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Amino Acid Spin Labels. An Application of Chelation Ability to a Nickel(II) Ion

Sayaka Osada, Kazuki Igarashi, Takashi Nogami, and Takayuki Ishida*
Department of Applied Physics and Chemistry, The University of Electro-Communications, Chofu, Tokyo 182-8585

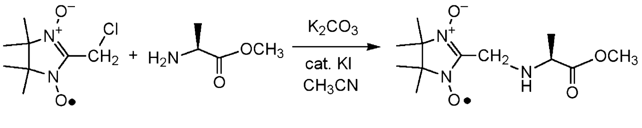
(Received March 10, 2010; CL-100226; E-mail: ishi@pc.uec.ac.jp)

NN–CH_2-substituted L-alanine methyl ester (NNCH_2–L-Ala–OMe) and β-alanine ethyl ester (NNCH_2–β-Ala–OEt) were prepared, where NN stands for 4,4,5,5-tetramethylimidazolin-1-oxyl 3-oxide. Their nickel(II) perchlorate complexes with a metal/ligand ratio of 1/2 were structurally and magnetically characterized.

Spin-label techniques have been utilized for probing supramolecular environments especially in biological functional systems,[1] and spin-labeled lipids, nucleic acids, carbohydrates as well as peptides have been exploited for that purpose.[1,2] Spin-labeled compounds also contribute to development of functional magnetic materials as air-stable spin carriers.[3,4] Amino acids are known to be good chelating agents to transition-metal ions,[5] and some proteins and peptides, such as valinomycin, are known to work as ionophores in biological systems.[6] Nitroxide oxygen atoms can ligate metal ions as well.[7] In the present study, we have developed novel amino acid spin labels, which turned out to be oily, and we moved to characterization of them as crystalline solids by complex formation with transition-metal ions.

The nitronyl nitroxide radical group (abbreviated as NN; 4,4,5,5-tetramethylimidazolin-1-oxyl 3-oxide)[8] has been well investigated, and the NN–CH_2– group could be substituted onto the amino group using a precursory NN–CH_2Cl reagent.[9] We supposed that α- and β-alanine derivatives would be a promising prototype for paramagnetic amino acids and peptides. The carboxylic acid moiety has been protected as an ester, and such esters are commercially available. According to the literature methods for NN–CH_2-substituted azacrowns,[10] substitution reaction of L-Ala–OMe•HCl with NN–CH_2Cl was conducted in acetonitrile in the presence of K_2CO_3 and a catalytic amount of KI. The mixture was purified through activated alumina column chromatography eluted with dichloromethane/acetonitrile, giving NNCH_2–L-Ala–OMe as a purple oily product in 53% yield (Scheme 1). A similar reaction using β-Ala–OEt•HCl afforded purple oil of NNCH_2–β-Ala–OEt in 62% yield. They can be stored in a refrigerator but slowly decompose in a few weeks.

They show major five lines in solution ESR with a relative intensity of 1/2/3/2/1, each of which is split into a 1/2/1 triplet (Figure 1). We assigned hyperfine splitting coupling constants in toluene at room temperature, as follows: $a_N = 0.732$ mT and $a_H = 0.170$ mT at $g = 2.0066$ for NNCH_2–L-Ala–OMe, and $a_N = 0.737$ mT and $a_H = 0.179$ mT at $g = 2.0066$ for NNCH_2–β-Ala–OEt. Polar solvents made a_N larger; $a_N = 0.800$ mT and $a_H = 0.183$ mT at $g = 2.0064$ in water for NNCH_2–L-Ala–OMe, and $a_N = 0.805$ mT and $a_H = 0.180$ mT for NNCH_2–β-Ala–OEt. Nitroxide ESR spectroscopy usually exhibits a solvent effect,[1] which is interpreted in terms of the contribution of a canonical structure of $>N^{+\bullet}–O^-$. Electro-spray ionization MS showed molecular ion signals as base peaks at m/z 295.1 ($M + Na^+$) for the former and m/z 309.1 ($M + Na^+$) for the latter.



Scheme 1. Synthetic scheme for NNCH_2–amino acids.

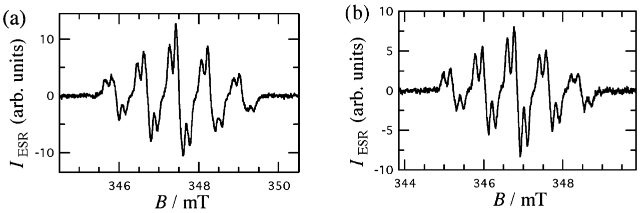


Figure 1. ESR spectra of (a) NNCH_2–L-Ala–OMe and (b) NNCH_2–β-Ala–OEt in water at room temperature.

Complexation reactions of NNCH_2–L-Ala–OMe and NNCH_2–β-Ala–OEt with $Ni(ClO_4)_2$ in dichloromethane–ethanol gave red platelet crystals of $[Ni(NNCH_2\text{–L-Ala–OMe})_2](ClO_4)_2 \cdot 1.5(CH_2Cl_2)$ (**1**) and red needles of $[Ni(NNCH_2\text{–}\beta\text{-Ala–OEt})_2](ClO_4)_2 \cdot 0.5(CH_2Cl_2)$ (**2**), respectively. Elemental analysis indicated the metal/ligand ratio of 1/2.[11] Other metal ions could afford coordination compounds, but the nickel(II) derivatives were suitable for X-ray crystallographic analysis.[12]

Each nickel ion (Figure 2) has an octahedral coordination structure and each ligand plays the role of a tridentate mer configured *O*,*N*,*O*-donor. The crystal of **1** is acentric in a monoclinic $P2_1$ space group, and the absolute configuration of (*S*)-alanine was confirmed by means of refining the Flack parameter[13] to a value of 0.13(3). A fused five-membered/six-membered chelate system is found for each ligand. As for **2**, a similar double mer configuration can be found with a fused six-/six-membered ring system. Each molecule is chiral, but there is an enantiomer in each unit cell due to a $P\bar{1}$ space group.

The direct coordination was found between the radical oxygen atom and the transition metal center (the O_{Rad}–Ni bond lengths of 2.036(11) and 2.034(10) Å for **1** and 2.010(7) and 2.025(5) Å for **2**). The ligating radical groups are located in a cis manner, which may lead to a through-space radical–radical exchange coupling. The O_{Rad}–Ni–O_{Rad} angles are 91.0(4) and 93.5(2)° for **1** and **2**, respectively, and the through-space $O_{Rad} \cdots O_{Rad}$ distances are 2.90(2) and 2.940(9) Å, respectively.

The magnetic susceptibilities of polycrystalline samples of **1** and **2** were measured on a SQUID magnetometer (Figure 3a). The $\chi_m T$ value of **1** approached null around 20 K on cooling. This behavior was reproduced when we applied an enantiomeric NNCH_2–D-Ala–OMe to this system. In contrast, the $\chi_m T$ value of **2** remained at 2 K. An approximately isosceles triangular model (Figure 3b) explains the behaviors of **1** and **2** under the exchange-coupling conditions with $|J_{Ni–Rad}| > |J_{Rad–Rad}|$ and $|J_{Ni–Rad}| < |J_{Rad–Rad}|$, respectively. The parameters were estimat-

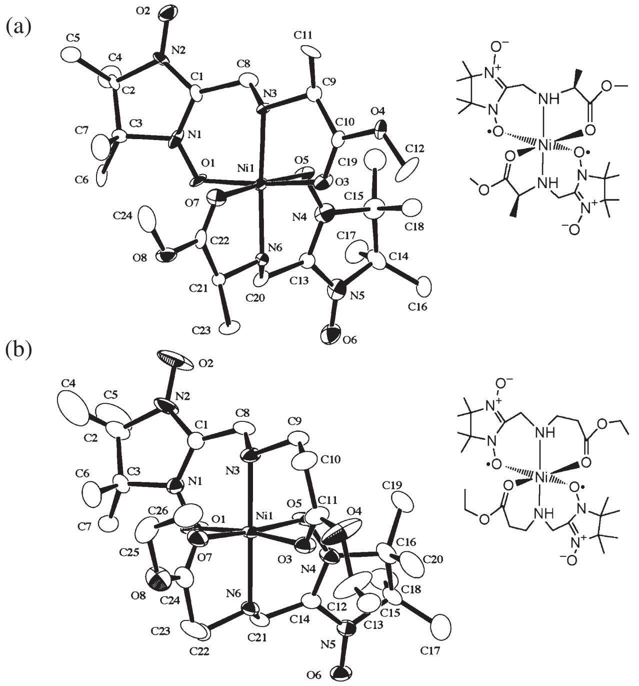


Figure 2. Molecular structures of (a) the [Ni(NNCH$_2$–L-Ala–OMe)$_2$]$^{2+}$ moiety in **1** and (b) the [Ni(NNCH$_2$–β-Ala–OEt)$_2$]$^{2+}$ moiety in **2**. Thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms are omitted for clarity. Structural formulas are also shown.

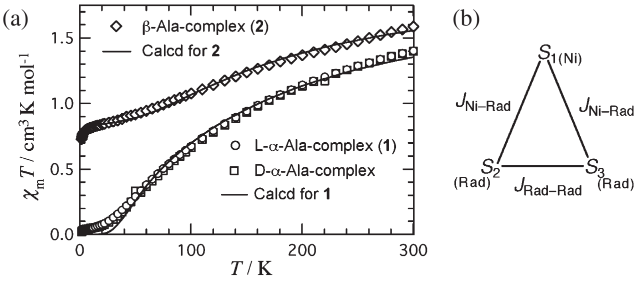


Figure 3. (a) Temperature dependence of $\chi_m T$ for **1** and **2**. Solid lines represent calculated curves. For the equation and parameters, see the text. (b) An exchange model consisting of one $S_{Ni} = 1$ and two $S_{Rad} = 1/2$ centers.

ed to be $J_{Ni\text{–}Rad} = -73(1)$ K and $J_{Rad\text{–}Rad} = -68(5)$ K with $g_{avg} = 2.1$ (fixed)[14] for **1** and $J_{Ni\text{–}Rad} = -45.2(7)$ K and $J_{Rad\text{–}Rad} = -90(1)$ K with $g_{avg} = 2.132(5)$ for **2** from the Heisenberg spin Hamiltonian $H = -2J_{Ni\text{–}Rad}(S_1 \cdot S_2 + S_1 \cdot S_3) - 2J_{Rad\text{–}Rad}S_2 \cdot S_3$.[15] According to the magneto–structure relationship between $J_{Ni\text{–}Rad}$ and the Ni–O–N–C$_\alpha$ torsion angle (ϕ),[7b] it is reasonable that the larger torsion of $\phi = 24.5(13)$ and 32.9(15)° in **1** gave the larger $|J_{Ni\text{–}Rad}|$, in comparison with $\phi = 18.0(9)$ and 30.2(10)° in **2**. The strong $J_{Rad\text{–}Rad}$ interaction is ascribed to the through-space overlap between the magnetic π^* orbitals.[16]

In conclusion, we have prepared two amino acid spin labels, and characterized their nickel(II) perchlorate complexes. These labels seem to be promising agents for a spin-probe method in biological systems as well as building blocks for bio-inspired materials chemistry including chiral magnets.[17]

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11 **1**: Anal. Calcd for $C_{24}H_{44}N_6O_{16}Cl_2Ni_1 \cdot (CH_2Cl_2)_{1.5}$: C, 32.95; H, 5.10; N, 9.04%. Found: C, 33.04; H, 5.14; N, 8.74%. IR (neat) 1660, 1456, 1067, 731, 621 cm^{-1}. **2**: Anal. Calcd for $C_{26}H_{48}N_6O_{16}Cl_2Ni_1 \cdot (CH_2Cl_2)_{0.5}$: C, 36.47; H, 5.66; N, 9.63%. Found: C, 36.42; H, 5.86; N, 9.26%. IR (neat) 1657, 1306, 1240, 1067, 621 cm^{-1}.

12 Selected data. **1**: $C_{26}H_{48}Cl_6N_6NiO_{16}$, monoclinic, $P2_1$, $a = 13.20940(10)$, $b = 16.4048(3)$, $c = 13.20380(10)$ Å, $\beta = 134.6560(14)°$, $V = 2035.31(4)$ Å^3, $Z = 2$, $D_{calcd} = 1.586$ g cm^{-3}, μ(Mo Kα) = 0.943 mm^{-1}, $R_{int} = 0.063$, $R(F)$ ($I > 2\sigma(I)$) = 0.0988, $R_w(F^2)$ (all data) = 0.1381, and $T = 100$ K for 8565 unique reflections. **2**: $C_{27}H_{50}Cl_4N_6NiO_{18}$, triclinic, $P\bar{1}$, $a = 11.2480(8)$, $b = 12.4290(7)$, $c = 15.6678(10)$ Å, $\alpha = 72.4190(16)$, $\beta = 78.818(3)$, $\gamma = 86.9210(14)°$, $V = 2048.4(2)$ Å^3, $Z = 2$, $D_{calcd} = 1.536$ g cm^{-3}, μ(Mo Kα) = 0.813 mm^{-1}, $R_{int} = 0.038$, $R(F)$ ($I > 2\sigma(I)$) = 0.1144, $R_w(F^2)$ (all data) = 0.1535, and $T = 106$ K for 8564 unique reflections. For details, see CCDC 768674 and 768675. The partial liberation of the solvent molecules may result in the final poor R factors.

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14 A treatment of variable g gave a somewhat large error; $g = 2.09(9)$, $J_{Ni\text{–}Rad} = -72(11)$ K, and $J_{Rad\text{–}Rad} = -70(30)$ K. The fixed value of 2.1 seems to be reasonable compared with that of **2**, and the parameters were better optimized after this assumption.

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16 The spatial arrangement cannot be described simply, but torsion angles around C1–N1–O1···O5 etc. seem to be important. The angles (27.9(8) and 18.2(9)°) of **2**, larger than those of **1** (24.8(10) and 17.6(13)°), may be related to more appreciable π^*–π^* orbital overlap.

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