Heteronuclear NMR Studies of Cobalt Corrinoids. 16. Indirect Detection of the ^{15}N NMR Resonances of the Axial 5,6-Dimethylbenzimidazole Nucleotide and of Corrin Ring N22 and N23 via the Heteronuclear Multiple-Quantum Coherence (HMQC) Experiment[1]

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Observation of the ^{15}N NMR resonances of cobalt corrinoids has provided new insights into the chemistry of these elegant and biologically important molecules.[2-8] Studies of the corrin side chain amide ^{15}N resonances, by polarization transfer[2,4] or by inverse-detected HMQC methodologies,[4,7,8] have led to observations of intramolecular hydrogen bonding interactions which would be difficult to observe by other methods. The successful application of this methodology has permitted the observation of the side chain amide ^{15}N resonances of cobalt corrinoids at natural abundance.

The ^{15}N NMR resonances of the axial nucleotide and corrin ring of Cbl's[9] have been observed only by direct detection of enriched samples.[4,5] To our knowledge, the inverse-detected HMBC experiment[10] has never been successfully employed for ^{15}N observation. We now report the successful use of the inverse-detected HMQC experiment to observe the B1 and B3 (Figure 1) ^{15}N NMR resonances of the axial nucleotide of Cbl's at natural abundance. In addition, this method permits the simultaneous observations of two corrin ring nitrogens which can be assigned to N22 and N23.

In the HMQC experiments, generation and inverse detection of the double quantum transition rely on a coupling-dependent transfer function.[11,12] Setting the HX coupling delay ($1/2J$) to 5.6 ms (i.e., J = 90 Hz) optimizes the transfer function for the one-bond coupling typical of amides and provides excellent 2D spectra of the corrin peripheral amide nitrogens and their attached protons.[4,7,8] In our previous direct-detected observations of the axial Bzm resonances in [B1, B3-$^{15}N_2$]CNCbl,[4] we observed two-bond coupling constants between the B2 hydrogen and the B1 and B3 nitrogens of about 5–7 Hz (J = 10.3 Hz for free dimethylbenzimidazole).[13] The simple expedient of increasing the coupling delay in the HMQC experiment to 68 ms (i.e., $J \approx$ 7 Hz) has now been found to permit the observation of the B1 and B3 ^{15}N resonances at natural abundance as shown for CNCbl in Figure 1. The chemical shifts observed are given in Table I.[14]

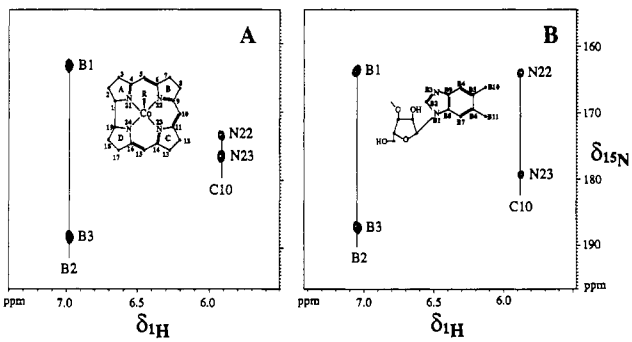

Figure 1. ^1H, ^{15}N HMQC spectra of (A) CNCbl and (B) 8-OH-CNCbl-*c*-COO^-, optimized for a H-N coupling constant of ca. 7 Hz. Insets: structure and numbering scheme of (A) the corrin ring and (B) the axial nucleotide.

The ^1H, ^{15}N HMQC spectrum of CNCbl in DMSO-d_6 generated in this manner consists solely of four cross-peaks occurring in the 160–190 ppm region of the ^{15}N dimension; i.e., it is entirely devoid of the resonances due to the amide nitrogens.[8] Two of the cross-peaks occur at a ^1H chemical shift of 6.97 ppm and at nitrogen chemical shifts very close to those of [B1, B3-$^{15}N_2$]CNCbl in H_2O.[4] As this ^1H chemical shift is within the range (6.92–7.10 ppm) of those of the B2 hydrogen of 14 base-on Cbl's,[1,16-21] these responses undoubtedly arise due to B2H–B1 and B2H–B3 coupling. The other two cross-peaks in this spectrum occur at a ^1H chemical shift of 5.93 ppm and at ^{15}N chemical shifts very close to those of two of the four corrin ring ^{15}N resonances previously observed by Kurumaya et al.[5,22] As the ^1H chemical shift of these responses falls within the range (5.90–6.10 ppm) of those previously observed for the C10 hydrogen in 14 base-on Cbl's,[1,16-21] these responses must result from significant vinylic coupling between C10H and N22 and N23.[23]

The B1 and B3 resonances of cyanocobalt corrinoids were previously assigned by analogy to literature assignments of the ^{15}N resonances of other nitrogen heterocycles.[4] The availability of a method for observing the Bzm ^{15}N resonances of cobalamins

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(9) Abbreviations: Cbl = cobalamin, CNCbl = cyanocobalamin (vitamin B_{12}), CN-13-epiCbl = cyano-13-epicobalamin, CN-8-epiCbl = cyano-8-epicobalamin, 8-OH-CNCbl-*c*-COO^- = Coα-(α-5,6-dimethylbenzimidazolyl)-Coβ-cyano-8-hydroxycobamic acid *a,b,d,e,g*-pentaamide (8-hydroxycyanocobalamin-*c*-monocarboxylate), α-ribazole = 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole, α-ribazole-3′-P = 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole-3′-phosphate, and Bzm = 5,6-dimethylbenzimidazole.
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(23) A recent study of the ^1H, ^{15}N, and 2D ^1H, ^{15}N chemical shift correlated NMR spectra of the ^{15}N-enriched CNCbl analog, cyano-5-hydroxybenzimidazoylcobamide,[24] found the C10–H–N22 and C10–H–N23 coupling constants to be 4.5 Hz. These authors also report the observation of small, but unresolved couplings between N21 and $C20H_3$, N21 and C3–H, and N24 and C19–H.
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Table I. ^{15}N NMR Chemical Shifts for the B1, B3, N22, and N23 Nitrogens of Base-on Cobalamins, Cyanocobalamin Analogs, and Free α-Ribazole[a]

	$\delta_{^{15}N}$, ppm[c]				$\delta_{^1H}$, ppm[d]	
compound[b]	B1	B3	N22	N23	B2H	C10H
CNCbl	163.2[e]	188.3[f]	173.4	176.4	6.97	5.93
CH_3Cbl	159.5	225.0	196.0	198.8	7.04	5.90
H_2OCbl	166.0	143.6	173.9	179.5	6.72	6.00
8-OH-CNCbl-*c*-COO^-	163.7	187.0	164.0	179.3	7.04	5.87
CN-13-epiCbl	163.5	187.2	170.7	170.7	7.14	5.77
CN-8-epiCbl	163.8	188.3	170.3	178.0	7.02	5.83
α-ribazole	160.2	243.2			8.29	
α-ribazoleH$^+$	172.1	162.7			9.56	

[a] Spectra measured at 27 °C in DMSO-d_6, by inverse-detected ^1H, ^{15}N HMQC, as described in ref 14. [b] Abbreviations are given in ref 9. [c] ^{15}N chemical shifts reported relative to NH_3(l). [d] ^1H chemical shifts relative to TSP. [e] Previously observed at 164.2 ppm by direct detection of [B1, B3-$^{15}N_2$]CNCbl in H_2O. [f] Previously observed at 187.7 ppm by direct detection of [B1, B3-$^{15}N_2$]CNCbl in H_2O.[4]

at natural abundance now permits a firm assignment based on the effects of variations of the trans axial ligand on these chemical shifts. Observation of the Bzm ^{15}N chemical shifts of CH_3Cbl and H_2OCbl (Table I) shows that in this series of three base-on RCbl's, in which the affinity of the pendent nucleotide for the cobalt center varies by 5 orders of magnitude,[25] the chemical shift of one of the two Bzm nitrogens varies by only 6.5 ppm while the chemical shift of the other nitrogen varies by 8.14 ppm. Clearly, the latter nitrogen resonances must be assigned to the B3 nitrogen.

In order to assign the two corrin ring ^{15}N resonances showing cross-peaks to the C10 hydrogen, several analogs of CNCbl were studied, including the C13[26,27] and C8[28] epimers and 8-OH-CNCbl-*c*-COO^-, in which the corrin ring is substituted with a hydroxyl group at C8 and the *c* amide has been hydrolyzed[32] (Table I). For CN-13-epiCbl, the X-ray crystal structure[34] reveals a distinct change in C-ring conformation relative to CNCbl, suggesting that the N23 chemical shift should be influenced by epimerization at C13. Indeed, the chemical shift of the more upfield of the corrin ring ^{15}N resonances of CNCbl is affected much less by C13 epimerization (shifted upfield by 2.7 ppm) than that of the more downfield resonance, which is shifted upfield by 5.7 ppm. Just the opposite occurs in CN-8-epiCbl, the more downfield resonance of CNCbl being displaced less (1.6 ppm downfield) than the more upfield one (3.1 ppm upfield) upon epimerization at C8. We therefore assign the more upfield of the two corrin ring ^{15}N resonances of CNCbl (173.4 ppm) to N22 and the more downfield one (176.4 ppm) to N23. These assignments are confirmed by observation of the N22 and N23 resonances of 8-OH-CNCbl-*c*-COO^- (Figure 1), in which the former is shifted upfield by 9.4 ppm while the latter is shifted downfield by 2.9 ppm. These assignments are in accord with those recently made by Hollenstein and Stupperich[24] from 2D ^1H, ^{15}N chemical shift correlation observations of the CNCbl analog cyano-5-hydroxybenzimidazoylcobamide, which was uniformly enriched in ^{15}N. The N22 and N23 resonances of H_2-OCbl and CH_3Cbl have then been assigned by analogy to CNCbl; i.e., N22 is assumed to be the more upfield of the two resonances.

The chemical shifts of the Bzm nitrogens of CNCbl observed here in DMSO-d_6 are remarkably similar to those previously observed in H_2O by direct detection of an enriched sample[4] (Table I). This is not the case for the free nucleoside in solution due to the large effect of solvent hydrogen bond donor acidity on the ^{15}N chemical shifts of nitrogen heterocycles. The downfield displacement of the B3 resonance of the detached nucleoside, α-ribazole, in DMSO-d_6 relative to H_2O (16.1 ppm)[4] is similar to that observed by Duthaler and Roberts[35] for the ^{15}N resonance of pyridine on transfer from H_2O to DMSO (18.1 ppm). The B1 nitrogen of α-ribazole undergoes a much smaller solvent dependent shift (1.7 ppm),[4] as would be anticipated. For the B3 protonated free nucleoside, α-ribazoleH$^+$, the solvent effect on the B3 chemical shift is much smaller (3.7 ppm downfield in DMSO), while the B1 resonance is hardly affected at all. The smaller solvent effect on the B3 chemical shift in α-ribazoleH$^+$ is in accord with the larger difference in solvent donor acidity, α, for H_2O (1.13) and DMSO (0.0), relative to the difference in solvent acceptor basicity, β (0.18 for H_2O and 0.76 for DMSO).[36]

While the ^{15}N resonances of the axial nucleotide of base-on CNCbl are clearly shielded from significant solvent effects, the B3 chemical shift must be strongly affected by the magnetic anisotropy of the cobalt atom. The chemical shift range exhibited by B3 in the base-on RCbl's studied here is very large (81.4 ppm), close to that for the chemical shift difference at this position between the protonated and free base species of the detached nucleoside in DMSO (80.5 ppm) and even exceeding this difference in H_2O (68.7 ppm). In addition, the B3 chemical shift for H_2OCbl falls outside the range of chemical shifts observed for the protonated and free base species of the detached nucleoside in either solvent.

The facile, indirect-detected observation of the B1 and B3 ^{15}N resonances of cobalt corrinoids demonstrated here may permit the observation of these resonances in cobalamins bound to proteins, although ^{15}N enrichment would certainly be desirable. Given the high sensitivity of the B3 chemical shift to electronic effects, axial Co–N bond distance alteration of Cbl's upon complexation with proteins should be readily detectable. In addition, this method, coupled with the sensitivity of the B3, N22, and N23 chemical shifts to changes in the upper axial ligand, will enable studies of cis and trans substituent effects in base-on Cbl's. Studies of both types are currently in progress.

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Supplementary Material Available: Text describing the HMQC experimental parameters and the preparation of 8-OH-CNCbl-*c*-COO^- and Table S1, listing the ^{13}C chemical shifts and assignments for 8-OH-CNCbl-*c*-COO^- (2 pages). Ordering information is given on any current masthead page.

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