

Site-Selective Recognition of Amino Acids by Co(III) Complexes Containing a $(N)(O)_3$-Type Tripodal Tetradentate Ligand

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The bis-*N*,*N*-carboxymethy-(*S*)-phenylalaninato carbonato cobalt(III) complex, $[Co(bcmpa)(CO_3)]^{2-}$, has been prepared as a simple model that enables the recognition of an amino acid (Haa) whose coordination behaviours in solution have been characterized by electronic absorption (AB), circular dichroism (CD) and ^{1}H NMR spectroscopies. The reaction of the $K_2[Co(bcmpa)(CO_3)]$ complex with amino acids (Haa) has predominantly afforded the [Co(bcmpa)(aa)] complex in the *trans*(*N*)-configuration mode, rather than in the *cis*(*N*)-form. By using amino acid derivatives with bulky substituents at their amino or carboxylate sites under a neutral condition, the reactions have been demonstrated to be initiated by coordination of the amino nitrogen site. Interestingly, the *cis*(*N*)-complex, which is isolated as a minor product, isomerizes to the *trans*(*N*)-form in the presence of active charcoal under pH 7 in an aqueous solution. The site-selective coordination of Haa to the $[Co(bcmpa)(CO_3)]^{2-}$ complex and the stereoselective isomerization of the $[Co(bcmpa)(aa)]^-$ complex have been explained to be regulated by weak non-covalent interactions within the ligands, whose origin has been discussed based on a detailed examination of the crystal structures of the *trans*(*N*)- and *cis*(*N*)-K[Co(bcmpa)(aa)] complexes.

Non-covalent interactions are essential for both specificity and high efficiency in biological systems. Enzyme–substrate, nucleobase–nucleobase, nucleic acid–protein, neurotransmitter–receptor, and other specific interactions are achieved via weak interactions, such as hydrogen bonding, electrostatic, hydrophobic, and aromatic ring stacking interactions.[1–4] The combination of various interacting groups leads to a sufficient "energy gain" and gives rise to specific binding of the substrate. At the active site, metalloenzyme-substrate binding may result in ligand–ligand interactions, which may then be considered to take place within the coordination sphere of the metal ion. Indeed, such interactions are known to play vital roles in enzyme catalysis. A classical example showing such weak interactions is the carboxypeptidase A–glycyl-(*S*)-tyrosine complex.[5,6] From such a viewpoint, by using metal complexes we have spectroscopically and structurally investigated weak interactions between their ligands,[7–15] and have recently succeeded to construct a novel amino acid-binding receptor based on weak non-covalent interactions.[16] The prepared molecular-recognition model is a Co(III) complex comprising the $(N)(O)_3$-type tripodal tetradentate ligand, bis-*N*,*N*-carboxymethyl-(*S*)-phenylalanine (H_3bcmpa), and a carbonato anion, which can site-specifically coordinate with an amino acid (Haa) in a *trans*(*N*)-form. The coordination specificity has been explained based on the X-ray crystal structures of the K_2[Co(bcmpa){(*S*)-leucine}] and K_2[Co(bcmpa){(*R*)-leucine}] complexes, which has suggested that the interactions between the coordination sites of bcmpa and aa ligands importantly contribute to the stability of their structures. Previously, Koine et al.[17–19] reported on the specific binding of amino acids to a *trans*(*N*)-form by using Co(III) complexes with similar $(N)(O)_3$-type tripodal tetradentate ligands, L-/D-alaninato-*N*,*N*-diacetate and nitrilotriacetate (nta), under a slightly basic condition. However, the specific preparation of *trans*(*N*)-form was not particularly significant, and the coordination mechanism was not explained in detail. Bernauer et al.[20,21] also found a similar behavior in the formation of Co(III) complexes with (*S*)-aspartic-*N*-monoacetic acid and amino acids, in which the individual complexes isolated, *trans*(*N*)- and *cis*(*N*)-isomers, indicated an isomerization leading to a mixture of *cis*(*N*)- and *trans*(*N*)-isomers. At this stage, it is very important to characterize the findings that the Co(III) complex with the ligand bcmpa, reported previously,[16] may have the ability to site-specifically recognize amino acids in an aqueous solution under neutral condition.

Here, reactions of the $K_2[Co(bcmpa)(CO_3)]$ with different amino acids or their derivatives with bulky substituent groups were performed under a neutral condition. We subsequently discovered a site-selective binding of Haa, a dominant preparation of the *trans*(*N*)-isomer, and a pronounced isomerization of *cis*(*N*)-form to *trans*(*N*)-one. Their molecular mechanisms were discussed on based on the X-ray crystal structure and solution structures obtained from UV-vis, ^{1}H NMR, and CD spectroscopies.

Experimental

Measurements. Electronic absorption and circular dichroism (CD) spectra were recorded on JASCO UVDEC-660 and JASCO J-500C spectrophotometers, respectively. All measurements were performed in an aqueous solution at room temperature. ^{1}H NMR spectra were taken with a JEOL JNM FX-400 spectrometer at 400 MHz in D_2O. The chemical shifts are shown in δ value (ppm)

from DSS as an internal standard. HPLC was observed with JASCO GULLIVER series, and the column was carried out on a finepak SIL NH2-5 or ODS.

Synthesis of the Co(III) Ternary Complexes with Various Amino Acids. A tripodal tetradentate ligand, H_3bcmpa, was prepared by the carboxymethylation of (*S*)-phenylalanine with bromoacetic acid in an aqueous alkaline solution, which was identified by its ^{1}H NMR spectrum and elemental analysis: ^{1}H NMR (D_2O) δ 7.34–7.44 (m, 5H, phenyl-H), 4.39 (dd, 1H, J = 8.5, 7.0 Hz, >CH–CH_2–), 4.08 (d, 2H, J = 17.4 Hz, –CH_2COO), 3.95 (d, 2H, J = 17.4 Hz, –CH_2COO), 3.42 (dd, 1H, J = 14.8, 7.0 Hz, >CH–CH_2–), 3.23 (dd, 1H, J = 14.8, 8.5 Hz, >CH–CH_2–); Anal. Calcd for $C_{13}H_{15}NO_6\cdot0.5H_2O$: C, 53.79; H, 5.56; N, 4.83%. Found: C, 54.05; H, 5.42; N, 4.97%.

Ternary complexes, K[Co(bcmpa)(aa)], were prepared according to a modified method from previous literature:[16] To 10 mL of a 0.2 M (1 M = 1 mol dm^{-3}) aqueous solution of K_2[Co(bcmpa)(CO_3)] prepared from K_3[Co(CO_3)$_3$] and H_3bcmpa was added an equimolar amount of amino acid neutralized with KOH. The resulting mixture was adjusted to pH 7.0 by adding 0.1 M HCl, and stirred at 50 °C for 12 h. The amino acids employed in the preparation were as follows: (*S*)-leucine ((*S*)-Hleu) (**1**), glycine (Hgly) (**2**), (*S*)-alanine ((*S*)-Hala) (**3**), (*S*)-phenylalanine ((*S*)-Hphe) (**4**), (*S*)-valine ((*S*)-Hval) (**5**), (*S*)-tryptophane ((*S*)-Htrp) (**6**), (*S*)-proline ((*S*)-Hpro) (**7**), and (*R*)-phenylalanine ((*R*)-Hphe) (**8**). After a treatment on an anion-exchange Sephadex column with an aqueous 0.1 M KCl solution, two diastereomers of Co(III) complexes were isolated; the first eluted band with reddish violet color was in the *trans*(*N*) configuration (**t**) and a secondary band with blue color was in the *cis*(*N*) form (**c**), whose isomers were determined based on the characteristic electronic absorption spectra. The coordination selectivity for the *trans*(*N*) and *cis*(*N*) complexes was followed by a HPLC measurement. All obtained complexes were identified by the electronic absorption, CD and ^{1}H NMR spectra and the elemental analyses, as follows:

trans(*N*)-K[Co(bcmpa){(*S*)-leu}]·3.5H_2O, **1t**.
Calcd for $C_{19}H_{24}CoKN_2O_8\cdot3.5H_2O$: C, 40.07; H, 5.49; N, 4.92%. Found: C, 39.78; H, 5.27; N, 4.91%.

cis(*N*)-K[Co(bcmpa){(*S*)-leu}]·4.5H_2O, **1c**.
Calcd for $C_{19}H_{24}CoKN_2O_8\cdot4.5H_2O$: C, 38.84; H, 5.66; N, 4.77%. Found: C, 38.67; H, 5.36; N, 4.76%.

trans(*N*)-K[Co(bcmpa)(gly)]·2.5H_2O, **2t**.
Calcd for $C_{15}H_{16}CoKN_2O_8\cdot2.5H_2O$: C, 36.37; H, 3.87; N, 5.66%. Found: C, 36.31; H, 3.80; N, 5.72%.

cis(*N*)-K[Co(bcmpa)(gly)]·3H_2O, **2c**.
Calcd for $C_{15}H_{16}CoKN_2O_8\cdot3H_2O$: C, 35.72; H, 4.40; N, 5.55%. Found: C, 35.66; H, 4.10; N, 5.50%.

trans(*N*)-K[Co(bcmpa){(*S*)-ala}]·3H_2O, **3t**.
Calcd for $C_{16}H_{18}CoKN_2O_8\cdot3H_2O$: C, 37.07; H, 4.67; N, 5.40%. Found: C, 37.15; H, 4.39; N, 5.46%.

cis(*N*)-K[Co(bcmpa){(*S*)-ala}]·3.5H_2O, **3c**.
Calcd for $C_{16}H_{18}CoKN_2O_8\cdot3.5H_2O$: C, 36.47; H, 5.08; N, 5.31%. Found: C, 36.22; H, 5.01; N, 5.26%.

trans(*N*)-K[Co(bcmpa){(*S*)-phe)]·3.5H_2O, **4t**.
Calcd for $C_{22}H_{22}CoKN_2O_8\cdot3.5H_2O$: C, 43.78; H, 4.84; N, 4.64%. Found: C, 43.60; H, 4.52; N, 4.70%.

cis(*N*)-K[Co(bcmpa){(*S*)-phe}]·2.5H_2O, **4c**.
Calcd for $C_{22}H_{22}CoKN_2O_8\cdot2.5H_2O$: C, 45.13; H, 4.65; N, 4.78%. Found: C, 45.44; H, 4.42; N, 4.87%.

trans(*N*)-K[Co(bcmpa){(*S*)-val}]·3H_2O, **5t**.
Calcd for $C_{18}H_{22}CoKN_2O_8\cdot3H_2O$: C, 42.02; H, 5.49; N, 5.45%. Found: C, 41.88; H, 5.30; N, 5.61%.

cis(*N*)-K[Co(bcmpa){(*S*)-val}]·4H_2O, **5c**.
Calcd for $C_{18}H_{22}CoKN_2O_8\cdot4H_2O$: C, 40.60; H, 5.68; N, 5.26%. Found: C, 40.61; H, 5.66; N, 5.11%.

trans(*N*)-K[Co(bcmpa){(*S*)-trp)]·2H_2O, **6t**.
Calcd for $C_{24}H_{23}CoKN_3O_8\cdot2H_2O$: C, 46.83; H, 4.42; N, 6.83%. Found: C, 46.50; H, 4.16; N, 6.82%.

trans(*N*)-K[Co(bcmpa){(*S*)-pro}]·3H_2O, **7t**.
Calcd for $C_{18}H_{20}N_2O_8CoK\cdot3H_2O$: C, 39.71; H, 4.81; N, 5.15%. Found: C, 39.56; H, 4.70; N, 5.01%.

trans(*N*)-K[Co(bcmpa){(*R*)-phe)]·2H_2O, **8t**.
Calcd for $C_{22}H_{22}CoKN_2O_8\cdot2H_2O$: C, 45.84; H, 4.55; N, 4.86%. Found: C, 46.01; H, 4.53; N, 4.97%.

X-ray Crystallographic Analysis of 2c. A single crystal of **2c** suitable for an X-ray diffraction measurement was obtained by slow diffusion of an aqueouos solution at room temperature for a few days. The crystal was mounted on a glass capillary, and the diffraction data were collected on a Rigaku RAXIS-IV imaging plate area detector using graphite-monochromated Mo $K\alpha$ radiation at room temperature. X-ray data for **2c**: $C_{30}H_{42}Co_2K_2N_4O_{21}$, M_r = 786.71, monoclinic, space group $P2_1$; a = 14.500(4), b = 6.9312(7), c = 20.471(3) Å, β = 103.25(2)°, V = 2002.5 Å^3; ρ_{calc} = 1.636 g cm^{-3}, Z = 2; $F(000)$ = 1012; μ(Mo $K\alpha$) = 11.18 cm^{-1}; 3760 reflections collected, 3170 ($I > 3\sigma(I)$) reflections observed; R_1 = 0.032; wR_2 = 0.043. All of the structures were solved by combining a direct method and Fourier techniques, and all of the non-hydrogen atoms were anisotropically refined by full-matrix least-squares calculations. Atomic-scattering factors and anomalous-dispersion terms were taken from the International Tables for X-ray Crystallography.[22] Hydrogen atoms were loctated from difference Fourier maps and refined isotropically with thermal parameters based on the corresponding atoms, [U(H) = 1.2U_{eq}(C,N,O)]. All calculations were carried out on a Japan SGI workstation computer using the teXsan program.[23] The complete data are deposited as Document No. 74035 at the Office of the Editor of Bull. Chem. Soc. Jpn. Crystallographic data have been also deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publiucation citation and the deposition number 159127.

Reactions of K_2[Co(bcmpa)(CO_3)] with *N*-/*O*-Protected Amino Acids. The reaction of K_2[Co(bcmpa)(CO_3)] with an *N*-protected amino acid, phthaloyl glycine, was carried out according to the above-mentioned method. A very trace amount of a pink-colored univalent negative charged complex, which was first adsorbed, was eluted with a 0.1 M KCl solution, and a blue-violet main band, which was adsorbed second, was eluted with a 0.4 M KCl solution. The former was the main product and the latter was the starting complex, K_2[Co(bcmpa)(CO_3)].

A reaction with *O*-protected amino acid, such as glycine benzyl ester, (*S*)-phenylalanine benzyl ester, glycine ethyl ester and (*S*)-phenylalanine ethyl ester, was also carried out at pH 7. Two product complexes with a univalent negative charge, adsorbed as reddish-violet and blue-violet bands on a QAE Sephadex column, were separately obtained in two equal amounts of fractions, which are referred to complexes **t** and **c**, respectively.

Isomerization of *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ to *trans*(*N*)-[Co(bcmpa)(aa)]$^-$. An aqueous solution (100 mL) of *cis*(*N*)-K[Co(bcmpa)(aa)] complex (3.9×10^{-3} mol) (aa = gly, (*S*)-ala and (*S*)-leu) containing active charcoal (0.02 g) adjusted at pH 7.0 (KH_2PO_4–KOH buffer) was vigorously stirred at 50 °C, in which the ionic strength was kept at 0.20 mol dm^{-3} by KCl. The reaction mixture was examined at an appropriate time by electronic absorption and CD spectra after removing the active charcoal.

Results and Discussion

Preparation of Co(III)-bcmpa-aa Ternary Complexes. Reactions of K_2[Co(bcmpa)(CO_3)] with several amino acids (**1–8**) were performed in both cases of the presence and absence of active charcoal. In the absence of active charcoal, the reaction gave univalent negative charged Co(III) ternary complexes, which were resolved as two bands by an anion exchange column. The lst (**t**) and 2nd (**c**) adsorbed bands exhibited the first d–d transition absorption peaks at about 510 nm (19.6 k cm^{-1}) and 570 nm (17.5 k cm^{-1}), respectively, which are characteristic of *trans*(*N*)- and *cis*(*N*)-[$Co(N)_2(O)_4$]-type complexes, respectively.[16–19,24–28] The complexes **t** and **c** were assigned to *trans*(*N*)- and *cis*(*N*)-[Co(bcmpa)(aa)]$^-$, respectively. The electronic absorption spectral data for some amino acid complexes are listed in Table 1. The formation reactions of *trans*(*N*)- and *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ in the absence of active charcoal were followed using HPLC, and in the cases of all amino acid complexes the *trans*(*N*)-species were predominantly obtained; the ratio of t and c, as described previously,[16] was about 6:1 at the stage of 30% conversion of the reaction. The yields of the ternary Co(III)-bcmpa-aa systems were 20–30% as a mixture of **t** and **c** after a reaction time of 12 hrs. The selectivity of the *cis*(*N*)-/*trans*(*N*)-species did not depend on the kinds of Haa, except for the cases of (*S*)-trp, (*S*)-pro and (*R*)-phe. The reaction of K_2[Co(bcmpa)(CO_3)] with (*S*)-trp, (*S*)-pro or (*R*)-phe gave only the *trans*(*N*)-species. Judging from a consideration of the CPK model, the reason that the *cis*(*N*)-K[Co(bcmpa)((*S*)-pro)] was not prepared may be explained by a steric repulsion between the G-ring protons of bcmpa and the (*S*)-proline ring, although we have no idea concerning the cases of (*S*)-trp and (*R*)-phe.

On the other hand, in the presence of active charcoal, the reaction time became a few-times faster compared with that in the absence of active charcoal; furthermore, the yields of the ternary Co(III)-bcmpa-aa systems increased up to 50–60%. Interestingly, the Co(III) complex obtained under this condition was only the *trans*(*N*)-[Co(bcmpa)(aa)]$^-$ species. Apparently, the active charcoal contributes to not only the promotion of complex formation, but also to isomerization from the *cis*(*N*)- to *trans*(*N*)-species. The coordination selectivity and isomerization were spectroscopically examined, as discussed below.

Reactions of K_2[Co(bcmpa)(CO_3)] with *N*-/*O*-Protected Amino Acids. An amino acid can bind with a metal ion as a didentate chelating ligand through the amino nitrogen and carboxyl oxygen atoms. In order to examine which atom, amino nitrogen or carboxyl oxygen atom initially binds with the central cobalt ion, reactions of K_2[Co(bcmpa)(CO_3)] with phthaloyl glycine or glycine ethyl ester, where the amino nitrogen or carboxyl oxygen is protected by bulky substituents, respectively, were carried out according to the same method as descrived above in the absence of active charcoal. The former hardly afforded a ternary Co(III) complex with phthaloyl glycine, although the starting complex, [Co(bcmpa)(CO_3)]$^{2-}$, was recovered. On the other hand, the latter gave a ternary Co(III) complex with not glycine ester, but glycine. It seems that the formation of the [Co(bcmpa)(gly)]$^-$ anion results in a hydrolysis of the ester carboxyl carbon activated by coordination to the metal ion. Interestingly, the obtained complex was dominant in the *cis*(*N*)-species more than ten times compared with the *trans*(*N*)-one. Although this reaction was also performed for glycine benzyl ester, (*S*)-phenylalanine ethyl ester, and (*S*)-phenylalanine benzyl ester, they gave the same results. The above findings suggest that the coordination of amino acid to Co(bcmpa) is initiated by an attack of the amino nitrogen atom to predominantly afford the *cis*(*N*)-isomer. Since the coordination sites around the Co(III) ion in the [Co(bcmpa)(CO_3)]$^{2-}$ complex are surrounded by anionic groups, and the total charge of the complex is also negative, an attack of amino acids to the Co(III) complex would be carried out by the amino group, rather than carboxyl one. The dominant preparation of the *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ complex suggests that this reaction proceeds during the course of hydrolytic cleavege of the ester, because in those cases with amino acids the formation of *trans*(*N*)-species was dominant.

Isomerization from *cis*(*N*)-Complex to *trans*(*N*)-One as Measured by CD Spectroscopy. The CD spectra for the ternary Co(bcmpa)(aa) systems exhibited d–d absorption peaks at 16–27 k cm^{-1}, which are given in Table 2. The *cis*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$ (**1c**) exhibited intense CD peaks at 17.6, 20.0, and 26.1 k cm^{-1} with signs of (−), (+), and (−), respectively, whereas the *trans*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$ (**1t**) showed intense absorption maxima at 16.3, 18.5, 20.7, and 26.9 k cm^{-1} with signs of (+), (+), (−), and (+), respectively.[17–19,26–28]

Remarkable changes at a constant pH of 7.0 were observed in the electronic absorption and CD spectra for *cis*(*N*)-K[Co(bcmpa)(aa)] complexes, depending on time in the pres-

Table 1. Electronic Absorption Spectral Data in d–d Transion Region of *trans*(*N*)- and *cis*(*N*)- [Co(bcmpa)(aa)]$^-$ Complexes[a)]

aa	*trans*(*N*)-Configuration (**t**)				*cis*(*N*)-Configuration (**c**)			
	Band 1		Band 2		Band 1		Band 2	
	σ_{1max}	log ε_1	σ_{2max}	log ε_2	σ_{1max}	log ε_1	σ_{2max}	log ε_2
(*S*)-leu (**1**)	19.6	2.24	26.8	2.25	17.6	2.31	25.9	2.21
gly (**2**)[b)]	19.6	2.21	26.7	2.22	17.6	2.31	25.9	2.22
(*S*)-ala (**3**)	19.6	2.25	26.8	2.25	17.6	2.30	26.1	2.23
(*S*)-phe (**4**)	19.6	2.23	26.8	2.23	17.9	2.30	25.7	2.24
(*S*)-val (**5**)	19.6	2.20	26.8	2.21	17.8	2.30	26.0	2.22
(*S*)-trp (**6**)	19.7	2.22	27.7	2.53	—[c)]		—[c)]	
(*S*)-pro (**7**)	19.4	2.20	26.5	2.25	—[c)]		—[c)]	
(R)-phe (**8**)	19.6	2.23	26.8	2.26	—[c)]		—[c)]	

a) $\sigma_{1max}/10^3$ cm^{-1}, log ε (ε/M^{-1} cm^{-1}). b) Ref. 16. c) *cis*(*N*)-Configuration was not detected.

Table 2. CD Spectral Data in d–d Transition Region of the *trans*(*N*)- and *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ Complexes[a)]

AA	*trans*(*N*)-Configuration (**t**)								*cis*(*N*)-Configuration (**c**)					
	Band 1						Band 2		Band 1				Band 2	
	σ_{1max}	$\Delta\varepsilon_1$	σ_{2max}	$\Delta\varepsilon_2$	σ_{3max}	$\Delta\varepsilon_3$	σ_{4max}	$\Delta\varepsilon_4$	σ_{5max}	$\Delta\varepsilon_5$	σ_{6max}	$\Delta\varepsilon_6$	σ_{7max}	$\Delta\varepsilon_7$
(*S*)-leu (**1**)	16.4	+0.17	18.5	+0.28	20.7	−1.02	26.7	+0.41	17.7	−0.49	19.8	+0.91	26.0	−0.24
gly (**2**)	16.3	+0.34	18.9	+0.18	—[b)]		—[b)]		17.0	+0.08	19.7	+0.24	25.9	−0.03
(*S*)-ala (**3**)	16.4	+0.32	18.5	+0.33	20.6	−0.88	26.8	+0.42	17.7	−0.33	20.0	+0.66	26.5	−0.14
(*S*)-phe (**4**)	16.4	+0.18	18.4	+0.31	20.6	−1.14	26.7	+0.43	17.8	−0.30	20.1	+0.70	26.1	−0.20
(*S*)-val (**5**)	16.4	+0.20	18.5	+0.30	20.6	−0.93	26.7	+0.41	17.7	−0.41	19.9	+0.78	26.2	−0.19
(*S*)-trp (**6**)	16.4	+0.11	18.5	+0.23	20.6	−1.12	26.7	+0.34						
(*S*)-pro (**7**)	16.1	+0.19	18.7	+0.44	20.8	−0.60	27.0	+0.40						
(*R*)-phe (**8**)	16.3	+0.59	—[b)]		20.5	−1.27	26.8	−0.30						

a) $\sigma_{max}/10^3$ cm^{-1}, $\Delta\varepsilon$/M^{-1} cm^{-1}. b) Not detected.

ence of a small amount of active charcoal, in which the spectra at the end point of the reaction were attributed to those of the corresponding *trans*(*N*)-[Co(bcmpa)(aa)]$^-$ complexes. Figure 1 shows the CD spectral change from *cis*(*N*)-[Co(bcmpa){(*S*)-leu)]$^-$ (**1c**) to *trans*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$ (**1t**) at 50 °C at pH 7. This spectral change proceeds with three isochromic points at 336, 425, and 533 nm, and the spectrum of the final product coincides completely to that of the *trans*(*N*)-isomer (**1t**). These findings indicate that this isomerization has been under way through an interconversion from *trans*(*N*)- to *cis*(*N*)-form without any side reactions. This clearly suggests that the *trans*(*N*)-isomer is thermodynamically stable compared with the *cis*(*N*)-one. Although similar isomerization has also been reported in the Co(III) complex with (*S*)-aspartic-*N*-monoacetic acid and different amino acids by Bernauer et al.,[20,21] the products led from each of the *trans*(*N*)- and *cis*(*N*)-complexes were a mixture of the *trans*(*N*)- and *cis*(*N*)-isomers. The clear isomerization from *trans*(*N*)- to *cis*(*N*)-species found in this system is quite significant from the viewpoint of the molecular-recognition mechanism occurring near to the metal centers in biological systems.

^{1}H NMR Spectra. ^{1}H NMR spectroscopy is a powerful method for detecting even weak interligand interactions in metal complexes. As is obvious from the spectral patterns for *trans*(*N*)-K[Co(bcmpa)(gly)] (**2t**) (Fig. 2(a)) and *cis*(*N*)-K[Co(bcmpa)(gly)] (**2c**) (Fig. 2(b)), the difference between the coordination surroundings around the *trans*(*N*)- and *cis*(*N*)-ternary complexes is clearly distinguished in the ^{1}H NMR spectra, which are listed in Tables 3 and 4, respectively. All proton signals of [Co(bcmpa)(aa)]$^-$ were assigned based on comparisons of those of [Co(nta)(aa)]$^-$,[17–19,26–29] as shown in Figs. 2(a) and 2(b).

The ^{1}H NMR peaks of R- and G-ring methylene protons of the bcmpa ligand in *trans*(*N*)-[Co(bcmpa)(aa)]$^-$ exhibited about 0.2 ppm down-field shifts compared with the corresponding proton peaks of *cis*(*N*)-[Co(bcmpa)(aa)]$^-$, which may be explained by the *trans* influence, which is affected by the difference in the coordination atoms of aa in the trans posi-

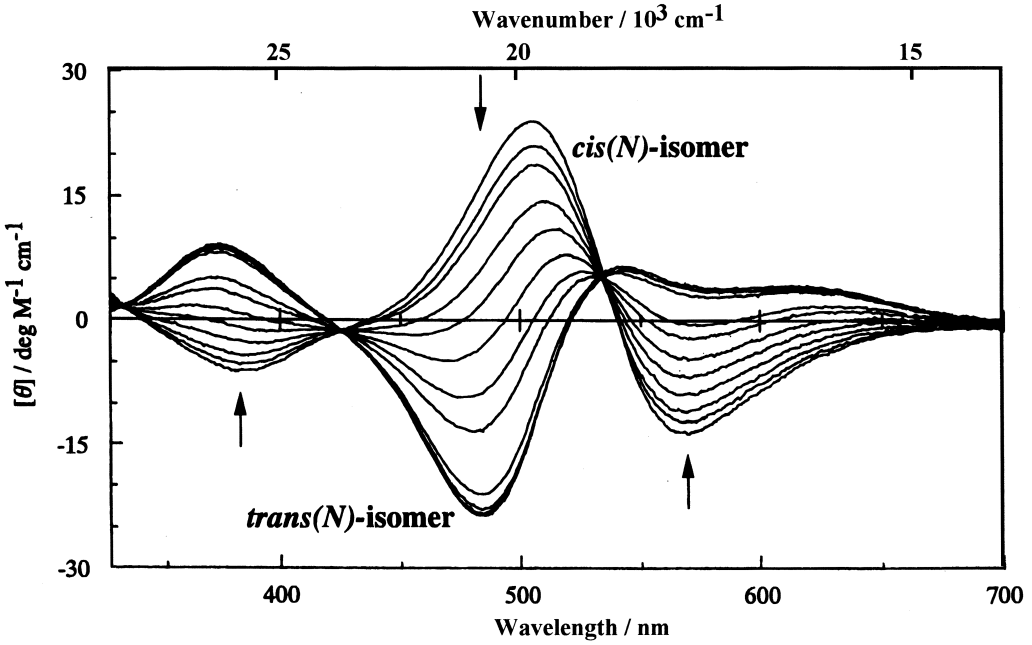


Fig. 1. CD spectral change with reaction time during the isomerization from complexes **1c** to **1t** in the presence of active charcoal (0.02 g/100mL). Time (min) = 0, 15, 35, 55, 85, 120, 150, 180, 245, 300, 360, 420. Experimental conditions: T = 50 °C, pH 7, [Co] = 3.9×10^{-3} M.

Table 3. ^{1}H NMR Chemical Shift Values of Methylene and Methine Protons of *trans*(*N*)-[Co(bcmpa)(aa)]$^-$ Complexes[a,b]

	(*S*)-leu (**1t**)[c]	gly (**2t**)	(*S*)-ala (**3t**)	(*S*)-phe (**4t**)	(*S*)-val (**5t**)	(*S*)-trp (**6t**)	(*S*)-pro (**7t**)	(*R*)-phe (**8t**)
α-H (aa)	3.85 (d,d)	3.75 (d)	3.93 (m)	4.11 (d,d)	3.86 (d)	4.20 (d,d)	4.24 (m)	4.06 (d,d)
	J = 10.4,	3.80 (d)		J = 9.6,	J = 3.1	J = 10.5,		J = 9.3,
	3.7	J = 17.3		4.7		5.0		4.7
R-ring H	4.58 (d),	4.60 (d),	4.59 (d),	4.56 (d),	4.57 (d),	4.49 (d),	4.63 (d),	4.57 (d)
(bcmpa)	3.93 (d)	3.94 (d)	3.94 (d)	3.91 (d)	3.91 (d)	3.86 (d)	3.98 (d)	3.91 (d)
	J = 18.3	J = 18.3	J = 18.0	J = 18.0	J = 17.9	J = 17.9	J = 18.1	J = 18.2
G-ring H	4.18 (d),	4.16 (d),	4.18 (d),	4.17 (d),	4.13 (d),	4.13 (d),	4.20 (d),	4.16 (d),
(bcmpa)	3.23 (d)	3.21 (d)	3.23 (d)	3.20 (d)	3.19 (d)	3.21 (d)	3.25 (d)	3.16 (d)
	J = 16.5	J = 16.6	J = 16.5	J = 16.5	J = 16.5	J = 16.5	J = 16.3	J = 16.4
α-H	5.04 (d,d)	5.01 (d,d)	5.03 (d,d)	5.03 (d,d)	4.98 (d,d)	5.00 (d,d)	5.00 (d,d)	5.04 (d,d)
(bcmpa)	J = 10.4,	J = 10.5,	J = 10.8,	J = 10.5,	J = 10.4,	J = 10.5,	J = 10.3,	J = 10.6,
	5.2	5.0	5.1	5.0	5.2	4.8	5.3	5.0
β-H	3.61 (d,d)	3.61 (d,d)	3.61 (d,d)	3.60 (d,d)	3.56 (d,d)	3.57 (d,d)	3.61 (d,d)	3.60 (d,d)
(bcmpa)	J = 15.1,	J = 15.1,	J = 15.0,	J = 15.2,	J = 15.3,	J = 15.0,	J = 15.0,	J = 15.2,
	5.2	5.0	5.1	5.0	5.2	4.8	5.3	5.0
	3.50 (d,d),	3.50 (d,d)	3.50 (d,d)	3.48 (d,d)	3.46 (d,d)	3.43 (d,d)	3.51 (d,d)	3.47 (d,d)
	J = 15.1,	J = 15.1,	J = 15.0,	J = 15.2,	J = 15.3,	J = 15.0,	J = 15.0,	J = 15.2,
	10.4	10.5	10.8	10.5	10.4	10.5	10.3	10.6

a) Chemical shift in ppm from DSS. b) J value in Hz. c) Ref. 16.

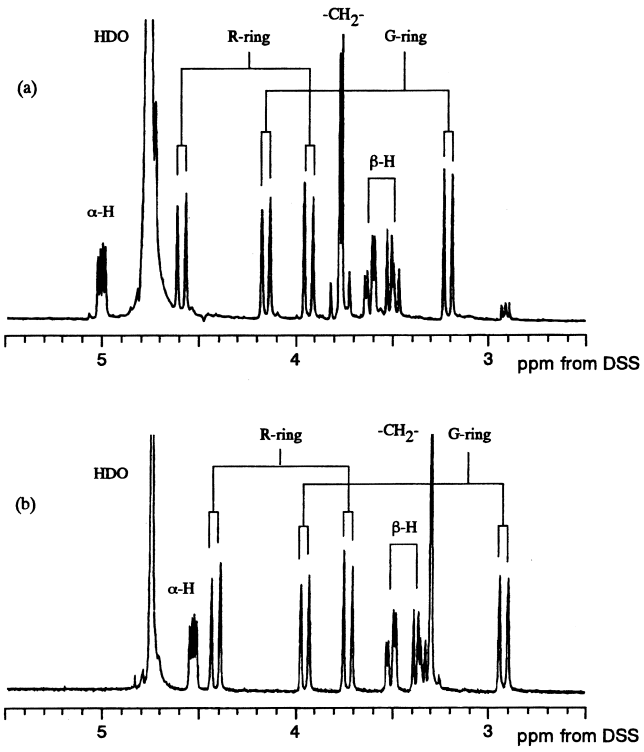


Fig. 2. ^{1}H NMR spectra for *trans*(*N*)-K[Co(bcmpa)(gly)] (**2t**) (a) and *cis*(N)-K[Co(bcmpa)(gly)] (**2c**) (b).

tion of the tertiary nitrogen atoms of bcmpa, amino nitrogen or carboxyl oxygen atom. The α-H of the coordinated aa also demonstrated a large down-field shift, which may be due to differences in the coordination forms of aa. However, the larger down-field shift observed in α-H of bcmpa of the *trans*(*N*)-species (δ ca. 5.0 ppm), compared with those of the *cis*(*N*)-species (δ ca. 4.5 ppm), can not be explained only by the trans influence, which is probably due to hydrogen bonding between the α-H atom of bcmpa and carboxyl oxygen of aa, as previously suggested from the crystal structures of the *trans*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$ and *cis*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$;[16] the distances between the α-carbon and carboxyl oxygen of (*S*)-leu in *trans*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$, 2.83 and 2.84 Å, are within the range of the usual hydrogen-bonding distances, and are significantly short compared with that between the α-carbon and amino nitrogen of (*S*)-leu in *cis*(*N*)-[Co(bcmpa){(*S*)-leu}]$^-$, 3.06 Å. Furthermore, one of the G-ring methylene protons is observed at a higher-field region (δ 0.8–1.0 ppm) in both the *trans*(*N*)- and *cis*(*N*)-species compared with those of the corresponding nta complexes, suggesting that they were shielded by the aromatic ring of the bcmpa side chain, even in an aqueous solution, judging from the crystal structures of **1**, established previously.[16]

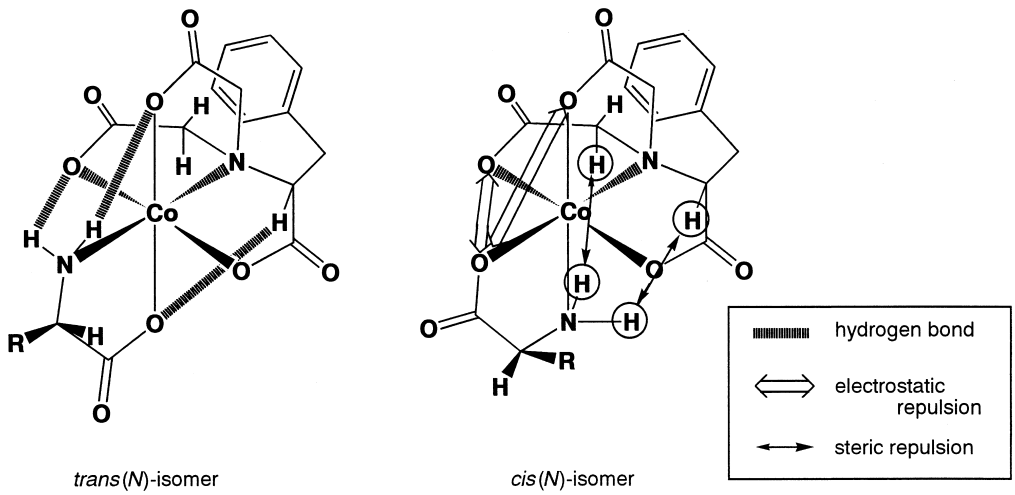
Crystal Struture of *cis*(*N*)-K[Co(bcmpa)(gly)] (2c). Fortunately, a single crystal of **2c** suitable for X-ray analysis was obtained. The crystal structure, although it contains two independent molecules of **2c** (Fig. 3) and five water molecules, is almost the same as that of *cis*(*N*)-K[Co(bcmpa)((*S*)-leu)] (**1c**) reported previously.[16] As expected, some steric and electrostatic repulsions were detected between bcmpa and gly. The former repulsions were found between the α-proton of bcmpa and amino hydrogens of gly: a large N(11a)–Co(a)–N(41a) (99.7(2)°) and N(11b)–Co(b)–N(41b) angles (100.6(2)°) and elongated C(11a)...N(41a) (3.19 Å) and C(11b)...N(41b) distances (3.11 Å). The latter one was observed between electronegative carboxylate oxygen atoms of bcmpa and aa; a longer Co(a)–O(33a) (1.909(5) Å) and Co(b)–O(23b) bonds (1.909(4) Å) and a slightly larger O(44a)–Co(a)–O(carboxylate of bcmpa, O(13a), O(23a) and O(33a)) (88.2(2), 92.1(2) and 95.4(2)°) and O(44b)-Co(b)-O(carboxylate of bcmpa, O(13b), O(23b) and O(33b)) (88.1(2), 89.4(2) and 98.4(2)°).

From the crystal structures of *trans*(*N*)-K[Co(bcmpa)((*S*)-leu)] (**1t**) and *cis*(*N*)-K[Co(bcmpa)((*S*)-leu)] (**1c**), reported previously,[16] and that of *cis*(*N*)-K[Co(bcmpa)(gly)] (**2c**), it is ap-

Table 4. ^{1}H NMR Chemical Shift Values of Methylene and Methine Protons of *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ Complexes[a,b]

	(*S*)-leu (**1c**)[c]	gly (**2c**)	(*S*)-ala (**3c**)	(*S*)-phe (**4c**)	(*S*)-val (**5c**)
α-H	3.33 (m)	not identified	3.48 (m)	3.67 (d,d)	3.38 (m)
				J = 7.9, 5.2	
R-ring H (bcmpa)	4.38 (d), 3.74 (d)	4.43 (d), 3.74 (d)	4.40 (d), 3.78 (d)	4.36 (d), 3.70 (d)	4.40 (d), 3.75 (d)
	J = 17.9	J = 18.0	J = 17.9	J = 18.2	J = 18.2
G-ring H (bcmpa)	4.25 (d), 2.96 (d)	3.95 (d), 2.92 (d)	4.09 (d), 2.96 (d)	4.04 (d), 2.90 (d)	4.25 (d), 2.91 (d)
	J = 17.0	J = 17.0	J = 17.0	J = 17.0	J = 17.0
α-H (bcmpa)	4.58 (d,d)	4.54 (d,d)	4.50 (d,d)	4.43 (d,d)	—[d]
	J = 9.5, 5.2	J = 10.1, 4.9	J = 10.1, 5.2	J = 9.5, 5.2	
β-H (bcmpa)	3.49 (d,d)	3.50 (d,d)	3.50 (d,d)	3.43 (d,d)	3.51 (d,d)
	J = 15.1, 5.2	J = 15.3, 4.9	J = 15.2, 5.2	J = 15.3, 5.2	J = 15.1, 4.6
	3.35 (d,d)	3.36 (d,d)	3.36 (d,d)	3.30 (d,d)	3.36 (d,d)
	J = 15.1, 9.5	J = 15.3, 10.1	J = 15.2, 10.1	J = 15.3, 9.5	J = 15.1, 10.8

a) Chemical shift in ppm from DSS. b) J value in Hz. c) Ref. 16. d) Not detected.



Scheme 1. Structures of *trans*(*N*)- and *cis*(*N*)-[Co(bcmpa)(aa)]$^-$complexes.

parent that the hydrogen bonding, steric, and electrostatic interactions contribute to the site-selective recognition of amino acids that the *trans*(*N*)-species is prefered to *cis*(*N*)-one.

Molecular Mechanism in *N*-/*O*-Recognition of Amino Acid by the Co(III) Ternary Complex. As described above, the facts that the [Co(bcmpa)(CO_3)]$^{2-}$ complex favors the attack of the amino nitrogen of aa over the carboxyl oxygen and that the [Co(bcmpa)(aa)]$^-$ complex prefers site-selectively *trans*(*N*)- to *cis*(*N*)-coordination for amino acids were characterized. In this experiment, clear evidence for the coordination selectivity of Haa to the Co(III) complex is quite unique, although isomerization of the Co(III) complexes of amino acids has been previously reported to proceed under a basic condition (pH > 9) by Bernauer et al.[20,21] However, in their system the products led from each of the *trans*(*N*)- and *cis*(*N*)-complexes were a mixture of *trans*(*N*)- and *cis*(*N*)-isomers. The study presented here was carried out under neutral condition, and was examined in both cases of the presence and absence of active charcoal. From a detailed examination of their ^{1}H NMR spectra and the X-ray structures of **1c**, **2t** and **2c**, described below, three structural features were revealed in the ligand-ligand interactions between bcmpa and aa, as shown in Scheme 1. At first, as suggested from the crystal structure of **1t**[16] and a large down-field shift of α-H's in the ^{1}H NMR spectra of *trans*(*N*)-K[Co(bcmpa)(aa)] complexes in comparison with those of *cis*(*N*)-K[Co(bcmpa)(aa)] complexes,[16] the hydrogen bonding interactions significantly contribute to the higher stability of the *trans*(*N*)-species **1t**. Secondly, an interesting structural feature was found in the crystal structures of *trans*(*N*)-K[Co(bcmpa){(*S*)-leu}] (**1t**) and *cis*(*N*)-K[Co(bcmpa){(*S*)-leu)] **1c**.[16] The angles N(11)–Co–N(41) (99.6(2)°) in **1c** and N(11a)–Co(a)–N(41a) (99.7(2)°) and N(11b)–Co(b)–N(41b) angles (100.6(2)°) in **2c** is significantly larger than the angle O(44)–Co–N(11) (92.3(3) and 93.3(2)°) in **1t**, indicating that a steric repulsion between the amino hydrogens of aa and G-ring methylene protons of bcmpa in **1c** and **2c** caused such an enlargement. The fact that the reaction of [Co(bcmpa)(CO_3)]$^{2-}$ with (*S*)-pro gives no *cis*(*N*)-form may suggest such a steric repulsion due to a large substituent group attached to the amino nitrogen of (*S*)-pro, although we can not give a clear interpretation for the reason that the *cis*(*N*)-species are not formed in the cases of (*S*)-trp and (*R*)-phe. Thirdly, an electrostatic repulsion may also contribute to the lability of the *cis*(*N*)-form, because the carboxylate oxygen of amino acid in the *cis*(*N*)-form is surrounded by three carboxyl oxygen atoms of bcmpa with negative charges. The above finding indicates

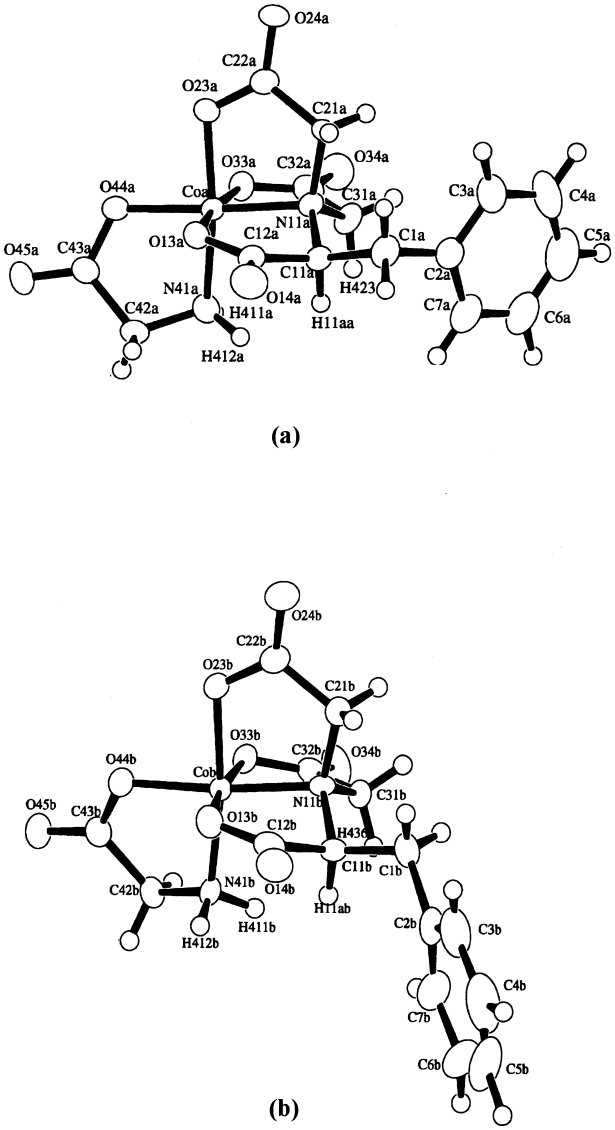


Fig. 3. ORTEP drawings of the two independent anion parts in the crystal structure of **2c**.

that the non-covalent interactions assembled on the ternary Co(III) complexes acted as a dominant factor for isomerizing *cis*(*N*)-[Co(bcmpa)(aa)]$^-$ to *trans*(*N*)-form, in which the *trans*(*N*)-species is thermodynamically more stable than the *cis*(*N*)-one.

We succeeded in constructing an interesting lower molecular weight model that can site-specifically recognize an amino acid through some non-covalent interactions. A detailed examination of molecular recognition mechanism revealed that the coordination of Haa to the $[Co(bcmpa)(CO_3)]^{2-}$ complex is initiated by an attack of the amino group, and that the geometry around the Co(III) ion terminates to the *trans*(*N*)-isomer, which is thermodynamically stable. The present findings suggest that the combination of non-covalent interactions make possible the recognition and discrimination of molecules, even if their bonds are not very strong. The high specificity of the substrate in an enzyme may be understood by an assembly of such weak interactions.

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References

1 B. Albert, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson, "Molecular Biology of the Cell," 3rd ed, Garland, New York (1994), p. 89.

2 E. Frieden, *J. Chem. Educ.*, **52**, 754 (1975).

3 W. P. Jencks, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **43**, 219 (1975).

4 O. Yamauchi, A. Odani, H. Masuda, and H.Sigel, "Stacking Interactions Involving Nucleotides and Metal Ion Complexes," In "Metal Ions in Biological Systems," ed by A. Sigel, and H. Sigel; Dekker, New York (1996), Vol. 32, p. 207.

5 W. N. Lipscomb, Jr.,G. N. Reeke, J. A. Hartsuck, F. A. Quiocho, and P. H. Bethge, *Phil. Trans. Roy. Soc. London, Ser. B*, **257**, 177 (1970).

6 D. W. Christianson, and W. L. Lipscomb, *Acc. Chem. Res.*, **22**, 62 (1989).

7 K. Jitsukawa, K. Iwai, H. Masuda, H. Ogoshi, and H. Einaga, *Chem. Lett.*, **1994**, 303.

8 K. Jitsukawa, K. Iwai, H. Masuda, H. Ogoshi, and H. Einaga, *J. Chem. Soc., Dalton Trans.*, **1997**, 3691.

9 T. Sugimori, K. Shibakawa, H. Masuda, A. Odani, and O. Yamauchi, *Inorg. Chem.*, **32**, 4951 (1993).

10 T. Sugimori, H. Masuda, and O. Yamauchi, *Bull. Chem. Soc. Jpn.*, **67**, 131 (1994).

11 T. Sugimori, H. Masuda, N. Ohata, K. Koiwai, A. Odani, and O. Yamauchi, *Inorg. Chem.*, **36**, 576 (1997).

12 T. Hayashi, T. Miyahara, N. Koide, Y. Kato, H. Masuda, and H. Ogoshi, *J. Am. Chem. Soc.*, **119**, 7281 (1997).

13 H. Kumita, K. Jitsukawa, H. Masuda, and H. Einaga, *Inorg. Chim. Acta*, **298**, 160 (1998).

14 M. Mizutani, I. Kubo, K. Jitsuakawa, H. Masuda, and H. Einaga, *Inorg. Chem.*, **38**, 420 (1999).

15 M. Mizutani, S. Tomosue, H. Kinoshita, K. Jitsukawa, H. Masuda, and H. Einaga, *Bull. Chem. Soc. Jpn.*, **72**, 981 (1999).

16 K. Jitsukawa, T. Morioka, H. Masuda, H. Ogoshi, and H. Einaga, *Inorg. Chim. Acta*, **216**, 249 (1994).

17 N. Koine, N. Sakota, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **42**, 1583 (1969).

18 N. Koine, N. Sakota, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **42**, 1779 (1969).

19 N. Koine, N. Sakota, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **43**, 1737 (1970).

20 G. Colomb and K. Bernauer, *Helv. Chim. Acta*, **60**, 459 (1977).

21 G. Colomb and K. Bernauer, *Helv. Chim. Acta*, **60**, 468 (1977).

22 J. A. Ibers and W. C. Hamilton, International Tables for X-ray Crystallography; Vol. IV, Kynoch Press, Birmingham, U. K. (1974).

23 teXsan, Crystal Structure Analysis Package, Molecular Structure Corporation, 1985 & 1992.

24 H. Yamatera, *Bull. Chem. Soc. Jpn.*, **31**, 95 (1958).

25 N. Matsuoka, J. Hidaka, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **40**, 1868 (1967).

26 K. Akamatsu, T. Komorita, and Y. Shimura, *Bull. Chem.*

Soc. Jpn., **54**, 3000 (1981).
27 K. Akamatsu, T. Komorita, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **55**, 140 (1982).
28 K. Akamatsu, T. Komorita, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **55**, 2390 (1982).
29 N. Koine, H. Yamaguchi, T. Tanigaki, J. Hidaka, and Y. Shimura, *Chem. Lett.*, **1974**, 993.