Supporting Information

The orbital ground state of the azide-substrate complex of human heme oxygenase is an indicator of distal H-bonding: Implications for the enzyme mechanism

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Contains 6 Figures and 1 Table. Total pages 7.

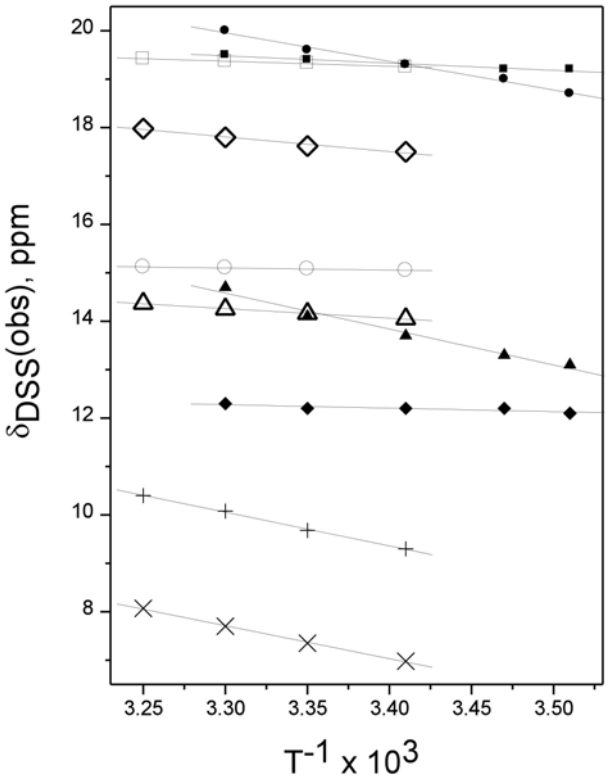

Figure S1. Curie plots (observed chemical shifts versus reciprocal absolute temperature) of the six DMDH methyls of hHO-DMDH-N_3 (closed markers for resolved methyls, and x and + symbols for unresolved methyls), and D140A-hHO-DMDH-N_3 (open markers), in ^2H_2O, 100 mM phosphate, pH 7.1

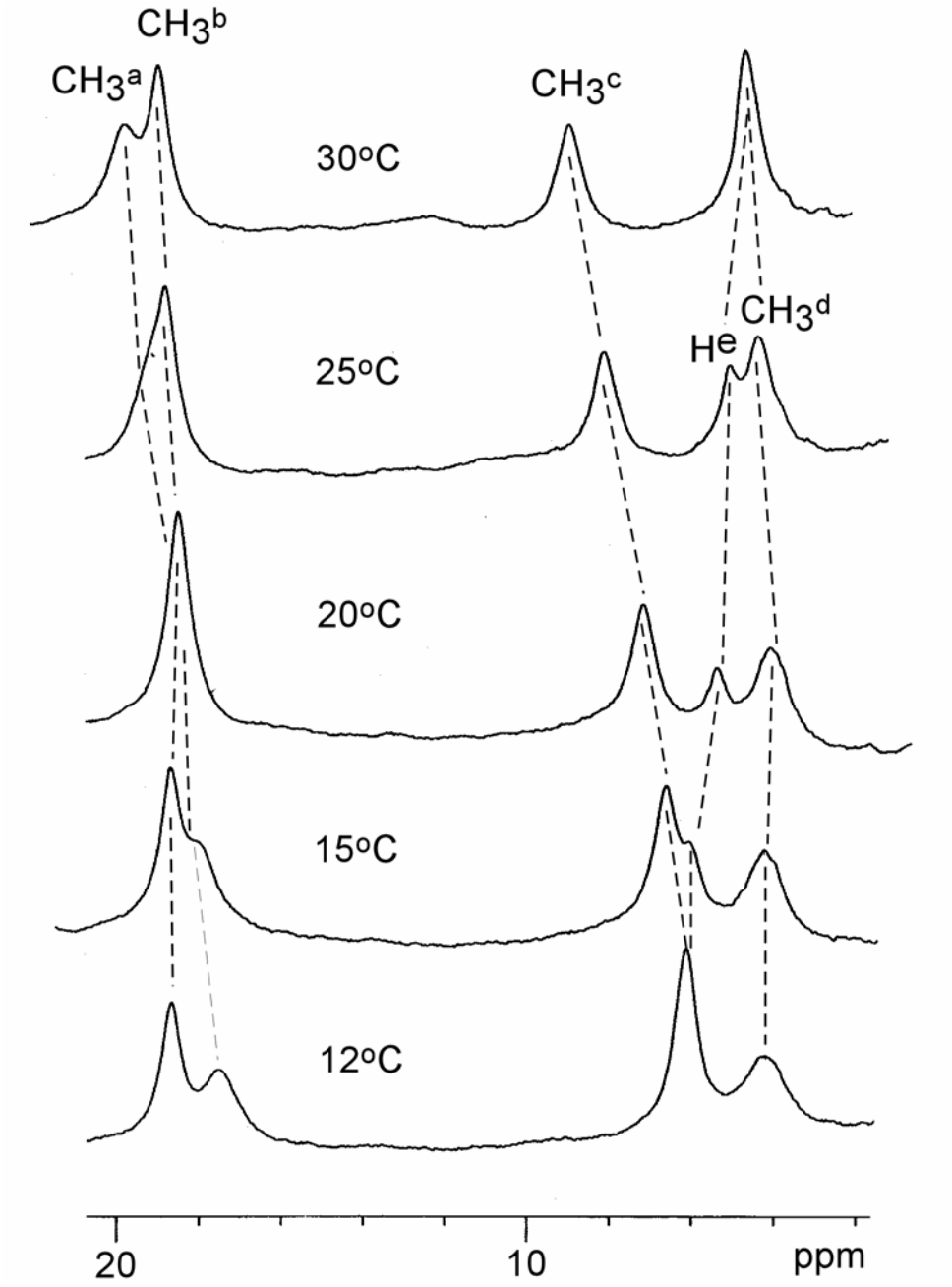

Figure S2. Low-field 600 MHz, ^{1}H NMR spectral window for the resolved DMDH methyls for D140A-hHO-DMDH-N_3 in $^{2}H_2O$, 100 mM phosphate, pH 7.1 as a function of temperatures that allows the resolution of each methyl peak.

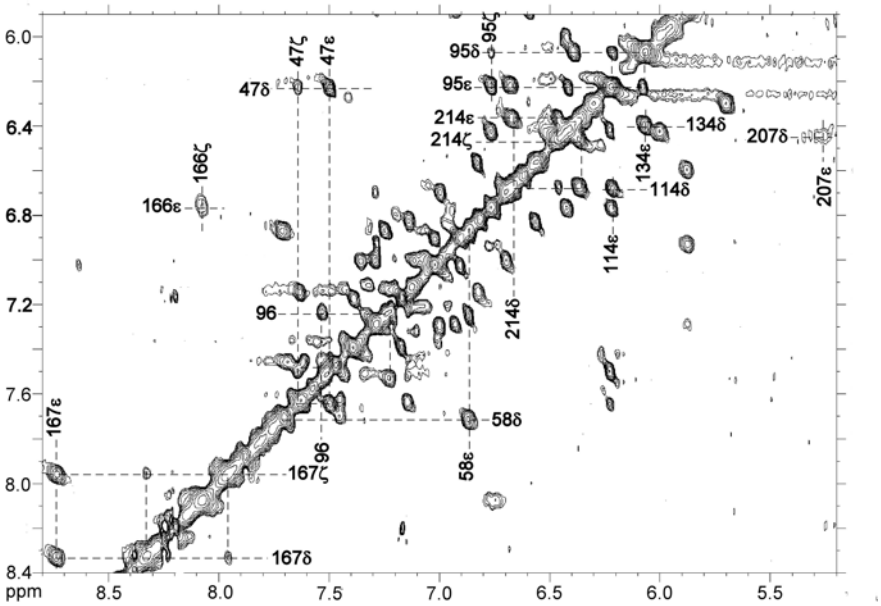

Figure S3. Portion of the 500 MHz ^{1}H NMR TOCSY spectrum (mixing time 20 ms, repetition rate 1.3 s^{-1}) of hHO-DMDH-N_3 in $^{2}H_2O$, 100 mM phosphate, pH 7.1, at 30°C illustrating the scalar contacts within aromatic residues. Cross peaks are labeled by residue number and ring position.

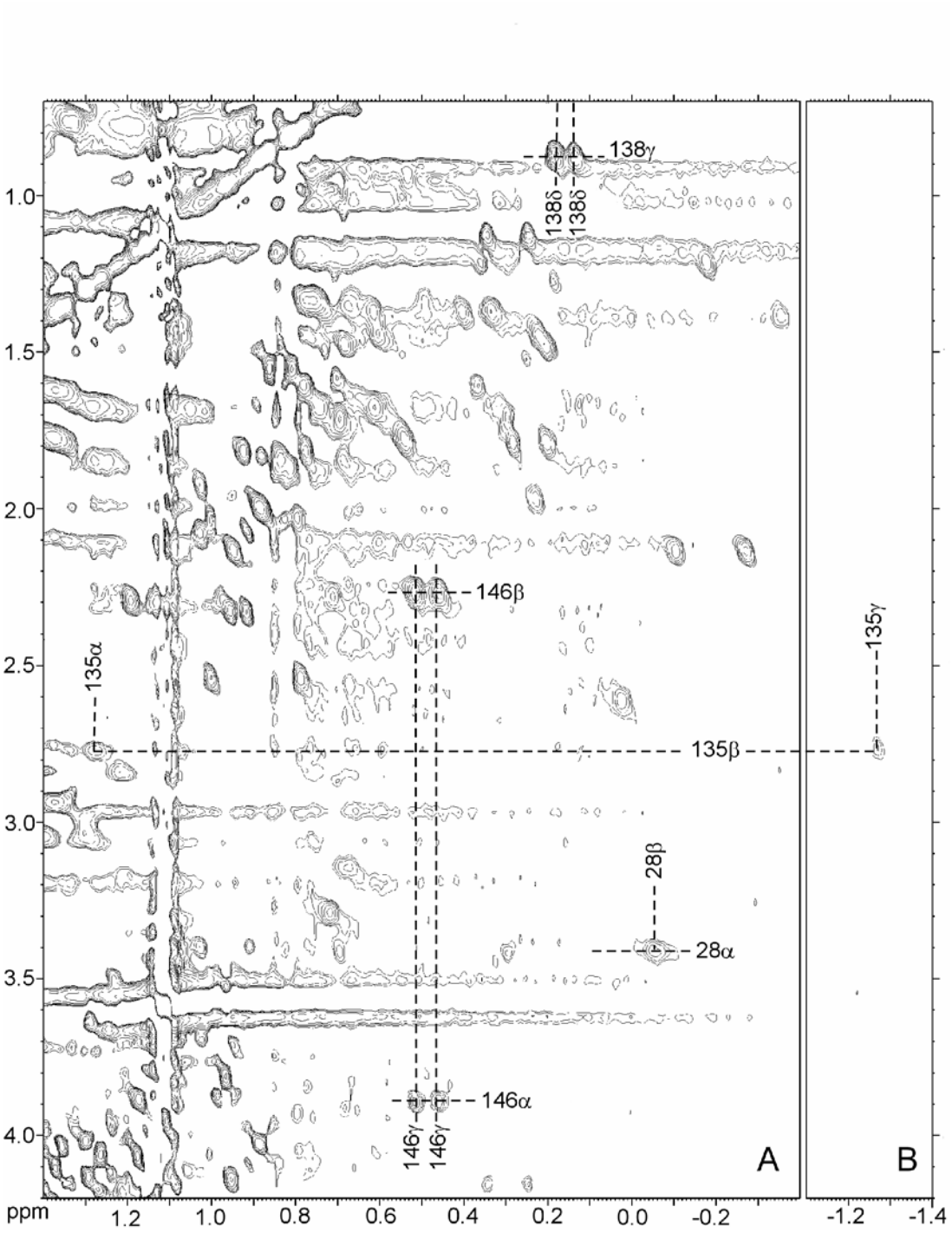

Figure S4. Portions of the 600 MHz ^{1}H NMR TOCSY spectrum (mixing time 20 ms, repetition rate 1.4 s^{-1}) illustrating the scalar contacts within key aliphatic residues of the active site; Ala28, Ile57, Leu138, Thr135, and Val146.

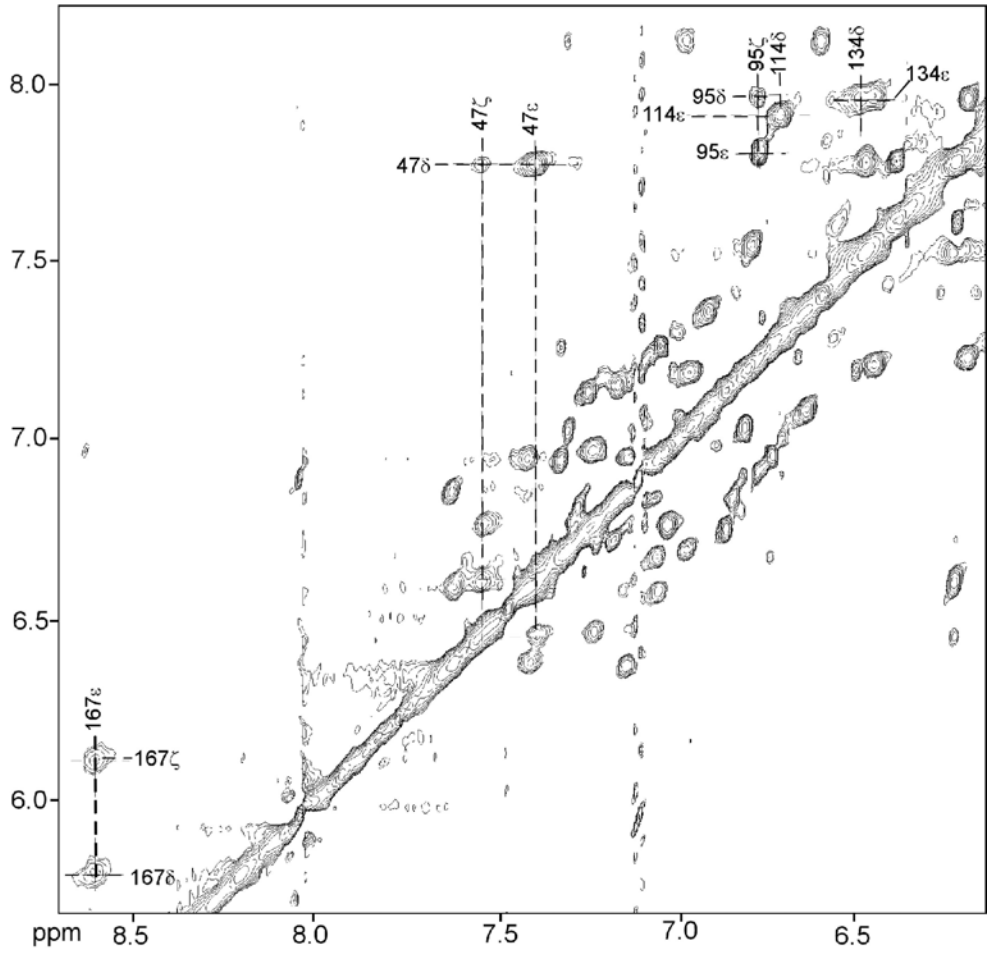

Figure S5. Portion of the 500 MHz ^{1}H NMR TOCSY spectrum (mixing time 50 ms, repetition rate 1.4 s^{-1}) of D140A-hHO-DMDH-N_3 in $^{2}H_2O$, 100 mM in phosphate, pH 7.1 at 30°C, illustrating scalar connection within aromatic rings.

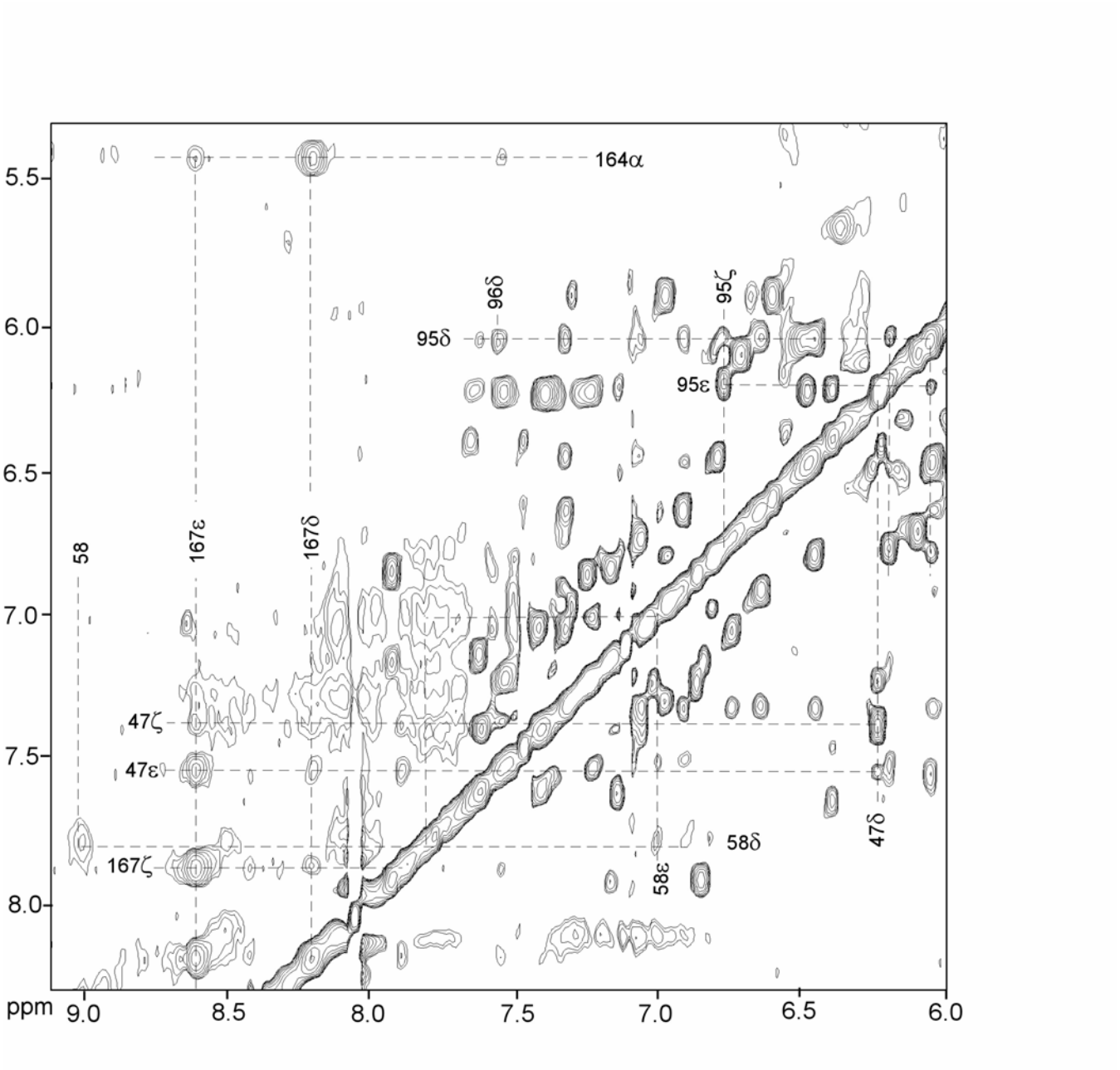

Figure S6. Portion of the 600 MHz ^{1}H NMR NOESY spectrum (mixing time 40 ms; repetition rate 1.5 s^{-1}) of D140A-hHO-DMDH-N_3 in $^{2}H_2O$, 100 mM in phosphate, pH 7.0 at 30°C illustrating the conserved contacts among the aromatic residues in the distal pocket.

Table S1. Chemical shifts for the distal aromatic cluster residues in DMDH-azide complexes of WT hHO and D140A-hHO

		WT hHO		D140A-hHO
Residue	Proton	Azide[a]	Cyanide[b]	Azide[a]
Phe47	$C_\delta Hs$	6.22	6.23	6.22
	$C_\varepsilon Hs$	7.49	7.45	7.41
	$C_\zeta H$	7.63	7.57	7.50
Tyr58	$C_\delta Hs$	7.68	7.16	7.79
	$C_\varepsilon Hs$	6.85	6.97	6.98
Phe95	$C_\delta Hs$	6.07	6.02	6.07
	$C_\varepsilon Hs$	6.26	6.16	6.20
	$C_\zeta H$	6.75	6.70	6.72
Trp96	$N_\varepsilon H$	11.70	11.71	
	$C_{\delta 1} H$	7.53	7.52	
	$C_{\zeta 2} H$	7.58	7.48	
Leu138	$C_\alpha H$	3.76	3.12	-
	$C_\beta Hs$	-0.35, 0.47	-0.40, 0.55	-
	$C_\delta H$	0.48	0.55	-
Leu164	$C_\alpha H$	5.47	5.38	5.40
	$C_{\delta 2} H_3$	1.34	0.93	1.29
Phe166	$C_\delta Hs$	5.98	5.93	5.96
	$C_\varepsilon Hs$	6.75	6.85	7.00
	$C_\zeta H$	7.50	8.24	8.05
Phe167	$C_\delta Hs$	8.34	8.16	8.14
	$C_\varepsilon Hs$	8.74	8.73	8.52
	$C_\zeta H$	7.96	8.01	8.82

a) Chemical shift, in ppm referenced to DSS via the residual solvent signals, in ^2H_2O, 50 mM in phosphate, pH 7.1 at 30°.

b) Chemical shift in ppm, referenced to DSS, in ^1H_2O, 50 mM in phosphate. . Data taken from Li et al.(18)