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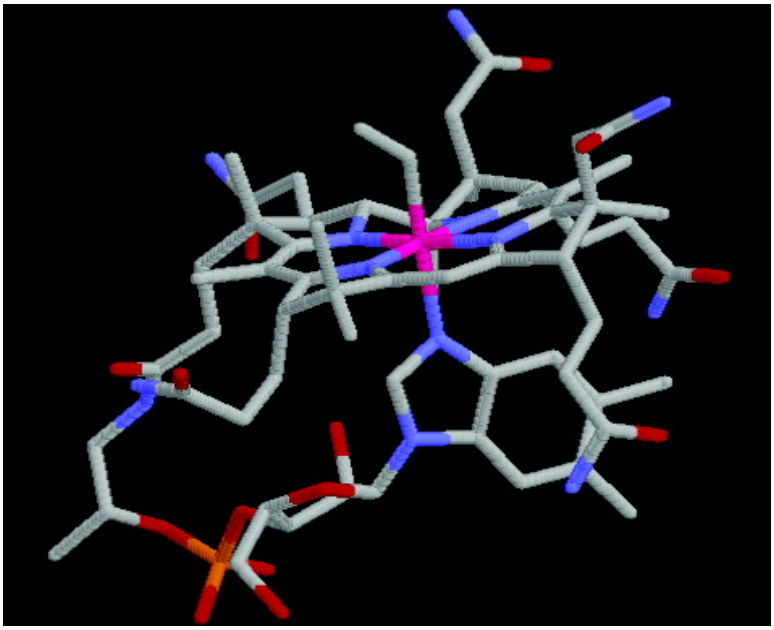

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Properties and Reactivity of Chlorovinylcobalamin and Vinylcobalamin and Their Implications for Vitamin B_{12}-Catalyzed Reductive Dechlorination of Chlorinated Alkenes

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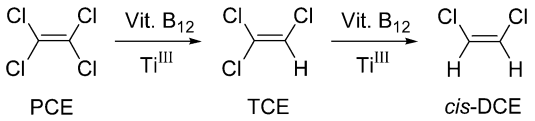
Abstract: Vitamin B_{12}-catalyzed reductive dechlorination of perchloroethylene (PCE) and trichloroethylene (TCE) is a potential strategy for cleanup of polluted environments. Presented are crystal structures of vinylcobalamin **2** and *cis*-chlorovinylcobalamin **1**. They show a strong resistance toward photolysis. Reduction of **2** is difficult, but reduction of **1** occurs readily and produces **2**. The mechanism of this latter reaction involves acetylene as an intermediate. These and other findings are discussed in the context of environmental studies on B_{12}-catalyzed dechlorination of PCE and TCE and investigations of the haloalkene reductive dehalogenases that catalyze similar reactions.

Perchloroethylene (PCE) and trichloroethylene (TCE) are persistent contaminants found in many terrestrial and groundwater environments.[1] Several anaerobic organisms use corrinoid-dependent enzymes to reductively dechlorinate these toxic compounds in a process that is coupled to energy metabolism.[2] Catalytic vitamin B_{12} (Figure 1) has also been used for abiotic dechlorination of PCE and TCE in the presence of titanium(III) citrate (eq 1).[3]



(1)

Mechanistic studies on this latter process suggest that the conversion of PCE to TCE occurs via electron transfer from cob(I)alamin to PCE,[3d,e,4] whereas further transformation of TCE to di- and monochlorinated ethylenes, acetylene, and ethene involves organocobalamins.[5] The latter hypothesis is supported by detection of di- and monochlorinated vinylcobalamins by mass spectrometry in dechlorination reactions, by model studies on chlorinated vinylcobaloximes, and by product distributions of TCE reduction with B12s vs outer-sphere reductants.[6−8] Prior

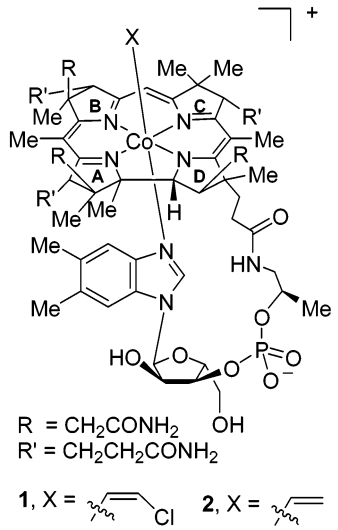


Figure 1. Structure of vitamin B_{12} (X = CN). Derivatives in which X is an alkyl group are collectively termed alkylcobalamins.

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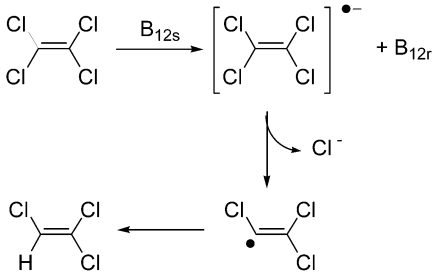
Scheme 1

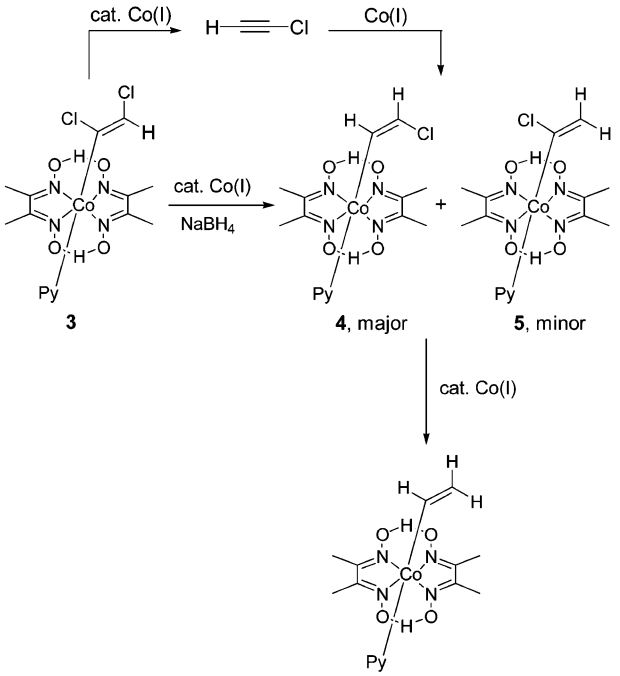


to our preliminary paper on *cis*-chlorovinylcobalamin **1**,[9] no information was available about the structure and chemical properties of chlorinated vinylcobalamins. Moreover, it was unclear whether these compounds could function as intermediates in the catalytic process since the Co−C bond in vinylcobalamins was expected to be too strong for homolytic cleavage. We recently found that chlorovinylcobalamin **1** is, however, readily reduced under the conditions of reductive dechlorination. We herein extend our preliminary findings to a detailed investigation of the properties of both chlorovinylcobalamin **1** and its nonchlorinated analogue **2**.

The mechanism of vitamin B_{12}-catalyzed dechlorination of PCE and TCE has been the focus of a number of recent studies. It is generally believed that the first step involves a dissociative electron transfer from cob(I)alamin (B_{12s}) to PCE, leading to chloride elimination and the formation of a trichlorovinyl radical (Scheme 1). Support for this mechanism comes from spectroscopic studies including stopped-flow UV−vis experiments that show the formation of cob(II)alamin (B_{12r}) when PCE and cob(I)alamin are reacted under single-turnover conditions.[3d,4] Furthermore, the results of studies with inter- and intramolecular radical traps are in line with the formation of chlorinated vinyl radicals.[3b,d,4] The redox potential of the Co(I)/Co(II) couple of vitamin B_{12}, −0.61 V,[10] is close to the calculated redox potential for one-electron reduction of PCE to chloride and a trichlorovinyl radical (−0.60 V),[11] lending further support for an electron-transfer mechanism. However, unlike the developing consensus on the mechanism for the conversion of PCE to TCE, the mechanism of B_{12}-catalyzed reductive dechlorination of TCE and the various isomers of dichloroethene (DCE) is not as clear. These compounds are less electron deficient than PCE, with calculated one-electron reduction potentials[11] of −0.67 V for TCE, −1.01 V for *cis*-DCE, and −0.96 V for *trans*-DCE questioning the feasibility of an electron-transfer pathway. Indeed, spectroscopic studies on the B_{12}-catalyzed reduction of some of these compounds by Schwarzenbach and co-workers have suggested the formation of organocobalamins.[3d,5] Furthermore, analysis of samples taken from dechlorination reactions by electrospray mass spectrometry revealed the presence of mono-, di-, and nonchlorinated vinylcobalamins.[6] These reports raise the question of how, if at all, such organocobalt complexes might be returned to the catalytically active cob(I)alamin state.

We[7,12] and others[8a] have provided initial insights into this question through model studies involving chlorinated vinylcobaloximes. Our investigations showed that in the presence of a Co(I) catalyst and a sacrificial reductant, dichlorovinylcobaloxime **3** could be converted into monochlorovinylcobaloximes **4** and **5**, which in turn could be reduced to vinylcobaloxime (Scheme 2).[7] Detailed mechanistic studies on the conversion of **3** into **4** and **5** indicated that the Co−C bond is broken and that chloroacetylene is an intermediate. In an independent study, McNeill and co-workers reported that treatment of **3** with strong reductants such as sodium naphthalenide yielded chloroacetylene, providing further support for the pathway in Scheme 2.[8a] These results on cobaloxime models were also in accord with kinetic studies that detected chloroacetylene as an intermediate in the vitamin B_{12}-catalyzed transformation of TCE to acetylene and ethene.[3e,g] Kinetic modeling in these reports suggested that one or more organocobalamins might be intermediates in this process. Whereas the consistency of these studies is gratifying, many questions still remain: Foremost, what are the structures of these proposed organocobalamin intermediates, and can Ti(III) indeed reduce them? The present study sought to extend the cobaloxime model studies to the actual vitamin B_{12} catalyst system.

Scheme 2



Results

Synthesis and Structural Characterization of Chlorovinylcobalamin and Vinylcobalamin. In our previous study, we showed that reaction of chloroacetylene with cob(I)aloxime leads to a mixture of two chlorovinylcobaloximes, **4** and **5**, with the *cis*-complex **4** produced predominantly at high pH.[7] The analogous reaction with cob(I)alamin as the nucleophile produced *cis*-chlorovinylcobalamin **1** exclusively in excellent yield (89%). Ethynylcobalamin was not observed, which has been proposed to be the major product in the reaction of bromoacetylene with cob(I)alamin on the basis of IR evidence.[13] No organocobal-

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Table 1. Selected Bond Lengths (Å) and Angles (deg) for *cis*-Chlorovinylcobalamin **1** (Mean of Four Structures in the Unit Cell) and Vinylcobalamin **2**[a]

	1	2		1	2
Co−CV_α	1.951(7)	1.911(7)	Co−N22	1.922(5)	1.919(5)
CV_α−CV_β	1.286(8)	1.319(10)	Co−N23	1.908(5)	1.910(6)
Co−NB3	2.144(5)	2.165(6)	Co−N24	1.893(5)	1.882(5)
Co−N21	1.894(5)	1.877(5)			
Co−CV_α−CV_β	139.8(7)	128.3(6)	N23−Co−NB3	85.5(3)	88.5(2)
CV_α−Co−NB3	176.0(4)	175.7(2)	N24−Co−NB3	94.8(3)	90.3(2)
N21−Co−N22	90.3(3)	90.5(2)	N21−Co−N23	172.2(3)	172.3(2)
N21−Co−N24	83.4(7)	83.1(2)	N22−Co−N24	171.2(3)	172.8(2)
N22−Co−N23	96.3(3)	96.3(2)	N22−Co−CV_α	85.0(4)	92.5(3)
N23−Co−N24	90.5(3)	90.3(2)	N23−Co−CV_α	96.4(3)	87.3(3)
N21−Co−CV_α	88.3(3)	88.7(3)	N24−Co−CV_α	88.8(4)	90.7(3)
N21−Co−NB3	90.1(3)	95.5(2)	fold angle[b]	5.7(9)	12.5(2)
N22−Co−NB3	91.3(3)	87.0(2)			

[a] For the numbering scheme see ref 17 and the Supporting Information of ref 9. [b] See the text.

amins could be isolated in the reactions of cob(I)alamin with PCE, TCE, and dichloroacetylene (vide infra for a possible explanation). The electrospray mass spectrum of **1** displayed ions at *m*/*z* 1390 $[M]^+$ and 695 $[M]^{2+}$, and analysis by ^{1}H NMR spectroscopy showed doublet resonances at 5.03 ppm ($^3J = 5.60$ Hz) and 5.73 ppm ($^3J = 5.60$ Hz), consistent with a *cis* relationship of the two vinyl protons, which was confirmed by X-ray crystallographic analysis.[9,14] The Co−C bond length in this structure is 1.951(7) Å (Table 1), shorter than the corresponding bonds in methylcobalamin (MeCbl; 1.983 Å)[15a] and adenosylcobalamin (AdoCbl; 2.049 Å)[15b] as expected for a bond featuring an sp^2-hybridized carbon. The Co−NB3 bond length to the axial benzimidazole ligand in **1** is 2.144(5) Å, significantly shorter than those in MeCbl (2.195 Å) and AdoCbl (2.234 Å), providing an example of the "inverse" or "anomalous" *trans* effect that is often observed in organocobalamins and -cobaloximes.[16] This effect is manifested in the Co−C and Co−NB3 bonds both shortening (or lengthening) upon changing the donor strength of the alkyl ligand in contrast to the usual thermodynamic *trans* effect. Similar to our previous report on the corresponding *cis*-chlorovinylcobaloxime,[7] the Co−C_α=C_β bond angle in **1** is very large, 139.8(7)° (Table 1), presumably because of steric interactions between the chlorine and the corrin ring and its β-substituents. Indeed, the upward fold angle,[17] defined as the dihedral angle between the best planes through rings A + B and C + D (Figure 1), which is a measure of steric interactions between the β-axial and equatorial groups, is 5.7(9)° in **1**. This value is by far the smallest fold angle reported for cobalamins which have values between 13.3° and 18.7° for adenosylcobalamin[15e] and aquocobalamin,[18] respectively. In the structure of **1**, the C_α=C_β bond of the vinyl ligand is positioned between the C and D rings of the corrin ring and makes a dihedral angle of −25(8)° with the Co−N23 bond. The chlorine atom is not within hydrogen-bonding distance to any side chains of the corrin macrocycle.

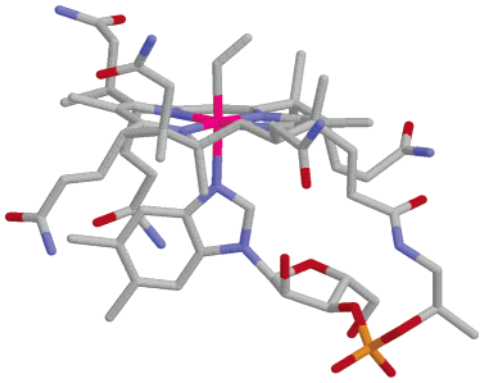

Figure 2. Structure of vinylcobalamin **2**. For details, see the Supporting Information.

Vinylcobalamin **2** was prepared from cob(I)alamin and acetylene[13] to compare the properties of the two complexes and assess the influence of chlorine substitution. Crystallization was achieved from 40% PEG, after examination of a series of conditions typically used for proteins. The X-ray structure of **2** (Figure 2) showed a Co−C bond length of 1.911(7) Å and a Co−NB3 bond length of 2.165(6) Å (Table 1). The vinyl group occupies a position similar to that of the chlorovinyl group in **1**.

The Co−C bond in **2** is significantly shorter than that in cobalamin **1**, presumably because of bond lengthening in **1** as a result of the steric repulsion between the chlorine and the β-substituents on the corrin ring. Indeed, the Co−C_α=C_β bond angle in **2** (128.3(6)°) is closer to the expected value for an sp^2-hybridized central carbon, and the fold angle, although still small, is significantly larger at 12.5(2)°. An additional factor that contributes to the longer bond length in **1** is the weaker σ-donating capacity of the chlorovinyl group compared to the vinyl group as indicated by the lower pK_a of the β-proton of vinyl chloride (31)[19] compared to that of ethylene (36−44).[20,21] To deconvolute these two factors, it would be valuable to have the *trans*-chlorovinylcobalamin structure for comparison, but

(14) In this work a new, more accurate structure of **1** was determined. Details are found in the CIF file for this structure in the Supporting Information.
(15) (a) Rossi, M.; Glusker, J. P.; Randaccio, L.; Summers, M. F.; Toscano, P. J.; Marzilli, L. G. *J. Am. Chem. Soc.* **1985**, *107*, 1729. (b) Lenhert, P. G. *Proc. R. Soc. London, Ser. A* **1968**, *303*, 45. (c) Zou, X.; Brown, K. L. *Inorg. Chim. Acta* **1998**, *267*, 305. Other values (Co−C, 1.979(4) Å; Co−NB3, 2.187(7) Å) have been reported for MeCbl: (d) Randaccio, L.; Furlan, M.; Geremia, S.; Slouf, M.; Srnova, I.; Toffoli, D. *Inorg. Chem.* **2000**, *39*, 3403−3413. Other values (Co−C, 2.023(10) Å; Co−NB3, 2.214(9)) have been reported for AdoCbl: (e) Bouquiere, J. P.; Finney, J. L.; Savage, H. F. J. *Acta Crystallogr., B* **1994**, *B50*, 566−578.
(16) (a) De Ridder, D. J. A.; Zangrando, E.; Buergi, H.-B. *J. Mol. Struct.* **1996**, *374*, 63. (b) Randaccio, L. *Comments Inorg. Chem.* **1999**, *21*, 327−376. (c) Zangrando, E.; Bresciani-Pahor, N.; Randaccio, L.; Charland, J.-P.; Marzilli, L. G. *Organometallics* **1986** *5*, 1938 (d) Marzilli, L. G.; Bayo, F.; Summers, M. F.; Thomas, L. B.; Zangrando, E.; Bresciani-Pahor, N.; Mari, M.; Randaccio, L. *J. Am. Chem. Soc.* **1987**, *109*, 6045−6052. For exceptions, see: Randaccio, L.; Geremia, S.; Stener, M.; Toffoli, D.; Zangrando, E. *Eur. J. Inorg. Chem.* **2002**, 93 and references therein.
(17) Glusker, J. P. In *B12*; Dolphin, D., Ed.; John Wiley: New York, 1982; Vol. 1, pp 23−106.
(18) Kratky, C.; Farber, G.; Gruber, K.; Wilson, K.; Dauter, Z.; Nolting, H. F.; Konrat, R.; Kräutler, B. *J. Am. Chem. Soc.* **1995**, *117*, 4654−4670.

unfortunately all attempts toward its synthesis have been unsuccessful to date. For comparison, the *cis*- and *trans*-chlorovinylcobaloximes have been crystallographically characterized, with Co−C bond lengths of 1.9468(17) Å for the former[7] and 1.931(3) Å for the latter.[8a]

The structures of **1** and **2** provide the first examples of organocobalamins in which the carbon ligand has sp^2 hybridization. A correlation has been observed between the length of the Co−NB3 bond and the so-called K_{Co}, the equilibrium constant for coordination of the benzimidazole to the metal.[22] This constant can be obtained from the $pK_{a,obs}$ for protonation of the dimethylbenzimidazole ligand.[23] UV−vis spectra of aqueous solutions of **1** and **2** showed the characteristic change from the yellow, base-off form to the red, base-on form upon increasing the pH. The titration data were fit to a two-state equation, providing $pK_{a,obs}$ values of 2.3 ± 0.2 for **1** and 2.4 ± 0.1 for **2**.[24] These pK_a values are significantly lower than those reported for EtCbl (4.16) and MeCbl (2.90) and higher than those of di- and trifluoromethylcobalamin (2.15 and 1.44, respectively).[25] Converting the pK_a values to pK_{Co} and $\Delta G°_{Co}$ values as described by Brown and co-workers[23] (see the Experimental Section) shows that the previously reported very good correlation between $-\Delta G°_{Co}$ and the Co−NB3 bond length for five structures ($r^2 = 0.990$)[22] is diminished but still quite good when including four more recently characterized structures (X in Figure 1 is CF_3, CHF_2, vinyl, chlorovinyl, $r^2 = 0.917$, Figure 3).[26]

Co−C Bond in Vinylcobalamins. The bond dissociation enthalpy (BDE) of the vinylcobalamins is expected to be higher than that of methylcobalamin (37 kcal/mol).[30] It did not prove feasible to use the elegant kinetic methods developed in the laboratories of Halpern[31,32] and Finke[33,34] to experimentally determine the Co−C bond strength because at the temperatures required for homolysis the cob(II)alamin product decomposed, precluding clean kinetics.[35] As an alternative means to determine the relative strength of the Co−C bond in these vinylcobalamins compared to methylcobalamin, the photolability of the Co−C bonds in these complexes was investigated.

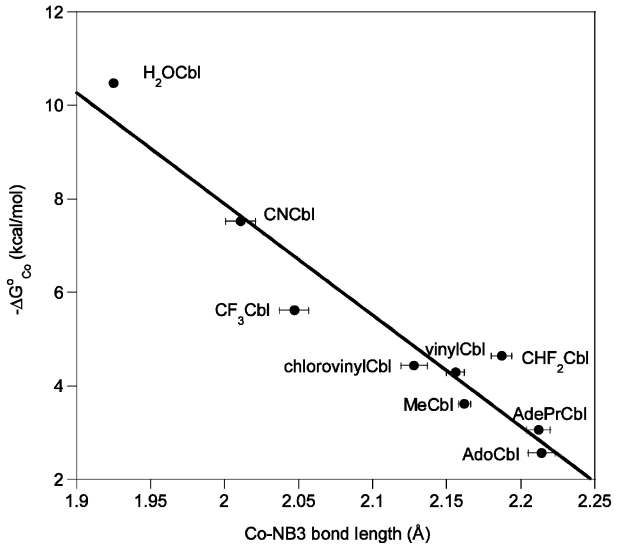

Figure 3. Empirical correlation between the Co−NB3 bond length and the free energy of formation of the base-on form (see the text). The solid line was obtained by nonlinear regression analysis. The slope of −24.1 ± 2.7 kcal mol^{-1} $Å^{-1}$ and intercept of 56.1 ± 5.8 kcal mol^{-1} are similar to those of a previous correlation with five structures.[22] The r^2 value is 0.917, with much of the error stemming from the two fluorinated cobalamins. When these data points are omitted, $r^2 = 0.984$. Data were taken from this work and the indicated references for CNCbl,[27] CNCbl(KCl),[15d] CF_3Cbl,[15c] CHF_2-Cbl,[28] MeCbl,[15d] AdePrCbl,[29] AdoCbl,[15e] and H_2OCbl.[18]

Both vinylcobalamins were photolyzed in aqueous solution in the presence of a 130 mM concentration of the water-soluble nitroxide radical trap 4-hydroxy-TEMPO (H-TEMPO) using white light from a 200 W light bulb. These reactions were considerably (~20-fold; see below) slower for the vinyl complexes than for methylcobalamin under identical conditions. For vinylcobalamin, a mixture of cob(II)alamin and aquocob(III)alamin products was observed. Aquocob(III)alamin is formed due to follow-up electron transfer between Co^{II} and the nitroxide trapping agent.[36] This latter reaction is slow[36b] relative to photolysis in the case of alkylcobalamins, but because of the longer reaction times for photolysis of vinylcobalamin and the requirement for higher concentrations of nitroxide to trap the vinyl radical, oxidation of cob(II)alamin by H-TEMPO was observed in the present case. Surprisingly, photolysis of (*Z*)-chlorovinylcobalamin in the presence of 130 mM H-TEMPO only showed aquocob(III)alamin and no cob(II)alamin even though the overall rates of photolysis of both compounds are comparable. DFT calculations[37] show that the standard redox potential of the chlorovinyl radical/anion in water (+0.29 V) is more positive than that of the Co(II)/Co(III) couple of B_{12} (0.20 V).[10] Therefore, the Co(III) species is likely produced by in-cage electron transfer from cob(II)alamin to the initially formed 2-(*Z*)-chorovinyl radical to yield aquocob(III)alamin and the 2-(*Z*)-chlorovinyl anion. The Co(III) formation is less pronounced for photolysis of vinylcobalamin because the standard

(19) Butin, K. P.; Beletskaya, I. P.; Kashin, A. N.; Reutov, O. A. *J. Organomet. Chem.* **1967**, *10*, 197−210. Note that this pK_a (MSAD scale) is actually for the proton *trans* to the chlorine atom. To the best of our knowledge, no data are available for the *cis*-proton.
(20) Reutov, O. A.; Beletskaya, I. P.; Butin, K. P. *CH-Acids. A guide to all existing problems of CH-acidity with new experimental methods and data, including indirect electrochemical, kinetic, and thermodynamic studies*; Pergamon: Oxford, 1978.
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(24) The pK_a values given are uncorrected for the presence of 5-coordinate species. Pratt and co-workers have further analyzed these types of pH dependencies by distinguishing between 5- and 6-coordinate complexes. In doing this, a $pK_{a,obs}$ of 2.4 for vinylcobalamin was corrected to a value of 1.9. See: (a) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2259−66. (b) Chemaly, S. M.; Pratt, J. M. *J. Chem. Soc., Dalton Trans.* **1980**, 2267−73.
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(30) Martin, B. D.; Finke, R. G. *J. Am. Chem. Soc.* **1990**, *112*, 2419−2420.
(31) Halpern, J.; Kim, S. H.; Leung, T. W. *J. Am. Chem. Soc.* **1984**, *106*, 8317−8319.
(32) Halpern, J. *Polyhedron* **1988**, *7*, 1483−1490.
(33) Hay, B. P.; Finke, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 4820−4829.
(34) Hay, B. P.; Finke, R. G. *Polyhedron* **1988**, *7*, 1469−1481.
(35) Alternatively, the rate of appearance of the TEMPO-trapped radicals may be used to determine the rate of Co−C bond cleavage (Fleming, P. E.; Daikh, B. E.; Finke, R. G. *J. Inorg. Biochem.* **1998** *69*, 45−57). Attempts along this line are currently ongoing.
(36) TEMPO and H-TEMPO have been shown previously to oxidize cob(II)alamin in water. In ethylene glycol this reaction is much slower and does not occur over the time period of the experiments described here. See also: (a) Finke, R. G.; Hay, B. P. *Inorg. Chem.* **1984**, *23*, 3041−3043. (b) Brown, K. L.; Zou, X.; Evans, D. R. *Inorg. Chem.* **1994**, *35* 5713−5720.
(37) Pratt, D. A.; van der Donk, W. A. *J. Am. Chem. Soc.*, in press.

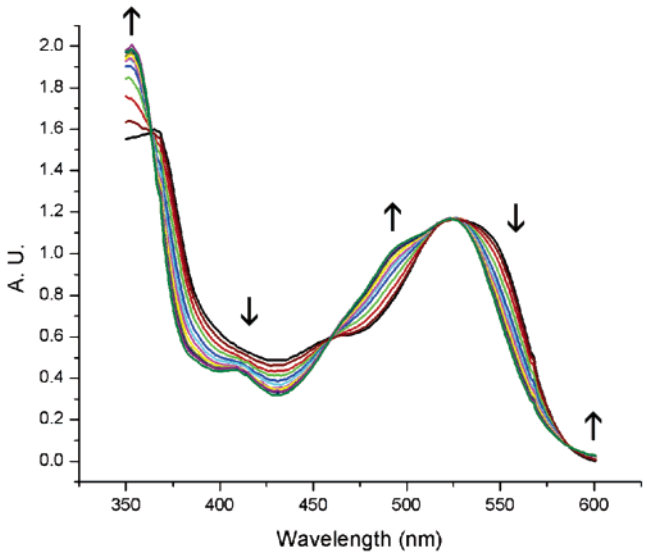


Figure 4. Spectral changes during photolysis of 150 μM (*Z*)-chlorovinylcobalamin in 0.1 M phosphate buffer, pH 7.0, over 60 min. The consumption of (*Z*)-chlorovinylcobalamin followed first-order kinetics (Figure S1, Supporting Information).

reduction potential of the vinyl radical is too negative for an analogous process (calculated $E° = -0.43$ V),[37] and hence, Co(III) formation in this case is due solely to oxidation of the originally formed cob(II)alamin by H-TEMPO. Consistent with this postulate, (*Z*)-chlorovinylcobalamin was readily photolyzed to aquocob(III)alamin with three isosbestic points in the region of 350−600 nm even in the absence of any H-TEMPO and under strictly anaerobic conditions (Figure 4).

Switching solvents to ethylene glycol omitted any interference from the follow-up oxidation of Co(II) by H-TEMPO in the photolysis of vinylcobalamin.[36] In this solvent, clean conversion of vinylcobalamin to cob(II)alamin was observed in 30 min with first-order kinetics (Figure 5A). Comparison of the observed first-order rate constant obtained as an average of five measurements (0.0029 ± 0.0005 s^{-1}) to that observed for methylcobalamin (Figure 5B) under identical experimental conditions (0.062 ± 0.03 s^{-1}) identifies a ca. 20-fold slower rate of photolysis for vinylcobalamin.

The relatively slow photoinduced Co−C bond scission of the vinylcobalamins is due either to the much stronger bond or to an unusually large cage effect that results in much more efficient geminate cage recombination for the vinylcobalamins compared to methylcobalamin.[38] Time-resolved studies on the photolysis of MeCbl, AdoCbl, and AdoCbi have shown that the geminate recombination rate can vary greatly,[39−41] accounting in part for their differences in quantum yields ($\phi = 0.20$ for AdoCbl, $\phi = 0.35$ for MeCbl, and $\phi = 0.045$ for AdoCbi).[40−42] Grissom and colleagues suggested that the higher efficiency of cage recombination in AdoCbl compared to MeCbl is the result of a more pyramidal Ado radical that is initially produced.[39] Using similar arguments, the initially produced sp^2-hybridized vinyl radical produced by homolytic cleavage of the Co−C bond in **1** and **2** is expected to give rise to more efficient recombination as it is directed entirely at the Co d_{z^2} orbital.

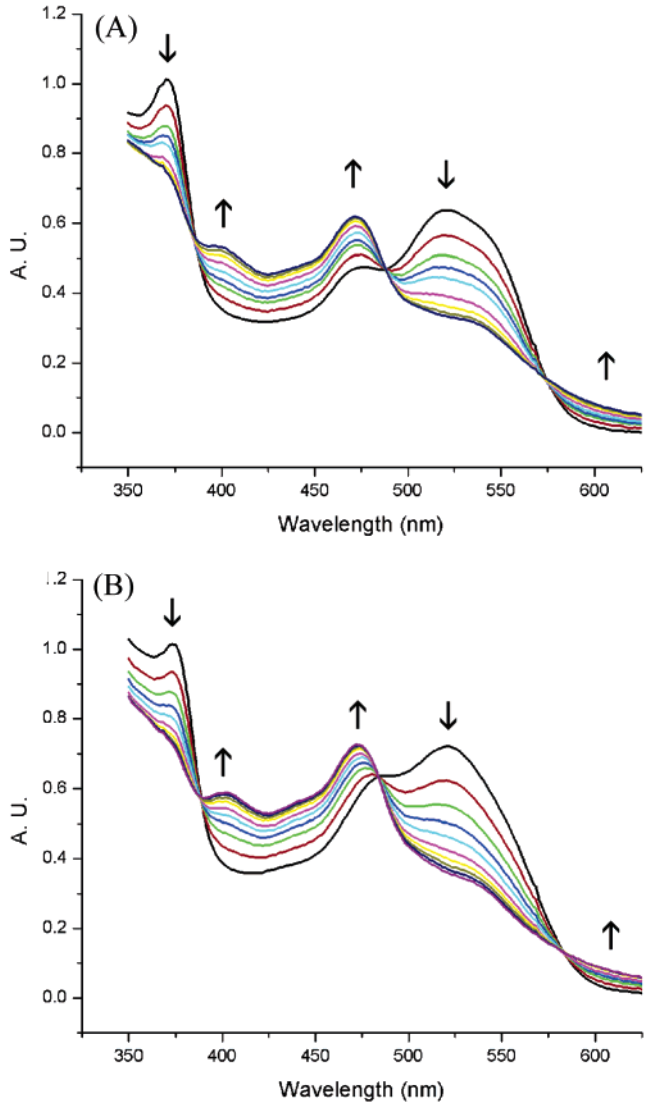


Figure 5. Spectral changes during photolysis of 100 μM (A) vinylcobalamin and (B) methylcobalamin in ethylene glycol containing 130 mM TEMPO over 30 min and 60 s, respectively.

Reductive Dechlorination of *cis*-Chlorovinylcobalamin. As anticipated, complex **1** proved very stable in aqueous solution at room temperature, questioning its possible involvement in the catalytic dechlorination process. However, when 140 μM **1** was treated anaerobically with excess titanium(III) citrate (5 mM), a slow decrease in the absorbance of **1** at 527 nm was observed concomitant with formation of cob(I)alamin at 384 nm.[43] After 1 h, the decrease in absorbance at 527 nm accelerated before leveling off, at which point vinylcobalamin **2** was isolated in 50% yield; complex **1** was completely consumed. In our preliminary paper we attributed the observed acceleration in the consumption of **1** after an initial lag to the

(38) Quantitative values for cage effects in B_{12} systems have been documented for several solvents. Adenosylcobinamide displayed a thermal fractional cage efficiency factor (F_c) between 0.94 and 1.0 in ethylene glycol at 110 °C (Garr C. D.; Finke, R. G. *J. Am. Chem. Soc.* **1992**, *114*, 10440−10445), adenosylcobalamin has an F_c between 0.4 and 1.0 in the same solvent (Garr C. D.; Finke, R. G. *Inorg. Chem.* **1993**, *32*, 4414−4421), and in water the F_c of cyanomethylcobinamides is between 0.049 and 0.12 (ref 36b).

(39) Lott, W. B.; Chagovetz, A. M.; Grissom, C. B. *J. Am. Chem. Soc.* **1995**, *117*, 12194−12201.

(40) Walker, L. A., Jr.; Jarrett, J. T.; Anderson, N. A.; Pullen, S. H.; Matthews, R. G.; Sension, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 3597−3603.

(41) Walker, L. A.; Shiang, J. J.; Anderson, N. A.; Pullen, S. H.; Sension, R. J. *J. Am. Chem. Soc.* **1998**, *120*, 7286−7292.

(42) Chen, E.; Chance, M. R. *Biochemistry* **1993**, *32*, 1480−1487.

(43) The cob(I)alamin peak is partially obscured by the absorbance of the excess titanium(III) citrate, preventing verification of the stoichiometry of chlorovinylcobalamin consumed and cob(I)alamin produced.

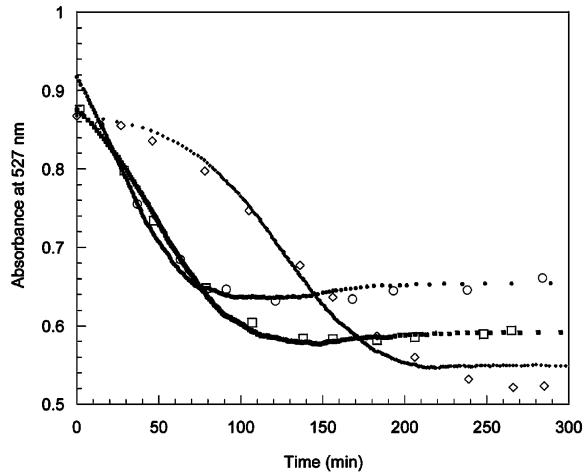
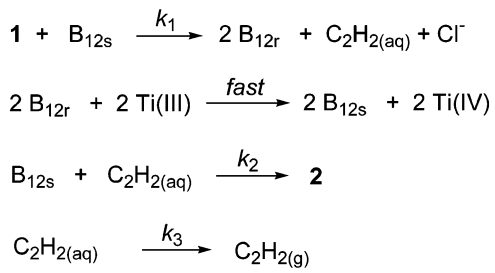


Figure 6. Time-dependent changes of the absorbance at 527 nm during anaerobic reaction of cobalamin **1** (0.14 mM) with 5 mM titanium(III) citrate at pH 9 in the presence of 0.0014 mM (tilted squares), 0.014 mM (squares), and 0.028 mM (circles) hydroxocobalamin. Also shown in smaller symbols are the calculated absorbance changes by simulation of the overall process according to Scheme 3 and the rate constants presented in the text.

Scheme 3



formation of cob(I)alamin via reductive Co−C bond cleavage. This species then acts as a catalyst for further dechlorination of the remaining **1**. To test this hypothesis, **1** was treated with excess titanium(III) citrate and catalytic amounts of cob(I)alamin (1%, 10%, or 20%). The latter two reactions did not display a significant lag, and increasing the concentration of cob(I)alamin enhanced the initial rate (Figure 6). The Co(I)-catalyzed reduction of **1** was unexpected since the potentials required to reduce alkylcobalamins ($E° < -1.5$ V)[44] are typically much more negative than that of the Co(I)/Co(II) couple of B_{12} (−0.61 V)[10] or the redox potential of titanium(III) citrate ($E° \approx -0.6$ V at pH 8).[6,45] A mechanistic model for the overall process is diagrammed in Scheme 3.[46] In this model, cob(I)alamin (vitamin B_{12s}) promotes reduction of **1** to generate two molecules of cob(II)alamin (B_{12r}) and acetylene, which we detected in this study by GC as discussed in a later section. The cob(II)alamin initially formed is rapidly reduced to cob(I)alamin by the excess titanium(III) citrate present. We estimated the rate of this latter reaction by independent stopped-flow experiments in which cob(II)alamin was reacted with excess titanium(III) citrate under pseudo-first-order conditions (data not shown). From these studies we deduce a second-order rate constant of $(16.9 \pm 0.2) \times 10^3$ M^{-1} min^{-1} for the reduction of Co(II) to Co(I); the reduction of hydroxocobalamin to cob(II)alamin under the same conditions was too fast to be measured. These rate constants are much larger than the rate constant for consumption of **1** (~4 M^{-1} s^{-1}; see below), and therefore, the cob(II)alamin produced initially is actually never observed as it is reduced rapidly to cob(I)alamin, which is observed spectroscopically. Cob(I)alamin reacts with acetylene to produce vinylcobalamin **2**,[5,13,47] in competition with escape of acetylene into the atmosphere (sublimation point −81 °C). Given the impediments that Scheme 3 introduces with respect to direct kinetic measurements, we attempted simulation of the observed kinetics according to Scheme 3. As shown in Figure 6, a set of parameters was found that simulated the observed absorbance changes quite accurately ($k_1 = 3.83$ M^{-1} s^{-1}, $k_2 = 4.0$ M^{-1} s^{-1}, $k_3 = 2.5 \times 10^{-4}$ s^{-1}).[48] Of special note is that the autocatalytic aspects are well reproduced for the reactions with 1% and 10% cob(I)alamin catalyst (sigmoidal curves), and that the same set of rate constants is able to reproduce the observed absorbance changes for three different experiments with varying amounts of cob(I)alamin catalyst. Also well reproduced are the final absorbances in these three independent experiments. The simulations predict that at $t = 6$ h about half of the cobalt species is present as vinylcobalamin and half as cob(I)alamin, which is consistent with the observed spectroscopic data. Vinylcobalamin **2** did not react to an appreciable extent when subjected to the same reaction conditions for a period of 6 h, explaining the constant absorbance at 527 nm after all chlorovinylcobalamin has been reduced.

Mechanism of Conversion of *cis*-Chlorovinylcobalamin to Vinylcobalamin. Several different mechanisms can be drawn for the reductive conversion of **1** to **2**. The spectroscopically observed production of cob(I)alamin suggests that the Co−C bond is broken in this transformation, a supposition that is further corroborated by the results of cyclic voltammetry (vide infra). Cleavage of the Co−C bond in the initial one-electron-reduced intermediate provides either the chlorovinyl radical and cob(I)alamin (Scheme 4, pathway a)[44a,b] or cob(II)alamin and the chlorovinyl anion,[44e] which may partition between protonation and chloride elimination (Scheme 4, pathway c). Concerted cleavage of the Co−C and C−Cl bonds would directly produce cob(II)alamin, acetylene, and chloride anion (pathway b). We performed a series of deuterium labeling experiments to interrogate the details of the reduction of **1**.

Treatment of pure, isolated complex **1** with cob(I)alamin/titanium(III) citrate in D_2O produced vinylcobalamin **2a** deuterium labeled at all three positions of the vinyl ligand as determined by ^1H NMR spectroscopy and ESI-MS (eq 2). When the transformation was stopped at ~50% conversion, the recovered complex **1** did not contain any deuterium, ruling out exchange of the label from solvent into the starting complex. Subjecting vinylcobalamin **2** to the reaction conditions in D_2O also did not result in incorporation of deuterium into the vinyl

(44) (a) Lexa, D.; Savéant, J. M. *J. Am. Chem. Soc.* **1978**, *100*, 3220. (b) Rubinson, K. A.; Itabashi, E.; Mark, H. B., Jr. *Inorg. Chem.* **1982**, *21*, 3571−3573. (c) Shepherd, R. E.; Zhang, S.; Dowd, P.; Choi, G.; Wilk, B.; Choi, S. C. *Inorg. Chim. Acta* **1990**, *174*, 249. (d) Kumar, V. T.; Birke, R. L. *Anal. Chem.* **1993**, *65*, 2428−2436. (e) Cobalamins containing strong electron-withdrawing groups have less negative potentials and produce Co(II) and an organoanion upon reduction: Zhou, D.-L.; Tinembart, O.; Scheffold, R.; Walder, L. *Helv. Chim. Acta* **1990**, *73*, 2225.

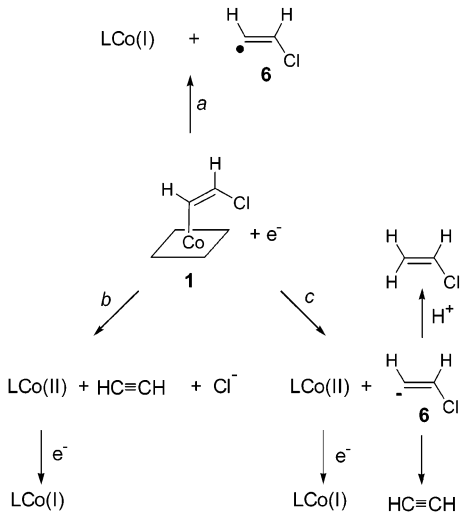
(45) Zehnder, A. J. B. Thesis no. 5716, ETH, Zurich, 1976; p 5716.

(46) In the Supporting Information of ref 9 we intended to present a similar scheme, but the oxidation states of the cobalamins were given incorrectly.

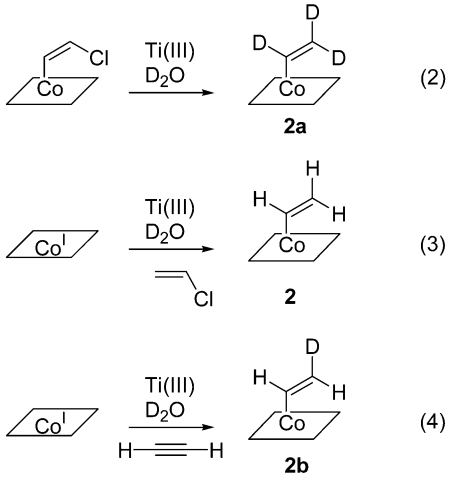
(47) Naumberg, M.; Duong, K. N. V.; Gaudemer, A. *J. Organomet. Chem.* **1970**, *25*, 231−242.

(48) These rate constants were obtained by iterative simulation of the three independent experiments using the chemical kinetics simulator (IBM, 1996). When a global fitting program was utilized to fit all three experiments simultaneously (Dynafit) (Kuzmic, P. *Anal. Biochem.* **1996**, *237*, 260−273), the following very similar rate constants were obtained: $k_1 = 3.44 \pm 0.096$ M^{-1} s^{-1}, $k_2 = 3.37 \pm 0.40$ M^{-1} s^{-1}, and $k_3 = (1.83 \pm 0.25) \times 10^{-4}$ s^{-1}.

Scheme 4



ligand. Thus, the deuterium incorporation must be occurring during the reductive dechlorination, possibly at the stage of the organic intermediate. To probe this possibility, cob(I)alamin was reacted with vinyl chloride in D_2O. ^1H NMR analysis of the product indicated the formation of **2** without any deuterium incorporation into the vinyl group (eq 3). Accordingly, vinyl chloride is not a reaction intermediate in the conversion of **1** to **2**. Cob(I)alamin was also reacted with acetylene in D_2O to generate **2b**, via *anti* addition (eq 4). At first glance, this also



seems to rule out acetylene as an intermediate in the conversion of **1** to **2**. However, the conditions of the reaction in eq 4, which involved purging a cob(I)alamin solution with a large amount of acetylene, do not accurately mimic the conditions in the conversion of **1** to **2**. Specifically, the rate of the formation of **2b** in eq 4 is significantly faster than that of **2a** in eq 2. At a lower concentration of acetylene, and hence a longer exposure time to the deuterated solvent, exchange of the alkyne protons with solvent can occur prior to reaction with cob(I)alamin. Indeed, fully deuterated complex **2a** was observed as the product in reactions between cob(I)alamin and acetylene when acetylene was kept over a solution of titanium(III) citrate/D_2O for several hours before addition of cob(I)alamin. The most definitive evidence that acetylene is indeed the intermediate in the transformation of **1** into **2** was obtained by GC analysis of the headspace of the reaction performed in a closed container, which

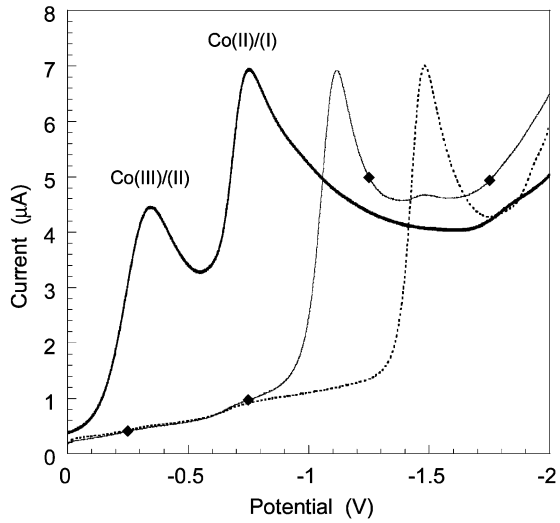


Figure 7. Linear scan voltammograms showing the peak potentials of 1 mM hydroxo-Cbl (solid line), **1** (solid line with tilted squares), and **2** (dashed line) at 25 °C and a scan rate of 0.1 V/s. Potentials were measured against an internal standard with a glassy carbon electrode (Experimental Section) and are reported vs a Ag^+/AgCl reference electrode (3 M KCl).

unambiguously showed formation of acetylene, whereas no evidence for vinyl chloride (bp −13 °C) was obtained. These findings on *cis*-chlorovinylcobalamin correlate well with previous model studies on dichlorinated vinylcobaloximes showing that reduction produces chloroacetylene.[7,8] Furthermore, these experimental findings are also in full agreement with recent DFT calculations that arrived at the same conclusion.[37] Collectively, these studies show that reduction of vinylcobalt complexes with a chlorine on the β-carbon gives rise to elimination products.

Electrochemistry. Our model studies on vinylcobaloxime and its chlorinated analogues showed that, with each additional chlorine substituent on the vinyl ligand, the peak potential for reduction shifted approximately 150 mV to less negative values.[7] To evaluate the effect of chlorination for vinylcobalamin, linear scan voltammograms were recorded of **1** and **2** using a glassy carbon electrode to minimize adsorption. The observed waves indicate that the influence of chlorine substitution is much larger in these complexes than in the cobaloxime models since the peak potential shifts from −1.48 V vs Ag^+/AgCl for vinylcobalamin to −1.11 V for *cis*-chlorovinylcobalamin (Figure 7).[49] Thus, one chlorine on the β-carbon brings the peak potential about halfway to that of the Co(I)/Co(II) couple of B_{12}. This finding has interesting implications with respect to dichloro- or trichlorovinylcobalamins. As mentioned, we have been unsuccessful in preparing these compounds in reactions of cob(I)alamin with PCE, TCE, or dichloroacetylene. Given the remarkable shift in peak potential upon introduction of one chlorine, we surmise that these complexes once formed will be consumed under the reaction conditions, because such multichlorinated vinylcobalamins will have reduction potentials that are close to or less negative than the Co(II)/Co(I) potential of B_{12} and the Ti(III)/Ti(IV) potential of titanium(III) citrate.

We reported in our preliminary paper that the reduction wave for **1** in cyclic voltammograms was completely irreversible at scan rates up to 1 V/s.[9] An anodic return wave was observed at −0.77 V for the oxidation of cob(I)alamin to cob(II)alamin, confirming that reduction of complex **1** leads to Co−C bond

(49) See the Experimental Section for conversion to potentials vs NHE.

cleavage. Similar observations were made for vinylcobalamin **2** at 100 mV/s. Surprisingly, however, cyclic voltammograms recorded at faster scan rates (1 V/s) resulted in a quasi-reversible wave ($i_a \approx i_c$, $\Delta E_p = 90$ mV). To the best of our knowledge, this is the first organocobalamin to give reversible reduction behavior at ambient temperature and without the need for ultrafast scan rates. The only other organocobalamin for which reversible electrochemistry has been reported is methylcobalamin, but reversibility required the experiment to be conducted at −20 °C and 200 V/s.[44a] We attribute the high stability of the one-electron-reduced intermediate to the stronger Co−C bond in vinylcobalamin compared to alkylcobalamins that have been investigated previously electrochemically.[50]

The origin of the different behavior of the two complexes, showing a quasi-reversible reduction wave for vinylcobalamin but not for *cis*-chlorovinylcobalamin under similar conditions, can be accounted for by a much faster follow-up reaction for the latter. The rates of dissociative electron-transfer processes are governed by a number of properties of the electron acceptor including its redox potential and the bond dissociation energy of the bond that is broken.[51] On the basis of the similar Co−C bond lengths and pK_a values for protonation of the dimethylbenzimidazole ligand, the two ground-state energies of the Co−C bonds in **1** and **2** are not very different and are unlikely to account for the different electrochemical behavior.[52] Rather, on the basis of the observed production of acetylene as well as the results of a recent theoretical study,[37] the higher lability toward reductive cleavage of the Co−C bond of **1** compared with **2** involves a change in the mechanism of Co−C bond cleavage. Most one-electron reductions of organocobalamins lead to formation of cob(I)alamin and the organic radical,[53] and this is also the case for vinylcobalamin.[37] In contrast, reduction of **1** results in chloride elimination from the one-electron-reduced intermediate and formation of acetylene (Scheme 4). Hence, the different types of chemical follow-up reactions for **1** and **2** are responsible for the observed differences in reversibility of the electrochemistry.

Discussion

cis-Chlorovinylcobalamin was obtained as the only product in the reaction of cob(I)alamin with chloroacetylene, in contrast to our previous observations with cob(I)aloxime, which provided two isomers (Scheme 2). This difference may be due to the different pK_a values of "hydridocob(I)aloxime" (∼10.5)[54] and the putative hydride of cob(I)alamin (∼1−1.5).[55,56] Hence, in the cobaloxime experiments performed at pH 10−11 some of the nucleophile was present as a cobalt hydride, which has been shown to give *syn* addition to acetylenes with opposite regiochemistry compared to deprotonated cob(I)aloxime.[57] Cob(I)alamin, however, is entirely present in the unprotonated form at alkaline pH, which results exclusively in *anti* addition,[47] giving *cis*-chlorovinylcobalamin as the only product. A second difference between the cobaloxime study and the current investigation is evident in the ease of reduction. The electrochemical experiments show that the presence of one chloride on the vinyl ligand shifts the peak potential by a remarkable 0.37 V. This effect is much more pronounced than observed for the corresponding cobaloximes ($\Delta E_p = 0.15$ V). Part of this shift in peak potentials arises from thermodynamic differences in the true reversible redox potentials between **1** and **2**, and part is caused by kinetic coupling in the case of complex **1**. That is, the peak potential of **1** is associated with an irreversible electrochemical process in which rapid chloride elimination perturbs the equilibrium of the electron-transfer step and shifts the observed peak potential to less negative values.[58] We have estimated this shift caused by a fast chemical follow-up reaction to be ∼120 mV.[9] On the other hand, complex **2** does not have the possibility of chloride elimination upon one-electron reduction. Its cyclic voltammogram still shows irreversible behavior at scan rates of <0.1 V/s, due to cleavage of the Co−C bond. However, as shown by the reversible waves seen at scan rates >1 V/s, this chemical follow-up reaction is relatively slow because of the rather strong Co−C bond.

As evidenced by MS, both di- and monochlorinated vinylcobalamins are formed during the reductive dechlorination of PCE.[6] The E_p of these complexes is expected to shift toward less negative potentials when the number of chlorides on the vinyl ligand is increased,[7] and hence, reduction of di- and trichlorinated vinylcobalamins will occur readily, allowing return of the catalyst to the active Co(I) form. In fact, for trichlorinated vinylcobalamin, the reduction potential will be close to or less negative than the Co^{I}/Co^{II} couple of B_{12}, explaining why trichlorinated vinylcobalamin has never been detected. On the other hand, vinylcobalamin **2** subjected to the same reaction conditions was not dealkylated over 6 h. The resistance of **2** toward reductive dealkylation prevents the rapid regeneration of the active catalyst, and this sequestering presents an obstacle for efficient B_{12}-catalyzed reductive dechlorination of chlorinated ethylenes.

Comparison with Environmental Studies. The results presented here provide some illuminating insights with respect to previous kinetic studies of the B_{12}-catalyzed dechlorination of PCE and TCE. Three studies showed that chloroacetylene is a precursor to acetylene.[3e−g] On the basis of kinetic modeling, Reinhard and co-workers proposed that ethynylcobalamin was formed upon reaction of cob(I)alamin with chloroacetylene and that ethynylcobalamin was subsequently converted to acetylene

(50) One alternative explanation that was suggested by an anonymous reviewer of ref 9 attributed the reversibility of the reduction of vinylcobalamin to transfer of the electron to the axial vinyl ligand instead of to the corrin ring. This scenario, however, is not consistent with DFT calculations (ref 37).

(51) Savéant, J. M. *Acc. Chem. Res.* **1993**, *26*, 455−461.

(52) Reductive cleavage of the Co−C bond in alkylcobalamins involves two unoccupied low-energy orbitals featuring a corrin orbital (π8) and the antibonding combination of the d_{z^2} and sp^3 orbitals on the metal and ligand, respectively (Salem, L.; Eisenstein, O.; Anh, N. T.; Burgi, H. B.; Devaquet, A.; Segal, G.; Veillard, A. *Nouv. J. Chim.* **1977**, *1*, 335−348). On the basis of electrochemical evidence, Scheffold and co-workers suggested that a fast electron transfer occurs from the electrode to the empty corrin-based orbital prior to an electronic transition to the σ* orbital associated with the Co−C bond. In this picture, the products of reductive cleavage are cob(I)alamin and an organic radical, and the precise energy level of the σ* orbital controls the rate of Co−C bond cleavage with a rate increase for ligands that can stabilize the organic radical (ref 44e). When the vinyl and 2-chlorovinyl radical products are compared, the latter is expected to be more stable due to both inductive and resonance effects (Nonnenberg, C.; van der Donk, W. A.; Zipse, H. *J. Phys. Chem. A* **2002**, *106*, 8708−8715), thereby possibly accounting for significantly different rates of Co−C bond cleavage in **1** and **2**. However, the observed similar rates of photochemical cleavage suggest that increased stabilization of the *cis*-chlorovinyl radical does not account for the greatly different redox behavior.

(53) Rubinson, K. A.; Itabashi, E.; Mark, H. B. J. *Inorg. Chem.* **1982**, *21*, 3571−3573.

(54) Schrauzer, G. N.; Holland, R. J. *J. Am. Chem. Soc.* **1971**, *93*, 1505−1506.

(55) Lexa, D.; Savéant, J. *J. Chem. Soc., Chem. Commun.* **1975**, 872−874.

(56) Rubinson, K. A.; Parekh, H. V.; Itabashi, E.; Mark, H. B., Jr. *Inorg. Chem.* **1983**, *22*, 458−463.

(57) Johnson, M. D.; Meeks, B. S. S. *J. Chem. Soc. B* **1971**, 185−189.

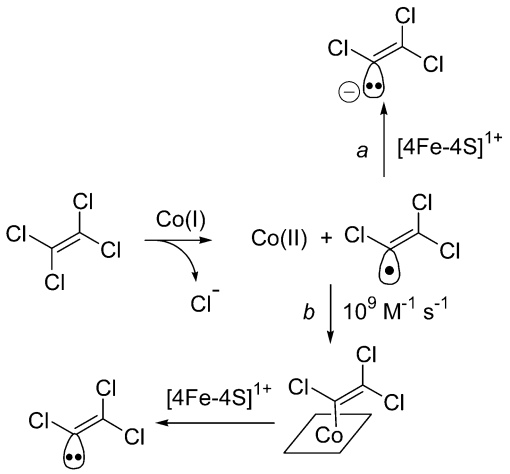
(58) (a) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964**, *36*, 706−723. (b) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1965**, *37*, 178−190.

by reductive Co−C bond cleavage.[3g] In this work we corroborate the overall pathway, but we show that it is *cis*-chlorovinylcobalamin **1** that is the actual link between chloroacetylene and acetylene. In the same study, kinetic modeling of the conversion of acetylene into ethene suggested half-lives of 251 and 1.4 h for vinylcobalamin and ethynylcobalamin, respectively, with the latter shown here to be *cis*-chlorovinylcobalamin. The two studies were conducted under quite different conditions, but qualitatively these half-lives correlate very well with the relative reactivities determined in this work for pure, isolated vinyl- and *cis*-chlorovinylcobalamins.

Comparison with Enzymatic Dechlorination. The reductive dechlorination of perchloroethylene is one of the few examples where the rate constants for an enzymatic reaction and a nonenzymatic cofactor-dependent reaction can be compared.[59] Using the k_{cat} (243 s^{-1}) and K_m (0.28 mM) values[60] reported for the enzyme from *Dehalospirillum multivorans*, the second-order rate constant for the enzymatic reaction (k_{cat}/K_m) can be calculated as 8.7×10^5 M^{-1} s^{-1}. The second-order rate constant for the nonenzymatic B_{12}-catalyzed reaction is 179 M^{-1} s^{-1} at pH 8.[61] Therefore, the enzyme achieves a rate enhancement of 4800-fold over the reaction with the cofactor itself, a value that is low compared to rate enhancements for other enzymes.[62] This relatively small rate acceleration is primarily the result of the high rate of the nonenzymatic process, which for this reason has received much attention for its potential in remediation.

Our findings suggest an interesting possibility for the enzymatic dechlorination. The dehalogenases characterized to date contain a corrin and two iron sulfur clusters.[63] When the fully reduced protein from *Dehalobacter restrictus* was reacted with PCE, Co(II) formation was observed, suggesting a one-electron-transfer mechanism[64] analogous to our stopped-flow results on the abiotic reaction.[4] Little is known regarding the steps following this electron transfer. It is conceivable that the initially formed trichlorovinyl radical is reduced to the anion by electron transfer from one of the reduced low-potential (∼−480 mV)[64,65] iron sulfur clusters (Scheme 5, pathway a). The findings in this work also offer an alternative explanation (Scheme 5, pathway b). After one-electron transfer and chloride elimination, cob(II)alamin and a trichlorovinyl radical would be juxtaposed within the active site. Cob(II)alamin has a very high affinity for organic radicals with rate constants for diffusive combination of $\sim 10^9$ M^{-1} s^{-1}.[39,66−69] In this instance, recombination would thus generate trichlorovinylcobalamin, which could subsequently be reduced by electron transfer from one of the reduced FeS clusters, resulting in Co(II) and a trichlorovinyl anion or Co(I) and a trichlorovinyl radical.[70] Scheffold and co-workers have shown that organocobalamins featuring ligands with a low pK_a favor the former pathway,[44e] and with a reported pK_a of 18 for TCE,[19] trichlorovinylcobalamin is expected to produce the trichlorovinyl anion. If this latter step is fast, then reacting the reduced form of the protein with PCE would lead to cob(II)alamin as the observed product in hand-mixed experiments, in agreement with the experimental data.[64] Mechanism b would have the advantage that it would limit the exposure of the active site to a highly reactive trichlorovinyl radical.

Scheme 5



Conclusions

In summary, chlorovinyl- and vinylcobalamins observed previously by mass spectrometry in abiotic B_{12}-catalyzed dechlorination of PCE and TCE can be generated by reaction of cob(I)alamin with chloroacetylene and acetylene, compounds detected in these processes.[3e,g] Return of the chlorinated vinylcobalamins to the active form of the catalyst is achieved via reductive dealkylation promoted by cob(I)alamin. This reductive dealkylation is particularly facile for multichlorinated vinylcobalamins, but not for vinylcobalamin. The mechanism of the reductive dechlorination of *cis*-chlorovinylcobalamin involves the formation of acetylene, by elimination of chloride upon electron transfer. Reduction of vinylcobalamin cannot follow such a mechanism, and as a result, the one-electron-reduced intermediate is remarkably stable due to the strong Co−C bond and electrochemical reduction becomes reversible. The large difference in peak potentials of chlorovinylcobalamin and vinylcobalamin suggests that a trichlorovinylcobalamin, if it were produced, would be rapidly reduced, precluding its observation.

Experimental Section

All NMR spectra were recorded on Varian U400 and U500 spectrometers. ^{1}H NMR spectra of cobalamins were referenced to the peak for CH_3OH at 3.31 ppm. Mass spectrometry (MS) experiments

(59) For other examples of organic cofactors or cofactor analogues that can catalyze the same reaction as enzymes that require them, see: (a) Lee, Y.; Sayre, L. M. *J. Am. Chem. Soc.* **1995**, *117*, 3096−3105. (b) Mure, M.; Klinman, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 8698−8706. (c) Doll, K. M.; Finke, R. G. *Inorg. Chem.* **2003**, *42*, 4849−4856. (d) Doll, K. M.; Bender, B. R.; Finke, R. G. *J. Am. Chem. Soc.* **2003**, *125*, 10877−10884. (e) A large number of small molecular weight metal complexes have been studied that catalyze the same reaction as the enzymes they aim to model. See: Iranzo, O.; Kovalesky, A. Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 1988−93 and references therein.

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(62) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93. In this report, rate enhancements for the enzymatic over the nonenzymatic reaction are given for a series of enzymes that do not use a cofactor. The rate enhancements are then calculated by comparing k_{cat} with k_{non}, which are both unimolecular rate constants.

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(70) Of course, as shown in AdoCbl-dependent enzymes, close proximity of cob(II)alamin and an organic radical does not need to lead to alkylcobalamin formation and will be determined by the active site geometry.

were carried out by the Mass Spectrometry Laboratory at the University of Illinois at Urbana-Champaign (UIUC). Compounds and solvents were obtained from Fisher, Aldrich, Acros, Baker, Fluka, Sigma, and Mallenkrodt. All manipulations were performed using Schlenk and inert glovebox techniques.

Synthesis of 1 and 2. Under an Ar atmosphere, a 100 mL round-bottom flask was charged with hydroxocobalamin (0.1 g, 0.072 mmol), cobalt nitrate (0.001 g, 0.003 mmol), and 9 mL of degassed, distilled H_2O. $NaBH_4$ (0.020 g, 0.53 mmol) dissolved in 0.5 mL of degassed, distilled H_2O was slowly injected into the cobalamin solution. After reduction was complete, chloroacetylene was generated as previously described,[71] and distilled into the cob(I)alamin solution at 25 °C for 2 h. (*Caution: Although no problems were encountered using chloroacetylene in this study, chloroacetylene may be explosive in the presence of oxygen!*) The cobalamin was extracted with phenol/CH_2Cl_2 (1:1) until the aqueous phase no longer contained any **1**. The phenol extracts were combined and were washed a minimum of three times with distilled H_2O (1/3 of the volume of the phenol solution). The phenol solution was then diluted with 1-butanol/CH_2Cl_2 (1:1; 10 times the volume of the phenol solution), and **1** was extracted into distilled H_2O until the phenolic phase no longer contained any cobalamin. Lyophilization of the aqueous solution resulted in the isolation of **1**. The complex was dissolved in 1 mM ammonium acetate buffer, pH 4, and further purified by RP-HPLC using an elution profile consisting of a gradient from 95:5 buffer/MeCN to 70:30 buffer/MeCN over 20 min on a preparative Vydac C18 protein and peptide column. Characteristic ^{1}H NMR signals (400 MHz, CD_3OD): δ (ppm) 5.03 (1 H, d, $J = 5.60$ Hz, vinyl H), 5.73 (1 H, d, $J = 5.60$ Hz, vinyl H). LRMS-ESI (*m*/*z*): 1392 ($M^+ + 1$), 696 ($M^{2+} + 1$). At pH 7, $\epsilon_{525\,nm} = 7 \times 10^3\ M^{-1}\ cm^{-1}$ and $\epsilon_{374\,nm} = 1 \times 10^4\ M^{-1}cm^{-1}$; at pH 1, $\epsilon_{460\,nm} = 4 \times 10^3\ M^{-1}\ cm^{-1}$. Cobalamin **2** was synthesized in an analogous fashion except acetylene was used instead of chloroacetylene as described in the literature.[13] Purification and crystallization were carried out as described for **1**. In the HPLC experiments complex **2** elutes before compound **1**. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 5.59 (1 H, dd, $J = 14.36$, 6.27 Hz, CoCH═). COSY (500 MHz) shows ═CHH resonances at 4.20 and 3.58 ppm. LRMS-ESI (*m*/*z*): 1358 ($M^+ + 1$), 680 ($M^{2+} + 1$). At pH 7, $\epsilon_{525\,nm} = 7 \times 10^3\ M^{-1}\ cm^{-1}$ and $\epsilon_{374\,nm} = 1 \times 10^4\ M^{-1}\ cm^{-1}$; at pH 1, $\epsilon_{460\,nm} = 5 \times 10^3\ M^{-1}\ cm^{-1}$. Crystallization of both **1** and **2** was achieved from 40% PEG. The crystals were mounted in mother liquor and 40% PEG 400 onto 0.2 and 0.3 mm cryoloops, respectively, and flash frozen in liquid nitrogen. Diffraction data for **1** were collected on a Bruker Proteum/M D8 diffractometer with graphite-monochromated Cu Kα radiation. The diffraction data for **2** were collected using a Siemens Platform/CCD diffractometer with graphite-monochromated Mo Kα radiation. The structures were solved by direct methods and refined against F2 (SHELX-97-2).[72,73] For further details see the tables of bond lengths and angles and the CIF file in the Supporting Information.

Determination of pK_a and pK_{Co}. The pK_a of complexes **1** and **2** was determined by recording their UV−vis spectra at a concentration of 0.1 mM in solutions of different pHs. All measurements were made in 50 mM glycine buffer. $K_{a,obs}$ values were obtained by least-squares regression analysis of the absorbance changes at 462 and 525 nm for the base-off and base-on forms, respectively, using the following equations: $A_{462} = [((A_{i,462})([H^+])/(K_a + [H^+])) + (A_{f,462} + K_a)/(K_a + [H^+])]$ and $A_{525} = [((A_{i,525})([H^+])/(K_a + [H^+])) + (A_{f,525} + K_a)/(K_a + [H^+])]$, where A_i and A_f are the absorbances for pure base-off and base-on forms, respectively. The $K_{a,obs}$ so obtained for the protonation of the dimethylbenzimidazole in complexes **1** and **2** was converted to K_{Co} values according to eq 5 previously derived by Brown and co-workers.[25] In this equation, K_{Bz} is the K_a value for α-ribazole (p$K_a = 5.56$ at 25 °C and 1.0 M ionic strength) and K_H is an equilibrium constant for two forms of the deprotonated base-off complex which has been determined to be 4.16.[23]

$$K_{a,obs} = (1 + K_H + K_{Co})K_{Bz} \quad (5)$$

Photolysis of Methyl-, Vinyl-, and *cis*-2-Chlorovinylcob(III)alamins. Degassed stock solutions of each organocobalamin (200−300 μM) were made up in either 0.1 M phosphate buffer, pH 7.0, or ethylene glycol. Degassed stock solutions (260 mM) of H-TEMPO and TEMPO were made up in phosphate buffer, pH 7.0, and ethylene glycol, respectively. Aliquots of 500 μL of one of the cobalamin solutions and 500 μL of either the H-TEMPO or TEMPO solution were combined in an argon-purged 1.5 mL airtight cuvette. Spectral changes, corrected for a baseline absorbance of 130 mM H-TEMPO or TEMPO, were recorded as a function of irradiation time. Samples were irradiated under argon with a 200 W light bulb positioned 5 cm from the cuvette for the specified time (generally up to 1 h). The results of representative experiments in buffer and ethylene glycol are shown in Figures 4 and 5, respectively. In buffer, the photolysis of vinylcob(III)alamin to cob(II)alamin was complicated by the subsequent formation of aquocob(III)alamin due to reaction with H-TEMPO. Photolysis of *cis*-2-chlorovinylcob(III)alamin resulted in the formation of aquocob(III)-alamin in the presence of H-TEMPO and in its absence. In ethylene glycol, the photolysis of methylcob(III)alamin and vinylcob(III)alamin yielded excellent first-order kinetics, and observed rate constants were obtained from plots of $\ln(A - A_\infty)$ versus time.

Experimental Procedure for Co(I)-Catalyzed Reductive Dealkylation. *cis*-Chlorovinylcobalamin **1** was dissolved in 50 mM Tris buffer (pH 9) at a concentration of 0.14 mM ($\epsilon_{527} = 6 \times 10^3\ M^{-1}\ cm^{-1}$). The appropriate quantities of hydroxocobalamin and titanium(III) citrate (5 mM final concentration) were added to the cobalamin solution, and the resulting solution's absorbance was monitored at 527 nm for 6 h in an airtight cuvette equipped with a septum cap. Initial rate studies were undertaken in a similar fashion except a solution of cob(I)alamin, obtained from a premixed solution of hydroxocobalamin and titanium(III) citrate, was added to the solution containing **1**. The differences in cobalamin concentrations in each sample due to addition of cob(I)-alamin and titanium(III) citrate introduced differences in absorbance at 527 nm. All initial absorbance measurements were therefore normalized to 0.9 unit.

Solvent-Labeling Studies. Preparation of Vinylcobalamin-d_3 2a. *cis*-Chlorovinylcobalamin **1** (0.1 g, 0.072 mmol) was dissolved in 200 mL of deuterated 50 mM Tris buffer (pH 9). Hydroxocobalamin (0.020 g, 0.014 mmol) and a deuterated titanium(III) citrate solution (5 mM final concentration) were added to the solution of **1**, and the resulting solution was allowed to stir for 6 h. The vinylcobalamin-d_3 was extracted with phenol/CH_2Cl_2 (1:1) until the aqueous phase no longer contained any **2a**. The phenol extracts were combined and were washed a minimum of three times with distilled H_2O (1/3 of the volume of the phenol solution). The phenol solution was then diluted with 1-butanol/CH_2Cl_2 (1:1; 10 times the volume of the phenol solution), and **2a** was extracted into distilled H_2O until the phenolic phase no longer contained any cobalamin. Lyophilization of the aqueous solution resulted in the isolation of **2a**. The complex was dissolved in 1 mM ammonium acetate buffer, pH 4, and further purified by RP-HPLC using an elution profile consisting of a gradient from 95:5 buffer/MeCN to 70:30 buffer/MeCN over 25 min to yield pure **2a** (0.044 g, 45%). LRMS-ESI (*m*/*z*): 1360 ($M^+ + 1$), 685 ($M^{2+} + 1$).

Detection of Acetylene by GC. A 27 mL test tube was charge with *cis*-chlorovinylcobalamin **1** (0.002 g, 0.0014 mmol), 6.66 mL of 50 mM Tris buffer (pH 9), and 0.091 mL of 369 mM titanium(III) citrate and sealed with a crimp seal septum under a nitrogen atmosphere. Periodically over a period of 5 h, a 0.4 mL sample of the headspace in the sealed tube was removed and replaced with an equal volume of N_2. The samples were analyzed with a Perkin-Elmer Autosystems GC

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instrument using a J&W Scientific GS-Q column (30 m × 0.53 mm). Authentic standards of acetylene and vinyl chloride were used to identify any volatile products. Only acetylene was detected.

Reaction of Cob(I)alamin with Acetylene in D_2O. Acetylene was allowed to equilibrate for 4 h in a sealed 250 mL round-bottom flask charged with 100 mL of a 50 mM Tris buffer in D_2O (pD 9.4) and 1.3 mL of a 367 mM solution of titanium(III) citrate in D_2O. Cob(I)alamin, generated by the reduction of hydroxocobalamin (0.08 g, 0.058 mmol) with 60 equiv of titanium(III) citrate in Tris buffer in D_2O, was injected into the flask. After the solution was stirred for 1 h, the vinylcobalamin-d_3 **2a** was extracted with phenol/CH_2Cl_2 (1:1) until the aqueous phase no longer contained any organocobalamins. The phenol extracts were combined and were washed a minimum of three times with distilled H_2O (1/3 of the volume of the phenol solution). The phenol solution was then diluted with 1-butanol/CH_2Cl_2 (1:1; 10 times the volume of the phenol solution), and **2a** was extracted into distilled H_2O until the phenolic phase no longer contained any cobalamin. Lyophilization of the aqueous solution resulted in the isolation of **2a**. The complex was dissolved in 1 mM ammonium acetate buffer, pH 4, and further purified by RP-HPLC using an elution profile consisting of a gradient from 95:5 buffer/MeCN to 70:30 buffer/MeCN over 25 min to yield pure **2a** (0.068 g, 85%). Analysis by ^{1}H NMR spectroscopy showed the absence of the signals associated with the protons on the vinyl group, and MS analysis showed an increase of 3 mass units compared to authentic **2**.

Electrochemistry. Cyclic voltammetry measurements were carried out using a CH Instruments 617A electrochemical analyzer with a glassy carbon working electrode, a Ag^+/AgCl reference electrode (3 M KCl, 0.213 V vs NHE), and a platinum wire counter electrode. All measurements were performed at a 1 mM concentration of each complex. The peak potentials measured in DMF given in the text are referenced vs the Ag^+/AgCl reference electrode. In addition, in a separate series of experiments, an internal reference system (ferrocene/ferrocenium ion) was used, which has been shown to remain constant regardless of the medium,[74,75] thereby allowing better comparison with other studies by eliminating problems of variable liquid junction potentials.[76−79] Under the conditions used, the reversible Fc/Fc^+ $E_{1/2}$ was 0.53 V vs the Ag^+/AgCl electrode ($\{E_p + E_c\}/2$). When using this as an internal reference system and taking the Fc^+/Fc couple as 0.40 V vs NHE (Koepp, H. M.; Wendt, H.; Strehlow, H. Z. *Z. Elektrochem.* **1960**, *64*, 483−491), the following peak potentials can be derived vs NHE: −1.23 V for **1** and −1.61 V for **2**.

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Supporting Information Available: CIF files of the structures of **1** and **2**, and a table with details of the crystallographic data and a plot of the rate of consumption of **1** upon photolysis in buffer (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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