166. Coenzyme F430 from Methanogenic Bacteria: Detection of a Paramagnetic Methylnickel(II) Derivative of the Pentamethyl Ester by ^{2}H-NMR Spectroscopy

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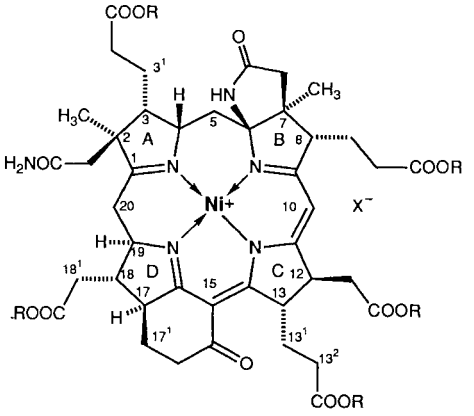
A methylnickel(II) derivative of coenzyme F430 (**1**) was proposed as an intermediate in the enzymic process catalyzed by methyl-CoM reductase. Indirect evidence points to formation of CH_3–F430M^{II} in the reaction of F430M^{I} (obtained from F430M^{II} (**2**)) with electrophilic methyl donors. The results presented here show, that such a compound does exist. A paramagnetic CD_3–Ni^{II} derivative **5b** of the pentamethyl ester **2** (F430M) of coenzyme F430 was prepared by *in situ* methylation with $(CD_3)_2Mg$ and characterized by its isotropically shifted ^{2}H-NMR spectrum. At –40°, the very broad D-signal of the axially coordinated CD_3 group is found at –490 ppm. Comparison with the ^{2}H- and ^{1}H-NMR spectra of methyl(tetramethylcyclam)nickel(II) derivatives **4** ([Ni^{II}(CH_3)(tmc)]CF_3SO_3 (**4a**) is the only isolated CH_3–Ni derivative of a N_4macrocyclic Ni^{II} complex) shows that the large isotropic shift to high field is characteristic for a Me group axially bound to the Ni center. The temperature dependence of the isotropic shift of the CD_3–Ni group in both **4b** and **5b** follows *Curie*'s law and yields ^{2}H hyperfine coupling constants of –0.65 (**4b**) and –0.85 MHz (**5b**), respectively. The ^{1}H-NMR spectrum indicates that, in contrast to the five-coordinate monochloro complex [Ni^{II}Cl(tmc)]$^+$, intermolecular exchange of the axial ligand in [Ni^{II}(CH_3)(tmc)]$^+$ **4a** is either slow at the NMR time scale or does not occur at all.

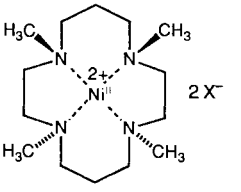
1. Introduction. – Coenzyme F430 [1], the hydrocorphinoid Ni^{II} complex **1**, is the prosthetic group of the enzyme methyl coenzyme M reductase [2a] which catalyses the last step of methane formation (see Scheme 1 in [2b]) [3] in methanogenic bacteria by a still unknown mechanism.

We reported earlier that the pentamethyl ester **2** (F430M = F430M^{II}) can be reversibly reduced to the Ni^{I} form F430M^{I} [4] and that the latter reacts in a 2:1 stoichiometry with electrophilic Me donors like MeI to give methane and the Ni^{II} form **2** [5]. Indirect evidence obtained through D-incorporation studies according to the *Scheme* points to formation of an intermediate from which the Me group can be dissociated by protonolysis to give methane and F430M^{II}. These observations are consistent with the properties expected for a methylnickel(II) derivative with the Me group directly bound to the central Ni ion.

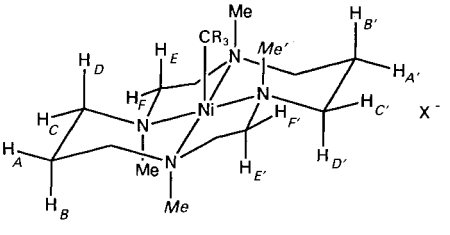
Alkyl-Ni^{II} derivatives were proposed as intermediates in the reaction of synthetic electrogenerated [Ni^{I}(cyclam)] derivatives with alkyl halides [6]. To our knowledge, the only *isolated* derivative of a square-planar tetraazamacrocyclic Ni^{II} complex for which the structure of an alkylnickel complex (structure **4a**) was proposed is the paramagnetic solid obtained by *D'Aniello* and *Barefield* [7] on methylation of [(*R*,*R*,*S*,*S*)-*N*,*N'*,*N''*,*N'''*-tetramethylcyclam]nickel(II) bis(trifluoromethanesulfonate) ([Ni^{II}(tmc)]$(CF_3SO_3)_2$; **3**, X = CF_3SO_3) with $(CH_3)_2Mg$. The structure assignment was based on elemental analysis, the fact that **4a** reacted with acids to give methane and [Ni^{II}(tmc)]$^{2+}$ **3**, and on the d–d



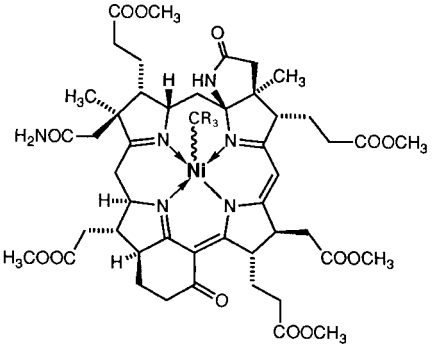
1 R = H; coenzyme F430
2 R = CH_3, X = CF_3SO_3 or ClO_4; F430M (= $F430M^{II}$)



3 $[Ni^{II}\{(R,R,S,S)\text{-tmc}\}]X_2$



4a R = H
b R = D

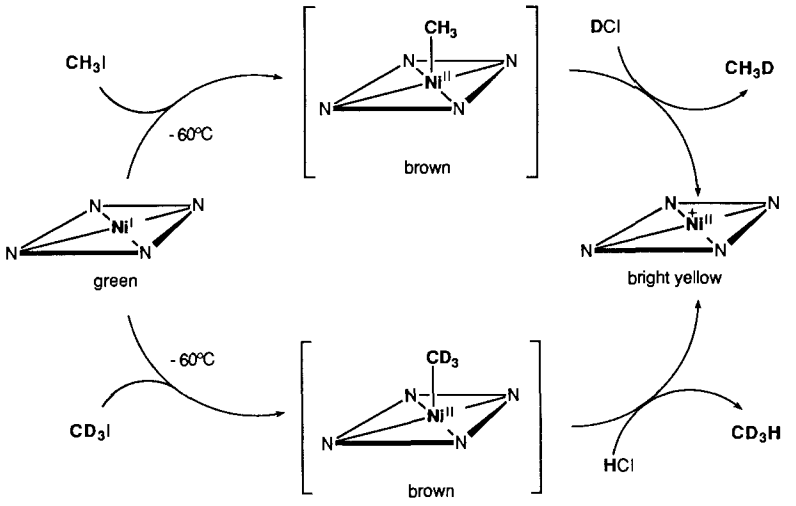


5a R = H; CH_3-$F430M^{II}$
b R = D; CD_3-$F430M^{II}$

absorption spectrum of **4a** which was similar to that of other five-coordinate complexes of **3** (*e.g.* $[Ni^{II}Cl(tmc)]^+$). In stoichiometric reactions of $[Ni^{I}\{(R,R,S,S)\text{-tmc}\}]^+$ with alkyl halides, *Bakac* and *Espenson* [8] observed transient UV/VIS spectra which they attributed to $[Ni^{II}(alkyl)(tmc)]^+$ complexes based on their similarity to the UV/VIS spectrum of **4a** as described by *D'Aniello* and *Barefield* [7].

Searching for a direct spectroscopic proof of the formation of the CH_3–Ni^{II} complex CH_3–$F430M^{II}$ (**5a**) in the reaction of $F430M^{I}$ with electrophiles, we were confronted with the problem that none of the conventional spectroscopic techniques seemed appropriate to demonstrate the presence of a C–Ni bond in hypothetical **5a**. In view of the pronounced tendency of $F430M^{II}$ (**2**) to form five- and six-coordinate high-spin complexes by adding ligands in the axial positions and in analogy to the paramagnetism of **4a** (2.78 μ_B [7]), CH_3–$F430M^{II}$ (**5a**) was expected to be a paramagnetic (high-spin d^8) compound, unsuitable for characterization by either ESR or high-resolution NMR. The d–d transitions in the UV/VIS absorption spectrum, which in the case of $[Ni^{II}(tmc)](CF_3SO_3)_2$ (**3**, X = CF_3SO_3) were found to shift considerably upon methylation, are hidden under much stronger π–π^* transitions of the ligand chromophore in F430M (**2**) which show only small differences between the low-spin and high-spin forms.

Scheme



Due to very efficient electron relaxation, square pyramidal and quasi-tetragonal high-spin Ni^{II} complexes often show well dispersed isotropically shifted NMR spectra [9]. The diamagnetic (fully assigned) ^{1}H-NMR spectrum (dry CD_2Cl_2) of pentamethyl ester **2** (X = ClO_4) gradually changes into the isotropically shifted spectrum of five- and then six-coordinate high-spin forms upon titration with ligands like Cl^- or imidazole. The spectra of six-coordinated **2** typically cover a range from *ca.* +60 to −60 ppm and contain regions where signals of many protons overlap[1]). Since, formally, half of the unpaired spin density resides in the d_{z^2}-type orbital of the central metal, protons of a CH_3 group directly bound to Ni^{II} in one of the axial positions would be expected to experience a large contact shift in both **4a** and **5a**. However, in the absence of any precedent information about the magnitude and direction of the isotropic shift for such an axially bound CH_3 group, we considered it doubtful, whether its presumably very broad line would be detectable among the signals of the macrocycle protons. This prompted us to use a CD_3 group (see **5b**) and ^{2}H-NMR for the initial attempts, which, although at the cost of sensitivity, promised straightforward assignment and in addition, narrower lines [11].

In order to test this expectation, we first investigated the isotropically shifted NMR spectra of *Barefield*'s compound **4a** as a model system and then applied the same method to study the *in situ* methylation of F430M (**2**).

2. Results. – 2.1. *Studies with the Model Compound [Ni^{II}{(R,R,S,S)-tmc}](CF$_3$SO$_3$)$_2$* (**3**, X = CF_3SO_3). *^{1}H-NMR Spectra of Five- and Six-Coordinate Forms of* **3** (X = CF_3SO_3). In CF_3COOD, the triflate salt **3** (X = CF_3SO_3) is diamagnetic and showed a well resolved ^{1}H-NMR spectrum [12]. All resonances are now assigned by homodecoupling and 1D-NOE-difference spectroscopy. Observation of 7 different chemical shifts

[1]) Because of the presence of 47 magnetic sites giving rise to strongly overlapping spectra for both the diamagnetic and the paramagnetic forms, the assignment of the spectrum of paramagnetic **2** proved to be an involved task, which made it necessary to use 2D-NMR techniques. The detailed results of this study will be reported in a separate communication [10].

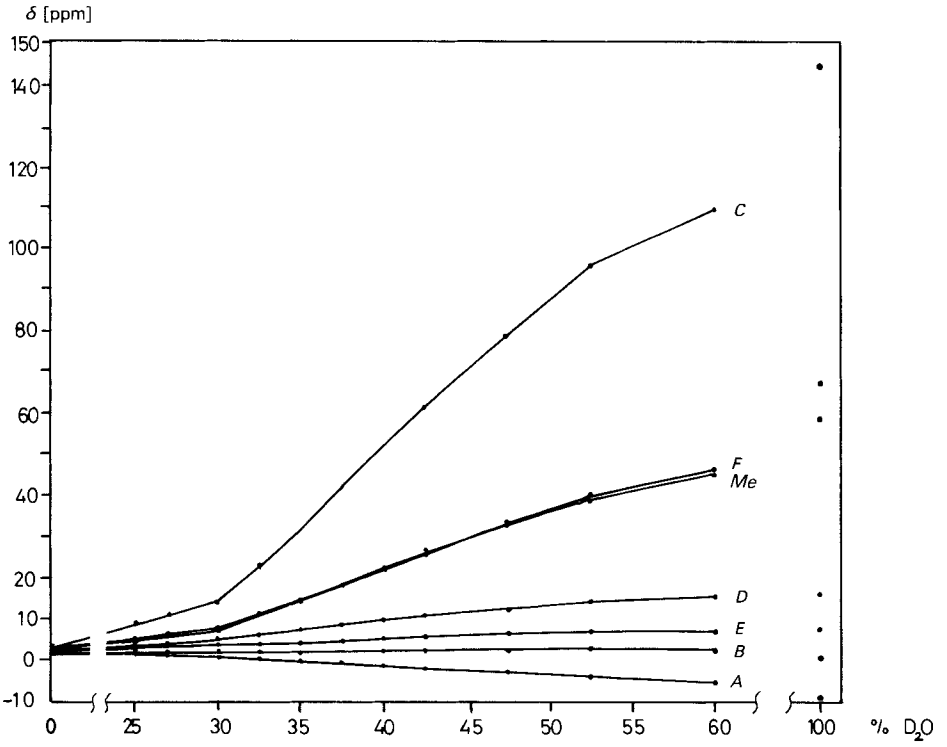


Fig. 1. *Titration of* $[Ni^{II}(tmc)](CF_3SO_3)_2$ (**3**, X = CF_3SO_3) *in* CF_3COOD *with* D_2O

confirm that, within the NMR time scale, the molecule has C_{2h} symmetry. Upon titration with D_2O, formation of the high-spin diaquo complex is observed, which causes a gradual shift and broadening of all resonances and permits to correlate the assignments in the diamagnetic spectrum with the isotropic shifts in neat D_2O as shown in *Fig. 1*. *Herron* and *Moore* showed, that in pure H_2O, the molar fraction of the diaquo complex of **3** is in the range from 0.53 (UV/VIS) to 0.74 (paramagnetic susceptibility) [13]. Since formation of the diaquo complex is not complete, the isotropic shifts measured in D_2O are averages between the shifts of the four- and fully six-coordinate species each weighted with the respective molar fraction[2]).

As described by *D'Aniello* and *Barefield* [7], $[Ni^{II}(tmc)](CF_3SO_3)_2$ (**3**, X = CF_3SO_3), which is insoluble in THF, dissolved upon addition of 1 equiv. of LiCl to give a green soln. of five-coordinate $[Ni^{II}Cl(tmc)]^+$. The ^{1}H-NMR spectrum (300 MHz) of this solution at 24.5° in (D_8)THF shows relatively narrow lines for magnetic sites *A*, *B*, *D*, and *E*, but extremely broad lines for signals *F*, *Me* and *C* (*Table 1;* for labels, see *Formula* **6**). Upon addition of a second equiv. of LiCl, the color of the solution changes to pale green and the ^{1}H-NMR spectrum (*Fig. 2a*) of the now present[3]) dichloro complex **6** shows relatively

[2]) Using a molar fraction of 0.74, the extrapolated isotropic shifts for the diaquo complex correspond closely to the shifts of the corresponding dichloro complex **6**.

[3]) The ^{1}H-NMR spectrum did not change upon addition of a large excess of LiCl, indicating, that formation of **6** was virtually complete with 2 equiv. of LiCl.

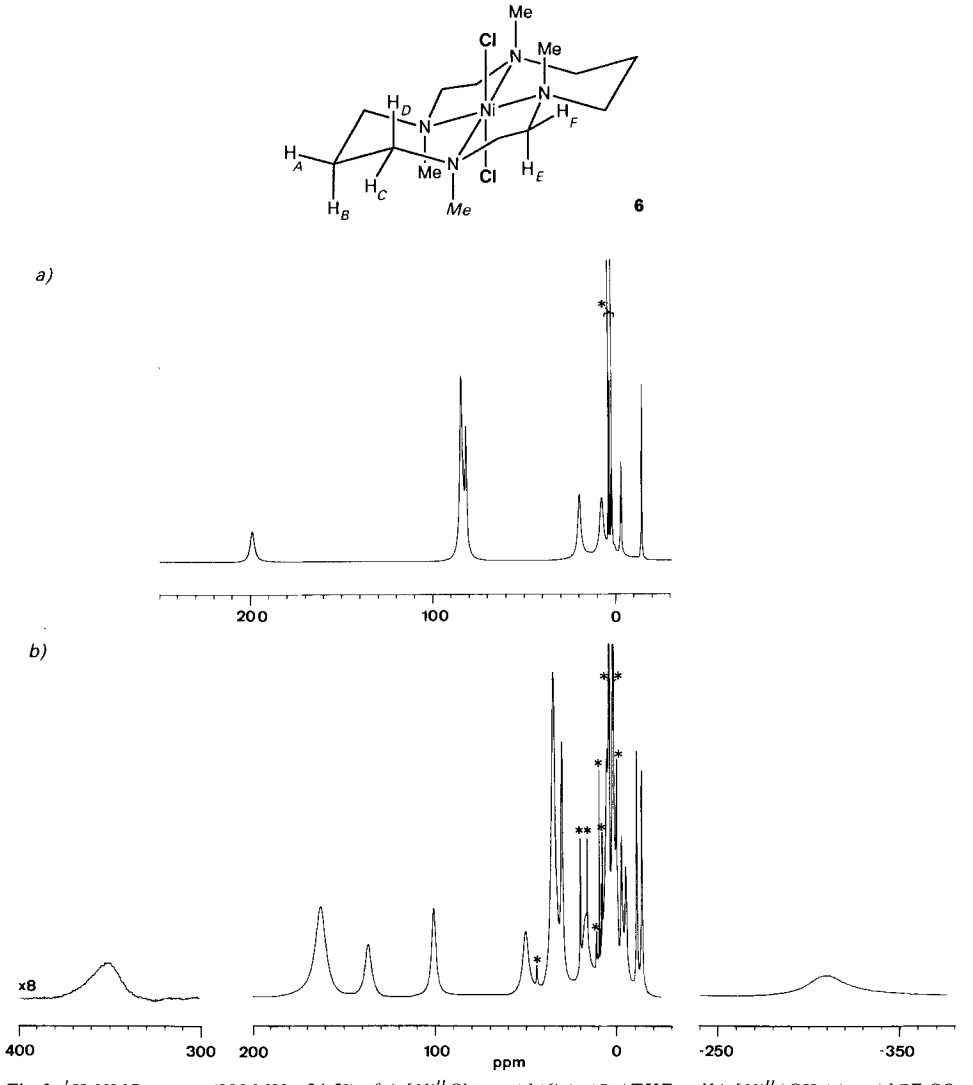


Fig. 2. *^{1}H-NMR spectra* (300 MHz, 24.5°) *of* a) *[$Ni^{II}Cl_2$(tmc)]* (**6**) *in (D_8)THF and* b) *[$Ni^{II}(CH_3)$(tmc)]CF_3SO_3* (**4a**) *in (D_5)pyridine* (⋆: signals from solvent and impurities)

narrow lines for all 7 magnetic sites. The highest possible symmetry of $[Ni^{II}Cl(tmc)]^+$ being C_s, 14 different chemical shifts would be expected in case of slow ligand exchange. The fact that only 7 lines are observed, three among them with very large line width, indicates that under these conditions, the rate of intermolecular ligand exchange is large enough to lead to fast exchange for those protons with smaller differences in isotropic shift between the two exchanging magnetic environments (*cf.* unprimed and primed labels in *Formula* **4**), whereas protons with large shift differences are just above coalescence.

^{2}H-NMR Spectrum of [$Ni^{II}(CD_3)$(tmc)]CF_3SO_3 (**4b**, X = CF_3SO_3). Reaction of a slight excess of $(CD_3)_2Mg$ with a suspension of finely powdered $[Ni^{II}(tmc)](CF_3SO_3)_2$ (**3**,

Table 1. *^{1}H-NMR Data of the Five- and Six-Coordinate $[Ni^{II}(tmc)]^{2+}$ Complexes*

Assignment	$[Ni^{II}(CH_3)(tmc)]^+$ **4a**[a]) δ[b]) ((D$_5$)pyridine)	$[Ni^{II}Cl(tmc)]^+$ δ((D$_8$)THF)	$[NiCl_2(tmc)]$ (**6**) δ((D$_8$)THF)
CH_3N (*Me*)	162.3	105[c])	83.4
	34.4		
$CH_2(\alpha)$ (*C*)	350	207[c])	198.4
	136.5[d])		
$CH_2(\alpha)$ (*F*)	100.4[d])	95[c])	81.1
	29.8[d])		
$CH_2(\alpha)$ (*D*)	50.0[d])	27.1	19.1
	4.2[d])		
$CH_2(\alpha)$ (*E*)	16.5[d])	12.0	7.0
	4.2[d])		
$CH_2(\beta)$ (*B*)	–3.3	1.0	–3.7
	–5.5		
$CH_2(\beta)$ (*A*)	–11.5	–14.8	–15.1
	–14.3		
CH_3Ni	–310		

[a]) Double entries refer to a pair of primed and unprimed magnetic sites in **4a**. [c]) Very broad lines (*ca.* 5–10 kHz).
[b]) Chemical shifts at 24.5° in ppm relative to external TMS. [d]) Tentatively assigned.

X = CF_3SO_3) in THF led to a green solution which was sealed in an NMR tube. *Fig. 3* shows the ^{2}H-NMR spectrum (46.05 MHz) obtained at three different temperatures. Besides the sharp line of excess $(CD_3)_2Mg$ at –2 ppm, the spectrum consists of a single broad line (line width = 360 Hz) for CD_3–Ni, which is strongly shifted to high field (–343 ppm at –20.3°). The temperature dependence of the shift (*Table 2* and *Fig. 4a*) obeys *Curie*'s law within experimental error. After opening the tube at –20° under Ar and addition of 50 µl of CF_3COOH/THF 1:10, the solution lost its color immediately, and red crystals of **3** (X = CF_3COO) formed. In the ^{2}H-NMR spectrum (–20.3°), the broad line at –343 ppm and the signal of $(CD_3)_2Mg$ have disappeared, while a weak signal assigned to CD_3H (0.2 ppm) is observed instead.

^{1}H-NMR Spectrum of $[Ni^{II}(CH_3)(tmc)]CF_3SO_3$ (**4a**). Using the same procedure, $[Ni^{II}(tmc)](CF_3SO_3)_2$ (**3**, X = CF_3SO_3) was methylated with $(CH_3)_2Mg$ in (D$_8$)THF. Because **4a** is much more soluble in pyridine than in THF, the ^{1}H-NMR spectrum (*Fig. 2b*, *Table 1*) was taken in (D$_5$)pyridine. At high field, the very broad signal (line width = 6 kHz) of the axial CH_3 group is found at the expected shift (–310 ppm at 24.5°). In the region from +350 to –15 ppm, where the protons of the equatorial macrocycle resonate, 12 isolated signals are detected as opposed to the 7 different chemical shifts observed in six-coordinate and fast-exchanging five-coordinate complexes of $[Ni^{II}(tmc)]^{2+}$. Experiments, in which the intensity of the solvent signals is minimized either by an inversion recovery sequence or by presaturation, show that two more signals are hidden underneath the strong absorptions of residual Et_2O and the solvent. The observation of a total of 14 magnetic sites reflects a reduction of the symmetry from 4-fold to 2-fold ($C_{2h} \rightarrow C_s$) and shows that intermolecular exchange of CH_3 groups is slow on the NMR time scale, or does not occur at all.

The four $CH_2(\beta)$ protons appear as two pairs of lines and are directly assignable from their small isotropic shift and narrow line width. Methylation of a sample of (D_{12})-**3**

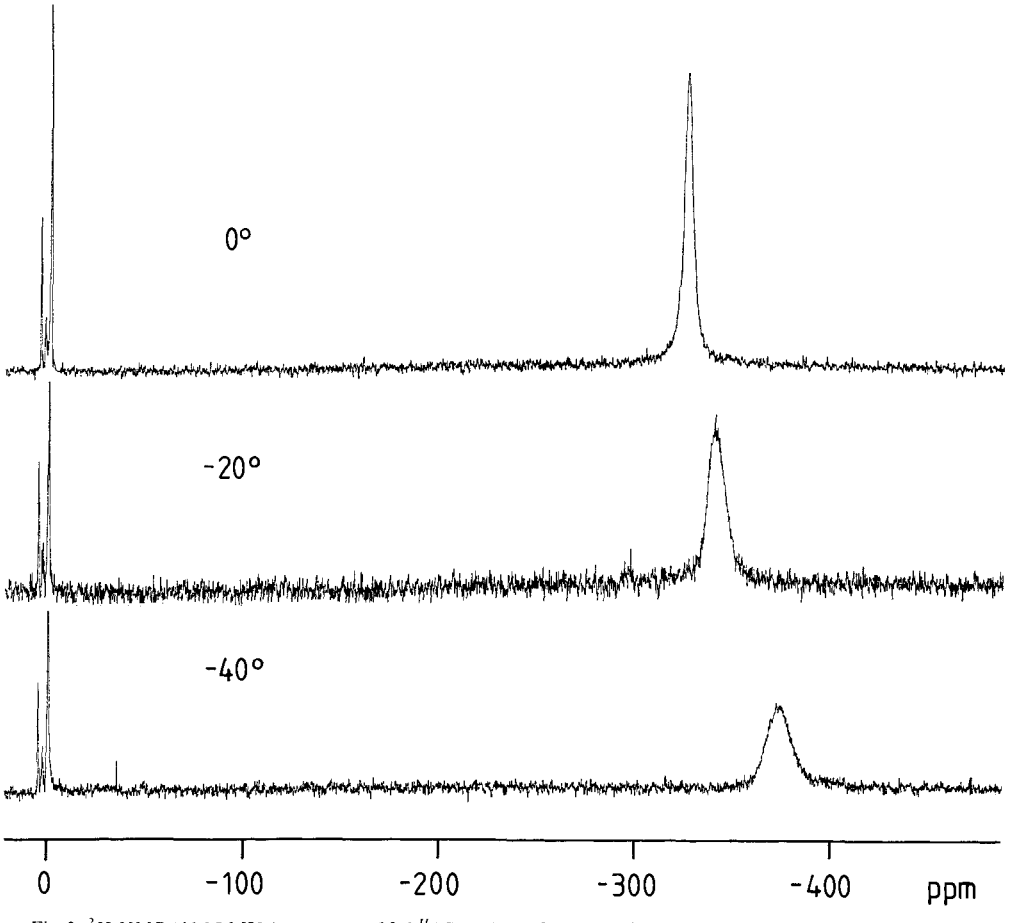


Fig. 3. *^{2}H-NMR* (46.05 MHz) *spectrum of* $[Ni^{II}(CD_3)(tmc)]CF_3SO_3$ (**4b**) *in THF at* a) *0.2°*, b) *−20°, and* c) *−40°*

Table 2. *Temperature Dependence of the* CD_3–Ni^{II} *^{2}H-NMR Signal of* **4b** *and* **5b**

$[Ni^{II}(CD_3)(tmc)]CF_3SO_3$ (**4b**)			CD_3–F430M^{II} (**5b**)[a])	
T [°C]	δ(^{2}H) [ppm][b])	Line width [Hz]	T [°C]	δ(^{2}H) [ppm][b])
21.0	–299	180		
0.1	–305	200		
–20.3	–343	360	–20.0	–440
–40.4	–366	530	–40.0	–490
–60.5	–393	680	–60.0	–535
–81.2	–458	935	–79.5	–590
–101.4	–520	1250		

[a]) The signal/noise ratio of the spectra of **5b** did not allow an accurate determination of the line width.
[b]) Referenced to internal C_6D_6.

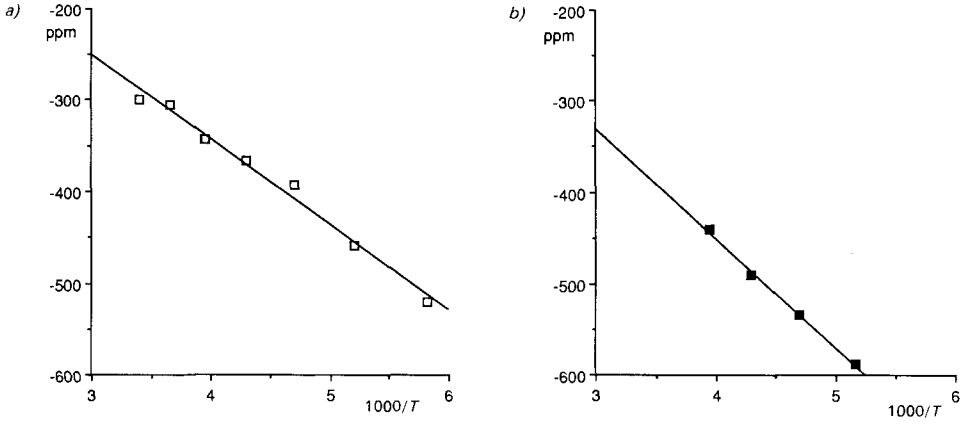


Fig. 4. *Temperature dependence of the* ^{2}*H-NMR chemical shifts* (in THF) *of* a) *[Ni^{II}(CD$_3$)(tmc)]CF$_3$SO$_3$* (**4b**) *and* b) *CD$_3$–F430M^{II}* (**5b**)

(X = CF_3SO_3) containing four CD_3N groups allows the assignment of the two signals at 162.3 and 34.4 ppm to CH_3N, because they are missing in the ^{1}H-NMR of (D_{12})-**4a**[4]). The $CH_2(\alpha)$ protons, which appear as signals *C*, *D*, *E*, and *F* in the spectrum of six-coordinate or fast-exchanging five-coordinate complexes, give rise to 8 signals in the spectrum of **4a** because, in the absence of fast exchange, the primed and unprimed positions in *Formula* **4** are no longer equivalent. These signals are tentatively assigned as pairs by analogy to the spectrum of **6** and based on the approximate correspondence of the average isotropic shift of each pair to the shift in the monochloro complex. Since no NOE's can be observed at all, the question whether it is the primed or the unprimed proton in each pair that shows the larger isotropic shift has to remain open.

2.2. *Studies with F430M^{II}* (**2**, X = CF_3SO_3). Upon reaction of 6.5 μmol of **2** (X = CF_3SO_3) with slightly less than 1 equiv. of $(CD_3)_2Mg$ (*Fig. 5a*) in a vacuum-sealed NMR tube at −78° (see *Exper. Part*), the color of the solution changed immediately from yellow (**2**) to brown, and a small amount of gas evolved. The ^{2}H-NMR spectrum of the mixture, measured at −40°, shows a single, very broad line (line width *ca.* 2.5 kHz) at −490 ppm for CD_3–Ni (**5b**), whereas the sharp signal of $(CD_3)_2Mg$ at −2.0 ppm has disappeared (*Fig. 5b*). The temperature dependence of the isotropic shift of the CD_3–Ni signal was investigated by measurements at 4 different temperatures (*Table 2, Fig. 4b*). Repeated measurements at −20° show, however, that the signal is slowly decaying at this temperature. When the tube was opened at −78° under Ar and 50 μl of 0.05M CF_3SO_3H in H_2O were added, the color changed immediately back to yellow, and the signal at high field had disappeared from the ^{2}H-NMR spectrum (*Fig. 5c*). After workup, > 95% of the original F430M (**2**) was recovered unchanged (UV/VIS, TLC).

When this ^{2}H-NMR experiment was repeated using different concentrations of F430M (**2**) and different reaction temperatures, the yield of CD_3–F430M^{II} (**5b**), as estimated from the integral ratios between the isotropically shifted signal and internal C_6D_6, showed large variations (0 to 60% based on **2**). In one experiment at −78° with a rather

[4]) The very large spectral widths which cause nonuniform excitation, do not allow to identify the CH_3 signals unequivocally by integration.

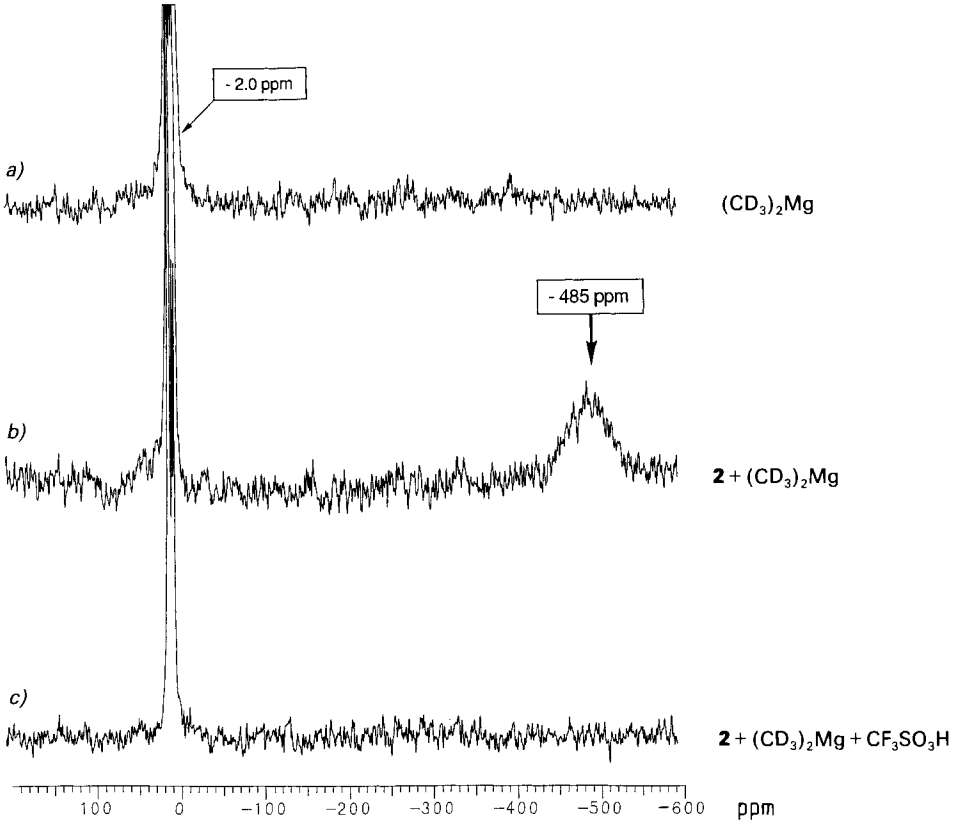


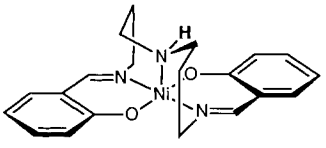
Fig. 5. *Methylation of F430M* (**2**): ^{2}*H-NMR spectra* (46.05 MHz, −40°) *of* a) *the* $(CD_3)_2Mg$ *reagent solution,* b) CD_3–*F430M*^{II} (**5b**) *after reaction at −78°, and* c) *the same system after addition of* CF_3SO_3H. All spectra were recorded and processed under identical conditions.

high concentration (c = 20 mM) of **2**, a fine dark brown precipitate formed after mixing, leaving a colorless solution which did not show the isotropically shifted ^{2}H-signal at all. However, in all experiments, recovery of **2** after acidic workup was nearly quantitative. These observations indicate, that deprotonation of **2** competes with CD_3 transfer to the Ni-atom even at low temperatures. Once formed, CD_3–F4340M^{II} (**5a**) decayed only very slowly below −20°, presumably by irreversible formation of methane after intramolecular proton transfer.

3. Discussion. – Qualitatively, the isotropically shifted NMR spectra of both, $[Ni^{II}(H_2O)_2(tmc)]^{2+}$ and $[Ni^{II}Cl_2(tmc)]$ (**6**) are similar to that of $[Ni^{II}Cl_2([14]aneN_4)]$ as reported by *Dei* [14]. Our assignments, which for $[Ni^{II}(H_2O)_2(tmc)]^{2+}$ are based on the correlation with the spectrum of the diamagnetic complex, agree with those of *Dei* [14], but differ for one pair of signals from the tentative assignment for the diaquo complex of **3** (X = CF_3SO_3) by *Merbach* and coworkers [15]. ^{1}H-NMR spectra of high-spin Ni^{II} complexes were generally interpreted in terms of predominant contact contribution to the isotropic shift. In the paramagnetic $[Ni^{II}([14]aneN_4)]^{2+}$ derivatives investigated so far, the

protons of CH_2 groups α to the N-atoms are shifted to low field, while the $CH_2(\beta)$ protons are moderately shifted to high field. Protons in equatorial positions of the macrocycle experience much stronger shifts than those in axial positions. This pattern was interpreted as a consequence of σ-spin delocalization which leads to positive spin density on the $CH_2(\alpha)$ protons causing downfield shifts. The upfield shifts of the $CH_2(\beta)$ protons were attributed to dominant spin-polarization effects [16].

In the ^{1}H- and ^{2}H-NMR spectrum of **4**, the signal of the Me group axially bound to Ni^{II} is drastically shifted to high field. Its chemical shift of -310 ppm (^{1}H, 24.5°) is comparable to that of the structurally analogous NH proton (*ca.* -330 ppm) of the axial secondary-amine ligand in **7**, as reported[5]) by *La Mar* and *Sacconi* [17], who attributed the shift to high field to a strong spin-polarization effect. The analogy with **7**, the strong isotropic shift, and the large line width of the CH_3 signal demonstrate the presence of a C–Ni bond in **4a**.



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In contrast to the macrocyclic model compound **3** ($X = CF_3SO_3$), several functional groups of the pentamethyl ester **2** of coenzyme F430, particularly the amide and lactam NH protons, are potentially reactive towards Me_2Mg. In fact, the observed formation of small amounts of methane upon reaction of Me_2Mg with **2** even at $-100°$, together with the fact that **2** was recovered quantitatively after protonation with acid, indicate that reversible deprotonation of **2** is the major competing reaction to methylation at the Ni-center. Only if a large excess of Me_2Mg was used, additional products resulting from attack on the ester groups were isolated.

The isotropic shift of the Ni-bound CD_3 group in **5b** is even larger and the line broader than for the model compound **4b**, indicating that more spin density is delocalized to the CD_3 C-atom. This points to a stronger Ni–C bond in the F430 derivative than in **4** although, due to the onset of intramolecular proton transfer; **5** is less stable at higher temperatures than **4**. Since, within experimental error, the temperature dependence of the CD_3–Ni isotropic shifts for both **4b** and **5b** follow *Curie*'s law, the electron-deuterium hyperfine coupling constants (A_c/h) can be estimated if pure *Fermi* contact shift is assumed. The resulting values[6]) are $A_c/h = -0.65$ MHz for **4b** and $A_c/h = -0.85$ MHz for **5b**.

4. Conclusions. – Our results show that NMR spectroscopy, particularly ^{2}H-NMR, is a very useful technique for the characterization of high-spin Me-Ni^{II} derivatives. The

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[5]) The isotropic shift of the N–H proton was not given in the original communication [17], but it is shown in Fig. 2.5, p. 26, of [9b].

[6]) Calculated using an average g factor of 1.97 deduced from $\mu = 2.78\ \mu_B$ [7] using the *Bloembergen-McConnel* approximation [18]. Neglecting possible isotope effects, these values correspond to proton hyperfine-coupling constants of -4.3 (**4a**) and -5.5 MHz (**5a**). From the *Curie* plot of the ^{1}H-NMR signal of **4a**, a proton hyperfine coupling constant of -4.35 MHz was determined.

characteristic, large high-field shift of the Me signal constitutes a direct proof for the presence of a Ni–C bond in these compounds. The observation of such a signal after *in situ* methylation of F430M shows, that CH_3–F430M^{II} (**5a**), which was postulated as an intermediate in the formation of methane, exists. However, generation of **5b** was not quantitative and the question, whether the CD_3 group is bound at the α- or β-side of the macrocycle is still open[7]). It remains to be demonstrated, using the technique reported here, that **5a** is indeed the intermediate observed in the reaction of F430M^{I} with electrophilic CH_3 donors such as CH_3I or methylsulfonium ions.

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Experimental Part

1. *General.* Et_2O (*Carlo Erba*, RPE grade) was distilled from Na/benzophenone, 1,4-dioxane (*Fluka, puriss. p.a.*) from Na, and THF (*Fluka, puriss. p.a.*) 3 times from K. (D_8)THF (*Merck;* > 99% D) was distilled from K and stored with a ball of K in a sealed *Schlenk* flask with a metal valve, where it was degassed by 3 freeze-thaw cycles; the solvent was transferred to the apparatus (see below, *Fig. 6*) or NMR tube by condensation at the vacuum line. CH_2Cl_2 (*Fluka, purum p.a.*) and CD_2Cl_2 (*Ciba-Geigy;* 99.5 atom-% D) were distilled form CaH_2 and C_6H_6 (*Fluka, purum p.a.*) and C_6D_6 (*Ciba-Geigy, ultra puriss.;* 99.95 atom-% D) from NaH. (D_5)Pyridine (*Dr. Glaser AG*, Basel; > 99.5 atom-% D) and CF_3COOD (*Dr. Glaser AG*, Basel; > 99.5 atom-% D) were used as received. CH_3I (*Fluka, purum*) and CD_3I (*Ciba-Geigy;* > 99 atom-% D) were freshly distilled. F430M (**2**, X = ClO_4) was prepared and purified according to the published procedure of *Pfaltz et al.* (1982) [1].

2. *F430M* (**2**, X = CF_3SO_3). A soln. of **2** (X = ClO_4) in CH_2Cl_2 was extracted 3 times with 0.5M aq. CF_3SO_3Na. The org. phase was evaporated and the residue redissolved in 0.5 ml of CH_2Cl_2 and washed with 2 drops of H_2O. After evaporation of CH_2Cl_2, traces of halogenated solvents, which adversely affect the reactions with Me_2Mg, were eliminated by 3-fold precipitation of **2** (X = CF_3SO_3) from THF with benzene. The resulting powder was dried *in vacuo* overnight.

3. *[(R,R,S,S)-1,4,8,11-Tetramethyl-1,4,8,11-tetraazacyclotetradecane]nickel(II) Bis(trifluoromethane sulfonate)* (= *[(R,R,S,S)-N,N′,N″,N‴-Tetramethylcyclam]nickel(II) Bis(trifluoromethanesulfonate);* [Ni^{II}(tmc)]$(CF_3SO_3)_2$; **3**, X = CF_3SO_3) was synthesized according to the published procedure [12] [19]. Blue crystals were obtained after recrystallization from aq. soln. Crystal water was removed by drying overnight at r.t./10^{-2} Torr, giving a pink brittle solid. Before reaction with Me_2Mg or LiCl, a very fine powder was prepared in a vibrating mill and dried further at 10^{-2} Torr overnight. ^{1}H-NMR (CF_3COOD; c = 33.4 mM; δ rel. to sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS); assignments based on NOE and ^{1}H,^{1}H-COSY; for labels, *cf. Formula* **6**): 1.92 (*m*, 2 H, H_A, overlap with H_B); 1.92 (*m*, 2 H, H_B); 2.21 (*d*, 4 H, H_F); 2.34 (*m*, 4 H, H_C); 2.96 (*s*, 12 H, CH_3N); 3.10 (*d*, 4 H, H_E); 3.45 (*m*, 4 H, H_D). ^{13}C-NMR (100 MHz, CF_3COOD; c = 33.4 mM; δ rel. to DSS; assignments based on DEPT): 22.92 ($CH_2(\beta)$); 46.98 (CH_3N); 60.96, 63.89 ($CH_2(\alpha)$).

[(R,R,S,S)-1,4,8,11-Tetra($^2H_3$*)methyl-1,4,8,11-tetraazacyclotetradecane]nickel(II) Bis(trifluoromethanesulfonate)* ((D$_{12}$)-**3**, X = CF_3SO_3) was prepared as described for **3** (X = CF_3SO_3) using CD_3I for the alkylation step.

4. *Dimethyl Magnesium and Di(*$^2H_3$*)methyl Magnesium* were prepared by addition of a slight excess of 1,4-dioxane to 1.25M MeMgI in Et_2O and intensive stirring at r.t. under N_2 for 24 h. After removal of the precipitated dioxane complex of MgI_2 by centrifugation, Me_2Mg was recrystallized 3 times from dry Et_2O at −78°. Finally, the white crystals were dissolved in Et_2O to give a 0.5M stock soln. in a *Schlenk* tube. ^{1}H-NMR ((D_8)THF, 24.5°): −1.73 (*s*, CH_3).

5. *NMR Spectroscopy. General.* ^{1}H-NMR spectra: *Bruker WM300;* 300 MHz spectra of paramagnetic compounds were taken with spectral widths of 80–166 kHz, 12-bit ADC, 90° pulse 4.2 µs; 32 K data points in the time domain; processing with an exponential line broadening of 5–20 Hz; 4000–12000 transients/spectrum;

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[7]) Because of the very large line width of the CD_3–Ni signal, the possibility that we observed a mixture of α-CD_3 and β-CD_3 forms can not be definitely excluded.

polynomial or sinusoidal baseline correction; referenced to external TMS. ^{2}H-NMR spectra: *Varian XL-300* (46.05 MHz); measured without lock (drift < 2 Hz/h); spectral width 50 kHz; 12-bit ADC, 16 K data points in the time domain; exponential line broadening 2–8 Hz; referenced to internal C_6D_6 (7.27 ppm). Temperature control: to within 0.15 K on both instruments; calibrated before and after the measurements with a Pt-100 thermometer in the place of the sample tube.

Titration of [Ni^{II}(tmc)](CF$_3$SO$_3$)$_2$ (**3**, X = CF_3SO_3) *in CF$_3$COOD with D$_2$O.* To a soln. of 15 mg of **3** (X = CF_3SO_3) in 0.6 ml of CF_3COOD, increasing amounts of D_2O were added and the ^{1}H-NMR recorded after each step (*Fig. 1*). Concentration was not maintained constant, the final volume being 1.5 ml. Finally, the ^{1}H-NMR of 8 mg of **3** (X = CF_3SO_3) in 0.6 ml of pure D_2O was taken.

^{1}H-NMR of [Ni^{II}Cl(tmc)]$^+$. To a suspension of 9.3 mg (15.2 μmol) of **3** (X = CF_3SO_3) in 0.7 ml of (D_8)THF, 60 μl of 0.255M LiCl (15.3 μmol) in (D_8)THF was added. After stirring at r.t. for 30 min a blue-green soln. of [Ni^{II}Cl(tmc)]$^+$ resulted. Traces of residual solids were removed by centrifugation before recording the ^{1}H-NMR at 24.5°.

^{1}H-NMR of [Ni^{II}Cl$_2$(tmc)] (**6**). To the soln. of [Ni^{II}Cl(tmc)]$^+$ (see above), a second equiv. of LiCl/(D_8)THF soln. was added, which resulted in a change of color from blue-green to pale green. After recording the ^{1}H-NMR, the soln. was allowed to stand at −20° overnight, during which time **6** crystallized as turquoise rhombic crystals. The mother liquor was decanted and the crystals partly redissolved in 0.225M LiCl/(D_8)THF (> 100-fold excess of Cl^-). Within experimental error, the ^{1}H-NMR of this soln. was identical to that of **3** (X = CF_3SO_3) in the presence of 2 equiv. of Cl^-.

^{1}H-NMR of [Ni^{II}(CH$_3$)(tmc)]CF$_3$SO$_3$ (**4a**, X = CF_3SO_3). While stirring vigorously under N_2, 40 μl of 0.63N $(CH_3)_2Mg$ in Et_2O (25.2 μmol of 'CH_3^-') was added to a suspension of 11.5 mg (18.8 μmol) of **3** (X = CF_3SO_3) in 1.0 ml of (D_8)THF. After 2 min of continued stirring, the suspension was separated into a faintly green soln. and a mixture of green and white ($Mg(CF_3SO_3)_2$) solids by centrifugation. The supernatant soln. was removed, and the solids were stirred with 0.7 ml of (D_5)pyridine under N_2. The green solid dissolved quantitatively and was separated from the remaining white precipitate by centrifugation. The clear, emerald green, supernatant soln. was transferred to an NMR tube and the ^{1}H-NMR (*Fig. 2b, Table 1*) was recorded at 24.5°. Using the vacuum-line procedure and apparatus (*Fig. 6*), solns. of **4a** in (D_8)THF were prepared and the ^{1}H-NMR recorded. For a given temp., the isotropic shifts in (D_8)THF were the same within experimental error as those in (D_5)pyridine. However, in our hands, **4a** was less stable in THF than in pyridine at r.t.

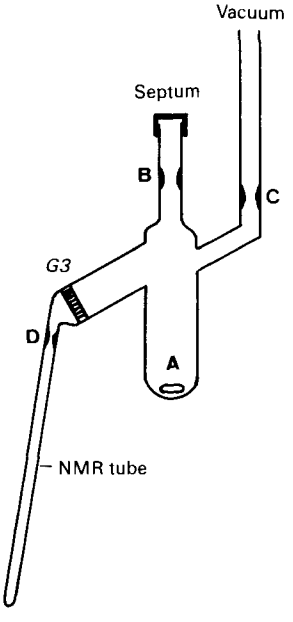

Fig. 6. *Preparation of NMR solutions.* For details, see text.

^{1}H-NMR of [$Ni^{II}(CH_3)\{(D_{12})tmc\}$]$CF_3SO_3$ ((D$_{12}$)-**4a**, X = CF_3SO_3). Starting from (D$_{12}$)-**3** (X = CF_3SO_3), the sample was prepared according to the procedure described above. The ^{1}H-NMR in (D$_5$)pyridine at 24.5° was identical with that of **4a**, with the exception of the lines at 162.3 and 34.4 ppm (CH_3N) which were missing.

^{2}H-NMR of [$Ni^{II}(CD_3)(tmc)$]CF_3SO_3 (**4b**, X = CF_3SO_3). To 100 mg (163 μmol) of finely powdered **3** (X = CF_3SO_3) in A of the apparatus shown in *Fig. 6*, 1 ml of THF was added and the resulting suspension degassed by 3 freeze-thaw cycles at a vacuum line (10^{-5} Torr). The system was flushed with purified N_2 and 234 μl (164 μmol 'CD_3^-') of 0.7N ($CD_3)_2Mg$ in Et_2O was added into A through the septum. The suspension in A was immediately frozen in liq. N_2, and, after evacuation of the whole system, side arms **B** and C were sealed. After warming to 20°, the mixture was stirred vigorously for *ca.* 10 min and then filtered into the NMR tube. The filtrate was emerald green, but an undefined amount of product had precipitated and remained on the filter together with small amounts of starting material **3** (X = CF_3SO_3). Finally, the clear soln. in the NMR tube was frozen and sealed off at D. The solubility of **4b** shows a pronounced temp. dependence. While the soln. in the NMR tube remained homogeneous down to *ca.* −20°, increasing amounts of the product precipitated as a blue-green solid if the temp. was lowered beyond.

^{2}H-NMR of CD_3–F430M^{II} (**5b**). A soln. of 6.5 μmol of F430M (**2**, X = CF_3SO_3) in the minimum amount of THF was transferred into A of an apparatus similar to that shown in *Fig. 6*, but without filter. The solvent was carefully distilled into a trap on the vacuum line, leaving a solid film of **2** which was dried at r.t./10^{-5} Torr overnight. The system was then flushed with dry and O_2-free N_2, and 0.55 ml of a dilute stock soln. of ($CD_3)_2Mg$ were added into the NMR tube. The concentration of the stock soln. had been adjusted using ^{2}H-NMR (integral ratio for the internal standard C_6D_6 and ($CD_3)_2Mg$) to give exactly 0.95 equiv. of 'CD_3^-' based on **2**. The reagent soln. in the NMR tube was degassed by 3 freeze-thaw cycles, and the system was sealed at positions **B** and C at 10^{-5} Torr. After melting, ⅔ of the liquid phase were internally distilled from the NMR tube to A to dissolve all **2**. The residual ($CD_3)_2Mg$ soln. was frozen in liq. N_2 and then the soln. of **2** was allowed to flow into the NMR tube, freezing on top of the solid ($CD_3)_2Mg$ soln. The NMR tube was sealed and the reaction induced by thawing and mixing the two layers at −78°. An immediate color change from yellow to brown was observed. The ^{2}H-NMR of **5b** was measured at −40° (*Fig. 5b*), then at −20°. *Ca.* 30 min after the reaction, the tube was opened at −78° under Ar and 50 μl of 0.05M CF_3SO_3H in H_2O were added. Upon mixing the acid with the still brown soln., the color changed immediately back to yellow. The ^{2}H-NMR was again measured at −40° (*Fig. 5c*) and −20° under identical conditions. A high-resolution ^{2}H-NMR of the diamagnetic region revealed a signal at 0.2 ppm which was attributed to CD_3H. To check for paramagnetic impurities, a spectrum of the stock soln. of ($CD_3)_2Mg$ was recorded under identical conditions (*Fig. 5a*).

Recovery of F430M (**2**, X = ClO_4) *from Methylation Reactions.* After quenching with acid (see above), the contents of the NMR tube were extracted 3 times with 2 ml of CH_2Cl_2 and 2 ml of 0.1M aq. $NaClO_4$/0.01M $HClO_4$. The org. phase was evaporated and the purity checked by TLC, ^{1}H-NMR, and UV/VIS (yield > 95%).

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