3+}$ ions. The double-stranded chelator may prove useful for studying the selective separation of lanthanide ions, and the development of functional peptides.

Key words chemical hardness; double-stranded amino acid; chelator; lanthanide ion; binding constant

Lanthanides (Ln) as heavy rare earth elements and Y are attracting the interest of chemists since complexes prepared by the reaction of lanthanide ions (Ln$^{3+}$) with chelators have a variety of applications, including probes for fluorometry,1–4) catalysts,5) sensitizing chromophores,6–8) and nanomaterials.9,10) Recently, lanthanide ions with unique physical and coordination properties have been used to develop lanthanide-antenna sensors as luminescent sensors for cellular imaging,11) and protein and DNA assays. As chemical and physicochemical properties, lanthanides are chemically similar to metal ions because of the shielding of progressive filled inner 4$s$ orbitals by the filled outer 5$d$ orbitals and the lanthanide contraction effect. The study of the stability of lanthanide-ligand complexes is interesting because the lanthanide contraction effect appears to vary directly with the ionic radius.

The development of chelator–Ln$^{3+}$ complexes has been of interest to investigators of the regioselective lanthanide-induced hydrolysis of phosphate esters, including DNA and RNA.11) Such compounds are potentially applicable to pharmacological fields12) as chemotherapeutic agents, such as the platinum anticancer drug cisplatin. Generally, chelator–Ln$^{3+}$ complexes have low solubility. The design of chelators, which have both hydrophobic parts (or regions) and hydrophilic parts in the same molecule, is required to increase the solubility of Ln$^{3+}$ ions. We envisage the development of highly soluble chelator–Ln$^{3+}$ complexes which express biological effects through coordination with the binding sites of drugs, proteins, and DNA. It is necessary to understand which ligands coordinate easily with Ln$^{3+}$; however, there is no convenient chemical method of examining the interaction between chelators and metal ions.

In the past several years, the development of theoretical models of metals and molecules based on electrophilicity ($\omega$) and chemical hardness ($\eta$) indexes for the characterization of chemical reactions, biological activity, and atoms has progressed steadily.13) The concepts of chemical hardness, absolute hardness [$\eta=1/2(\partial E/\partial N)$] and absolute electronegativity [$\chi=-(\partial E/\partial N)$],14,15] which have been associated with the theory of chemical reactivity in molecules, are being applied to the design of chelators and chemicals.16,17) $N$ is the total electron number and $E$ is total electron energy.

To develop chelators, we have focused on a convenient model using quantum chemical $\eta$–$\chi$ diagrams for chelators and lanthanide ions based on the chemical hardness concept, $\eta$ and $\chi$, as described above. The $\eta$–$\chi$ diagram shows that phenol and Cat (catechol) are appropriate for the reaction of a ligand with Ln$^{n+}$ ions since Ln$^{n+}$ is chemically harder than phenol and Cat.

Here, we report a new chelator (1) that conjugated Cat to bis(tert-butoxycarbonyl (Boc)-L-Phe)-N,N-ethylenedioxy-bis(ethylene), which should support a $\beta$-sheet-like conformation.18,19) The chelator could expect flexible conformation due to a backbone conjugated with two amino acid residues to the N-terminus of a spacer, $–\text{O}–\text{CH}_2–\text{CH}_2–\text{O}–$, and its binding site is Cat, not an amido group. Chelator 1 provides a coordinate bond complex with Ln$^{3+}$ of the largest ionic radius in $10\text{mM} \ N(2\text{-hydroxyethyl})\text{pipеразине-}N’\text{-2\text{-ethanesulfonic}} \ \text{acid} \ \text{(HEPES)} \ \text{buffer} \ \text{in 70\% MeCN (pH 4.8)}$. Indeed, the binding constants of 1–Ln$^{3+}$ complexes are about $10^4$–$10^6 \text{M}^{-1}$. Therefore, we show that compound 1 analogs with ligand Cat are excellent chelators for Ln$^{3+}$. Complexes of chelator 1 with Ln$^{3+}$ may be applied in the pharmacological field as chemotherapeutic agents and luminescent sensors, which are a subject of many recent studies.

In addition, the quantum chemical $\eta$–$\chi$ diagram is a useful tool for the design of chelators, and we will deal with the synthesis and characterization of the complexes of the chelator with Ln$^{3+}$. To rationalize the structure of chelator–Ln$^{n+}$ complexes, we also discussed the structural properties of complexes of the chelator with La$^{3+}$ and Lu$^{3+}$ using the B3LYP level.

Results and Discussion

Design of an Active Site: Quantum Chemical $\eta$–$\chi$ Diagrams for Lanthanide Ions

The relation between the ionization potential ($\partial p$) and electron affinity (EA) of metal ions (M$^{n+}$) is shown in Eq. 1. The values of absolute hardness ($\eta$)
and absolute electronegativity ($\chi$) were calculated using data published previously.\(^{20}\)

Coordinate $r(\chi, \eta)$ as the electronic structure of $\text{Ln}^{3+}$ was calculated from Eqs. 1, 2, and 3:

$$
\text{Ln}^{3+}: \text{Ln}^{3+}(\text{Ea}) \rightarrow \text{Ln}^{3+}(\mu) + e^{-}
$$

$$
-\mu = (\mu + \text{Ea}) / 2 = \chi
$$

$$
\eta = (\mu + \text{Ea}) / 2
$$

Where $\mu$ is chemical potential. Here, we found that the $r(\chi, \eta)$ of lanthanide ions, $\text{Ln}^{3+}$, having 4f electrons, has no linear relationship between absolute hardness and absolute electronegativity. In the diagram of $r(\chi, \eta)$, $\text{Sc}^{3+}$ ($4f = 0$), $\text{Y}^{3+}$ ($4f = 0$), $\text{La}^{3+}$ ($4f = 0$), and $\text{Gd}^{3+}$ ($4f = 7$) have a two-order correlation between $\eta$ and $\chi$, and we found that the correlation curve was fitted with $\eta = -0.0308\chi + 3.2518\chi - 61.171$ (correlation coefficient ($r$): $r = 0.9994$); however, $\text{Ce}^{3+}$ ($4f = 1$)–$\text{Eu}^{3+}$ ($4f = 6$) and $\text{Dy}^{3+}$ ($4f = 9$)–$\text{Lu}^{3+}$ ($4f = 14$) groups did not show a linear relationship between $\eta$ and $\chi$ (Fig. 1). The graph has no two-order or three-order correlations; however, the behavior of $\text{Gd}^{3+}$ is similar to that of $\text{Sc}^{3+}$, $\text{Y}^{3+}$, and $\text{La}^{3+}$ although it has 4f electrons. This may be explained by the relativity theory for heavy rare earth elements.\(^{21}\)

Hughes et al. reported that although the effects of spin orbital coupling of $\text{Gd}^{3+}$ can be neglected, the spin orbital coupling and orbital moments of other $\text{Ln}^{3+}$ ions with 4f electrons are important to obtain estimates of magnetic moments and magnetic ordering temperatures.\(^{22}\) Therefore, heavy rare earth elements and related elements, $\text{Sc}^{3+}$, $\text{Y}^{3+}$, $\text{La}^{3+}$, and $\text{Gd}^{3+}$, which can be neglected in terms of the effect of relativity theory, are determined using the $r(\chi, \eta)$ diagram. For example, the $\eta$ of $\text{Ce}^{3+}$ is 8.28 and that of $\text{Gd}^{3+}$ is 11.71. $\text{Ce}^{3+}$ is chemically softer and $\text{Gd}^{3+}$ is harder than other $\text{Ln}^{3+}$ ions with 4f electrons. The $\eta$ of lanthanum ions, $\text{La}^{3+}$, is 14.875 and $\text{La}^{3+}$ lack three electrons, ($5d^{10}(6s)^2$), since the electron configuration of a neutral La is ($4d^{10}(5s)^2(5p)^6$). $\text{Eu}^{3+}$ has the electron configuration ($4d^{10}(5f)^2(5p)^6$).

To achieve the design and synthesis of functional chelators, which selectively bind with chemically soft $\text{Eu}^{3+}$ and $\text{Gd}^{3+}$ ions, we discuss a method for the development of chelators using $r(\chi, \eta)$ diagrams of $\text{La}^{3+}$ ions and ligands (L) (2–10). As a soft-based chelator is favorable for binding soft-acid metal $\text{M}^{n+}$ ions according to the hardness concept, our target is a chemically soft ligand. In addition, our aim is to design a $\text{L} – \text{Ln}^{3+}$ complex in which the binding force between the ligand and $\text{M}^{n+}$ ion is weak; that is, ligand exchange is active. The interaction of $\text{Ln}^{3+}$ ions with the ligand as a donor is a result of an overlap of orbitals or of electrostatic binding of two $\text{Ln}^{3+}$ ions and L (Eq. 4).

$$
\text{L} + \text{Ln}^{3+} \rightleftharpoons \text{L} – \text{Ln}^{3+}
$$

We show a $r(\chi, \eta)$ diagram plotting the $\eta$ and $\chi$ of several ligands calculated using the density functional theory B3LYP with a 6-31G(d) basis set (Fig. 1). When $\text{Ln}^{3+}$ forms a complex with L, electrons flow from L to $\text{Ln}^{3+}$. The quantitative charge transfer ($\Delta Q$) and stabilization energy ($\Delta E$) were calculated using Eqs. 5 and 6.\(^{14}\)

$$
\Delta Q = \frac{\text{(L} – \text{Ln}^{3+})}{2\text{(L} – \text{Ln}^{3+})}
$$

$$
\Delta E = \frac{\text{(L} – \text{Ln}^{3+})}{4\text{(L} – \text{Ln}^{3+})}
$$

According to Eqs. 5 and 6, the softer the target chelator, the less favorably it binds with $\text{Ln}^{3+}$ ions. In the diagram in Fig. 1a, the Cat (7) is a softer chelator than 18-crown-6-ether (2), en (3), ethylenediaminetetraacetic acid (EDTA) (4), 12-ane-4 (5), and calixarene (8), and the $\chi$ of Cat is smaller than that of 3, 4, or 5. In making up the electrostatic Cat–$\text{Eu}^{3+}$ complex of Cat with $\text{Eu}^{3+}$, the stabilization energy $\Delta E$ is $-20.48$ (eV), as listed in Table 1. The value is larger than that of 2–6 and 8–10. These values are expressed as the binding force of the chelators with $\text{La}^{3+}$ and $\text{Eu}^{3+}$ ions. Moreover, the $\Delta Q$ of the 7–$\text{Eu}^{3+}$ complex is $-1.317$. This indicates that electrons easily withdraw $\text{Eu}^{3+}$ from 7. Calculated $\Delta Q$ and $\Delta E$ values indicate that the stabilization of ligand–$\text{Eu}^{3+}$ complexes decreases in the following order: 7–$\text{Eu}^{3+}$< 8–$\text{Eu}^{3+}$< 10–$\text{Eu}^{3+}$< 9–$\text{Eu}^{3+}$< 6–$\text{Eu}^{3+}$< 3–$\text{Eu}^{3+}$< 4–$\text{Eu}^{3+}$< 5–$\text{Eu}^{3+}$ and >2–$\text{Eu}^{3+}$ (Table 1). This indicates that ligand 6 would form a stable solvated complex with $\text{Ln}^{3+}$ ions in solution; therefore, we designed and synthesized a novel chelator (1), in which a chemically soft ligand 7 was conjugated to two terminals in the molecule. Although the spacer –HN–$\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{NH}–$ of 1 is as chemically hard as ligand 2 or 5, 1 joined to 7 is a chemi-

![Fig. 1. Plot of $r(\chi, \eta)$ as a Coordinate Diagram of Electronic Structure for Alkaline (M$^+$) and Lanthanide Ions (L$^{3+}$) (a), and Structures of Several Chelators (2–10) (b)](image-url)
Conjugated 2,3-bis(benzyloxy)benzoic acid was afforded by treatment with trifluoroacetic acid (TFA) to yield the free compound.

**Synthesis and Chemical Properties**

The synthesis of the target double-stranded amino acid chelators is shown in Fig. 2. Boc-L-Phe-OH was conjugated to 1,8-(ethylene-dioxy)bis(ethylamine) to yield a Boc-protected bis(Boc-L-Phe)-N,N-(ethylenedioxy)bis(ethylamine) (11a) and 11a was purified using silica gel chromatography with CHCl₃ and 3% MeOH/CHCl₃. Removal of the Boc groups by trifluoroacetic acid (TFA) afforded the free compound (12a) in good yield. Conjugated 2,3-bis(benzyloxy)benzoic acid was prepared from 2,3-dihydroxybenzoic acid, with the N-terminal of 12a afforded by treatment with DEPC (phosphoroxyanidate) on dry N,N-dimethylformamidine (DMF). Deprotection of the Bzl (benzyl) group in 13a using 5% Pd/C–H₂ gave a new peptide chelator (14a) in 65% yield. Both compounds, 14a and 14b, gave satisfactory results in IR, ¹H-NMR, ¹³C-NMR, ¹H–¹H correlation spectroscopy (COSY), ¹³C–¹H COSY NMR, and FAB-MS analyses.

The δH-1 of 14a observed at 4.82 ppm in CDCl₃–DMSO-d₆ (volume ratio; 0.5/0.2) is coupled with an amido proton (NH₃CO–) of 8.48 ppm and the observed ³Jα, NH is 7.9 Hz. The value indicates that 12a has a β-sheet (7.0—10.0 Hz)-like conformation. The observed ³Jα, NH value of δH-1 of 15a conjugated with benzoic acid is 8.0 Hz, which is also an acceptable coupling constant for a β-sheet-like conformation.

**Table 1. Calculated Hardness (η), Electronegativity (χ), Quantitative of Charge Transfer (ΔQ) and Stabilization Energy (ΔE) for Chelators and Chelator-Lu³⁺ Complexes**

| Chelator | η
| ΔQ Lu³⁺ complex (eV) | ΔE Lu³⁺ complex (eV) | ΔQ Eu³⁺ complex (eV) | ΔE Eu³⁺ complex (eV) |
|----------|------------------|------------------|------------------|------------------|
| 2        | 3.063            | 0.839            | 13.21            | 1.201            | -18.47          |
| 3        | 4.172            | 0.855            | 13.94            | 1.218            | -19.39          |
| 4        | 3.522            | 0.864            | 13.41            | 1.264            | -19.15          |
| 5        | 3.837            | 0.878            | 14.37            | 1.264            | -20.20          |
| 6        | 3.063            | 0.883            | 13.57            | 1.314            | -19.68          |
| 7        | 2.703            | 0.895            | 14.26            | 1.317            | -20.48          |
| 8        | 3.063            | 0.892            | 14.05            | 1.317            | -20.24          |
| 9        | 3.063            | 0.886            | 13.86            | 1.307            | -19.95          |
| 10       | 3.063            | 0.851            | 13.20            | 1.235            | -18.70          |

*a* At the B3LYP/6-31G(d) level.  
*b* Coordinate r(€, η) of electronic state of Lu³⁺ and Eu³⁺ is r(34.57, 14.88) and r(33.81, 8.89), respectively.

**Fig. 2.** Synthesis of Several Double-Stranded Amino Acid Chelators  
**Keys:** (i) 90% TFA at 4 °C. (ii) Cat, DEPC, and TEA in dry DMF. (iii) 5% Pd/C–H₂ in MeOH.

**Fig. 3.** Circular Dichroism Spectra of Double-Stranded Chelators

Circular dichroism spectra of (a) 14a, b and (b) 15a, b in 70% CH₃CN (10 mM HEPES buffer, pH 4.8) at 23±0.1 °C. Concentration of 14a, b and 15a, b: 1.50×10⁻⁶ M.
Hildebrand plot analysis.\textsuperscript{21)} Figures 4a–d show the spectral change in UV/Vis titration studies on the binding of \textit{14a} with \textit{Y}\textsuperscript{3+}, \textit{La}\textsuperscript{3+}, \textit{Eu}\textsuperscript{3+}, or \textit{Lu}\textsuperscript{3+}. UV/Vis spectra of the chelator \textit{14a} were measured in 10 mM HEPES buffer in 70% MeCN (pH 4.8) and the first excited wavelength (\(\lambda_{\text{max}}\)) was 313 nm.

The absorbance of a colorless solution of \textit{14a} at 313 nm decreased on the addition of \textit{Eu}\textsuperscript{3+} from 2.60 to 129.0 \times 10\textsuperscript{-5} M under a fixed concentration of \textit{14a} (8.067 \times 10\textsuperscript{-5} M). However, the absorbance of the new wavelength of the \textit{14a}–\textit{Eu}\textsuperscript{3+} complex at 340 nm (\(\lambda_{\text{max}}\)) increased on the addition of \textit{Eu}\textsuperscript{3+} (Fig. 4c). As the \textit{14a}–\textit{Eu}\textsuperscript{3+} complex has a 1:1 molar ratio, the 1:1 binding constant (\(K_{\text{MY}}\)) of \textit{14} for \textit{Ln}\textsuperscript{3+} is calculated from the Benesi–Hildebrand plot. A charge in absorbance (OD) is only induced by the formation of a 1:1 complex between \textit{14} ([\(C_{\text{U}}\)], M) and \textit{Ln}\textsuperscript{3+} ion ([\(D_{\text{L}}\)], M), and the OD intensity (OD) at maximum wavelength (\(\lambda_{\text{max}}\)) can be expressed by the following equations:

\[
\frac{[C_{\text{U}}]}{[D_{\text{L}}]} = \frac{1}{\left[D_{\text{L}}\right]}K_{\text{MY}} \cdot \Delta \varepsilon + \frac{1}{\Delta \varepsilon}
\]

\[
\frac{1}{\Delta \varepsilon} = y\text{-intercept and } \frac{1}{\Delta \varepsilon}K_{\text{MY}} = \text{slope}
\]

Figure 4e shows Benesi–Hildebrand plots of the absorbance at 350 nm or 340 nm as a function of \textit{Y}\textsuperscript{3+} or \textit{Eu}\textsuperscript{3+} concentrations at a fixed concentration of \textit{14a} according to Eqs. 7 and 8. From the slope, the \(K_{\text{MY}}\) of the \textit{14a}–\textit{Y}\textsuperscript{3+} and \textit{14a}–\textit{Eu}\textsuperscript{3+} complexes was 2.12 and 4.37 \times 10\textsuperscript{4} M\textsuperscript{-1}, respectively. The binding constants of other complexes are shown in Fig. 5. Stabilization increases in the following order: \textit{14a}–\textit{Lu}\textsuperscript{3+} < \textit{14a}–\textit{Eu}\textsuperscript{3+} < \textit{14a}–\textit{Y}\textsuperscript{3+} < \textit{14a}–\textit{Eu}\textsuperscript{3+} < \textit{14a}–\textit{La}\textsuperscript{3+}.

The result indicates that the \(K_{\text{MY}}\) value of the complex between \textit{14a} and \textit{Ln}\textsuperscript{3+} is about 10\textsuperscript{8}–10\textsuperscript{9} M\textsuperscript{-1}, similar to that of \textit{Ln}\textsuperscript{3+} with calix[6]arene analogs.\textsuperscript{24} In order to compare the binding ability of the enantiomer \textit{14b}–\textit{Eu}\textsuperscript{3+} complex with the \textit{14a}–\textit{Eu}\textsuperscript{3+} complex, the binding constant \(K_{14b-\text{Eu}^{3+}}\) was measured by similar method; however, the \(K_{14b-\text{Eu}^{3+}}\) of \textit{d}-form \textit{14b} was almost the same value with \textit{l}-form \textit{14a}.

The shift of the second absorption from 313 to 317 nm of the complexes of \textit{14a} with \textit{La}\textsuperscript{3+} was much smaller than that of complexes of \textit{14a} with \textit{Y}\textsuperscript{3+} and \textit{Lu}\textsuperscript{3+} (Figs. 4a, b, d). The \(K_{MY}\) of \textit{14a}–\textit{La}\textsuperscript{3+} is 3.43 \times 10\textsuperscript{6} M\textsuperscript{-1}, the largest in the Benesi–Hildebrand plot. Although the absorption shift of the \textit{14a}–\textit{La}\textsuperscript{3+} complex was small, the \(K_{MY}\) value was the largest in this study (Fig. 5). The \(\lambda_{\text{max}}\) of \textit{14a}–\textit{Eu}\textsuperscript{3+} (\(\lambda_{\text{max}} 340\text{ nm}\)) shifted to a larger value than that of chelator \textit{14a} (\(\lambda_{\text{max}} 313\text{ nm}\)). The shifts of \textit{14a}–\textit{Ln}\textsuperscript{3+} complexes decreased in the following order: \textit{14a}–\textit{Lu}\textsuperscript{3+} > \textit{14a}–\textit{Y}\textsuperscript{3+} > \textit{14a}–\textit{Eu}\textsuperscript{3+} > \textit{14a}–\textit{La}\textsuperscript{3+}.

The reason for the difference in \(K_{MY}\), for instance \textit{14a}–\textit{Eu}\textsuperscript{3+} and \textit{14a}–\textit{La}\textsuperscript{3+} complexes, is as follows. A large shift in \(\lambda_{\text{max}}\) indicates that the complex is polarized in the excited
state and ionized; however, a small shift means that the complex is greatly polarized as in the case of the 14a–La$^{3+}$ complex. In the case of 14a–Eu$^{3+}$, the large shift of $\lambda_{\text{ex}}$ in the excited state is associated with little polarization of the complex; therefore, 14a and related chelators would be applicable to the separation of Ln$^{3+}$ ions in solution. In addition, 14a emits fluorescence with a $\lambda_{\text{ex}}$ of about 425 nm at $\lambda_{\text{em}}$ of 540 nm in 10 mM HEPES buffer in 70% MeCN (pH 4.8) (data not shown). Here, 15a did not bind with the Ln$^{3+}$ ions used in this study since the shift and increase in intensity at $\lambda_{\text{ex}}$ of the 15a–Ln$^{3+}$ complex was not observed by UV/Vis titration. This shows that catechol ligands provide the function of 14, not amide groups.

**Molar Ratio and Structure** The molar ratio of the complex between Ln$^{3+}$ and the chelator was analyzed by continuous variation (a)$^{25}$ and molar ratio (b) methods (Figs. 6a, b).

![Graph](image)

**Fig. 6.** Determination of Compositional Ratio of the 14a–Eu$^{3+}$ Complex with the Continuous Variation Method (a) and Molar Ratio Method (b)

(a) Ratio of [14a/Eu$^{3+}$]: 0.11, 0.25, 0.43, 0.67, 1.0, 1.5, 2.33, and 4.0 in 70% MeCN (10 mM HEPES, pH 4.8). (b) Ratio of [14a/Eu$^{3+}$]: 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0 and 5.0; at $\lambda_{\text{em}}$ of 340 nm at pH 4.8. Concentration of 14a and Eu$^{3+}$: 2.34 x 10$^{-5}$ M and 1.17 x 10$^{-5}$ M.

The continuous variation method gives the result for plots of [14a]/[Eu$^{3+}$] vs. absorbance. The molar ratio was the result for plots of [14a]/[Eu$^{3+}$] vs. absorbance for equilibrium under a fixed concentration of Eu$^{3+}$ (1.17 x 10$^{-5}$ M) at pH 4.8. With the continuous variation method (Fig. 6a), the plots gave two linear slopes in the ratio of concentration of [14a]/[Eu$^{3+}$]: 5.74 x 10$^{-5}$/5.16 x 10$^{-4}$—4.59 x 10$^{-5}$/1.15 x 10$^{-4}$. It was found that the 14a–Eu$^{3+}$ complex has a 1:1 ratio since the intersection of the two slopes of correlation coefficient $r=0.974$ and 0.999 give a value of n/m=1 for altitude. Similar results were provided by the molar ratio method (Fig. 6b).

The molar ratio (14a: Eu$^{3+}$ = 1:1) is useful to estimate the chemical structure of the 14a–Ln$^{3+}$ complex. Generally, Ln$^{3+}$ ions yield complexes with coordination numbers of 8—12. To clarify the optimized structure satisfying conditions such as a 1:1 ratio and coordination number of 8, we estimated the optimized structure of 14a (Fig. 7a) and the 14a–La$^{3+}$ complex (Fig. 7c) using the ab initio molecular orbital (MO) method. Figure 7 shows the results of B3LYP hybrid density functional methods with Stuttgart–Dresden–Bonn (SDD) effective core potentials (ECP)$^{26}$ basis set using the Gaussian 03 program$^{27}$; however, the SDD ECP basis set was used for Ln$^{3+}$ ions. C, H, N, and Cl atoms were optimized at the 6-31G(d) basis set level. The calculated mean O(4)–La$^{3+}$ and HO(3)–La$^{3+}$ bond lengths in the 14a–La$^{3+}$ complex are about 2.306 and 2.747 Å, respectively (Table 2), and the O–La$^{3+}$ bond length is close to the observed length of the O–La$^{3+}$ bond (2.443 Å) in TREN-1,2-HOIQO–La$^{3+}$.$^{28}$ The O(4)–Lu$^{3+}$ bond length (2.202 Å) in the 14a–Lu$^{3+}$ complex is also similar to the observed length of the O–Lu$^{3+}$ bond (2.287 Å) in the TREN-1,2-HOIQO–Lu$^{3+}$ complex.$^{28}$ The results indicate that the structures of 14a–La$^{3+}$ and

![Image](image)

**Fig. 7.** Optimized Structure of 14a and the 14a–La$^{3+}$ Complex

Structure of (a) 14a and (c) the 14a–La$^{3+}$ complex shown as a ball and spoke model. At the B3LYP level using the SDD basis set for La, and an all-electron 6-31G(d) basis set for the remaining atoms.

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*a) At the B3LYP/6-31G(d) level. b) At B3LYP/6-31G(df), and SDD basis set was used for the optimization of La$^{3+}$ and Lu$^{3+}$. 
forms the random conformation (16) to the folding conformation (17). Next, the chemically soft acid Eu\(^{3+}\) interacts with a chemically soft base, catecholhydroxide of 14a, and the complex (18) is stabilized by the valance, in both ionic size and chemical hardness. Molecular orbital (MO) calculations using the B3LYP method provided the geometry of 14a in which two catechols were opposite; however, binding of K\(^+\) (or Li\(^+\)) to the spacer \(-\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{O}–\) would fix the flexible conformation of 14a. As expected, the two catechols formed binding sites of 14a, which are face to face and close. This provides a new functional chelator for controlling the binding of 14a with Ln\(^{3+}\) in the presence of alkaline metal ions. Finally, this results indicates that chelator 14 is active in displacing the ligand in the solution and a dynamic flexible−rigid equilibrium is produced in K\(^+\) (or Li\(^+\)) and Ln\(^{3+}\) solutions.

**Conclusion**

We reported the synthesis and chemical properties of a series of new double-stranded phenylalanine chelators 14 conjugated to catechol. The interactions between double-stranded amino acid chelators (or other ligands) and lanthanide series ions (Ln\(^{3+}\)) were discussed using \(\eta−\chi\) diagrams as coordinates of electron structure \(r(\chi, \eta)\) based on the chemical hardness theory. According to chemical hardness, 14 is a chemically soft base chelator containing a chemically hard base \(-\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{O}–\text{CH}_2\text{CH}_2\text{O}–\) spacer. The diagram suggested that catechol ligand provides stable chelators for Ln\(^{3+}\) ions, but not Li\(^+\) and K\(^+\) ions; therefore, 14−conjugated catechol produced a stable solvated 14−Ln\(^{3+}\) complex. Indeed, binding constants of the 14−Ln\(^{3+}\) complex were \(10^6−10^8\) M\(^{-1}\). Interestingly, the order of binding stability is identical to the order of the lanthanide contraction effect: \(\text{Lu}^{3+}<\text{Eu}^{3+}<\text{Y}^{3+}\). Spectrophotometric titration also provided evidence that the binding constant for Eu\(^{3+}\) and 14a increased in the presence of a chemical hard ion, Li\(^+\) (Na\(^+\) or K\(^+\)) (Fig. 5). This result suggested that 14 is ligand displacement active, and that the motion of the molecular skeleton is flexible in solution. Our results indicate the \(\eta−\chi\) diagram is a useful tool for developing functional chelators. Further studies of the isolation of the 14−Ln\(^{3+}\) complex and inhibitory effect on cell growth are in progress.

**Experimental**

**General Methods** The general approach to the synthesis of the compounds (11, 12) was described in our previous papers.\(^{15,18}\) The peptide and related ligands were detected on TLC (thin-layer chromatography) plates using iodine vapor or UV (ultraviolet spectrum) absorption. Silica gel column chromatography was performed on silica gel 60N (100 mesh, neutral; Kanto Chemical Co., Japan). Solvent systems were as follows, A: CHCl\(_3\)−MeOH (20:1), and B: CHCl\(_3\)−MeOH (10:1). UV/vis (ultraviolet/visible) spectra were measured with a JASCO V-530 spectrophotometer, pH was measured with a TOA pH instrument (Model HM-60G). Specific rotations were measured with a JASCO DIP-140 digital polarimeter. NMR (nuclear magnetic resonance, as \(^{1}H\)-NMR, \(^{13}C\)-NMR) spectra were obtained with a JEOL α-500 or a Bruker AV300 spectrometer, and NMR samples were dissolved in DMSO-\(d_6\)/CDCl\(_3\) (volume ratio: 0.5 ml/0.2 ml) with TMS (tetramethylsilane) as an internal reference. FAB-MS (fast atom bombardment mass spectrometry) spectral data were obtained on a JEOL LMS-HX110 spectrometer, and relevant data were tabulated as m/z.

**Synthesis of Bis(Cat-t-Phe)\(~\text{N}-(\text{ethylendioxy})\text{bis(ethylamine)}\) (14a). Bis(t-Phe)\(~\text{N}-(\text{ethylendioxy})\text{bis(ethylamine)}\) (12a) CDI (1.1‘-carbonyldimidazole) (4.45 g, 26 mmol) was added to a solution of Boc-t-Phe-OH (5.08 g, 19.2 mmol) in dry CHCl\(_3\) (40 ml). The solution was stirred for 1 h at room temperature, 2.2‘-(ethylendioxy)bis(ethylamine) (1.26 g,

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8.5 mmol) was added, and the mixture was stirred overnight. This solution was evaporated dry. After the addition of aq. MeOH, the sediment was collected, washed with aq. MeOH, 5% citric acid, 5% NaHCO₃, and water, and dried in vacuo. The resulting product, bis[(boc- ϑ-Phe)-N-4(ethylendioxy)bis(ethylamine)] (11a), a colorless solid, was obtained in 80% yield. The crude product was purified by chromatography on silica gel column (41 g) and eluted in CHCl₃ and 3% MeOH/CHCl₃ (stepwise elution); mp 92—93 °C from MeCN. R(A) = 0.37. FAB-MS (nitrobenzylalcohol, NBA) m/z: 643 (M+H⁺).

To 11a (4.57 g, 5.04 mmol) was added 90% TFA (26 ml). The mixture was stirred for 1 h in an ice bath. After a 5% NaHCO₃, and 1 M NaOH workup, product 12a was extracted with CHCl₃, the organic layer was washed with water and dried with Na₂SO₄, and 12a was obtained in 75.0% yield; R(B) = 0.16. Oily. 1H-NMR (CDCl₃/DMF-d₇, 5.00 ppm) δ: 3.33 (1H, m, –O–CH₂–), 3.35–3.55 (1H, m, –O–CH₂–), 4.08 (1H, br d, J = 6.9, 9.8 Hz, –NH–), 5.5–5.8 (5H, m, aromatic protons). 13C-NMR (CDCl₃/DMF-d₇, 5.00 ppm) δ: 36.83, 41.06, 56.30, 68.49, 69.92, 126.34, 128.28, 129.19, 131.18, and 134.74. FAB-MS (NBA) m/z: 443 (M+, [M+Na⁺]).

Bis[Cat-1-Phe]-N-4(ethylendioxy)bis(ethylamine) (14a) DEPC (phosphorochloridate) (0.83 g, 5.1 mmol) was added to a solution of 2,3-bis(benzoylato)benzoic acid (1.21 g, 3.63 mmol), and 12a (0.697 g, 1.58 mmol) and 14a (n-butyllithium, 4 mmol) at 4 °C in DMF (20 ml). The mixture was stirred first at 4 °C for 1 h, and then at room temperature overnight. Ice water was added and the solution extracted several times with CHCl₃. The combined organic extracts were dried over anhydrous Na₂SO₄, and evaporated dry. The crude residue was chromatographed on silica gel (41 g) with CHCl₃, and 3% MeOH/CHCl₃, (stepwise elution) as eluants. 13a was obtained as a colorless solid in 83.0% yield; R(A) = 0.66; HR-FAB-MS (NBA) m/z: 1075.4863 (Calcd for C₄₃H₄₅N₅, O₁₀ + M+H⁺, 1075.4857). The compound was a yellow solid.

Regarding the effect of K⁺ ions on the formation of a molecular complex of Eu³⁺ with 14a, the binding constant of Eu³⁺-14a was obtained using a prepared concentration of 14a: 7.14×10⁻⁵ M, [K⁺]: 7.7×10⁻⁵ M, and [Eu³⁺]: 0.0, 1.2, 2.3, 4.6, 7.0, 11.6, 18.2, 23.4, 34.8, 46.4, 69.6, 92.8 and 116.0×10⁻⁵ M.

Continuous Variation Method Stock solutions of Eu³⁺ (5.74×10⁻⁵ M) and 14a (5.74×10⁻⁵ M) were prepared in 10 mM HEPES (70% MeCN, pH 4.8). Before the measurement, the Eu³⁺ and 14a solutions were mixed and a standard solution was prepared with a [Eu³⁺]/[14a] ratio of 0.11, 0.25, 0.43, 0.67, 1.00, 1.50, 2.33, or 4.00. In all the spectra of 14a-Eu³⁺ complex in each [14a]/[Eu⁺] ratio, absorbance (OD) was measured at maximum bands.

Molar Ratio Method Stock solutions of Eu³⁺ (2.34×10⁻⁵ M) and 14a (2.34×10⁻⁵ M) were prepared in 10 mM HEPES (70% MeCN, pH 4.8). To a solution of Eu³⁺ (1.17×10⁻⁵ M) was added a solution of 14a (2.34×10⁻⁵ M) to prepare [14a]/[Eu⁺] with a molar ratio of 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, and 5.0 in all spectra of the 15a-Eu³⁺ complex in each [14a]/[Eu⁺] ratio, absorbance (OD) was measured at 340 nm.

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