

# Studies on the mechanism of the reaction between 5'-deoxyadenosylcobinamide and cyanide†

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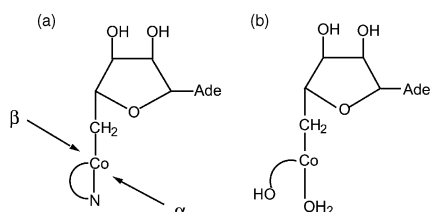
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The reaction between 5'-deoxyadenosylcobinamide (AdoCbi<sup>+</sup>OH<sup>-</sup>) and cyanide (NaCN or tetrabutylammonium cyanide) to form dicyanocobinamide, adenine and 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol has been studied by <sup>1</sup>H NMR and UV-visible spectroscopies in aqueous solution at pH (pD) 11.0 and in 92% *N,N*-dimethylformamide (DMF)–8% D<sub>2</sub>O. Saturation kinetics are observed by UV-visible spectroscopy at high cyanide concentrations and data were fitted to a mechanism involving rapid formation of an (β-adenosyl)(α-cyano)cobinamide intermediate, (β-Ado)(α-CN)Cbi ( $K_{CN} = 5.6$  (H<sub>2</sub>O) and  $6.9$  M<sup>-1</sup> (D<sub>2</sub>O), 25.0 °C,  $I = 1.0$  M (NaClO<sub>4</sub>);  $K_{CN} = 4.6$  (H<sub>2</sub>O) and  $3.8$  M<sup>-1</sup> (D<sub>2</sub>O), 25.0 °C,  $I = 2.0$  M (NaClO<sub>4</sub>),  $K_{CN} = 2.8 \times 10^3$  M<sup>-1</sup>, 25.0 °C,  $I = 0.50$  M ((TBA)ClO<sub>4</sub>)), followed by solvent-assisted, rate-determining cleavage of the Co–Ado bond of (β-Ado)(α-CN)Cbi ( $10^2k_2 = 2.91$  (H<sub>2</sub>O) and  $2.17$  s<sup>-1</sup> (D<sub>2</sub>O), 25.0 °C,  $I = 1.0$  M (NaClO<sub>4</sub>);  $10^2k_2 = 2.55$  (H<sub>2</sub>O) and  $2.45$  s<sup>-1</sup> (D<sub>2</sub>O), 25.0 °C,  $I = 2.0$  M (NaClO<sub>4</sub>),  $10^5k_2 = 6.7$  s<sup>-1</sup>, 92% DMF–8% D<sub>2</sub>O, 25.0 °C,  $I = 0.50$  M ((TBA)ClO<sub>4</sub>)). Deviations in the plots of the observed rate constant versus cyanide concentration in aqueous solution at high cyanide concentrations is attributed to medium effects, and only CN<sup>-</sup>, not HCN, reacts with AdoCbiOH. Evidence for rapid formation of (β-adenosyl)(α-cyano)cobinamide from AdoCbiOH plus cyanide is also obtained by investigating the <sup>1</sup>H NMR chemical shifts of the signals in the aromatic region immediately after mixing AdoCbiOH and cyanide as a function of cyanide concentration (D<sub>2</sub>O, 25.0 °C;  $K_{CN}' = 5.7$  and  $3.3$  M<sup>-1</sup> at  $I = 1.0$  (NaClO<sub>4</sub>) and  $2.0$  M (NaClO<sub>4</sub>), respectively). A comparison of rate and equilibria constants for the reaction between AdoCbiOH and 5'-deoxyadenosylcobalamin is made.

## Introduction

The reaction between cyanide and the B<sub>12</sub> corrinoids has been known since the first corrinoids were isolated in the 1960s, and was used as a method of characterisation before <sup>1</sup>H NMR spectroscopy and X-ray crystallography became widely available.<sup>1,2</sup> What originally brought this reaction to our attention<sup>3</sup> was the considerable discussion in the literature over several decades regarding the mechanism of the reaction between coenzyme B<sub>12</sub> (5'-deoxyadenosylcobalamin, AdoCbl) and cyanide to form dicyanocobalamin, adenine and 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol;<sup>1c,3-7</sup> specifically, does cyanide first substitute at the β or α site of the corrinoid (Fig. 1a). We

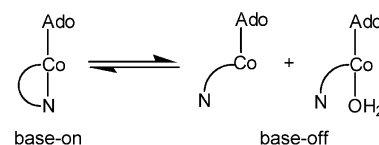


**Fig. 1** Schematic representation of (a) 5'-deoxyadenosylcobalamin (AdoCbl), showing the two possibilities for approach of the CN<sup>-</sup> ligand (upper = β, lower = α) with respect to the corrin ring of the cobalamin and (b) 5'-deoxyadenosylcobinamide (AdoCbi<sup>+</sup>).

recently answered this question by examining the reaction in 92% *N,N*-dimethylformamide (DMF)–8% D<sub>2</sub>O.<sup>3b</sup> Under these conditions, unlike in aqueous solution,<sup>3a</sup> an intermediate, (β-adenosyl)(α-cyano)cobalamin, could be unequivocally identified by both UV-visible and <sup>1</sup>H NMR spectroscopies, thus

demonstrating that cyanide first substitutes at the α (or lower, Fig. 1a) site of AdoCbl. The formation of (β-alkyl)(α-cyano)cobamides from the reaction between alkylcobalamins or alkylcobinamides and cyanide has been reported for a range of alkylcobamides.<sup>8</sup>

In this paper a similar study has been carried out on the reaction between cyanide and 5'-deoxyadenosylcobinamide, AdoCbiOH, a well known model compound of AdoCbl. A schematic representation of 5'-deoxyadenosylcobinamide is given in Fig. 1b. Such a study is useful, as it provides a way of further probing the interpretation of the AdoCbl plus CN<sup>-</sup> kinetic data, and to our knowledge is the first mechanistic study on the reaction between an alkylcobinamide and cyanide. (It has been proposed that alkylcobinamides exist in both five-coordinate (no OH<sub>2</sub> ligand at the α axial site) and six-coordinate forms.<sup>9</sup>) Although AdoCbl and AdoCbiOH are very similar in structure, there are several important differences which should be reflected in their reaction with cyanide: (1) AdoCbl can exist in a “base-on” (the 5,6-dimethylbenzimidazole, DMBI, is intramolecularly coordinated to the α axial site of the corrinoid, Fig. 2) and “base-off” form (a solvent molecule is instead coordinated at the α site or the α site is unoccupied).<sup>1</sup> In aqueous solution ≈99% of AdoCbl exists in its base-on form at 25 °C.<sup>10</sup> EXAFS studies indicate that base-off AdoCbl is mainly five-coordinate at 25.0 °C.<sup>9b</sup> A base-on form does not exist for AdoCbiOH. (2) The overall charge on



**Fig. 2** “Base-on” and “base-off” forms of AdoCbl. 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).

† Electronic supplementary information (ESI) available: kinetic plots. See <http://www.rsc.org/suppdata/dt/b1/b109292h/>

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AdoCbl is zero, while AdoCbi<sup>+</sup> is cationic (+1). And thirdly, (3) a comparison of both rate and equilibria constants between the two systems provides a further check on the proposed mechanism<sup>3b</sup> for the reaction between corrinoids and CN<sup>-</sup>.

In this study the reaction between AdoCbiOH and CN<sup>-</sup> has been investigated in both aqueous solution and 92% DMF–8% D<sub>2</sub>O by UV-visible and <sup>1</sup>H NMR spectroscopies. Comparisons between rate and equilibrium constants with those obtained for the reaction between AdoCbl and cyanide<sup>3a,b</sup> are made.

## Experimental

5'-Deoxyadenosylcobinamide hydroxide (AdoCbiOH) was prepared by a modification of a literature procedure.<sup>11</sup> Specifically, desalting by phenol extraction was replaced by an Amberlite XAD-2 column separation. The product (from 250 mg AdoCbl starting material) was dissolved in a solution of NaCl (≈25 mg) in 50 mL of H<sub>2</sub>O and loaded onto a ≈5 × 8 cm XAD-2 column prepared in H<sub>2</sub>O which had been pre-washed with EtOH, MeOH, CH<sub>3</sub>CN and H<sub>2</sub>O. The column was washed until the eluent tested negative for Cl<sup>-</sup> (AgNO<sub>3</sub>) and the product eluted with 4 : 1 EtOH : H<sub>2</sub>O. The resulting product was found to be ≈97% pure (two separate syntheses were ≈96% and ≈98% pure, respectively), by examination of the aromatic region of the <sup>1</sup>H NMR spectrum (in D<sub>2</sub>O, referenced internally with TSP; four signals at 5.63(d), 6.98, 8.03 and 8.24 ppm) in agreement with literature values.<sup>11a</sup> 5'-Deoxyadenosylcobalamin (≈98%), tetrabutylammonium cyanide ((TBA)CN, 96%), sodium cyanide (97% and 99.99%, according to the manufacturer the latter NaCN was 97.9% by titration with AgNO<sub>3</sub>; control experiments established that the results were independent of which NaCN was used) and 5'-deoxyadenosine (97%) were obtained from Aldrich. (CAUTION: cyanide salts are highly toxic.) Adenine (≥99%) and tetrabutylammonium perchlorate ((TBA)ClO<sub>4</sub>, ≥99%) were purchased from Fluka. Cyanocobinamide (CNCbiOH) was prepared according to a literature procedure<sup>12</sup> and found to be ≈90% pure by integration of the <sup>1</sup>H NMR signals in the aromatic region for a solution of CNCbiOH in NaCN (CNCbi<sup>+</sup> + CN<sup>-</sup> → (CN)<sub>2</sub>Cbi; δ((CN)<sub>2</sub>Cbi, H-ClO)<sup>13</sup> = 5.88 ppm (D<sub>2</sub>O, 4.00 × 10<sup>-2</sup> M NaCN, 0.100 M CAPS,<sup>14</sup> pD 11.0, I = 1.0 M (NaClO<sub>4</sub>)), in agreement with a literature value<sup>15</sup>). 8,5'-Anhydroadenosine was prepared by a modified published procedure.<sup>3b</sup> All other chemicals were of AR grade and purchased from either Sigma (CAPS, CHES, TES, TAPS buffers)<sup>14</sup> or BDH (NaClO<sub>4</sub>). Distilled water was purified through a Milli-Q ultra-pure water system.

Solution pH was measured at 25.0 ± 0.1 °C using an Orion 710A pH meter equipped with an Orion 91-01 glass electrode and an Orion 90-20 double junction reference electrode. A 1.60 M NH<sub>4</sub>NO<sub>3</sub>–0.200 M NaNO<sub>3</sub> solution adjusted to pH 7.0 (conc. HNO<sub>3</sub>) was used as the outer filling solution of the reference electrode, so that measurements in a ClO<sub>4</sub><sup>-</sup> medium were possible. The electrodes were standardised using BDH pH 6.98 and 10.00 buffers. Solution pH was adjusted using conc. NaOH or conc. HClO<sub>4</sub> as necessary. Measurements in alkaline solution were carried out under an argon atmosphere. Solutions of pH 12.0 were prepared from a NaOH solution of known concentration.

(TBA)CN was stored under nitrogen and handled in a glove bag under positive nitrogen pressure due to its hygroscopic nature. Buffers (0.100 M) were used in aqueous solutions for pH ≤ 11 and control reactions in the absence of buffers showed that they had no effect on reaction rates. AdoCbiOH and AdoCbl solutions were protected from light (*i.e.*, red-light-only conditions) due to their known light sensitivity.<sup>1</sup> All solutions were filtered through a millipore filter (0.45 μm).

UV-Visible spectra were measured using a Cary 1E spectrophotometer equipped with a thermostatted cell compartment

(±0.1 °C) and operating with Varian WinUV Bio software, version 2.00 (1999). The wavelengths chosen to collect kinetic data were as follows: AdoCbi<sup>+</sup> + CN<sup>-</sup> in H<sub>2</sub>O–D<sub>2</sub>O, 367 or 579 nm; AdoCbiOH + CN<sup>-</sup> in DMF–D<sub>2</sub>O solvent mixtures, 625 nm; AdoCbl + CN<sup>-</sup> in H<sub>2</sub>O, 367 nm. A wavelength >600 nm was chosen to analyse the reaction between AdoCbiOH and CN<sup>-</sup> in DMF–D<sub>2</sub>O solvent mixtures, since under these conditions both (TBA)CN and AdoCbiOH undergo slow decomposition reactions. Data were collected for at least six half-lives and fitted to a single exponential rate equation using the data fitting program OLIS-KINFIT (On-Line Instrument Systems, Inc., Jefferson, GA, USA, 1989).

<sup>1</sup>H NMR spectra were obtained on a Varian Inova 500 MHz spectrometer equipped with a 5 mm thermostatted (25.0 ± 0.2 °C) probe. Solutions were prepared in D<sub>2</sub>O and/or DMF-d<sub>7</sub> and TSP was added as an internal reference.

Data fits were carried out using Microcal Origin, version 3.5. All errors are reported as one standard deviation of the mean value.

### Determination of the acid dissociation constant for HCN

The acidity constant of HCN was determined in duplicate [once each with both sources (97% and 99.99%) of NaCN; the two values agreed to within 0.01 pH units] by potentiometric titration: 40.00 mL of a ≈1 × 10<sup>-2</sup> M NaCN (9.75 × 10<sup>-3</sup> and 0.0104 M, respectively, I = 1.0 M (NaClO<sub>4</sub>)) was titrated with 0.100 or 0.200 mL aliquots of a HClO<sub>4</sub> solution (0.219 and 0.214 M, respectively, I = 1.0 M (NaClO<sub>4</sub>)), giving a mean pK<sub>a(HCN)</sub> = 8.95 ± 0.03 (25.0 °C, I = 1.0 M (NaClO<sub>4</sub>)).<sup>16</sup>

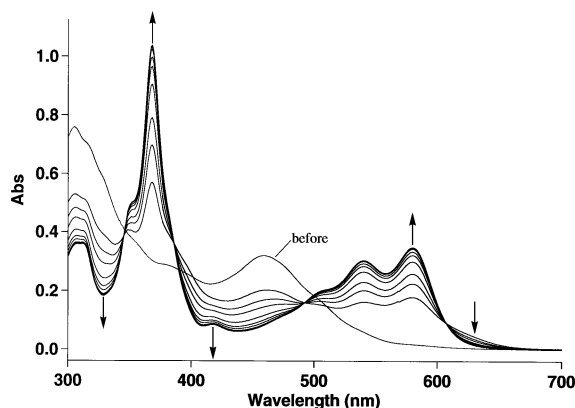
### Determination of the percentage of adenine product by HPLC

A predried sample of AdoCbiOH (13.1 mg, 4.89 × 10<sup>-4</sup> M, dried overnight under vacuum (0.1 torr) at 40 °C) was dissolved in 20.00 mL of a cyanide solution (0.100 M NaCN, pH 11.0, I = 1.0 M (NaClO<sub>4</sub>)) and allowed to react to completion under red-light-only conditions (≈1 h). A sample for HPLC measurements was prepared by adjusting the solution to pH ≈7 (conc. NaOH), and 1.00 mL of solution diluted to 10.00 mL with phosphate buffer (2.85 × 10<sup>-2</sup> M), pH 7. An adenine solution (5.07 × 10<sup>-5</sup> M) was prepared in phosphate buffer (2.57 × 10<sup>-2</sup> M) and the two samples analysed by HPLC immediately following one another. HPLC were recorded using a Waters Alliance 2690 Separation Module operating with a Waters 996 Photodiode Array Detector and a Waters C18, 3.5 μm, 4.6 × 7.55 mm column in 10% CH<sub>3</sub>CN–90% (2.85 × 10<sup>-2</sup> M phosphate buffer) at 1.2 mL min<sup>-1</sup> flow rate, λ = 260 nm. Each sample was analysed twice. Comparison of the peak areas of the adenine (2.97 min) in the two samples gave the percentage conversion of AdoCbiOH in the presence of 0.100 M NaCN = 100 ± 2%.

## Results

### The reaction between AdoCbiOH and cyanide in aqueous solution

The reaction between AdoCbiOH and cyanide (NaCN) was initially examined in aqueous solution by UV-visible spectroscopy. Fig. 3 gives typical spectra before and after mixing (cycle time = 0.400 min) an AdoCbiOH solution (≈1 × 10<sup>-4</sup> M, pH 11.0, I = 1.0 M (NaClO<sub>4</sub>)) with an equal volume of a NaCN solution (0.200 M, pH 11.0, I = 1.0 M (NaClO<sub>4</sub>)) using a tandem (split-cell) cuvette. The spectrum before mixing is that of AdoCbi<sup>+</sup> (λ<sub>max</sub> = 305, 377 and 458 nm, in agreement with literature values).<sup>11a</sup> (Note that pH 11.0 ± 0.1 was chosen since at pH 11.0, >99% of the cyanide exists as CN<sup>-</sup> rather than HCN (pK<sub>a(HCN)</sub> = 8.95 ± 0.03, 25.0 °C, I = 1.0 M (NaClO<sub>4</sub>)); details are given in the Experimental section entitled "Determination of the acid dissociation constant for HCN".) The spectrum at the completion of the reaction corresponds to



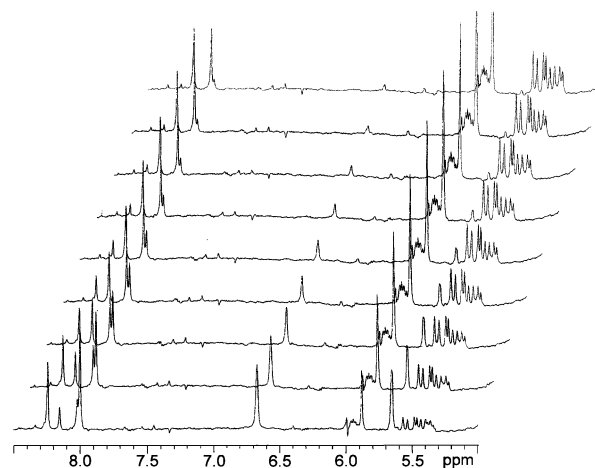
**Fig. 3** UV-Visible spectra recorded before and after mixing (tandem cuvette) AdoCbiOH ( $\approx 1 \times 10^{-4}$  M, H<sub>2</sub>O, 0.100 M CAPS, pH 11.0,  $I = 1.0$  M (NaClO<sub>4</sub>)) with an equal volume of NaCN (0.200 M, H<sub>2</sub>O, 0.100 M CAPS, pH 11.0,  $I = 1.0$  M (NaClO<sub>4</sub>)) at 25.0 °C. Cycle time = 0.400 min.

dicyanocobinamide, (CN)<sub>2</sub>Cbi ( $\lambda_{\text{max}} = 312, 368, 418, 540$  and 580 nm). Confirmation of this was obtained by observing an identical spectrum for the product of the reaction (= (CN)<sub>2</sub>Cbi)<sup>1c</sup> between CNCbi<sup>+</sup> and CN<sup>-</sup>. A colour change from yellow to blue/black was immediately observable ( $\leq 2$  s) upon mixing AdoCbiOH and cyanide. Close examination of the first few spectra after mixing reveals that the species present immediately after mixing absorbs significantly at  $\approx 630$  nm; that is, an intermediate is observable, since neither the reactant AdoCbi<sup>+</sup> or product (CN)<sub>2</sub>Cbi absorb significantly at this wavelength. Two intermediates are possible, ( $\beta$ -adenosyl)-( $\alpha$ -cyano)cobinamide, ( $\beta$ -Ado)( $\alpha$ -CN)Cbi, or cyanocobinamide, CNCbi<sup>+</sup>, corresponding to substitution of the first cyanide at the  $\alpha$  or  $\beta$  sites of AdoCbi<sup>+</sup>, respectively. However, previous studies suggest that an absorbance at  $\lambda > 600$  nm is associated with the  $\gamma$  band of a ( $\beta$ -alkyl)( $\alpha$ -CN)cobinamide intermediate; that is, ( $\beta$ -Ado)( $\alpha$ -CN)Cbi, rather than CNCbi<sup>+</sup>.<sup>3b,8a-c,e</sup> This was confirmed by measuring the UV-visible spectrum of CNCbi<sup>+</sup> ( $\lambda_{\text{max}}$  at 322(s), 356, 404, 499(s), 526 nm (pH 11.0,  $I = 1.0$  M (NaClO<sub>4</sub>)), in agreement with literature values,<sup>17</sup> actually a mixture of  $\beta$  and  $\alpha$  isomers,<sup>8d</sup> no significant absorbance for  $\lambda > 600$  nm).

After mixing AdoCbiOH and cyanide, sharp isosbestic points at 345, 387, 495 and 615 nm are observed, meaning that the intermediate is cleanly converted to (CN)<sub>2</sub>Cbi. Data at a selected wavelength (e.g. 367 nm, where the largest absorbance changes were observed) can be fitted to a single first-order rate equation (see Fig. A, ESI), giving  $k_{\text{obs}} = (9.76 \pm 0.01) \times 10^{-3} \text{ s}^{-1}$ .

The reaction was also examined by <sup>1</sup>H NMR spectroscopy, using D<sub>2</sub>O as the solvent. Fig. 4 gives <sup>1</sup>H NMR spectra of the aromatic region for the reaction between AdoCbiOH and CN<sup>-</sup> at intervals of 1.52 min after dissolving AdoCbiOH in a cyanide solution ( $4.00 \times 10^{-2}$  M NaCN, pD 11.0, D<sub>2</sub>O,  $I = 1.0$  M (NaClO<sub>4</sub>)). The initial spectrum consists of a  $\approx 3 : 1$  mixture of reactants (signals at 5.65 (d, A11), 6.67 (C10), 8.00 (A8), and 8.24 ppm (A2)) and products (the remaining signals).<sup>13</sup> (It will later be shown that under these conditions ( $[\text{CN}^-] = 4.00 \times 10^{-2}$  M), the “reactant” signals contain contributions from both AdoCbi<sup>+</sup> and ( $\beta$ -Ado)( $\alpha$ -CN)Cbi). Product signals increase at a comparable rate to the rate of decrease of the signals of the reactants as a function of time, and correspond to the known chemical shifts of (CN)<sub>2</sub>Cbi (5.88 ppm, in agreement with a literature value),<sup>15</sup> adenine (8.02, 8.15 ppm)<sup>3a</sup> and 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol (5.39, 5.43, 5.46, 5.48, 5.53, 5.57 and multiplets at 5.32–5.36 and 5.9–6.0 ppm).

A rate constant for the reaction between AdoCbiOH and CN<sup>-</sup> could be obtained from the data shown in Fig. 4 by examining the rate of decrease or increase of reactant or product signal areas, respectively. A plot of  $\ln(\text{peak area of the$

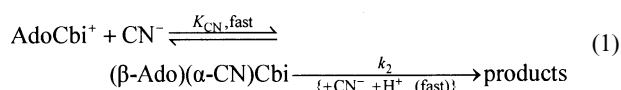


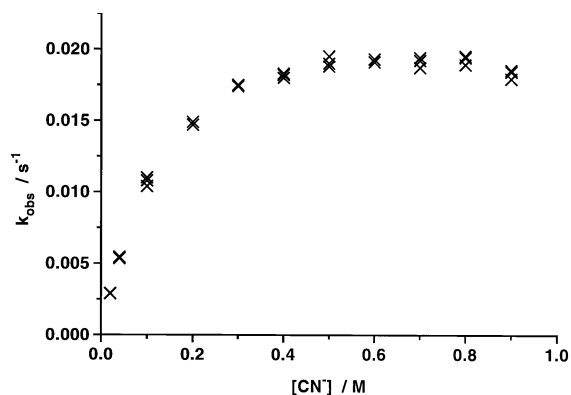
**Fig. 4** <sup>1</sup>H NMR spectra for the reaction between AdoCbiOH and CN<sup>-</sup> (D<sub>2</sub>O,  $[\text{AdoCbi}^+]_t \approx 1 \times 10^{-3}$  M,  $[\text{NaCN}]_f = 4.00 \times 10^{-2}$  M, D<sub>2</sub>O, 0.100 M CAPS, pD 11.0  $\pm$  0.1, 25.0 °C,  $I = 1.0$  M (NaClO<sub>4</sub>;  $f = \text{final concentration}$ ) at times (relative to the first spectrum; from bottom to top) 0, 1.52, 3.04, 4.56... min. Peaks can be assigned to AdoCbi<sup>+</sup>/ $\beta$ -Ado)( $\alpha$ -CN)Cbi (5.65 (d, All), 6.67 (C10), 8.00 (A8), 8.24 ppm (A2)), (CN)<sub>2</sub>Cbi (5.88 ppm (C10)), adenine (8.02, 8.15 ppm), 1-cyano-D-erythro-2,3-dihydroxy-4-pentenol (5.39, 5.43, 5.46, 5.48, 5.53, 5.57 ppm and multiplets at 5.32–5.36 and 5.9–6.0 ppm).

“reactant” signal at 8.24 ppm) versus time gave a linear plot and a corresponding first-order rate constant,  $k_{\text{obs}} = (4.3 \pm 0.2) \times 10^{-3} \text{ s}^{-1}$  (plot not shown; reported  $k_{\text{obs}}$  is the mean of two separate experiments). This value is in good agreement with a value obtained by UV-visible spectroscopy under the same conditions,  $4.69 \times 10^{-3} \text{ s}^{-1}$  (except that D<sub>2</sub>O rather than H<sub>2</sub>O was used as the solvent for the NMR experiment), especially given that  $k_{\text{obs}}$  would be expected to show some solvent dependence. Indeed, this will shortly be demonstrated to be the case.

Importantly, the reaction between AdoCbiOH and cyanide proceeds *via* clean ( $\geq 98\%$ ) heterolytic Co–C cleavage, since only Co–C heterolytic cleavage products were observed by <sup>1</sup>H NMR spectroscopy (*i.e.*, adenine + cyanohydrin), rather than the Co–C homolytic cleavage products 5'-deoxyadenosine and/or 8,5'-anhydroadenosine. (<sup>1</sup>H NMR chemical shifts in the aromatic region for 5'-deoxyadenosine and 8,5'-anhydroadenosine are at 6.04(d), 8.24 and 8.28 ppm and 6.19 and 8.20 ppm, respectively ( $4.00 \times 10^{-2}$  M NaCN, pD 11.0,  $I = 1.0$  M (NaClO<sub>4</sub>)).) HPLC has also been demonstrated to be a useful technique for quantification of the nucleoside products arising from Ado–Co bond cleavage.<sup>3a,7,18</sup> The products of the reaction between AdoCbiOH ( $4.89 \times 10^{-5}$  M) and NaCN (0.100 M) were therefore analysed by HPLC, confirming that only Co–C heterolysis occurs, since  $1.00 \pm 0.02$  mol equiv. adenine was produced per AdoCbiOH (see the section entitled “Determination of the percentage of adenine product by HPLC” in the Experimental section).

The concentration dependence of the observed rate constant was examined in order to obtain more information on the mechanism of the reaction. Fig. 5 gives a plot of  $k_{\text{obs}}$  versus  $[\text{CN}^-]$  (pH 11.0  $\pm$  0.1, H<sub>2</sub>O,  $I = 1.0$  M (NaClO<sub>4</sub>)). It is clear from this figure that  $k_{\text{obs}}$  increases with increasing CN<sup>-</sup> concentration, reaching a limiting value for  $[\text{CN}^-] \geq 0.5$  M. Such curvature is typical of saturation kinetics in which a rapid pre-equilibrium precedes the rate-determining step. Given that UV-visible spectroscopy measurements clearly indicate that ( $\beta$ -Ado)( $\alpha$ -CN)Cbi is rapidly formed upon the addition of CN<sup>-</sup> to AdoCbi<sup>+</sup>, which then reacts further to form the products, the most plausible mechanism is:





**Fig. 5** Plot of observed rate constant ( $k_{\text{obs}}$ ) versus  $\text{CN}^-$  concentration for the reaction between AdoCbiOH and  $\text{CN}^-$  ([AdoCbiOH]  $\approx 1 \times 10^{-4}$  M,  $\text{H}_2\text{O}$ , 0.100 M CAPS, pH  $11.0 \pm 0.1$ ,  $25.0^\circ\text{C}$ ) at  $[\text{NaCN}] = 4.00 \times 10^{-2}$ – $0.900$  M,  $I = 1.0$  M ( $\text{NaClO}_4$ ).

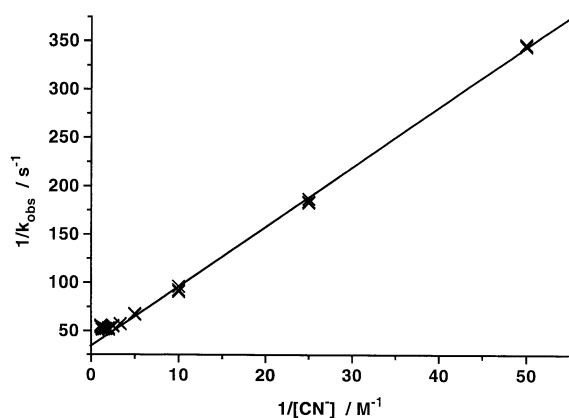
with a rate equation

$$k_{\text{obs}} = k_2 K_{\text{CN}} [\text{CN}^-] / (1 + K_{\text{CN}} [\text{CN}^-]) \quad (2)$$

Note that cyanide is not involved in the rate-determining step. The data given in Fig. 5 also hint that  $k_{\text{obs}}$  may actually decrease for  $[\text{CN}^-] > 0.8$  M. To determine whether or not this decrease is real (*i.e.*, beyond experimental error), and, if real, whether it results from medium effects or a changeover in mechanism at high  $\text{CN}^-$  concentrations, the same reaction was examined at a total ionic strength of 2.0 rather than 1.0 M, so that  $\text{CN}^-$  concentrations up to 1.9 M could now be examined. This data is given in Fig. B in the ESI. Under these conditions, the observed rate constant only begins to decrease when  $[\text{CN}^-]$  approaches its highest values ( $[\text{CN}^-] \geq 1.5$  M), rather than for  $[\text{CN}^-] > 0.8$  M as would be expected if a change in mechanism was responsible for the decrease. It is therefore likely that the decrease at high  $\text{CN}^-$  concentrations is a result of medium effects. Rearranging eqn. (2) gives:

$$1/k_{\text{obs}} = 1/k_2 K_{\text{CN}} [\text{CN}^-] + 1/k_2 \quad (3)$$

The lowest  $\text{CN}^-$  concentration for which medium effects become important can therefore be determined by a plot of  $1/k_{\text{obs}}$  versus  $1/[\text{CN}^-]$ , since the data should fit a straight line when medium effects are negligible. Fig. 6 gives this plot for the

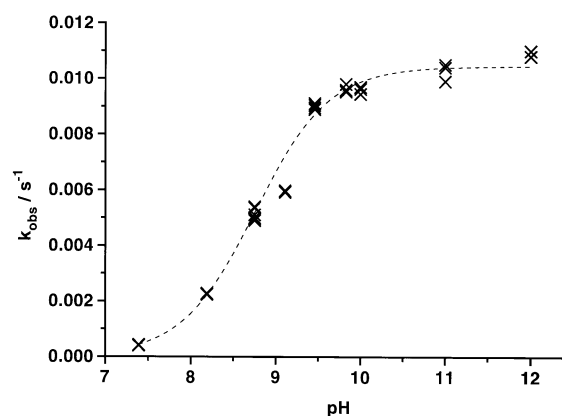


**Fig. 6** Plot of  $1/k_{\text{obs}}$  versus  $1/[\text{CN}^-]$  for the data given in Fig. 5. The line represents the best fit of the data at the five lowest  $\text{CN}^-$  concentrations to eqn. (3), giving  $k_2 = (2.91 \pm 0.10) \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 5.6 \pm 0.1 \text{ M}^{-1}$ .

data in Fig. 5. A similar plot at  $I = 2.0$  M is given in Fig. C in the ESI. Values of  $k_2$  and  $K_{\text{CN}}$  corresponding to the best fit of the data to eqn. (3) at the five lowest  $\text{CN}^-$  concentrations are  $k_2 = 2.91 \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 5.6 \text{ M}^{-1}$  ( $25.0^\circ\text{C}$ ,  $I = 1.0$  M

( $\text{NaClO}_4$ )). This means that at the conditions used in the  $^1\text{H}$  NMR experiment shown in Fig. 4,  $\approx 20\%$  ( $\beta\text{-Ado}$ )( $\alpha\text{-CN}$ )Cbi is rapidly formed upon dissolving AdoCbiOH in  $4.00 \times 10^{-2}$  M  $\text{CN}^-$ , since  $[(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}]/[\text{AdoCbi}^+] = K_{\text{CN}}[\text{CN}^-] = 5.6 \times 0.04 = 0.22$ . The experimental data deviates from linear behaviour at  $[\text{CN}^-] > 0.3$  M ( $I = 1.0$  M, Fig. 6) and  $[\text{CN}^-] > 1.0$  M ( $I = 2.0$  M, Fig. C, ESI); hence medium effects are important for  $[\text{CN}^-] > 0.3$  M and  $[\text{CN}^-] > 1.0$  M for  $I = 1.0$  and 2.0 M, respectively. Deviations from ideal behaviour are known to occur when a reactant concentration (in this case  $\text{CN}^-$ ) begins to make a significant contribution to the total ionic strength of the solution; that is, when  $[\text{CN}^-] \ll [\text{ClO}_4^-]$  is no longer true.<sup>19</sup>

The pH dependence of the observed rate constant for the reaction between AdoCbiOH and  $\text{CN}^-$  was examined. Fig. 7



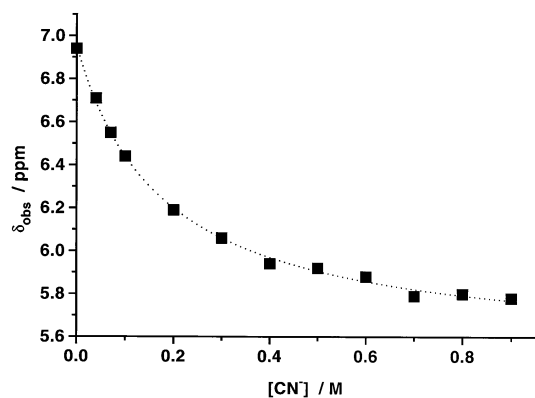
**Fig. 7**  $k_{\text{obs}}$  versus pH for the reaction between AdoCbiOH and  $\text{CN}^-$  ( $[\text{CN}^-] + [\text{HCN}] = 0.100$  M,  $\text{H}_2\text{O}$ ,  $25.0^\circ\text{C}$ ,  $I = 1.0$  M ( $\text{NaClO}_4$ )). The dotted line represents a simulated fit to eqn. (4) in the text, with  $K_{\text{a}}(\text{HCN}) = 1.12 \times 10^{-9}$  M,  $k_2 = 2.91 \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 5.6 \text{ M}^{-1}$ .

gives a plot of  $k_{\text{obs}}$  versus pH at a total cyanide concentration of 0.100 M. According to the mechanism proposed in eqn. (1), if the total cyanide concentration,  $[\text{CN}]_{\text{T}} (= [\text{CN}^-] + [\text{HCN}])$  is constant, assuming that only  $\text{CN}^-$ , not  $\text{HCN}$ , reacts, then:

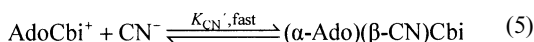
$$k_{\text{obs}} = \left( \frac{k_2 K_{\text{CN}} K_{\text{a}}(\text{HCN}) [\text{CN}]_{\text{T}}}{K_{\text{a}}(\text{HCN}) + a_{\text{H}^+}} \right) / \left( 1 + \frac{K_{\text{CN}} K_{\text{a}}(\text{HCN}) [\text{CN}]_{\text{T}}}{K_{\text{a}}(\text{HCN}) + a_{\text{H}^+}} \right) \quad (4)$$

The dotted line in Fig. 7 is a simulated fit with  $K_{\text{a}}(\text{HCN}) = 1.12 \times 10^{-9}$  M ( $\text{p}K_{\text{a}}(\text{HCN}) = 8.95$ , see section entitled ‘‘Determination of the acid dissociation constant for HCN’’ in the Experimental section),  $k_2 = 2.91 \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 5.6 \text{ M}^{-1}$ ; that is, the values of  $k_2$  and  $K_{\text{CN}}$  determined under the same conditions (in  $\text{H}_2\text{O}$ ,  $I = 1.0$  M ( $\text{NaClO}_4$ )) by UV-visible spectroscopy kinetic studies. The excellent fit of the data gives further support to the proposed mechanism and the assumption that only  $\text{CN}^-$  reacts with AdoCbi $^+$ .

If AdoCbi $^+$  and ( $\beta\text{-Ado}$ )( $\alpha\text{-CN}$ )Cbi are in fast exchange with respect to the NMR time scale, then the ratio of  $[\text{AdoCbi}^+]/[(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}]$  at a specific  $\text{CN}^-$  concentration could also be determined by examination of the initial ‘‘reactant’’ chemical shifts in the aromatic region immediately after mixing AdoCbiOH with cyanide, prior to the formation of the products. Fig. 8 gives a plot of the C10 signal of  $\{\text{AdoCbi}^+ + (\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}\}$  as a function of  $\text{CN}^-$  concentration at  $I = 1.0$  M. A similar plot was also obtained at  $I = 2.0$  M (Fig. D, ESI). The chemical shifts of the other three  $^1\text{H}$  signals of the Ado ligand which resonate in the aromatic region (A2, A8 and A11) were only slightly altered by the addition of  $\text{CN}^-$  ( $\Delta\delta = -0.04$ ,  $+0.10$  and  $-0.01$  ppm, respectively). Assuming that the change in chemical shift upon the addition of  $\text{CN}^-$  can be attributed to the formation of ( $\beta\text{-Ado}$ )( $\alpha\text{-CN}$ )Cbi with an associated equilibrium constant  $K_{\text{CN}}'$



**Fig. 8** Plot of  $^1\text{H}$  NMR chemical shift *versus*  $[\text{CN}^-]$  for the corrin C10 signal of  $\{\text{AdoCbi}^+ + (\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}\}$  ( $\text{D}_2\text{O}$ , 0.100 M CAPS, pD 11.0, 25.0  $^\circ\text{C}$ ,  $I = 1.0$  M ( $\text{NaClO}_4$ )). The data has been fitted to eqn. (6) in the text, giving  $K_{\text{CN}'} = 5.7 \pm 0.4 \text{ M}^{-1}$  and  $\delta_{\text{AdoCNCbi}} = 5.5 \pm 0.8$  ppm.



and that exchange between  $\text{AdoCbi}^+$  and  $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$  is fast with respect to the NMR time scale, it can be shown that

$$\delta_{\text{obs}} = (\delta_{\text{AdoCbi}} + K_{\text{CN}'}[\text{CN}^-]\delta_{\text{AdoCNCbi}})/(1 + K_{\text{CN}'}[\text{CN}^-]) \quad (6)$$

where  $\delta_{\text{obs}}$  is the observed chemical shift at a specific  $\text{CN}^-$  concentration,  $\delta_{\text{AdoCbi}}$  and  $\delta_{\text{AdoCNCbi}}$  are the chemical shifts of  $\text{AdoCbi}^+$  and  $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ , respectively, and  $K_{\text{CN}'}$  is the equilibrium constant corresponding to eqn. (5). Both sets of data (Fig. 8 and Fig. D, ESI) were fitted to eqn. (6) and the best fit of the data is superimposed on the experimental data ( $\cdots$ ). Equilibrium constants ( $K_{\text{CN}'}$ ) of  $5.7 \pm 0.4$  and  $3.29 \pm 0.05 \text{ M}^{-1}$  were obtained for  $I = 1.0$  and 2.0 M, respectively. Control experiments in the absence of  $\text{CN}^-$  showed that the C10  $^1\text{H}$  chemical shifts of  $\text{AdoCbi}^+$  in the aromatic region are only marginally altered by the addition of  $\text{ClO}_4^-$  ( $\Delta\delta_{\text{C10}} \leq 0.05$  ppm, 0–2.0 M  $\text{NaClO}_4$ ); that is, the change in chemical shift observed upon the addition of  $\text{CN}^-$  can be principally attributed to changing  $[\text{CN}^-]$  rather than to changing  $[\text{ClO}_4^-]$  (the latter being adjusted so as to maintain constant ionic strength).

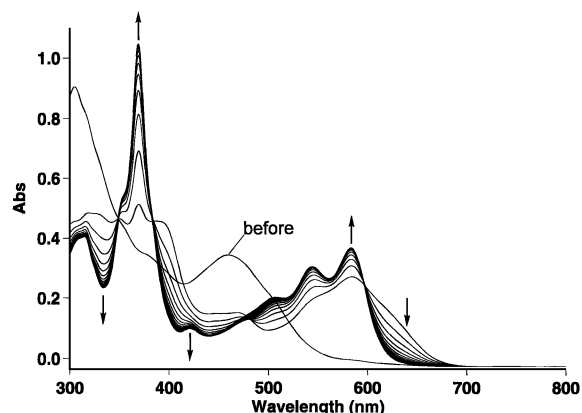
In order to be able to directly compare values of  $K_{\text{CN}'}$  and  $K_{\text{CN}}$  determined by UV-visible and  $^1\text{H}$  NMR spectroscopies, respectively, the concentration dependences of the observed rate constant for the reaction between  $\text{AdoCbiOH}$  and  $\text{CN}^-$  were re-determined (UV-visible spectroscopy) in  $\text{D}_2\text{O}$ , pD 11.0 at both  $I = 1.0$  and 2.0 M (ESI, Figs. E and F). The respective inverse plots of  $1/k_{\text{obs}}$  *versus*  $1/[\text{CN}^-]$  are given in Figs. G and H in the ESI. Values of  $k_2$  and  $K_{\text{CN}}$  corresponding to the best fit of the data at the five lowest  $\text{CN}^-$  concentrations are  $k_2 = (2.17 \pm 0.08) \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 6.9 \pm 0.4 \text{ M}^{-1}$  at  $I = 1.0$  M, and  $k_2 = (2.45 \pm 0.04) \times 10^{-2} \text{ s}^{-1}$  and  $K_{\text{CN}} = 3.8 \pm 0.1 \text{ M}^{-1}$  at  $I = 2.0$  M. The values of  $K_{\text{CN}}$  are not significantly different from the  $K_{\text{CN}'}$  values determined by  $^1\text{H}$  NMR spectroscopy ( $K_{\text{CN}'} = 5.7 \pm 0.4 \text{ M}^{-1}$ ,  $K_{\text{CN}} = 6.9 \pm 0.4 \text{ M}^{-1}$  ( $\text{D}_2\text{O}$ ,  $I = 1.0$  M ( $\text{NaClO}_4$ ));  $K_{\text{CN}'} = 3.29 \pm 0.05 \text{ M}^{-1}$ ,  $K_{\text{CN}} = 3.8 \pm 0.1 \text{ M}^{-1}$  ( $\text{D}_2\text{O}$ ,  $I = 2.0$  M ( $\text{NaClO}_4$ )).

Activation parameters ( $\Delta H^\ddagger = 53 \pm 4 \text{ kJ mol}^{-1}$ ,  $\Delta S^\ddagger = -97 \pm 14 \text{ J mol}^{-1} \text{ K}^{-1}$ ) were determined for the rate-determining step ( $k_2$ ) by examining the temperature dependence of the reaction (15.5, 20.5, 25.0, 30.1 and 34.5  $^\circ\text{C}$ ) at low  $\text{CN}^-$  concentrations ( $2.00 \times 10^{-2}$ –0.300 M). The rate constant at each temperature was taken from the intercept ( $= 1/k_2$ ) of linear plots of  $1/k_{\text{obs}}$  *versus*  $1/[\text{CN}^-]$  (see ESI Fig. I for Eyring plot).

#### The reaction between $\text{AdoCbiOH}$ and cyanide in 92% DMF–8% $\text{D}_2\text{O}$

The reaction between  $\text{AdoCbiOH}$  and tetrabutylammonium cyanide, (TBA)CN, was examined in 92% DMF–8%  $\text{D}_2\text{O}$ . It

has previously been established that under these conditions, cyanide exists as  $\text{CN}^-$  (*i.e.*, no HCN).<sup>3b</sup> A colour change occurs (from yellow to blue/black) immediately ( $\leq 2$  s later) upon the addition of  $\text{CN}^-$  to  $\text{AdoCbiOH}$  in 92% DMF–8%  $\text{D}_2\text{O}$ . From Fig. 9 it can be seen that the UV-visible spectrum obtained



**Fig. 9** UV-Visible spectra recorded before and directly after mixing (tandem cuvette)  $\text{AdoCbiOH}$  ( $\approx 1 \times 10^{-4}$  M, 92% DMF–8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA) $\text{ClO}_4$ )) with an equal volume of (TBA)CN ( $8.00 \times 10^{-2}$  M, 92% DMF–8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA) $\text{ClO}_4$ )) at 25.0  $^\circ\text{C}$ . Cycle time = 10.0 min.

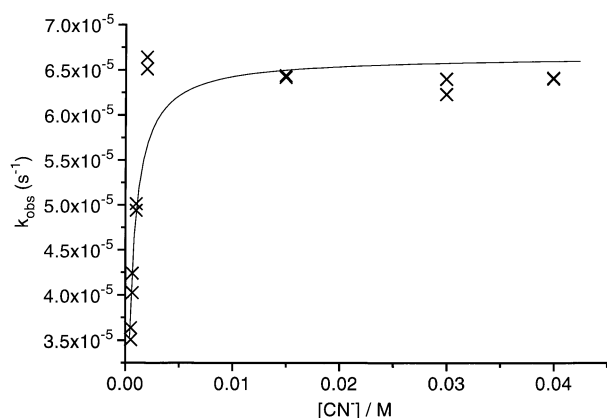
directly after mixing ( $\approx 15$  s later) a solution of  $\text{AdoCbiOH}$  ( $\approx 1 \times 10^{-4}$  M, 92% DMF–8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA) $\text{ClO}_4$ )) with a (TBA)CN solution ( $8.00 \times 10^{-2}$  M, 92% DMF–8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA) $\text{ClO}_4$ )) using a tandem cuvette was very different ( $\lambda_{\text{max}} = 318, 352, 369, 469$  and 584 nm) from the spectrum before mixing; that is, the spectrum of  $\text{AdoCbi}^+$  ( $\lambda_{\text{max}} = 306, 377(\text{s}), 460$  nm). This means that within the time required to acquire the first spectrum after mixing,  $\text{AdoCbiOH}$  has once again already reacted with cyanide to produce a new species. Following the reaction by UV-visible spectroscopy at longer times affords the addition of a second cyanide to the cobinamide intermediate to give dicyanocobinamide ( $\lambda_{\text{max}} = 315, 369, 421, 507, 545$  and 584 nm; confirmed by running the spectrum of  $\text{CNCbi}^+$  in  $4.00 \times 10^{-2}$  M (TBA)CN ( $\text{CNCbi}^+ + \text{CN}^- \rightarrow (\text{CN})_2\text{Cbi}$ ; 92% DMF–8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA)CN)). A good fit was obtained upon fitting the data at 625 nm to a first-order rate equation, giving  $k_{\text{obs}} = (6.40 \pm 0.05) \times 10^{-3} \text{ s}^{-1}$  (data not shown). The spectrum directly after mixing is very different from  $\text{CNCbi}^+$  (corresponding to substitution of the first  $\text{CN}^-$  at the  $\beta$  site of  $\text{AdoCbi}^+$ ;  $\lambda_{\text{max}}(\text{CNCbiOH}) = 323(\text{s}), 355, 405, 498, 527, 579(\text{s})$  nm in 92% DMF–8%  $\text{D}_2\text{O}$ ), and once again  $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$  is the reaction intermediate, as a result of  $\text{CN}^-$  first substituting at the  $\alpha$  site of  $\text{AdoCbi}^+$ .

The above reaction could also be followed by  $^1\text{H}$  NMR spectroscopy in DMF- $d_7$ - $\text{D}_2\text{O}$  under otherwise identical conditions. Four signals in the aromatic region were observed immediately upon dissolving  $\text{AdoCbiOH}$  in a solution of (TBA)CN (5.62, 5.71(d), 8.17 and 8.24 ppm,  $4.00 \times 10^{-2}$  M (TBA)CN, 92% DMF- $d_7$ -8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA) $\text{ClO}_4$ )), and correspond to the expected number of signals from  $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ ; three from the adenosyl ligand (A2, A8, A11(d)) and one from the corrin ring (C10). The chemical shifts in the aromatic region for the intermediate are substantially different from the chemical shifts of the two isomeric forms of  $\text{CNCbi}^+$  ( $\alpha$ - and  $\beta$ - $\text{CNCbi}^+$  have signals (C10) in the aromatic region at 6.37 ( $\alpha$  or  $\beta$ ) and 6.46 ( $\beta$  or  $\alpha$ ) ppm in 92% DMF- $d_7$ -8%  $\text{D}_2\text{O}$ ,  $I = 0.50$  M ((TBA)CN)). In short, this data provides independent evidence that once again  $(\beta\text{-Ado})(\alpha\text{-CN})\text{Cbi}$ , rather than one or both of the isomers of  $\text{CNCbi}^+$ , is the mono-cyano reaction intermediate. The signal areas of the intermediate decreased at the same rate as the rate of increase in the signals of the products, which were identified as  $(\text{CN})_2\text{Cbi}$  (5.83 ppm; confirmed by running the  $^1\text{H}$  NMR spectrum of  $\text{CNCbi}^+$

in  $4.00 \times 10^{-2}$  M (TBA)CN (92% DMF–8% D<sub>2</sub>O,  $I = 0.50$  M ((TBA)ClO<sub>4</sub>) and adenine (7.74, 8.02 ppm<sup>3b</sup>). The low signal-to-noise ratio made the cyanohydrin signals difficult to distinguish from the noise. A plot of ln(peak area of the intermediate signal at 8.24 ppm) versus time gave a straight line corresponding to an observed rate constant =  $(7.1 \pm 0.1) \times 10^{-5}$  s<sup>-1</sup> (plot not shown). This value is in good agreement with the value obtained by UV-visible spectroscopy ( $(6.40 \pm 0.05) \times 10^{-5}$  s<sup>-1</sup>), keeping in mind that once again solvent isotope effects may mean that the two values are not identical.

If the intermediate contains a Co–C bond, the rate of conversion of the intermediate to (CN)<sub>2</sub>Cbi should be considerably enhanced by exposing the solution to light, due to the known light sensitivity of Co–C corrinoid bonds.<sup>1</sup> This was much easier to test in 92% DMF–8% D<sub>2</sub>O compared with in aqueous solution, since the conversion of the intermediate to products is considerably slower in 92% DMF–8% D<sub>2</sub>O (in D<sub>2</sub>O,  $k_2 = 2.17 \times 10^{-2}$  s<sup>-1</sup> ( $I = 1.0$  M (NaClO<sub>4</sub>); in 92% DMF–8% D<sub>2</sub>O,  $k_2 = 6.7 \times 10^{-5}$  s<sup>-1</sup> ( $I = 0.50$  M ((TBA)ClO<sub>4</sub>)). The <sup>1</sup>H NMR spectrum of a solution of the intermediate in (TBA)CN ( $4.11 \times 10^{-2}$  M) which was exposed to indirect sunlight for ≈30 min showed that all the intermediate had been converted to (CN)<sub>2</sub>Cbi within this time (plus Ado–Co homolysis products; no attempt was made to identify and quantitate these). In the absence of light, this reaction takes ≈14 h (=  $5t_{1/2}$ ); hence the intermediate has a light-sensitive Co–C bond.

The concentration dependence of the observed rate constant for the reaction between AdoCbiOH and CN<sup>-</sup> was also examined by UV-visible spectroscopy in 92% DMF–8% D<sub>2</sub>O. Fig. 10



**Fig. 10** Plot of observed rate constant versus CN<sup>-</sup> concentration for the reaction between AdoCbiOH and (TBA)CN ([AdoCbiOH] ≈  $1 \times 10^{-4}$  M, [CN<sup>-</sup>] =  $5.00 \times 10^{-4}$ – $4.00 \times 10^{-2}$  M, 92% DMF–8% D<sub>2</sub>O,  $I = 0.50$  M ((TBA)ClO<sub>4</sub>), 25.0 °C). The data has been fitted to eqn. (2) in the text, giving  $k_2 = (6.7 \pm 0.2) \times 10^{-5}$  s<sup>-1</sup> and  $K_{CN} = (2.8 \pm 0.4) \times 10^3$  M<sup>-1</sup>.

summarises these results. Once again  $k_{obs}$  is seen to increase with increasing CN<sup>-</sup> concentration. Fitting the data in Fig. 10 to eqn. (2) gives the fit shown, with  $K_{CN} = (2.8 \pm 0.4) \times 10^3$  M<sup>-1</sup> and  $k_2 = (6.7 \pm 0.2) \times 10^{-5}$  s<sup>-1</sup>.

#### Dependence of the observed rate constant and the “isosbestic wavelengths” on the D<sub>2</sub>O–DMF solvent composition

The dependence of the observed rate constant and the “isosbestic wavelengths” for the reaction between AdoCbiOH and CN<sup>-</sup> ( $5.00 \times 10^{-2}$  M (TBA)CN) was examined as a function of D<sub>2</sub>O–DMF solvent composition, and the results are summarised in Table 1. (Upon close examination of the spectra it could be seen that the “isosbestic points” were not 100% clean in DMF–D<sub>2</sub>O solvent mixtures, due to the slow decomposition of AdoCbi<sup>+</sup> and (TBA)CN in DMF.) UV-Visible and <sup>1</sup>H NMR spectroscopic data suggest that cleavage of the Co–C bond of the intermediate is rate-determining in aqueous solution and in

**Table 1** Dependence of the observed rate constant and isosbestic points for the reaction between AdoCbiOH and cyanide on the percentage of D<sub>2</sub>O in DMF–D<sub>2</sub>O solvent mixtures ( $5.00 \times 10^{-2}$  M (TBA)CN, 25.0 °C)

% D <sub>2</sub> O	Mean $10^4 k_{obs}^a/s^{-1}$	“Isosbestic points”/nm
100	101 ± 2	345, 381, 486, 598
50	25.0 ± 0.1	346, 383, 483, 599
25	5.53 ± 0.04	347, 384, 483, 598
15	1.61 ± 0.01	348, 384, 483, 598
8	0.507 ± 0.009	348, 384, 483, 598
4	0.155 ± 0.002	349, 384, 478, 598

<sup>a</sup> Average of three values. All values agreed within 5%.

92% DMF–8% D<sub>2</sub>O. This is reflected in the wavelengths of the isosbestic points, which are only marginally affected ( $\Delta\lambda_{max} \leq 8$  nm) upon changing the solvent from 100% D<sub>2</sub>O through to 92% DMF–8% D<sub>2</sub>O. The observed rate constant (which contains contributions from both  $K_{CN}$  and  $k_2$ , eqn. (2)), decreases systematically by over two orders of magnitude in changing the solvent from 100% D<sub>2</sub>O to 96% DMF–4% D<sub>2</sub>O.

## Discussion

Examination of the UV-visible and <sup>1</sup>H NMR spectra for the reaction between AdoCbiOH and NaCN in aqueous solution and in 92% DMF–8% D<sub>2</sub>O immediately after mixing the reactants demonstrated that under both conditions AdoCbiOH reacts with CN<sup>-</sup> to give dicyanocobinamide, adenine and 1-cyano-D-erythro-2,3-dihydroxy-4-pentanol via a (β-Ado)-(α-CN)Cbi intermediate. The observed rate constant was found to increase with increasing CN<sup>-</sup> concentration, to reach a limiting value at higher CN<sup>-</sup> concentrations (Figs. 5 and 10 and Figs. B, E and F, ESI); that is, saturation kinetics are observed. The data were interpreted in terms of rapid formation of an (β-Ado)(α-CN)Cbi intermediate prior to rate-determining Ado–Co bond cleavage, eqn. (1), followed by fast substitution of the second CN<sup>-</sup> and subsequent rearrangement to give the products. Other authors have also observed that formation of (β-alkyl)(α-CN)cobinamide complexes from RCbi plus cyanide is rapid.<sup>8d</sup> The pH dependence of the observed rate constant in water could be solely accounted for by the change in the CN<sup>-</sup> concentration as a function of pH. If significant amounts of six-coordinate (β-Ado)(α-OH)Cbi were present at higher pH conditions, the observed rate constant would be expected to decrease at higher pH, since hydroxy groups have been shown to be substitution inert for a range of cobamides.<sup>20</sup> This is clearly not observed (Fig. 7), which suggests that AdoCbiOH is essentially base-off or that the α-OH<sub>2</sub> ligand of (β-Ado)-(α-OH<sub>2</sub>)Cbi<sup>+</sup> does not deprotonate under the conditions used in this study. The proposed mechanism for the reaction between AdoCbiOH and cyanide is given in Scheme 1.

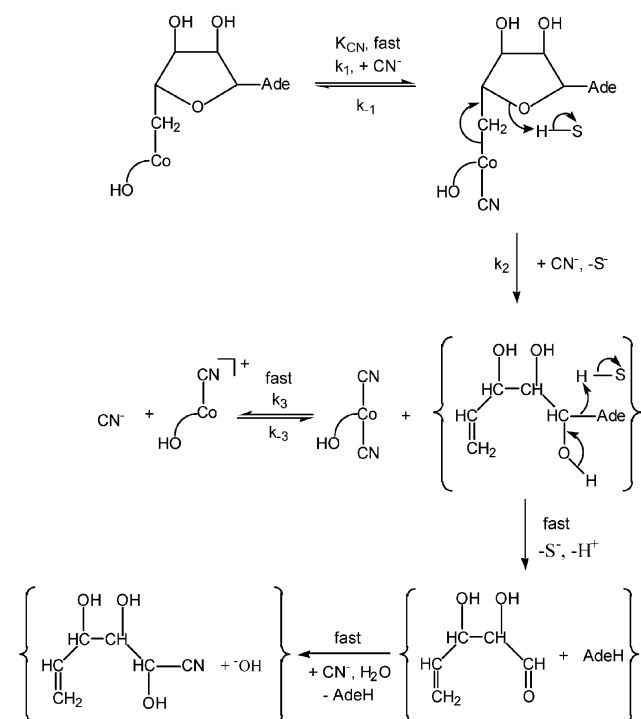
Evidence for fast exchange between AdoCbi<sup>+</sup> and (β-Ado)-(α-CN)Cbi with respect to the NMR time scale prior to rate-determining Co–C bond cleavage of the intermediate in aqueous solution was obtained by examining the C10 <sup>1</sup>H NMR chemical shifts of the reactant as a function of CN<sup>-</sup> concentration. From this data (Fig. 8 and Fig. D in the ESI),  $K_{CN}'$  (eqns. (5) and (6)), could be determined. The values of  $K_{CN}'$  were not significantly different from the  $K_{CN}$  value determined at the same conditions by UV-visible spectroscopy ( $K_{CN}' = 5.7 \pm 0.4$  M<sup>-1</sup>,  $K_{CN} = 6.9 \pm 0.4$  M<sup>-1</sup> (D<sub>2</sub>O,  $I = 1.0$  M (NaClO<sub>4</sub>));  $K_{CN}' = 3.29 \pm 0.05$  M<sup>-1</sup>,  $K_{CN} = 3.8 \pm 0.1$  M<sup>-1</sup> (D<sub>2</sub>O,  $I = 2.0$  M (NaClO<sub>4</sub>)). Indeed, excellent fits of the UV-visible kinetic data were obtained when fixing the value of  $K_{CN} = K_{CN}'$  (Figs. J and K, ESI).

Deviation of data for the reaction between AdoCbiOH and CN<sup>-</sup> at high CN<sup>-</sup> concentrations in aqueous solution (Fig. 5 and Figs. B, E and F, ESI) was attributed to the CN<sup>-</sup>

**Table 2** Rate and equilibria constants for the reaction between AdoCbiOH and cyanide in aqueous solution (25.0 °C, pH/pD 11.0,  $I = 1.0\text{--}2.0$  M (NaClO<sub>4</sub>)) and in 92% DMF–8% D<sub>2</sub>O (25.0 °C,  $I = 0.50$  M (TBA)ClO<sub>4</sub>)

Solvent	$I/M$	$K_{CN}^a/M^{-1}$	$K_{CN}^b/M^{-1}$	$k_2/s^{-1}$
H <sub>2</sub> O	1.0	5.6		$2.91 \times 10^{-2}$
H <sub>2</sub> O	2.0	4.6		$2.55 \times 10^{-2}$
D <sub>2</sub> O	1.0	6.9	5.7	$2.17 \times 10^{-2}$
D <sub>2</sub> O	2.0	3.8	3.3	$2.45 \times 10^{-2}$
DMF–D <sub>2</sub> O	0.50	$2.8 \times 10^3$		$6.7 \times 10^{-5}$

<sup>a</sup> Determined by UV-visible spectroscopy. <sup>b</sup> Determined by <sup>1</sup>H NMR spectroscopy.



**Scheme 1** Proposed mechanism for the reaction between AdoCbiOH and cyanide in aqueous solution and in DMF–D<sub>2</sub>O solvent mixtures.

concentration becoming significant with respect to the total contribution of anionic species to the ionic strength. Further support for this can be obtained from studying the reaction between AdoCbiOH and CN<sup>-</sup> ( $[CN^-] = 2.00 \times 10^{-3}\text{--}0.300$  M) as a function of temperature. At each temperature, a plot of  $1/k_{obs}$  versus  $1/[CN^-]$  was constructed, and the value of  $k_2$  calculated from the  $y$  axis intercept (eqn. (3)). The corresponding Eyring plot is linear (Fig. 1, ESI;  $\Delta H^\ddagger = 53 \pm 4$  kJ mol<sup>-1</sup> and  $\Delta S^\ddagger = -97 \pm 14$  J mol<sup>-1</sup> K<sup>-1</sup>), which gives support to the method used to determine  $k_2$  values; that is, from  $k_{obs}$  values at low CN<sup>-</sup> concentrations, and also supports the idea that medium, rather than some other chemical or mechanistic, effects are responsible for the deviations at high CN<sup>-</sup> concentrations.

Rate and equilibria constants for the reaction between AdoCbiOH and cyanide in aqueous solution and in 92% DMF–8% D<sub>2</sub>O are summarised in Table 2. The values of  $K_{CN}$  in aqueous solution ( $4\text{--}7$  M<sup>-1</sup>) are similar in magnitude to a previously reported value for formation of (β-ethyl)(α-cyano)cobinamide from ethylcobinamide plus cyanide ( $4.3 \pm 0.2$  M<sup>-1</sup> at 25.0 °C<sup>8d</sup>). From Table 2 it can be seen that the rate constant  $k_2$  corresponding to cleavage of the Co–Ado bond of (β-Ado)(α-CN)Cbi is ≈400 times larger in aqueous solution compared with 92% DMF–8% D<sub>2</sub>O. There is a gradual decrease in the reaction rate upon changing the solvent from 100% D<sub>2</sub>O to 96% DMF–4% D<sub>2</sub>O, Table 1. This supports our previous suggestion based on a study on the reaction between AdoCbl and cyanide

that Co–C bond cleavage of (β-alkyl)(α-cyano)cobamides is solvent-assisted.<sup>3b</sup> Also supporting this is the observation that  $k_2$  is slightly smaller (≈5–15%, Table 2) when D<sub>2</sub>O rather than H<sub>2</sub>O is the solvent. Solvent-assisted bond cleavage, in which transfer of a proton from the solvent assists in C–N,<sup>21a</sup> Si–N,<sup>21b</sup> or M–C<sup>21c</sup> (M = Si, Sn) bond cleavage, has also been proposed for other systems.

From Table 2 it can also be seen that the equilibrium constant for formation of the intermediate,  $K_{CN}$ , is ≈2 orders of magnitude larger in 92% DMF–8% D<sub>2</sub>O compared with in water. We attribute this to the enhanced nucleophilicity of cyanide in an apolar, diprotic solvent (*i.e.*, DMF) compared with aqueous solution. For biomolecular reactions involving small anions, rate enhancements of several orders of magnitude can occur when changing from a polar protic to an apolar diprotic solvent due to the increased activity of the anion as a result of its greatly reduced hydrogen bonding interactions with the solvent.<sup>22</sup>

A comparison can be made between rate and equilibria constants for the reaction between AdoCbl or AdoCbiOH with cyanide. In aqueous solution, a linear dependence on the observed rate constant with respect to cyanide concentration ( $[CN^-] \leq 0.500$  M) was interpreted in terms of rate-determining addition of CN<sup>-</sup> to AdoCbl ( $k_1/K_{Co} = 7.4 \times 10^{-3}$  M<sup>-1</sup> s<sup>-1</sup>).<sup>3a</sup> This result is unexpected, given that averaged {AdoCbi<sup>+</sup> + (β-Ado)(α-CN)Cbi} signals are observed by <sup>1</sup>H NMR spectroscopy, meaning that addition of CN<sup>-</sup> to AdoCbi<sup>+</sup> is very rapid (25 °C). Conversion of AdoCbl between its base-on and base-off forms is also fast.<sup>10</sup> We are therefore currently re-investigating the reaction between AdoCbl and cyanide at much higher cyanide concentrations, to see if there is any evidence for curvature on the plot of  $k_{obs}$  versus cyanide concentration at much higher cyanide concentrations; that is, evidence for rapid formation of an (β-Ado)(α-CN)Cbl intermediate.

It is also informative to compare the kinetics of the reaction between AdoCbi<sup>+</sup> plus CN<sup>-</sup> in 92% DMF–8% D<sub>2</sub>O with that of the reaction between AdoCbl and CN<sup>-</sup> in the same solvent mixture reported in our earlier paper<sup>3b</sup> (see the ESI for further discussion on the assumption  $1 \ll K_{Co}$  made in treating the AdoCbl + CN<sup>-</sup> data). The kinetic behaviour for the two reactions are very similar; that is, plots of  $k_{obs}$  versus CN<sup>-</sup> show curvature, from which values for the equilibrium constant for formation of the mono-cyano intermediate from the reactants ( $K_{CN} = 2.7 \times 10^3$  M<sup>-1</sup> for AdoCbi<sup>+</sup> and  $K_{CN}/K_{Co} = 1.2 \times 10^2$  M<sup>-1</sup> for AdoCbl) and the rate constant for cleavage of the Co–C bond of the mono-cyano intermediate ( $k_2$ ) can be extracted. The rate constant  $k_2$  for the two cobamide reactants (AdoCbi<sup>+</sup> and AdoCbl) are similar ( $6.7 \times 10^{-5}$  and  $9.3 \times 10^{-5}$  s<sup>-1</sup>, respectively), giving weight to the proposed mechanism in Scheme 1. The ratio of the equilibrium constants for formation of the intermediate,  $K_{CN}$  (for AdoCbi<sup>+</sup>) versus  $K_{CN}/K_{Co}$  (for AdoCbl) is ≈12, suggesting that  $K_{Co}$  for AdoCbl ≈ 12 (± 5) in 92% DMF–8% D<sub>2</sub>O. This value is in excellent agreement with the value of  $K_{Co}$  predicted on the basis of UV-visible spectroscopy measurements (= 12, see ESI for details).

## Summary

Rate and equilibria constants have been determined for the reaction between AdoCbiOH and cyanide in both aqueous solution and in 92% DMF–8% D<sub>2</sub>O. A (β-Ado)(α-CN)cobinamide intermediate is rapidly formed under all conditions, followed by rate-determining solvent-assisted cleavage of the Co–C bond of the intermediate.

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