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Copper(II) Complexes of the Schiff Bases derived from Chloramphenicol[1)]

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Schiff bases (**3**, **4**) were obtained by condensation of D-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (**2**) with salicylaldehyde and acetylacetone. Copper(II) chelates (**5** and **6**) were synthesized from **3** and **4**, respectively, and their chemical structures were determined by elemental analysis, molecular weight measurement, and infrared and nuclear magnetic resonance spectroscopies. From the temperature dependence of their magnetic susceptibilities, **5**, **6** and the phenylcarbamate (**7**) derived from **5** were inferred to be tetramers having cubane-type structures. The phenylcarbamate (**9**) derived from **6** was presumed to have a dimeric structure.

Keywords——chloramphenicol; D-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol; Cu(II) complex; phenylcarbamoyl derivative; ^{1}H-NMR; magnetic susceptibility; tetramer; cubane-type structure; square-planar binuclear complex

Chloramphenicol (**1**) is known to lose its antimicrobial activity when the electron-withdrawing dichloroacetyl group on the nitrogen atom is removed to give D-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (**2**). The authors sought to synthesize copper(II) complexes of Schiff bases of **2** in the hope of obtaining compounds with antimicrobial activity, because an electron-withdrawing effect at the nitrogen atom should be caused by the C=N double bond formation and coordination of the imino nitrogen to a metal ion.

The Schiff base (**3**) derived from **2** and salicylaldehyde was obtained as yellow needles (mp 178—180°) and the Schiff base (**4**) was obtained from **2** and acetylacetone as light yellow needles (mp 150°). These Schiff bases were assigned the structures **3** and **4** on the basis of elemental analysis, infrared (IR) and nuclear magnetic resonance (NMR) spectra.

On heating with copper(II) acetate in ethanol, the Schiff bases **3** and **4** gave the corresponding copper(II) complexes (**5**) as greenish-blue plates and **6** as a greenish-blue crystalline powder, respectively. Elemental analysis revealed that both complexes contain copper(II) and the Schiff base **3** or **4** in a molar ratio of 1:1.

The Schiff bases have four possible coordinating groups; primary and secondary alcoholic hydroxyl groups, a phenolic hydroxyl group, and aldoimine nitrogen in **3**, and primary and secondary alcoholic hydroxyl groups, an enolic hydroxyl group, and a ketoimine nitrogen in **4′**, to which **4** was presumed to isomerize on chelate formation.

Since only three of these four groups can take part in chelate formation owing to the steric requirements, the authors attempted to obtain from **5** an O-acyl derivative, in which a free hydroxyl group might be acylated and thus be distinguishable by physico-chemical methods. Although benzoylation and acetylation catalyzed by pyridine and sodium carbonate were unsuccessful, phenyl isocyanate reacted with **5** to give the phenylcarbamoyl complex (**7**) as a greenish-blue crystalline powder. To remove copper(II) from **7**, its chloroform solution

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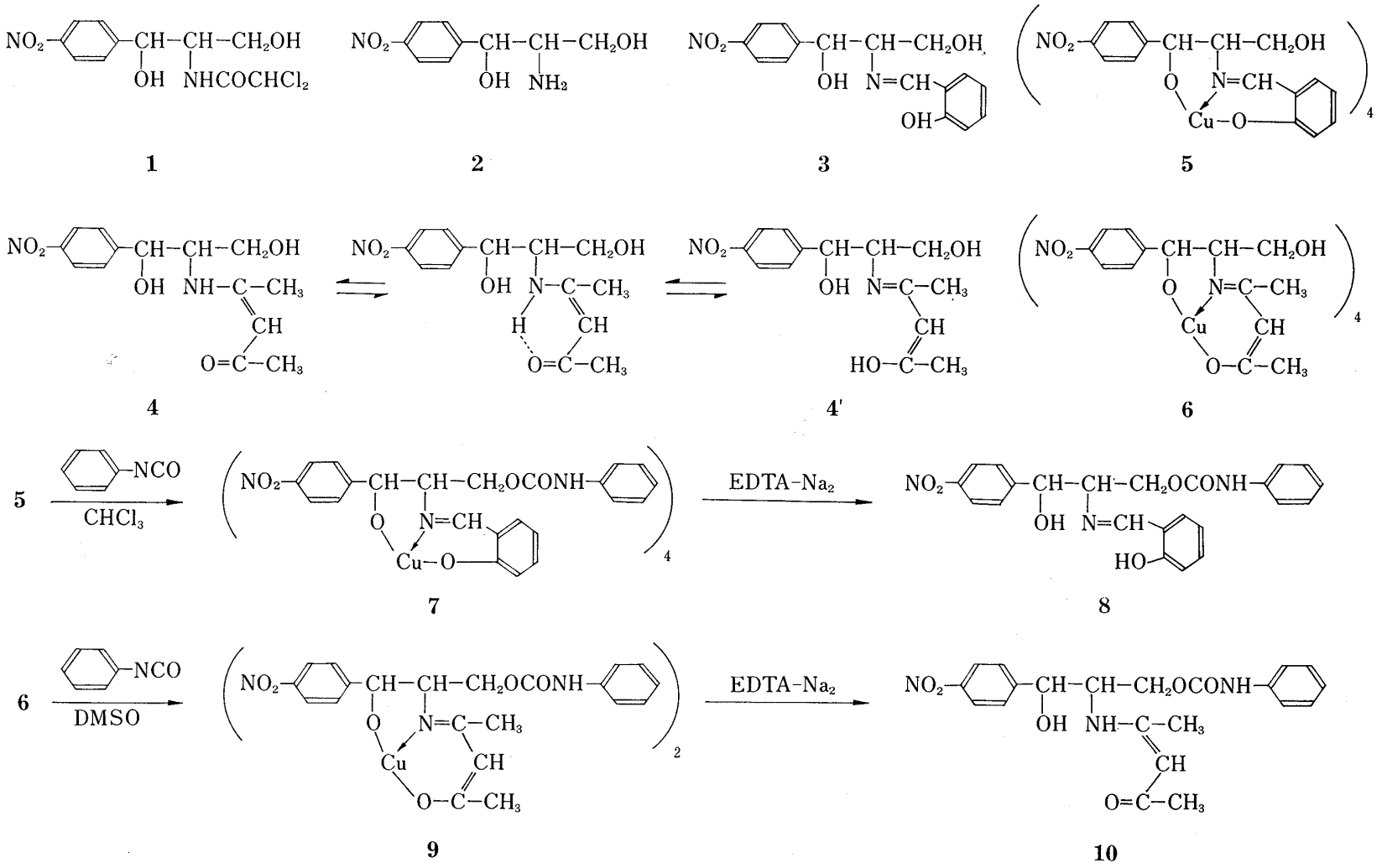
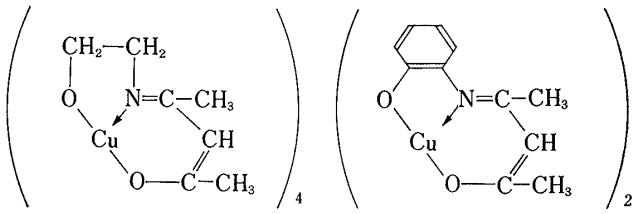


Chart 1

was shaken with an aqueous solution of the disodium salt of EDTA. Yellow prismatic crystals (**8**) (mp 106—108°) were isolated from the organic layer. The compound **8** was determined to be the phenylcarbamoyl derivative of **3** by elemental analysis and from the IR spectrum. Comparison of the NMR spectra of **3** and **8** disclosed that the signal at 4.7 ppm due to the primary hydroxyl group was absent in **8** and that the signals of the two protons on the carbon atom bearing the primary hydroxyl group were shifted to lower magnetic field (3.4 ppm in **3** and 4.17 and 4.45 ppm in **8**), while that of the proton on the carbon atom bearing the secondary hydroxyl group remained unchanged (5.0 ppm in **3** and **8**). These findings indicate that the phenylcarbamoyl group had reacted at the primary hydroxyl group that had not participated in coordination (structure **8**). Consequently, the Schiff base **3** is concluded to react with copper(II) as a terdentate ligand, coordinating through the imino nitrogen and two oxygens from the secondary alcoholic and phenolic hydroxyl groups.

Similarly, the phenylcarbamoyl complex (**9**) was derived from **6** as a pale blue crystalline powder, from which the phenylcarbamoyl derivative (**10**) of **4** was obtained as colorless needles (mp 158°). The structure of **10** was determined from the results of elemental analysis, IR and NMR spectra, and it was concluded that the Schiff base **4'** coordinated to copper(II) through the imino nitrogen and the oxygens of the secondary alcoholic and enolic hydroxyl groups.

On the other hand, magnetic susceptibility measurements at room temperature by the Gouy method gave effective magnetic moments, μ_{eff} of 1.73 B.M. for **5** and 1.66 B.M. for **6**, which are rather low for the monomeric structures expected from the analytical compositions of the complexes. The values suggest the existence of magnetic exchange between copper(II) ions as a result of cluster formation. The molecular weights of **5** and **6**, measured by the vapor pressure osmotic method in chloroform, were found to be 1500 and 1300, respectively, which are in accordance with the calculated values of 1510.8 and 1422.8 for the corresponding tetramers. Therefore, the Schiff base complexes **5** and **6** are confirmed to have tetrameric structures expressed by the formulas **5** and **6**, respectively, both in solution and in the solid state. The



11 **12**

Chart 2

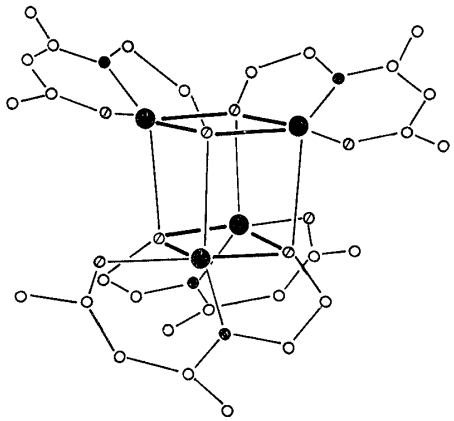


Fig. 1a. Molecular Structure of the Cu(II) Complex of 2-(2-Hydroxyethyl)imino-4-oxopentane (**11**)

Cu ●, C ○, N •, O ◐.

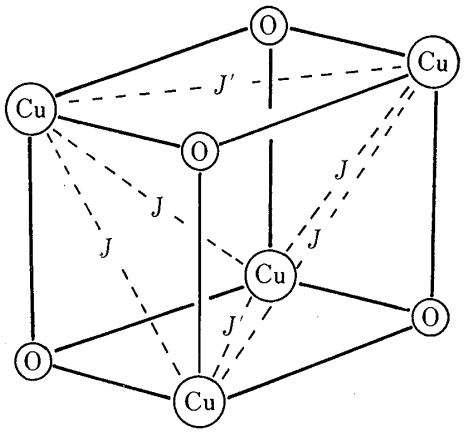


Fig. 1b. Cubane-Type Skeleton

phenylcarbamoyl complex (**7**) had a μ_{eff} value of 1.53 B.M. and its molecular weight was determined to be 1540 in chloroform, which might be consistent with a tetrameric structure having a molecular weight of 1988.

On the other hand, **9** was presumed to have a dimeric structure, because **9**, after precipitation from the chloroform solution in which it reacted with phenyl isocyanate, gave a μ_{eff} value of 1.19 B.M. and a molecular weight of 950 measured in dioxane, a value reasonably close to that of the dimer (994).

As regards the skeletal structures of the complexes, 2-(2-hydroxyethyl)imino-4-oxopentane is known to form a tetrameric copper(II) complex (**11**) whose structure has been determined by X-ray analysis (Fig. 1a).[3] The authors assumed a similar skeleton (Fig. 1b) for **5**, **6** and **7**, because their ligands, **3**, **4** and **8** are closely related to the ligand in **11**. Figure 2 shows the temperature dependence of the magnetic susceptibilities of **5**, **6** and **7** measured by the Faraday method and the theoretical magnetic susceptibilities for tetrameric skeletons calculated according to Eq. 1 derived by Hatfield,[4] with $g=2.09$, $2J/k=-100$ K, $2J'/k=60$ K, and $N\alpha=60$ cgs emu for **5** (heavy solid line) and with $g=2.02$, $2J/k=-160$ K, $2J'/k=155$ K and $N\alpha=60$ cgs emu for **6** (light solid line). The temperature dependence of the magnetic susceptibility of **7** was reproduced by Eq. 1 with $g=2.02$, $2J/k=-180$ K, $2J'/k=170$ K and $N\alpha=60$ cgs emu (broken line), which confirms that **7** has a structure similar to those of **5** and **6**.

$$\chi_A = \frac{3g^2\beta^2}{32T} \times \frac{10+4\exp[-2(J+J')/kT]+2\exp(-4J/kT)}{5+6\exp[-2(J+J')/kT]+3\exp(-4J/kT)+\exp[-(J-J')/kT]+\exp[2(J-J')/kT]} + N\alpha \quad \text{(Eq. 1)}$$

where J and J' denote the exchange integrals between copper(II) ions as shown in Fig. 1b, g is the g-value and $N\alpha$ is the temperature-independent paramagnetism.

The agreement between the calculated and the experimental values is not good in lower temperature region, but it is satisfactory at temperatures higher than 60 K.

Considering that the ligand structure and magnetic behavior of **9** resemble those of the Cu(II) complex of 2-(2-hydroxyphenyl)imino-4-oxopentane (**12**) which has been shown to

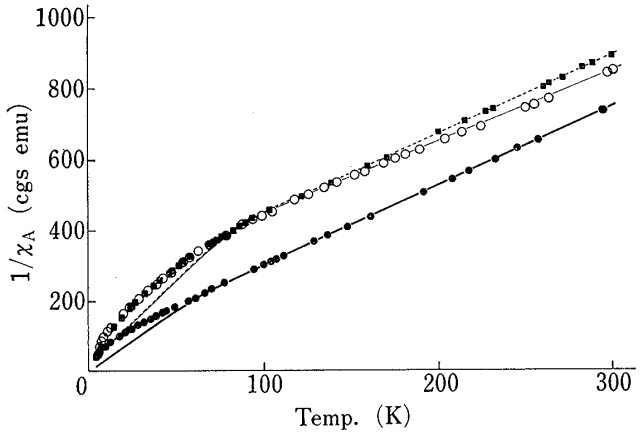


Fig. 2. The Temperature Dependence of the Magnetic Susceptibilities of Complexes **5** (●), **6** (○) and **7** (■)

The theoretical susceptibilities were calculated from Eq. 1 with $g=2.09$, $2J/k=-100$ K, $2J'/k=60$ K and $N\alpha=60$ cgs emu (——), with $g=2.02$, $2J/k=-160$ K, $2J'/k=155$ K and $N\alpha=60$ cgs emu (——), and with $g=2.02$, $2J/k=-180$ K, $2J'/k=170$ K and $N\alpha=60$ cgs emu (----), respectively.

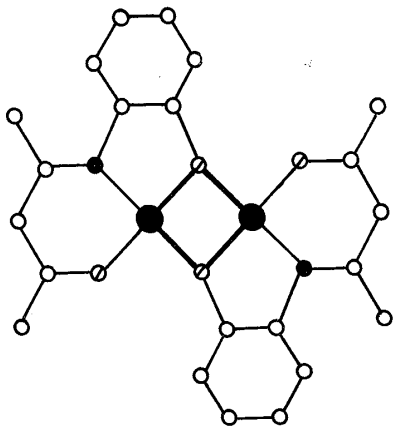


Fig. 3. Molecular Structure of the Cu(II) Complex of 2-(2-Hydroxyphenyl)imino-4-oxopentane (**12**)

Cu ●, C ○, N •, O ⓞ.

3) J.A. Bertrand, J.A. Kelley, and C.E. Kirkwood, *Chem. Commun.*, **1968**, 1329.
4) W.E. Hatfield and G.W. Inman, Jr., *Inorg. Chem.*, **8**, 1376 (1969).

have a dimeric structure (Fig. 3) by X-ray analysis,[5] the structure around copper(II) in **9** may be similar. This is regarded as a structure unit constituting the cubane-type tetramer.

Further support for the dimeric structure was provided by the temperature dependence of magnetic susceptibility of **9**, which approximately obeys the Bleaney–Boweres equation[6] for dimers (Fig. 4).

It may be concluded, therefore, that **5**, **6** and **7** involve spin-spin interactions between the copper(II) ions and that they have tetrameric molecular structures with the skeleton shown in Fig. 1b. Although it is difficult to explain the deviation of the theoretical magnetic susceptibilities from the observed ones at low temperatures, this might arise from distortion of the cubane-type structure due to steric hindrance arising from the bulkiness of the ligands, as judged from CPK models.

The complex **9** also involves a spin-spin interaction between the copper(II) ions and has been inferred to have the dimeric structure shown in Fig. 3. It is presumed that the cubane-type structure of **6** was split to give the square-planar binuclear copper(II) complex (**9**) during the phenylcarbamate formation.

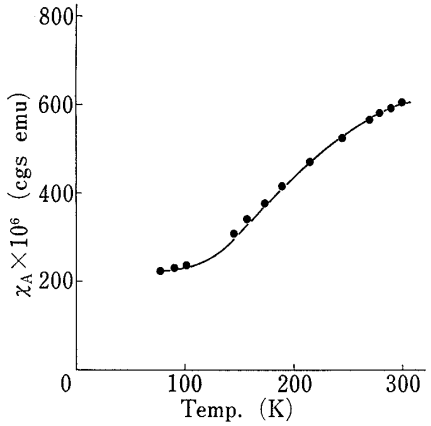


Fig. 4. The Temperature Dependence of the Magnetic Susceptibility of Complex **9**

Observed values are shown by solid circles. The curve represents the theoretical curve calculated from the Bleaney-Boweres equation $\left(\chi_A=\frac{Ng^2\beta^2}{3kT}\times\frac{1}{1+\frac{1}{3}\exp(J/kT)}+Na\right.$, with $g=2.17$ and $J/k=650$ K$\left.\right)$, and is corrected for the presence of the monomer (2.5%) which obeys the Curie law ($\chi_A=Ng^2\beta^2/4kT$, with $g=2.15$).

Contrary to our original expectation, the Schiff bases **3** and **4** as well as their copper(II) complexes **5** and **6** did not show any inhibitory effects on fourteen kinds of Gram-positive and -negative microorganisms tested.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus, model MP-S3, and are uncorrected. Optical rotation were measured with a Union PN 101 automatic digital polarimeter in a 1 cm tube.

NMR spectra were measured with a Varian XL-100-12 spectrometer at 100 MHz using TMS as an internal standard. IR spectra were measured in Nujol mulls with a Hitachi EPI-S infrared spectrometer and ultraviolet and visible spectra were measured with a Hitachi 124 spectrophotometer. Molecular weights were determined with a vapor pressure osmometer (model 301A).

The magnetic susceptibility was determined at room temperature by means of a Gouy magnetic apparatus with a Mettler H 51 AR microbalance and a Tokyo Giken WM-III electromagnet in a field of about 9000 G. The temperature dependence of magnetic susceptibility was measured in the range of 4.2—300K by the Faraday method.

D-*threo*-2-(2-Hydroxybenzylidene)amino-1-(4-nitrophenyl)-1,3-propanediol (3)——According to the literature,[7] a solution of D-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (**2**) (3 g) and salicylaldehyde (1.58 g) in benzene (120 ml) was refluxed for 2.5 hr. The precipitate deposited after cooling was collected by filtration and recrystallized from ethanol to give **3** (3.6 g, 80%) as yellow needles. mp 178—180°. $[\alpha]_D^{25}$ −207.3° (c=1.02, DMSO). UV-visible λ_{max}^{MeOH} nm (ε): 272 (16170), 463 (336). IR ν_{max}^{Nujol} cm^{-1}: 3310 (OH), 1628 (–CH=N–), 1510, 1350 (NO_2). NMR (DMSO-d_6) δ: 3.40—3.75 (3H, m, $-C\underline{H}_2OH$ and $-\underset{N=}{C\underline{H}}$), 4.75 (1H, bt, J=3 Hz, primary OH), 5.00 (1H, t, J=4 Hz, $-\underset{OH}{C\underline{H}}-$), 5.75 (1H, d, J=4 Hz, secondary OH), 6.7—8.25 (8H, aromatic H), 8.30 (1H, s, $-C\underline{H}$=N–), 13.55 (1H, bs, phenolic OH).

5) G.A. Barclay, C.M. Harris, B.F. Hoskins, and E. Kokot, *Proc. Chem. Soc.*, **1961**, 264.
6) B. Bleaney and K.D. Boweres, *Proc. Roy. Soc.*, **A18**, 451 (1952).
7) A. Pedrazzori and S. Tricerri, *Helv. Chim. Acta*, **34**, 533 (1951).

D-*threo*-1-(4-Nitrophenyl)-2-(4-oxo-2-pent-2-enyl)amino-1,3-propanediol (4)——Acetylacetone (5.03 g) was added to a solution of **2** (7.12 g) in ethanol (70 ml). The mixture was refluxed for 2.5 hr and then evaporated to dryness *in vacuo*. The residue was washed with ether and recrystallized from ethanol, yielding **4** (5.54 g, 54.7%) as light yellow needles. mp 150°. $[\alpha]_D^{25}$ −703.5° (c=0.995, DMSO). UV λ_{max}^{MeOH} nm (ε): 274 (12690), 312 (19860). IR ν_{max}^{Nujol} cm^{-1}: 3310 (OH), 1590 (–CH=N–), 1505, 1345 (NO_2). NMR (DMSO-d_6) δ: 1.52 (3H, s, =C–C<u>H</u>$_3$), 1.84 (3H, s, C<u>H</u>$_3$–CO), 3.58 (2H, m, –C<u>H</u>$_2$–OH), 3.64 (1H, m, –C<u>H</u>(NH)–), 4.76 (1H, s, =C<u>H</u>–), 4.94 (1H, d, primary OH), 5.02 (1H, d, –C<u>H</u>(OH)–), 5.92 (1H, d, secondary OH), 7.63—8.16 (4H, m, aromatic H), 10.90 (1H, d, –C<u>H</u>(NH)–). *Anal.* Calcd. for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.17; N, 9.52. Found: C, 57.06; H, 6.03; N, 9.47.

Tetrakis-[D-*threo*-1,3-dihydroxypropane-1-(4-nitrophenyl)-2-(2-hydroxybenzylideneaminato)copper(II)] (5)——A mixture of **3** (2 g) and copper(II) acetate dihydrate (0.78 g) in ethanol (20 ml) was refluxed for 10 min and evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ (20 ml), and the solution was shaken with saturated aqueous $NaHCO_3$ and then with water, dried over Na_2SO_4 and evaporated down. The residue was washed with ethanol and dried *in vacuo* to give **5** (1.5 g, 62.8%) as greenish-blue plates. mp 236—238° (dec.). $[\alpha]_D^{25}$ −90.0° (c=0.1, DMSO). UV-visible λ_{max}^{DMSO} nm (ε): 270 (17850), 362 (19420), 622 (147). IR ν_{max}^{Nujol} cm^{-1}: 3130 (OH), 1640 (–CH=N–), 1525, 1350 (NO_2). Molecular magnetic susceptibility $\chi_M = 1118 \times 10^{-6}$; effective magnetic moment μ_{eff}=1.73 B.M. *Anal.* Calcd. for $(C_{16}H_{14}CuN_2O_5)_4$: C, 50.83; H, 3.74; Cu, 16.83; N, 7.42. Found: C, 50.59; H, 3.70; Cu, 16.51; N, 7.30.

Tetrakis-[D-*threo*-1,3-dihydroxypropane-1-(4-nitrophenyl)-2-(4-oxo-2-pentaneiminato)copper(II)] (6)——Copper(II) acetate dihydrate (2.5 g) was added to a solution of **4** (3 g) in ethanol (30 ml). By the same procedure as for **5**, the complex **6** (2.69 g, 74.3%) was obtained as a greenish-blue crystalline powder after recrystallization from ethanol. mp 202° (dec.). $[\alpha]_D^{25}$ −240.0° (c=0.1, DMSO). UV-visible λ_{max}^{DMSO} nm (ε): 278 (28800), 320 (22600), 597 (108). IR ν_{max}^{Nujol} cm^{-1}: 1590 (–CH=N–), 1500, 1335 (NO_2). Molecular magnetic susceptibility $\chi_M = 996 \times 10^{-6}$; effective magnetic moment μ_{eff}=1.66 B.M. *Anal.* Calcd. for $(C_{14}H_{16}CuN_2O_5)_4$: Cu, 17.87; N, 7.88. Found: Cu, 17.59; N, 7.62.

Tetrakis-[D-*threo*-1-hydroxy-1-(4-nitrophenyl)-3-(N-phenylcarbamoyl)oxypropane-2-(2-hydroxybenzylideneaminato)copper(II)] (7)——Phenyl isocyanate (0.54 g) diluted with $CHCl_3$ (10 ml) was added dropwise to a solution of **5** (1.46 g) in $CHCl_3$ (15 ml) with stirring and ice-cooling. After stirring at room temperature for 2 hr then at 40° for 1 hr, the resulting blue solution was washed with water, dried over Na_2SO_4 and evaporated down. The residue was recrystallized from aqueous DMSO to give dark-green prisms which did not show a sharp melting point (80—140°). Elemental analysis suggested that 1 mol of the product included 2 mol each of DMSO and H_2O. Therefore, the crystals were dissolved in $CHCl_3$ and shaken with water several times to remove DMSO. The chloroform solution was dried over Na_2SO_4 and evaporated down to give greenish-blue crystalline powder (0.75 g). mp 146—150° (dec.). $[\alpha]_D^{28}$ +90° (c=1, DMSO). UV-visible λ_{max}^{DMSO} nm (ε): 275 (17300), 371 (5960), 632 (62). IR ν_{max}^{Nujol} cm^{-1}: 1723 (–CONH–), 1625 (–CH=N–), 1523, 1346 (NO_2), 1212 (–OCONH–). $\chi_M = 781 \times 10^{-6}$, μ_{eff}=1.53 B.M. *Anal.* Calcd. for $(C_{23}H_{19}CuN_3O_6)_4 \cdot CHCl_3$: C, 52.91; H, 3.63; N, 7.98. Found: C, 52.77; H, 3.75; N, 7.78.

D-*threo*-1-Hydroxy-2-(2-hydroxybenzylidene)amino-1-(4-nitrophenyl)-3-(N-phenylcarbamoyl)oxypropane (8)——The resulting blue solution of **7** (1 g) in $CHCl_3$ (15 ml) was shaken with a solution of EDTA–Na_2 $2H_2O$ (2 g) in water (15 ml). The blue color moved gradually from the organic layer to the aqueous layer. The yellow chloroform solution was washed with water, dried over Na_2SO_4 and evaporated down. The crystalline residue was dissolved in $CHCl_3$ and a few drops of n-hexane was added to the solution until it became slightly turbid. After cooling, **8** (0.6 g) was obtained as yellow prismatic crystals including $CHCl_3$. mp 106—108°. $[\alpha]_D^{14}$ −50.25° (c=0.995, MeOH). UV-visible λ_{max}^{MeOH} nm (ε): 258 (20110), 318 (5500), 404 (590). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3340 (OH), 1720 (–CONH–), 1620 (–CH=N–), 1503, 1350 (NO_2), 1204 (–OCONH–). NMR (DMSO-d_6) δ: 3.85 (1H, m, –C<u>H</u>(N=)–; when the peak at 5.10 ppm was irradiated, the multiplet changed to a doublet of doublets, J=4, 8 Hz), 4.17 (1H, dd, J=8, 11 Hz, A part of ABX system –CH–C<u>H</u>$_2$–OCO–), 4.45 (1H, dd, J=4, 11 Hz, B part of ABX system –CH–C<u>H</u>$_2$OCO–), 5.10 (1H, t, J=4 Hz, –C<u>H</u>(OH)–), 6.03 (1H, d, J=4 Hz, secondary OH), 6.75—8.30 (13H, aromatic H), 8.25 ($CHCl_3$), 8.40 (1H, s, –CH=N–), 9.54 (1H, s, –CONH–), 13.25 (1H, s, phenolic OH). *Anal.* Calcd. for $C_{23}H_{21}N_3O_6 \cdot CHCl_3$: C, 51.96; H, 4.00; N, 7.57. Found: C, 51.98; H, 3.87; N, 7.70.

Bis-[D-*threo*-1-hydroxy-1-(4-nitrophenyl)-3-(N-phenylcarbamoyl)oxypropane-2-(4-oxo-2-pentaneiminato)-copper (II)] (9)——Phenyl isocyanate (1.2 g) diluted with $CHCl_3$ (30 ml) was added dropwise to a solution of **6** (3 g) in $CHCl_3$ (60 ml) with stirring and ice-cooling. After stirring at room temperature for 1 hr then at 40° for 1 hr, the pale blue powdery precipitate was separated by filtration. The powder was recrystallized from aqueous DMSO to afford a pale blue crystalline powder (2.04 g). mp 230° (dec.). $[\alpha]_D^{26}$ +358° (c=1, DMSO). UV-visible λ_{max}^{DMSO} nm (ε): 284 (12930), 318 (12370), 606 (92). IR ν_{max}^{Nujol} cm^{-1}: 1730, 1717 (–CHNH–),

1600 (–CH=N–), 1505, 1343 (NO_2), 1213 (–OCONH–). $\chi_M = 312 \times 10^{-6}$, $\mu_{eff} = 1.19$ B.M. *Anal.* Calcd. for $(C_{21}H_{21}CuN_3O_6)_2$: C, 53.10; H, 4.46; N, 8.85. Found: C, 52.74; H, 4.47; N, 8.65.

D-*threo*-1-Hydroxy-1-(4-nitrophenyl)-2-(4-oxo-2-pent-2-enyl)amino-3-(N-phenylcarbamoyl)oxypropane (10)——A solution of **9** (2 g) in DMSO (5 ml) was mixed with a solution of EDTA–$Na_2 \cdot 2H_2O$ (4 g) in H_2O (50 ml) and stirred. $CHCl_3$ (50 ml) was added to the resulting blue solution and the mixture was shaken vigorously. The colorless chloroform layer was separated from the blue aqueous layer, washed with water, dried over Na_2SO_4 and evaporated down. The gummy residue was solidified in an ice box and recrystallized from a mixture of $CHCl_3$ and *n*-hexane to give colorless fine needles (1.43 g). mp 157—158°. $[\alpha]_D^{26}$ −410° ($c=1$, MeOH). UV λ_{max}^{MeOH} nm (ε): 280 (15330), 311 (21800). IR ν_{max}^{Nujol} cm^{-1}: 3440, 3300 (OH), 1703 (–CONH–), 1617 (–C=CH–), 1517, 1353 (NO_2), 1221 (–OCONH–). NMR (DMSO-d_6) δ: 1.55 (3H, s, =C–CH_3), 1.87 (3H, s, CH_3CO–), 4.02 (1H, m, –$\underset{\text{NH–}}{\underline{\text{C}}}$H–), 4.33 (2H, dd, $J=6$, 10 Hz, –CH_2OCO–), 4.80 (1H, s, =CH–), 5.06 (1H, d, secondary OH), 6.80—8.25 (9H, m, aromatic H), 9.63 (1H, s, –CONH–), 10.87 (1H, d, –$\underset{\text{NH}}{\underline{\text{C}}}$H–). *Anal.* Calcd. for $C_{21}H_{23}N_3O_6$: C, 61.01; H, 5.61; N, 10.16. Found: C, 60.88; H, 5.50; N, 10.13.

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