Supplementary Material (ESI) for Dalton Transactions

Supporting Information for

NMR Spectroscopy and Molecular Modelling Studies of Nitrosylcobalamin: Further Evidence that the Deprotonated, Base-off Form is Important for Nitrosylcobalamin in Solution

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Table S1. Correlation Table for NMR Connectivities for NOCbl Observed by Total Correlation Spectroscopy (TOCSY), Rotating Frame NOE (ROESY), and Heteronuclear Multiple-Bond Correlation (HMBC) Spectroscopy[a]

Resonance	TOCSY	ROESY	HMBC
C53		C13, C54, C56'	C14, C15, C16
C35		C3, C36, *c*H_A	C4, C5, C6
C25		C18, C19, C20, C26", *a*H_A, *a*H_S, B4	C1, C2, C3, C26
C54		C18, C53, C55", *g*H_A, *g*H_S	C16, C17, C18, C55
Pr3	Pr1', Pr1", Pr2	Pr1', Pr1", Pr2, C55", *f*H	Pr1, Pr2
C36		C8, C35, B10, *c*H_S, *c*H_A	C6, C7, C8, C37
C47		C10, C13, C46, C48", C49', C49"	C11, C12, C13, C46
B10	B7	C36, B4	
B11	B4	B7 B5	
C20		C18, C19, C25, C30', B4, B7	C1, C2, C19
C30'	C3, C31"	C3, C35 C3	
C30"	C3	C31' C31	
C41'	C8, C41", C42"	C8, C10, C41", C42", *d*H_A	C8, C42
C41"	C8, C41', C42"	C8, C10, C41'	
C48'	C13, C48", C49"	C10, C13, C47, C48", C49'	C12, C13, C50
C48"	C13, C48', C49', C49"	C10, C13, C47, C48", C49'	C12, C13, C14, C50
C46		C10, C13, C47	C11, C12, C13, C47
C55'	C55"	C18, C55", *f*H	C16, C57
C55"	C55'	C18, C54, C55', *f*H	C16, C17, C56, C57
C42'	C8, C42"	C8, C42", *d*H_A	C8, C41, C43
C42"	C8, C41', C41", C42'	C41', C42'	

C31'	C3	C3, C20, C25, C26', C36, *a*H_A	C3, C30, C32
C31"	C3, C30', C30"	C3, C26", C36, *b*H_A, *b*H_S	C3, C30, C32
C56'	C56"	C54, C55", *f*H	C17, C55, C56
C56"	C56'	C53, R4, *f*H	C565, C57
C49'	C13, C48", C49"	C47, C49", *e*H_S, *e*H_A	C13, C48, C50
C49"	C13, C48', C48", C49'	C47, C49', C53, *e*H_A, *e*H_S	C13, C48, C50
C60'	C19	C55', *g*H_A, *g*H_S	
C60'	C19	C55', *g*H_A, *g*H_S	
C18	C19	C19, C20, C25, C55', C55", Pr3, *g*H_A, *g*H_S	C1, C17, C19, C54, C55
C26'		*a*H_A, *a*H_S C1, C2, C3, C25, C27	
C26"		C25, C31', *a*H_A	C1, C2, C3, C25, C27
C37'	C37"	C8, C36, C37", B10, *c*H_A, *c*H_S	C7, C8, C36, C38
C37"	C37'	C8, C36, C37'	C6, C7, C8, C36
Pr1'	Pr1", Pr2, Pr3	Pr1", Pr2, Pr3, *f*H	C57, Pr3
Pr1"	Pr1', Pr2, Pr3	Pr1', Pr2, Pr3, *f*H	Pr2, C57
C13	C48', C48", C49', C49"	C46, C47, C48', C48", C49', C49", C53	C11, C12, C14, C15, C46, C48
C8	C41', C41", C42', C42"	C10, C36, C37", C41', C42', *c*H_A, *c*H_S, *d*H_A	C6, C7, C9, C10, C37, C41, C42
C3	C30', C30", C31', C31"	C26", C30', C31', C31", C35, *a*H_A, *a*H_S *b*H_A, *b*H_S	C1, C2, C4, C26, C30, C31
R5'	R2, R4, R5"	R3, R4, R5"	
R5"	R2, R3, R4, R5'	R3, R4, R5'	R3
R2	R1, R4, R5', R5"	R1, R5", B7	R1, R4
Pr2	Pr1', Pr1", Pr3	Pr1', Pr1", Pr3, *f*H	Pr1
R3	R1, R2, R4, R5', R5"	R1, R5', R5"	
C19	C18, C60', C60"	C18, C25, B10. Pr3	C1, C2, C18, C60
R4	R2, R3, R5', R5"	C30", R1, R5', R5", B7	
R1	R2, R3	R2, R3, R4, B7	

C10		C8, C41', C41", C46, C47, C48"	C8, C9, C11, C12
B7	B4, B11	R1, R2, R4, B7, B11	B8, B9, B11
B4	B7, B10	C25, C35, B10	B8, B9
B2		C30', C30", R1, R4	B8, B9
*d*H$_S$	*d*H$_A$	*d*H$_A$	
*c*H$_S$	*c*H$_A$	C8, C35, C36, C37', *c*H$_A$	
*d*H$_A$	*d*H$_S$	C8, C41', *d*H$_S$	
*e*H$_S$	*e*H$_A$	C49', C49", C53, *e*H$_A$	C50
*b*H$_S$	*b*H$_A$	C3, C3", *b*H$_A$	
*a*H$_S$	*a*H$_A$	C3, C25, C26', *a*H$_A$	
*g*H$_S$	*g*H$_A$	C18, C54, C60", *g*H$_A$	
*e*H$_A$	*e*H$_S$	C49', C49", C53, *e*H$_S$	
*c*H$_A$	*c*H$_S$	C8, C35, C36, C37', *c*H$_S$	
*b*H$_A$	*b*H$_S$	C3, C31", *b*H$_S$	
*a*H$_A$	*a*H$_S$	C3, C25, C26', C26", C31', *a*H$_S$	
*g*H$_A$	*g*H$_S$	C18, C54, C60, *g*H$_S$	
*f*H	Pr1', Pr1"	C55', C56', C56", Pr1', Pr1", Pr2, Pr3	

[a] In 0.15 M phosphate buffer, 90% H_2O/10% D_2O, using pulsed field gradient solvent suppression. Chemical shifts are relative to internal TSP. Prime and double prime denote the downfield and upfield members, respectively, of pairs of diastereotopic methylene protons. The *syn* and *anti* amide H's are designated *a*H$_S$, *a*H$_A$, *b*H$_S$, *b*H$_A$, etc., respectively, where *a*, *b*, etc., refer to the standard side chain lettering.

Table S2. Observed nOe's, their assigned strength, and the average, standard deviation, maximum, minimum and median H–H distances during a 500 ps molecular dynamics simulation at 300 K of the base-on and base-off forms of NOCbl with sampling every 10 fs. The nOe's that violate the distance criteria in both base-on and base-off forms are highlighted in blue; those that show a violation only in the base-on form are highlighted in yellow, whilst those that show a violation in only the base-off form are highlighted in orange. Violations are further indicated in ***bold italicized text***.

		Base-on					Base-off				
nOe	**Strength**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**
C46, C13	M	2.594	0.134	3.252	1.927	2.591	2.588	0.170	3.351	2.018	2.582
C25, C3	M	3.010	0.116	3.537	2.475	3.010	3.020	0.154	3.701	2.473	3.019
C41", C8	M	3.093	0.047	3.336	2.853	3.093	3.092	0.062	3.369	2.668	3.092
C25, C18	S	2.973	0.198	3.764	2.113	2.977	2.919	0.234	3.862	2.155	2.906
C25, C31"	S	2.942	0.637	4.210	1.848	2.616	2.562	0.291	4.300	1.882	2.523
C25, C26'	M	2.687	0.135	3.662	2.084	2.680	2.680	0.178	3.430	2.089	2.678
C41", B10	M	3.467	0.250	4.631	2.287	3.470	3.005	0.490	6.069	1.792	2.945
C25, C30'	M	2.361	0.178	3.673	1.794	2.337	2.338	0.214	3.607	1.782	2.309
C47, C13	M	3.097	0.143	3.828	2.491	3.083	3.045	0.161	4.007	2.340	3.040
C47, C49'	M	2.301	0.149	3.234	1.707	2.287	2.376	0.211	4.043	1.739	2.352
C47, C49"	M	2.432	0.504	4.537	1.738	2.260	2.697	0.605	5.568	1.782	2.471
Pr3, Pr2	S	2.514	0.083	2.938	2.124	2.511	2.646	0.263	3.282	2.091	2.552
C47, C42'	M	3.207	0.310	4.625	2.069	3.201	2.733	0.378	5.721	1.819	2.684
C36, C8	M	3.008	0.112	3.610	2.455	3.009	3.050	0.165	4.114	2.370	3.044
C36, C41'	M	2.368	0.147	3.158	1.844	2.357	2.315	0.176	3.618	1.797	2.298
C36, C35	M	3.600	0.107	4.246	1.862	3.595	2.566	0.569	5.422	1.756	2.343
C36, C37'	S	2.440	0.165	3.979	1.889	2.423	2.378	0.180	3.815	1.912	2.361
C42', C41'	M	2.584	0.084	3.002	2.155	2.584	2.617	0.124	3.247	2.069	2.610
C36, B10	S	2.473	0.189	3.553	1.791	2.461	2.772	0.345	5.657	1.861	2.741
C36, C41"	M	2.443	0.159	3.427	1.822	2.434	2.476	0.233	4.331	1.786	2.451
C56", R4	W	4.531	0.562	6.938	2.420	4.413	***5.923***	***0.732***	***10.675***	***4.030***	***5.883***
C37", C8	M	2.760	0.146	3.703	2.042	2.754	2.928	0.190	4.330	2.147	2.923
Pr3, Pr1'	M	2.589	0.135	3.453	2.042	2.585	2.847	0.510	4.121	1.993	2.635
C48", C13	M	3.089	0.051	3.393	2.814	3.089	3.086	0.089	3.363	2.182	3.093
C30', C3	M	2.672	0.085	3.150	2.218	2.673	2.733	0.118	3.149	2.260	2.736
C30', C35	S	2.643	0.236	3.989	1.902	2.634	2.886	0.354	5.791	1.909	2.857
C42', C8	S	2.648	0.136	3.377	2.061	2.641	2.607	0.167	3.627	2.033	2.602

		Base-on					Base-off				
nOe	**Strength**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**
C41', C42"	M	3.099	0.047	3.353	2.880	3.099	3.076	0.093	3.345	2.277	3.084
C26', C3	S	2.550	0.132	3.133	1.983	2.547	2.555	0.172	3.198	1.854	2.551
C3, C26"	S	***3.527***	***0.065***	***3.889***	***3.248***	***3.526***	***3.528***	***0.082***	***3.870***	***3.148***	***3.526***
C37', C8	M	3.585	0.088	3.973	2.532	3.584	3.534	0.105	3.905	2.941	3.535
C53, C13	S	2.424	0.260	3.826	1.781	2.374	2.405	0.301	3.745	1.773	2.352
C56', C18	S	***3.363***	***0.167***	***3.973***	***2.520***	***3.382***	2.562	0.199	3.876	1.921	2.551
Pr3, Pr1"	M	2.683	0.208	4.063	2.173	2.651	2.781	0.272	3.993	2.095	2.733
C53, C18	W	***5.081***	***0.432***	***6.637***	***4.400***	***4.927***	***5.243***	***0.426***	***6.465***	***4.306***	***5.112***
C35, C3	S	2.855	0.208	3.681	1.876	2.862	2.342	0.308	3.879	1.742	2.253
C31', C3	S	2.600	0.207	3.712	1.933	2.583	2.491	0.162	3.230	1.966	2.484
C18, C19	M	3.012	0.052	3.273	2.729	3.013	3.028	0.066	3.286	2.709	3.029
Pr1", Pr2	M	3.087	0.070	3.327	2.376	3.094	3.090	0.066	3.386	2.415	3.092
Pr1', Pr2	M	2.563	0.128	3.312	2.190	2.545	2.545	0.116	3.225	2.097	2.542
R5", R3	M	3.081	0.208	3.707	2.176	3.107	2.605	0.228	4.143	1.958	2.581
R5', R3	W	3.811	0.087	4.192	2.456	3.814	3.005	0.230	4.217	2.119	3.012
R5", R4	M	2.532	0.088	3.003	2.180	2.526	3.092	0.102	3.368	2.102	3.102
R5', R4	M	2.498	0.083	2.888	2.077	2.500	2.573	0.132	3.263	2.116	2.564
R4, R3	W	3.096	0.052	3.353	2.645	3.097	3.083	0.063	3.361	2.771	3.082
R1, R2	S	2.448	0.073	5.886	2.127	2.448	2.458	0.090	2.828	2.114	2.457
C10, C8	S	2.528	0.078	3.735	2.134	2.527	2.574	0.101	2.990	2.186	2.573
C10, C42'	S	2.644	0.194	3.801	2.002	2.632	2.594	0.262	4.676	1.901	2.566
C10, C47	S	2.823	0.184	3.676	2.031	2.833	2.748	0.203	3.632	2.000	2.743
B7, R1	S	2.569	0.173	6.834	2.024	2.573	2.616	0.216	3.761	1.875	2.603
R1, R3	S	2.887	0.149	3.681	2.323	2.872	2.738	0.173	3.524	2.121	2.732
B7, R2	M	2.869	0.346	4.534	2.043	2.801	2.791	0.368	4.453	1.735	2.743
B7, R4	VW	***5.670***	***0.209***	***6.413***	***2.321***	***5.652***	***5.686***	***0.253***	***6.411***	***4.415***	***5.715***
C54, C19	M	3.039	0.363	4.474	2.061	2.982	2.872	0.333	4.521	1.911	2.830
B2, R1	W	3.790	0.100	4.171	3.317	3.788	3.802	0.126	4.231	3.041	3.811
B2, R4	M	2.972	0.323	4.186	1.922	2.980	3.178	0.351	4.965	2.098	3.165
fH, R3	W	***5.086***	***0.381***	***6.774***	***3.539***	***5.078***	3.040	0.587	6.981	1.775	2.986
fH, Pr2	M	2.781	0.166	3.513	2.032	2.788	2.971	0.393	4.091	2.017	2.860
fH, R4	W	3.554	0.488	6.573	2.059	3.518	5.743	0.524	8.413	3.370	5.738

		Base-on					Base-off				
nOe	**Strength**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**	**average /Å**	**σ /Å**	**max /Å**	**min /Å**	**median /Å**
fH, Pr1'	M	2.549	0.126	3.253	2.178	2.533	2.799	0.231	3.275	2.194	2.773
fH, Pr1"	M	3.052	0.078	3.303	2.337	3.062	2.819	0.288	3.280	2.132	2.982
C54, C18	M	3.543	0.159	4.102	2.711	3.562	3.663	0.150	4.575	2.626	3.668
C54, C60"	M	2.464	0.167	3.493	1.854	2.453	2.448	0.278	4.187	1.824	2.406
C54, C53	S	2.392	0.233	3.715	1.705	2.390	2.454	0.442	4.113	1.691	2.297

Table S3. Comparsion of the solid state structure* and the molecular modelling consensus structure of base-on NOCbl.

Bond Lengths /Å				
(a) Cooordination sphere				
		Consensus	Solid State	|Cons-Solid state|
Co	N21	1.880	1.857	0.023
Co	N24	1.894	1.888	0.006
	Average	***1.887***	***1.873***	
Co	N22	1.921	1.909	0.012
Co	N23	1.929	1.915	0.014
	Average	***1.925***	***1.912***	
Co	NB3	2.063	2.123	0.060
Co	N(O)	1.925	1.928	0.003
N(O)	O	1.175	1.183	0.008
			1.143	0.032
			1.201	0.026
	Average		***1.176***	
(b) C–N				
C1	N21	1.482	1.496	0.014
C19	N24	1.447	1.473	0.026
	Average	***1.465***	***1.485***	
C4	N21	1.303	1.291	0.012
C16	N24	1.302	1.309	0.007
	Average	***1.303***	***1.300***	
C6	N22	1.378	1.394	0.016
C9	N22	1.361	1.338	0.023
C11	N23	1.370	1.345	0.025
C14	N23	1.373	1.380	0.007
	Average	***1.371***	***1.364***	
(c) Corrin double bonds				
C4	C5	1.405	1.442	0.037
C5	C6	1.405	1.368	0.037
C9	C10	1.391	1.394	0.003
C10	C11	1.392	1.350	0.042

C14	C15	1.406	1.326	0.080
C15	C16	1.409	1.467	0.058
	Average	***1.402***	***1.391***	

(d) Corrin single bonds

C1	C2	1.570	1.582	0.012
C2	C3	1.543	1.573	0.030
C3	C4	1.512	1.527	0.015
C6	C7	1.526	1.549	0.023
C7	C8	1.548	1.573	0.025
C8	C9	1.498	1.512	0.014
C11	C12	1.508	1.546	0.038
C12	C13	1.538	1.543	0.005
C13	C14	1.518	1.562	0.044
C16	C17	1.529	1.531	0.002
C17	C18	1.563	1.571	0.008
C18	C19	1.537	1.572	0.035
C19	C1	1.536	1.506	0.030
	Average	***1.533***	***1.550***	

Bond Angles /deg

			Consensus	Solid State	|Cons-Solid state|
N21	Co	N24	84.2	83.4	0.8
N21	Co	N22	90.7	90.0	0.7
N21	C4	C3	109.9	112.1	2.2
N21	C1	C2	98.5	102.8	4.3
N21	Co	N(O)	90.9	92.2	1.3
N21	Co	N3B	92.9	90.1	2.8
N22	Co	N23	94.9	96.6	1.7
N22	C6	C7	108.8	107.9	0.9
N22	C9	C10	126.8	125.8	1.0
N22	Co	N(O)	87.2	87.2	0.0
N22	Co	N3B	87.1	87.2	0.1
N23	Co	N24	90.2	90.3	0.1
N23	C11	C12	110.3	110.0	0.3
N23	C14	C15	126.0	129.1	3.1
N23	Co	N(O)	89.2	91.3	2.1
N23	Co	N3B	87.3	87.2	0.1
N24	C16	C17	110.4	112.0	1.6
N24	C19	C18	106.6	103.4	3.2
N24	Co	N(O)	90.0	89.0	1.0

N24	Co	N3B	94.6	96.9	2.3
Co	N21	C1	114.9	117.1	2.2
Co	N23	C11	124.8	122.1	2.7
Co	N24	C16	133.9	133.1	0.8
C1	C19	N24	106.6	107.9	1.3
C1	C2	C3	103.9	101.4	2.5
C2	C3	C4	98.7	101.2	2.5
C4	C5	C6	122.9	120.8	2.1
C4	N21	C1	114.8	112.5	2.3
C4	N21	Co	129.4	130.1	0.7
C5	C4	N21	124.6	125.2	0.6
C5	C6	N22	123.1	122.8	0.3
C6	N22	Co	126.6	127.5	0.9
C6	N22	C9	108.7	110.1	1.4
C6	C7	C8	102.0	101.0	1.0
C7	C8	C9	99.1	99.1	0.0
C8	C9	N22	108.9	112.2	3.3
C9	N22	Co	123.7	122.3	1.4
C9	C10	C11	123.2	124.8	1.6
C10	C11	N23	125.5	127.1	1.6
C11	N23	C14	109.6	112.6	3.0
C11	C12	C13	99.0	101.2	2.2
C12	C13	C14	101.9	102.3	0.4
C13	C14	N23	107.8	107.4	0.4
C14	C15	C53	115.3	120.8	5.5
C14	N23	Co	125.6	125.3	0.3
C15	C16	N24	121.3	121.3	0.0
C16	C15	C53	122.0	118.3	3.7
C16	C15	C14	122.8	120.8	2.0
C16	N24	C19	113.0	111.8	1.2
C16	C17	C18	104.3	101.5	2.8
C17	C18	C19	103.2	101.4	1.8
C19	N24	Co	112.0	114.6	2.6
C19	C1	N21	103.0	103.7	0.7
C35	C5	C4	117.4	117.4	0.0
C35	C5	C6	120.4	121.8	1.4

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Figure S1. Distance between (A) Co and NB3 and (B) between C20 and NB3 during a 250 ps dynamics simulation at 300 K of the base-off form of NOCbl. Co···NB3 distances of between 3 and 4 Å are indicative of the base-off species in which DMB is perpendicular to the corrin (Figure 6D) and distances of around 7 Å are indicative of the base-off species with DMB parallel to the corrin (Figure 6C).