Probing the nature of the Co(III) ion in the cobalamins: Kinetics of the ligand substitution reactions of iodocobalamin, and the deactivation of the metal towards ligand substitution in aqua-10-nitrosocobalamin

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Electronic Supplementary Information:

NMR data for 10-NO-H_2OCbl$^+$ and primary kinetic data for the ligand substitution reactions of ICbl

Table S1. ^{1}H and ^{13}C NMR Assignments for 10-NO-H_2OCbl^{+} [a]

Group	$\delta_{^{13}C}$, ppm	$\delta_{^{1}H}$, ppm	Group	$\delta_{^{13}C}$, ppm	$\delta_{^{1}H}$, ppm
C35	17.87	2.58	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	0.40	B6	138.22	
B10	22.51	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	41.80	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	

Group	δ_{13C}, ppm	δ_{1H}, ppm	Group	δ_{13C}, ppm	δ_{1H}, ppm
C2	50.01		R7		5.59
C12	53.80		$d\mathrm{H_A}$		6.33
C7	54.85		$d\mathrm{H_S}$		6.67
C8	56.77	3.65	$b\mathrm{H_S}$		6.89
C13	58.05	3.43	$e\mathrm{H_S}$		6.98
C3	58.85	4.43	$c\mathrm{H_S}$		6.98
C17	61.11		$g\mathrm{H_S}$		7.07
R5	63.05	3.75, 3.91	$a\mathrm{H_S}$		7.07
R2	71.29	4.28	$c\mathrm{H_A}$		7.33
R3	75.33[e]	4.74	$b\mathrm{H_A}$		7.62
Pr2	75.39[f]	4.29	$e\mathrm{H_A}$		7.67
C19	76.25	4.69	$a\mathrm{H_A}$		7.76
R4	84.77[g]	4.06	$g\mathrm{H_A}$		7.91
C1	88.29		*f*H		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 $a\mathrm{H_S}$, $a\mathrm{H_A}$, $b\mathrm{H_S}$, $b\mathrm{H_A}$, 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.) 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 S2. Comparison of the ^{13}C resonances of H_2OCbl^+, 10-Cl-H_2OCbl^+ and 10-NO-H_2OCbl^+.

Group	H_2OCbl^+, ppm	10-Cl-H_2OCbl^+, ppm	δ_1, ppm	10-NO-H_2OCbl^+, 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	-0.20	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
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	62.92	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

Group	H_2OCbl^+, ppm	10-Cl-H_2OCbl^+, ppm	δ_1, ppm	10-NO-H_2OCbl^+, ppm	δ_2, ppm
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	107.75	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
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

Table S3. Comparison of the Side Chain Amide N-H Chemical Shifts of 10-NO-H_2OCbl^+ to Cbl's[a]

N-H	$\delta\ ^1H$, ppm		
	Average[b]	H_2O-10-NOCbl	$\Delta\ \delta\ ^1H$[c]
aH_A	7.81 ± 0.08	7.76	0.05
aH_S	7.11 ± 0.07	7.07	0.04
bH_A	7.62 ± 0.04	7.62	0.00
bH_S	6.89 ± 0.04	6.89	0.00
cH_A	7.53 ± 0.04	7.33	0.20[d]
cH_S	7.01 ± 0.13	6.98	0.03
dH_A	6.33 ± 0.11	6.33	0.00
dH_S	6.45 ± 0.09	6.67	-0.22
eH_A	7.76 ± 0.01	7.67	0.09
eH_S	7.03 ± 0.03	6.98	0.05
*f*H	8.20 ± 0.05	8.17	0.03
gH_A	7.92 ± 0.04	7.91	0.01
gH_S	7.08 ± 0.05	7.07	0.01

[a]Cbl's = AdoCbl, CH_3Cbl, CNCbl, CF_3Cbl, CN-10-ClCbl, H_2OCbl^+, and H_2O-10-$ClCbl^+$, except for the c amide N-H's where H_2OCbl^+, and H_2O-10-$ClCbl^+$ are omitted from the comparison.

[b]Average chemical shift shown is the average and standard deviation of the shifts of the Cbl's.

[c]$\Delta\ \delta\ ^1H = \delta_{ave} - \delta_{H_2O\text{-}10\text{-}NOCbl^+}$.

[d]For H_2OCbl^+, $\Delta\ \delta\ ^1H$ was -0.63 for *c*H_A and –0.15 for *c*H_S.

Table S4. Primary kinetic data for the substitution of I^- in iodocobalamin by incoming L (μ = 2.2 mol dm^{-3}, $NaClO_4$; pH 7.00, 0.1 mol dm^{-3} MOPS).

L	T /^{o}C	[L] /mol dm^{-3}	k_{obs}	L	T /^{o}C	[L] /mol dm^{-3}	k_{obs}
Imidazole	10.0	0.1003	0.2105	N_3^-	25.0	0.1001	6.19
		0.1983	0.4743			0.2006	9.34
		0.2942	0.6764			0.3065	14.07
		0.4003	0.866			0.4016	16.45
		0.5013	0.9851			0.6000	22.00
	15.0	0.1003	0.3925			0.7990	27.43
		0.1983	0.8712			0.9025	28.73
		0.2942	1.2699			0.9990	29.30
		0.4003	1.595		30.0	0.2006	15.5
		0.5013	1.7605			0.3065	20.9
		0.5483	1.7502			0.4016	26.7
	20.0	0.1003	0.6847			0.5002	30.6
		0.1983	1.4710			0.6997	37.6
		0.2942	2.2512			0.7990	43.9
		0.4003	2.7778			0.9025	46.2
		0.5013	3.1466	$S_2O_3^{2-}$	10.0	0.0959	0.1582
		0.5483	3.1918			0.1003	0.1660
		0.6074	3.2436			0.2010	0.3385
		0.1003	0.6847			0.2512	0.4083
		0.1983	1.4710			0.2993	0.4752
		0.2942	2.2512			0.3498	0.6003
	25.0	0.1003	1.2876		15.0	0.0959	0.2710
		0.1983	2.7327			0.1003	0.2970
		0.2942	4.0041			0.2010	0.5370
		0.4003	4.8923			0.2512	0.6317
		0.5013	5.5882			0.2993	0.8586
		0.5483	5.7308			0.3498	0.9599
		0.6074	5.9727		20.0	0.0959	0.4718
		0.6533	6.0930			0.1003	0.4925
		0.6989	6.2171			0.2010	0.9148
		0.7926	6.3424			0.2512	1.1370
		0.9017	6.2580			0.2993	1.3400
N_3^-	10.0	0.2006	2.058			0.3498	1.6340
		0.3065	3.002		25.0	0.0959	0.7623
		0.4016	3.717			0.1003	0.7931
		0.5002	4.198			0.2010	1.3420
		0.6997	4.989			0.2512	1.8690
		0.7990	5.435			0.2993	2.2300

L	T /^{o}C	[L] /mol dm^{-3}	k_{obs}	L	T /^{o}C	[L] /mol dm^{-3}	k_{obs}
N_3^-	15.0	0.1001	2.293	$S_2O_3^{2-}$	25.0	0.3498	2.3870
		0.2006	3.521				
		0.3065	4.713				
		0.4016	5.974				
		0.7990	9.933				
		0.9025	10.42				