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Heteronuclear NMR Studies of Cobalamins. 11. ^{15}N NMR Studies of the Axial Nucleotide and Amide Side Chains of Cyanocobalamin and Dicyanocobamides[1]

In previous studies[2,3] spectroscopic and thermodynamic evidence has been found for the occurrence of the so-called "tuck-in" species of base-off, but benzimidazole deprotonated cobalamins in which the free-base benzimidazole ligand is associated with a corrin ring side chain, probably via a hydrogen-bonded interaction with an amide N–H. On the basis of tentative ^{13}C NMR assignments, a comparison of the ^{13}C NMR spectra of dicyanocobalamin ($(CN)_2Cbl$), which contains a pendent, deprotonated benzimidazole nucleotide, and dicyanocobinamide ($(CN)_2Cbi$), from which the nucleotide has been removed, allowed a tentative assignment of the hydrogen-bond donor in the tuck-in species as the e side chain amide. We now present further observations and characterization of the tuck-in species of $(CN)_2Cbl$ by ^{15}N NMR spectroscopy. These results represent the first observation of the ^{15}N NMR spectrum of the benzimidazole nucleotide of cobalamins. We also now report the first NMR observation of the amide protons of cobalamins and their connectivity to the amide nitrogens.

Figure 1 shows the ^{15}N NMR spectrum of (CN)Cbl (Figure 1A) and $(CN)_2Cbl$ (Figure 1B) 50% enriched in ^{15}N in the benzimidazole nucleotide. Chemical shifts, line widths, and ^{15}N–^{1}H coupling constants are collected in Table I, along with data for the protonated, base-off species of (CN)Cbl, the protonated, detached nucleoside (α-ribazole cation[7]), and the free-base, detached nucleotide (α-ribazole 3′-phosphate dianion[8]). Assignments of the ^{15}N resonances of the free base and protonated, detached nucleoside (or nucleotide) are based on analogies to the assignments for *N*-methylimidazole[10] and *N*-methylbenzimidazole[11] and the effects of protonation on the ^{15}N resonances of *N*-methylimidazole[10] and pyridine.[12] Assignment of the ^{15}N resonances of the base-off species of (CN)Cbl follows directly, and the assignment of the downfield resonance of base-on (CN)Cbl to the B3 nitrogen (i.e., the coordinated nitrogen) is confirmed by the influence of the metal atom's quadrupolar relaxation on the line width of this resonance.

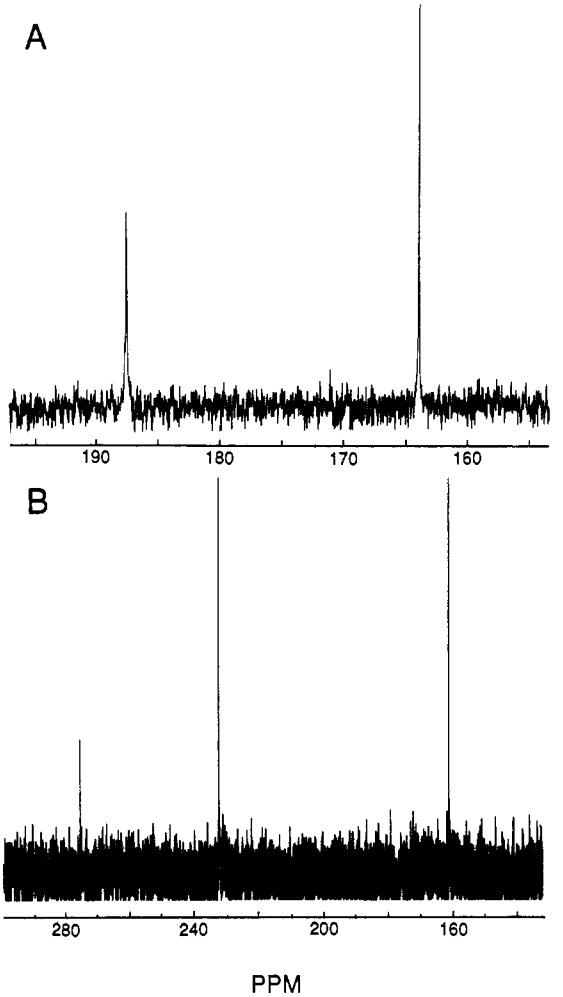

Figure 1. (A) ^{15}N NMR spectrum of [B1,B3-$^{15}N_2$](CN)Cbl, 10 mM in water, locked to D_2O in a concentric insert (Wilmad). A total of 3244 transients were collected into a 32K data set over a sweep width of 10000 Hz by using an inverse-gated decoupling sequence on a Bruker MSL 300 NMR spectrometer (30.415 MHz). The recycle time was 45 s. Chemical shifts are downfield relative to external $NH_3(l)$. (B) ^{15}N NMR spectrum of [B1,B3-$^{15}N_2$]$(CN)_2Cbl$, 10 mM in 0.3 M aqueous KCN, locked to D_2O in a concentric insert (Wilmad). A total of 3408 transients were collected into a 32K data set as in (A). The recycle time was 20 s. The small peak at 275.4 ppm is the nitrogen of free cyanide (at natural abundance).

Coordination of the B3 nitrogen of the nucleotide to the cobalt atom causes a 40.5 ppm upfield shift of the ^{15}N resonance (relative to that of the detached, free-base nucleotide), or 59% of the effect of B3 protonation (a 68.2 ppm upfield shift), while a similar comparison of B1 shows 43% of the effect of B3 protonation.[13]

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Table I. ^{15}N NMR Data for the Nucleotides of Cyanocobamides and Free α-Ribazole Species[a]

species	B1[b] δ,[c] ppm	B1[b] J,[d] Hz	B1[b] $\Delta\nu_{1/2}$,[e] Hz	B3[b] δ,[c] ppm	B3[b] J,[d] Hz	B3[b] $\Delta\nu_{1/2}$,[e] Hz
CNCbl,[f] base-on	164.22	6.1	1.7	187.71	6.9[g]	3.3
CNCbl,[h] base-off	172.31	5.5		159.43	5.2	
$(CN)_2$Cbl[i]	161.25	6.7	2.1	232.31	10.4	2.4
α-ribazole,[j] cation	171.70			158.98		
α-ribazole 3′-P,[k] dianion	158.51			227.16		

[a] Spectra measured at 25 °C in water, locked to D_2O (concentric insert), by inverse gated or undecoupled sequence at 30.415 MHz on a Bruker MSL 300 spectrometer or at 50.693 MHz on a Bruker AM 500 spectrometer. [b] From standard Cbl numbering.[4] B1 is benzimidazole nucleotide N_1 (the glycoside N) and B3 is benzimidazole nucleotide N_3 (the liganding nitrogen). [c] Chemical shifts were determined relative to external CH_3NO_2 and are reported relative to NH_3(l) ($\delta_{CH_3NO_2}$ = 380.23).[5] [d] Two-bond H–C–N coupling constant to benzimidazole B2 hydrogen. [e] Width at half-height, by Gaussian line fits with 0.6 Hz line broadening. [f] Sample was 10 mM in [B1,B3-$^{15}N_2$](CN)Cbl, 50 atom % ^{15}N, in water. [g] Lamm et al.[9] report a value of 10.3 Hz for the two-bond coupling constant in free dimethylbenzimidazole. [h] Sample was 10 mM [B1,B3-$^{15}N_2$](CN)Cbl, 50 atom % ^{15}N, in 0.52 M H_2SO_4 (ca. 50% base-off).[6] [i] Sample was 10 mM [B1,B3-$^{15}N_2$]-(CN)Cbl, 50 atom % ^{15}N, in 0.3 M KCN. [j] Sample was 0.255 M 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole[7] in water, pH 2.45 (adjusted with HCl). The pK_a of α-ribazole is 5.56 at 25 °C.[7] [k] Sample was 0.242 M 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole 3′-phosphate in 10% D_2O, pH 8.63 (adjusted with KOH). The second macroscopic pK_a of the zwitterion (pH_I = 2.94) is 6.27.

This effect compares favorably to the previously determined value of 44% of the effect of protonation from comparisons of the ^{13}C NMR spectra of the coordinated and free nucleotide.[8] Importantly, the chemical shifts of both nitrogens of the nucleotide of the protonated, base-off species are virtually identical with those of the detached, protonated nucleoside, confirming our previous conclusion (from ^{13}C NMR) of a lack of interaction between the pendent nucleotide and the remainder of the structure in the protonated, base-off species.[2] In contrast, the ^{15}N resonances of both B1 and B3 of the base-off but benzimidazole-deprotonated $(CN)_2$Cbl are shifted *downfield* relative to those of the detached, free-base nucleotide, the B3 resonance, by 5 ppm.

While it is clear that de novo formation of a hydrogen bond to a nitrogen heterocycle acceptor (like protonation of such an acceptor) should cause an upfield shift of its ^{15}N resonance,[17,18] the downfield shift of the B3 resonance of base-off dicyanocobalamin is in accord with a change of hydrogen-bond donor from water (i.e., in the free nucleotide) to an amide N–H. Thus, the ^{15}N resonance of pyridine[15] and that of N3 of *N*-methylimidazole[11] show regular downfield shifts upon transfer through a series of solvents of decreasing hydrogen bond donor strength (i.e., decreasing values of α, the solvent hydrogen bond donor acidity[19]). In fact, application of the Taft linear solvation energy relationship[20] (or the similar treatment of Kolling[21]) to the ^{15}N chemical shift of pyridine[12,22] predicts a 5–10 ppm downfield shift of the ^{15}N resonance on transfer from water (α = 1.13[20]) to formamide (α = 0.66[20]). Furthermore, transfer of *N*-methylimidazole from water to methylene chloride, in which it exists primarily as an intermolecularly hydrogen-bonded dimer, also causes a 5 ppm downfield shift of the N3 ^{15}N resonance.[17] Thus, the downfield shift of the B3 resonance of base-off $(CN)_2$Cbl relative to the free-base nucleotide is in accord with the existence of $(CN)_2$Cbl largely as the hydrogen-bonded tuck-in species.[2,3]

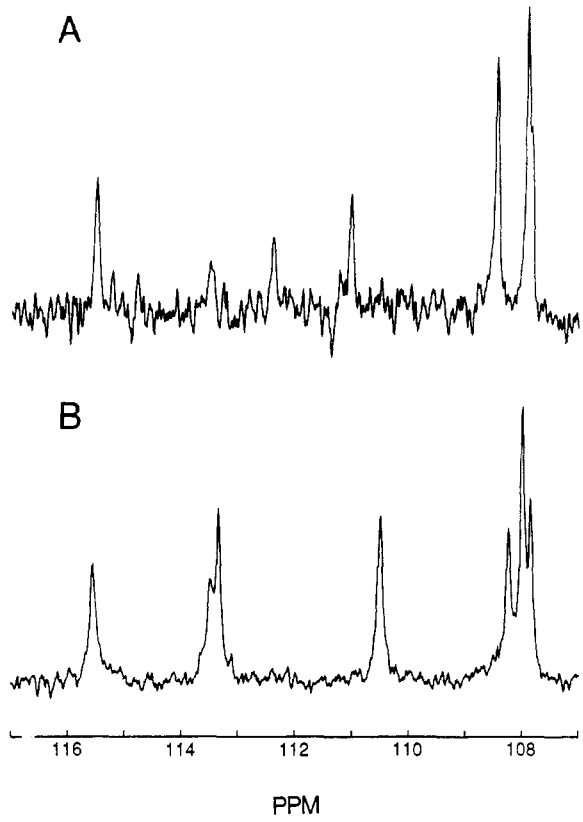

Figure 2. (A) ^{15}N NMR spectrum of the amide region of $(CN)_2$Cbl, 33 mM in 0.1 M NaCN in DMSO-d_6. A total of 44 034 transients were accumulated, by using the DEPT sequence with a $\pi/4$ proton read pulse, into a 32K data set over a 6000 Hz sweep width on a Bruker MSL 300 NMR spectrometer (30.415 MHz). The recycle time was 2.72 s. Chemical shifts are downfield relative to external NH_3(l). (B) ^{15}N NMR spectrum of the amide region of $(CN)_2$Cbi, 43 mM in 0.2 M NaCN in DMSO-d_6. A total of 31 124 transients were collected as described in (A).

We have also investigated the amide side chain ^{15}N chemical shifts of (CN)Cbl, $(CN)_2$Cbl, and $(CN)_2$Cbi (Figure 2) via the distortionless enhancement by polarization transfer sequence[23] in DMSO-d_6. In addition, $(CN)_2$Cbi, $(CN)_2$Cbl, and the b-, d-, and e-monocarboxylic acid derivatives[24] of the latter were observed by ^1H-detected, ^1H, ^{15}N heteronuclear multiple-quantum coherence spectroscopy,[25,26] which permits not only enhanced observation of the heteronuclei but selective observation of the attached protons and correlation of the ^1H and ^{15}N resonances. The data are collected in Table II, along with the proton chemical shift temperature gradients for the amide protons of $(CN)_2$Cbi and $(CN)_2$Cbl. Partial assignment of the ^{15}N resonances can be made[27] by observation of the missing ^{15}N and ^1H resonances in the HMQC maps of the b-, d-, and e-monocarboxylic acid derivatives of $(CN)_2$Cbl, since the three principle monocarboxylic acid derivatives obtained by limited acid-induced hydrolysis of CNCbl[28,29] have been thoroughly characterized by ^{13}C NMR

(13) These values ignore the effect of the magnetic anisotropy of the metal atom on the ^{15}N chemical shifts. If we correct for this effect by using McConnell's equation,[14] the known geometry of (CN)Cbl in the solid state,[15,16] and our previous value for the magnetic anisotropy of the cobalt atom in base-on (CN)Cbl ($\Delta\chi = -3.4 \times 10^{29}$ cm^3 molecule^{-1}),[8] "partial protonation" values of 55% using B3 ($\Delta\sigma$ = 3.0 ppm) and 45% using B1 ($\Delta\sigma$ = 0.3 ppm) are obtained.

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Table II. ^{15}N and ^{1}H NMR Data for the Side-Chain Amides of Cyanocobamides[a]

(CN)Cbl		$(CN)_2$Cbi[b]		$(CN)_2$Cbl[b]		$(CN)_2$Cbl-d-COO^-[b]		$(CN)_2$Cbl-b-COO^-[b]		$(CN)_2$Cbl-e-COO^-[b]	
δ_{15N},[c] ppm	amide[d]	δ_{15N},[e] ppm	δ_{1H},[e,f] ppm	δ_{15N},[e] ppm	δ_{1H},[e,f] ppm	δ_{15N},[e] ppm	δ_{1H},[e] ppm	δ_{15N},[e] ppm	δ_{1H},[e] ppm	δ_{15N},[e] ppm	δ_{1H},[e] ppm
106.70	d	107.85	6.69	107.83	6.70			107.84	6.71	107.73	6.71
			(−5.46)		(−5.12)						
			7.13		7.11				7.17		7.46
			(−5.13)		(−4.86)						
107.39	b	107.95	6.84	107.89	6.85	107.96	6.83			108.10	6.86
			(−5.36)		(−5.08)						
			7.47		7.46		7.46				7.26
			(−3.79)		(−3.75)						
109.77	c	108.35	6.72	108.57	6.92	108.63	6.86	108.53	6.91		
			(−5.47)		(−6.16)						
			7.07		7.14		7.42		7.15		
			(−4.50)		(−3.39)						
110.24		110.60	7.10	111.23	7.32	111.21	7.31	111.23	7.31	110.62	7.28
			(−5.44)		(−4.10)						
			7.89		7.87		7.81		7.90		7.79
			(−2.60)		(−4.14)						
112.80	f	113.47[g]	7.97	112.65[g]	8.31	112.57	8.31	112.75	8.32	113.20	8.34
			(−5.88)		(−4.14)						
113.85		113.63	6.99	113.57	7.00	113.62	6.98	113.50	6.98	113.45	7.00
			(−5.47)		(−5.32)						
			7.60		7.60		7.59		7.62		7.58
			(−4.81)		(−4.59)						
115.78		115.84	7.07	115.88	7.08	115.70	7.06	115.70	7.06	115.62	7.08
			(−4.50)		(−4.77)						
			7.91		7.85		7.78		7.77		7.76
			(−3.65)		(−4.99)						

[a] In DMSO-d_6. ^{15}N chemical shifts were referenced to external CH_3NO_2 but are reported relative to NH_3(l) ($\delta_{CH_3NO_2}$ = 380.23[5]). ^{1}H chemical shifts were determined relative to external TSP. [b] Samples ca. 50 mM in 0.1 M NaCN. [c] By DEPT at 30.415 MHz on a Bruker MSL 300 NMR spectrometer at 25 °C. [d] Reference 27. [e] By ^{1}H-detected ^{1}H, ^{15}N HMQC at 32 °C on a Bruker AM 500 NMR spectrometer (50.693 MHz). [f] Proton chemical shift gradient × 10^3, ppm/°C, in parentheses. Thermal gradients were determined relative to internal TSP from one-dimensional ^{1}H observations at 5 °C increments between 20 and 60 °C on a GE QE 300 NMR spectrometer at 300.669 MHz. [g] Resonance inverts in DEPT spectra with a $3\pi/4$ read pulse.

spectroscopy.[30,31] In addition, the f side chain ^{15}N resonance is readily identified by its inversion in DEPT spectra utilizing a $3\pi/4$ read-out pulse, as well as its correlation to only a single ^{1}H resonance in the HMQC maps.

Comparison of the amide ^{15}N resonances of (CN)Cbl and $(CN)_2$Cbi shows that removal of the benzimidazole nucleotide has a much larger effect on the chemical shifts of the downward-projecting b, d, e, and f side chain amides ($\Delta\delta = \delta_{(CN)_2Cbi} - \delta_{(CN)Cbl}$ = 0.56, 1.15, −1.42, and 0.67, respectively) than on the upward-projecting a, c, and g side chain amides ($\Delta\delta$ = 0.36, −0.22, and 0.06), as would be expected. This suggests that the b, d, e, and f amides are significantly affected by the magnetic anisotropy of the heterocyclic nucleotide in base-on (CN)Cbl and that the b, d, and f amide nitrogens are in the shielding region of the benzimidazole field, while the e amide nitrogen is in the deshielding region. Careful inspection of the X-ray crystal structures of base-on cobalamins[32,33] suggests that this may indeed by the case.

Comparison of the ^{15}N and ^{1}H amide resonances of $(CN)_2$Cbi and $(CN)_2$Cbl shows that three of the amides undergo significant chemical shift changes. The f (nucleotide loop) amide nitrogen undergoes an 0.8 ppm upfield shift in $(CN)_2$Cbl relative to $(CN)_2$Cbi, while its proton undergoes an 0.34 ppm downfield shift and a 30% reduction of its thermal gradient. The e amide nitrogen undergoes a very small (0.2 ppm) downfield shift, while its upfield (presumable anti[34,35]) proton also undergoes an 0.2 ppm downfield shift. Strangely, its downfield (presumably syn[34,35]) proton undergoes a 25% reduction in thermal gradient. Finally, the ^{15}N resonance of the acetamide side chain amide resonating near 111 ppm is shifted downfield 0.6 ppm and its upfield proton is shifted 0.22 ppm downfield and experiences a 25% decrease in its thermal gradient.[36] In the dicyano derivative of the d monocarboxylic acid ($(CN)_2$Cbl-d-COO^-) the downfield proton of the e amide undergoes a 0.3 ppm downfield shift (relative to $(CN)_2$Cbl), while a similar effect is seen for the downfield proton of the d amide in $(CN)_2$Cbl-e-COO^-. Considering the proximity of the d and e side chains, such effects are not unreasonable. Interestingly, in $(CN)_2$Cbl-e-COO^- the acetamide nitrogen resonating near 111 ppm is shifted upfield (as in $(CN)_2$Cbi) and the f amide ^{15}N resonance is shifted downfield, nearly to its position in $(CN)_2$Cbi. Strangely, the amide protons of these groups are unaffected.

The effects of changes in hydrogen bonding on amide ^{15}N chemical shifts are complicated, as evidenced by solvent effects on the ^{15}N resonances of peptides.[37-41] For instance, when actinomycin D is shifted through a series of solvents of decreasing hydrogen bond acceptor strength (i.e., decreasing values of β, the solvent hydrogen bond acceptor basicity[42]) but increasing donor strength (i.e., increasing α), all of its ^{15}N resonances shift downfield.[39] However, such downfield shifting may be due entirely

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to effects of hydrogen-bond donation to the amide carbonyl,[38-41] since the tertiary amides of actinomycin D also undergo such a shift.[39] In alumichrome, shifting the solvent from DMSO (an excellent acceptor but not a donor) to trifluoroethanol (an excellent donor) causes a downfield shift of the ^{15}N resonances of four amides whose N-H's are solvent protected but whose carbonyls are exposed, while a single amide whose amide proton is external but whose carbonyl is buried undergoes an upfield shift.[40] Thus, in the current case, where the amide hydrogen bond donor of the tuck-in species sees a decrease in acceptor strength as the acceptor is changed from DMSO (in $(CN)_2$Cbi) to benzimidazole N3[43] (in $(CN)_2$Cbl), and there is presumably no donation to its carbonyl oxygen in either state, an upfield shift of its ^{15}N resonance would be expected. Indeed, application of the Taft linear solvation energy relationship to the solvent effect on the ^{15}N resonance of formamide[47] predicts an upfield shift of ca. 2 ppm on transfer from DMSO to pyridine. However, this calculation ignores the influence of the magnetic anisotropy of the nitrogen heterocycle, which would be expected to deshield the ^{15}N resonance of the hydrogen-bond donor in the tuck-in species due to its edgewise approach.

Hydrogen bonding effects on amide proton chemical shifts are similarly complicated.[45-47] It is now known that formation of intramolecular hydrogen bonds in peptide amides can cause an upfield or downfield shift of the amide proton resonance (relative to the solvated species in water) depending on the H···O internuclear distance.[47] However, application of the Taft linear solvation energy relationship[20] to the amide ^1H chemical shifts of *N*-methylacetamide in 10 solvents[48] predicts a small (0.05-0.2 ppm) upfield shift of the amide proton upon transfer from DMSO to pyridine. Again, however, this treatment ignores the effect of the magnetic anisotropy of the benzimidazole moiety, which would surely deshield the amide proton significantly. The significant decrease in the amide proton chemical shift thermal gradient of the upfield protons of the e amide and the acetamide resonating near 111 ppm upon formation of the tuck-in species is consistent with the formation of an intramolecular hydrogen bond in DMSO solution to either of these amides.[45,49]

The observations summarized in Table II and discussed above are consistent with the possibility that either the e amide or the acetamide whose nitrogen resonates near 111 ppm is the donor in the tuck-in species,[50] while the other amide is involved in a hydrogen-bonded interaction with the f amide in either $(CN)_2$Cbl or $(CN)_2$Cbi. Alternatively, the chemical shift changes at the f amide could be due entirely to conformational effects upon removal of the nucleotide. In this case, either the e amide or the acetamide whose ^{15}N resonance is near 111 ppm is the hydrogen-bond donor and the chemical shift effects at the other amide are due to its proximity to the benzimidazole in the tuck-in species. The ^{15}N chemical shifts of $(CN)_2$Cbl-e-COO^- suggests the former interpretation, since hydrolysis of the c amide appears to cause a loss of the interactions causing the nitrogen chemical shift effects at both the f amide and the acetamide resonating near 111 ppm. This suggests that the e amide is the donor in the tuck-in species and that a hydrogen-bonded interaction between the f amide (as acceptor) and the acetamide resonating near 111 ppm (presumably the g acetamide) in $(CN)_2$Cbi is prevented from forming in the tuck-in species of $(CN)_2$Cbl. However, since the proton resonances in $(CN)_2$Cbl-e-COO^- do not confirm this interpretation, caution must be exercised in drawing any conclusions. Attempts to resolve these issues by a complete ^{13}C, ^1H, and ^{15}N assignment of $(CN)_2$Cbl are currently in progress.

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(43) While nitrogen heterocycles are better bases than DMSO ($pK_a \sim 0$[40]), they are weaker hydrogen bond acceptors (e.g. $\beta = 0.76$ for DMSO but 0.64 for pyridine[20]).
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(50) Inspection of models shows that hydrogen-bond formation from benzimidazole N3 to any of the three acetamides is feasible without development of significant strain in the nucleotide loop.

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First Examples of Six-Coordinate Homoleptic Complexes with Monodentate Arenethiolate Ligands. Synthesis and Structural Characterization of $[Ph_4P]_2[Nb(SPh)_6]$, $Na(THF)_3Nb(SPh\text{-}pMe)_6$, $[(15\text{-crown-}5)Na][Ta(SPh)_6]$, and $Nb_2(\mu_2\text{-}SPh)_4(SPh)_2Cl_2(C_2H_5CN)_2$

Recent synthetic and crystallographic studies of early-transition-metal complexes with aliphatic or aromatic thiolate ligands indicate that the chemistry of these M/S compounds (M = Zr,[1] Nb,[2] Ta[2a,3]) may be as extensive as that of the Mo/S and W/S systems. Coordination of aliphatic thiolate ligands to early-transition-metal ions often is followed by C-S bond cleavage that generates the S^{2-} ligand.[2b-d] The latter is incorporated in monomeric or oligomeric complexes in either terminal or bridging coordination modes. The C-S bond cleavage appears to be a heterolytic intramolecular process, facilitated by β-proton elimination.[4] Not unexpectedly, this reaction does not readily occur with benzenethiolate ligands although an example of C-S bond cleavage of benzenethiolate is known.[2c] Among the known thiolate complexes of "mixed"-ligand complexes that contain thiolate ligands, and S^2-ligands generated by C-S bond cleavage reactions,

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