NMR Coupling versus NMR Chemical Shift Information in Metallobiochemistry. High-Resolution One-Bond $^{1}H-^{13}C$ Coupling Constants Obtained by a Sensitive Reverse Detection Method

Scott J. Moore, Marian Iwamoto, and Luigi G. Marzilli*

Department of Chemistry, Emory University, Atlanta, Georgia 30322

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Imidazole rings are involved in acid/base chemistry, catalysis, H-bonding, and metal complexation throughout biochemistry; these rings are frequently targets for anticancer drugs and carcinogens. However, interpreting the changes in ^{13}C NMR shifts of these rings is often difficult. We explore the use of high-resolution one-bond $^{1}H-^{13}C$ coupling constants ($^{1}J_{CH}$) for the identification of electronic changes within imidazole rings of samples containing ^{13}C in natural abundance. The reverse detection method used, called *J*-coupled heteronuclear multiple quantum coherence (JHMQC) spectroscopy, employs a modified HMQC pulse sequence. The method was evaluated with B_{12} models of the type $Me_3BzmCo(DH)_2$(R or X), where Me_3Bzm = 1,5,6-trimethylbenzimidazole and DH = the monoanion of dimethylglyoxime. $^{1}J_{CH}$ values of $Me_3BzmCo(DH)_2CH_3$ obtained from both JHMQC and standard coupled 1D ^{13}C NMR spectra led to similar values, but the JHMQC method gave better resolution and much higher signal-to-noise ratios. The $^{1}J_{CH}$ values for the endocyclic carbons and the *N*-methyl group of the Me_3Bzm in five models fell between those of the free and protonated Me_3Bzm ligands. Thus, donation of electron density from Me_3Bzm to the Co center typically increases $^{1}J_{CH}$ values with respect to the free ligand. Values of $^{1}J_{CH}$ for several ^{13}C NMR signals correlated with both EP, a reported measure of electron-donating ability of R or X, and Co−N bond lengths from X-ray structures. For the assessment of electronic properties of the metal center, the $^{1}J_{CH}$ values appear to be more reliable parameters than the traditionally used ^{13}C shifts, especially for C's close to the metal. Moreover, $^{1}J_{CH}$ values for the ^{13}C signals for $Co-^{13}C$ were observed in several models; the ^{13}C signals for these carbons attached to the quadrupolar cobalt are too broad for $^{1}J_{CH}$ determination by the traditional 1D method. The JHMQC method developed here is thus very versatile and can provide information on any type of molecule showing resolved CH signals.

Introduction

Imidazole rings, present in nucleic acids, proteins, antibiotics, and cofactors, are involved in acid/base chemistry, catalysis, H-bonding, and metal complexation; these rings are frequently the molecular target of anticancer drugs and carcinogens. However, interpretation of the changes in ^{13}C NMR shifts of these heterocyclic compounds is often difficult. We hypothesized that coupling constants for the CH group linking the ring nitrogens would afford a more reliable means of assessing the nature of the involvement of the imidazole ring. In this report, we describe a reverse detection method for obtaining high-resolution one-bond $^{1}H-^{13}C$ coupling constants ($^{1}J_{CH}$), and we test the use of $^{1}J_{CH}$ with one type of model molecule of biological interest. The method in its present form is not applicable to molecules with many overlapping signals. However, it is useful for assessing the properties of large molecules with resolved aromatic signals and smaller molecules which can act as models for understanding spectral and bonding changes in larger molecules.

Reverse detection methods[1−10] are used in most studies to detect and assign signals of heteronuclei like ^{13}C, ^{31}P, or ^{15}N through the signal of a spin-coupled proton; reverse detection even facilitates ^{1}H NMR assignments in some cases. For example, NMR studies employing reverse detected heteronuclear methods, such as HMQC (heteronuclear multiple quantum coherence) and HMBC (heteronuclear multiple bond correlation) spectroscopies, permit the complete assignment of ^{1}H and ^{13}C signals of anticancer antibiotics[11−13] and cobalamins[4,14−20] and

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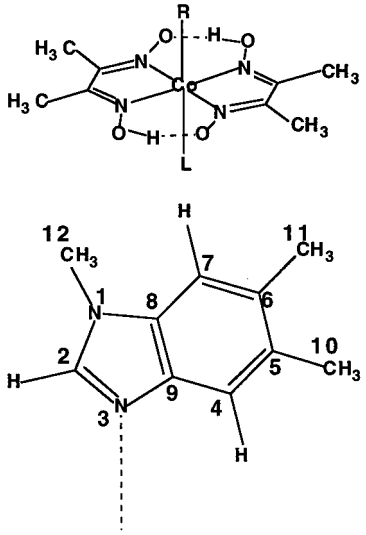
many of the ^{13}C and ^{31}P signals of oligonucleotides.[21−23] The methods are also useful in assigning signals of relatively small molecules with overlapping complicated spin systems, such as those found in many types of coordination compounds.[24,25] Although assignment of the signals of heteronuclei is very valuable in probing the properties of biomolecules, the full potential of reverse detection methods has not been realized since the coupling information itself is either acquired with low resolution or eliminated during acquisition. Because the magnitude of ^1H-X coupling depends on the nature of the bonding in the biomolecule, much useful information about the properties of the biomolecules is thus lost.

Anticipating that $^1J_{CH}$ information could contribute to many areas of active investigation, we selected as a test case one important class of biomolecules for which shift information has in many cases not been very useful. This class is widespread in biological systems, namely species containing an adduct between a metal center and an imidazole ring. The biomolecules in this class of compounds differ in the type of heterocycle (imidazole, purine, and benzimidazole). The shift of the signal of the carbon between the two imidazole ring N's (C2 in imidazoles and benzimidazoles and C8 in purines) is not very informative, either because it is shifted very little by the metal coordination or because it has a very small dependence on changes in the groups attached to the metal. For each type of heterocycle, we cite one example of the limited information available from shifts. The histidine residue of bleomycin binds to metals, and the Zn complex has been used to probe the binding of metal complexes of this anticancer antibiotic to DNA.[26] However, the changes in the shifts of the imidazole carbons from those of the free ligand are very small.[11] The clinically very useful cis-type Pt anticancer drugs bind to DNA through interaction with the N7's of two adjacent guanines.[27,28] This interaction is powerful enough to distort DNA structure, a feature believed to be associated with anticancer activity.[21,22,28−31] However, there are only very small shifts of the C8 signals.[21] Finally, although the differences between the Co−N(axial 5,6-dimethylbenzimidazole, DMBz) bond lengths in vitamin B_{12} and the B_{12} coenzymes are large (∼0.2 Å),[32] there is little difference in the C2 shifts.[16]

It is not surprising that there are confounding relationships between shift and the nature of the metal interactions since shifts are affected by through-space effects as well as the metal-dependent changes in electronic properties of the biomolecule.[33,34] The carbon between the two N's is of necessity close to the metal, and thus these shifts can be influenced by the metal and the adjacent ligands, including the highly anisotropic macrocycles common in metal cofactors, namely porphyrins and corrins.

Chart 1



To evaluate our hypothesis that coupling constants (which primarily reflect through-bond effects) should not suffer from the problems illustrated for shifts, we needed a well-understood system but one that nevertheless suffered from the complications mentioned above for interpreting shifts. We selected B_{12} model complexes of the type $Me_3BzmCo(DH)_2$(R or X), where DH = the monoanion of dimethylglyoxime and Me_3Bzm = 1,5,6-trimethylbenzimidazole (Chart 1). These complexes are very well defined structurally and are of interest for interpreting cobalamin chemistry.[34] Well-defined relationships exist between the NMR spectral characteristics of these models and cobalamins.[16] Other well-defined relationships have been found for such organocobalt species. For example, the Co−C stretching band was detected first by FT Raman spectroscopy on cobaloxime models, and later the band for methylcobalamin was found at an almost identical frequency.[35−38]

Background on the NMR Method. Measurement of high-resolution, first-order $^1H-^{13}C$ coupling constants of biomolecules is not easily achieved with 1D ^{13}C detection methods due to the low sensitivity and low natural abundance of the ^{13}C isotope. Because coupling constants measured with 2D experiments have low resolution, 1D ^1H-detected experiments are preferable. A sensitive 1D experiment can be devised from any one of the many reported $^1H[^{12}C]$ signal suppression 2D techniques introduced to observe selectively the $^1H[^{13}C]$ signals.[1,7,8,39,40] We chose to modify the convenient Sklenár/Bax[7] sequence (Figure 1) which utilizes optimization of a short

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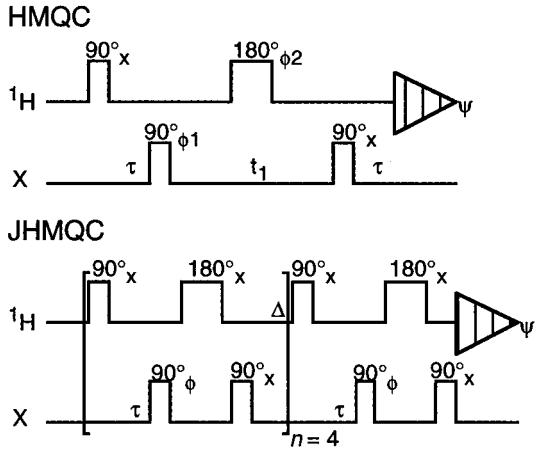

Figure 1. Top: 2D sequence of Sklenár and Bax.[7] The delay τ is set according to the one-bond heteronuclear coupling constant: $\tau = 1/(2^1J_{XH})$. $\phi1 = +x, +y, -x, -y$; $\phi2$ is incremented by 90° four times per t_1 value; $\psi = x, x, -x, -x$. The receiver phase is inverted four times per t_1 value. Data in odd- and even-numbered scans are stored in separate memory locations. Bottom: Modified 1D version of the Sklenár/Bax sequence (JHMQC). The bracketed portion of the sequence is cycled four times. The delay τ is set according to the one-bond heteronuclear coupling constant: $\tau = 1/(2^1J_{XH})$. Δ is optimized for the T_1 relaxation rates of the ^{1}H[^{13}C] protons. $\phi = +x, -y, -x, +y$; $\psi = x, y, -x, -y$.

repetition time dependent upon the shorter spin−lattice relaxation time of the ^{1}H[^{13}C] relative to the ^{1}H[^{12}C] nuclei. Although similar pulse sequences such as spin-echo difference spectroscopy are currently available on newer NMR spectrometers, we present this sequence as an easily implemented alternative for older instruments.

Our objective of obtaining data with high digital resolution requires the use of a long acquisition time (T_{ac}). Other techniques commonly used to enhance spectral digitization, such as zero-filling and interpolation, do not improve the intrinsic resolution of the data collected.[41] A T_{ac} long enough to allow for the measurement of $^1J_{CH}$ to ±0.2 Hz would result in a repetition time too long for ^{1}H[^{12}C] signal suppression with the 2D Sklenár/Bax[7] sequence. In our modified 1D sequence, *J*-coupled heteronuclear multiple quantum coherence (JHMQC, Figure 1), we used a long T_{ac} to achieve both good suppression of the ^{1}H[^{12}C] signals and a high-resolution spectrum.

Experimental Section

Materials. The Me_3Bzm complexes were prepared as described previously.[34] In $CDCl_3$, $ReOCl_3(OPPh_3)$ forms Me_3BzmH^+ on standing. Complex purity was confirmed by NMR. Deuterated solvents were from Cambridge Isotope Laboratories, Inc.

NMR Spectroscopy. ^{1}H 1D data were collected at 599.64 MHz on a General Electric GN-600 Omega spectrometer at 25 °C with a ~12 500 Hz window, a 30° pulse width, and 32K data points. The large window was necessary for observation of the bridging O--H−O signal at ~18−19 ppm. Sample concentrations were 50−100 mM in $CDCl_3$, and chemical shifts (ppm) were referenced to TMS unless otherwise noted.

The ^{13}C NMR shifts of the protonated Me_3Bzm and the adamantyl (adam) complex in $CDCl_3$ were obtained on a General Electric GN-600 Omega spectrometer and a General Electric QE−300 spectrometer, respectively. All other ^{13}C NMR shifts in $CDCl_3$ were taken from Charland et al.[34]

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Table 1. $^1J_{CH}$ and *S*/*N* Values Obtained from the JHMQC and Coupled ^{13}C NMR Spectra of $Me_3BzmCo(DH)_2CH_3$

	JHMQC		1D ^{13}C	
position	$^1J_{CH}$ (Hz)	*S*/*N*	$^1J_{CH}$ (Hz)	*S*/*N*
B2	208.21	40.9	208.30	6.3
B4	163.32	50.7	163.75	3.8
B7	159.24	62.4	158.90	4.7
B10[a]	126.24	199	126.48	4.3
B11[a]	125.97	199	126.63	4.3
B12	140.35	178	140.50	5.9
oxime CH_3	129.43	603	129.55	33.3

[a] B10 and B11 *S*/*N* values are reported for the most intense peak in the overlapping signals.

Proton and Carbon Signal Assignments. The typical numbering system for cobalamin Me_3Bzm protons (BH#) and carbons (B#) is given in Chart 1 and has been applied to the cobaloximes here and elsewhere.[34] Matrices for HMQC spectra, used for assignment of the overlapping pairs of signals for BH2 and BH4 in the JHMQC spectra,[34] were 256 × 2K data points with 32−64 acquisitions per t_1. Four dummy scans were applied at the beginning of the experiment, and a relaxation delay of 800 ms−1.5 s was used prior to each scan. The data were processed with Felix 2.30 (Hare Research) on a Silicon Graphics Iris Indigo computer. A square sine bell filter was applied in both dimensions with a 0−45° phase shift. The second dimension was zero-filled to 2K data points prior to Fourier transformation. In some cases, both dimensions were zero-filled to 4K data points for improved peak definition.

Data for determining $^1J_{CH}$ with the JHMQC sequence (vide infra) were collected with a spectral window of ~5000−6000 Hz and 32K data points. Typically, 500−1000 scans were collected. τ was set to 3.29 ms, and the delay Δ was optimized for the T_1 relaxation rates of the ^{1}H[^{13}C] protons. The Δ values were determined for each complex by visually inspecting trial spectra for ^{1}H[^{12}C] cancellation. For spectrometer stabilization, a 0.5 ms delay was inserted before and after each ^{1}H 180° pulse. Data were zero-filled to 64K (although the additional 32K points were not included in the digital resolution calculations) and apodized with an exponential multiplication (line broadening = 0.5−1.0 Hz). After Fourier transformation, the spectrum was viewed in either the phase-sensitive or the more convenient magnitude mode, with identical results. ^{1}H−^{13}C pairs were identified. Through a peak-picking routine, the chemical shifts in hertz of the paired peaks were recorded and the coupling constant was subsequently calculated. The digital resolution associated with each peak in an ^{1}H-[^{13}C] satellite pair was added to determine the error in measuring the coupling constant (i.e., (±0.1 Hz) + (±0.1 Hz) = ±0.2 Hz). The total measuring time for the high-resolution 1D JHMQC sequence for 800 scans on a 50 mM sample was ~3 h, although usable data were obtained in 32−64 scans (10−15 min).

Coupling constants acquired using the JHMQC method were compared to data acquired via a 1D coupled ^{13}C NMR spectrum using a 100 mM $Me_3BzmCo(DH)_2CH_3$ sample in $CDCl_3$ as a test sample. Each spectrum was acquired by setting the number of scans (na) such that the total experiment time was 8 h (na = 2260 and 26 000 for the JHMQC and 1D ^{13}C, respectively). The JHMQC was acquired using a 5263.16 Hz spectral width and 32K data points, yielding an error of ±0.16 Hz for $^1J_{CH}$ values. The coupled ^{13}C NMR spectrum was acquired with a 29 411.76 Hz spectral width and 32K data points, yielding a coupling constant error of ±0.90 Hz. Signal-to-noise (*S*/*N*) values were determined using a subroutine in the GN-600 Omega software. In each spectrum, a 1 ppm wide area of noise was automatically selected from a specified region containing no peaks (4.5−6.5 and 40−60 ppm for JHMQC and ^{13}C, respectively).

Results

Method. One-bond ^{1}H−^{13}C coupling constants ($^1J_{B\#H}$) of $Me_3BzmCo(DH)_2CH_3$ in $CDCl_3$ were determined from the coupled 1D ^{13}C NMR spectrum and also the JHMQC method (Table 1). The two sets of coupling constants are quite

Table 2. $^1J_{CH}$ (Hz)[a] for Free Me_3Bzm, Protonated Me_3Bzm, and $Me_3BzmCo(DH)_2R$ Complexes

	free	adam	CH_2CH_3	CH_3	CH_2NO_2	Cl	free H^+
$^1J_{B2H}$	203.7	207.2	208.0	208.2	209.1	209.9	218.6
$^1J_{B4H}$	158.8	163.6	163.3	163.3	163.7	164.8	166.5
$^1J_{B7H}$	157.5	158.5	159.0	159.2	159.8	160.4	163.2
$^1J_{B10H}$	126.2	125.9	126.3	126.2	126.7	126.6	overlap
$^1J_{B11H}$	126.1	125.8	126.1	126.0	126.5	126.5	overlap
$^1J_{B12H}$	139.4	139.8	140.3	140.4	140.9	141.2	143.4

[a] Values are reported to one decimal place although values to two places were used in our calculations.

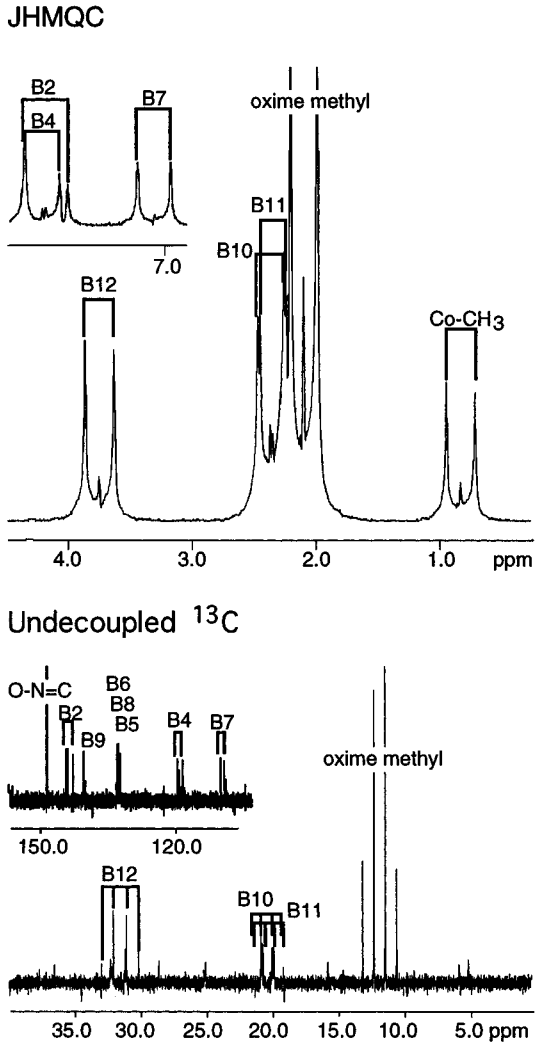

Figure 2. Top: JHMQC spectrum of $Me_3BzmCo(DH)_2CH_3$ (100 mM) at 25 °C after 2260 scans. Bottom: 1D coupled ^{13}C NMR spectrum of the same sample at 25 °C after 26 000 scans.

comparable, with a maximum difference of 0.66 Hz, a value within the error range of the ^{13}C-detected method (±0.90 Hz). The $^1H-^{13}C$ coupling constants for the remaining complexes (Table 2) were obtained at 25 °C via JHMQC in $CDCl_3$.

Figure 2 illustrates the greater *S*/*N* observed for the JHMQC spectrum when compared to the 1D coupled ^{13}C spectrum acquired over the same time period. The oxime methyl doublet in the JHMQC has an *S*/*N* = 603 for the more intense member of the doublet. In the ^{13}C spectrum, the oxime methyl signal is split into a quartet by the three hydrogens, with the most intense peak having an *S*/*N* = 33.3. The JHMQC reached an *S*/*N* = 33.3 for this peak after seven scans requiring ~90 s. Comparison of the B2 doublet in each spectrum suggests that only 53 scans (~12 min) with the JHMQC sequence are needed to achieve the *S*/*N* in the ^{13}C spectrum. *S*/*N* ratios for the other signals are presented in Table 1. The signals for the B10 and B11 $^1H[^{13}C]$ pairs are distinct and easily identified in the JHMQC spectrum. However, in the ^{13}C spectrum, quartets are expected for these positions. The upfield and downfield members of these quartets are indistinguishable from the baseline noise in the ^{13}C spectrum. Moreover, the B10 and B11 signals are difficult to distinguish from each other because of the decreased resolution of the B10 and B11 signals resulting from the larger spectral width of the ^{13}C experiment.

Table 3. Adjusted Coefficients of Determination (r_a^2)[a] for $^1J_{CH}$ vs Inductive Parameter

	r_a^2			r_a^2	
	EP	Co−N_L[b]		EP	Co−N_L[b]
$^1J_{B2H}$	0.9629	0.9937	$^1J_{B10H}$	0.6634	0.7056
$^1J_{B4H}$	0.5753	0.4324	$^1J_{B11H}$	0.8848	0.8167
$^1J_{B7H}$	0.9558	0.9975	$^1J_{B12H}$	0.9587	0.9809

[a] Linear regressions were carried out using Microsoft Excel 5.0. Coefficients of determination (r^2, the fraction of the total variance of the data set that is represented by the least-squares fit) were adjusted to reflect the small number of data points (*n*) according to the equation[49] $r_a^2 = 1 - (1 - r^2)(n - 1)/(n - 2)$. As *n* increases, r_a^2 approaches r^2. A correlation was considered to be significant when $r_a \geq 0.959$, i.e., a significance level (α) of 0.01 for $n = 5$. [b] Co−N_L data were obtained from ref 34.

Coupling Constants. The $^1J_{CH}$ values determined in this study are presented in Table 2. We explored whether the $^1J_{B\#H}$ values correlate with the electronic parameter (EP).[34,43,44] EP reflects the electron-donating ability of the axial R (or X) ligand as measured by the change in ^{13}C shift of the γC in pyCo(DH)$_2$(R or X) models relative to the R = CH_3 complex. The strongest correlations found (for $^1J_{B2H}$, $^1J_{B7H}$, and $^1J_{B12H}$; Table 3) have an excellent level of statistical significance with more than 95% of the variance represented by the least-squares line. The measured coupling constants $^1J_{B2H}$, $^1J_{B7H}$, $^1J_{B10H}$, $^1J_{B11H}$, and $^1J_{B12H}$ all lie on the regression line within the limits of the digital resolution (±0.2 Hz) except $^1J_{B2H}$ for R = adam.

Correlations of $^1J_{B\#H}$ with the Co−N_L distance are instructive. This parameter is a measure of the trans influence and reflects the electron-donating ability of R or X. All five data points in the line-fitting procedure with Co−N_L for $^1J_{B2H}$, $^1J_{B7H}$, and $^1J_{B12H}$ fall on their respective lines within the limits of the digital resolution of the JHMQC method, with >98% of the variance modeled by the regression line. The regression line in a similar fit of EP against Co−N_L is only moderately significant, describing ~94% of the variance.

Co−^{13}C NMR Shift Assignment. Generally the Co−^{13}C NMR signal is difficult to detect in a 1D experiment. Scalar coupling between the quickly relaxing quadrupolar ^{59}Co nucleus (100% abundant) and the ^{13}C nucleus decreases the transverse relaxation time, T_2, of the ^{13}C.[45] Consequently, the ^{13}C NMR signal for Co−^{13}C is broadened, as also reported for complexes with other quadrupolar metals.[46,47] In the JHMQC spectra of complexes containing a Co−$CH_{(n=1-3)}Y_{3-n}$ moiety, the $^1J_{CH}$ was readily observed. This finding supports the argument that the broadening of the ^{13}C NMR signal is a result of transverse

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relaxation since the other possibility, a short longitudinal relaxation time (T_1), would preclude observation of spin coupling. These observations suggested that broadened ^{13}C signals could be assigned via the HMQC method. Indeed, the ^{13}C signals of the Co-bound C were found in the HMQC spectra of complexes with several R groups as follows: CH_2NO_2, 63.7 ppm; CH_3, 13.1 ppm; CH_2CH_3, 27.1 ppm.

Discussion

Technique. In the JHMQC method, the Sklenár/Bax sequence was repeated five times prior to acquisition: four repetitions in the preparation period, followed by a final repetition for data collection (Figure 1). Each repetition in the preparation period is followed by a delay time (Δ) that when properly set allows for the full relaxation of the $^1H[^{13}C]$ signals but not the more slowly relaxing $^1H[^{12}C]$ signals. The latter signals are essentially randomized after a few cycles. Four repetitions proved ample for good cancellation. In this JHMQC sequence, T_{ac} is no longer limited by the T_1's of the ^{13}C-bound protons; therefore, T_{ac} is lengthened by increasing the number of points collected. With this modification, high resolution is achieved without sacrificing the good cancellation of the ^1H-$[^{12}C]$ signals.

Because of the relative ease of using the Sklenár/Bax sequence, the JHMQC pulse sequence can be implemented very quickly; the JHMQC sequence avoids using techniques such as homospoil pulses, spin lock fields, or gradients. Incorporation of the other types of $^1H[^{12}C]$ suppression methods cited above was not evaluated in our collection of high-resolution data.

The JHMQC technique was assessed by a comparison to coupling constants measured from a coupled 1D ^{13}C NMR spectrum of $Me_3BzmCo(DH)_2CH_3$. The coupling constants measured via the two techniques (Table 1) agreed within the range of experimental error for the 1D ^{13}C spectrum. The more striking comparison between the two methods was the much improved *S*/*N* obtained via the JHMQC method. The JHMQC sequence was shown to achieve the same *S*/*N* as the ^{13}C NMR spectrum for the B2 doublet in ~2.5% of the time. Furthermore, the lower multiplicity of JHMQC signals is an added advantage. For example, the intensity of the methyl signals in the ^{13}C NMR spectrum was distributed among the four peaks of the quartet, whereas in the JHMQC spectrum the intensity was distributed between only two peaks. Comparison of the B10 and B11 signals between the methods demonstrated the advantage of the lower multiplicity obtained with the JHMQC approach (Figure 2).

Applications. All $^1J_{CH}$ values for the five cobalt complexes were between those of Me_3Bzm and Me_3BzmH^+ (the smallest and largest $^1J_{CH}$, respectively) with the exception of $^1J_{B10H}$ and $^1J_{B11H}$ for the adam complex (Table 2). From these data, it is apparent that the coupling constant was influenced more strongly by protonation than by coordination. The proton has a strong electron-withdrawing effect, and properties of heterocycles are influenced more strongly by binding of the proton than by binding of a metal center.[31]

The value of $^1J_{CH}$ is influenced in a clear way by the binding of either H^+ or $Co^{III}(DH)_2$(R or X) to Me_3Bzm. In contrast, ^{13}C chemical shifts are influenced in a much more complicated manner by the binding of these species to Me_3Bzm (Supporting Information). For example, some signals shift upfield (B4, B8, B9), whereas others shift downfield (B5, B6, B7, B12).[34] Unlike $^1J_{CH}$ values of the ring and *N*-methyl signals, the ^{13}C chemical shifts for the complexes do not fall so nicely between the respective signals of Me_3Bzm and its protonated form. Thus, $^1J_{CH}$ values are a more reliable method for examining the effects of binding interactions.

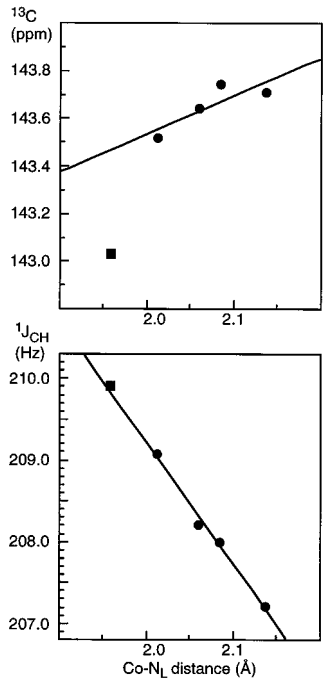

Figure 3. B2 shift (top) and $^1J_{CH}$ (bottom) versus Co−N_L distance.[34] The solid line represents the least-squares fit of the data for the alkylcobaloximes (circles). The value for the chlorocobaloxime is represented by the square.

It is worth examining the dependence of $^1J_{CH}$ values of coordinated Me_3Bzm on the nature of the trans ligand, since the relative magnitudes of the trans influence of R or X are well established. As the trans influence of R or X increased, the magnitude of $^1J_{CH}$ for all the carbon signals decreased (Table 2). The sensitivity of $^1J_{CH}$ to R or X followed the order $^1J_{B10H} < {}^1J_{B11H} < {}^1J_{B12H} < {}^1J_{B4H} < {}^1J_{B7H} < {}^1J_{B2H}$. However, the chemical shift patterns are not so clear (Supporting Information). As the trans influence of R or X increases (i.e., the Me_3Bzm ligand becomes more weakly bound to resemble free Me_3Bzm), one would expect the shifts to approach the free Me_3Bzm values. For almost all signals exhibiting significant changes on coordination (B4−9, B12), this behavior is found. B2 exhibits rather unusual shift behavior. For the X = Cl complex, B2 has a shift closest in value to that of free Me_3Bzm, exactly the opposite of the result expected. In contrast, the dependence of the direction of B2 shifts on trans influence is normal and similar to that for B4, B8, and B9. These shift trends could reflect many effects and cannot be attributed primarily to through-bond phenomena.

Comparison of the relationship of $^1J_{B2H}$ and of EP to Co−N_L further demonstrates the advantage of using $^1J_{CH}$ over shift-based measures of inductive electronic effects. Plots readily reveal that $^1J_{B2H}$ is better than EP in accounting for the weak trans influence of Cl (Figures 3 and 4). Regression statistics for the line describing EP with Co−N_L suggest a moderately significant correlation. The EP parameter was based on shifts of a specially selected carbon well removed from the cobalt. However, we find a stronger relationship of $^1J_{CH}$ with Co−N_L (Table 3), even for B2, a carbon whose chemical shift changes have been troublesome to explain. Thus the coupling constant is a better indicator of the electronic changes in the coordinated ligand. Indeed, a recent study has indicated that several factors may contribute to the shifts upon which EP was based.[48]

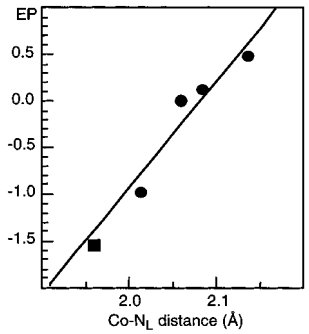

Figure 4. Least-squares fit of alkylcobaloxime EP values (circles) as a function of Co−N_L distance.[34] The value for the chlorocobaloxime is represented by the square.

In summary, $^1J_{CH}$ values can now be conveniently obtained by the JHMQC method, which provides signals with lower multiplicity, much improved digital resolution, and superior *S*/*N* compared to the 1D coupled ^{13}C experiment. Since high-resolution coupling constants for a variety of types of compounds, including non-metal compounds, are now conveniently assessable, we suggest that they be used in many types of investigations previously using the shifts of ^{13}C or other nuclei. Indeed here we have shown that the JHMQC method can provide information on signals for which shift data are not readily obtained. Furthermore, we have found that, even when shifts are used very carefully and successfully to obtain information on the properties of the coordinated ligands resulting from changes in the trans influence, a superior result is obtained with coupling constants.

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Supporting Information Available: A table of Me_3Bzm ^{13}C NMR shifts and the HMQC spectrum of $Me_3BzmCo(DH)_2CH_2CH_3$ showing the assignment of the Co−C ^{13}C signal (2 pages). Ordering information is given on any current masthead page.

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