Supporting Information

^{1}H NMR study of the effect of variable ligand on heme oxygenase electronic and molecular structure

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Two tables of chemical shifts for all assigned residues and six Figures (TOCSY spectra for aromatic and upfield residues, NOESY spectra for the H-bond network, for His C_{β}Hs in $^{2}H_{2}O$ and Curie plot comparison with *Nm*HO-PH-CN). Total 16 Pages

Table S1. Substrate chemical shifts for *Nm*HO-PH-X*

	$X = N_3^-$	$X^{\dagger} = CN^-$
$1CH_3$	18.83	7.90
$2H_\alpha$	9.50	14.48
$2H_\beta$s		-5.58, -6.62
$3CH_3$	8.05	21.40
$4H_\alpha$	11.95	8.06
$4H_\beta$s	-6.42, -6.81	-2.80, -3.38
$5CH_3$	18.33	9.62
$6H_\alpha$s	10.00, 2.51	16.92, 1.14
$6H_\beta$s		-1.02, -3.38
$7H_\alpha$s	9.95, 6.12	13.06, 5.73
$7H_\beta$s		-1.10, -2.17
$8CH_3$	20.25	10.33

*In ppm, referenced to DSS via the solvent resonance; ^1H_2O 50(100) mM in phosphate for azide (cyanide), pH 7.1 at 25°C.

†Taken from Ref.: Y. Liu, X. Zhang, T. Yoshida, G. N. La Mar, Biochemistry 43 (2004) 10112-10126.

Table S2. Chemical shift data for assigned residues in *Nm*HO-PH-X.[*]

		X = N_3^-		X = CN^-	Shift differences	
		δ_{DSS}(obs)[†]	δ_{dip}(calc)[±]	δ_{DSS}(obs)[¶]	$\Delta\delta_{DSS}$(obs)[§]	$\Delta\delta_{dip}$(calc)[‖]
Phe 11	NH	7.60	-0.09	7.50	0.10	0.03
	$C_\alpha H$		-0.07	4.37	0.03	-0.02
	$C_\delta Hs$	7.05	-0.11	7.03	0.02	0.00
	$C_\varepsilon Hs$	6.88	-0.13	6.90	-0.02	-0.03
	$C_\zeta H$	6.67	-0.15	6.76	-0.09	-0.04
Ala12	NH	9.04	-0.16	8.96	0.08	0.05
	$C_\alpha H$	3.38	-0.26	3.30	0.08	0.06
	$C_\beta H_3$	1.17	-0.29	1.02	0.15	0.11
Leu15	$C_\gamma H$	0.54	-0.12	0.57	-0.03	-0.03
	$C_{\delta 1}H_3$	-0.65	-0.28	-0.65	0.00	-0.03
	$C_{\delta 2}H_3$	0.11	-0.14	0.21	-0.10	-0.10
Asp18	NH	8.60	0.34	8.64	-0.04	-0.02
	$C_\alpha H$	4.91	0.43	4.97	-0.06	-0.05
	$C_{\beta 1}H$	2.87	0.29	2.95	-0.08	-0.09
	$C_{\beta 2}H$	2.78	0.26	2.85	-0.07	-0.03
Thr19	NH	7.98	0.75	8.04	-0.06	-0.10
	$C_\alpha H$	5.28	0.93	5.52	-0.24	-0.20
	$C_\beta H$	5.23	1.03	5.75	-0.52	-0.50
	$C_\gamma H_3$	1.41	0.43	1.70	-0.29	-0.20
	$O_\eta H$	5.60	0.36	5.90	-0.28	-0.30
Thr20	NH	8.38	1.30	8.37	0.01	-0.09
	$C_\alpha H$	6.38	3.45	6.30	0.08	-0.15
	$C_\beta H$	5.25	1.37	5.14	0.11	0.01

	$C_{\gamma}H_3$	2.41	2.11	2.27	0.14	0.10
Ala21	NH	9.27	1.36	9.36	-0.09	-0.12
	$C_{\alpha}H$	5.23	1.43	5.31	-0.08	-0.11
	$C_{\beta}H_3$	1.97	0.89	2.04	-0.07	-0.09
Val22	NH	9.14	1.40	9.44	-0.30	-0.24
	$C_{\alpha}H$	4.60	1.11	4.87	-0.27	-0.21
	$C_{\beta}H$	2.44	0.79	2.72	-0.28	-0.23
	$C_{\gamma1}H_3$	1.04	1.15	1.56	-0.52	-0.44
	$C_{\gamma2}H_3$	1.17	0.60	1.40	-0.23	-0.20
His23	NH	10.54	3.10	11.08	-0.54	-0.57
	$C_{\alpha}H$	6.25	4.82	7.37	-1.12	-1.38
	$C_{\beta1}H$	12.50		11.53	**	
	$C_{\beta2}H$	9.54		10.75	**	
Asp24	NH	11.07	4.13	11.41	-0.34	-0.27
	$C_{\alpha}H$	6.44	3.65	6.80	-0.36	-0.20
	$C_{\beta1}H$	3.52	1.59	3.65	-0.13	-0.10
	$C_{\beta2}H$	4.14	2.02	4.30	-0.16	-0.13
Ser25	NH	8.60	1.85	8.77	-0.17	-0.17
Val26	NH	8.28	1.42	8.33	-0.05	-0.08
	$C_{\alpha}H$	3.12	-0.27	2.80	0.32	0.27
	$C_{\beta}H$	1.57	0.64	1.60	-0.03	0.05
	$C_{\gamma1}H_3$	-0.98	-2.41	-1.96	0.98	0.94
	$C_{\gamma2}H_3$	0.43	-0.09	0.51	-0.08	-0.13
Val30	NH	8.38	-0.87	7.87	0.51	0.52
	$C_{\alpha}H$	2.92	-0.70	2.46	0.46	0.42
	$C_{\beta}H$	1.40	-1.64	0.40	1.00	0.90
	$C_{\gamma1}H_3$	0.40	-0.97	-0.68	1.04	0.67

	$C_{\gamma 2}H_3$	0.44	-1.49	-0.21	0.65	0.93
Val33	NH	6.84	-0.34	6.54	0.30	0.18
	$C_{\alpha}H$	4.18	-0.19	4.05	0.13	0.11
	$C_{\beta}H$	1.78	-0.21	1.62	0.16	0.13
	$C_{\gamma 1}H_3$	0.45	-0.23	0.29	0.16	0.13
	$C_{\gamma 2}H_3$	-0.34	-0.34	-0.55	0.21	0.20
Tyr42	$C_{\varepsilon}Hs$	6.94	0.20	6.79	0.15	0.08
	$O_{\eta}H$	9.20	0.32	9.16	0.04	0.07
Phe45	$C_{\delta}Hs$	7.12	-0.09	6.77	0.35	0.27
	$C_{\varepsilon}Hs$	7.83	-0.03	7.23	0.60	0.48
	$C_{\zeta}Hs$	8.15	0.37	7.34	0.79	0.61
Leu48	$C_{\alpha}H$	3.65	-0.20	3.50	0.15	0.12
	$C_{\gamma}H$	1.15	-0.42	0.80	0.35	0.19
	$C_{\delta 1}H_3$	-0.25	-0.42	-0.50	0.25	0.28
Gln49	$N_{\varepsilon 1}H$	10.97	0.55	10.14	0.83	0.35
	$N_{\varepsilon 2}H$	7.76	0.75	7.35	0.41	0.56
Phe52	$C_{\delta}Hs$	6.53	-0.52	6.55	-0.02	-0.03
	$C_{\varepsilon}Hs$	6.50	-0.81	6.71	-0.21	-0.20
	$C_{\zeta}H$	6.08	-1.02	6.41	-0.33	-0.32
His53	$C_{\delta 1}H$	7.66	0.18	7.44	0.22	0.09
	$C_{\varepsilon 2}H$	8.08	0.38	7.97	0.11	-0.02
	$N_{\varepsilon 1}H$	12.22	0.33	12.13	0.09	0.14
Tyr60	$C_{\varepsilon}Hs$	6.60	-0.12	6.65	-0.05	-0.08
	$O_{\eta}H$	10.36	-0.05	10.43	-0.07	-0.08
Trp110	$C_{\delta 1}H$	7.14	-0.23	7.27	-0.13	-0.12
	$N_{\varepsilon 1}H$	11.40	-0.19	11.52	-0.12	-0.11
	$C_{\varepsilon 3}H$	6.80	-0.38	6.48	-0.15	-0.13

	$C_{\zeta 2}H$	7.17	-0.20	7.27	-0.10	-0.09
	$C_{\zeta 3}H$	6.40	-0.33	6.48	-0.08	-0.08
	$C_{\eta 2}H$	6.30	-0.25	6.38	-0.08	-0.08
Leu111	$C_{\gamma}H$	1.32	-0.34	1.43	-0.11	-0.12
	$C_{\delta 1}H_3$	0.68	-0.24	0.68	0.00	-0.08
	$C_{\delta 2}H_3$	0.05	-0.24	0.13	-0.08	-0.05
Tyr112	$C_{\delta}Hs$	6.46	-0.84	6.57	-0.11	0.12
	$C_{\varepsilon}Hs$	6.37	-0.45	6.62	-0.24	-0.37
	$O_{\eta}H$	12.0	-0.83	11.68	0.32	0.38
Cys113	NH	7.2	-0.89	7.40	-0.20	-0.36
	$C_{\alpha}H$	1.46	-2.16	2.60	-1.14	-0.98
	$C_{\beta 1}H$	1.75	-1.08	2.32	-0.57	-0.47
	$C_{\beta 2}H$	1.15	-1.00	2.02	-0.87	-0.67
	$S_{\gamma}H$	-0.90	-2.52	-0.19	-0.71	-0.58
Ala114	NH	7.56	-1.02	8.04	-0.48	-0.37
	$C_{\alpha}H$	3.30	-1.09	3.71	-0.41	-0.40
	$C_{\beta}H_3$	1.45	-0.50	1.60	-0.15	-0.19
Glu115	NH	8.73	-0.80	9.07	-0.34	-0.24
	$C_{\alpha}H$	3.70	-0.12	3.97	-0.27	-0.13
Gly116	NH	7.63	-1.72	7.9	-0.32	-0.30
	$C_{\alpha 1}H$	0.76	-3.86	0.49	0.27	-0.03
	$C_{\alpha 2}H$	-0.08	-2.04	0.55	-0.63	-0.40
Asn118	NH	8.30	-1.29	8.46	-0.16	-0.24
Leu119	$C_{\alpha}H$	4.35	1.05	4.72	-0.37	0.09
	$C_{\beta 1}H$	-1.55	-0.98	-1.1	-0.4	0.96
	$C_{\beta 2}H$	-0.7	-0.26	-0.95	0.25	-0.42
	$C_{\gamma}H$	0.06	-1.01	-0.40	0.46	0.72

	$C_{\delta 1}H_3$	-0.86	-0.30	-1.69	-0.83	0.30
	$C_{\delta 2}H_3$	-0.08	-1.89	-0.63	0.55	1.01
Ala120	NH		6.08	13.0		0.49
Ala121	NH	15.53	8.28	15.32	0.21	-1.01
	$C_{\alpha}H$	8.55	8.90	8.52	0.03	-1.04
	$C_{\beta}H_3$	4.95	4.85	5.44	-0.49	-0.33
Ala122	NH		3.25	9.73		-1.07
	$C_{\alpha}H$		1.30	4.93		-0.45
	$C_{\beta}H_3$		0.69	1.98		-0.55
Phe123	NH	7.75	1.05	8.83	-1.58	-1.30
	$C_{\alpha}H$	3.93	-0.03	4.51	-0.58	-0.58
	$C_{\beta 1}H$	2.22	-0.77	3.46	-1.23	-1.24
	$C_{\beta 2}H$	2.13	-1.11	3.27	-1.16	-1.00
	$C_{\delta}Hs$	6.22	-0.83	7.07	-0.85	-0.42
	$C_{\varepsilon}Hs$	6.47	-0.71	6.94	-0.47	-0.18
	$C_{\zeta}H$		-0.61	7.15		-0.11
Leu124	NH	8.05	2.15	8.09	-0.04	-0.65
Phe125	NH	10.10	1.38	10.00	0.10	-0.28
	$C_{\alpha}H$	4.40	0.77	4.47	-0.07	-0.09
	$C_{\delta}Hs$	7.42	0.66	7.50	-0.08	-0.12
Tyr133	NH	6.96	0.14	6.97	0.01	-0.01
	$C_{\alpha}H$	5.36	0.16	5.34	0.02	0.00
	$C_{\beta}H$	2.36	0.21	2.32	0.04	0.01
	$C_{\beta}H'$	3.54	0.20	3.50	0.04	0.00
	$C_{\delta}Hs$	6.68	0.13	6.64	0.04	0.01
	$C_{\varepsilon}Hs$	6.53	0.08	6.57	-0.04	0.03
His137	NH	7.57	0.21	7.54	0.03	-0.01

	$C_{\alpha}H$	4.95	0.16	4.92	0.03	0.00
	$C_{\beta}H$	3.07	0.14	3.05	0.02	-0.01
	$C_{\beta}H'$	3.32	0.14	3.25	0.08	0.00
	$C_{\delta}H$	7.16	0.11	7.12	0.04	0.00
Gly138	NH	10.45	0.18	10.43	0.02	0.02
	$C_{\alpha 1}H$	3.92	0.18	3.87	0.05	0.02
	$C_{\alpha 2}H$	3.49	0.18	3.5	-0.05	0.03
Ala139	NH	7.86	0.28	7.82	0.04	0.00
	$C_{\alpha}H$	4.92	0.44	4.82	0.10	0.03
	$C_{\beta}H_3$	1.42	0.44	1.36	0.06	0.00
Arg140	NH	12.50	0.34	12.34	0.16	0.01
	$C_{\alpha}H$	4.21	0.40	4.18	0.03	-0.01
	$C_{\beta 2}H$	1.87	0.33	1.84	0.03	0.00
	$N_{\varepsilon}H$	10.10	-0.15	9.93	0.17	0.01
His141	NH	11.00	0.48	10.91	0.09	0.02
	$C_{\alpha}H$	4.57	0.60	4.46	0.09	0.00
	$C_{\varepsilon 1}H$	7.97	0.45	7.78	0.19	0.12
	$C_{\delta 2}H$	6.98	0.90	6.75	0.23	0.12
	$N_{\delta 1}H$	13.75	0.46	13.56	0.19	0.06
Leu142	NH	7.38	0.78	7.23	0.15	-0.01
	$C_{\alpha}H$	5.42	1.70	5.30	0.12	-0.00
Ala143	NH	6.38	0.78			-0.06
	$C_{\alpha}H$	4.30	0.73			-0.09
	$C_{\beta}H_3$	1.32	0.52			-0.04
Asp147	NH	11.05	0.18	11.04	0.01	-0.01
	$C_{\beta 1}H$	2.78	0.07	2.74	0.04	0.03
	$C_{\beta 2}H$	2.97	0.11	2.94	0.03	0.00

Trp153	$C_{\alpha}H$	4.52	-0.20	4.34	0.18	0.13
	$C_{\delta1}H$	6.77	-0.55	6.37	0.40	0.33
	$N_{\varepsilon1}H$	9.45	-0.85	8.67	0.78	0.48
	$C_{\varepsilon3}H$	7.20	-0.42	7.09	0.11	0.17
	$C_{\zeta2}H$	6.92	-1.13	6.43	0.49	0.45
	$C_{\zeta3}H$	5.86	-0.85	5.87	-0.01	0.10
	$C_{\eta2}H$	6.24	-1.25	6.09	0.15	0.19
Val157	NH	8.39	-0.25	8.28	0.11	0.10
	$C_{\alpha}H$	2.70	-0.30	2.65	0.05	0.09
	$C_{\beta}H$	1.40	-0.27	1.28	0.12	0.12
	$C_{\gamma1}H_3$	0.24	-0.27	0.05	0.19	0.12
	$C_{\gamma2}H_3$	-0.35	-0.44	-0.42	0.07	0.19
Asn161	$N_{\delta1}H$	7.25	-0.14	7.22	0.03	0.04
	$N_{\delta2}H$	6.24	-0.16	6.16	0.08	0.06
Ala180	NH	8.08	-0.24	8.28	-0.20	-0.22
	$C_{\alpha}H$	2.12	-0.38	2.28	-0.16	-0.17
	$C_{\beta}H_3$	-0.08	-0.56	0.26	-0.34	-0.30
Phe181	NH	7.45	-0.32	7.82	-0.37	-0.36
	C_{δ}Hs	6.39	-0.30	7.25	-0.86	-0.71
	$C_{\varepsilon}H$	5.85	-0.88	7.72	-2.42	-2.43
	$C_{\zeta}H$		0.21	8.87		-2.85
Phe183	NH	8.27	-0.25	8.39	-0.12	-0.12
	$C_{\beta}H$	3.04	0.34	3.09	-0.05	-0.11
	$C_{\beta}H'$	3.43	0.23	3.48	-0.05	-0.06
	C_{δ}Hs	7.32	-0.27	7.36	-0.04	-0.04
	C_{ε}Hs	7.13	-0.21	7.11	0.02	-0.01
	$C_{\zeta}H$	6.75	-0.18	6.77	-0.02	0.00

Tyr184	NH	7.93	-0.49	7.97	-0.04	-0.10
	$C_\beta H$	2.40	-0.87	2.44	-0.04	0.01
	$C_\beta H$	1.96	-0.97	1.92	0.03	0.07
	$C_\delta Hs$	5.65	-1.01	5.20	0.45	0.21
	$C_\varepsilon Hs$	5.98	-1.08	5.35	0.63	0.52
	$O_\eta H$	8.23	-0.73	7.44	0.79	0.69
Val187	NH	7.79	-0.26	7.73	0.06	0.04
	$C_\alpha H$	3.24	-0.19	3.13	0.11	0.05
	$C_\beta H$	1.23	-0.33	1.11	0.12	0.08
	$C_{\gamma 1}H_3$	0.54	-0.22	0.45	0.09	0.08
	$C_{\gamma 2}H_3$	-0.12	-0.23	-0.18	0.06	0.03
His207	$C_\alpha H$	3.80		4.04		
	$C_\beta H$	1.64		2.22		
	$C_\beta H'$	1.89		2.43		
	$C_\delta H$	6.16		6.81		
Arg208	$C_\alpha H$	3.83		3.84		

*In ^1H_2O 50 mM in phosphate, pH 7.1 at 25°C.

†Chemical shifts, in ppm, referenced to DSS via the solvent resonance.

‡Dipolar shift, in ppm, at 25°C for *Nm*HO-PH-N_3, calculated by the optimized orientation and anisotropies of the paramagnetic susceptibility tensor as described in Figure 6.

¶Chemical shift, in ppm, referenced to DSS, as reported previously (Y. Liu, X. Zhang, T. Yoshida, G. N. La Mar, Biochemistry 43 (2004) 10112-10126).

§$\Delta\delta_{DSS}$(obs), as described in Eq. (5).

||$\Delta\delta_{dip}$(calc), as described in Eq. (4).

**Possesses large contribution from contact shifts.

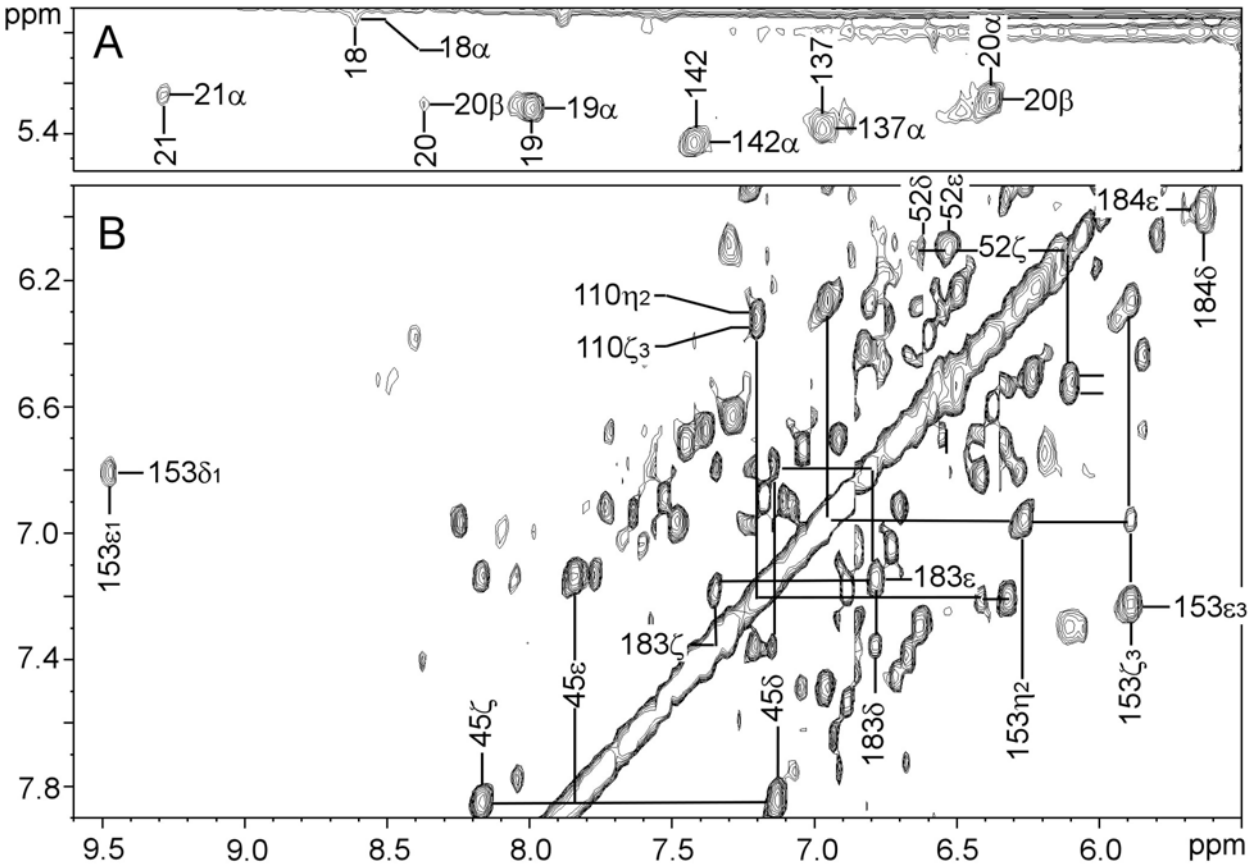

Figure S1. Portions of the lowfield 500 MHz ^{1}H NMR TOCSY spectrum (mixing time 28 ms, repetition rate 1 s^{-1}) for *Nm*HO-PH-N_3 in $^{1}H_2O$, 50 mM phosphate, pH 7.1 at 25°C, illustrating scalar connections: (**A**) within hyperfine shifted proximal helix residues Asp18-Ala21, His137 and Leu142; and (**B**) aromatic rings of Phe45, Phe52, Trp110, Phe123, Trp153, Phe156 and Phe183.

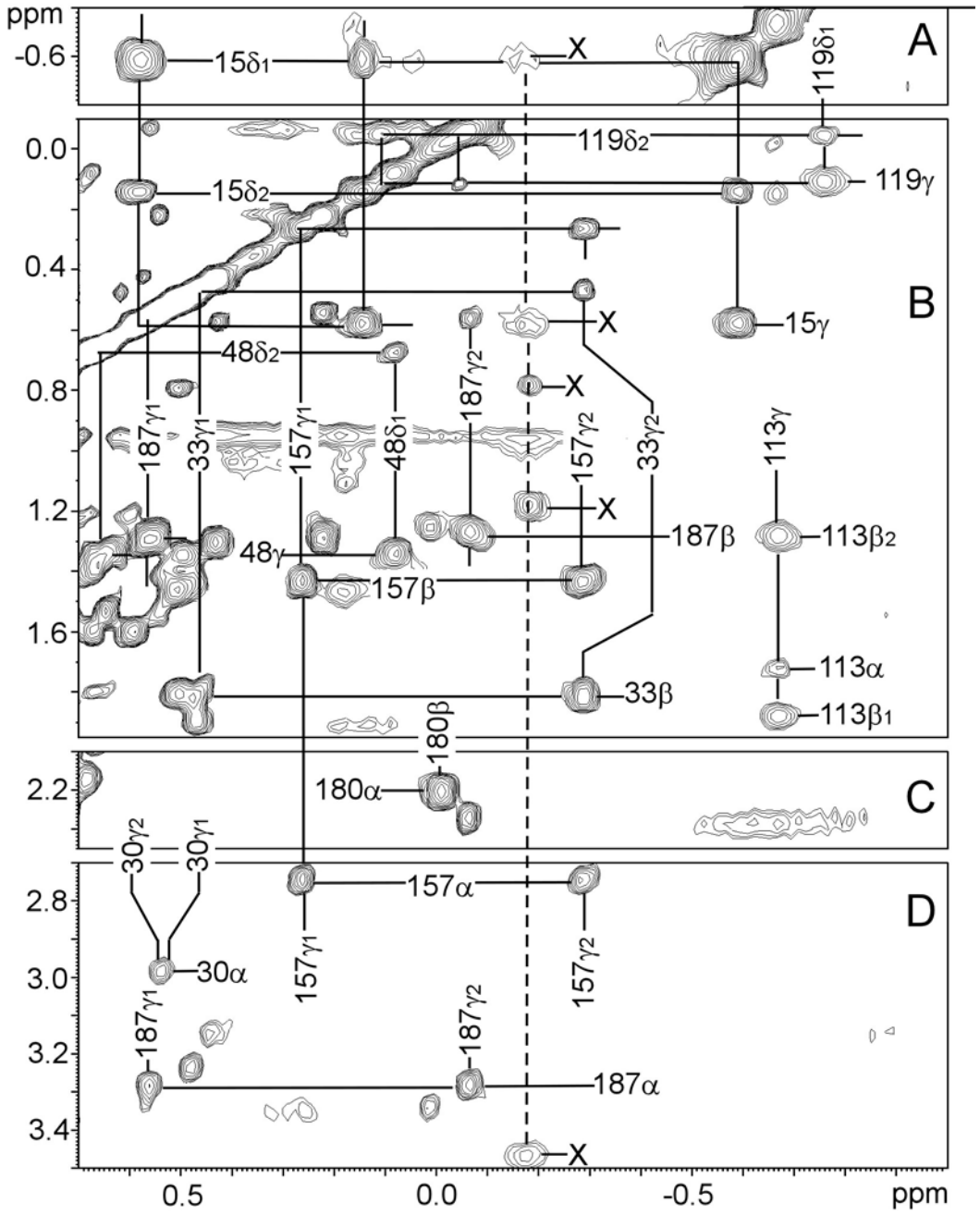

Figure S2. Upfield portions of the 500 MHz ^{1}H NMR TOCSY spectrum (mixing time 30 ms, repetition rate 1 s^{-1}) of *Nm*HO-PH-N_3 in $^{1}H_2O$, 50 mM in phosphate, pH 7.1 at 34°C, illustrating the scalar contacts for the aliphatic side chains for: Leu15 (**A**, **B**), an unidentified, exchange broadened residue X with peaks marked X (**A**, **B**, **D**), Val33, Leu48, Cys113 and Leu119 (**B**); Ala180 (**C**) and Val30 (**D**); and Val157 and Val187 (**B**,**D**).

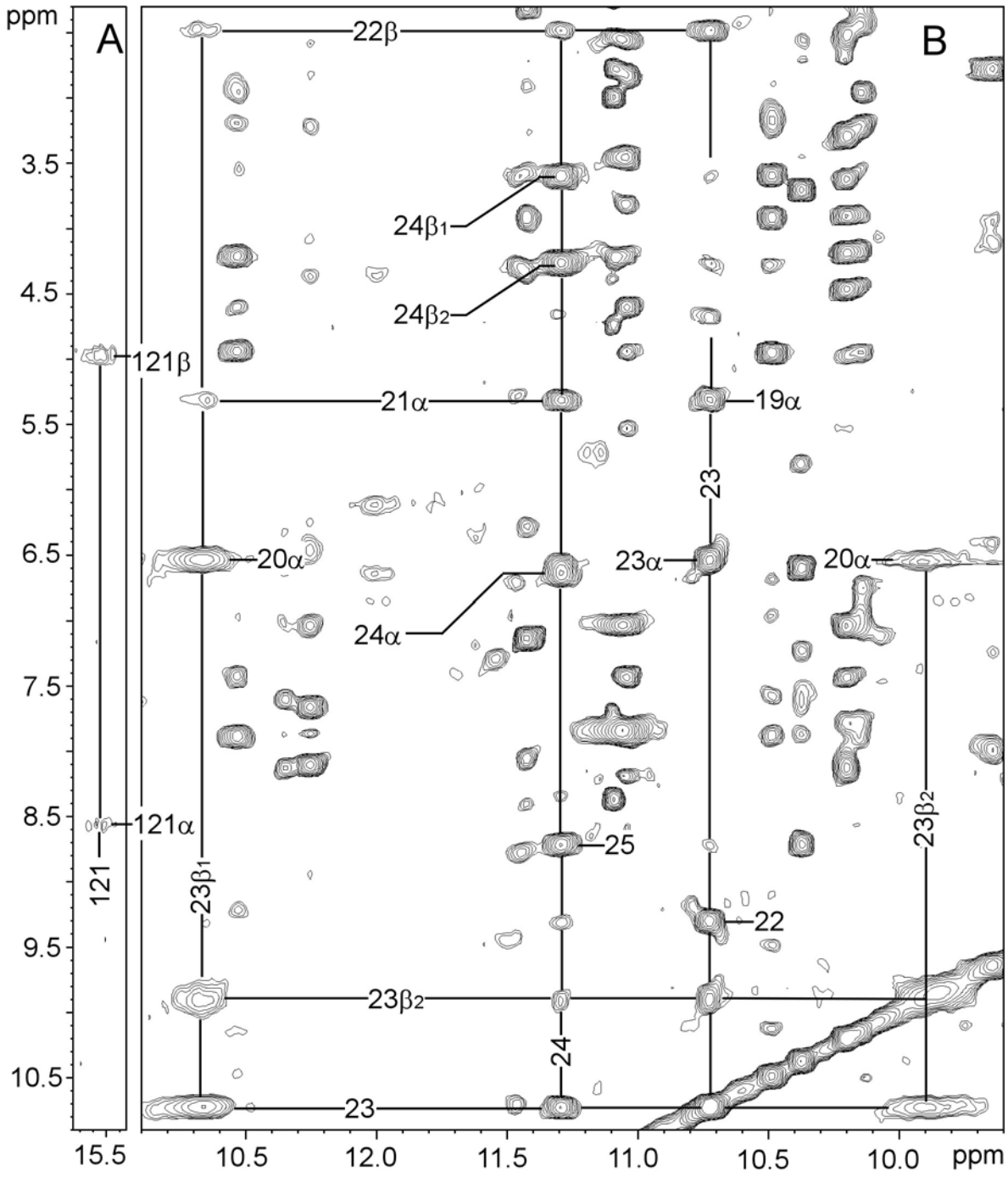

Figure S3. (**A**) Portions of the 600 MHz ^{1}H NOESY spectrum (mixing time 40 ms, repetition rate 2 s^{-1}) of *Nm*HO-PH-N_3 in $^{1}H_2O$, 50 mM phosphate, pH 7.0 at 25°C, illustrating key contacts for the relaxed resonances (**A**) Ala121, and (**B**) the low-field shifted backbone of the axial His23 and its sequential contacts to Asp24 $C_\alpha H$ and Thr20.

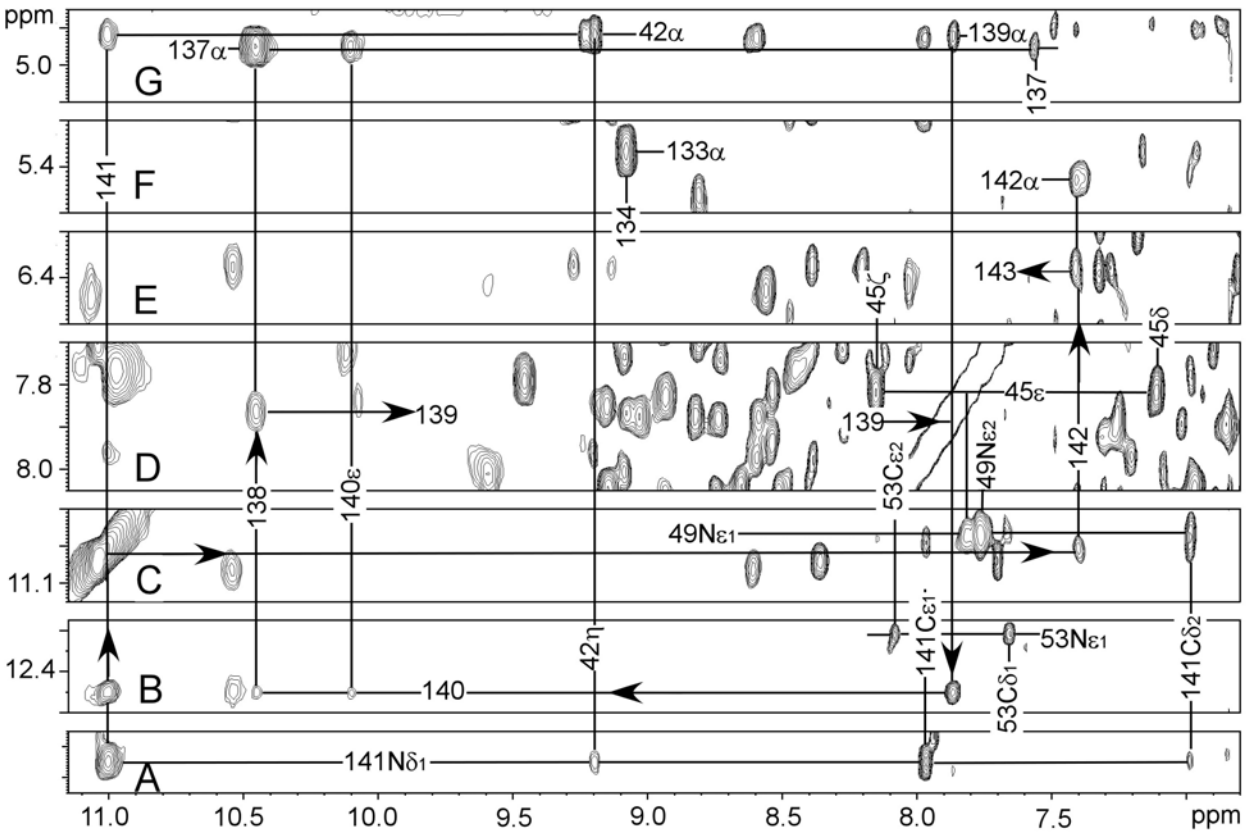

Figure S4. Portions of the 600 MHz ^{1}H NMR NOESY spectrum illustrating: (**A**) the intra His141 and the expected Tyr42 to His141 contact; (**B-F**), the sequential N_i-N_{i+1} contacts for Gly138 - Ala143, and (**B**), intra-His53 ring and intra-Gln49 $N_\varepsilon H_2$ contacts and (**C**) the Phe45 to Gln49 contact.

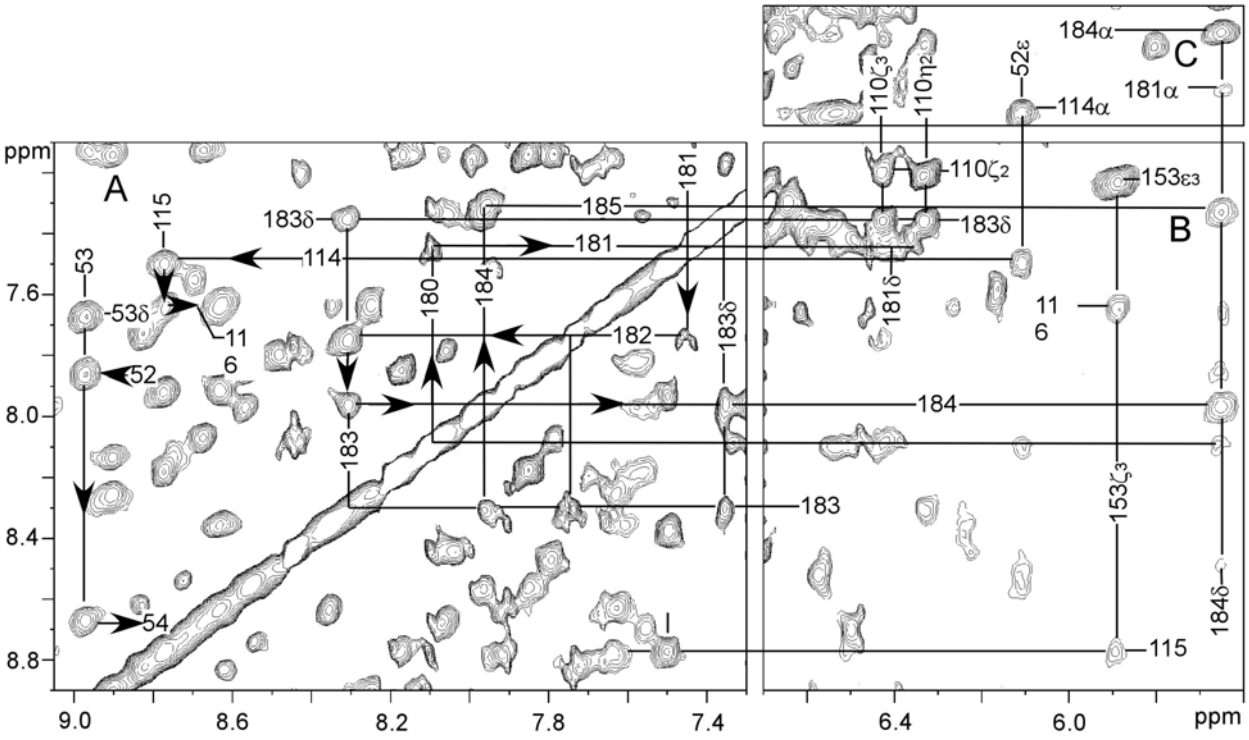

Figure S5. Portions of the 600 MHz ^{1}H NMR NOESY spectrum (mixing time 40 ms, repetition rate 1 s^{-1}) for *Nm*HO-PH-N_3 in $^{2}H_2O$, 50 mM phosphate, pH 7.1 at 25°C, illustrating sequential helical N_i-N_{i+1} (**A**), α_i-N_i (**C**) and α_i-N_{i+3} (**C**) contacts for slowly exchanging peptide NHs involving residues Phe52-Ala55, Ala114-Gly116, Ala180-Lys185 and Val187, and the contacts between these NHs and aromatic rings (**B**, **D**). The sequential N_i-N_{i+1} for the three helical fragments are indicated by arrows.

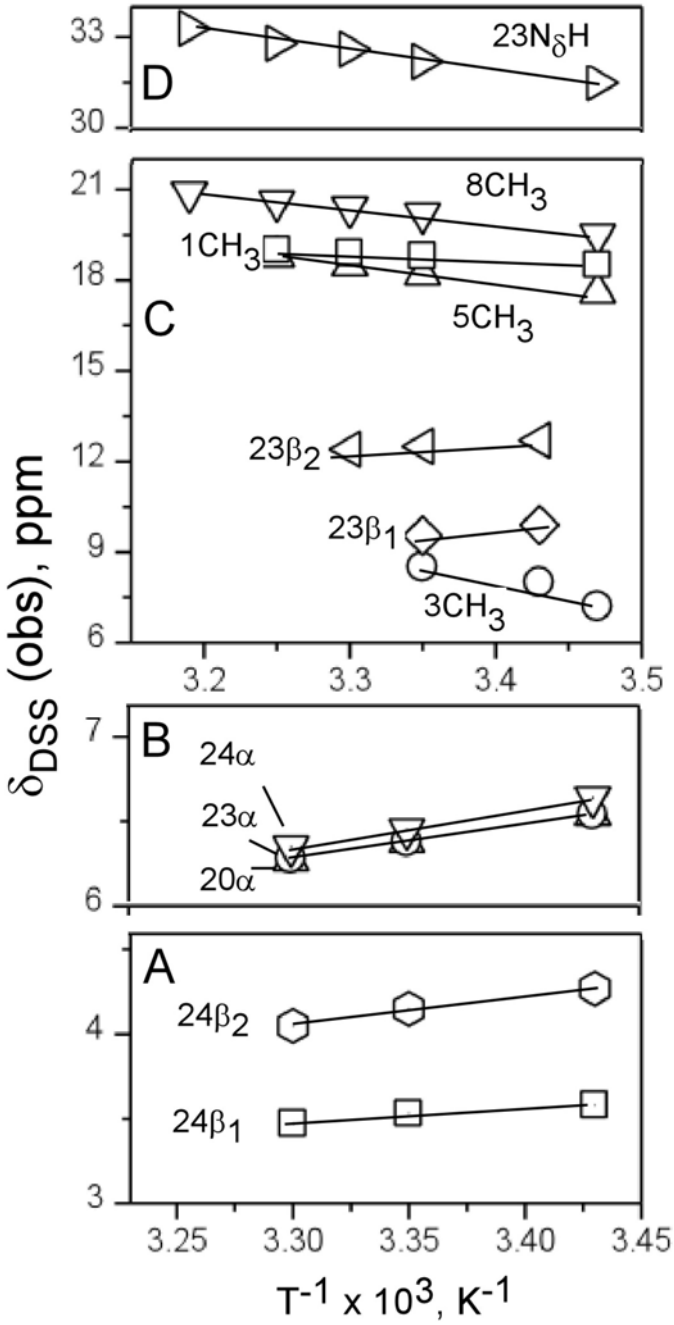

Figure S6 Plot of δ_{DSS}(obs) versus reciprocal absolute temperature (Curie-plot) for the heme methyls, His23 C_βHs and N_δH, and non-ligated residue Thr20 and Asp24 protons for *Nm*HO-PH-N_3.