

Mechanistic insight from activation parameters for the reaction between co-enzyme B₁₂ and cyanide: further evidence that heterolytic Co–C bond cleavage is solvent-assisted†

Mohamed S. A. Hamza,^{‡a} Andrew G. Cregan,^b Nicola E. Brasch^{*b} and Rudi van Eldik^{**a}

^a Institute for Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany

^b Research School of Chemistry, The Australian National University, Canberra ACT 0200, Australia

Received 14th October 2002, Accepted 11th December 2002

First published as an Advance Article on the web 20th January 2003

The potential involvement of the solvent in heterolytic Co–C bond cleavage for vitamin B₁₂ derivatives has been probed by measuring for the first time activation parameters for heterolytic Co–C bond cleavage. If a water molecule is part of the transition state, negative entropies and volumes of activation should be observed. Conversely, if Co–C bond cleavage is a purely dissociative process, these parameters will be positive. Activation parameters have been determined for the reactions of co-enzyme B₁₂ (5'-deoxyadenosylcobalamin, AdoCbl) and the corresponding cobinamide, 5'-deoxyadenosylcobinamide (AdoCbi⁺), with cyanide, since previous studies have shown that these reactions proceed at convenient rates, Co–C heterolytic bond cleavage is rate-determining and the reaction proceeds cleanly (that is, negligible homolytic cleavage). In addition, the kinetics of the reaction of AdoCbl with cyanide in aqueous solution were re-investigated at high concentrations of sodium cyanide (up to 3.00 M) at pH 11.0 and *I* = 5.0 M (NaClO₄) using UV-visible and ¹H NMR spectroscopy. A significant kinetic saturation was obtained at high CN[−] concentrations and the limiting rate constant at 25.0 °C was found to be $(2.21 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$, with an overall formation constant for the (β-Ado)(α-CN)Cbl[−] intermediate, $K_{\text{CN}}/K_{\text{Co}}$, of $0.259 \pm 0.007 \text{ M}^{-1}$. ΔH^\ddagger , ΔS^\ddagger and ΔV^\ddagger under these conditions were found to be $55 \pm 1 \text{ kJ mol}^{-1}$, $-98 \pm 3 \text{ J K}^{-1} \text{ mol}^{-1}$ and $-5.7 \pm 0.3 \text{ cm}^3 \text{ mol}^{-1}$, respectively. ΔV^\ddagger for the reaction of AdoCbi⁺ and CN[−] at pH 11.0 and 35.0 °C was found to be $-4.5 \pm 0.4 \text{ cm}^3 \text{ mol}^{-1}$, while ΔV^\ddagger for the reaction of AdoCbl and AdoCbi⁺ with tetrabutylammonium cyanide in 92% DMF–8% D₂O at 35 °C was found to be -8.2 ± 0.3 and $-8.8 \pm 0.7 \text{ cm}^3 \text{ mol}^{-1}$, respectively. The activation parameters data obtained in the present study support the earlier suggestion that the rate determining step for the reaction of AdoCbl and AdoCbi⁺ with CN[−] which involves heterolytic cleavage of the Co–C bond of the (β-Ado)(α-CN)Cbl[−] and (β-Ado)(α-CN)Cbi intermediates, respectively, is a solvent-assisted, β-elimination process, in which the solvent partially protonates the ribosyl oxygen of the intermediate.

Introduction

The reaction between β-alkyl derivatives of vitamin B₁₂, alkylcobalamins and cyanide has been known since the B₁₂ derivatives were first isolated and characterised over four decades ago. The mechanism of the reaction between one of the two co-enzyme forms of vitamin B₁₂, adenosylcobalamin (5'-deoxyadenosylcobalamin, co-enzyme B₁₂, AdoCbl, Fig. 1) and cyanide has especially attracted attention in the literature.¹ On the basis of finding one kinetically observable step, no detectable intermediate(s), a linear dependence of the observed rate constant on the cyanide concentration and significantly negative activation entropy and activation volume values for the rate-determining step, we initially suggested that rate-determining associative attack by cyanide occurs at the β-5'-deoxyadenosyl site rather than at the α-dimethylbenzimidazole site of co-enzyme B₁₂.^{1a} However, studies on the mechanism of the reaction between alkylcobalamins and cyanide for a wide range of alkylcobalamins have shown that other alkylcobalamins react with cyanide to form (β-alkyl)(α-cyano)cobalamin intermediates, which can, in many cases, undergo heterolytic Co–C cleavage and react with a second cyanide to form dicyanocobalamin, and the corresponding β-alkyl Co–C cleavage products (Scheme 1).² In addition, more recent detailed kinetic studies

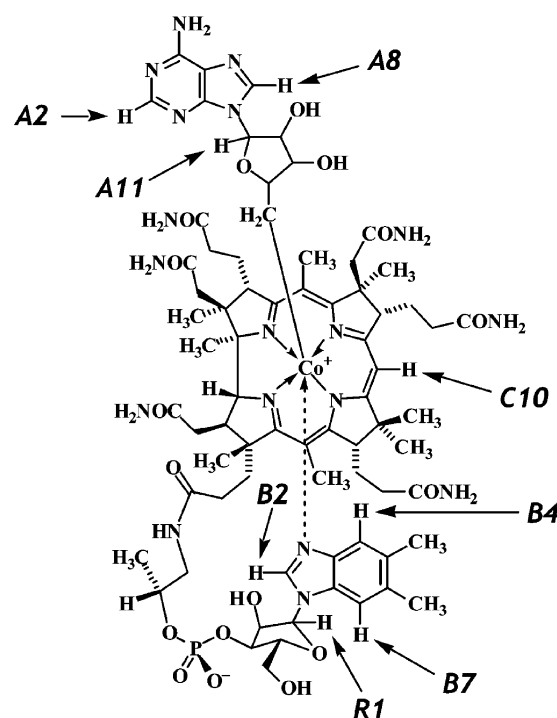
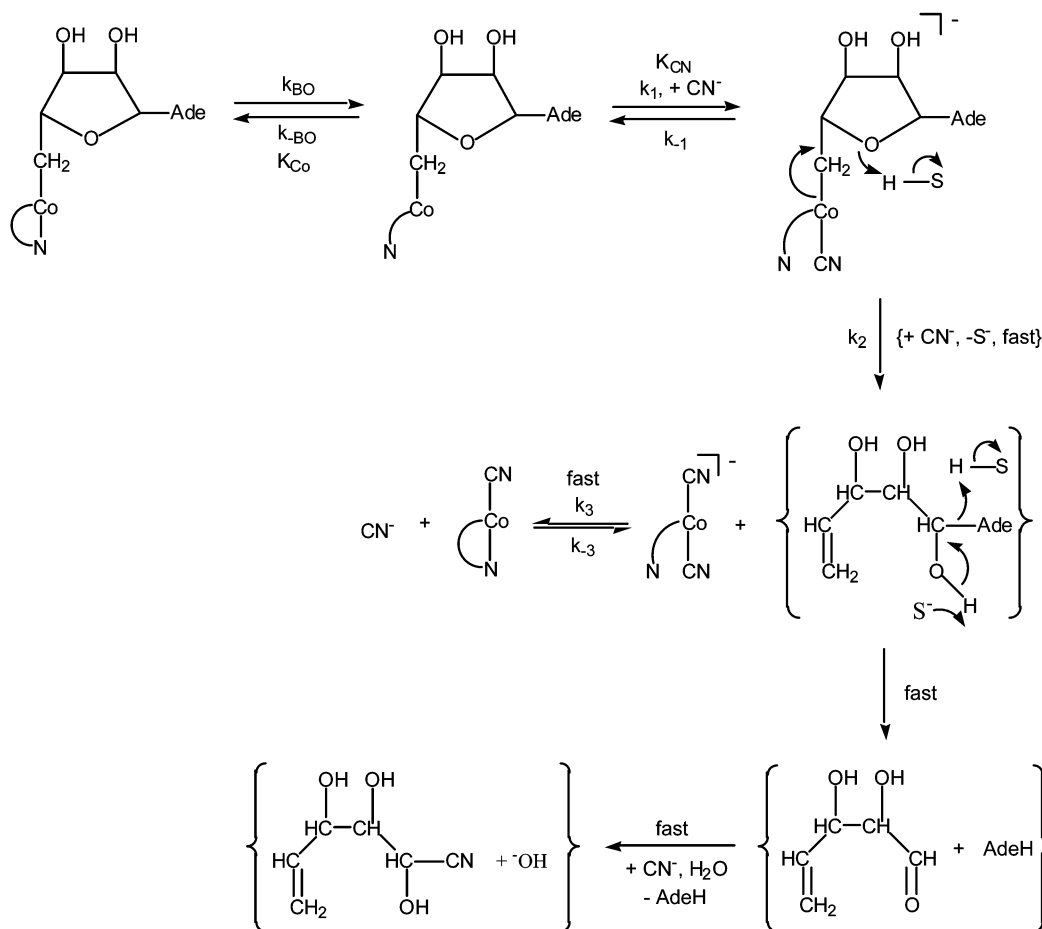


Fig. 1 The structure of adenosylcobalamin. The protons that resonate in the aromatic region are labeled.

† Electronic supplementary information (ESI) available: NMR and kinetic data. See <http://www.rsc.org/suppdata/dt/b2/b210068a/>

‡ On leave from the Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt.



Scheme 1 Proposed mechanism for the reaction of alkylcobalamins with cyanide.

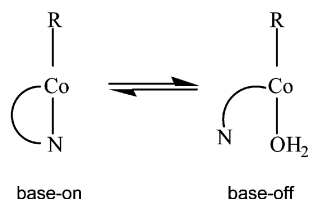


Fig. 2 “Base-on” and “base-off” forms of alkylcobalamins. The base-off form can exist in a five coordinate (no ligand coordinated at the α axial site) and six coordinate form (a solvent molecule occupies the α axial site).

have shown that alkylcobalamins must be converted to their base-off forms (Fig. 2) prior to reacting with cyanide to form the mono-cyano intermediate.^{1g,2d} Activation parameter measurements indicate that formation of the base-off alkylcobalamin (k_{BO} of Scheme 1) is a dissociative (I_d or D) process, as would be expected for this system.^{1g,2d}

Recently we investigated the mechanism of the reaction between AdoCbl and cyanide in 92% DMF–8% D_2O .^{1f} Under these conditions, a (β -5'-deoxyadenosyl)(α -cyano)cobalamin intermediate was observable by 1H NMR and UV-visible spectroscopy and the intermediate was light-sensitive, confirming that it contains a Co–C bond. A mechanism was proposed in which fast substitution of CN^- at the α axial site to form (β -Ado)(α -CN)Cbl $^-$ precedes rate-determining, solvent-assisted Co–C bond heterolysis. Rapid addition of a second cyanide and rearrangement gave the products, dicyanocobalamin, adenine and 1-cyano-D-erythro-2,3-dihydroxy-4-pentanol. Thus, under these conditions, Co–C bond cleavage at the β axial site of the (β -Ado)(α -CN)Cbl $^-$ intermediate is the rate-determining step, rather than addition of the first cyanide as found in aqueous medium.^{1a} Interestingly, a solvent-assisted bond cleavage involving transfer of a proton from the solvent

has previously been proposed for a number of systems (C–N,^{3a} Si–N,^{3b} M–C (M = Si, Sn)^{3c}), but to our knowledge this is the first study in which direct kinetic evidence for the involvement of the solvent in the formation of the transition state preceding metal–carbon bond cleavage has been obtained.

Alternative explanations for the discrepancy between the mechanisms for the reactions between cyanide and AdoCbl, and between cyanide and other alkylcobalamins (that is, associative or dissociative) were recently discussed.^{1g} EXAFS studies indicate AdoCbl is mainly present in solution as a five-coordinate complex (*i.e.*, the α axial site is vacant), due to the large *trans* influence of the β -5'-deoxyadenosyl group on the α -5,6-dimethylbenzimidazole group.⁴ This could favour an associative substitution mechanism in aqueous solution. However, a more detailed comparison of the second-order rate constants found for the cyanation reaction of a series of modified RCbl's (R = *n*-Pr, Me, CF_2H , CH_2CN , CF_3 , CN^-), showed that this interpretation cannot be correct.^{1g}

Recently complementary investigations on the reaction between a model compound for adenosylcobalamin, 5'-deoxyadenosylcobinamide (AdoCbi $^+OH^-$, Fig. 3), and cyanide in both aqueous solution and in DMF– D_2O solvent mixtures were carried out.⁵ For this system, it was found that fast addition of cyanide to form a (β -Ado)(α -CN)cobinamide intermediate precedes rate-determining, Co–C bond heterolysis, regardless of the DMF– D_2O solvent ratio. Given the similarity in the structures of AdoCbl and AdoCbi $^+$ (Fig. 1; R = 5'-deoxyadenosyl, and Fig. 3), it seems unlikely that the mechanism of the reaction between AdoCbl and cyanide in aqueous solution would be different from that for AdoCbi $^+ + CN^-$. Importantly, there were also serious discrepancies in the activation parameter data.^{1g}—specifically, with the exception of AdoCbl, the rate-determining step for formation of the mono-cyano intermediate exhibited dissociative activation parameters for all

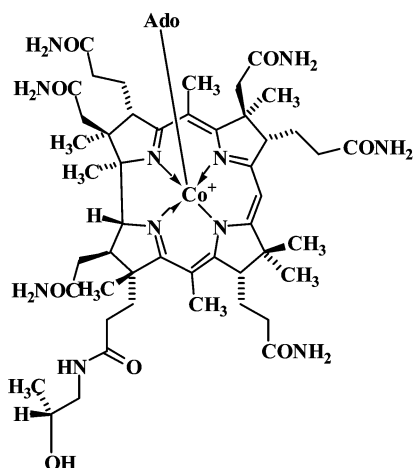


Fig. 3 The structure of 5'-deoxyadenosylcobinamide, AdoCbi⁺. The cobinamide derivatives are model compounds of cobalamins in which the α -ribose-3'-phosphate of the cobalamin has been cleaved by hydrolysis of the P–O bond.

alkylcobalamins. This led us to question our interpretation of the AdoCbl + CN[−] kinetic data in our earlier study,^{1a} and UV-visible spectra obtained immediately upon mixing AdoCbl with CN[−] up to 3.0 M CN[−] have subsequently shown that the reaction between AdoCbl and CN[−] also proceeds *via* a mechanism of the type shown in Scheme 1.^{1g}

In this study the kinetics of the reaction between AdoCbl and CN[−] have been re-investigated in aqueous solution using a much larger range of cyanide concentrations than in our earlier study.^{1a} These results allow us to directly compare rate and equilibrium constants for the reactions between AdoCbl and cyanide and AdoCbi⁺ and cyanide in aqueous solution, thus providing an excellent means of further testing our proposed general mechanism for the reaction between alkylcobalamins (including adenosylcobalamin) and alkylcobinamides and cyanide. In addition, activation parameters have been determined for the first time for the Co–C heterolytic bond cleavage process of an alkylcobalamin ((β -Ado)(α -CN)Cbl[−]) and an alkylcobinamide ((β -Ado)(α -CN)Cbi[−]) in aqueous solution, under conditions where the Co–C bond heterolysis is rate-determining and there is no interference (or minimal interference) from the pre-equilibrium formation of the mono-cyano intermediate. Importantly, the determination of activation parameters for Co–C heterolysis provides us with a novel way to further probe the suggested involvement of the solvent in the transition state of the Co–C bond cleavage process. Activation parameters for the Co–C bond cleavage step of the cyanation of AdoCbl and AdoCbi⁺ have also been determined in 92% DMF–8% D₂O, and show how the solvent controls the rate and mechanism of this step.

Experimental

Adenosylcobalamin (AdoCbl, >98%) was purchased from Aldrich and sodium perchlorate (>98%) from BDH. Tetrabutylammonium perchlorate (>99%) tetrabutylammonium cyanide (~97%) and dimethylformamide were from Fluka. CAPS buffer (>98%) was provided by Sigma. All solutions were made in D₂O at a total ionic strength of 5.0 M (NaClO₄) except otherwise stated. Distilled water was purified through an ultra-pure water system.

All pH measurements were carried out at 25.0 °C using an Orion Model 710A pH meter in conjunction with an Orion 9101 BN glass electrode and an Orion 900200 double junction reference electrode, or a Mettler Delta 350 pH meter with a combined glass electrode. The electrodes were standardised using BDH 6.98 and 10.00 buffer solutions. Measurements in alkaline solution were carried out under a nitrogen or argon

atmosphere. Solution pH was adjusted using NaOH solution. The pD of the D₂O solutions was calculated using the relationship pD = pH + 0.40.⁶ No correction was made for the Na⁺ concentration.

¹H NMR spectra were recorded on an Inova 500 MHz NMR spectrometer equipped with a 5 mm thermostated (25.0 ± 0.2 °C) probe. All solutions were prepared in D₂O and referenced externally to a solution of TSP (3-trimethylsilyl propionate) in D₂O.

All kinetic measurements were carried out under pseudo-first order conditions; that is, the nucleophile concentration was at least in ten fold excess. An excellent fit to a single first-order rate equation was found for all kinetic data for at least 5 half-lives (OLIS-KINFIT fitting program). UV-visible spectra at ambient pressures were recorded on a Cary 1E spectrophotometer equipped with a 8 × 6 cell changer and operated with Win UV Bio software (version 2.00) or a Shimadzu UV-2101 spectrophotometer. The cell blocks of both spectrophotometers were thermostated (± 0.1 °C).

Kinetic measurements under high pressure were carried out using either a home-made high pressure unit connected with the Shimadzu spectrophotometer or a high pressure stopped-flow instrument.⁷ At least three kinetic runs were recorded under all conditions, and the reported rate constants represent the mean values.

All reported errors represent one standard deviation of the mean value. Data fits were carried out using the non-linear least squares fitting program Origin, version 3.5.

Kinetic measurements on the reaction between AdoCbl and NaCN

The reaction between AdoCbl and CN[−] was studied at [NaCN] = 0.150–3.00 M, pH 12.0, *I* = 5.0 M (NaClO₄). Each measurement was done in triplicate. In a typical experiment, 0.800 mL of a NaCN solution (0.300–2.40 M NaCN) and 0.800 mL of a ~1.00 × 10^{−4} M AdoCbl solution were mixed in a tandem cuvette and the absorbance monitored at 367 nm. For NaCN ≥ 1.40 M, 10.0 μL of *ca.* 1.30 × 10^{−2} M AdoCbl solution was injected into 3.00 mL of a NaCN solution and the absorbance monitored at 367 nm. Similar procedures were used to determine rate constants for the reaction between AdoCbl and CN[−] in D₂O (pD 12.0, *I* = 5.0 M (NaClO₄)).

¹H NMR Spectroscopy measurements on the reaction between AdoCbl and NaCN

Experiments were carried out at pD 12.0, *I* = 5.0 M (NaClO₄). ~12 mg of solid AdoCbl was dissolved in a NaCN solution ([NaCN] = 0–3.00 M), filtered, (45 μm filter) and a ¹H NMR spectrum immediately recorded.

Results and discussion

Kinetic measurements

In order to obtain additional support for the mechanism of the reaction between AdoCbl and CN[−] in aqueous solution as proposed in Scheme 1, the dependence of the observed rate constant of the reaction between AdoCbl and CN[−] was studied as a function of CN[−] concentration by UV-visible spectroscopy, up to CN[−] concentrations of 3.00 M. At each CN[−] concentration, the data gave an excellent fit to a first-order rate equation. The results are presented in Fig. 4.

Curvature is observed, typical for saturation kinetics in which a rapid pre-equilibrium precedes rate-determining Co–C bond cleavage as proposed in Scheme 1. (Note that for [CN[−]] ≤ 0.500 M, the plot is essentially linear, as found in our earlier study.^{1a}) We have recently provided UV-visible spectroscopic evidence that the intermediate is (β -Ado)(α -CN)Cbl[−],^{1g} since the intermediate absorbs strongly in the 580–620 nm

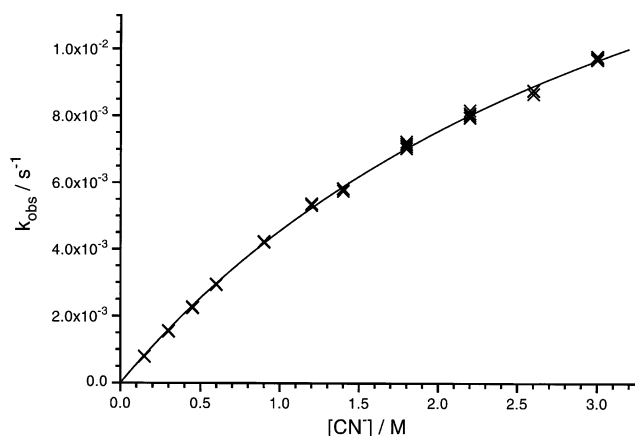


Fig. 4 Plot of observed rate constant (k_{obs}) versus CN^- concentration for the reaction between AdoCbl and CN^- at 25.0 °C ($[\text{AdoCbl}] \sim 5 \times 10^{-5} \text{ M}$, $[\text{NaCN}] = 0.100\text{--}3.00 \text{ M}$, H_2O , 0.100 M CAPS, pH 11.0, $I = 5.0 \text{ M}$ (NaClO_4)). The best fit of the data to eqn. (1) gave $k_2 = (2.21 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$ and $K_{\text{CN}}/K_{\text{Co}} = 0.259 \pm 0.007 \text{ M}^{-1}$.

region, which is characteristic of $(\beta\text{-alkyl})(\alpha\text{-CN})\text{cobalamin}$ and $(\beta\text{-alkyl})(\alpha\text{-CN})\text{cobinamide}$ complexes.^{1f,1g,2a,2d,5} The intermediate also decomposes in light (*i.e.*, it contains a light-sensitive Co–C bond.^{1b})

The rate law for a mechanism of the type shown in Scheme 1 is

$$k_{\text{obs}} = k_2(K_{\text{CN}}/K_{\text{Co}})[\text{CN}^-]/(1 + (K_{\text{CN}}/K_{\text{Co}})[\text{CN}^-]) \quad (1)$$

where $K_{\text{CN}}/K_{\text{Co}}$ is the overall formation constant for the $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ intermediate, as defined in Scheme 1. The best fit of the data in Fig. 4 to eqn. (1) gave $k_2 = (2.21 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$ and $K_{\text{CN}}/K_{\text{Co}} = 0.259 \pm 0.007 \text{ M}^{-1}$ (H_2O , pH 11.0, $I = 5.0 \text{ M}$ (NaClO_4), 25.0 °C). In an earlier paper, the equilibrium constant ratio for formation of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$, $K_{\text{CN}}/K_{\text{Co}}$, was determined in H_2O , by measuring UV-visible spectra of solutions of AdoCbl upon immediate addition of NaCN, as a function of CN^- concentration, giving $K_{\text{CN}}/K_{\text{Co}} = 0.6 \pm 0.1 \text{ M}^{-1}$ (pH 11.0, 25 °C).^{1g} As expected, this value of $K_{\text{CN}}/K_{\text{Co}}$ is somewhat different and has a larger associated error since the ionic strength was not controlled, whereas the measurements in this study have been carried out maintaining the total ionic strength at 5.0 M (NaClO_4).

The kinetics of the reaction between AdoCbl and CN^- were also studied in D_2O using the same methodology, giving $k_2 = (1.93 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$ and $K_{\text{CN}}/K_{\text{Co}} = 0.25 \pm 0.01 \text{ M}^{-1}$ (D_2O , pD 11.0, $I = 5.0 \text{ M}$ (NaClO_4), 25.0 °C). The corresponding plot of observed rate constant *versus* cyanide concentration and the best fit of the data to eqn. (1) are given in Fig. A in the Supporting Information. These data are useful since they allow us to compare these results with those from the $\{\text{AdoCbl}^+ + \text{CN}^-\}$ system (see later). In addition, the equilibrium constant ratio $K_{\text{CN}}/K_{\text{Co}}$ can also be determined in D_2O using ^1H NMR spectroscopy (see below), thus providing a further method to check the proposed mechanism.

Determination of $K_{\text{CN}}/K_{\text{Co}}$ by ^1H NMR spectroscopy

The reaction between AdoCbl and CN^- can also be followed by ^1H NMR spectroscopy. Indeed, this method has previously been used by us to study the reaction between AdoCbl and CN^- in 92%DMF–8% D_2O .^{1f} Upon dissolving AdoCbl in D_2O , 8 signals are observed in the aromatic region attributable to the A2, A8 and A11 proton resonances of the 5'-deoxyadenosyl ligand, the C10 proton of the corrin ring, and the B2, B4, B7 and R1 resonances of the nucleotide (Fig. 1).⁸ If NaCN is added to the solution, the peaks in the aromatic region are shifted downfield (and broaden), with their chemical shifts being dependent on the specific CN^- concentration. When the

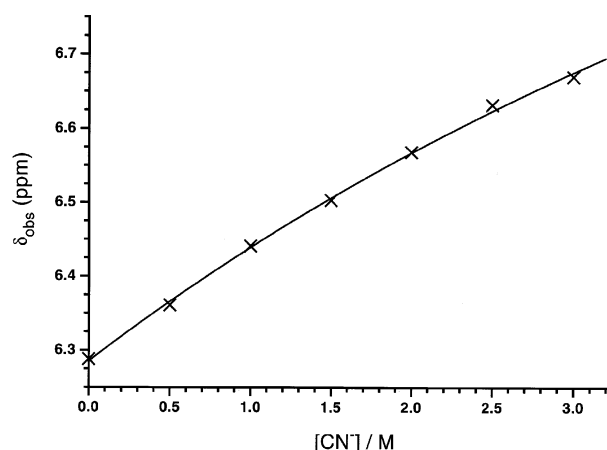
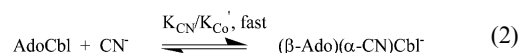


Fig. 5 Plot of ^1H NMR chemical shift *versus* $[\text{CN}^-]$ for the B4 nucleotide signal of $\{\text{AdoCbl} + (\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-\}$ (D_2O , 0.100 M CAPS, pD 11.0, 25.0 °C, $I = 5.0 \text{ M}$ (NaClO_4)). The data has been fitted to eqn. (3) in the text, giving $K_{\text{CN}}/K_{\text{Co}}' = 0.10 \pm 0.03 \text{ M}^{-1}$, $\delta_{\text{AdoCbl}} = 6.29 \pm 0.01 \text{ ppm}$ and $\delta_{\text{AdoCNCbl}} = 8.0 \pm 0.3 \text{ ppm}$.

reaction mixture is monitored at longer times, the peak areas of these signals decrease until no longer observable, while new peaks grow in at a comparable rate. The new peaks can be attributed to the products adenine (7.97, 8.12 ppm^{9a}) the B2, B4, B7, R1 and C10 resonances of dicyanocobalamin (8.29, 7.44, 7.34, 6.31(d) and 5.76 ppm⁹), and the cyanohydrin 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol (5.31, 5.33, 5.35, 5.38, 5.39, 5.41 ppm and a multiplet at 5.9–6.0 ppm^{9a}). Observed rate constants for the reaction between AdoCbl and CN^- can also be determined from ^1H NMR peak areas, although this was not done in this study since we have previously found that more accurate data for the reaction between B_{12} derivatives and cyanide is obtained by UV-visible spectroscopy.^{1f,5}

Fig. 5 gives the observed chemical shift for the B4 signal⁸ of AdoCbl (actually a mixture of AdoCbl and $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$) as a function of CN^- concentration. Assuming that AdoCbl and the intermediate, $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$, are in fast exchange on the ^1H NMR time scale; that is



then

$$\delta_{\text{obs}} = (\delta_{\text{AdoCbl}} + (K_{\text{CN}}/K_{\text{Co}}')[\text{CN}^-]\delta_{\text{AdoCNCbl}}) / (1 + (K_{\text{CN}}/K_{\text{Co}}')[\text{CN}^-]) \quad (3)$$

where δ_{obs} is the observed chemical shift at a specific CN^- concentration, δ_{AdoCbl} and δ_{AdoCNCbl} are the chemical shifts of AdoCbl and $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ respectively, and $K_{\text{CN}}/K_{\text{Co}}'$ is the overall formation constant of the intermediate determined by ^1H NMR spectroscopy corresponding to eqn. (2). The best fit of the data in Fig. 5 to eqn. (3) gives $K_{\text{CN}}/K_{\text{Co}}' = 0.10 \pm 0.03 \text{ M}^{-1}$, as shown by the solid line in Fig. 5. Alternatively, the data can be fitted assuming $K_{\text{CN}}/K_{\text{Co}}' = 0.25 \text{ M}^{-1}$; that is, the value of $K_{\text{CN}}/K_{\text{Co}}$ obtained by UV-visible spectroscopy. Using this value of $K_{\text{CN}}/K_{\text{Co}}'$ also results in a very good fit of the experimental data (see Fig. B of the Supporting Information). This indicates that the value of $K_{\text{CN}}/K_{\text{Co}}'$ obtained by ^1H NMR spectroscopy should be regarded as an estimate only. The same conclusion is reached when examining the standard deviations of the values in Fig. 5, which are large. The results are summarized along with other available data in Table 1.

Activation parameter measurements

In order to further probe the possible involvement of the solvent in formation of the transition state for Co–C heterolysis of

Table 1 Rate and equilibrium constants for the reaction between AdoCbl or AdoCbiOH and cyanide at 25.0 °C

Cobamide	Solvent	I/M	$K_{\text{CN}}^a/\text{M}^{-1}$	$K_{\text{CN}}'^b/\text{M}^{-1}$	$(K_{\text{CN}}/K_{\text{Co}})^a/\text{M}^{-1}$	$(K_{\text{CN}}/K_{\text{Co}})^b/\text{M}^{-1}$	k_2/s^{-1}	Ref.
AdoCbl	H ₂ O	5.0			0.259		2.21×10^{-2}	— ^d
AdoCbl	D ₂ O	5.0			0.25	0.10	1.93×10^{-2}	— ^d
AdoCbl	H ₂ O	— ^c			0.6			1g
AdoCbl	DMF–D ₂ O	0.50			2.2×10^2		9.3×10^{-5}	1f
AdoCbi ⁺	H ₂ O	1.0	5.6				2.91×10^{-2}	4
AdoCbi ⁺	H ₂ O	2.0	4.6				2.55×10^{-2}	4
AdoCbi ⁺	D ₂ O	1.0	6.9	5.7			2.17×10^{-2}	4
AdoCbi ⁺	D ₂ O	2.0	3.8	3.3			2.45×10^{-2}	4
AdoCbi ⁺	DMF–D ₂ O	0.50	2.8×10^3				6.7×10^{-5}	4

^a Determined by UV-visible spectroscopy. ^b Determined by ¹H NMR spectroscopy. ^c No added electrolyte. ^d This study.

Table 2 Activation parameters for the heterolytic Co–C cleavage of (β-Ado)(α-CN)Cbl[−] and (β-Ado)(α-CN)Cbi

Cobamide	Solvent	$\Delta H^\ddagger/\text{kJ mol}^{-1}$	$\Delta S^\ddagger/\text{J K}^{-1} \text{mol}^{-1}$	$\Delta V^\ddagger/\text{cm}^3 \text{mol}^{-1}$
AdoCbl	H ₂ O	55 ± 1^a	-98 ± 3^a	-5.7 ± 0.3^a
AdoCbl	DMF–D ₂ O	90.0 ± 0.5^b	-20.5 ± 0.2^b	-8.2 ± 0.3
AdoCbi ⁺	H ₂ O	53 ± 4^c	-97 ± 14^c	-4.5 ± 0.4
AdoCbi ⁺	DMF–D ₂ O	85 ± 3	-39 ± 10	-8.8 ± 0.7

^a Contain contributions from $1/K_{\text{Co}}$ and K_{CN} . ^b Ref. 1f. ^c Ref. 4.

the (β-Ado)(α-CN)Cbl[−] intermediate, activation parameters were determined. These values were obtained at the highest CN[−] concentration that was experimentally feasible; that is, at 4.00 M CN[−] concentrations. Using a value of $K_{\text{CN}}/K_{\text{Co}} = 0.45 \text{ M}^{-1}$ (the mean value of 0.31 and 0.6 M^{−1}), this means that the majority (~64%) of the complex formed prior to the rate-determining Co–C bond cleavage exists as (β-Ado)(α-CN)Cbl[−] under these conditions.¹⁰ Hence, the Co–C bond heterolysis process should dominate the activation parameter measurements.

The reaction between AdoCbl and 4.0 M CN[−] at pH 11.0 was studied as a function of temperature and pressure and the results are reported in Fig. C (Supporting Information) and Fig. 6, respectively. The activation parameters are summarized

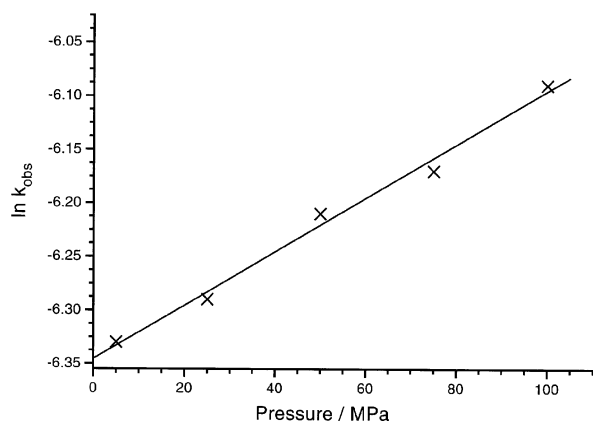


Fig. 6 Plot of $\ln(k_{\text{obs}})$ versus pressure for the reaction between $\sim 8 \times 10^{-5} \text{ M}$ AdoCbl and 4.00 M NaCN in H₂O at pH 11.0 and 3.0 °C. From the slope of the plot, $\Delta V^\ddagger = -5.7 \pm 0.3 \text{ cm}^3 \text{mol}^{-1}$.

in Table 2. ΔH^\ddagger and ΔS^\ddagger were found to be $55 \pm 1 \text{ kJ mol}^{-1}$ and $-98 \pm 3 \text{ J K}^{-1} \text{mol}^{-1}$, respectively, whereas ΔV^\ddagger was calculated to be $-5.7 \pm 0.3 \text{ cm}^3 \text{mol}^{-1}$ at 3.0 °C. We suggested earlier on the basis of the effect of the solvent (*i.e.*, D₂O versus 92%DMF–8%D₂O) on the magnitude of the rate constant for Co–C heterolytic bond cleavage that a solvent molecule is involved in the transition state for this process.^{1f} If this is indeed the case, it is reasonable to expect $\Delta V^\ddagger(k_2)$ to have a small negative value, resulting from partial binding of a solvent molecule and heterolysis of the Co–C bond, as experimentally observed. Note that if the solvent was not involved in the bond cleavage

process, dissociative activation parameters (*i.e.*, a significantly positive value of ΔV^\ddagger) would be expected for the heterolysis process. Similar arguments can be adopted to account for the negative activation entropy found for this reaction in aqueous solution. Hence, both the volume and entropy of activation measurements provide further support that Co–C heterolytic cleavage of the intermediate is indeed a solvent-assisted, β-elimination process, in which the solvent partially protonates the ribosyl oxygen of the 5'-deoxyadenosyl ligand of the intermediate.

The effect of pressure on the observed rate constant was also studied for the reaction of AdoCbi⁺ with 0.500 M CN[−] at pH 11.0, $I = 1.0 \text{ M}$ (NaClO₄) and 35.0 °C, for which ΔV^\ddagger was found to be $-4.5 \pm 0.4 \text{ cm}^3 \text{mol}^{-1}$ (Fig. D, Supporting Information), under conditions where Co–C heterolysis of the (β-Ado)(α-CN)Cbi intermediate has been previously shown to be the rate-determining step (*i.e.*, $k_{\text{obs}} = k_2$).⁵ This result also suggests partial binding of the solvent molecule prior to Co–C bond heterolysis of (β-Ado)(α-CN)Cbi.

In our earlier study, the activation parameters for the reaction between AdoCbl and cyanide in H₂O were studied at low cyanide concentration ($7.50 \times 10^{-2} \text{ M}$), and ΔH^\ddagger , ΔS^\ddagger and ΔV^\ddagger were reported to be $53.0 \pm 0.6 \text{ kJ mol}^{-1}$, $-127 \pm 3 \text{ J K}^{-1} \text{mol}^{-1}$ and $-10.0 \pm 0.4 \text{ cm}^3 \text{mol}^{-1}$, respectively.^{1a} From eqn. (1) it can be seen at low cyanide concentrations, $k_{\text{obs}} = k_2(K_{\text{CN}}/K_{\text{Co}})[\text{CN}^-]$, or, in terms of the proposed mechanism in Scheme 1, $\Delta V^\ddagger(k_{\text{obs}}) = \Delta V^\ddagger(k_2) + \Delta V^\ddagger(1/K_{\text{Co}}) + \Delta V^\ddagger(K_{\text{CN}})$, where $\Delta V^\ddagger(1/K_{\text{Co}})$ and $\Delta V^\ddagger(K_{\text{CN}})$ represent the reaction volumes for formation of the base-off AdoCbl and the subsequent formation of the intermediate (β-Ado)(α-CN)Cbl[−] complex, respectively. If it is assumed that $\Delta V^\ddagger(k_2)$ for AdoCbl $\sim \Delta V^\ddagger(k_2)$ for AdoCbi⁺, which seems quite reasonable, given the excellent agreement between rate constants for the two systems (see later), then for the reaction between AdoCbl and cyanide, $(\Delta V^\ddagger(1/K_{\text{Co}}) + \Delta V^\ddagger(K_{\text{CN}}))$ can be estimated to be $-5.5 \pm 0.8 \text{ cm}^3 \text{mol}^{-1}$ (*i.e.*, the difference between -10.0 and $-4.5 \text{ cm}^3 \text{mol}^{-1}$). These steps involve decoordination of the 5,6-dimethylbenzimidazole at the α axial site accompanied by bond formation with the entering cyanide ligand, such that it is reasonable to expect an overall small negative value for $(\Delta V^\ddagger(1/K_{\text{Co}}) + \Delta V^\ddagger(K_{\text{CN}}))$, due to the larger volume decrease associated with the coordination of free cyanide as compared to the volume increase associated with the dechelation of the α-dimethylbenzimidazole. Thus, on the basis of these arguments, and the mechanistic interpretation presented here, we conclude that the significantly negative volume

of activation observed before (and interpreted as evidence for associative binding of cyanide^{1a}) results from approximately equal negative contributions for both the rapid formation of the $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ intermediate and the subsequent solvent assisted heterolysis reaction.

Our earlier work on the reactions of AdoCbl and AdoCbi⁺ with cyanide in 92%DMF–8%D₂O was also extended by studying pressure dependence of the reaction under conditions where the heterolytic cleavage of the Co–C bond is the rate-limiting step, and for which thermal activation parameters have been reported. (We have previously established that the “pH” of 92%DMF–8%D₂O is sufficiently high enough to ensure cyanide exists as CN[−], not HCN.^{1f}) The results, which are presented in Figs. E and F of the Supporting Information, reveal that $\Delta V^\ddagger(k_2)$ equals -8.2 ± 0.3 and -8.8 ± 0.7 cm³ mol^{−1} at 35.0 °C for heterolytic cleavage of the Co–C bond of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ and $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$, respectively. Once again the values of ΔV^\ddagger clearly support a solvent-assisted, β -elimination process in which the solvent partially protonates the ribosyl oxygen of the 5'-deoxyadenosyl ligand, since otherwise a significantly positive volume of activation would be expected. The effect of temperature on the reaction of AdoCbi⁺ with 0.04 M cyanide in 92%DMF–8%D₂O ($I = 0.5$ M) was also studied and the data are reported in Fig. G of the Supporting Information. ΔH^\ddagger and ΔS^\ddagger in this case were found to be 85 ± 3 kJ mol^{−1} and -39 ± 10 J K^{−1} mol^{−1}, respectively. These data are in good agreement with those reported previously for the reaction of AdoCbl and cyanide in the same medium.^{1f}

Comparison of rate, equilibrium constants and activation parameters for the reactions between cyanide and AdoCbl or AdoCbi⁺

Tables 1 and 2 summarize all the data we have measured for the reactions between {AdoCbl and CN[−]} and {AdoCbi⁺ and CN[−]}.^{1f,1g,5} The rate constant, k_2 , for Co–C bond heterolytic cleavage of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$, is much larger in aqueous solution (2.21×10^{-2} and 1.93×10^{-2} s^{−1} in H₂O and D₂O, respectively (Table 1)), compared with in 92%DMF–8%D₂O (9.3×10^{-5} s^{−1}). This is also the case for the rate constants for heterolytic cleavage of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ in aqueous solution *versus* 92%DMF–8%D₂O (Table 1), and we previously interpreted this as indicating that water must be involved in the formation of the transition state for Co–C heterolysis for these systems.⁵ Note that the values of k_2 for $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ and $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ in aqueous solution are very similar ($(1.93\text{--}2.21) \times 10^{-2}$ s^{−1} *versus* $(2.17\text{--}2.91) \times 10^{-2}$ s^{−1}, respectively), providing further support for the proposed mechanistic scheme for the cyanation of B₁₂ derivatives in aqueous solution (Scheme 1). Not unexpectedly, the values of k_2 for these two systems are also very similar in 92%DMF–8%D₂O, as previously discussed.⁵

From Table 1 it can also be seen that the ratio of the equilibrium constants for formation of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ ($K_{\text{CN}}/K_{\text{Co}}$) is over two orders of magnitude smaller in aqueous solution ($0.10\text{--}0.6$ M^{−1}) compared with in 92%DMF–8%D₂O (2.2×10^2 M^{−1}). This is attributed to the much smaller value of $K_{\text{CN}}(\text{AdoCbl})$ in aqueous solution compared with in 92%DMF–8%D₂O, since CN[−] is a much less potent nucleophile in aqueous solution due to increased interactions between CN[−] and the solvent. The value of K_{CN} for formation of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ is also much larger in 92%DMF–8%D₂O compared with in aqueous solution (Table 1) for the same reasons.⁵ The ratio of the equilibrium constants $K_{\text{CN}}(\text{AdoCbi}^+)$ to $K_{\text{CN}}/K_{\text{Co}}(\text{AdoCbl})$ in aqueous solution ($3.3\text{--}6.9$ M^{−1} *versus* $0.10\text{--}0.6$ M^{−1}) is ~ 10 . Given the similarity in the structures of base-off AdoCbl and AdoCbi⁺, then it is reasonable to assume that $K_{\text{CN}}(\text{AdoCbl}) \sim K_{\text{CN}}(\text{AdoCbi}^+)$, allowing one to estimate K_{Co} to be in the range 10–70. This range of value is in excellent agreement with values reported for K_{Co} obtained by UV-visible and NMR spectroscopic measurements.^{11,12} A similar argument

was previously employed when comparing $K_{\text{CN}}(\text{AdoCbi}^+)$ to $K_{\text{CN}}/K_{\text{Co}}(\text{AdoCbl})$ in 92%DMF–8%D₂O, and once again a reliable estimate of K_{Co} could be obtained; that is, one that agreed favourably with a value of K_{Co} determined by UV-visible spectroscopic measurements.⁵

The close parallels between the rate constants for Co–C heterolytic cleavage of $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$ and $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ in either aqueous solution or 92%DMF–8%D₂O are also reflected by the activation parameters for the heterolysis of the Co–C bond, which are summarized in Table 2.¹³ In Table 2, the very negative activation entropies and the significantly negative activation volumes clearly support the participation of a solvent (water) molecule in the transition state of the process, preceding rate-determining cleavage of the Co–C bond in both solvents (aqueous solution or 92%DMF–8%D₂O). Since the rate of this reaction is significantly slower in an aprotic solvent, the more negative activation volumes found for this reaction in 92%DMF–8%D₂O may indicate a later transition state; that is, where the participation of the solvent (water) in the transition state causes a larger volume collapse, due to partial ionization of the solvent molecule in order for a proton to stabilize the ribosyl oxygen of the 5'-deoxyadenosyl ligand. Ionization of water is accompanied by a large volume collapse (*ca.* -22 cm³ mol^{−1}¹⁴) as a result of a drastic increase in electrostriction surrounding the H⁺ and OH[−] ions. Thus the partial protonation of the ribosyl oxygen and the subsequent partial ionization to facilitate Co–C bond cleavage will lead to a significant decrease in partial molar volume that will offset the expected volume increase associated with the cleavage of the Co–C bond. Alternatively, the more negative activation volumes observed for the heterolysis reaction in 92%DMF–8%D₂O may result from a medium effect, due to the change in polarity of the solvent in going from more polar D₂O to a less polar solvent in 92%DMF–8%D₂O. Charge creation associated with the partial ionization of a solvent (water) molecule will result in larger electrostriction effects in a less polar solvent (*i.e.* a more negative activation volume).

To summarise, activation parameters have been determined for the reaction between AdoCbl and AdoCbi⁺ and cyanide in aqueous solution and in 92%DMF–8%D₂O. Negative entropies and volumes of activation give further support to a mechanism involving Co–C bond heterolysis of the $(\beta\text{-Ado})(\alpha\text{-CN})\text{-cobamide}$ intermediates *via* a solvent-assisted, β -elimination process, in which the solvent partially protonates the ribosyl oxygen. We have also re-examined the reaction between AdoCbl and cyanide in aqueous solution and have finally been able to resolve the mechanistic inconsistencies resulting from the participation of an intermediate species, *viz.* $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^-$, for which no spectroscopic evidence could be detected under the experimental conditions employed before.^{1a} By going to extreme experimental conditions (up to 4.00 M cyanide), and by studying related reactions and systems,^{1f,1g,2d,5} it was possible to pinpoint this intermediate spectroscopically and to resolve the apparent mechanistic discrepancies.

Acknowledgements

The authors gratefully acknowledge financial support from the Deutsche Forschungsgemeinschaft through SFB 583 “Redox active metal complexes” (M. S. A. H. and R. vE.), the Alexander von Humboldt Foundation (M. S. A. H.). M. S. A. H. thanks the Ain Shams University for sabbatical leave.

References and notes

- (a) N. E. Brasch, M. S. A. Hamza and R. van Eldik, *Inorg. Chem.*, 1997, **36**, 3216 and references therein; (b) J. M. Pratt, *Inorganic Chemistry of Vitamin B₁₂*, Academic Press, London, New York, 1972; (c) A. W. Johnson and N. Shaw, *J. Chem. Soc. A*, 1962, 4608; (d) H. P. C. Hogenkamp, J. E. Rush and C. A. Swenson, *J. Biol. Chem.*, 1965, **240**, 3641; (e) C. D. Garr, J. M. Sirovatka and

- R. G. Finke, *J. Am. Chem. Soc.*, 1996, **118**, 11142; (f) N. E. Brasch and R. J. Haupt, *Inorg. Chem.*, 2000, **39**, 5469; (g) M. S. A. Hamza, X. Zou, K. L. Brown and R. van Eldik, *J. Chem. Soc., Dalton Trans.*, 2002, 3832; (h) ref. 1(b), p. 244.
- 2 (a) W. W. Reenstra, R. H. Abeles and W. P. Jencks, *J. Am. Chem. Soc.*, 1982, **104**, 1016; (b) F. Nome, M. C. Rezende, C. M. Sabóia and A. Clemente da Silva, *Can. J. Chem.*, 1987, **65**, 2095; (c) K. L. Brown, *J. Am. Chem. Soc.*, 1987, **109**, 2277; (d) M. S. A. Hamza, X. Zou, K. L. Brown and R. van Eldik, *Inorg. Chem.*, 2001, **40**, 5440; (e) S. F. Brodie, A. G. Cregan, R. van Eldik, N. E. Brasch, *Inorg. Chim. Acta*, in press.
- 3 (a) T. J. Broxton and L. W. Deady, *J. Org. Chem.*, 1974, **39**, 2767; (b) Z. Lasocki and M. Witekowa, *J. Organomet. Chem.*, 1984, **264**, 49; (c) C. Eaborn, I. D. Jenkins and G. Seconi, *J. Organomet. Chem.*, 1977, **131**, 387.
- 4 M. D. Wirt and M. R. Chance, *J. Inorg. Biochem.*, 1993, **49**, 265.
- 5 N. E. Brasch, A. G. Cregan and M. E. Vanselow, *J. Chem. Soc., Dalton Trans.*, 2002, 1287.
- 6 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.
- 7 R. van Eldik, W. Gaede, S. Wieland, J. Kraft, M. Spitzer and D. A. Palmer, *Rev. Sci. Instrum.*, 1993, **64**, 1355.
- 8 For ^1H NMR assignments of AdoCbl and a complete labeling scheme, see: A. M. Calafat and L. G. Marzilli, *J. Am. Chem. Soc.*, 1993, **115**, 9182.
- 9 (a) Chemical shifts in 2.00 M NaCN. The exact chemical shifts are dependent on the cyanide concentration; (b) K. L. Brown, H. B. Brooks, B. D. Gupta, W. J. Coux and R. Timkovich, *Inorg. Chem.*, 1991, **30**, 3430.
- 10 $\%(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbl}^- = 100(K_{\text{CN}}/K_{\text{Co}}[\text{CN}^-])/(1 + K_{\text{CN}}/K_{\text{Co}}[\text{CN}^-])$.
- 11 (a) R. A. Firth, H. A. O. Hill, B. E. Mann, J. M. Pratt, R. G. Thorp and R. J. P. Williams, *J. Chem. Soc. A*, 1968, 2419; (b) ref. 1(h), pp. 246–247.
- 12 K. L. Brown, J. M. Hakimi and D. W. Jacobsen, *J. Am. Chem. Soc.*, 1984, **106**, 7894.
- 13 Note that the temperature and pressure dependence of the observed rate constant for acid-catalysed heterolytic cleavage of AdoCbl and AdoCbi $^+$ has been studied by Balt and co-workers.^{13a,b} However, the reported activation parameters contain contributions from both protonation and Co–C cleavage: (a) L. E. H. Gerards and S. Balt, *Recl. Trav. Chim. Pays-Bas*, 1992, **111**, 411; (b) L. E. H. Gerards and S. Balt, *Recl. Trav. Chim. Pays-Bas*, 1994, **113**, 137.
- 14 Y. Kitamura and T. Itoh, *J. Solution Chem.*, 1987, **16**, 715.