acetal to a Lewis acid rather than to an α-enone. These results hence provide a rationale for the above mechanism on the basis of energetics.

In summary, the mechanism of the Mukaiyama reaction has never been examined from the standpoint of electron transfer. We conclude here that the Lewis acid mediated electron transfer from hindered ketene silyl acetals to α-enones plays an essential role in connecting quaternary carbon centers. The conclusion sheds light on a novel facet of the ketene silyl acetal chemistry, and consequently relevant reactions should be reexamined along this line, particularly in terms of stereochemistry.[18]

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Supplementary Material Available: ^1H and ^{13}C NMR spectral data of **3a-c** and **7a,b**, results of the crossover reaction employing other ketene silyl acetals and an α-enone, and a figure exhibiting relations of the log k_{et} values with $E°_{red}$ or $E°_{ox}$ (3 pages). Ordering information is given on any current masthead page.

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Variable-Temperature Magnetic Circular Dichroism Spectroscopy as a Probe of the Electronic and Magnetic Properties of Nickel in Jack Bean Urease

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Urease catalyzes the hydrolysis of urea to yield ammonia and carbamate, which spontaneously hydrolyzes to form carbonic acid and a second molecule of ammonia.[1] The best characterized example is jack bean urease, which is a hexamer of identical 90 770-Da subunits,[2] each containing one catalytic site and two Ni(II) ions, which are required for activity.[3] Although information concerning the Ni(II) active site is now beginning to emerge, its structure and electronic properties are still poorly characterized. Weak absorption bands superimposed on the scattering background of the globular protein have been assigned to the spin-allowed d-d transitions of octahedral Ni(II),[4] and studies using X-ray absorption spectroscopy (XAS) are consistent with pseudooctahedral coordination by O or N donor atoms.[5]

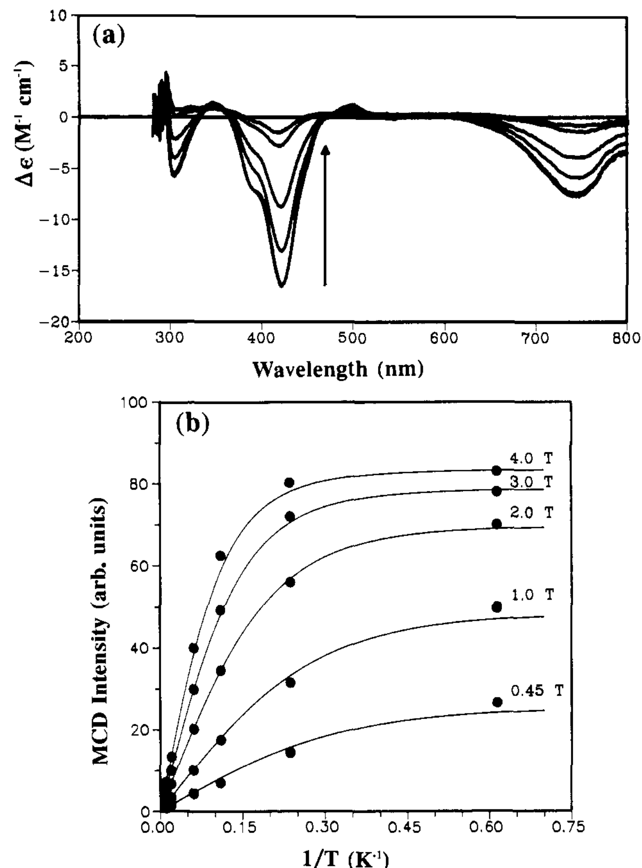

Figure 1. Variable-temperature MCD spectra of native jack bean urease. The urease sample, in 20 mM phosphate buffer containing 65% (v/v) glycerol, pH 6.6, was 2.8 mM in Ni. (a) MCD spectra with a magnetic field of 4.5 T at 1.63, 4.22, 9.1, 16, 49, and 91 K. The arrow indicates the direction of change in the MCD intensity with increasing temperature. (The spectra at 1.63 and 4.22 K are almost superimposed.) (b) Temperature dependence of the MCD intensity at 420 nm at 4.0, 3.0, 2.0, 1.0, and 0.45 T. Solid lines are the best fit to eq 6 of ref 14, with $g_\perp = 7.3$ and $\delta = 4.1$ cm^{-1}.

Evidence for a binuclear Ni(II) active site has come from magnetic susceptibility studies of jack bean urease which indicate that ~80% of the Ni(II) is in an antiferromagnetically exchange coupled binuclear active site ($J = -6.3$ cm^{-1}, using an isotropic exchange Hamiltonian $H = -2J\mathbf{S}_1 \cdot \mathbf{S}_2$) with the remaining ~20% as magnetically isolated high spin ($S = 1$) Ni(II).[6] On binding of the competitive inhibitor 2-mercaptoethanol (2-ME, $K_I = 0.72 \pm 0.26$ mM at 25 °C[3b]) to the jack bean enzyme, a large decrease in paramagnetism is observed, and this has been ascribed to the formation of a strongly antiferromagnetically coupled binuclear Ni(II) center.[6] In contrast, subsequent magnetic susceptibility studies of native *Klebsiella aerogenes* urease were interpreted in terms of a mixture of low-spin ($S = 0$) and high-spin ($S = 1$) Ni(II) without invoking an exchange-coupled binuclear center.[7]

Here we report the results of variable-temperature MCD studies[8] of native and 2-ME-inhibited jack bean urease. The enzyme samples used in this work were prepared as previously reported,[6] contained 65% (v/v) glycerol, exhibited specific activities >75% of the maximum reported[9] (~2700 IU/mg), and contained

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~2 Ni atoms/subunit. The results demonstrate the potential of this technique for monitoring the ligand-field transitions and investigating the ground-state properties of paramagnetic Ni(II) in urease and provide unambiguous evidence for the presence of a binuclear Ni(II) center with strong antiferromagnetic exchange coupling ($J = -40$ cm^{-1}) in the 2-ME-inhibited enzyme.

The variable-temperature MCD spectra of native jack bean urease exhibit two negative bands centered at 420 and 745 nm that increase in intensity with decreasing temperature (Figure 1a). No additional MCD bands were observed out to 1000 nm. By analogy to the variable-temperature MCD spectra of a range of octahedral Ni(II) complexes (e.g., $[Ni(H_2O)_6]^{2+}$, $[Ni(NH_3)_6]^{2+}$, and $[Ni(en)_3]^{2+}$),[10] these bands are assigned to the two highest energy d–d transitions of Ni(II) in an octahedral ligand field, i.e., $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ at 420 nm and $^3A_{2g} \rightarrow {}^3T_{1g}(F)$ at 745 nm. The energies of these transitions are consistent with Dq = 800–830 cm^{-1} and B = 900–950 cm^{-1}, which predicts that the lowest energy d–d transition, $^3A_{2g} \rightarrow {}^3T_{2g}$, will occur at ~1300 nm (outside the spectral range of these measurements). These ligand-field parameters are characteristic of octahedral Ni(II) centers with predominantly oxygenic coordination.[11]

MCD magnetization data collected at both 420 and 745 nm approach magnetic saturation with increasing field (0–4.5 T) at 1.6 K. Such behavior is incompatible with an antiferromagnetically coupled binuclear Ni center, since it dictates that a near degenerate state lies lowest in energy.[12] Indeed, the observed magnetization data can be fit to a good approximation using theoretical expressions developed for a randomly oriented isolated non-Kramers doublet, assuming x,y polarization and $g_\perp = 0$,[13] see Figure 1b. At both wavelengths the best fit parameters were $g_\parallel = 7.3$ with the rhombic zero-field splitting, δ = 3.7–4.1 cm^{-1}. A $g_\parallel$ value of 7.3 is too large for a $M_s = \pm 1$ doublet and is more indicative of a $M_s = \pm 2$ doublet, which could only arise from a ferromagnetically exchange coupled binuclear Ni(II) center. While a major component of ferromagnetically coupled binuclear nickel centers is inconsistent with the magnetic susceptibility data,[6] a minor component that dominates the low-temperature MCD spectrum is possible. This raises the possibility that the heterogeneity of the active-site nickel centers, apparent in the magnetic susceptibility data, may arise from a mixture of antiferromagnetically and ferromagnetically coupled binuclear Ni(II) centers, rather than a mixture of antiferromagnetically coupled binuclear and magnetically isolated high-spin Ni(II) ions.

The MCD spectrum of 2-ME-inhibited jack bean urease, Figure 2a, is remarkable for the anomalous temperature dependence.[14] The spectrum consists of negative, temperature-dependent MCD bands centered at 324, ~380 (shoulder), 422, and 750 nm that *increase* in intensity with increasing temperature up to 150 K. By analogy with the native enzyme, the bands at 422 and 750 nm are assigned to the two highest energy Ni(II) d–d bands. Consequently the nature and magnitude of the Ni(II) ligand field are not significantly perturbed by 2-ME binding, which is in accord with the available absorption[6] and XAS[5c] data. Since XAS studies indicate direct thiolate S coordination of Ni in 2-ME-bound jack bean urease,[5c] the new MCD bands at 324 and 380 nm (shoulder) are attributed to thiolate S → Ni(II) charge-transfer transitions.

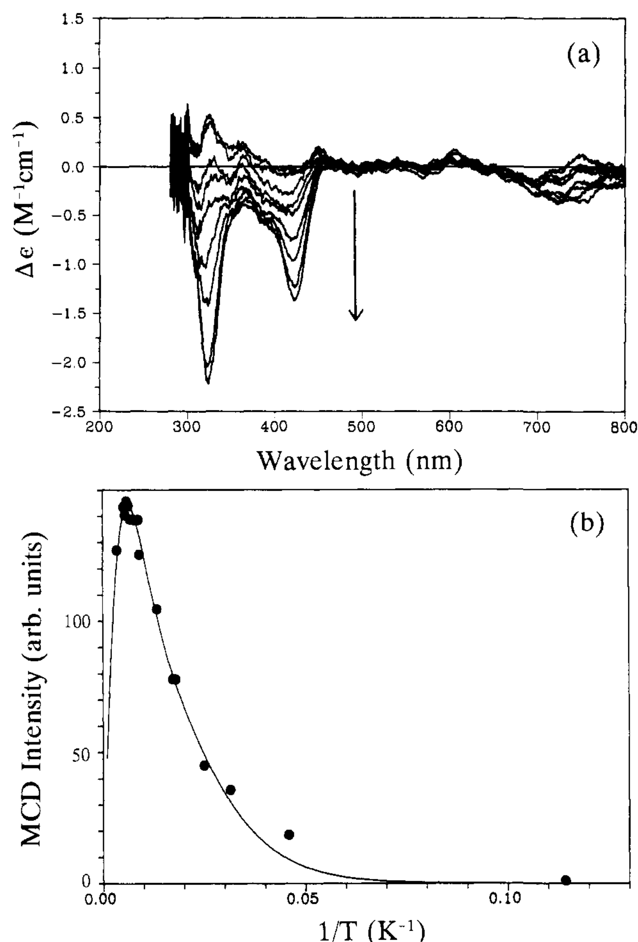

Figure 2. Variable-temperature MCD spectra of 2-mercaptoethanol-inhibited jack bean urease. The urease sample, in 20 mM phosphate buffer containing 65% (v/v) glycerol, pH 6.6, was 3.7 mM in Ni and 40 mM in 2-mercaptoethanol. (a) MCD spectra with an applied magnetic field of 4.5 T at 4.2, 8.8, 22, 32, 40, 56, 75, 115, and 150 K. The arrow indicates the direction of change in the MCD intensity with increasing temperature. (The spectra at 4.2 and 8.8 K are almost superimposed.) The residual contribution from native jack bean urease has been subtracted from the spectrum at each temperature. (b) Temperature dependence of the MCD intensity at 422 nm. Solid line is the best fit to eq 1, using C_1 = 9084, C_2 = 61124 (arbitary units), and $J = -40$ cm^{-1}.

The anomalous temperature dependence can *only* be reconciled with an antiferromagnetically coupled binuclear Ni(II) center, i.e., S = 0, 1, and 2 states separated in energy by $-2J$ and $-4J$, respectively, with the nondegenerate state lowest in energy. As shown in Figure 2b, the MCD intensity, ΔA, can be fit to a Boltzmann population distribution over a three-level system:

$$\Delta A = \alpha_1 C_1/kT + \alpha_2 C_2/kT \quad (1)$$

where α_1 and C_1 and α_2 and C_2 and are the fractional populations and C terms originating from the 3-fold-degenerate $S = 1$ and 5-fold-degenerate $S = 2$ excited states, respectively. Best fits at both wavelengths were obtained with $J = -40 \pm 5$ cm^{-1}.[15] A singlet–triplet splitting of 80 cm^{-1} is almost an order of magnitude greater than the zero-field splitting expected for high-spin Ni(II) in an approximately octahedral coordination environment (typically 0–10 cm^{-1}), which rules out a mononuclear Ni(II) species as the origin of MCD bands and justifies the neglect of zero-field

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$$H = -2J\mathbf{S}_1\cdot\mathbf{S}_2 + D[S_{1z}^2 + S_{2z}^2 - \tfrac{2}{3}S(S+1)] + g\beta H(S_1 + S_2)$$

where the subscripts 1 and 2 refer to the different nickels and D is the axial zero-field-splitting parameter, predicts a singlet state lowest in energy for $J < 0$ for any value of D.

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(15) This value of J is in excellent agreement with recent high-temperature magnetic susceptibility studies of 2-ME-inhibited jack bean urease, which were best fit with $J = -35 \pm 10$ cm^{-1} for the antiferromagnetically coupled binuclear Ni(II) center. Alvarez, M. L.; Wilcox, D. E., unpublished results.

splitting in eq 1. A binuclear Ni(II) complex with such large exchange interaction is without precedence in inorganic chemistry and may be a consequence of a bridging thiolate. The synthesis and characterization of appropriate binuclear model compounds is required to evaluate this proposal.

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Triplex Formation of Oligonucleotides Containing 2′-*O*-Methylpseudoisocytidine in Substitution for 2′-Deoxycytidine

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Recently, studies of sequence-specific triplex formation of short synthetic oligonucleotides (or their analogues) have been of prime interest.[1-6] In this report, a synthesis of a new oligonucleotide analogue containing 2-amino-5-(2-*O*-methyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (2′-*O*-methylpseudoisocytidine or **1**) in substitution for 2′-deoxycytidine and the triplex formation of this oligonucleotide analogue with a target duplex at neutral pH are reported.

A triplex can be formed as a triad consisting of a homopyrimidine strand, a homopurine strand, and a homopyrimidine strand.[7-11] The third strand (i.e., the second pyrimidine strand) is located in the major groove of a duplex with Watson-Crick base pairing.[12-16] Thymines or cytosines in the third strand form Hoogsteen type hydrogen bondings with adenines or guanines in the homopurine strand, respectively. Since protonation of cytosine bases in acidic conditions is essential in order to provide the second hydrogen bonding between the protonated cytosine and guanine in the Hoogsteen pair of the triad (Figure 1, upper-left panel), this C-G-C+ triad is unstable in physiological conditions.[17-19] This requirement limits the formation of triplex in living cells since the cellular pH is usually above pH 7.0.

To overcome this limitation, we designed and synthesized an oligonucleotide containing **1** which may form Hoogsteen type base pairings through hydrogen bondings with guanine in neutral and basic conditions (Figure 1, upper-right panel). As indicated in the figure, this nucleoside **1** already contains one hydrogen at the N-3 position for hydrogen bonding with the guanine in the Hoogsteen pair of the triad. Also, methoxy substitution at 2′-position of pyrimidine nucleosides in a third strand stabilized the triplex formation.[20]

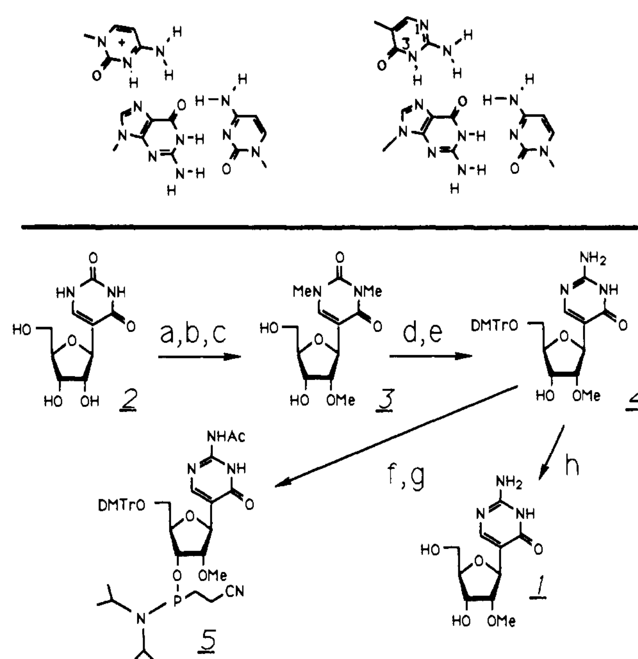

Figure 1. The hydrogen-bonding schemes of triplexes of CGC+ (upper left) and CG1 (upper right). The lower panel is a scheme for the synthesis of **1** and **5**: **2** was obtained from Kyowa Hakko, U.S.A., Inc. (New York, NY). (a) Protection of the 3′ and 5′ hydroxyl groups according to Pankiewicz et al.[28] (b) Methylation of the 2′-hydroxyl group; iodomethane (40 equiv), Ag_2O (10 equiv), 25 °C, 7 days.[22] (c) Deprotection of the silyl group; Bu_4NF (2.5 equiv), THF, 25 °C, 1 h; 58% from the 3′- and 5′-protected compound. (d) Protection of the 5′-hydroxyl group; dimethoxytrityl (DMT) chloride (1.05 equiv), pyridine, 25 °C, 12 h; 94%. (e) Conversion of the base moiety; guanidine (100 equiv), sodium ethoxide (70 equiv), absolute ethanol, reflux for 1 h;[29] 61%. The α-anomer was not detected. (f) Protection of the amino group; acetic anhydride, DMF, 25 °C, 12 h, 78%. (g) (2-Cyanoethyl)-*N*,*N*-diisopropylphosphonamidous chloride (1.5 equiv),[30] $EtiPr_2N$ (2 equiv), CH_2Cl_2, 25 °C, 30 min; 71%. (h) Acetic acid (80%), 25 °C, 12 h; 78%.

A scheme for the synthesis of **1**[21] and its amidite synthon **5** is shown in the lower panel of Figure 1. An octamer 5′-(TT1TT1TT)3′ (**a**) was synthesized on a DNA synthesizer (Applied Biosystem). After deblocking and purification, the oligomer a of this preparation showed one sharp peak by HPLC analysis. After hydrolysis of the oligomers by snake venom phosphodiesterase and alkaline phosphatase to nucleosides, the nucleoside composition for each oligomer was confirmed by HPLC.

We studied a triplex formation of the oligomer **a** with an undecameric target duplex 5′d(AAGAAGAAGAA)3′-5′d-(TTCTTCTTCTT)3′ (**d**). Both 5′d(TTCTTCTT)3′ (**b**) and 5′(TTCmTTCmTT)3′ (**c**, Cm = 2′-*O*-methylcytidine[22]) were used as controls for the third strand. An octamer **a** or **b** or **c** was mixed with the duplex **d** in a buffer, and the thermally induced transitions of the helices in each mixture was studied by measurement of the UV absorption at 260 nm at pH 7 (Figure 2, upper panel). Both the duplex **d** alone and the mixtures consisting of **b** and **d** or of **c** and **d** showed only one transition (T_m = 42 °C, Figure 2, lower panel) which was attributable to the melting of the duplex **d** itself. On the other hand, the mixture of **a** and **d** showed two transitions

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