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Kinetic and thermodynamic studies of the ligand substitution reactions of the cobalamins

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APPENDIX 1

NMR SPECTROSCOPIC DATA FOR AQUA-10-NITROSOCOBALAMIN

Table A1.1 ^{1}H and ^{13}C NMR Assignments for aqua-10-nitrosocobalamin[a]

Group	$\delta_{^{13}C}$	$\delta_{^{1}H}$	Group	$\delta_{^{13}C}$	$\delta_{^{1}H}$
C35	17.87	22.58	R1	89.93	6.32[h]
C53	18.14	2.66	C15	109.65	
C54	18.32	1.32	C5	113.06	
C25	19.51	1.42	B7	114.31	7.26
C47	20.74	1.45	B4	118.70	6.40
C36	21.30	1.94	B8	132.21	
Pr3	21.58[b]	1.27[c]	C10	132.77	
B11	21.87	2.26	B5	136.10	
C20	22.41	2.27	B9	139.10	
C30	28.68	2.01, 2.14	B2	143.80	6.80
C41	29.56	1.08, 1.89	C6	165.68	
C48	31.12	1.95, 2.10	C14	166.41	
C46	32.20	1.28	C11	170.88	
C60	33.90	2.70, 2.77	C9	171.28	
C42	34.93	1.09, 2.04	C38	177.08	
C55	35.05	1.87, 2.70	C57	177.14	
C56	35.41	2.10, 2.63	C61	178.14	
C49	37.10	2.66	C27	178.51	
C31	37.47	2.55, 2.62	C43	179.00	
C18	41.40	2.87	C32	180.24	
C37	44.12	2.12, 2.51	C50	180.30	
C26	44.90	2.43, 2.63	C16	182.24	
Pr1	48.00[d]	2.93, 3.63	C4	183.62	

Table A1.1 *Continued*

Group	$\delta_{^{13}C}$	$\delta_{^{1}H}$	**Group**	$\delta_{^{13}C}$	$\delta_{^{1}H}$
C2	50.01		R7		5.59
C12	53.80		dH$_A$		6.33
C7	54.85		dH$_S$		6.67
C8	56.77	3.65	bH$_S$		6.89
C13	58.05	3.43	eH$_S$		6.98
C3	58.85	4.43	cH$_S$		6.98
C17	61.11		gH$_S$		7.07
R5	63.05	3.75, 3.91	aH$_S$		7.07
R2	71.29	4.28	cH$_A$		7.33
R3	75.33[e]	4.74	bH$_A$		7.62
Pr2	75.39[f]	4.29	eH$_A$		7.67
C19	76.25	4.69	aH$_A$		7.76
R4	84.77[g]	4.06	gH$_A$		7.91
C1	88.29		fH		8.17

[a]In 90% H_2O/10%D_2O, 25 ˚C. Chemical shifts are relative to internal TSP. The *syn* and *anti* amide H's are designated aH$_S$, aH$_A$, bH$_S$, bH$_A$, etc., respectively, where *a*, *b*, etc. , refer to the standard side chain lettering. [b] $^3J_{PC}$ = 1.5 Hz. [c] $J_{Pr2\text{-}Pr3}$ = 6.0 Hz. [d] $^3J_{PC}$ = 4.7 Hz. [e] $^2J_{PC}$ = 9.9 Hz. [f] $^2J_{PC}$ = 3.8 Hz. [g] $^3J_{PC}$ = 6.9 Hz. [h] $^3J_{R1,R2}$ = 2.5 Hz.

Table A1.2 Comparison of the ^{13}C resonances of aquacobalamin, aqua-10-chlorocobalamin and aqua-10-nitrosocobalamin.

Group	**Aquacobalamin**	**Aqua-10-chlorocobalamin**		**Aqua-10-nitrosocobalamin**	
	ppm	**ppm**	**δ_1, ppm**	**ppm**	**δ_2, ppm**
C35	18.11	18.83	0.72	17.87	–0.24
C53	17.96	18.13	0.17	18.14	0.18
C54	19.26	19.03	–0.23	18.32	–0.94
C25	20.28	20.30	0.02	19.51	–0.77
C36	23.81	23.86	0.05	21.30	–2.51
Pr3	21.62	21.72	0.10	21.58	–0.04
B11	21.81	21.72	–0.09	21.87	0.06
B10	22.52	22.54	0.02	22.51	–0.01
C20	22.41	22.23	–0.18	22.41	0.00
C47	22.18	24.63	2.45	20.74	–1.44
C41	29.02	29.85	0.83	29.56	0.54
C30	29.02	29.09	0.07	28.68	–0.34
C48	30.64	31.25	0.61	31.12	0.48
C46	34.84	32.13	–2.71	32.20	–2.64
C42	33.99	33.99	0.00	34.93	0.94
C55	35.23	35.14	–0.09	35.05	–0.18
C56	35.57	35.37	–0.320	35.41	–0.16
C60	33.90	33.99	0.09	33.90	0.00
C49	37.21	37.52	0.31	37.10	–0.11
C31	37.50	38.18	0.68	37.47	–0.03
C18	42.21	42.27	0.06	41.80	–0.41
C37	48.21	48.15	–0.06	44.12	–4.09
C26	45.95	45.88	–0.07	44.90	–1.05
Pr1	47.95	47.96	0.01	48.00	0.05
C2	50.25	49.96	–0.29	50.01	–0.24

Table A1.2 *Continued*

C12	50.90	53.36	2.46	53.80	2.90
C7	53.59	54.20	0.61	54.85	1.26
C13	56.42	57.92	1.50	58.05	1.63
C8	60.02	59.98	–0.04	56.77	–3.25
C3	59.68	59.41	–0.27	58.85	–0.83
C17	61.75	61.39	–0.36	61.11	–0.64
R5	62.85	62.95	0.07	63.05	0.20
R2	71.20	71.32	0.12	71.29	0.09
Pr2	75.39	75.51	0.12	75.39	0.00
R3	75.50	75.56	0.06	75.33	–0.17
C19	77.73	77.33	–0.40	76.25	–1.48
R4	84.84	84.80	–0.04	84.77	–0.07
C1	87.67	87.58	–0.09	88.29	0.62
R1	90.02	89.91	–0.11	89.93	–0.09
C10	97.58	106.84	9.26	132.77	35.19
C15	106.85	1.7075	0.90	109.65	2.80
C5	110.63	112.15	1.52	113.06	2.43
B7	114.50	114.42	–0.08	114.31	–0.19
B4	118.15	118.35	0.20	118.70	0.55
B8	131.82	131.94	0.12	132.21	0.39
B5	136.41	136.26	–0.15	136.10	–0.31
B6	138.52	138.44	–0.08	138.22	–0.30
B9	138.70	138.76	0.06	139.10	0.40
B2	144.07	143.95	–0.12	143.80	–0.27
C6	166.75	166.25	–0.50	165.68	–1.07
C14	168.53	168.18	–0.35	166.41	–2.12
C9	177.24	176.74	–0.50	171.28	–5.96
C11	181.49	177.75	–3.74	170.88	–10.61
C38	178.12	177.27	–0.85	177.08	–1.04
C57	177.29	177.08	–0.21	177.14	–0.15

Table A1.2 *Continued*

C16	183.85	182.51	–1.34	182.24	–1.61
C4	183.99	183.27	–0.72	183.62	–0.37
C61	178.12	178.18	0.06	178.14	0.02
C27	178.48	178.51	0.03	178.51	0.03
C43	179.44	179.55	0.11	179.00	–0.44
C32	180.24	180.27	0.03	180.24	0.00
C50	180.60	180.53	–0.07	180.30	–0.30

$\delta_1 = \delta_{H_2O\text{-}10\text{-}ClCbl^+} - \delta_{H_2OCbl^+}$ and $\delta_2 = \delta_{H_2O\text{-}10\text{-}NOCbl^+} - \delta_{H_2OCbl^+}$

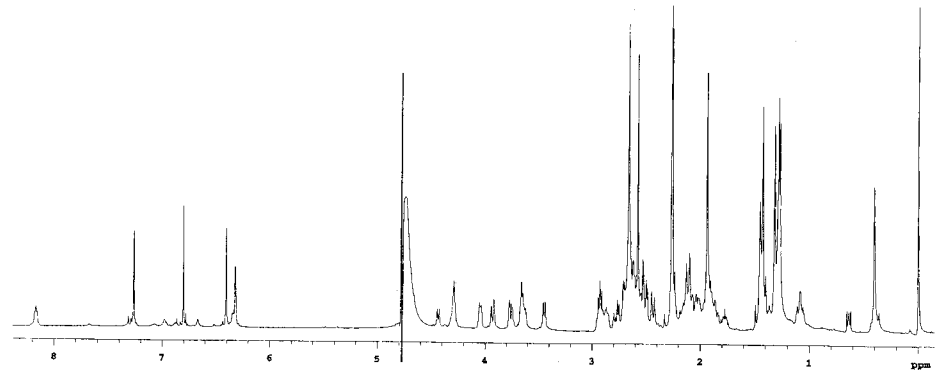


Figure A1.1 ^{1}H NMR spectrum of aqua-10-nitrosocobalamin.

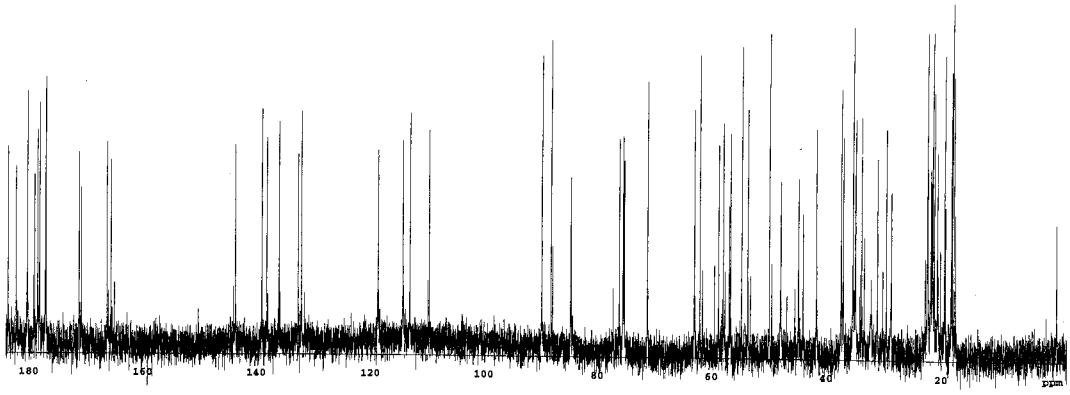


Figure A1.2 ^{13}C NMR spectrum of aqua-10-nitrosocobalamin.

The parameter data for all of the NMR spectra can be found on the CD.

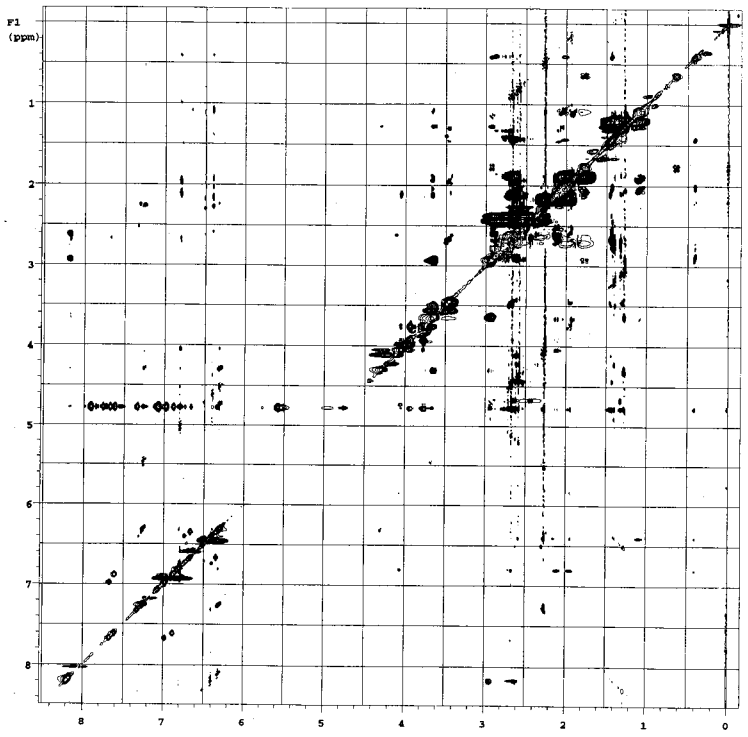


Figure A1.3 ROESY spectrum of aqua-10-nitrosocobalamin.

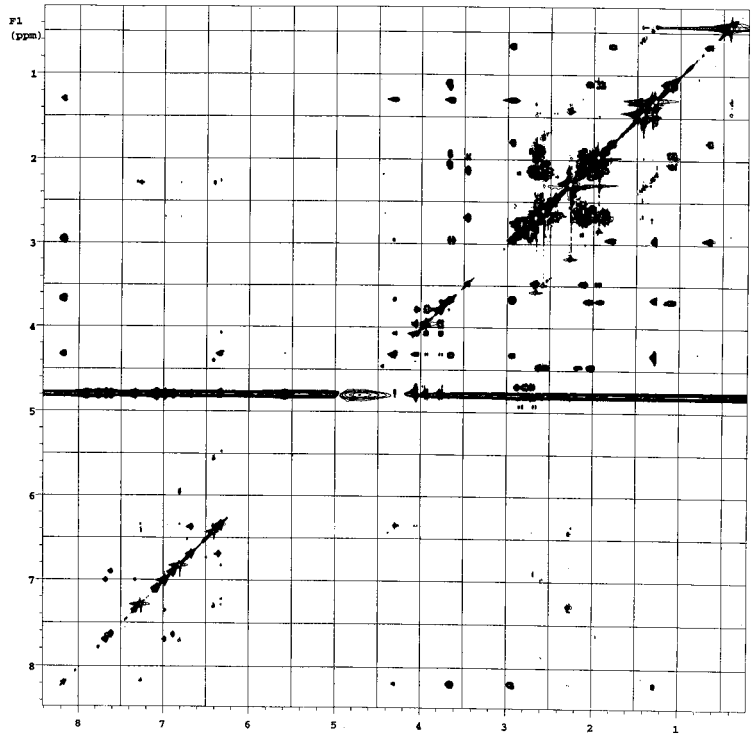


Figure A1.4 TOCSY spectrum of aqua-10-nitrosocobalamin.

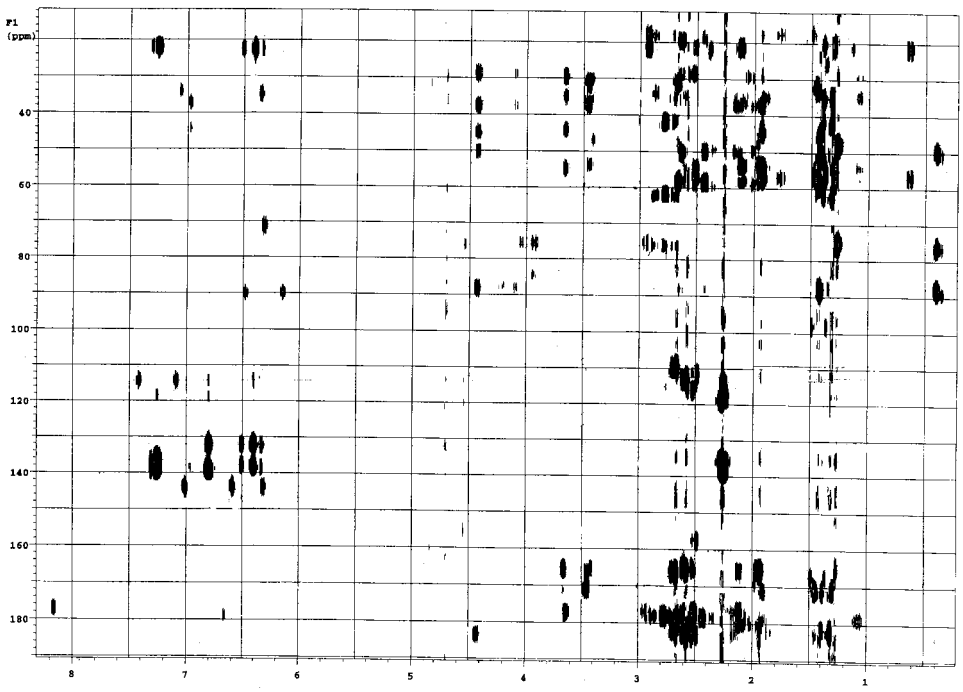


Figure A1.5 HMBC spectrum of aqua-10-nitrosocobalamin.

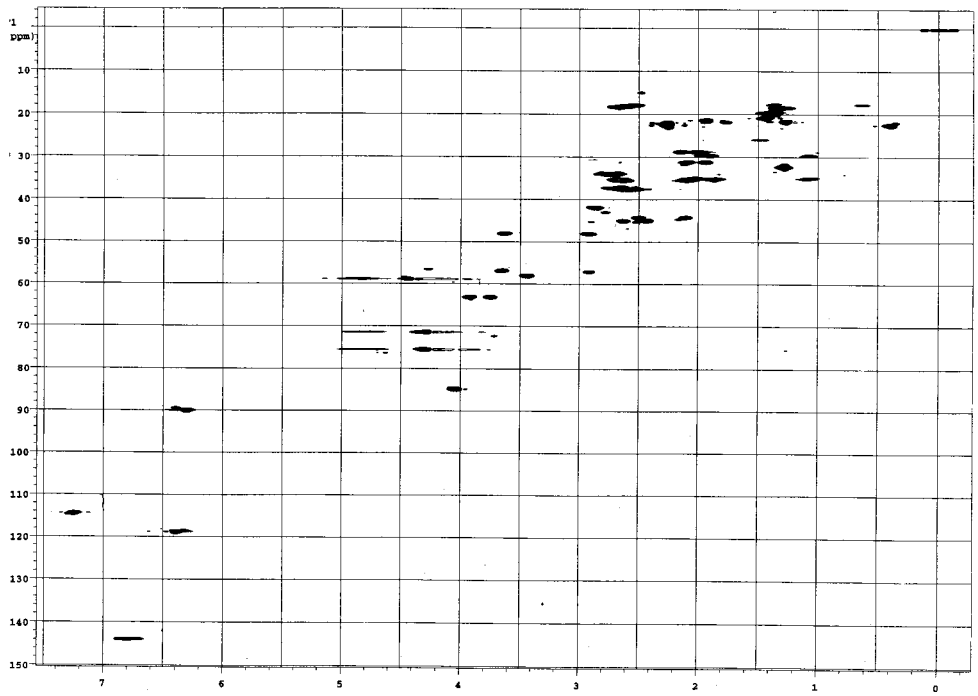


Figure A1.6 HMQC spectrum of aqua-10-nitrosocobalamin.

APPENDIX 2

Figure A2.1 The FAB-mass spectra of aqua-10-nitrosocobalamin. The molecular ion peak $(MH)^+$ occurs at 1374.6 (below) and $(MNa)^+$ occurs at 1396.6 (above), thus giving a molecular weight of 1373.6.

APPENDIX 3

DERIVATION OF THE EQUATIONS FOR A DISSOCIATIVE AND DISSOCIATIVE INTERCHANGE MECHANISM WHEN k_{-2} AND k_{-4}, RESPECTIVELY, ARE SIGNIFICANT (CHAPTERS 4, 5, 6 and 9).

$$\text{dmbzim - Co - H}_2\text{O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{dmbzim - Co + H}_2\text{O} \qquad \text{(A2.1)}$$

$$\text{dmbzim - Co + L} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{dmbzim - Co - L} \qquad \text{(A2.2)}$$

The rate law for the mechanism is

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_2[\text{dmbzim - Co}][\text{L}] - k_{-2}[\text{dmbzim - Co - L}] \qquad \text{(A2.3)}$$

Applying the steady-state approximation to dmbzim-Co gives:

$$\begin{aligned}[\text{dmbzim - Co}] &= 0 \\ &= k_1[\text{dmbzim - Co - H}_2\text{O}] + k_{-2}[\text{dmbzim - Co - L}] - k_{-1}[\text{dmbzim - Co}] \\ &\qquad - k_2[\text{dmbzim - Co}][\text{L}] \qquad \text{(A2.4)}\end{aligned}$$

then,

$$[\text{dmbzim - Co}] = \frac{k_1[\text{dmbzim - Co - H}_2\text{O}] + k_{-2}[\text{dmbzim - Co - L}]}{k_{-1} + k_2[\text{L}]} \qquad \text{(A2.5)}$$

and,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] =$$

$$\frac{k_1k_2[\text{dmbzim - Co - H}_2\text{O}][\text{L}] + k_2k_{-2}[\text{dmbzim - Co - L}][\text{L}]}{k_{-1} + k_2[\text{L}]} - k_{-2}[\text{dmbzim - Co - L}]$$

$$= \frac{1}{k_{-1} + k_2[\mathrm{L}]} \{k_1 k_2[\text{dmbzim - Co - } \mathrm{H_2O}][\mathrm{L}] + k_2 k_{-2}[\text{dmbzim - Co - L}][\mathrm{L}]$$

$$- k_{-1} k_{-2}[\text{dmbzim - Co - L}] - k_2 k_{-2}[\text{dmbzim - Co - L}][\mathrm{L}]\}$$

$$= \frac{k_1 k_2[\text{dmbzim - Co - } \mathrm{H_2O}][\mathrm{L}] - k_{-2} k_{-1}[\text{dmbzim - Co - L}]}{k_{-1} + k_2[\mathrm{L}]} \qquad \text{(A2.6)}$$

Since there is no spectroscopic evidence for the existence of free metal ions then,

$$[\text{dmbzim - Co}]_{\text{total}} \approx [\text{dmbzim - Co - } \mathrm{H_2O}] + [\text{dmbzim - Co - L}]$$

and since $[\text{dmbzim - Co}]_{\text{total}} \approx 0$

thus,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = \frac{k_1 k_2[\text{dmbzim - Co - } \mathrm{H_2O}][\mathrm{L}] - k_{-2} k_{-1}[-\text{dmbzim - Co - } \mathrm{H_2O}]}{k_{-1} + k_2[\mathrm{L}]}$$

$$= \frac{[\text{dmbzim - Co - } \mathrm{H_2O}](k_1 k_2[\mathrm{L}] + k_{-2} k_{-1})}{k_{-1} + k_2[\mathrm{L}]} \qquad \text{(A2.7)}$$

and since under pseudo first-order conditions,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_{\text{obs}}[\text{dmbzim - Co - } \mathrm{H_2O}] \qquad \text{(A2.8)}$$

finally,

$$k_{\text{obs}} = \frac{k_1 k_2[\mathrm{L}] + k_{-2} k_{-1}}{k_{-1} + k_2[\mathrm{L}]} \qquad \text{(A2.9)}$$

In the case of an I_d mechanism:

$$\text{dmbzim - Co - } H_2O + L \underset{}{\overset{K}{\rightleftharpoons}} \{H_2O \cdots \text{dmbzim - Co} \cdots L\} \tag{A.210}$$

$$\{H_2O \cdots \text{dmbzim - Co} \cdots L\} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{dmbzim - Co - L} + H_2O \tag{A2.11}$$

so,

$$K = \frac{[H_2O \cdots \text{dmbzim - Co} \cdots L]}{[\text{dmbzim - Co - } H_2O][L]} \tag{A2.12}$$

Let

$$\begin{aligned}[\text{dmbzim - Co - } H_2O]_{\text{total}} &= [\text{dmbzim - Co - } H_2O] + [H_2O \cdots \text{dmbzim - Co} \cdots L] \\ &= \frac{[H_2O \cdots \text{dmbzim - Co} \cdots L]}{[L]K} + [H_2O \cdots \text{dmbzim - Co} \cdots L] \\ &= [H_2O \cdots \text{dmbzim - Co} \cdots L]\left(\frac{1 + K[L]}{K[L]}\right)\end{aligned} \tag{A2.13}$$

Hence,

$$[H_2O \cdots \text{dmbzim - Co} \cdots L] = \frac{K[L][\text{dmbzim - Co - } H_2O]_{\text{total}}}{1 + K[L]} \tag{A2.14}$$

Now,

$$\begin{aligned}\frac{d}{dt}[\text{dmbzim - Co - L}] &= k_4[H_2O \cdots \text{dmbzim - Co} \cdots L] - k_{-4}[\text{dmbzim - Co - L}] \\ &= \frac{Kk_4[\text{dmbzim - Co - } H_2O]_{\text{total}}[L]}{1 + K[L]} - k_{-4}[\text{dmbzim - Co - L}]\end{aligned}$$

Since there is no spectroscopic evidence for the existence of free metal ions then,

$$[\text{dmbzim - Co}]_{\text{total}} \approx [\text{dmbzim - Co - H}_2\text{O}]_{\text{total}} + [\text{dmbzim - Co - L}]$$

and since $[\text{dmbzim - Co}]_{\text{total}} \approx 0$

thus,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = \frac{Kk_4[\text{dmbzim - Co - H}_2\text{O}]_{\text{total}}[\text{L}]}{1 + K[\text{L}]} - k_{-4}[-\text{dmbzim - Co - H}_2\text{O}]_{\text{total}}$$

$$= [\text{dmbzim - Co - H}_2\text{O}]_{\text{total}}\left(\frac{Kk_4[\text{L}]}{1 + K[\text{L}]} + k_{-4}\right) \qquad \text{(A2.15)}$$

and since under pseudo first-order conditions,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_{\text{obs}}[\text{dmbzim - Co - H}_2\text{O}] \qquad \text{(A2.16)}$$

$$k_{\text{obs}} = \frac{k_4 K[\text{L}]}{1 + K[\text{L}]} + k_{-4} \qquad \text{(A2.17)}$$

DERIVATION OF THE EQUATIONS FOR A DISSOCIATIVE AND DISSOCIATIVE INTERCHANGE MECHANISM WHEN k_{-2} AND k_{-4}, RESPECTIVELY, ARE INSIGNIFICANT (CHAPTERS 4, 5, 6 and 9).

$$\text{dmbzim - Co - H}_2\text{O} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{dmbzim - Co} + \text{H}_2\text{O} \qquad \text{(A2.18)}$$

$$\text{dmbzim - Co} + \text{L} \overset{k_2}{\rightleftharpoons} \text{dmbzim - Co - L} \qquad \text{(A2.19)}$$

The rate law for the mechanism is

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_2[\text{dmbzim - Co}][\text{L}] \qquad \text{(A2.20)}$$

Applying the steady-state approximation to dmbzim-Co gives

$$\begin{aligned} &\frac{d}{dt}[\text{dmbzim - Co}] = 0 \\ &= k_1[\text{dmbzim - Co - H}_2\text{O}] - k_{-1}[\text{dmbzim - Co}] - k_2[\text{dmbzim - Co}][\text{L}] \end{aligned} \qquad \text{(A2.21)}$$

then,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = \frac{k_1 k_2[\text{dmbzim - Co - H}_2\text{O}][\text{L}]}{k_{-1} + k_2[\text{L}]} \qquad \text{(A2.22)}$$

and since under pseudo first-order conditions,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_{\text{obs}}[\text{dmbzim - Co - H}_2\text{O}] \qquad \text{(A2.23)}$$

Finally,

$$k_{\text{obs}} = \frac{k_1 k_2 [\text{L}]}{k_{-1} + k_2 [\text{L}]} \tag{A2.24}$$

In the case of an I_d mechanism:

$$\text{dmbzim - Co - } H_2O + \text{L} \overset{K}{\rightleftharpoons} \{H_2O \cdots \text{dmbzim - Co} \cdots \text{L}\} \tag{A2.25}$$

$$\{H_2O \cdots \text{dmbzim - Co} \cdots \text{L}\} \overset{k_4}{\rightleftharpoons} \text{dmbzim - Co - L} + H_2O \tag{A2.26}$$

so,

$$K = \frac{[H_2O \cdots \text{dmbzim - Co} \cdots \text{L}]}{[\text{dmbzim - Co - } H_2O][\text{L}]} \tag{A2.27}$$

Let

$$\begin{aligned} [\text{dmbzim - Co - } H_2O]_{\text{total}} &= [\text{dmbzim - Co - } H_2O] + [H_2O \cdots \text{dmbzim - Co} \cdots \text{L}] \\ &= \frac{[H_2O \cdots \text{dmbzim - Co} \cdots \text{L}]}{[\text{L}]K} + [H_2O \cdots \text{dmbzim - Co} \cdots \text{L}] \\ &= [H_2O \cdots \text{dmbzim - Co} \cdots \text{L}]\left(\frac{1 + K[\text{L}]}{K[\text{L}]}\right) \end{aligned} \tag{A2.28}$$

Hence,

$$[H_2O \cdots \text{dmbzim - Co} \cdots \text{L}] = \frac{K[\text{L}][\text{dmbzim - Co - } H_2O]_{\text{total}}}{1 + K[\text{L}]} \tag{A2.29}$$

Now,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_4[\text{H}_2\text{O}\cdots\text{dmbzim - Co}\cdots\text{L}]$$

$$= \frac{Kk_4[\text{dmbzim - Co - H}_2\text{O}]_{\text{total}}[\text{L}]}{1 + K[\text{L}]} \qquad \text{(A2.30)}$$

and since under pseudo first-order conditions,

$$\frac{d}{dt}[\text{dmbzim - Co - L}] = k_{\text{obs}}[\text{dmbzim - Co - H}_2\text{O}] \qquad \text{(A2.31)}$$

$$k_{\text{obs}} = \frac{k_4 K[\text{L}]}{1 + K[\text{L}]} \qquad \text{(A2.32)}$$

APPENDIX 4

EQUATION FOR THE BINDING OF AN AXIAL LIGAND BY AQUACOBALAMIN AND AQUA-10-CHLOROCOBALAMIN (CHAPTER 8).

Consider the equilibrium:

$$\text{dmbzim - Co - } H_2O + L \overset{K}{\rightleftharpoons} \text{dmbzim - Co - L} + H_2O \qquad \text{(A3.1)}$$

The coordination constant *K* is as follows:

$$K = \frac{[\text{dmbzim - Co - L}]}{[\text{dmbzim - Co - } H_2O][L]} \qquad \text{(A3.2)}$$

The fractions of dmbzim-Co-H_2O and dmbzim-Co-L are as follows:

$$f_{\text{dmbzim-Co-}H_2O} = \frac{1}{1 + K[L]} \qquad \text{(A3.4)}$$

$$f_{\text{dmbzim-Co-L}} = \frac{K[L]}{1 + K[L]} \qquad \text{(A3.5)}$$

The total absorbance at the monitoring wavelength is:

$$A_T = f_{\text{dmbzim-Co-}H_2O}(A_0) + f_{\text{dmbzim-Co-L}}(A_1) \qquad \text{(A3.6)}$$

where A_0 and A_1 are the absorbances of the complexes dmbzim-Co-H_2O and dmbzim-Co-L, respectively.

Substituting Equations A3.4 and A3.5 Into A3.6, gives Equation A3.7, which gives the total absorbance at the monitoring wavelength.

$$A_T = \frac{A_0 + A_1 K[L]}{1 + K[L]} \tag{A3.7}$$

APPENDIX 5

DETERMINATION OF EQUATIONS FOR THE CORRECTION OF THE OBSERVED RATE CONSTANTS FOR pH AND THE PROTONATION OF THE EXOGENOUS LIGAND (CHAPTERS 4, 5, 6 and 9).

Consider the following equilibria:

$$LH^+ \overset{K_L}{\rightleftharpoons} L + H^+ \qquad \text{(A4.1)}$$

$$\text{dmbzm-Co-}H_2O \overset{K_{Co}}{\rightleftharpoons} \text{dmbzm-Co-}OH^- + H^+ \qquad \text{(A4.2)}$$

Hence,

$$K_L = \frac{[L][H^+]}{[LH^+]} \qquad \text{(A4.3)}$$

$$K_{Co} = \frac{[\text{dmbzm-Co-}OH^-][H^+]}{[\text{dmbzm-Co-}H_2O]} \qquad \text{(A4.4)}$$

The fractions of the complex dmbzm-Co-H_2O and L are given by:

$$f_{\text{dmbzim-Co-}H_2O} = \frac{1}{1 + \frac{K_{Co}}{[H^+]}} \qquad \text{(A4.5)}$$

$$f_L = \frac{1}{1 + \frac{[H^+]}{K_L}} \qquad \text{(A4.6)}$$

Now the observed rate constant is obtained from the following equation:

$$\text{dmbzm - Co - } H_2O + L \underset{}{\overset{k_{obs}}{\rightleftharpoons}} \text{dmbzm - Co - L} + H_2O \qquad \text{(A4.7)}$$

and so,

$$k_{obs} = \frac{[\text{dmbzm - Co - L}]}{[\text{dmbzm - Co - } H_2O][L]} \qquad \text{(A4.8)}$$

The rate constant, k, occurs from the fractions of the complex dmbzm-Co-H_2O and the ligand L only. Hence,

$$k_{obs} = k \times f_{\text{dmbzim-Co-}H_2O} \times f_L \qquad \text{(A4.9)}$$

Substituting Equations A4.5 and A4.6 into Equation A4.9 gives:

$$k = k_{obs}\left(1 + \frac{K_{Co}}{[H^+]}\right)\left(1 + \frac{[H^+]}{K_L}\right) \qquad \text{(A4.10)}$$

which rearranges to:

$$k = k_{obs}(1 + 10^{pH - pK_{Co}})(1 + 10^{pK_L - pH}) \qquad \text{(A4.11)}$$

APPENDIX 6

DETERMINATION OF EQUATIONS FOR THE CORRECTION OF THE OBSERVED BINDING CONSTANTS FOR pH AND THE PROTONATION OF THE EXOGENOUS LIGAND (CHAPTER 8).

Consider the following equilibria:

$$LH^+ \overset{K_L}{\rightleftharpoons} L + H^+ \qquad \text{(A5.1)}$$

$$\text{dmbzm-Co-}H_2O \overset{K_{Co}}{\rightleftharpoons} \text{dmbzm-Co-}OH^- + H^+ \qquad \text{(A5.2)}$$

Hence,

$$K_L = \frac{[L][H^+]}{[LH^+]} \qquad \text{(A5.3)}$$

$$K_{Co} = \frac{[\text{dmbzm-Co-}OH^-][H^+]}{[\text{dmbzm-Co-}H_2O]} \qquad \text{(A5.4)}$$

The fractions of the complex dmbzm-Co-H_2O and L are given by:

$$f_{\text{dmbzim-Co-}H_2O} = \frac{1}{1 + \frac{K_{Co}}{[H^+]}} \qquad \text{(A5.6)}$$

$$f_L = \frac{1}{1 + \frac{[H^+]}{K_L}} \qquad \text{(A5.7)}$$

The coordination constant, *K*, occurs from the fractions of the complex dmbzm-Co-H_2O and the ligand L only. Hence,

$$K_{obs} = K \times f_{dmbzim\text{-}Co\text{-}H_2O} \times f_L \quad \text{(A5.8)}$$

Substituting Equations A5.6 and A5.7 into Equation A5.8 gives:

$$K = K_{obs}\left(1+\frac{K_{Co}}{[H^+]}\right)\left(1+\frac{[H^+]}{K_L}\right) \quad \text{(A5.9)}$$

which rearranges to:

$$K = K_{obs}(1+10^{pH-pK_{Co}})(1+10^{pK_L-pH}) \quad \text{(A5.10)}$$

APPENDIX 7

CALCULATION OF THE OUTER-SHERE CONSTANT USING THE FUOSS-EIGEN EQUATION (CHAPTER 9)

$$K_{\mathrm{E}} = \frac{4\pi}{3} \mathrm{a}^3 N_{\mathrm{A}} \mathrm{e}^{-V/RT} \tag{A6.1}$$

where,

$$V = \frac{q_1 q_2 N_{\mathrm{A}} \mathrm{e}^2}{4\pi \varepsilon r} \tag{A6.2}$$

and,

$$\varepsilon = \varepsilon_0 \varepsilon_{\mathrm{r}} \tag{A6.3}$$

hence for a spherical aquacobalamin with a net charge of +2,

$$V = \frac{(+2)(-1)(1.602\times10^{-19}\,\mathrm{C})^2 (6.022\times10^{23}\,\mathrm{mol^{-1}})}{4\pi\,(8.854\times10^{-12}\,\mathrm{J^{-1}C^2m^{-1}})(78)(5\times10^{-10}\,\mathrm{m})}$$

$$= -7123\ \mathrm{J\ mol^{-1}} \tag{A6.4}$$

Now,

$$K_{\mathrm{E}} = \frac{4\pi}{3}(5\times10^{-10}\,\mathrm{m})^3 (6.022\times10^{23}\,\mathrm{mol^{-1}}) \exp\left[\frac{-(-7123\mathrm{Jmol^{-1}})}{(8.315\mathrm{JK^{-1}mol^{-1}})(298\mathrm{K})}\right]$$

$$= 5.5\ \mathrm{M^{-1}} \tag{A6.5}$$

and for a spherical aquacobalamin with a net charge of +1,

$$V = \frac{(+1)(-1)(1.602\times10^{-19}\,\mathrm{C})^2(6.022\times10^{23}\,\mathrm{mol^{-1}})}{4\pi\,(8.854\times10^{-12}\,\mathrm{J^{-1}C^2m^{-1}})(78)(5\times10^{-10}\,\mathrm{m})}$$

$$= -3561\ \mathrm{J\ mol^{-1}} \qquad \text{(A6.6)}$$

Now

$$K_{\mathrm{E}} = \frac{4\pi}{3}(5\times10^{-10}\,\mathrm{m})^3(6.022\times10^{23}\,\mathrm{mol^{-1}})\exp\left[\frac{-(-3561\mathrm{J mol^{-1}})}{(8.315\mathrm{JK^{-1}mol^{-1}})(298\mathrm{K})}\right]$$

$$= 1.3\ \mathrm{M^{-1}} \qquad \text{(A6.7)}$$

APPENDIX 8

DATA FOR CRYSTAL STRUCTURE OF OCNCOBALAMIN

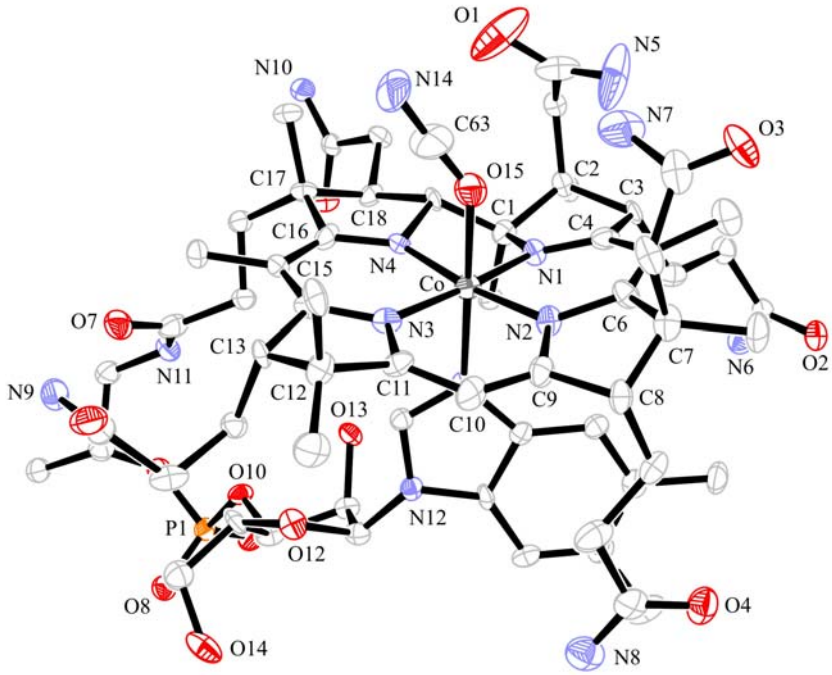


Figure A8.1 ORTEP diagram of NCOcobalamin (ellipsoids drawn at the 30% probability level). Hydrogen atoms and water moledcules have been removed for clarity. The binding shown through oxygen is just a representation as the data was not good enough to determine through which atom the OCN^- binds.

Intensity data were collected at -150 °C on a Bruker SMART 1K CCD area detector diffractometer with graphite monochromated Mo-K_α radiation (50kV, 30mA). The collection method involved ω-scans of width 0.3°. Data reduction was carried out using the program SAINT+.[1] The crystal structure was solved by direct methods using SHELXTL[2]. Non-hydrogen atoms were first refined isotropically followed by anisotropic refinement by full matrix least-squares calculations based on F^2 using SHELXTL. Hydrogen atoms were positioned geometrically and allowed to ride on their respective parent atoms. Diagrams and publication material were generated using SHELXTL, ORTEP-3[3] and SCHAKAL-97.[4]

Table A8.1. Crystal data and structure refinement for OCNcobalamin

Empirical formula	C63 H86 Co N14 O27.25 P
M_r	1565.36
Temperature	123(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 16.255(3) Å; α= 90°.
	b = 20.689(3) Å; β= 90°.
	c = 23.962(5) Å; γ = 90°.
Volume	8059(3) $Å^3$
Z	4
Density (calculated)	1.290 mg m^{-3}
Absorption coefficient	0.316 mm^{-1}
F(000)	3288
Crystal size	0.48 x 0.44 x 0.23 mm^3
Theta range for data collection	1.51 to 25.00°.
Index ranges	$-12<h<14$; $-24<k<24$; $-28<l<11$
Reflections collected	22849
Independent reflections	10504 [*R*(int) = 0.1867]
Completeness to theta = 25.00°	80.5 %
Absorption correction	None
Max. and min. transmission	0.9310 and 0.8633
Refinement method	Full-matrix least-squares on F_2
Data / restraints / parameters	10504 / 0 / 990
Goodness-of-fit on F_2	1.300
Final *R* indices [I>2sigma(I)]	*R*1 = 0.1208, *wR*2 = 0.3027
R indices (all data)	*R*1 = 0.1509, *wR*2 = 0.3502
Absolute structure parameter	-0.09(4)
Largest diff. peak and hole	2.154 and –1.004 e $Å^{-3}$

Table A8.2 Some bond lengths [Å] and angles [°] for OCNcobalamin

Bond lengths [Å]	
N(1)–Co	1.891(10)
N(2)–Co	1.901(7)
N(3)–Co	1.921(10)
N(4)–Co	1.893(7)
N(13)–Co	1.970(8)
O(15)–Co	1.951(8)
Angles [°]	
N(1)–Co–N(4)	82.1(4)
N(1)–Co–N(2)	91.7(4)
N(4)–Co–N(2)	172.2(4)
N(1)–Co–N(3)	172.6(3)
N(4)–Co–N(3)	90.7(4)
N(2)–Co–N(3)	95.6(4)
N(1)–Co–O(15)	92.0(4)
N(4)–Co–O(15)	87.9(3)
N(2)–Co–O(15)	87.7(3)
N(3)–Co–O(15)	89.3(4)
N(1)–Co–N(13)	91.6(4)
N(4)–Co–N(13)	94.5(3)
N(2)–Co–N(13)	90.2(3)
N(3)–Co–N(13)	87.4(4)
O(15)–Co–N(13)	175.9(4)
C(63)–O(15)–Co	142.7(12)

The rest of the data can be found on the CD.

Figure A8.2 Hydrogen bonding between the OCN ligand and neighbouring hydrogen donors. Intramolecular bonding occurs with an amide group and intermolecular hydrogen bonding can be seen between two of the H_2O molecules. Once again the binding shown through oxygen is only a representation.

Table A8.3 Hydrogen bonding distances between the donor and acceptor atoms in Figure A8.2.

Donor···Acceptor Atoms	**Distance /Å**
N7···O15	3.176
O12W···N14	2.954
O9W···N14	3.134

REFERENCES FOR APPENDIX 8

1. Bruker SAINT+. Version 6.02 (includes XPREP and SADABS). Bruker AXS Inc., Madison, Wisconsin, USA, 1999.
2. Bruker SHELXTL. Version 5.1. (includes XS, XL, XP, XSHELL) Bruker AXS Inc., Madison, Wisconsin, USA, 1999.
3. E. Keller SCHAKAL-97. University of Freiberg, Germany, 1997.
4. C. K. Johnson, M. N. Burnett and L. J. Farrugia, ORTEP3, Windows Version. University of Glasgow, Scotland 1997.