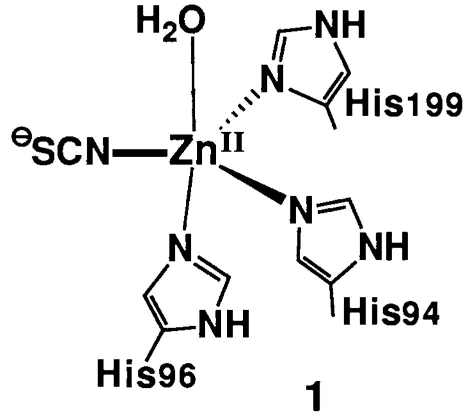
CHEMISTRY LETTERS, pp. 787-790, 1992.

A Versatile Macrocyclic [12]aneN_3 for Interconversion of Tetrahedral and Trigonal Bipyramidal Zinc(II) Complexes. Relevance to Four- $\rightleftharpoons$ Five-Coordinate Geometries of Zinc(II) in Carbonic Anhydrase

Eiichi KIMURA,* Tohru KOIKE, Mitsuhiko SHIONOYA, and Motoo SHIRO†
Department of Medicinal Chemistry, Hiroshima University School
of Medicine, Kasumi 1-2-3, Minami-ku, Hiroshima 734
† Rigaku Corporation, Matsubara-cho 3-9-12, Akishima, Tokyo 196

X-Ray crystal study of [12]aneN_3-$Zn^{II}(NCS)_2$ has revealed a 5-coordinate, trigonal bipyramidal structure with an equatorial Zn^{II}-NCS^- bond closer and linear, and an apical Zn^{II}-NCS^- bond longer and bent, and the new configuration of [12]aneN_3, which is reversed from those known for 4-coordinate, tetrahedral Zn^{II} complexes.

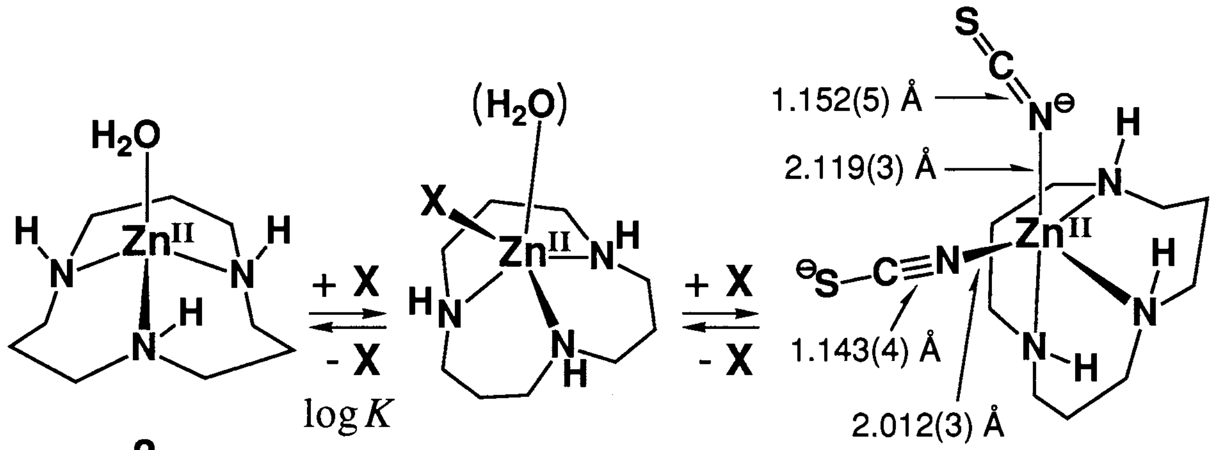
Carbonic anhydrases (CA) are a family of zinc enzymes that contain Zn^{II}-(imidazole)$_3$ complexes at the active centers, catalyzing the interconversion of carbon dioxide and bicarbonate via a possible five-coordinate intermediate.1-3) Thiocyanate anion, SCN^- is one of the typical CA inhibitors,4) which is due to the strong binding to the Zn^{II} ion ($\log K = 2.8$ at 25 °C).5) Recent X-ray crystal analysis (to 1.9 Å resolution) of the SCN^- binding to CA at pH 8.5 showed the Zn^{II} ion in an ill-defined five-coordinate complex **1** with SCN^- (extraordinarily short Zn–N bond distance 1.9 Å and almost linear Zn-NCS) and a water (Zn–O 2.2 Å) bound.6) More detailed structural and chemical characterization of the five-coordinate Zn^{II} complexes would be essential in defining the intrinsic properties of the Zn^{II} ion in the enzymes.



1

SCN^- Inhibition mechanism of *Carbonic Anhydrase*

Recently, we have discovered that a tetrahedral Zn^{II}-OH_2 triamine complex **2** with a 12-membered macrocyclic triamine 1,5,9-triazacyclododecane ([12]aneN_3) is a good model for the active center of CA kinetically (in acetaldehyde hydration and carboxylic ester hydrolyses) as well as thermodynamically (in anion bindings with **2** (e.g. X = OH^-, halogen ions, carboxylates, phosphates, etc.)).[7-9] Of particular interest was the similarly strong affinity of **2** to SCN^- ion into **3** ($\log K = 2.4$ at 25 °C and $I = 0.20$).[7]



2
[*up, up, up*]

3

4 (X = SCN^-)
[*down, up, up*]

We now have isolated Zn^{II}[12]ane$N_3$$(NCS)_2$ **4** as colourless powder by mixing [12]aneN_3 (390 mg, 2.3 mmol) and $Zn(NCS)_2$ (450 mg; > 90% purity, Kanto Chemical Co.) in 30 cm^3 of 98% EtOH at 50 °C in 72% yield (580 mg). The product was recrystallized from aqueous 50% MeOH to give colorless crystals of **4** for X-ray analysis.[10] X-Ray analysis of **4** has shown a *five-coordinate structure* (Fig. 1) with two bound SCN^- ions.[11] The Zn^{II} ion is 0.095 Å above the N(1), N(5), N(16) trigonal plane to an apical SCN^- ion. Two novel features with **4** have been found.

One is the unequivalent two Zn^{II}–NCS^- bonds in a trigonal bipyramidal structure, despite **4** resulting from the SCN^- replacement reaction at the symmetrical **2**: the equatorial Zn-NCS^- bond length is very short (Zn-N(16) 2.012(3) Å) and the Zn-N-C angle is more linear (Zn-N(16)-C(17) 171.3(3)°), and the apical Zn–NCS^- bond length is longer (Zn–N(13) 2.119(3) Å) and the Zn-N(13)-C(14) angle is bent at 152.2(3)°.[12] This fact implies that the five-coordinate, trigonal bipyramidal structure is one of the *intrinsic properties* of Zn^{II} ion, where a strong σ donating ligand such as SCN^- comes at an equatorial position. The infrared CN stretching frequencies ν_{CN} of 2107 and 2062 cm^{-1} are assigned to the equatorial shorter N≡C length of 1.143(4) Å and the apical N=C length of 1.152(5) Å, respectively. The equatorial NCS^- ion forms three intermolecular hydrogen bondings S(18)··· HN with three adjacent Zn^{II}[12]aneN_3 complexes.

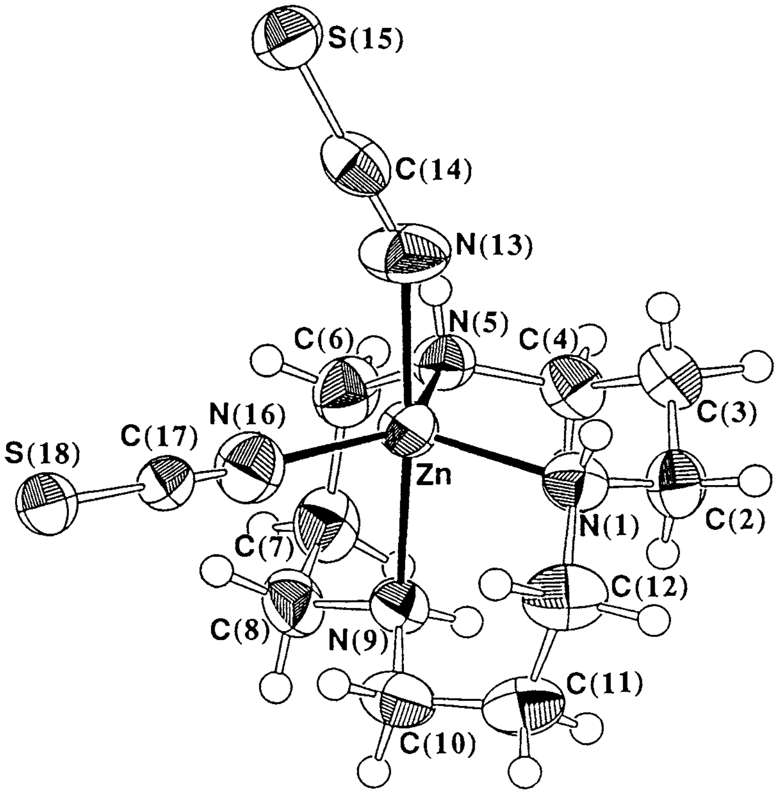


Fig. 1. ORTEP drawing (50% probability) of **4**.

The second remarkable feature is a hitherto unreported configuration (the NH hydrogens are [*down, up, up*]) of the Zn-bound ligand [12]aneN$_3$, which is inversed at one NH (to accommodate a SCN$^-$) from the one (the NH hydrogens are all [*up*]) in symmetrical tetrahedral complexes (*e.g.* [12]aneN$_3$-Zn^{II}-OH$^-$,7) see **2**, and [12]aneN$_3$-Zn^{II}-Br$^-$ 13)). A CPK molecular model indicates the [*down, up, up*] configuration is favorable only for five-coordinate, trigonal pyramidal geometry. The strong binding of the first incoming NCS$^-$ ion would break the [*up, up, up*] configuration set for the tetrahedral geometry. The barrier for the configurational interconversion would not be so high as to hinder the four- $\rightleftarrows$ five-coordinate transformation in solution, but would be high enough to allow the occurrence of **4** in a solid state. **4** exhibited only three ^{13}C NMR signals assigned to two kinds of CH$_2$ and one SCN$^-$ ion, and symmetrical ^{1}H NMR signals in MeOH at 27 °C,10) indicating the fast equilibrium for **4** $\rightleftarrows$ **3** (or the H$_2$O-less, four-coordinated complex). In D$_2$O solution of **4** the CN stretching absorption occurs broadly only at 2063 cm^{-1}.

In the CA catalysis, a similar fast interconversion between OH$^-$ and HCO$_3^-$ bindings to the Zn^{II} ion through a five-coordinate intermediate has been proposed in the catalytic cycle.2) The 12-membered macrocyclic triamine complex is thus well illustrating the four- $\rightleftarrows$ five-coordinate transformation of Zn^{II} in the enzyme.

References

1) S. Lindoskog, "Zinc Enzymes," Brikäuser, Boston, MA (1986), Chap. 22, p. 307; E. A. Eriksson, T. A. Jones, and A. Liljas, *ibid.*, Chap. 23, p. 317; Y. Pocker, N. Janjic, and C. H. Miao, *ibid.*, Chap. 25, p. 341.

2) a) K. K. Kannan, M. Petef, K. Fridborg, H. Cid-Dersdener, and S. Lövgren, *Febs Letter*, **73**, 115 (1977). b) J. Liang and W. N. Lipscomb, *Biochemistry*, **26**, 5293 (1987). c) K. M. Merz, Jr., R. Hoffmann, and M. J. S. Dewar, *J. Am. Chem. Soc.*, **111**, 5636 (1989).

3) A. E. Eriksson, T. A. Jones, and A. Liljas, *Proteins*, **1988**, 274.

4) D. N. Silverman and S. Lindskog, *Acc. Chem. Res.*, **21**, 30 (1988).

5) Y. Pocker and T. L. Deits, *J. Am. Chem. Soc.*, **104**, 2424 (1982).

6) A. E. Eriksson, P. M. Kylsten, T. A. Jones, and A. Liljas, *Proteins*, **1988**, 283.

7) E. Kimura, T. Shiota, T. Koike, M. Shiro, and M. Kodama, *J. Am. Chem. Soc.*, **112**, 5805 (1990).

8) E. Kimura and T. Koike, *Comments Inorg. Chem.*, **11**, 285 (1991).

9) T. Koike and E. Kimura, *J. Am. Chem. Soc.*, **113**, 8935 (1991).

10) IR (KBr pellet): 3495, 3254, 3235, 2930, 2919, 2107 (SCN^-), 2062 (SCN^-), 1456, 1426, 1381, 1368, 1264, 1115, 1098, 1017, 972, 890 cm^{-1}. ^1H NMR (CD_3OD; TMS reference, at 27 °C): δ 1.63 (3 H, ttd, J = 2, 9, 16 Hz, CCHC), 2.04 (3 H, ttd, J = 2, 9, 16 Hz, CCHC), 2.87 (6 H, ddd, J = 2, 9, 13 Hz, CCHC), 3.19 (6 H, ddd, J = 2, 9, 13 Hz, CCHC). ^{13}C NMR (CD_3OD; TMS reference, at 27 °C): δ 26.8, 51.6, 135.8; (D_2O; DSS reference, at 27 °C): δ 27.9, 53.1, 136.6. Anal. Found: C, 37.31; H, 5.96; N, 19.83%. Calcd for $C_{11}H_{21}N_5S_2Zn$: C, 37.44; H, 6.00; N, 19.85%.

11) Crystal data: $C_{11}H_{21}N_5S_2Zn$, M = 352.82, monoclinic, space group $P2_1/c$, a = 8.400(5), b = 13.157(5), c = 14.713(4) Å, β = 91.62(3)°, V = 1625(1) Å^3, Z = 4, D_c = 1.442 g cm^{-3}, crystal size 0.3 × 0.3 × 0.3 mm^3, μ(Cu Kα) = 44.50 cm^{-1}, 2554 data collected at 296 K on Rigaku AFC-5R diffractometer. The data were collected for Lorentz-polarization, extinction and absorption. The structure was solved by the heavy atom method and refined anisotropically by using a total of 2069 reflections ($I > 3\sigma(I)$) to give R = 0.031 and R_W = 0.049. The program used was TEXSAN-TEXRAY structure analysis package (Moleqular Structure Corporation, 1985). Selective bond distances (Å), intermolecular hydrogen bond distances (Å) and bond angles (°): Zn-N(1) 2.033(3), Zn-N(5) 2.050(3), Zn-N(9) 2.203(3), Zn-N(13) 2.119(3), Zn-N(16) 2.012(3), N(13)-C(14) 1.152(5), C(14)-S(15) 1.621(4), N(16)-C(17) 1.143(4), C(17)-S(18) 1.624(3); S(18) ··· N(1) [$-x, -y, -z$] 3.501(3), S(18) ··· N(5) [$x-1, y, z$] 3.523(3), S(18) ··· N(9) [$-x, {}^1/_2+y, {}^1/_2-z$] 3.580(3); N(1)-Zn-N(5) 106.8(1), N(1)-Zn-(16) 119.6(1), N(5)-Zn-N(16) 132.9(1), N(1)-Zn-N(9) 86.5(1), N(1)-Zn-N(13) 98.6(1), N(5)-Zn-N(9) 85.3(1), N(5)-Zn-N(13) 90.9(1), N(9)-Zn-N(13) 174.4(1), N(9)-Zn-N(16) 89.8(1), N(13)-Zn-N(16) 89.8(1).

12) In Ref. 7, the pH-metric determination of the SCN^- affinity to **2** in 100% aqueous solution demonstrated that only one SCN^- ion binds to the Zn^{II} ion in **3** at 25 °C and [SCN^-] ≤ 0.20 mol dm^{-3}. Thus, the apical SCN^- in **4** would be labile and dissociate.

13) P. M. Schaber, J. C. Fettinger, M. R. Churchill, D. Nalewajek, and K. Fries, *Inorg. Chem.*, **27**, 1641 (1988).

(Received February 12, 1992)