F430 Model Chemistry. A Reexamination of the [1,4,7,10,13-Pentaazacyclohexadecane-14,16-dionato(2−)]nickel(II)-Induced Formation of Methane from Methyl Coenzyme-M

Zhong Zhang, Jeffrey L. Petersen, and Alan M. Stolzenberg*

Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506

Received March 8, 1996[⊗]

The report that the nickel(II) complex of the macrocyclic ligand 1,4,7,10,13-pentaazacyclohexadecane-14,16-dione, L, cleaves $CH_3SCH_2CH_2SO_3^-$ to CH_4 and $HSCH_2CH_2SO_3^-$ was reexamined. $CH_3SCH_2CH_2SO_3^-$, or cofactor methyl coenzyme-M, carries the methyl group in the final step of methanogensis in methanogenic bacteria. The cleavage of the cofactor was reproduced when the nickel complex of L synthesized from unpurified, technical grade tetraethylenepentamine was used. However, authentic samples of the nickel complex were found to be incapable of carrying out the cleavage reaction. $NiL(OAc)_2$ prepared from L synthesized from pure tetraethylenepentamine crystallizes in the monoclinic space group $P2_1/c$ ($Z = 4$) with unit cell dimensions $a = 8.234(1)$ Å, $b = 13.439(2)$ Å, $c = 18.915(2)$ Å, $\beta = 95.370(10)°$, and $V = 2083.9(5)$ Å^3. The structure was refined to $R = 0.037$ on F_o^2 on the basis of 2754 reflections with $I > 2\sigma(I)$. The nickel atom is coordinated in a meridional fashion by the three secondary nitrogen atoms of L with the remaining three coordination sites occupied by one chelating η^2-acetate and one nonchelating η^1-acetate. Neither this N_3O_3 octahedral form, which prevails in neutral aqueous solution (presumably with waters replacing the acetates as the oxygen donors), nor the five-coordinate, square pyramidal, amide coordinated form, which prevails in basic solution, effect cleavage of methyl coenzyme-M. Upon reexamination, the ligand prepared from technical grade tetraethylenepentamine was found to be contaminated with 1,4,7,10-tetraazacyclotridecane-11,13-dione, L′, which crystallizes in the triclinic space group $P\bar{1}$ ($Z = 2$) with unit cell dimensions $a = 8.658(2)$ Å, $b = 8.663(2)$ Å, $c = 8.888(2)$ Å, $\alpha = 69.11(3)°$, $\beta = 83.51(3)°$, $\gamma = 62.49(3)°$, and $V = 551.3(2)$ Å^3. The structure was refined to $R = 0.045$ on F^2 on the basis of 1277 reflections with $I > 2\sigma(I)$. The Ni complex of L′, $NiH_{-2}L'$, does not cleave methyl coenzyme-M. Solutions of physical mixtures of $NiL(OAc)_2$ and $NiH_{-2}L'$ can reproduce the features of the UV−vis spectra observed during cleavage of methyl coenzyme-M but cannot cleave methyl coenzyme-M. The compound(s) or cooperative interactions between compounds in the impure material that are responsible for the cleavage reaction have not been isolated or identified. Nonetheless, based upon our observations, the originally proposed mechanism that involves NiL as the active complex is incorrect and cannot be taken as a precedent for the cleavage of methyl coenzyme-M by the enzyme methyl coenzyme-M reductase.

Methyl coenzyme-M reductase (MCR)[1,2] contains the nickel-hydrocorphinoid complex F430.[3−5] The enzyme catalyzes the reduction of methyl coenzyme-M ($CH_3-S-CoM$) by the thiol cofactor $H-S-HTP$[6] to afford methane and the heterodisulfide $CoM-S-S-HTP$.[7,8] Active MCR exhibits two distinct Ni^I EPR signals, which are designated MCR-red1 (axial) and MCR-red2 (rhombic).[9−11] Addition of the substrate $CH_3-S-CoM$ converts MCR-red2 to MCR-red1 whereas addition of $H-S-CoM$ leads to an increase in the MCR-red2 signal.[11] Other distinct EPR signals are observed in the presence of inhibitory, halogenated substrate analogues of both $CH_3-S-CoM$ and $H-S-HTP$.[12] Thus, the substrates either bind directly to Ni^I-F430 or close enough to change the environment of the Ni(I)-atom.

The involvement of Ni(I) in MCR has led to considerable interest in the chemistry of the Ni^I forms of F430 and Ni isobacteriochlorins,[13−19] the only tetrapyrrolic compounds that afford isolable Ni(I) complexes when reduced.[20−22] Although

---

[⊗] Abstract published in *Advance ACS Abstracts*, July 1, 1996.

(1) Rouviere, P. E.; Wolfe, R. S. *J. Biol. Chem.* **1988**, *263*, 7913−7916.

(2) Friedmann, H. C.; Klein, A.; Thauer, R. K. *FEMS Microbiol. Rev.* **1990**, *87*, 339−348.

(3) Pfaltz, A.; Jaun, B.; Faessler, A.; Eschenmoser, A.; Jaenchen, R.; Gilles, H. H.; Diekert, G.; Thauer, R. K. *Helv. Chim. Acta* **1982**, *65*, 828−865.

(4) Livingston, D. A.; Pfaltz, A.; Schreiber, J.; Eschenmoser, A.; Ankel-Fuchs, D.; Moll, J.; Jaenchen, R.; Thauer, R. K. *Helv. Chim. Acta* **1984**, *67*, 334−351.

(5) Faerber, G.; Keller, W.; Kratky, C.; Jaun, B.; Pfalz, A.; Spinner, C.; Kobelt, A.; Eschenmoser, A. *Helv. Chim. Acta* **1991**, *74*, 697−716.

(6) Abbreviations: CoM, 2-mercaptoethanesulfonic acid anion; methyl CoM, (2-methylthio)ethanesulfonic acid anion; HS-HTP, (7-mercaptoheptanoyl)threonine phosphate; F430M, the pentamethyl ester of F430; OEiBC, mixture of *ttt*- and *tct*-2,3,7,8-tetrahydro-2,3,7,8,12,-13,17,18-octaethylporphyrin dianion; L, 1,4,7,10,13-pentaazacyclohexadecane-14,16-dione; L′, 1,4,7,10-tetraazacyclotridecane-11,13-dione; dien, diethylenetriamine; trien, triethylenetetramine; tetren, tetraethylenepentamine.

(7) Bobik, T. A.; Olson, K. D.; Noll, K. M.; Wolfe, R. S. *Biochem. Biophys. Res. Commun.* **1987**, *149*, 455−460.

(8) Ellermann, J.; Hedderich, R.; Boecher, R.; Thauer, R. K. *Eur. J. Biochem.* **1988**, *172*, 669−677.

(9) Albracht, S. P. J.; Ankel-Fuchs, D.; Van der Zwaan, J. W.; Fontijn, R. D.; Thauer, R. K. *Biochim. Biophys. Acta* **1986**, *870*, 50−57.

(10) Albracht, S. P. J.; Ankel-Fuchs, D.; Boecher, R.; Ellermann, J.; Moll, J.; Van der Zwaan, J. W.; Thauer, R. K. *Biochim. Biophys. Acta* **1988**, *955*, 86−102.

(11) Rospert, S.; Boecher, R.; Albracht, S. P. J.; Thauer, R. K. *FEBS Lett.* **1991**, *291*, 371−375.

(12) Rospert, S.; Voges, M.; Berkessel, A.; Albracht, S. P. J.; Thauer, R. K. *Eur. J. Biochem.* **1992**, *210*, 101−107.

(13) Jaun, B.; Pfaltz, A. *J. Chem. Soc., Chem. Commun.* **1986**, 1327−1329.

(14) Jaun, B.; Pfaltz, A. *J. Chem. Soc., Chem. Commun* **1988**, 293−294.

(15) Lin, S. K.; Jaun, B. *Helv. Chim. Acta* **1991**, *74*, 1725−1738.

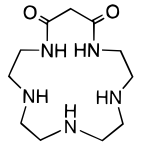
(16) Lin, S. K.; Jaun, B. *Helv. Chim. Acta* **1992**, *75*, 1478−1490.

(17) Stolzenberg, A. M.; Stershic, M. T. *J. Am. Chem. Soc.* **1988**, *110*, 5397−5403.

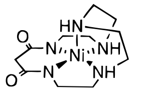
S0020-1669(96)00263-7 CCC: $12.00

Ni^I−F430M and Ni^I(OEiBC) react rapidly and catalytically with alkyl halides, alkyl-*p*-toluenesulfonates, and alkyl-sulfonium ions in nonaqueous solvents to afford alkanes and alkenes via the intermediacy of alkyl−nickel species, little[18] or no[14] methane is formed upon reaction with methyl CoM or other methyl thioethers. It is not clear whether the limited solubility of the salts of methyl CoM in nonaqueous solvents or the rapid reaction of Ni(I) with the ammonium counterion to afford H_2 is responsible for the observed lack of reactivity in these systems. However, Ni^I−tetraazamacrocyclic complexes are only somewhat more effective in producing methane from methyl CoM in aqueous solution.[23] Yields vary from trace levels of methane at pH 7.4 to over 10% at pH 9.4.

In striking contrast to the apparent inability of Ni^I complexes to effect C−S bond cleavage is the single existing report of a Ni^{II} coordination complex that reacts directly with methyl CoM. The nickel complex of the ligand 1,4,7,10,13-pentaazacyclohexadecane-14,15-dione, L, was reported to cleave methyl CoM to methane and H−S−CoM in a slow, stoichiometric process.[24,25] The mechanism advanced for this reaction invokes a Ni(III)/Ni(II) redox couple rather than the Ni(II)/Ni(I) couple that is implied by the biochemical evidence. Cleavage of methyl CoM is proposed to cause concurrent oxidation of Ni(II) to Ni(III). Subsequent re-reduction to Ni(II) occurs by oxidation of H_2O to O_2. As H−S−CoM forms, it coordinates to and deactivates the nickel complex and is also partially oxidized to CoM−S−S−CoM by O_2 generated in the reaction. Reaction ceases after 1.2 equiv of methyl CoM is consumed unless an external oxidant is added to convert the H−S−CoM ligand to disulfide.



L



$NiH_{-2}L$

Other reported chemistry of NiL is equally remarkable. Potentiometric titrations established that aqueous solutions of Ni(II) and L undergo multiple speciation equilibria.[26−28] The ligand and metal do not asssociate below roughly pH 5. The blue solutions of slightly higher pH contain a complex in which Ni(II) is thought to coordinate to the three secondary amino groups of the ligand. As the pH is increased from 8 through 10, the solution color turns pink as the two ligand amide groups sequentially deprotonate and coordinate to the nickel atom. The changes are sluggish, suggesting that substantial conformational rearrangement of the ligand must occur. The high-spin, five-coordinate, square-pyramidal complex $Ni^{II}H_{-2}L$[29,30] that results is oxidized to a brown Ni(III) complex at the unusually low redox potential of +0.24 V vs SCE.[27] More remarkably, $Ni^{II}H_{-2}L$ forms a 1:1 complex with O_2.[27,28] Uptake of O_2 is reversible for the 15-ethyl- or benzyl-substituted complexes, but unsubstituted $Ni^{II}H_{-2}L$-O_2 decomposes with concurrent hydroxylation of an external substrate or of L at position 15.[26,31,32] Thus, aerobic solutions of $Ni^{II}H_{-2}L$ oxidize benzene to phenol[31] and oxidatively cleave DNA.[33] $Ni^{II}H_{-2}L$ is also a very active catalyst for the dismutation of superoxide.[34]

We were struck by several inconsistencies between the reports of Drain et al.[24,25] and those of other workers.[26−33] The Ni(II) complex of L that cleaves methyl CoM is reported to be a green hygroscopic solid that affords green solutions in both water and ethanol. The mechanism proposed for this reaction involves steps that are imbalanced by charge and ignores the protonation equilibria of the ligand amide groups and the concomitant structural changes of the complex. Suprisingly, the initial rate of formation of methane is reported to be nearly independent of pH (40% decrease) from pH 4.0 to 9.0 and to increase slightly when the reaction is conducted under an O_2 atmosphere instead of argon. Finally, it is questionable whether $Ni^{III}H_{-2}L$ is thermodynamically or kinetically capable of oxidizing water to O_2.

The importance of the report of the cleavage of methyl CoM by NiL as a unique precedent for the chemistry of MCR and the inconsistencies that we noted led us to reinvestigate this system. The results that we report in this paper establish that some chemistry leading to cleavage of methyl CoM does occur in this system, but that it is not brought about by NiL. Thus, the mechanism proposed by Drain et. al.[24,25] is incorrect.

Results and Discussion

In initial experiments, we carefully followed the procedure of Drain et al. to prepare the ligand L and its nickel complex. The procedure, which involves several modifications to Kimura's original method, is described in an unpublished manuscript.[35] An important detail is that unpurified, technical grade (75% pure) tetraethylenepentamine, tetren, was used as a starting material in the synthesis of L. The authors stated that purification of tetren[36] was a tedious, low yield procedure and asserted that L prepared from technical grade material could be successfully purified by a sequence of steps that included flash chromatography, extraction, and recrystallization and was obtained with greater net conversion of material (10% yield).

The crystalline L that we obtained had physical and spectroscopic properties that agreed with literature reports.[24−26,28] Anaerobic reaction of L with $Ni(OAc)_2·4H_2O$ in absolute ethanol resulted in a green solution that afforded a green,

---

(18) Lahiri, G. K.; Schussel, L. J.; Stolzenberg, A. M. *Inorg. Chem.* **1992**, *31*, 4991−5000.

(19) Lahiri, G. K.; Stolzenberg, A. M. *Inorg. Chem.* **1993**, *32*, 4409−4413.

(20) Stolzenberg, A. M.; Stershic, M. T. *J. Am. Chem. Soc.* **1988**, *110*, 6391−6402.

(21) Renner, M. W.; Furenlid, L. R.; Barkigia, K. M.; Forman, A.; Shim, H. K.; Simpson, D. J.; Smith, K. M.; Fajer, J. *J. Am. Chem. Soc.* **1991**, *113*, 6891−6898.

(22) Furenlid, L. R.; Renner, M. W.; Smith, K. M.; Fajer, J. *J. Am. Chem. Soc.* **1990**, *112*, 1634−1635.

(23) Zilbermann, I.; Golub, G.; Cohen, H.; Meyerstein, D. *Inorg. Chim. Acta* **1994**, *227*, 1−3.

(24) Drain, C. M.; Sable, D. B.; Corden, B. B. *Inorg. Chem.* **1988**, *27*, 2396−2398.

(25) Drain, C. M.; Sable, D. B.; Corden, B. B. *Inorg. Chem.* **1990**, *29*, 1428−1433.

(26) Chen, D.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1991**, *30*, 1396−1402.

(27) Kimura, E.; Sakonaka, A.; Machida, R.; Kodama, M. *J. Am. Chem. Soc.* **1982**, *104*, 4255−4257.

(28) Kimura, E.; Machida, R.; Kodama, M. *J. Am. Chem. Soc.* **1984**, *106*, 5497−5505.

(29) Kushi, Y.; Machida, R.; Kimura, E. *J. Chem. Soc., Chem. Commun.* **1985**, 216−218.

(30) Machida, R.; Kimura, E.; Kushi, Y. *Inorg. Chem.* **1986**, *25*, 3461−3466.

(31) Kimura, E.; Machida, R. *J. Chem. Soc., Chem. Commun.* **1984**, 499−500.

(32) Chen, D.; Martell, A. E. *J. Am. Chem. Soc.* **1990**, *112*, 9411−9412.

(33) Cheng, C.-C.; Rokita, S. E.; Burrows, C. J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 277−278.

(34) Kimura, E.; Sakonaka, A.; Nakamoto, M. *Biochim. Biophys. Acta* **1981**, *678*, 172−179.

(35) Sable, D. B.; Edelman, M. A.; Drain, C. M.; Corden, B. B.; see footnote 13 in ref 25.

(36) Stapleton, I. W. *Aust. J. Chem.* **1985**, *38*, 633−636.

hygroscopic solid upon evaporation. Attempts to purify the solid by recrystallization were unsuccessful. This precluded a meaningful characterization of the solid by elemental analysis or X-ray crystallography and confirmation that it had the composition $NiH_{-2}L\cdot H_2O$, as claimed. The correctness of this claim is open to question given that the found composition (C, 40.10; H, 6.90; N, 20.30; Ni, 17.04) is not in satisfactory agreement with the calculated composition (C, 39.79; H, 6.98; N, 21.09; Ni, 17.68), which was incorrectly stated in the original report.[25] The solid dissolved in deoxygenated distilled water to afford a green solution. Addition of excess ammonium methyl CoM resulted in a color change to brown and production of significant amounts of methane. Given the preliminary nature of this experiment, we did not carefully calibrate the GC method and reaction vessel volumes used for detection and quantification of methane. Nonetheless, the amount of methane produced appeared to be substantially less than one equivalent. The UV−vis spectra of the green solid dissolved in pH 9.5 borate buffer and of the final reaction mixture were similar, but not identical, to those in the original report. The principal difference was that we observed an inflection point rather than a well-resolved band at 290 nm in the spectrum of the initial complex. Thus, we successfully reproduced the essential features of the observations reported by Drain et al.[24,25]

In subsequent experiments, we examined if the synthesis of L from purified tetren proceeded with improved facility or yield in our hands. Tetren was purified by repeated recrystallization of its pentahydrochloride salt.[37] Although the yield of L obtained from the reaction of tetren and diethylmalonate increased, the net conversion of tetren to L decreased substantially as a consequence of the poor recovery of pure tetren from its salt.

A blue, crystalline nickel complex was obtained from the reaction of $Ni(OAc)_2\cdot 4H_2O$ with L synthesized from purified tetren. The reaction was repeated in ethanol of varying water content and was performed both aerobically and anaerobically to confirm that the disparity in products did not result from a difference in reaction conditions. L synthesized from purified tetren afforded the blue complex in all cases. Neutral aqueous solutions of the complex were blue and had UV−vis spectra similar to but redshifted 15 nm with respect to that of dilute 1:1 solutions of nickel(II) salts and diethylenetriamine, dien. Consistent with previous reports, the solution turned pink when the pH was increased above 9 and turned brown and eventually yellow if also exposed to oxygen or air. Anaerobic solutions of the nickel complex did not react with excess ammonium methyl CoM over the pH range of about 6−13. Detectable quantities of methane ($<10^{-9}$ mol in the reaction vessel volume or 10^{-5} equiv) and the brown color that is characteristic of the final reaction mixture were not observed, even after several months. Given the lack of color change upon addition of methyl CoM, it is questionable whether the thioether coordinates to the nickel complex to any appreciable extent. Aqueous solutions prepared by the in situ reaction of the ligand L and 1 equiv of either nickel(II) acetate, chloride, or nitrate had UV−vis spectra similar to that of the crystalline nickel complex and also failed to react with ammonium methyl CoM.

Table 1. Crystallographic Data for $Ni(C_{11}H_{23}N_5O_2)(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$ and $C_9H_{18}N_4O_2$

	$NiL(OAc)_2$	L′
formula	$C_{15}H_{29}N_5NiO_6$	$C_9H_{18}N_4O_2$
fw	434.14	214.27
a, Å	8.234(1)	8.658(2)
b, Å	13.439(2)	8.663(2)
c, Å	18.915(2)	8.888(2)
α, deg		69.11(3)
β, deg	95.37(1)	83.51(3)
γ, deg		62.49(3)
V, Å^3	2083.9(5)	551.3(2)
Z	4	2
space group	$P2_1/c$	$P\bar{1}$
T, K	295(2)	295(2)
λ, Å	0.71073	0.71073
ρ_{calc}, g/cm^3	1.384	1.291
μ, cm^{-1}	9.70	0.94
R1	0.0374	0.0452
wR2	0.0835	0.1051

Table 2. Atomic Coordinates for the Non-Hydrogen Atoms of $Ni(C_{11}H_{23}N_5O_2)(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$

atom	x	y	z	U(eq),[a] Å^2
Ni	0.39790(5)	0.29895(3)	0.35902(2)	0.03740(13)
O(1)	0.9401(3)	0.1332(2)	0.3517(2)	0.0991(11)
O(2)	0.4899(3)	−0.0780(2)	0.35273(12)	0.0604(6)
O(3)	0.3596(2)	0.18302(15)	0.27713(10)	0.0455(5)
O(4)	0.2109(3)	0.31796(15)	0.27027(10)	0.0500(5)
O(5)	0.5775(3)	0.25660(15)	0.43480(10)	0.0461(5)
O(6)	0.6362(5)	0.3921(2)	0.4972(2)	0.1119(13)
N(1)	0.2183(3)	0.2386(2)	0.41769(14)	0.0445(6)
N(2)	0.3370(4)	0.4303(2)	0.40124(14)	0.0500(7)
N(3)	0.5556(3)	0.3815(2)	0.30165(13)	0.0452(6)
N(4)	0.6954(3)	0.1653(2)	0.29579(12)	0.0466(6)
N(5)	0.4778(3)	0.0665(2)	0.41122(13)	0.0461(6)
C(1)	0.1612(5)	0.3227(3)	0.4595(2)	0.0640(10)
C(2)	0.1691(5)	0.4193(3)	0.4188(2)	0.0615(9)
C(3)	0.3723(5)	0.5051(2)	0.3486(2)	0.0622(10)
C(4)	0.5427(5)	0.4845(2)	0.3288(2)	0.0595(9)
C(5)	0.7265(4)	0.3510(3)	0.2993(2)	0.0622(10)
C(6)	0.7455(4)	0.2549(3)	0.2602(2)	0.0626(10)
C(7)	0.7969(4)	0.1112(2)	0.3388(2)	0.0511(8)
C(8)	0.7355(4)	0.0167(3)	0.3697(2)	0.0567(9)
C(9)	0.5549(4)	−0.0011(2)	0.37634(14)	0.0435(7)
C(10)	0.3049(4)	0.0600(2)	0.4208(2)	0.0486(8)
C(11)	0.2466(4)	0.1481(2)	0.4615(2)	0.0515(8)
C(12)	0.2503(4)	0.2367(2)	0.2446(2)	0.0466(8)
C(13)	0.1704(5)	0.2039(3)	0.1740(2)	0.0897(15)
C(14)	0.6474(4)	0.3027(2)	0.4878(2)	0.0518(8)
C(15)	0.7505(5)	0.2414(3)	0.5406(2)	0.0628(9)

[a] U(eq) is defined as one-third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

The X-ray crystal structure of the blue nickel complex established that it has composition $NiL(OAc)_2$. Crystallograpic data, atomic coordinates, and selected bond lengths and angles are presented in Tables 1−3, respectively. The molecular structure and atom numbering scheme are shown in Figure 1. The Ni atom has a distorted octahedral geometry. The three secondary amine nitrogen atoms of ligand L coordinate to nickel in a meridional fashion. The remaining coordination sites are occupied by one chelating and one unidentate acetate ligand. The Ni−N bond lengths of the trans disposed N(1) and N(3) atoms are typical of a secondary amines coordinated to high-spin, octahedral Ni(II).[38] In contrast, the Ni−N(2) bond, which is trans to one oxygen atom of the η^2-acetate, is significantly shorter and may be shorter than the corresponding bond to the center nitrogen in other *mer*-Ni^{II}(dien) complexes.[39] A search of the Cambridge Structural Database reveals that η^2-carboxylate complexes of Ni are relatively rare and η^2,η^1-bis(carboxylate) complexes are unprecedented.[40] Carboxylates coordinated to Ni in an η^2-fashion typically have nearly equivalent Ni−O distances of roughly 2.10 Å and an O−Ni−O angle of about 62°.[41,42] In some cases, the two Ni−O distances are inequivalent

---

(37) Jonassen, H. B.; Frey, F. W.; Schaafsma, A. *J. Phys. Chem.* **1957**, *61*, 504−505.

(38) Orpen, A. G.; Brammer, L.; Allen, F. H.; Kennard, O.; Watson, D. G.; Taylor, R. *J. Chem. Soc., Dalton Trans.* **1989**, S1−S83.

(39) Biagini, S.; Cannas, M. *J. Chem. Soc. A* **1970**, 2398−2408.

(40) Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.* **1983**, *16*, 146−153.

Table 3. Selected Bond Lengths (Å) and Angles (deg) for $NiL(OAc)_2$

Ni−N(1)	2.094(3)	Ni−O(3)	2.199(2)
Ni−N(2)	2.020(3)	Ni−O(4)	2.184(2)
Ni−N(3)	2.087(2)	Ni−O(5)	2.042(2)
O(3)−C(12)	1.267(4)	O(5)−C(14)	1.271(3)
O(4)−C(12)	1.250(4)	O(6)−C(14)	1.219(4)
N(1)−Ni−O(3)	92.11(9)	O(3)−Ni−O(4)	59.81(7)
N(1)−Ni−O(4)	88.31(10)	O(3)−Ni−O(5)	110.10(8)
N(1)−Ni−O(5)	91.21(10)	O(4)−Ni−O(5)	169.86(8)
N(1)−Ni−N(2)	85.15(11)	C(12)−O(3)−Ni	89.2(2)
N(1)−Ni−N(3)	170.40(10)	C(12)−O(4)−Ni	90.3(2)
N(2)−Ni−O(3)	150.82(10)	C(14)−O(5)−Ni	131.5(2)
N(2)−Ni−O(4)	91.05(10)	O(4)−C(12)−O(3)	120.5(3)
N(2)−Ni−O(5)	99.00(10)	O(4)−C(12)−C(13)	119.6(3)
N(2)−Ni−N(3)	86.03(11)	O(3)−C(12)−C(13)	119.9(3)
N(3)−Ni−O(3)	93.75(9)	O(6)−C(14)−O(5)	124.1(3)
N(3)−Ni−O(4)	88.07(9)	O(6)−C(14)−C(15)	119.5(3)
N(3)−Ni−O(5)	93.90(9)	O(5)−C(14)−C(15)	116.4(3)

and one may be as long as 2.19 Å.[43−45] The two nearly equivalent Ni−O distances of the η^2-acetate in $NiL(OAc)_2$ are the longest and the O−Ni−O angle is the smallest known for this structure type. As expected, the N(2)−Ni−O(5) angle opposite the constrained O(3)−Ni−O(4) angle is significantly greater than 90°.[46] At 99.0° it is the largest angle between cis ligands in the complex. The short Ni−O bond length and inequivalent C−O bond lengths of the η^1-acetate are typical of unidentate carboxylates coordinated to Ni in such compounds as $Ni(OAc)_2{\cdot}4H_2O$.[47,48]

The amide groups of L are well removed from the Ni atom and do not interact with it. Both amide groups are planar and have a trans configuration with respect to the macrocycle backbone. The amide planes are inclined relative to the macrocycle and to each other, and the amide hydrogen atoms are directed toward opposite faces of the macrocycle ring. To the extent that the structure of $NiL(OAc)_2$ resembles that of the complex in neutral aqueous solution (ignoring the probable replacement of carboxylate oxygen donor atoms by water), the reason that the formation of square pyramidal $NiH_{-2}L$ is sluggish when the pH is raised is apparent. The three secondary amine nitrogen atoms of L must isomerize from *mer* to *fac* sites, and the conformation of L must change to bring the amide groups coplanar and closer to N(1) and N(3).

The coordination geometry at nickel in $NiL(OAc)_2$ is not imposed by the macrocycle ring. $Ni(dien)(OAc)_2$, which we prepared by reaction of $Ni(OAc)_2{\cdot}4H_2O$ with 1 equiv of dien in ethanol, has an analogous structure.[49] The bond lengths and angles around nickel in the two complexes are indistinguishable within the considerable errors of the dien structure. We were unable to obtain single crystals of $Ni(dien)(OAc)_2$ of sufficient quality for a high precision structure determination because of competing formation of $[Ni(dien)_2](OAc)_2$ during crystallization.

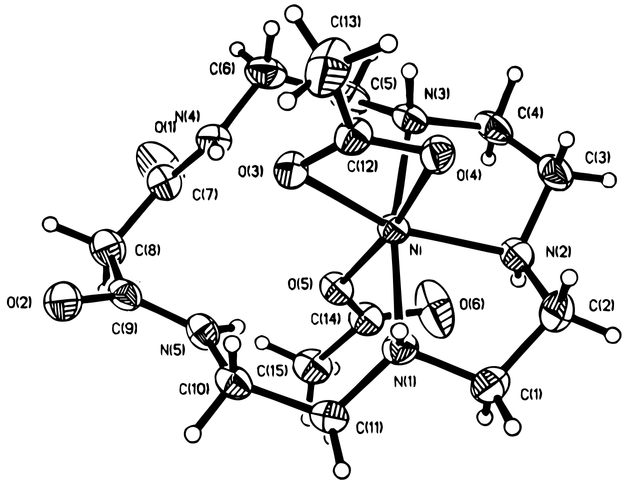


Figure 1. ORTEP diagram of the molecular structure of $Ni(C_{11}H_{23}N_5O_2)(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$.

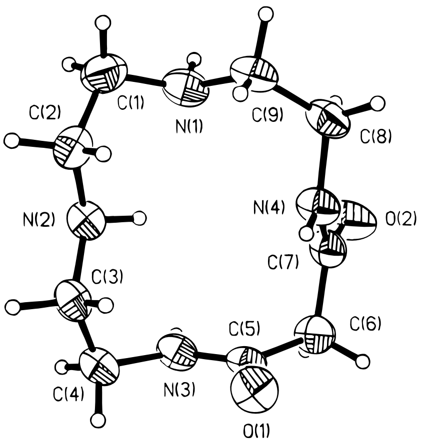
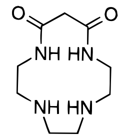


Figure 2. ORTEP diagram of the molecular structure of L′, $C_9H_{18}N_4O_2$. The crystal used in the structure determination was obtained from a sample of "purified" L synthesized from technical grade tetraethylenepentamine.

We returned to the original sample of L prepared from technical grade tetren with the intention of using it to prepare another sample of the green NiL complex. The white, needle-shaped crystals of L had lost crystallinity while stored during the intervening months. Recrystalliztion of the sample gave both needles and clear, well-formed parallelepipeds. An X-ray structure determination of the parallelepipeds revealed that they contained 1,4,7,10-tetraazacyclotridecane-11,13-dione, L′. Thus, the "purified" ligand prepared from technical grade tetren is a mixture that contains at least L and L′, if not other compounds.



The molecular structure and atom numbering scheme of L′ are shown in Figure 2. Crystallograpic data and atomic coordinates are presented in Tables 1 and 4, respectively. The bond lengths and angles of L′ are unremarkable and thus are not presented here.

---

(41) Hursthouse, M. B.; New, D. B. *J. Chem. Soc., Dalton Trans.* **1977**, 1082−1085.

(42) Whimp, P. O.; Bailey, M. F.; Curtis, N. F. *J. Chem. Soc. A* **1970**, 1956−1963.

(43) Wages, H. E.; Taft, K. L.; Lippard, S. J. *Inorg. Chem.* **1993**, *32*, 4985−4987.

(44) Smith, G.; Shariff, S. M.; O'Reilly, E. J.; Kennard, C. H. L. *Polyhedron* **1989**, *8*, 39−43.

(45) Aratake, Y.; Ohba, M.; Sakiyama, H.; Tadokoro, M.; Matsumoto, N.; Okawa, H. *Inorg. Chim. Acta* **1993**, *212*, 183−190.

(46) Connolly, J. A.; Kim, J. H.; Banaszczyck, M.; Drouin, M.; Chin, J. *Inorg. Chem.* **1995**, *34*, 1094−1099.

(47) Cramer, R. E.; van Doorne, W.; Dubois, R. *Inorg. Chem.* **1975**, *14*, 2462−2466.

(48) Downie, T. C.; Harrison, W.; Raper, E. S.; Hepworth, M. A. *Acta Cryst.* **1971**, *B27*, 706−712.

(49) Crystallographic data for $Ni(C_4H_{13}N_3)(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$: triclinic, $P1$, $a = 8.783(2)$ Å, $b = 8.898(2)$ Å, $c = 9.621(5)$ Å, $\alpha = 88.70(2)°$, $\beta = 78.03(3)°$, $\gamma = 64.25(2)°$, $V = 660.6(3)$ Å^3, $Z = 2$, $\rho_c = 1.408$ g/cm^3, $T = 295(2)$ K. Full-matrix anisotropic refinement (on F_o^2) of 147 parameters converged with R1 = 0.0843 and wR2 = 0.1889 for 1482 data with $I > 2\sigma(I)$ and GOF = 1.008. Typical esds are 0.008 Å for Ni−E bond lengths and 0.3° for E−Ni−E angles (E = O, N).

Table 4. Atomic Coordinates for the Non-Hydrogen Atoms of $C_9H_{18}N_4O_2$

atom	*x*	*y*	*z*	*U*(eq),[a] Å^2
O(1)	0.1559(2)	0.0134(2)	0.9052(2)	0.0504(5)
O(2)	−0.2644(2)	0.5183(3)	0.5564(2)	0.0738(7)
N(1)	−0.4233(3)	0.1202(3)	0.6740(3)	0.0494(6)
N(2)	−0.0822(3)	−0.1605(3)	0.7033(2)	0.0430(5)
N(3)	0.1391(2)	0.0106(3)	0.6565(2)	0.0425(5)
N(4)	−0.2547(3)	0.2940(3)	0.7880(2)	0.0438(5)
C(1)	−0.3931(3)	−0.0694(4)	0.7087(3)	0.0552(7)
C(2)	−0.2163(3)	−0.2073(3)	0.7929(3)	0.0488(6)
C(3)	0.0880(3)	−0.2637(3)	0.7911(3)	0.0479(6)
C(4)	0.2129(3)	−0.1895(3)	0.7026(3)	0.0492(6)
C(5)	0.1116(3)	0.0961(3)	0.7616(3)	0.0357(5)
C(6)	0.0137(3)	0.3052(3)	0.6924(3)	0.0483(6)
C(7)	−0.1806(3)	0.3815(3)	0.6727(3)	0.0421(6)
C(8)	−0.4401(3)	0.3473(3)	0.7862(3)	0.0498(6)
C(9)	−0.4759(3)	0.1861(3)	0.8094(3)	0.0466(6)

[a] *U*(eq) is defined as one-third of the trace of the orthogonalized $\mathbf{U}_{ij}$ tensor.

The macrocyclic dioxotetraamine ligand L′ and its nickel complex $NiH_{-2}L'$ have been reported previously, but the characterization data reported for the ligand were limited to the melting point and a partial description of the ^1H NMR and UV−vis spectra.[50−53] Authentic samples of L′ prepared from diethyl malonate and purified triethylenetetramine, trien, were found to be identical to the L′ obtained above. In contrast to L, reaction of L' with nickel acetate in ethanol affords directly the neutral, amide deprotonated complex $NiH_{-2}L'$. In aqueous solution, the complex is amide deprotonated at pH > 6 and is present solely as a yellow, low-spin square-planar complex.[53] Solutions of $NiH_{-2}L'$ did not react with excess ammonium methyl CoM at any pH.

Examination of the ^1H and ^{13}C NMR spectra and calculations of the elemental analyses of pure L, of pure L′, and of physical mixtures of the two compounds established that the presence of L′ in L is hard to detect. In both $CDCl_3$ and D_2O, the overall appearance of the ^1H spectra of the two compounds are quite similar with respect to the number, multiplet pattern, and the chemical shifts of the peaks. The amide, amine, and malonyl protons are exchangable so their chemical shifts and integrations can be affected by the water content of the solvent. Thus, the expected differences in integrations can be hard to observe. The ^{13}C chemical shifts of the corresponding carbon atoms in L and L′ are similar, but are resolvable. However, peaks due to L′ would not be observed in a routine spectrum of L with a typical signal to noise ratio unless the quantities of L and L′ in the sample were nearly comparable. Futhermore, the lesser solubility of L′ in $CDCl_3$ could result in enrichment of L in the NMR sample relative to the bulk. The C, H, and N analysis of L, either as the anhydrous ligand or the monohydrate, is not particular sensitive to the presence of L′. The largest calculated decrease in percent by weight for these elements in a mixture that contains 20% L′ is 0.21% (N) for the anhydrous ligand and 0.29% (C) for the monohydrate. These differences are within the error tolerances considered acceptable for agreement of calculated and found analyses.

UV−vis spectral data, Table 5, support the conclusion that the ligand prepared by Drain et al. was a mixture of L and L′. The green NiL complex was reported to have a band at 290 nm that has an ϵ_M of 1664 M^{-1} cm^{-1}.[24,25,35] Neither authentic NiL^{2+} in neutral aqueous solution nor $NiH_{-2}L$ in borate buffer has a well-resolved band at that wavelength or indeed any band with ϵ_M greater than 220 M^{-1} cm^{-1}. However, solutions of both L′ and $NiH_{-2}L'$ have extremely strong bands at 289 nm. Physical mixtures that contain roughly 1 part $NiH_{-2}L'$ and 5 parts NiL^{2+} give solutions that appear green and reproduce the main features of the spectrum that we observed for the "green NiL complex" in borate buffer.

Table 5. Absorption Spectral Data for Ligands and Complexes[a]

compound	λ_{max}, nm (ϵ, M^{-1} cm^{-1})			
L′	289 (6027)			
$NiH_{-2}L'$	289 (6510)	411 (82.5)		
$NiH_{-2}L'$[b]	289 (7550)	407 (186)		
L	287 (175)			
$NiL(OAc)_2$		375 (18.9)	610 (11.1)	>900 (>19)
$Ni(dien)(OAc)_2$		360 (11.5)	584 (7.2)	>900 (>12)
$NiH_{-2}L$[b]	280 (214)[c]	345 (41.7)	526 (15.2)	
$NiH_{-2}L$ + Na(HSCoM)[b]	290	336	404	517
$Ni^{II}L$ (green)[b,d]	290 (1664)	340 (120)		
$Ni^{II}L$ + Na(HSCoM)[b,d]		333 (2217)	405 (754)	510 (297)

[a] Solvent was unbuffered deionized water, except as noted. [b] Borate buffer, pH = 9.5. [c] Inflection point. [d] Data from ref 24.

Addition of coenzyme-M thiol to $NiH_{-2}L$ in pH 9.5 borate buffer resulted in an immediate color change from pink to brown and a UV−vis spectrum that resembled that of the final reaction mixture after cleavage of methyl CoM. The molar extinctions of the spectral features could not be determined because the complex began to precipitate before the endpoint of the coordination equilibrium was reached. However, the marked increase in absorbance of the solution suggests that the extinctions are probably an order of magnitude larger than those of $NiH_{-2}L$. Interestingly, the spectrum is unique to coenzyme-M thiol. Attempts to titrate $NiH_{-2}L$ with other neutral or charged thiols including thiophenol, 2-mercaptoethanol, L-cysteine, and the sodium salt of 3-mercaptopropionic acid resulted in immediate precipitation of the complex.

Although the reported spectra can be reproduced with physical mixtures of the Ni complexes of L and L′, the reported reactivity cannot be reproduced. No methane was detected in anaerobic reactions of methyl CoM with a 5:1 mixture of NiL^{2+} and $NiH_{-2}L'$ at any pH examined. Similarly, all control experiments with Ni salts alone or with Ni in the presence of trien or tetren failed to produce methane.

The known chemistry of $NiH_{-2}L$ with oxygen suggested that methane could result from the cleavage of methyl CoM by a series of reactions with oxygen derived species. Methyl radicals, and ultimately methane, are produced by attack of hydroxyl radicals on dimethyl sulfoxide and methionine.[54−56] The reaction of the latter compound is presumed to involve initial oxidation to methionine sulfoxide. A similar process involving the thioether methyl CoM could account for the observed formation of methane (but not for the apparent formation of CoM thiol). The ability of $NiH_{-2}L$ to bind oxygen to form a Ni(III) superoxide complex,[27,28,31] to dismute superoxide,[34] and to utilize oxygen to cleave DNA[33] and to hydroxylate substrates[31] including its own ligand[26,32] raised the possibility that free or coordinated superoxide, hydroxyl radical, and/or peroxide could be present in its aerobic solutions.

---

(50) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1979**, 325−329.
(51) Kimura, E.; Koike, T.; Machida, R.; Nagai, R.; Kodama, M. *Inorg. Chem.* **1984**, *23*, 4181−4188.
(52) Fabbrizzi, L.; Licchelli, M.; Perotti, A.; Poggi, A.; Soresi, S. *Isr. J. Chem.* **1985**, *25*, 112−117.
(53) Kodama, M.; Kimura, E. *J. Chem. Soc., Dalton Trans.* **1981**, 694−700.
(54) Gilbert, B. C.; Hodgeman, D. K. C.; Norman, R. O. C. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1748−1752.
(55) Taniguchi, H.; Takagi, H.; Hatano, H. *J. Phys. Chem.* **1972**, *76*, 135−138.
(56) Veltwisch, D.; Janata, E.; Asmus, K.-D. *J. Chem. Soc., Perkin Trans. 2* **1980**, 146−153.

Reaction of excess ammonium methyl CoM with either NiL(OAc)$_2$ or a 5:1 mixture of NiL(OAc)$_2$ and NiH$_{-2}$L′ in aerobic aqueous solution did not result in production of detectable quantities of methane. This result was independent of the solution pH or replacement of air with such oxidants as pure oxygen gas, H_2O_2, KO_2 in DMSO or in DMF containing excess [18]crown-6, $Na_2S_2O_8$, or potassium peroxymonosulfate (Oxone). Interestingly, addition of methyl CoM to an aerobic solution of NiH$_{-2}$L resulted in a color change from brown to pink, but no methane formed. Solutions of NiL(OAc)$_2$ are excellent catalysts for decomposition of peroxide.

Attempts to identify the agent(s) in the material prepared from technical grade tetren that causes or promotes the cleavage of methyl CoM have been unsuccessful to date. Many compounds may be present in addition to the two that we isolated and characterized. TLC of tetren reveals that it has three major components and one minor component.[36] Numerous linear and cyclic oligomers of the diester and the polyamine components may form during the reaction. Neither the crude product from the reaction of diethyl malonate and technical grade tetren nor the fractions rejected in the purification of L from the crude product have shown any activity when combined with nickel salts and ammonium methyl CoM in aqueous solution. Unfortunately, the unknown composition of these fractions makes it impossible to control the stoichiometric ratio of nickel to ligand(s). A more peculiar observation is that we have not succeeded in reproducing the synthesis of the impure crystalline L from technical grade tetren. Since pure L was synthesized from purified tetren in our laboratory, all batches of L that we prepared from technical grade tetren have afforded the blue NiL(OAc)$_2$ complex rather than the green NiL complex. One possibility is that the composition of the technical grade tetren has changed since the bottle was first opened. Alternatively, the presence of crystals of pure L in the lab may seed the formation of pure L from material prepared from technical grade tetren and disfavor the formation of the impure polymorph that contained the agent responsible for the chemistry of interest.[57] Efforts to isolate and identify the agent(s) continue in our labs.

Conclusions

The cleavage of methyl CoM to methane reported by Drain et al.[24,25] was reproduced with nickel complexes of L synthesized from unpurified, technical grade tetren. NiL(OAc)$_2$ was prepared from L synthesized from pure tetren and was structurally characterized by X-ray crystallography. This authentic NiL complex does not cleave methyl CoM.[58] Upon reexamination, the ligand prepared from technical grade tetren was found to be contaminated with 1,4,7,10-tetraazacyclotridecane-11,13-dione, L′. The Ni complex of L′, NiH$_{-2}$L′, does not cleave methyl CoM. Solutions of physical mixtures of NiL(OAc)$_2$ and NiH$_{-2}$L′ can reproduce the observed spectra, but cannot cleave methyl CoM. The agent(s) or interactions in the impure material that cleaves methyl CoM has not been identified. Nonetheless, the mechanism originally proposed by Drain et al.,[24,25] which involves NiL as the active complex, is incorrect. Thus, the proposed mechanism and the supposed involvement of a Ni(III)/Ni(II) redox couple cannot be taken as a precedent for the cleavage of methyl CoM catalyzed by the enzyme methyl coenzyme-M reductase.

Experimental Section

Materials and Methods. Reagent grade diethyl malonate, diethylenetriamine, 2-mercaptoethanesulfonic acid, sodium salt (Na^+ $HSCoM^-$), and triethylenetetramine tetrahydrochloride and technical grade tetraethylenepentamine and triethylenetetramine were purchased from Aldrich. Deionized water was purified with a Corning Mega-Pure System immediately before use. All other reagents or solvents were HPLC or reagent grade. Ammonium 2-(methylthio)ethanesulfonate was prepared by literature methods.[59]

Absorption spectra were recorded on a Perkin-Elmer Lambda 4C spectrophotometer. ^1H NMR (270 MHz) and ^{13}C NMR (67.9 MHz) spectra were recorded on a JEOL Eclipse 270 spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 spectrophotometer. Head-space gases were analyzed on a Hewlett-Packard 5890A gas chromatograph. The valve system for sampling, columns and conditions employed, and calibration methods were described previously.[18]

Tetraethylenepentamine Pentahydrochloride. A 150 g sample of technical grade tetraethylenepentamine was mixed with 300 mL of 95% ethanol. The stirred solution was cooled to 0 °C in a ice bath, and 180 mL of concentrated HCl was added dropwise over a 1.5 h period. A white precipitate formed in the yellow solution during the course of the addition. The precipitate was collected by filtration, recrystallized three times from water and ethanol, washed with ether, and dried in a vacuum oven at 40 °C for 48 h. Yield: 234 g, 85%. ^1H NMR (D_2O), δ: 3.33−3.20 (m).

Tetraethylenepentamine. A 50 g sample of tetraethylenepentamine pentahydrochloride was dissolved in a minimal quantity of deionized water. The solution was placed in a 500 mL flask and cooled to 0 °C. Then 200 mL of a 4.5 M ethanolic solution of NaOH was added dropwise to the stirred solution over a 2 h period. The resulting solution was stirred at room temperature for 10 h. The solvent was removed by evaporation, precipitated NaCl was removed by filtration, and the tetraethylenepentamine filtrate was dried in vacuo for 2 days. Yield: 14 g, 57%. The recovery of product decreases greatly if a drying agent is used. ^1H NMR (D_2O), δ: 2.50−2.42 (m). ^{13}C NMR ($CDCl_3$), δ: 41.5, 49.1, 49.2, 52.2.

Triethylenetetramine. Purification was by the same procedure as above. Yield: 61%. ^1H NMR (D_2O), δ: 2.57−2.49 (m). ^{13}C NMR (D_2O), δ: 39.9, 47.7, 50.4.

1,4,7,10,13-Pentaazacyclohexadecane-14,16-dione, L. Purified tetraethylenepentamine (14.1 g, 74.5 mmol) and diethyl malonate (12 g, 75.0 mmol) were added to 1.3 L of absolute ethanol in a 2 L, three-neck round bottom flask fitted with a reflux condenser. The ethanol solution was refluxed under a nitrogen atmosphere for 10 days. The solvent was removed by evaporation, and the resulting yellow oil was purified by flash chromatography on silica gel (40 μm mesh). Elution was initiated with chloroform. The polarity of eluent was increased gradually to a composition of 10:2:1 $CHCl_3$:CH_3OH:NH_4OH. Fractions that had similar R_f values by TLC were combined. Solvents were evaporated from the individual combined fractions and ^1H NMR spectra were obtained of the residual materials in $CDCl_3$ solution. Fractions that had a ^1H peak near 8 ppm were combined and dissolved in 30 mL of dry, hot CH_3CN in a 100 mL flask. The flask was sealed and placed in a refrigerator. After 10 days, the white needles that formed were collected by filtration and dried under vacuum. Yield: 2.3 g (15%). IR (KBr), ν, cm^{-1}: 1662, 1567, 1454. IR (D_2O), ν, cm^{-1}: 1660, 1450. ^1H NMR ($CDCl_3$), δ: 1.8 (br s, 3 H, amine); 2.75 (m, 12 H, methylene); 3.2 (s, 2 H, malonyl methylene); 3.41 (m, 4 H, methylene adjacent to amide); 7.84 (br t, 2 H, amide). ^1H NMR (D_2O), δ: 2.52 (s, 8 H, methylene); 2.58 (m, 4 H, methylene); 3.25 (m, 4 H, methylene adjacent

---

(57) Dunitz, J. D.; Bernstein, J. *Acc. Chem. Res.* **1995**, *28*, 193−200.

(58) A reviewer questioned whether differences in counterion or coordinated anion or ligand between the well-defined NiL(OAc)$_2$ complex and the NiL complex used in the work of Drain et al. could be responsible for the observed lack of reactivity of NiL(OAc)$_2$ toward methyl CoM. We consider this improbable. The similarity of the UV−vis spectra of aqueous solutions of NiL(OAc)$_2$ and of aqueous solutions containing L and nickel salts of other anions suggests that anions are not coordinated to nickel. Water is presumed to occupy the coordination sites on the high-spin octahedral nickel ion not occupied by L. The acetate anions are certainly not both coordinated when the amide groups of L deprotonate and coordinate to nickel at higher pH. Thus, inhibition by acetate is unlikely. The presence or absence of anions or ligands in Drain's NiL complex is uncertain. The similarity of the spectra of a mixture of NiH$_{-2}$L′ and NiL(OAc)$_2$ and of Drain's NiL complex in borate buffer implies that the latter is also in the five-coordinate, amide-deprotonated form at this pH. Given the near pH independence of the reactivity of Drain's NiL toward methyl CoM, this argues against the presence of a ligand that promotes reactivity.

(59) Gunsalus, R. P.; Romesser, J. A.; Wolfe, R. S. *Biochemistry* **1978**, *17*, 2374−2377.

to amide). ^{13}C NMR ($CDCl_3$), δ: 38.8 (methylene adjacent to amide); 43.1 (malonyl methylene); 48.3, 48.6, 48.9 (methylene); 168.4 (amide).

1,4,7,10-Tetraazacyclotridecane-11,13-dione, L′. The ligand was prepared from diethyl malonate and purified triethylenetetramine by a procedure similar to that for L. The reaction was refluxed for 3 days. Yield: 17%. IR (KBr), ν, cm^{-1}: 1654, 1576, 1534. ^{1}H NMR ($CDCl_3$), δ: 1.90 (br s, 2 H, amine); 2.67 (m, 8 H, methylene); 3.20 (s, 2 H, malonlyl methylene); 3.36 (m, 4 H, methylene adjacent to amide); 7.63 (br t, 2 H, amide). ^{1}H NMR (D_2O), δ: 2.48 (near s, 4 H, methylene); 2.53 (m, 4 H, methylene); 3.08 (s, 2 H, malonyl methylene), 3.19 (m, 4 H, methylene adjacent to amide). ^{13}C NMR (D_2O), δ: 39.6 (methylene adjacent to amide); 46.5 (malonyl methylene); 47.1, 47.9 (methylene); 168.2 (amide) ^{13}C NMR (D_2O), δ: 39.4 (methylene adjacent to amide); 45.8, 46.4 (methylene); 169.6 (amide).

NiL(OAc)$_2$. $Ni(OAc)_2 \cdot 4H_2O$ (0.3 g, 1.2 mmol) was dissolved in 50 mL of absolute ethanol in a 100 mL Schlenk flask, and the resulting solution was degassed. 1,4,7,10,13-Pentaazacyclohexadecane-14,16-dione (0.33 g, 1.2 mmol) was added, and the solution was refluxed under nitrogen. After 3 h, the deep blue solution was cooled to room temperature and the solvent was removed. The crude product was dissolved in 10 mL of ethanol, filtered, and recrystallized by adding 50 mL of diethyl ether. Light blue crystals of NiL(OAc)$_2$ were collected by filtration and dried in vacuo. The crystals appear to be affected by humidity. Yield: 0.55 g, 89%. IR (KBr), ν, cm^{-1}: 1651, 1614, 1565, 1447.

NiH$_{-2}$L′. The complex was prepared by reaction of equivalent amounts of $Ni(OAc)_2 \cdot 4H_2O$ and L′ in absolute ethanol by a procedure similar to that for NiL(OAc)$_2$. The product is a yellow hygroscopic powder. IR (KBr), ν, cm^{-1}: 1576 (br).

Ni(dien)(OAc)$_2$. A 0.41 g (1.65 mmol) sample of $Ni(OAc)_2 \cdot 4H_2O$ was dissolved in 100 mL of absolute ethanol, and the solution was degassed. Addition of 187 μL (1.73 mmol) of diethylenetriamine by syringe resulted in the immediate development of a deep blue color. The solvent was removed by evaporation, and the resulting blue powder was dried under vacuum overnight. The crude product was purified three times by adding diethyl ether to a chloroform solution of the complex. Single crystals were grown by layering diethyl ether above a chloroform solution of the purified complex. After this was allowed to stand under a nitrogen atmosphere, blue crystals of Ni(dien)(OAc)$_2$ and some pink crystals of Ni(dien)$_2$(OAc)$_2$ formed on the wall of the test tube. The latter complex predominates when the above reaction is run at higher concentration or with a large excess of dien. Ni(dien)(OAc)$_2$ is very hygroscopic and decomposes in air.

X-ray Structural Analysis of $Ni(C_{11}H_{23}N_5O_2)(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$, NiL(OAc)$_2$. Single crystals of NiL(OAc)$_2$ suitable for X-ray diffraction analysis were obtained by slow evaporation of an ethanol solution on a Schlenk vacuum line. A light blue crystal was sealed under nitrogen in a capillary tube and then optically aligned on the goniostat of a Siemens P4 automated X-ray diffractometer. The corresponding lattice parameters and orientation matrix for the monoclinic unit cell were determined from a least-squares fit of the orientation angles for 24 reflections ($20° < 2\theta < 30°$) at 22 °C. The refined lattice parameters and other pertinent crystallographic information are summarized in Table 1.

Intensity data were measured with graphite-monochromated Mo Kα radiation ($\lambda = 0.710\,73$ Å) and variable ω scans. Background counts were measured at the beginning and at the end of each scan with the crystal and counter kept stationary. The intensities of three standard reflections were measured periodically during data collection and gave no indication of crystal decay or sample movement. The data were corrected for Lorentz−polarization, and the symmetry-equivalent reflections were averaged.

The molecular structure determination was initiated with direct methods (SHELXTL PLUS operating on a Silicon Graphics IRIS Indigo workstation), and all non-hydrogen atoms were revealed by difference Fourier methods. Idealized positions for all of the hydrogen atoms were included as fixed contributions using a riding model with isotropic temperature factors set at 1.2 times that of the adjacent carbon. The positions of the methyl hydrogens were optimized by a rigid rotating group refinement with idealized tetrahedral angles. Full-matrix least-squares refinement, based upon the minimization of $\sum w_i|F_o^2 - F_c^2|^2$, with $w_i^{-1} = [\sigma^2(F_o^2) + (0.0427P)^2 + 0.49P]$ where $P = (\max(F_o^2,0) + 2F_c^2)/3$, was performed with SHELXL-93[60] operating on a Silicon Graphics IRIS Indigo workstation. After convergence, the final discrepancy indices[61] were R1 = 0.0374, wR2 = 0.0835, and GOF = 1.029 for 3397 reflections with $I > 2\,\sigma(I)$. A final difference electron density map did not reveal any significant residuals of electron density. The refined positional parameters for NiL(OAc)$_2$ with equivalent isotropic displacement parameters are provided in Table 2, and selected interatomic distances and bond angles are listed in Table 3.

X-ray Structural Analysis of $C_9H_{18}N_4O_2$, L′. A colorless crystal of L′ that was obtained from recrystallization of L prepared from technical grade tetren was optically aligned on the goniostat of a Siemens P4 automated X-ray diffractometer. The corresponding lattice parameters and orientation matrix for the triclinic unit cell were determined from a least-squares fit of the orientation angles for 25 reflections ($19° < 2\theta < 30°$) at 22 °C. The refined lattice parameters and other pertinent crystallographic information are summarized in Table 1. Details of the structure solution and refinement are as above. After convergence, the final discrepancy indices were R1 = 0.0452, wR2 = 0.1051, and GOF = 1.055 for 3397 reflections with $I > 2\,\sigma(I)$. A final difference electron density map did not reveal any significant residuals of electron density. The refined positional parameters for L′ with equivalent isotropic displacement parameters are provided in Table 4.

General Procedure for Reaction of Complexes with Methyl CoM. 10 mL of deionized water was placed in a 50 mL reaction tube equipped with a Kontes Teflon vacuum valve and a side arm with an O-ring joint that was connected to a vacuum line. Three or more freeze−pump−thaw cycles were used to deoxygenate the water. The water was frozen, and 0.092 mmol of the nickel complex (40.0 mg for NiL(OAc)$_2$) and 0.035 mmol of ammonium methyl CoM (60.0 mg) were added under heavy nitrogen flush. The reaction vessel was evacuated and then filled with the desired gas. The O-ring joint was then connected to a sampling adapter, which consisted of short tube (small volume) with an O-ring joint at one end, a standard taper joint fitted with rubber septum at the other, and a side arm with a stopcock. The space between the Teflon valve and the stopcock was evacuated. The water was permitted to thaw and magnetic stirring started to initiate the reaction. After a suitable interval, the Teflon valve was opened to expand the headspace gas into the adapter. The gas was sampled through the septum and characterized by GC.

The procedure was modified slightly for experiments conducted in basic solution or in which an oxidant was added. NiL(OAc)$_2$ was added separately to the frozen borate buffer or NaOH solution. The pink (or brown if air was present) color of the five-coordinate NiH$_{-2}$L was allowed to develop after thawing, the solution was refrozen, ammonium methyl CoM was added, and the procedure described above was resumed. If an oxidant was used, the contents of the tube after addition of ammonium methyl CoM were thawed, stirred to make the solution uniform, and refrozen before the oxidant was added. *Caution!* the amount of H_2O_2 or KO_2 added must be limited to prevent overpressurization of the sealed tube by the O_2 evolved.

Acknowledgment. We thank the National Institutes of Health (Grant GM 33882) for support of this research. Financial support for the acquisition of the Siemens P4 X-ray diffractometer was provided by the Chemical Instrumentation Program of the National Science Foundation (Grant CHE 9120098). We thank Barry Corden for providing a copy of the unpublished manuscript (ref 35) that described their synthetic procedures.

Supporting Information Available: Tables S-1 through S-8 giving crystal and structure refinement data, thermal parameters, hydrogen atom coordinates, and bond lengths and angles for NiL(OAc)$_2$ and L′ (9 pages). Ordering information is given on any current masthead page.

IC960263N

---

(60) SHELXL-93 is a FORTRAN-77 program (Professor G. Sheldrick, Institut for Anorganische Chemie, University of Gottingen, D-37077, Gottingen, Germany) for single-crystal X-ray structural analyses.

(61) The discrepancy indices were calculated from the expressions $R1 = \sum||F_o| - |F_c||/\sum|F_o|$ and $wR2 = [\sum(w_i(F_o^2 - F_c^2)^2)/\sum(w_i(F_o^2)^2)]^{1/2}$ and the standard deviation of an observation of unit weight (GOF) is equal to $[\sum(w_i(F_o^2 - F_c^2)^2)/(n - p)]^{1/2}$, where n is the number of reflections and p is the number of parameters varied during the last refinement cycle.