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HETERONUCLEAR NMR STUDIES OF COBALT CORRINOIDS—17. CHARACTERIZATION OF NEOPENTYLCOBINAMIDE AND NEOPENTYL-13-EPICOBINAMIDE BY ^{1}H AND ^{13}C NMR SPECTROSCOPY: INFERRED CORRIN RING CONFORMATIONS FROM CHEMICAL SHIFT DIFFERENTIALS*†

KENNETH L. BROWN‡ and DANIEL R. EVANS

Department of Chemistry, Box CH, Mississippi State University, Mississippi State, MS 39762, U.S.A

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Abstract—While the ^{1}H and ^{13}C NMR spectra of neopentylcobalamin (NpCbl) and its epimer at corrin ring C(13), neopentyl-13-epiCbl (Np-13-epiCbl), are extremely broad, apparently due to chemical exchange between the base-on and base-off species, the cobinamide derivatives, neopentyl cobinamide ($NpCbi^{+}$) and neopentyl-13-epicobinamide ($Np\text{-}13\text{-}epiCbi^{+}$), have sharp, well-resolved NMR spectra. The ^{1}H and ^{13}C NMR spectra of $NpCbi^{+}$ and $Np\text{-}13\text{-}epiCbi^{+}$ have now been completely assigned by modern two-dimensional NMR methodologies. Comparison of the ^{13}C spectra of these two complexes shows that significant chemical shift differences occur at a variety of corrin ring and peripheral carbon atoms and are not localized near the site of epimerization. Similarly, comparison of the ^{13}C NMR spectra of $NpCbi^{+}$ and 5′-deoxyadenosylcobinamide ($AdoCbi^{+}$) shows differences at many corrin ring and peripheral carbons. A first attempt at discerning differences in corrin ring conformation from such differences in ^{13}C chemical shift has been made by comparing the X-ray crystal structures and ^{13}C NMR spectra of 5′-deoxyadenosylcobalamin (coenzyme B_{12}, AdoCbl) and cyanocobalamin (vitamin B_{12}, CNCbl). After elimination of carbon atoms whose chemical shifts are likely to be significantly affected by differences in the inductive effect of the Ado and CN ligands, and after consideration of differential anisotropic shielding effects in the two complexes due to the presence or absence of the Ado ligand, the difference in magnetic anisotropy of the central cobalt atom, the change in position of the axial nucleotide and differences in the magnetic anisotropy of the corrin ligand, 15 peripheral carbon atoms [C(2), C(18), C(20), C(25), C(26), C(30), C(36), C(37), C(41), C(46), C(47), C(48), C(54), C(55), C(60)] emerge as candidate "reporter" carbons whose ^{13}C chemical shifts may be useful in deducing conformational differences in cobalt corrinoids. Application of this method to adeninylpropylcobalamin (AdePrCbl), for which the X-ray crystal structure and absolute NMR assignments are known, correctly predicts the gross conformational differences between the corrin ring of AdePrCbl and that of CNCbl. Use of these reporter carbon chemical shifts suggests that in $NpCbi^{+}$, the fold angle, defined as the angle between the "northern" and "southern" planes of the corrin ring, is reduced relative to $AdoCbi^{+}$. Comparison of the chemical shifts of the reporter carbon atoms in $NpCbi^{+}$ and $Np\text{-}13\text{-}epiCbi^{+}$ suggests that the fold angle in the former is larger than that for the latter.

The enzymatic "activation" of adenosylcobalamin (AdoCbl,† coenzyme B_{12}, Fig. 1), in which the thermal homolysis of the carbon–cobalt bond is catalysed by a factor of about 10^{12},[2,3] remains one of

* For Part 16, see ref. 1.

† IUPAC-IUB nomenclature is used throughout (*Biochemistry* 1974, **13**, 1555). All compounds are assumed to possess organic ligands on the β (or upper) face unless stated otherwise. Abbreviations: AdoCbl, 5′-deoxyadenosylcobalamin (coenzyme B_{12}); $AdoCbi^{+}$, 5′-deoxyadenosylcobinamide; CNCbl, cyanocobalamin (vitamin B_{12}); NpCbl, neopentylcobalamin; $NpCbi^{+}$, neopentylcobinamide; Np-13-epiCbl, neopentyl-13-epicobalamin; $Np\text{-}13\text{-}epiCbi^{+}$, neopentyl-13-epicobinamide; AdePrCbl, adeninylpropylcobalamin; Bzm, 5,6-dimethylbenzimidazole.

‡ Author to whom correspondence should be addressed.

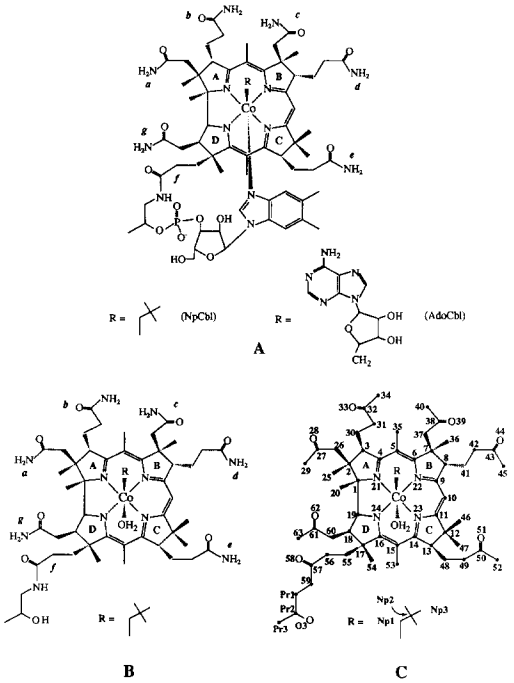


Fig. 1. (A) Structure of 5′-deoxyadenosylcobalamin (AdoCbl) and neopentylcobalamin (NpCbl). (B) Structure and (C) numbering scheme of neopentylcobinamide ($NpCbi^+$). In neopentyl-13-epicobinamide (Np-13-epiCbi$^+$) the configuration at C(13) is inverted and the *e* propionamide side chain projects "above" the corrin ring plane.

the outstanding problems of major importance in cobalamin chemistry and biochemistry. Despite many years of intensive effort studying the structure, reactivity and enzymology of alkylcobalt corrinoids, little is known about how AdoCbl-requiring enzymes achieve such accelerations or even about candidate mechanisms for catalysing Co—C bond homolysis.

AdoCbl itself undergoes thermal decomposition rather slowly ($t_{1/2}$ = 17 h at 85°C in aqueous solution at pH 7.0[5]) and, due to the presence of a β-oxygen substituent in its organic ligand,[4–7] suffers heterolytic cleavage in competition with Co—C bond homolysis.[2,3,8–10] As a result, the thermolysis of sterically strained RCbls such as secondary alkyl-, neopentyl- and benzylCbl, which lack β heteroatom substituents and consequently do not undergo competitive Co—C bond heterolysis, has been widely studied as a model of AdoCbl homolysis.[11–21] Many such RCbls are markedly more reactive towards thermal Co—C bond homolysis than AdoCbl. For instance, the base-on species of NpCbl, recently dubbed the "prototype" model of AdoCbl homolysis,[21] is nearly 2.0×10^5-fold (7.2 kcal) more thermally labile than base-on AdoCbl in aqueous solution at 25°C.[3,22] While we do not agree with Waddington and Finke[21] that NpCbl exhibits "10^6 of the 10^{12} enzymic activation of coenzyme B_{12}'s cobalt–carbon bond,"* we do very much agree that it is crucially important to attempt to understand the factors causing the 7–8 kcal labilization of the Co—C bond of NpCbl relative to that of AdoCbl, since such an understanding could, in principle, provide the necessary background for understanding the enzymatic acceleration of AdoCbl homolysis.

It is widely thought that conformational distortions of the corrin macrocycle are of fundamental importance in the thermal stability of the Co—C bond and the enzymatic activation of AdoCbl.[3,13–15,19,21,23–28] In the absence of an X-ray crystal structure of NpCbl[21] for direct comparison of its solid state structure to that of AdoCbl,[29–31] we have undertaken an NMR study in an attempt to infer conformational differences between these complexes, since the ^1H and ^{13}C NMR spectra of AdoCbl have been assigned unambiguously,[32] and the conformational sensitivity of ^{13}C NMR chemical shifts offers the potential of evaluating corrin ring conformational differences from chemical shift differences.[33–36] Unfortunately, both the ^1H and ^{13}C NMR spectra of NpCbl at neutral pH proved to be extremely broad, preventing the acquisition of sufficiently high resolution spectra for absolute assignments. Since the base-off analogue, $NpCbi^+$, has sharp NMR spectra, this broadening in NpCbl is apparently due to exchange between the base-on and base-off species, as NpCbl is 36% base-off in neutral aqueous solution at 25°C.[22]† In fact, even in the absence of substantial exchange broadening, this phenomenon would prevent the direct comparison of the NMR spectra of NpCbl and AdoCbl, since the latter is virtually completely base-on in neutral solution at 25°C.[2,3] We have consequently studied the NMR spectra of $NpCbi^+$ (Fig. 1), the base-off analogue of NpCbl in which the axial nucleotide has been removed by hydrolysis of the phosphodiester linkage. While the base-off species of RCbls (and the analogous $RCbi^+$s) are known to be some 10^2–10^3-fold less reactive toward thermal Co—C bond homolysis than the base-on species,[12,15,19,37]‡ the reactivity of $NpCbi^+$ still

* In ethylene glycol, the solvent in which Waddington and Finke compare the reactivity of base-on NpCbl and base-on AdoCbl, the relative reactivity at 25°C is 7.5×10^5.[21]

† In ethylene glycol at 25°C, Waddington and Finke[21] report $K_{off\text{-}on}$ = 0.36, i.e. NpCbl is 73.5% base-off.

‡ The base-on species of NpCbl is 835-fold more reactive than $NpCbi^+$ at 25°C in aqueous solution.[19,22]

exceeds that of $AdoCbi^+$ by 3.8×10^4.[19,22] In addition, the ^1H and ^{13}C NMR spectra of $AdoCbi^+$ have been assigned unambiguously,[38] permitting a direct NMR comparison between two species whose reactivity towards Co—C bond homolysis differs by 6.2 kcal mol^{-1} (at 25°C). Most importantly, a recent analysis of substitutent effects on ^{15}N NMR chemical shifts of a series of base-on cobalamins of the type YCH_2Cbl[39] has permitted the estimation of the inductive, resonance and steric substituent constants[40,41] of the Y substituent of AdoCbl. The values obtained for these substituent constants, $\sigma_I^Y = 0.06, \sigma_R^Y = -0.20$ and $E_s^Y = -1.53$, suggest that, electronically, the Y substituent of AdoCbl is quite similar to the Y substituent of NpCbl ($\sigma_I^Y = -0.01$,[42] $\sigma_R^Y = -0.18$[43]), so that the major difference in the substituent effect of the Ado and Np groups is likely to be steric ($E_s^Y = -2.78$ for Np[43]*). Thus, a comparison of the ^{13}C chemical shifts of $AdoCbi^+$ and $NpCbi^+$ should be minimally affected by electronic differences between the organic ligands.

Our earlier work on benzyl- and NpCbl thermolysis[19,20] has suggested the importance of entropic activation of these complexes for Co—C bond dissociation and has pointed to the corrin ring side chains as an important source of such entropy. In this view, restriction of the rotational freedom of motion of the *a*, *c* and *g* acetamide side chains (Fig. 1) by the bulky organic ligand is partly relieved in the transition state for Co—C bond cleavage, providing an entropic driving force for bond scission. In order to explore this hypothesis, we are interested in Cbl analogues with altered numbers, and/or structures of upwardly projecting side chains. As a first effort in this direction, we have synthesized and studied the thermolysis of the NpCbl analogue, Np-13-epiCbl, an epimer in which the configuration at corrin ring carbon C(13) is inverted so that the *e* propionamide side chain projects above the β-face. The kinetics and activation parameters for the thermolysis of this epimer are the subject of another report.[22] Since corrin conformational changes can presumably substantially affect the steric interactions of the upwardly projecting side chains with the bulky organic ligand, questions regarding conformational differences between NpCbl and Np-13-epiCbl are important. Once again, the NMR spectra of Np-13-epiCbl are badly broadened, apparently due to exchange between the base-on and base-off species (at 25°C in neutral aqueous solution, Np-13-epiCbl is 33% base-off[22]). We have consequently synthesized and studied the ^1H and ^{13}C NMR spectra of Np-13-epiCbi^+, which gives excellent high resolution NMR spectra, for direct comparison to those of $NpCbi^+$. By comparing the known X-ray crystal structures and carbon chemical shifts of AdoCbl, CNCbl and the AdoCbl analogue, AdePrCbl, in which the Ado ribose moiety is replaced by a propylene group, tentative conclusions can be drawn concerning the relative corrin ring conformations of $NpCbi^+$, Np-13-epiCbi^+ and $AdoCbi^+$.

EXPERIMENTAL

CNCbl was from Roussell. Factor B, a mixture of the diastereomeric (cyano)(aqua)-cobinamides, was obtained by a modification[44] of the procedure of Renz.[45] AdoCbl,[34] $AdoCbi^+$,[34] $NpCbi^+$ [22] and Np-13-epiCbi^+ [22] were prepared as described previously and AdePrCbl was prepared as described by Pagano *et al.*[46]

NMR samples, *ca* 20 mM in corrinoid, were prepared in D_2O and contained TSP as an internal chemical shift reference. In order to prevent the thermal decomposition of $NpCbi^+$ and Np-13-epiCbi^+, NMR samples were made anaerobic by three freeze–pump–thaw cycles and the NMR tubes were sealed under vacuum since, in the absence of a radical trap, alkylcobalt corrinoids are exceedingly stable with respect to Co—C bond homolysis.[21,22] Samples carefully prepared in this fashion were indefinitely stable.

One-dimensional ^{13}C NMR spectra of $NpCbi^+$, Np-13-epiCbi^+, $AdoCbi^+$, AdoCbl, AdePrCbl and CNCbl were obtained on a GE QE 300 NMR spectrometer operating at 75.61 MHz. Two-dimensional NMR spectra of $NpCbi^+$ were obtained on a JEOL Alpha 500 NMR spectrometer operating at 500.00 MHz (^1H) and 125.65 MHz (^{13}C). For the double quantum filtered homonuclear *J*-correlated spectrum (COSY),[47] data were collected into a 1024×512 data matrix and processed as a 512×512 matrix. Sixteen scans, preceded by 16 dummy scans, were collected over a sweep width of 4500.45 Hz at each t_1 increment of 222.2 ms. The hypercomplex homonuclear Hartmann–Hahn (HOHAHA)[48] data

* The relatively small value of E_s^Y for the bulky Ado group has been attributed to the fact that in AdoCbl, the Ado ligand is constrained to lie above the C–D ring junction (i.e. over the "southern" half of the corrin) by the "sentinel" methyl groups C(46) and C(54).[28] Thus, if the Ado ligand is indeed prevented from free rotation about the C—Co bond in solution, its steric interactions with the upward projecting acetamide side chains (and consequently its apparent steric size) may be substantially smaller than expected from its steric bulk.

were similarly collected using eight scans, preceded by 64 dummy scans, using a spin-lock period of 80 ms. The absorption mode NOE (NOESY)[49] data were collected similarly using eight scans preceded by eight dummy scans, and a mixing time of 250 ms. The spin-locked NOE (or rotating-frame Overhauser enhancement spectroscopy, ROESY)[50] data were collected analogously using eight scans, preceded by 32 dummy scans, and a spin-lock period of 200 ms. The data were plotted in two colours to allow the distinction between positive contours (direct NOEs) and negative contours (relayed NOEs and Hartmann–Hahn artefacts). Data for the heteronuclear single-quantum coherence (HSQC)[51] spectrum were collected into a 512×256 data matrix which was zero-filled to 512×512 for processing. Thirty-two scans, preceded by 32 dummy scans, were collected over sweep widths of 4500.45 Hz (^{1}H) and 14,001.68 Hz (^{13}C). The t_1 increment was 26 ms and the coupling delay was optimized for a C–H coupling of 145 Hz. The ^{1}H-detected heteronuclear multiple-quantum coherence (HMBC)[52] spectrum was generated using a 512×512 data matrix collecting 128 scans following 64 dummy scans, over sweep widths of 4500.45 Hz (^{1}H) and 29 994.0 Hz (^{13}C). The t_1 increment was 33 ms and the coupling delay was optimized for a C–H coupling of 8.3 Hz ($\Delta_2 = 60$ ms).

The two-dimensional NMR spectra of Np-13-epiCbi$^+$ were obtained on a Bruker AMX 300 NMR spectrometer operating at 300.135 MHz (^{1}H) and 75.475 MHz (^{13}C). The double quantum filtered COSY data were collected into a 1024×256 data matrix but processed as 512×512. Sixty-four scans were collected, after four dummy scans, at each 333.2 ms t_1 increment over a sweep width of 3012 Hz in both dimensions. The HOHAHA data were collected similarly using a 70 ms spin-lock period. The ROESY spectrum was collected similarly, except that 256 scans were obtained following four dummy scans. The ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC)[53] data were collected into a 2048×256 data matrix processed as a 512×512 matrix. One hundred and twenty eight scans, preceded by four dummy scans, were collected at each t_1 increment of 33.2 ms over sweep widths of 4504.5 Hz (^{1}H) and 30 190.0 Hz (^{13}C). The HMBC data were collected similarly, but over 3012.1 and 22 642.5 Hz sweep widths, using 1024 scans, preceded by four dummy scans, at each t_1 increment of 44.2 ms.

* Primes and double primes denote the downfield and upfield signals, respectively, of diastereotopic methylene protons.

RESULTS

The ^{1}H NMR spectrum of NpCbi$^+$ was assigned using the general strategies that have been used previously for cobalt corrinoid NMR assignments from two-dimensional NMR data.[32,34–36,38] The COSY (Fig. 2) and HOHAHA (Fig. 3) spectra were used to identify the coupled spin systems C(3)—C(30)—C(31), C(8)—C(41)—C(42), C(13)—C(48)—C(49), C(18)—C(19)—C(60), C(55)—C(56) and Pr(1)—Pr(2)—Pr(3). The latter could be absolutely assigned since the Pr(3) resonance (1.15 ppm) is the only three-proton doublet in the ^{1}H spectrum.

In order to assign the other spin systems, as well as protons that are not spin coupled to other protons, proton NOE information is required to establish "through-space" connectivities. For molecules the size of cobalamins, the traditional absorption-mode NOE experiment (NOESY) often results in weak crosspeaks, since the rotational correlation time for the molecule may be close to the reciprocal of the Larmor precession frequency.[32,34] The spin-locked NOE experiment (ROESY), however, produces crosspeaks that are always positive and increase in intensity with slower molecular motion.[50a] Crosspeaks arising from relayed NOEs are readily identified in a ROESY spectrum since they are of opposite phase from those arising from direct NOEs. In the case of NpCbi$^+$ at 11.75 T, both the NOESY and ROESY spectra contained a large number of crosspeaks, but each contained crosspeaks that were missing in the other.

The utility of this NOE information in making unambiguous proton NMR assignments is readily seen in the NOESY spectrum of NpCbi$^+$ (Fig. 4). Thus, the proton assigned to C(19), in addition to having crosspeaks with the C(18) and C(60) protons, also has NOE crosspeaks to C(25), C(55′), and C(56′).* This clearly distinguishes it from, for instance, the C(3) proton, which in addition to crosspeaks with the C(30) and C(31) protons, also has NOE crosspeaks with the C(25) and C(35) methyls and with C(26′). The NMR crosspeaks observed in all four homonuclear experiments are listed in Table SI, available as supplementary material, along with the HMBC correlations described below.

Once the ^{1}H spectrum of NpCbi$^+$ had been assigned, the ^{13}C resonances of the protonated carbons were readily assignable from the HSQC experiment, in which heteronuclear crosspeaks occur only between carbons and their attached protons. The non-protonated carbons were assigned from the HMBC spectrum in which the crosspeaks arise from two- and three-bond C–H couplings, but those

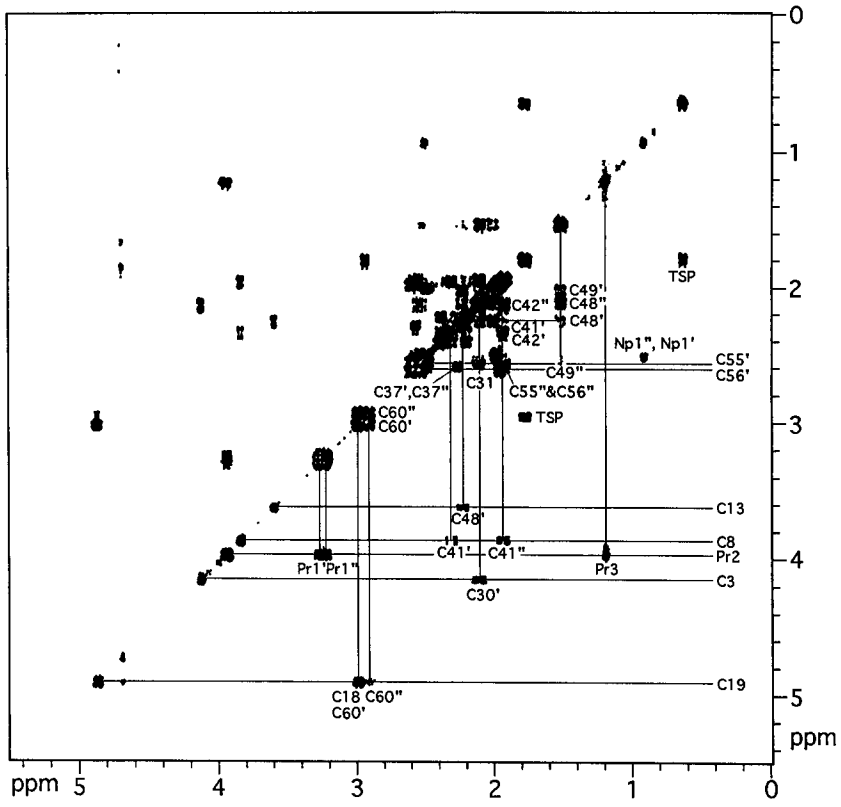


Fig. 2. COSY spectrum of NpCbi$^+$, showing crosspeaks due to direct spin–spin coupling in the Pr(1)—Pr(2)—Pr(3), C(3)—C(30)—C(31), C(8)—C(41)—C(42), C(13)—C(48)—C(49), C(18)—C(19)—C(60) and C(55)—C(56) spin systems.

due to one-bond C–H couplings are specifically suppressed. In addition to permitting the assignment of the non-protonated ring carbons and side chain carbonyl carbons, the HMBC spectrum also provides confirmation of proton NMR assignments.

The ^{1}H and ^{13}C resonances of the neopentyl ligand were assigned as follows. The Np(3) methyl protons are readily identified at −0.13 ppm as a nine-proton singlet resonance in the one-dimensional ^{1}H spectrum, which correlates with a ^{13}C resonance at 30.19 ppm in the HSQC spectrum. These protons have an NOE crosspeak only to the C(46) methyl protons in the ROESY spectrum. The Np(1′) and Np(1″) protons are assignable due to crosspeaks with the Np(3) carbon in the HMBC spectrum and have the anticipated spin–spin crosspeaks in the COSY and HOHAHA spectra. These protons correlate with a ^{13}C resonance at 45.39 ppm in the HSQC spectrum. The Np(2) carbon resonance (40.99 ppm) was assigned by default, as this unprotonated carbon had no correlations in any of the experiments. The final ^{1}H and ^{13}C NMR assignments for NpCbi$^+$ are summarized in Table 1, along with the differences in ^{13}C chemical shift between NpCbi$^+$ and AdoCbi$^+$. In order to provide as accurate a chemical shift comparison as possible, the ^{13}C NMR spectrum of AdoCbi$^+$ was obtained on the same instrument and under the same conditions used for the one-dimensional ^{13}C spectrum of NpCbi$^+$. This ^{13}C NMR spectrum of AdoCbi$^+$ was assigned according to Pagano *et al.*,[38] and the observed chemical shifts are listed in Table SII, available as supplementary material.

The ^{1}H and ^{13}C NMR spectra of Np-13-epiCbi$^+$ were assigned analogously from COSY, HOHAHA, ROESY, HMQC and HMBC spectra (not shown) obtained at 7.05 T. In this case, the ROESY spectrum contained considerably more information than the NOESY spectrum and so it was used exclusively to determine through-space connectivities. All of the NMR correlations observed for Np-13-epiCbi$^+$ are summarized in Table SIII, available as supplementary material, and the final ^{1}H and ^{13}C assignments are given in Table 2, along with the differences in chemical shift between NpCbi$^+$ and Np-13-epiCbi$^+$.

A comparison between the ^{13}C chemical shifts of AdoCbi$^+$ and NpCbi$^+$ is shown in structure **1**. For

Table 1. ^{1}H and ^{13}C NMR assignments for NpCbi^{+} [a]

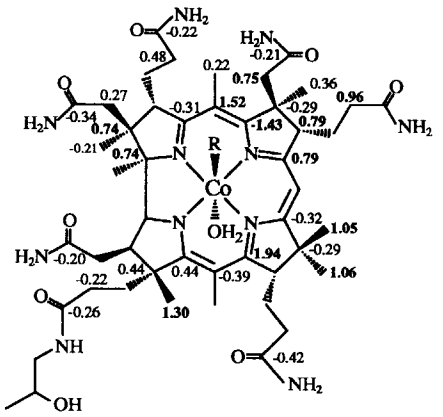
Atom	$\delta_{^{13}C}$ (ppm)	$\delta_{^{1}H}$ (ppm)	$\Delta\delta = \delta_{NpCbi} - \delta_{AdoCbi}$	
			$\Delta\delta_{^{13}C}$ (ppm)[b]	$\Delta\delta_{^{1}H}$ (ppm)[c]
C(53)	17.94	2.47	0.14	0.01
C(35)	18.02	2.39	0.22	−0.07
C(25)	18.60	1.47	−0.21	−0.05
C(54)	21.49	1.49	1.30	0.25
C(36)	21.57	1.85	0.36	−0.02
Pr(3)	21.96	1.15	0.07	−0.06
C(47)	22.66	1.62	1.06	−0.04
C(20)	26.32	0.80	−0.03	−0.11
C(30)	27.97	1.87, 2.06	0.48	−0.16, −0.12
C(48)	28.11	1.96, 2.18	−0.17	−0.11, −0.24
C(41)	28.85	1.88, 2.27	0.14	−0.04, −0.27
Np(3)	30.19	−0.13		
C(49)	33.74	1.47, 2.05	−0.01	−0.36, −0.21
C(55)	33.74	1.93, 2.45	−0.10	0.09, 0.08
C(56)	34.44	1.93, 2.55	−0.22	−0.13, 0.01
C(42)	34.54	2.15, 2.34	0.96	−0.18, −0.09
C(46)	34.80	1.12	1.05	0.20
C(60)	34.94	2.87, 2.95	0.17	0.24, 0.22
C(31)	37.43	2.51	0.00	−0.11
Np(2)	40.99			
C(18)	41.41	2.94	−0.17	0.02
Np(1)	45.39	0.93, 2.48		
C(26)	45.54	2.52, 2.62	0.27	0.10, −0.16
C(37)	46.02	2.27, 2.58	0.75	0.48, 0.26
C(2)	48.02		−0.09	
C(12)	48.47		−0.29	
Pr(1)	48.54	3.24, 3.29	0.00	−0.04, −0.01
C(7)	52.25		−0.29	
C(13)	54.63	3.62	−0.09	0.12
C(3)	57.42	4.15	−0.15	−0.17
C(8)	58.47	3.86	0.79	−0.01
C(17)	61.72		0.44	
Pr(2)	68.62	3.96	−0.04	−0.02
C(19)	77.20	4.90	−0.02	0.13
C(1)	90.09		0.74	
C(10)	99.71	7.00	−0.09	−0.06
C(15)	109.28		−0.39	
C(5)	112.10		1.52	
C(6)	164.39		−1.43	
C(14)	167.23		1.94	
C(9)	175.10		0.79	
C(38)	177.02		−0.21	
C(57)	177.48		−0.26	
C(27)	178.24		−0.34	
C(11)	178.26		−0.32	
C(4)	178.35		−0.31	
C(61)	178.53		0.20	
C(16)	178.87		0.44	
C(50)	180.25		−0.42	
C(43)	180.32		−0.19	
C(32)	180.42		−0.22	

[a] In D_2O, chemical shifts are relative to internal TSP.
[b] This work, ^{13}C chemical shifts assigned according to ref. 38.
[c] Reference 38.

Table 2. ^{1}H and ^{13}C NMR assignments for Np-13-epiCbi^{+} [a]

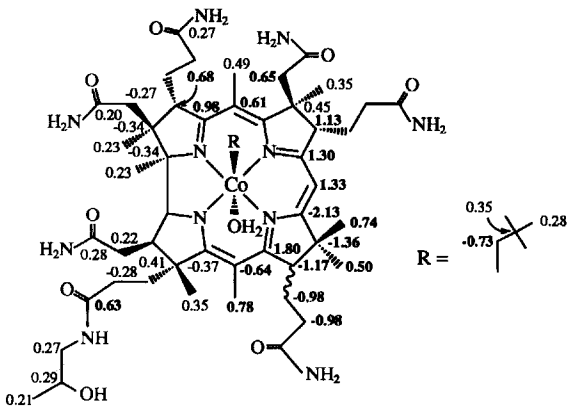
Atom	$\delta_{^{13}C}$ (ppm)	$\delta_{^{1}H}$ (ppm)	$\Delta\delta = \delta_{NpCbi} - \delta_{Np\text{-}13\text{-}epiCbi}$	
			$\Delta\delta_{^{13}C}$ (ppm)	$\Delta\delta_{^{1}H}$ (ppm)
C(53)	17.16	2.59	0.78	−0.12
C(35)	17.53	2.51	0.49	−0.12
C(25)	18.36	1.60	0.23	−0.13
C(54)	21.14	1.65	0.35	−0.16
C(36)	21.22	1.97	0.35	−0.12
Pr(3)	21.76	1.26	0.21	−0.11
C(47)	22.16	1.74	0.50	−0.12
C(20)	26.08	0.89	0.23	−0.09
C(30)	27.97	2.00, 2.20	0.00	−0.13, −0.14
C(41)	28.79	2.08, 2.40	0.16	−0.20, −0.13
C(48)	29.09	1.75, 1.89	−0.98	0.21, 0.29
Np(3)	29.91	0.00	0.28	−0.13
C(55)	33.59	2.06, 2.48	0.15	−0.13, −0.03
C(46)	34.05	1.43	0.74	−0.31
C(42)	34.36	2.18	0.18	−0.03, −0.16
C(56)	34.72	2.09, 2.41	−0.28	−0.16, 0.14
C(49)	34.72	2.65	−0.98	−1.18, −0.60
C(60)	34.72	2.98, 3.06	0.22	−0.11, −0.11
C(31)	37.28	2.64	0.15	−0.13
Np(2)	40.64		0.35	
C(18)	41.26	3.04	0.15	−0.10
C(37)	45.37	2.32, 2.69	0.65	−0.05, −0.11
C(26)	45.81	2.69, 2.79	−0.27	−0.17, −0.17
Np(1)	46.11	0.97, 2.79	−0.73	−0.04, −0.31
C(2)	48.07		−0.02	
Pr(1)	48.27	3.30, 3.35	0.27	−0.06, −0.06
C(12)	49.83		−1.36	
C(7)	51.81		0.45	
C(13)	55.81	3.55	−1.17	0.07
C(3)	56.74	4.20	0.68	−0.05
C(8)	57.34	3.95	1.13	−0.09
C(17)	61.31		0.41	
Pr(2)	68.33	4.03	0.29	−0.07
C(19)	77.19	4.97	0.01	−0.07
C(1)	90.44		−0.34	
C(10)	93.38	7.03	1.33	−0.03
C(15)	109.93		−0.64	
C(5)	111.49		0.61	
C(6)	164.37		0.02	
C(14)	165.43		1.80	
C(9)	173.80		1.30	
C(38)	177.10		0.08	
C(57)	176.85		0.63	
C(27)	178.04		0.20	
C(11)	180.39		−2.13	
C(4)	177.38		0.98	
C(61)	178.25		0.28	
C(16)	179.24		−0.37	
C(50)	180.16		0.09	
C(43)	180.28		0.04	
C(32)	180.16		0.27	

[a] In D_2O, chemical shifts are relative to internal TSP.



$\Delta\delta = \delta_{NpCbi} - \delta_{AdoCbi}$

1



$\Delta\delta = \delta_{NpCbi} - \delta_{Np\text{-}13\text{-}epiCbi}$

2

spectra recorded on the same instrument under identical conditions, we consider chemical shift differences $\geqslant 0.20$ ppm to be clearly significant.[54] Chemical shift differences $\geqslant 0.5$ ppm (highlighted as boldface in **1**) must clearly be considered to be large. While a number of the significant and large chemical shift differences occur in the "southern" half of the structure, in the immediate vicinity of the Ado ligand in the X-ray structure of AdoCbl,[28,31]* many such differences also occur in the "northern" half, remote from the putative position of the Ado ligand. Given the apparent similarity of the electronic effects of the Ado and Np ligands,[39] these chemical shift differences strongly suggest significant conformational differences between $AdoCbi^+$ and $NpCbi^+$.

A similar comparison of the ^{13}C chemical shifts of $NpCbi^+$ and Np-13-epiCbi^+ is shown in **2**. We note the following regarding the NMR comparison of these two complexes. (1) There are significant and large differences in chemical shift at the Np carbons between $NpCbi^+$ and Np-13-epiCbi^+. This suggests that the inversion of configuration in the C ring distorts the inner coordination sphere of the metal atom, changing the electronic interaction between the metal and the α carbon of the organic ligand. (2) The differences in ^1H chemical shift between the diastereotopic protons of the α methylene group of the organic ligand (Tables 1 and 2) are very large, both for $NpCbi^+$ (1.55 ppm) and for Np-13-epiCbi^+ (1.82 ppm). These differences exceed those for base-on (0.98 ppm)[32] and base-off (1.08 ppm)[55] AdoCbl, and for $AdoCbi^+$ (inexplicably, 0.31 ppm),[38] as well as those in all other known cases[35,46] except for the α diastereomers, α-AdoCbl (1.99 ppm)[34] and α-$AdoCbi^+$ (1.95 ppm),[34] in which the Ado ligand occupies the lower (or α) axial ligand position. (3) While there are many large differences in ^{13}C chemical shift in and about the C ring close to the site of epimerization, there are also many significant and large chemical shift differences quite remote from C(13). Since the axial ligands are the same in both complexes, these chemical shift differences strongly suggest that, in addition to conformational shift differences anticipated in the C ring,[56-58] there are more global conformational differences between $NpCbi^+$ and Np-13-epiCbi^+ as well.

DISCUSSION

To date, all conformational knowledge of cobalt corrinoids has come from X-ray crystallographic studies. Unfortunately, growing crystals of suitable quality of such species is difficult, and structural refinement is tedious and time consuming due to the large numbers of solvent molecules found in such crystals. More importantly, no one has ever successfully crystallized base-off cobalamins or cobinamides and so there is no conformational information available for these species. In contrast, given modern methods and access to high field NMR spectrometers, absolute assignments of the ^{13}C NMR spectra of cobalt corrinoids are now relatively straightforward to obtain. While differences in ^{13}C chemical shift of the corrin ring and its attach-

* Pagano *et al.*[38] have argued from NOE observations that the conformation of the Ado moiety relative to the corrin ring in base-on and base-off AdoCbl, and in $AdoCbi^+$, are very similar. Similarly, Bax *et al.*[55] have argued from NOE observations that the orientation of the Ado moiety relative to the corrin in AdoCbl is roughly the same in solution and in the solid state.

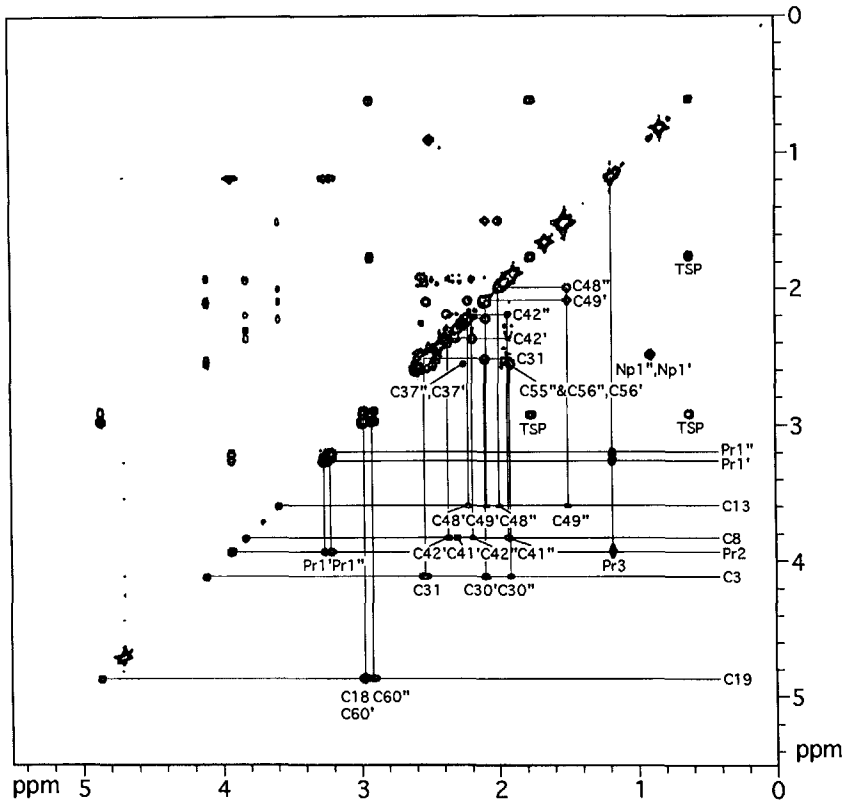


Fig. 3. HOHAHA spectrum of NpCbi$^+$, showing crosspeaks due to relayed as well as direct spin–spin coupling in the Pr(1)—Pr(2)—Pr(3), C(3)—C(30)—C(31), C(8)—C(41)—C(42), C(13)—C(48)—C(49), C(18)—C(19)—C(60) and C(55)—C(56) spin systems.

ments, such as those shown in **1** and **2**, have been taken as an indication of differences in corrin ring conformation,[35,36,46,54] there has been no method for relating chemical shift changes at specific locations to specific differences in conformation.

Carbon chemical shifts are dominated by the paramagnetic shielding term,[59] which in turn is largely determined by the hybridization of the carbon atom and atoms to which it is bonded.[59,60]* Thus, carbon chemical shifts are extremely sensitive to conformation[60,62] and have been widely used for conformational analysis in cyclic hydrocarbons,[61,63] heterocycles,[64] mono- and oligosaccharides,[65] steroids[66] and macromolecules.[60g,67] We now report an initial attempt to observe a relationship between ^{13}C chemical shift changes and corrin ring conformational differences in order to try to draw tentative conclusions regarding differences in conformation between AdoCbi$^+$ and NpCbi$^+$ and between NpCbi$^+$ and Np-13-epiCbi$^+$ based on the observed differences in ^{13}C chemical shift between these pairs of complexes. Since it is not yet possible to determine *a priori* how a given change in corrin ring conformation will affect the hybridization of a given carbon atom and that of the atoms to which it is bonded, any such relationship must, by necessity, be strictly empirical, a methodology for which there is ample precedent in ^{13}C NMR spectroscopy.[59a,b,60j,64a,c,68]

We have utilized a comparison of CNCbl and AdoCbl, two of the three cobalamins for which both the X-ray crystal structures[29–31,56] and the absolute ^{13}C NMR assignments[32,69,70] are known. Such a comparison is difficult because of the following. (1) The large difference in electronic effect of the Ado and CN axial ligands is expected to have substantial effects on the inner corrin carbon atom chemical shifts, in addition to any effects due to conformational differences between the two com-

* It is interesting to note that Wilcox and Gleiter's[60j] correlation of calculated ^{13}C chemical shifts for the bridge carbons of bicyclic molecules show that a change in 2*p* orbital population reflected by as little as a 1% change in 2*s* population changes the chemical shift by over 11 ppm. Similarly, Schleyer and co-workers' calculations[61] on tetramethylcyclobutene dication show that the methyl ^{13}C chemical shift changes by 6.5 ppm upon puckering the ring.

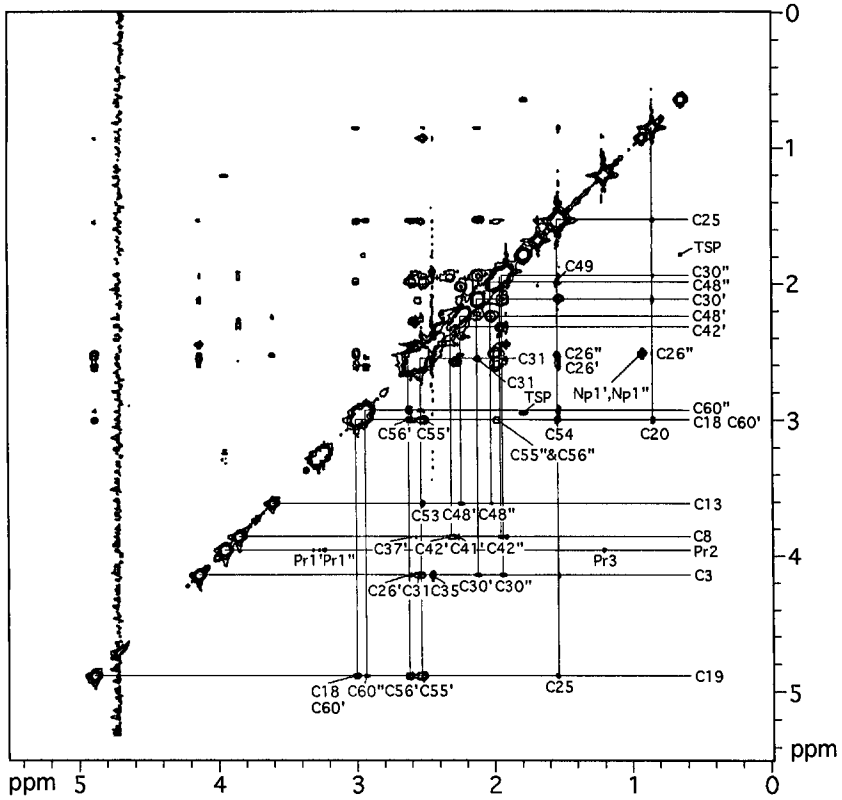


Fig. 4. Upfield portion of the NOESY spectrum of $NpCbi^+$, showing numerous through-space connectivities used to assign the spin systems identified in the COSY and HOHAHA spectra, as well as the protons that are not spin-coupled to other protons.

plexes. At present, there is no acceptable quantitative or semi-quantitative means of evaluating this electronic effect on ^{13}C chemical shifts, and atoms subject to this effect are useless as discriminators of conformational differences. (2) The Ado ligand is expected to have a substantial magnetic anisotropy associated with its ring current, which can affect the chemical shifts of nearby carbon atoms.[32,38,39,46,55] The anisotropy of the CN ligand is much smaller and, because of its greater distance from any of the other carbon atoms in CNCbl, is unlikely to affect any ^{13}C chemical shifts significantly. (3) The magnetic anisotropy of the cobalt atom in cobalamins is significant and varies significantly with the upper axial ligand.[33] Thus, variation in the magnetic anisotropy of the cobalt atom between AdoCbl and CNCbl can cause significant differences in anisotropic shielding of certain carbon atoms, in addition to any effect of conformational differences. (4) In the X-ray structure of CNCbl,[56] the axial Bzm is tilted about 7° towards the B and C rings from its orientation in AdoCbl,[31] changing the distance between the Bzm rings and other carbon atoms in the structure. Since the Bzm ring current must also generate a significant magnetic anisotropy, the chemical shifts of carbon atoms in its vicinity will see differential anisotropic shielding between AdoCbl and CNCbl. (5) The conjugated double bond system of the corrin ring is also likely to be a significant source of anisotropic shielding for certain carbon atoms. Since electron density in this conjugated system will be affected by the inductive effect of the upper axial ligand and differences in the inner coordination sphere geometry, corrin ring magnetic anisotropy will also be different in AdoCbl and CNCbl. Despite these difficulties, we believe that the following analysis suggests that some qualitative discriminators of conformational differences can be gleaned from a comparison of the X-ray structures and ^{13}C NMR spectra of AdoCbl and CNCbl if the "reporter" carbon atoms are chosen judiciously.

There is known to be a differential corrin fold angle about the Co · · · C(10) axis [the angle between the "northern" plane, defined by N(21), C(4), C(5), C(6), N(22), C(9) and C(10), and the "southern" plane defined by N(24), C(16), C(15), C(14), N(23), C(11) and C(10)] in CNCbl (17.7°) relative to that in AdoCbl (14.6°).[28] Analysis of the ^{13}C chemical shifts shows that this conformational difference may be discerned by comparison of the relative ^{13}C chemical shifts. Table 3 shows the differential ^{13}C

Table 3. Relative ^{13}C chemical shifts for the non-axial ligand carbons of AdoCbl and CNCbl

Atom	$\Delta\delta_{^{13}C}$ (ppm)[a]	Atom	$\Delta\delta_{^{13}C}$ (ppm)[a]
C(35)	0.27	C(8)	−1.22
C(53)	0.51	C(3)	−0.72
C(54)	0.86	C(17)	−1.72
C(25)	0.15	Pr(2)	0.09
C(36)	−0.23	C(19)	−1.09
Pr(3)	−0.21	C(1)	0.38
C(20)	1.39	C(10)	−0.09
C(47)	1.74	C(15)	0.05
C(41)	−0.09	C(5)	−1.96
C(30)	0.42	C(6)	−1.60
C(48)	−0.61	C(14)	−1.76
C(46)	−0.02	C(9)	−3.32
C(55)	−0.95	C(11)	−2.16
C(56)	0.31	C(38)	−0.05
C(60)	−0.36	C(57)	0.56
C(42)	0.25	C(16)	−3.06
C(49)	0.60	C(4)	−4.13
C(31)	0.42	C(61)	0.47
C(18)	0.69	C(27)	0.65
C(37)	−0.38	C(43)	0.36
C(26)	0.32	C(12)	−1.65
Pr(1)	−0.44	C(2)	−0.69
C(7)	−1.26	C(32)	0.41
C(13)	−0.79	C(50)	0.11

[a] $\Delta\delta_{^{13}C} = \delta_{AdoCbl} - \delta_{CNCbl}$. Chemical shifts determined in this work, but assigned according to refs 32 (AdoCbl) and 69 (CNCbl), as corrected in ref. 70.

chemical shifts, $\Delta\delta^{obs}_{AdoCbl/CNCbl} = \delta^{obs}_{AdoCbl} - \delta^{obs}_{CNCbl}$, for the non-axial ligand carbon atoms. In order to provide the most accurate chemical shift comparison, the ^{13}C chemical shifts of AdoCbl and CNCbl were measured on the same instrument and under the same conditions used for the one-dimensional ^{13}C spectra of NpCbi$^+$ and Np-13-epiCbi$^+$, and are listed in Table SII, available as supplementary material. Assignments were made according to the literature.[32,69,70]

Inspection of the data in Table 3 shows that there are both positive and negative chemical shift differentials between AdoCbl and CNCbl. This is to be expected, since, in addition to conformational effects, ^{13}C chemical shift differentials will be sensitive to the electronic and anisotropic effects detailed above. In choosing carbon atoms as potential reporters of corrin ring conformational changes, it is obviously advantageous to use those with relatively large chemical shift differences and those with $\Delta\delta_{obs}$ values below the level of significance (0.2 ppm) should generally be rejected. However, clearly the inner corrin ring carbons involved in the conjugated double bond system [C(4), C(5), C(6), C(9), C(10), C(11), C(14), C(15), C(16)] must be eliminated due to the expectation that their relative chemical shifts are largely determined by differences in electronic effects of the Ado and CN ligands. In addition, C(1) and C(19) must be eliminated since they are both directly bonded to coordinating nitrogens, which makes them especially sensitive to ligand electronic effects.

While it is tempting to consider the potential of the carbons C(3), C(35), C(7), C(8), C(12), C(13), C(53) and C(17) as conformational reporters, since all but C(35) are all characterized by large chemical shift differences ($|\Delta\delta^{obs}_{AdoCbl/CNCbl}| = 0.51$–$1.72$ ppm), these carbon atoms are directly bound to the conjugated ring system and most certainly are not sufficiently insulated from the electronic effects generated by the conjugated system.

This leaves the corrin ring substituent carbons and corrin ring carbons C(2) and C(18). Since the side chains are free to rotate in solution, one would expect that the chemical shifts depend upon the conformation of the corrin as well as the conformation of the side chain in solution. As a result of this expected mobility, it is probably wise to exclude the carbonyl carbons and those methylenes α to the carbonyls. Of the remaining carbons, only C(2), C(18), C(20), C(25), C(26), C(30), C(36), C(37), C(41), C(46), C(47), C(48), C(54), C(55) and C(60) are sufficiently insulated from the ligand-induced electronic effects and the motional freedom of the side chain carbonyls to be considered candidate reporter carbons. All of these carbons are at least three bonds removed from the central metal atom and are not directly bound to the extended conjugated corrin macrocycle.

In order to further evaluate these carbons as potential conformational reporters, it is necessary to estimate the magnitude of the anticipated differential anisotropic shielding effects on their ^{13}C chemical shifts described above. In order to estimate shielding effects due to the differential anisotropy of the central metal atom, we have utilized our earlier estimates[33,71]* of $\Delta\chi$, the magnetic ani-

* In our earlier work on the cobalt anisotropy of RCbls,[33] the assignments of the ^{13}C resonances of B(5) and B(6) were interchanged.[55] Recalculation of the values of $\Delta\chi$ using the correct assignments shows that this interchange of assignments has a minor effect on the calculated values of $\Delta\chi$ (e.g. $\Delta\chi = -3.88 \times 10^{-29}$ cm^3 molecule^{-1} for CNCbl rather than -3.35×10^{-29} cm^3 molecule^{-1}, $\Delta\chi = -16.0 \times 10^{-29}$ cm^3 molecule^{-1} for AdoCbl instead of -14.3×10^{-29} cm^3 molecule^{-1}). These minor differences do not affect the conclusions arrived at in this paper.

sotropy of the cobalt atom of AdoCbl and CNCbl, the geometry of these complexes from their X-ray structures, and McConnell's equation[72] [eq. (1)], where θ is the angle between the dipole axis of the

$$\Delta\delta^{Co}(\text{ppm}) = -(\Delta\chi(1-3\cos^2\theta)) \times 10^6/3r^3 \quad (1)$$

quadrapolar nucleus (which was defined by the cobalt-coordinated axial ligands) and a vector from cobalt to the subject atom, and r is the magnitude of that vector.

Anisotropic shielding due to the presence of the Ado ligand in AdoCbl was estimated using the ring current model of Giessner-Prettre *et al.*[73] for adenine and the neutron crystal structure of AdoCbl.[31] It was determined that the differential anisotropy introduced by the altered position of the benzimidazole ligand was not significant for any of the candidate reporter carbons and thus this effect was not included in the analysis.

Table 4 shows the anisotropic shieldings for the candidate "reporter" carbons in AdoCbl and the corrected chemical shift differentials for the AdoCbl/CNCbl comparison. It should be noted that three [C(25), C(41) and C(46)] of the 15 candidate "reporter" carbon chemical shift differentials are not significant, although two [C(41) and C(46)] of these three "become" significant after correcting for the differential anisotropy,* and thus can be included in this analysis. Inclusion of C(25) as a potential reporter carbon atom is discussed below.

In order to test the utility of these reporter carbons for predicting corrin ring conformational changes, we now examine 9-adeninylpropylcobalamin (AdePrCbl), an AdoCbl analogue in which the ribose moiety of the organic ligand is replaced by a propylene group, the only other cobalamin for which both the X-ray crystal structure and the absolute ^{13}C NMR assignments are known.[46] The fold angle for AdePrCbl is smaller than that observed for AdoCbl and has a value of 10.9°.[46] It should be noted that the adenine moiety of AdePrCbl, the plane of which is roughly parallel with the corrin ring, lies over the D ring of the corrin, rotated approximately 120° clockwise from its position in AdoCbl.[46]

In order to provide a precise chemical shift comparison, the ^{13}C chemical shifts of AdePrCbl (Table SII, available as supplementary material) were measured on the same instrument and under the same conditions used for the other complexes studied here. The values of the chemical shift differentials, $\Delta\delta^{obs}_{AdePrCbl/CNCbl} = \delta^{obs}_{AdePrCbl} - \delta^{obs}_{CNCbl}$, for the 15 candidate reporter carbons are listed in Table 5. In order to estimate the differential shielding effect of the central cobalt atom in AdePrCbl, the magnetic anisotropy, $\Delta\chi$, of the cationic cobalt of AdePrCbl was calculated using eq. (1), as described previously.[33] The value obtained, -16.7×10^{-29} cm^3 molecule^{-1}, is quite close to that obtained for AdoCbl, in agreement with the conclusion of Pagano *et al.*[46] that the electronic effects of the AdePr and Ado ligands are quite similar. Anisotropic shielding due to the presence of the adenine ligand in AdePrCbl was estimated using the ring current model of Giessner-Prettre *et al.*[73] for adenine and the X-ray crystal structure of AdePrCbl.[46] It was determined, as in the case for AdoCbl and CNCbl, that the differential anisotropy introduced by the altered position of the benzimidazole ligand was not significant for any of the candidate reporter carbons. The total differential shielding, $\Delta\delta^{anis}_{AdePrCbl}$, is shown in Table 5 and represents the total amount of anisotropic shielding/deshielding due to the presence of the cobalt and adenine functionalities.

This comparison of the chemical shifts of the "reporter" carbons of AdePrCbl and CNCbl (Table 5) shows that for those observed chemical shift differentials that are significant the same sign is observed, atom for atom, as is observed in the AdoCbl/CNCbl comparison. In short, the "reporter" carbon chemical shift differences for the AdePrCbl/CNCbl comparison (Table 5) correctly predict the gross difference in corrin ring conformation between these two complexes, i.e. a smaller corrin ring fold angle in AdePrCbl than in CNCbl.

The success of this analysis in predicting the corrin ring conformational difference between β-AdePrCbl and CNCbl suggests that it is reasonable to attempt to apply the conformational "rules" implicit in Table 4 to the comparison of the ^{13}C chemical shifts of AdoCbi$^+$ and NpCbi$^+$ (Table 1 and structure **1**). The absence of the axial nucleotide significantly affects the chemical shift of C(55) in cobinamides,[32,38,70] making it unclear whether or not C(55) can function as a reporter carbon. For the AdoCbi$^+$/NpCbi$^+$ comparison, C(2), C(18), C(20), C(41), C(48), C(55) and C(60) all have $\Delta\delta$ values below our generally acceptable level of significance. The signs of the $\Delta\delta$ values of five [C(26), C(30), C(46), C(47) and C(54)] of the remaining eight reporter carbons all predict that the fold angle of NpCbi$^+$ will be smaller than that observed in AdoCbi$^+$. Thus, the net result would appear to predict a downward pucker of the corrin ring in NpCbi$^+$ relative to AdoCbi$^+$. Such a downward flex of the corrin ring would tend to reduce the steric interactions between the bulky Np group and the upwardly projecting acetamide side chains. Presumably, such steric interactions are less severe in AdoCbi$^+$, despite the size of the Ado ligand, simply

Table 4. Observed and corrected chemical shifts and chemical shift differentials of the reporter carbons for AdoCbl/CNCbl

Atom	δ^{obs}_{AdoCbl} [a]	$\Delta\delta^{obs}_{AdoCbl/CNCbl}$ [b]	$\Delta\delta^{anis}_{AdoCbl}$ [c]	δ^{corr}_{AdoCbl} [d]	$\Delta\delta^{corr}_{AdoCbl/CNCbl}$ [e]
C(2)	49.05	−0.74	0.73	48.32	−1.32
C(18)	42.27	0.69	0.76	41.52	0.09
C(20)	23.24	1.39	0.19	23.05	1.28
C(25)	19.52	0.15	0.33	19.19	−0.11
C(26)	45.82	0.32	0.27	45.55	0.10
C(30)	28.98	0.42	0.37	28.61	0.14
C(36)	21.35	−0.23	0.31	21.04	−0.48
C(37)	44.94	−0.38	0.16	44.78	−0.47
C(41)	28.43	−0.09	0.34	28.09	−0.37
C(46)	33.84	−0.02	−0.55	34.39	0.63
C(47)	23.59	1.74	0.06	23.53	1.75
C(48)	29.98	−0.61	−0.02	30.00	−0.53
C(54)	19.28	0.86	0.41	18.87	0.49
C(55)	34.35	−0.77	0.35	34.00	−1.01
C(60)	34.45	−0.36	0.36	34.09	−0.66

[a] Chemical shifts determined in this work, but assigned according to ref. 32 (AdoCbl).

[b] $\Delta\delta^{obs}_{AdoCbl/CNCbl} = \delta^{obs}_{AdoCbl} - \delta^{obs}_{CNCbl}$.

[c] $\Delta\delta^{anis}_{AdoCbl} = \Delta\delta^{Co}_{AdoCbl} + \Delta\delta^{ade}_{AdoCbl}$, where $\Delta\delta^{Co}_{AdoCbl}$ was estimated using McConnell's equation [eq. (1)[72]] and the cobalt anisotropy previously determined for AdoCbl[33] (see footnote p. 2971) and $\Delta\delta^{ade}_{AdoCbl}$ is the differential chemical shift due to anisotropic shielding or deshielding by the adenine moiety in AdoCbl. $\Delta\delta^{ade}_{AdoCbl}$ was estimated using the ring current model for adenine[73] and the crystal structure geometry of AdoCbl.[31]

[d] $\delta^{corr}_{AdoCbl} = \delta^{obs}_{AdoCbl} - \Delta\delta^{anis}_{AdoCbl}$.

[e] $\Delta\delta^{corr}_{AdoCbl/CNCbl} = \delta^{corr}_{AdoCbl} - \delta^{corr}_{CNCbl}$ [where $\delta^{corr}_{CNCbl} = \delta^{obs}_{CNCbl} - \Delta\delta^{anis}_{CNCbl}$, and $\Delta\delta^{anis}_{CNCbl} \leqslant 0.07$ for all of the listed atoms and was calculated by eq. (1) using $\Delta\chi = -3.88 \times 10^{-29}$ cm^3 molecule^{-1}].

because the Ado ligand is constrained to lie over the southern half of the corrin,[28] primarily by the sentinel methyl groups C(46) and C(54),* and is not free to rotate about the Co—C bond.†

Turning to the NMR comparison of NpCbi$^+$ and Np-13-epiCbi$^+$ (Table 2 and structure **2**), 10 of the 15 reporter carbons (Table 2) have significant chemical shift differentials, and four of these qualify as large chemical shift differentials. It should be noted that there is a gross difference between the conformation of the C rings between CNCbl and CN-13-epiCbl which may serve to exclude those members of the C ring from this empirical analysis. For example, the observed value of the C(14)—C(13)—C(12)—C(11) torsional angle is 20.5° in CN-13-epiCbl as opposed to 28.6° in CNCbl.[28,58] As a result of this epimerization, "reporter" carbon atoms C(46), C(47) and C(48) must be excluded from the analysis since they are not directly comparable to those of the normal compound. The remaining reporter carbons are suggestive of a fold angle for NpCbi$^+$ that is larger than that observed in Np-13-epiCbi$^+$. Interestingly, Glusker[28] has shown that the corrin fold about the Co···C(10) axis is more severe in the X-ray structure of CN-13-epiCbl[58] (21.8°) than in CNCbl (17.7°). The current analysis suggests that this may not be the case for C(13) epimerization in NpCbi$^+$,

* The C(46) methyl protons undergo a smaller upfield shift of 0.33 ppm in AdoCbl[32] relative to CNCbl,[74] suggesting that the anisotropic shielding by the Ado ligand has been overestimated, perhaps due to the partial population of another configuration in solution.

† Bax *et al.*,[55] based on NOE observations, suggest that the Ado ligand of AdoCbl may be able to rotate counter clockwise (when viewed from above) by about 50° in solution. However, NOE data do not suggest that free rotation about the Co—C bond of AdoCbl is possible.

Table 5. Observed and corrected chemical shifts and chemical shift differentials of the reporter carbons for AdePrCbl/CNCbl

Atom	$\delta^{obs}_{AdePrCbl}$ [a]	$\Delta\delta^{obs}_{AdePrCbl/CNCbl}$ [b]	$\Delta\delta^{anis}_{AdePrCbl}$ [c]	$\delta^{corr}_{AdePrCbl}$ [d]	$\Delta\delta^{corr}_{AdePrCbl/CNCbl}$ [e]
C(2)	49.01	−0.78	0.60	48.41	−1.23
C(18)	42.08	0.50	0.35	41.73	0.30
C(20)	23.08	1.23	0.37	22.71	0.94
C(25)	19.31	−0.06	0.35	18.96	−0.33
C(26)	45.40	−0.10	0.04	45.36	−0.09
C(30)	28.90	0.34	0.36	28.54	0.05
C(36)	21.24	−0.34	0.31	20.93	−0.59
C(37)	44.89	−0.43	0.38	44.51	−0.77
C(41)	28.39	−0.13	0.19	28.20	−0.26
C(46)	34.31	0.45	0.42	33.89	0.13
C(47)	23.08	1.23	0.32	22.76	0.98
C(48)	30.38	−0.21	0.23	30.15	−0.38
C(54)	18.95	0.53	0.00	18.95	0.57
C(55)	34.53	−0.59	0.10	34.43	−0.62
C(60)	34.42	−0.39	−0.06	34.48	−0.27

[a] Chemical shifts determined in this work, but assigned according to ref. 46 (AdePrCbl).

[b] $\Delta\delta^{obs}_{AdePrCbl/CNCbl} = \delta^{obs}_{AdePrCbl} - \delta^{obs}_{CNCbl}$.

[c] $\Delta\delta^{anis}_{AdePrCbl} = \Delta\delta^{Co}_{AdePrCbl} + \Delta\delta^{ade}_{AdePrCbl}$, where $\Delta\delta^{Co}_{AdePrCbl}$ was estimated using McConnell's equation [eq. (1)[72]] and the cobalt anisotropy previously determined for AdePrCbl,[33] (see footnote p. 2971) and $\Delta\delta^{ade}_{AdePrCbl}$ is the differential chemical shift due to anisotropic shielding or deshielding by the 9-adeninylpropyl ligand in AdePrCbl. $\Delta\delta^{ade}_{AdePrCbl}$ was estimated using the ring current model for adenine[73] and the X-ray crystal structure geometry of AdePrCbl.[46]

[d] $\delta^{corr}_{AdePrCbl} = \delta^{obs}_{AdePrCbl} - \Delta\delta^{anis}_{AdePrCbl}$.

[e] $\Delta\delta^{corr}_{AdePrCbl/CNCbl} = \delta^{corr}_{AdePrCbl} - \delta^{corr}_{CNCbl}$ [where $\delta^{corr}_{CNCbl} = \delta^{obs}_{CNCbl} - \Delta\delta^{anis}_{CNCbl}$, and $\Delta\delta^{anis}_{CNCbl} \leqslant 0.07$ for all of the listed atoms and was calculated by eq. (1) using $\Delta\chi = -3.88 \times 10^{-29}$ cm^3 molecule^{-1}].

and suggests that the introduction of the *e* side chain to the β-face of the molecule sterically congests the environment of the Np ligand and that the corrin responds to this stress by flexing downward relative to NpCbi$^+$.

CONCLUSION

An attempt has been made to extract information about corrin ring conformational differences in cobalt corrinoids from differences in ^{13}C chemical shifts by a comparison of the crystal structures and ^{13}C NMR spectra of AdoCbl and CNCbl. After attempting to account for various differences in anisotropic shielding of carbon nuclei in the two complexes, 15 candidate carbon atoms [C(2), C(18), C(20), C(25), C(26), C(30), C(36), C(37), C(41), C(46), C(47), C(48), C(54), C(55) and C(60)] emerge as candidate "reporters" of conformational differences. Application of this analysis to AdePrCbl correctly predicts the qualitative difference in corrin ring conformation between AdePrCbl and CNCbl.

Application of this analysis to the ^{13}C NMR spectra of AdoCbi$^+$ and NpCbi$^+$ suggests that the corrin ring fold angle is smaller in the latter than in the former. This is presumably in response to the bulky Np ligand and its steric interactions with the upwardly projecting side chains. Moreover, a comparison of the ^{13}C chemical shifts of the reporter carbons of NpCbi$^+$ and Np-13-epiCbi$^+$ suggests that, in the former, the corrin ring fold angle is larger than for the latter. Once again, we believe this to be in response to the increased steric congestion of the β-face of the molecule, due, in this case, to the epimerization of the *e* side chain at C(13).

Supplementary materials available from the author. Table SI, the correlation table for NMR connectivities for NpCbi$^+$, Table SII, ^{13}C chemical shifts for AdoCbl, AdePrCbl, CNCbl and

AdoCbi$^+$, and Table SIII, the correlation table for the NMR connectivities of Np-13-epiCbi$^+$ (12 pages) are available on request from the corresponding author (K. L. B.).

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