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Hydrolytically Active Tetranuclear Nickel Complexes with Structural Resemblance to the Active Site of Urease

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Reaction of the new asymmetric ligand 2-(*N*-isopropyl-*N*-((1-methylimidazolyl)methyl)aminomethyl)-6-(*N*-carboxylmethyl-*N*-((1-methylimidazolyl)methyl) aminomethyl)-4-methylphenol (ICIMP) with nickel perchlorate and diphenylacetic acid leads to the formation of tetranuclear nickel complexes, whose crystal structures reveal that they consist of dimers of dimers in which each Ni_2 unit has a coordination environment that is similar to the active site of urease. One complex has been shown to coordinate urea and catalyze the hydrolysis of an organophosphate monoester.

Urease, which hydrolyzes urea to ammonia and carbamic acid, is an important enzyme in both agriculture and medicine.[1,2] It was the first enzyme to be crystallized[3] and also the first enzyme that was shown to contain nickel.[4−6] Several crystal structures of urease from two different organisms have recently been published.[7−10] These reveal that the active site of the enzyme contains two nickel atoms which are bridged by a carbamylated lysine (Figure 1).

A number of proposals for the mechanism of urease have been put forth,[1,5,10−12] and studies of model complexes for the active site of the enzyme have received new impetus by the structural determination of urease.[13−21] In order to prepare structural and functional mimics of urease and use these to investigate its mechanism, we have synthesized a new phenolate-based polydentate ligand with imidazole and carboxylate donor moieties, viz., 2-(*N*-isopropyl-*N*-((1-methylimidazolyl)methyl)aminomethyl)-6-(*N*-carboxylmethyl-*N*-((1-methylimidazolyl)methyl)aminomethyl)-4-methylphenol (ICIMP).[22] The synthesis of the ligand is outlined in Scheme 1. An overall yield of 26%, based on 1-methylimi-

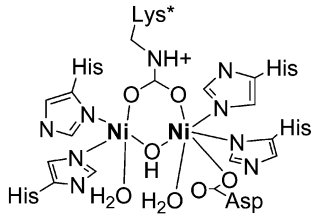

Figure 1. Schematic depiction of the structure of the active site of *Bacillus pasteurii* urease.[9]

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(22) NMR ^{1}H ($CDCl_3$), δ (ppm): 1.14 (d, 6H, *i*-Pr), 2.19 (s, 3H, phenol−Me), 3.07 (m, 1H, *i*-Pr), 3.33 (s, 2H, CH_2CO_2), 3.41 (s, 3H, CH_3Im−*i*-Pr), 3.63 (s, 3H, CH_3Im−CO_2), 3.74 (bs, 4H, CH_2Ph), 3.82 (s, 2H, ImCH_2−*i*-Pr), 3.84 (s, 2H, ImCH_2−CO_2), 6.80 (s, 1H, ImH−*i*-Pr), 6.81 (s, 1H, ImH−CO_2), 6.84 (d, 1H, ImH−CO_2), 6.89 (s, 1H, ImH−*i*-Pr), 6.93 (s, 1H, Ph), 6.99 (s, 1H, Ph). FAB-MS *m*/*z* (rel intensity, %) 455 (MH^+, 40), 302 (M − N(CH_2MeIm)(*i*-Pr), 60), 286 (M − N(CH_2MeIm)(CH_2CO_2H), 60). Anal. Calcd for $C_{24}H_{34}N_6O_3$·HCl: C, 58.70; H, 7.18; N, 17.12; Cl, 7.22. Found: C, 56.68; H, 7.11; N, 15.72; Cl, 5.98.

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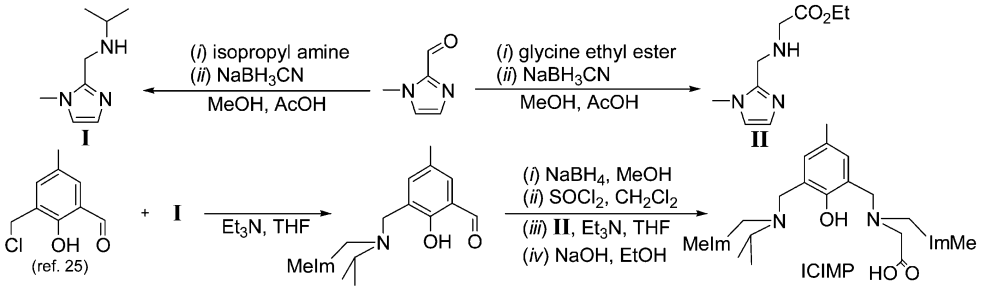
Scheme 1. Synthesis of ICIMP



dazole-2-carbaldehyde, was obtained. ICIMP is a member of a rapidly growing group of asymmetric ligands that has been developed in recent years.[20−21,23−25] To our knowledge, it is the first ligand of this kind to contain carboxylate donors.

Addition of an ethanolic solution of nickel perchlorate to a mixture of ICIMP, diphenylacetic acid, and sodium methoxide dissolved in hot absolute ethanol (2Ni(II):1ICIMP:2Ph_2Ac:4NaOMe) leads to the formation of a green solution. After 1 h of stirring, a blue-green precipitate is slowly formed. Mass spectrometry indicates that the precipitate contains a tetranuclear complex, $[Ni_4(ICIMP)_2(Ph_2Ac)_2]^+$, and microanalysis is consistent with the formulation $[Ni_4(ICIMP)_2(Ph_2Ac)_2][ClO_4]_2$ (**1**).[26] The complex could be recrystallized by dissolving it in DMF and allowing *tert*-butyl methyl ether to diffuse into the solution. The resulting blue crystals of $[Ni_4(ICIMP)_2(Ph_2Ac)_2(DMF)_2][ClO_4]_2 \cdot 2.5DMF$ (**2**)[27] exhibited a mass spectrum similar to that of **1**. Compound **2** was also characterized by microanalysis and single-crystal X-ray diffraction.[28]

If the supernatant from the above-mentioned reaction in ethanol is incubated with urea (2Ni(II):1.2urea), a second tetranuclear complex which contains coordinated urea, $[Ni_4(ICIMP)_2(Ph_2Ac)_2(urea)(H_2O)][ClO_4]_2$, is formed. Single blue crystals of $[Ni_4(ICIMP)_2(Ph_2Ac)_2(urea)(H_2O)][ClO_4]_2 \cdot 0.5EtOH \cdot H_2O$ (**3**)[29] could be grown, and its crystal structure has been determined.

The molecular structures of **2** and **3** (Figure 2) are very similar and may be described as dimers of dimers.[14,30] Each dimer consists of two nickel atoms which are bridged by one ICIMP ligand and one diphenylacetate. The vacant coordination sites of each Ni_2 unit are filled by the carboxylate group of the ICIMP ligand of the opposite Ni_2 moiety and one molecule of solvent or substrate (i.e., urea, cf. Figure 2). In complex **2**, the additional ligands are two DMF molecules, while one urea molecule and one water molecule are coordinated in complex **3**. Coordination of urea has previously been established for several model complexes for urease.[13−19] An interesting feature of the general structure of the compounds is the asymmetric arrangement of the interaction between the two Ni_2 units. In one Ni_2 unit, the ligand carboxylate group from the neighboring dimer bridges the two nickel atoms, while in the second dimer, the corresponding carboxylate moiety chelates one nickel (Figure 2c). Thus, the two Ni_2 units that are found in each tetranuclear complex represent two structural isomers that are related via a "carboxylate shift".[31]

The coordination environments of each Ni_2 unit in **2** and **3** are closely related to that of the active site of urease. The polydentate ligand provides coordinating moieties to mimic all the coordinating amino acid residues in the active site of urease except for the carbamylated lysine, which in each Ni_2 unit is modeled by diphenylacetic acid. The carboxylate from the neighboring Ni_2 moiety coordinates at the sites occupied by water molecules in the crystal structures of the enzyme (cf. Figure 1). All nickel atoms have N_2O_4 coordination, and one nickel atom in each dimer has a loosely bound solvent molecule, which indicates a possible site for urea coordination. As in the crystal structure of *Bacillus pasteurii* urease,[9] the bridging groups are one carboxylate and one OH/OR moiety.

In **2** and **3**, the "bridging" dimer [Ni(1), Ni(2)] is resemblant of the active site of urease with respect to the nature of the ligand coordination. Here, the open coordination site is located on the nickel atom that does not have carboxylate coordination from its own ligand (cf. Ni1 in the *B. pasteurii* crystal structure, Figure 1). In **3**, urea binds via

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(26) FAB-MS *m/z* (rel intensity, %): 1662 ($Ni_4(ICIMP)_2(Ph_2Ac)_2ClO_4$, 40), 779 ($Ni_2(ICIMP)(Ph_2Ac)$, 100). ES-MS *m/z* (rel intensity, %): 1662.1 ($Ni_4(ICIMP)_2(Ph_2Ac)_2ClO_4$, 40), 779.3 ($Ni_2(ICIMP)(Ph_2Ac)$, 100). Anal. Calcd for $C_{80}H_{98}Cl_2N_{12}Ni_4O_{20}$: C, 51.84; H, 5.33; N, 9.07. Found: C, 48.53; H, 4.95; N, 9.43.
(27) FAB-MS *m/z* (rel intensity, %): 1662 ($Ni_4(ICIMP)_2(Ph_2Ac)_2ClO_4$, 40), 779 ($Ni_2(ICIMP)(Ph_2Ac)$, 100). Anal. Calcd for $C_{89.5}H_{117.5}Cl_2N_{16.5}Ni_4O_{22.5}$: C, 51.43; H, 5.67; N, 11.06. Found: C, 51.13; H, 5.22; N, 10.22.
(28) Crystal data: $C_{179}H_{235}N_{33}O_{45}Cl_4Ni_8$, M = 4180.33, triclinic, a = 16.6097(3) Å, b = 16.8816(4) Å, c = 20.5384(5) Å, α = 76.4019(15)°, β = 79.1574(16)°, γ = 61.2237(9)°, V = 4887.23(19) Å^3, T = 120 K, space group $P\bar{1}$ (No. 2), Z = 2, μ(Mo Kα) = 0.71073 Å, 32713 reflections measured, 16032 unique reflections (R_{int} = 0.0502). The final R value was 0.0573 and $R_w(F^2)$ 0.1277 for $I > 2\sigma$. Corresponding values for all data: R = 0.0893 and $R_w(F^2)$ = 0.1461.
(29) FAB-MS *m/z* (rel intensity, %): 1662 ($Ni_4(ICIMP)_2(Ph_2Ac)_2ClO_4$, 30), 779 ($Ni_2(ICIMP)(Ph_2Ac)$, 100). Anal. Calcd for $C_{78}H_{97}Cl_2N_{14}Ni_4O_{21.5}$: C, 49.82; H, 5.20; N, 10.43. Found: C, 49.36; H, 5.22; N, 9.46. Crystal data: $C_{75}H_{181}Cl_6N_{12}Ni_6O_{12}$, M = 2008.28, triclinic, a = 13.9016(2) Å, b = 15.5577(2) Å, c = 21.0623(3) Å, α = 77.4506(5)°, β = 89.7781(5)°, γ = 71.9627(6)°, V = 4218.06(10) Å^3, T = 120 K, space group $P\bar{1}$ (No. 2), Z = 2, μ(Mo Kα) = 0.71073 Å, 63001 reflections measured, 16412 unique reflections (R_{int} = 0.0448). The final R value was 0.0566 and $R_w(F^2)$ 0.1483 for $I > 2\sigma$. Corresponding values for all data: R = 0.0763 and $R_w(F^2)$ = 0.1639.
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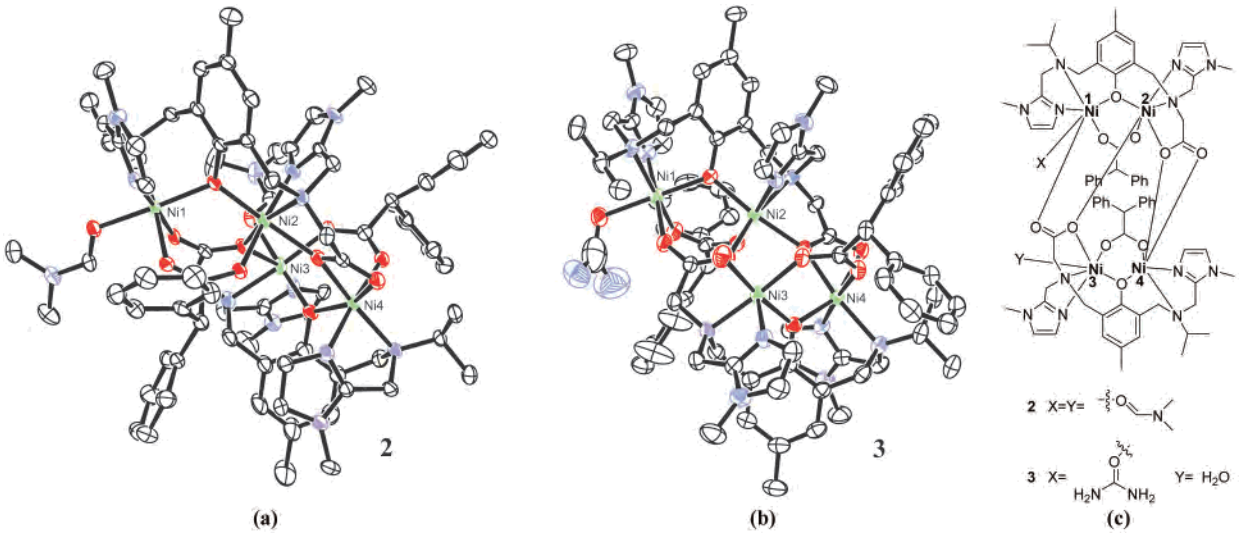

Figure 2. (a) ORTEP representation of compound **2**. Selected interatomic distances and angles: Ni(1)−$O_{phenolate}$ 2.012(3), Ni(1)−O_{Ph2Ac} 2.044(3), Ni(1)−O_{DMF} 2.092(3), Ni(2)−$O_{phenolate}$ 2.013(3), Ni(2)−O_{Ph2Ac} 2.069(3), Ni(3)−$O_{phenolate}$ 2.012(3), Ni(3)−O_{Ph2Ac} 2.004(3), Ni(3)−O_{DMF} 2.144(3), Ni(4)−$O_{phenolate}$ 2.008(3), Ni(4)−O_{Ph2Ac} 2.024(3), Ni(1)−Ni(2) 3.471, Ni(3)−Ni(4) 3.513, Ni(1)−Ni(3) 5.842, Ni(2)−Ni(3) 3.811, Ni(2)−Ni(4) 4.039, Ni(1)−$O_{phenolate}$−Ni(2) 119.12(14), Ni(3)−$O_{phenolate}$−Ni(4) 121.81(15), Ni(1)−Ni(2)−Ni(3)−Ni(4) 145.6. (b) ORTEP representation of compound **3**. Selected interatomic distances and angles: Ni(1)−$O_{phenolate}$ 1.992(3), Ni(1)−O_{Ph2Ac} 2.055(3), Ni(1)−O_{urea} 2.102(3), Ni(2)−$O_{phenolate}$ 2.010(3), Ni(2)−O_{Ph2Ac} 2.074(3), Ni(3)−$O_{phenolate}$ 2.006(3), Ni(3)−O_{Ph2Ac} 2.004(3), Ni(3)−O_{H2O} 2.184(3), Ni(4)−$O_{phenolate}$ 2.002(3), Ni(4)−O_{Ph2Ac} 2.011(3), Ni(1)−Ni(2) 3.468, Ni(3)−Ni(4) 3.492, Ni(1)−Ni(3) 5.757, Ni(2)−Ni(3) 3.794, Ni(2)−Ni(4) 4.004, Ni(1)−$O_{phenolate}$−Ni(2) 120.13(13), Ni(3)−$O_{phenolate}$−Ni(4) 121.19(13), Ni(1)−Ni(2)−Ni(3)−Ni(4) 150.9. (c) Schematic representation of the connectivity in compounds **2** and **3**.

its carbonyl oxygen to the open coordination site in the bridging dimer. This suggests that the initial step in the catalytic cycle of urease is the coordination of the urea carbonyl to Ni1 in the enzyme. The "chelated" dimer [Ni(3), Ni(4)] shows similarities to the *B. pasteurii* urease in that it has the same number of bridges between the nickel centers, which makes the Ni−Ni distances comparable to those found in urease.[7,9] The Ni−Ni distance is 3.51 (**2**), 3.49 (**3**) Å in the chelated Ni_2 unit vs 3.47 Å (**2** and **3**) in the bridged unit.

In order to assess the capacity of these compounds to act as functional models for hydrolytic metalloenzymes,[24] their ability of catalyzing the hydrolysis of organophosphate esters is currently being studied.[32] Complex **1** is a suitable candidate for a hydrolysis catalyst, as it is believed to possess vacant coordination sites (or loosely bound solvent molecules) where suitable substrates may bind. The rate of internal hydrolysis of 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP)[33] at pH 8.0 and 25° was increased by a factor of 21 in the presence of **1**, compared to the uncatalyzed reaction (5.1×10^{-6} M/h vs 2.4×10^{-7} M/h). Relatively moderate turnover numbers (10−15) were recorded for the reaction, possibly due to inhibition by the phosphate product. The fact that **1** catalyzes hydrolysis despite the lack of a bridging hydroxide might indicate that such a moiety is not crucial to urease catalysis.[10]

A dinuclear compound that is tentatively assigned the formula $[Ni_2(ICIMP)(Ph_2Ac)_2]$[34] may be synthesized by repeating the above-mentioned reaction using an excess of diphenylacetic acid; however, attempts to cleave the tetranuclear compounds into dinuclear complexes by reaction with diphenylacetic acid have thus far proven unsuccessful.

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Supporting Information Available: Supporting material concerning crystal structures and details concerning the hydrolysis reaction (CIF and PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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