

Supporting Online Material

Valence Tautomerism in Synthetic Models of Cytochrome P450

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Materials and Methods

The diphenyl disulfide ligand was purchased from Sigma Aldrich and purified via silica column in 100% CH₂Cl₂. Then, the resulting solid was recrystallized at -33°C in the minimum amount of CH₂Cl₂ and layered with methanol. [Fe(TPP)(Cl)] (1), [Fe(TPP)₂O] (2) and [Fe(TPP)] (3) were synthesized and purified as previously reported. The deuterated version of the benzylic thiolate complex is synthesized according to a previous report. The low temperature (LT) is 77 K unless otherwise indicated in the figure captions. DMPO was dissolved in dry and degassed THF and 100 equivalents of DMPO were added to the 1 mM solution of the aliphatic complex.

Synthesis of diphenyl disulfide-d₅: Thiophenol-d₅ was synthesized from bromobenzene-d₅ (purchased from Cambridge Isotope Laboratories) using a modified literature procedure.⁴ For the first step, the formation of the corresponding phenyl magnesium bromide ligand was carried out using the following procedure. All glassware was dried in the oven prior to the reaction and the ether was dried over CaH₂ and distilled under nitrogen. In a two-neck 50 mL round bottom flask equipped with a condenser, the magnesium turnings (0.90 g, 37.0 mmol) in 11 mL of dry ether were sparged with argon gas. Then, the bromobenzene-d₅ (2.38 g, 14.7 mmol) was added dropwise to the solution (solution becomes cloudy) and then heated to a reflux for one hour (solution turns brown in color). From this point, the synthesis was completed according to the literature procedure (4). The crude thiophenol-d₅ was oxidized to the corresponding diphenyl disulfide ligand according to a previous report (5) and purified in the same way as the non-deuterated ligand (see above). ¹³C-NMR: (CDCl₃, 126 MHz) 136.9 (s), 128.6 (t, J_{CD} = 24.6 Hz), 127.2 (t, J_{CD} = 24.6 Hz), 126.7 (t, J_{CD} = 24.6 Hz).

Synthesis of ³⁴S-thiophenol: This procedure is modified from a previous report.⁽⁴⁾ A 3.0 M solution of PhMgBr (1.42 mL, 4.27 mmol; purchased from Sigma Aldrich) in ether was added to

a 2-neck 100 mL round bottom flask under argon. To this solution, 145.2 mg $^{34}\text{S}_8$ (4.27 mmol) was slowly added at room temperature. The reaction was allowed to stir overnight (~15 hours) under argon to afford white precipitate of $\text{Ph}^{34}\text{SMgBr}$. Then, the crude thiolate was extracted with 10% NaOH (4 x 10 mL) and the combined aqueous layers were acidified with 3M HCl. The acidified solution was extracted with diethyl ether (5 x 25 mL) and then dried over Na_2SO_4 . The filtered solution was concentrated under a stream of nitrogen gas to give a yellow oil, which was used without further purification. Yield (approx., based on $^1\text{H-NMR}$): ~160 mg (1.43 mmol, ~33%) $^1\text{H-NMR}$ (CD_2Cl_2 , 500 MHz): $\delta = 3.548$ (s, 1H); 7.137-7.285 (m, 5H); $^{13}\text{C-NMR}$ (CD_2Cl_2 , 126 MHz): $\delta = 125.5$ (s), 129.1 (s), 129.2 (s), 130.9 (s). MS (EI) m/z: $[\text{M}]^+$ calcd. for $\text{C}_6\text{H}_6^{34}\text{S}$ 112.0; found, 112.0.

Synthesis of ^{34}S -diphenyldisulfide: This procedure is modified from a previous report.⁽⁵⁾ First, ~160 mg (1.43 mmol) ^{34}S -thiophenol was dispersed in 3 mL of water in air. Then, 207 μL diethylamine (2.0 mmol) was added to the solution, which was allowed to stir overnight. As the solution stirred it became cloudy. The next day the solution was allowed to stand at room temperature for several days (~1 week) until a white precipitate formed. The precipitated solid was filtered off and washed with cold methanol to give pure product. Yield: 147 mg (0.66 mmol, 93%) $^1\text{H-NMR}$ (CD_2Cl_2 , 400 MHz): 7.235-7.517 (m, 10H); $^{13}\text{C-NMR}$ (CD_2Cl_2 , 126 MHz): $\delta = 127.6$ (s), 127.8 (s), 129.4 (s), 137.2 (s). MS (EI) m/z: $[\text{M}]^+$ calcd. for $\text{C}_{12}\text{H}_{10}^{34}\text{S}_2$ 222.0; found, 222.0.

Synthesis of $[\text{Fe}(\text{TPP})(\text{SPh})]$: A 147 mg (0.19 mmol) portion of $[\text{Fe}(\text{TPP})]$ was dissolved in 5 mL of dry, degassed toluene. Then, 135 mg (0.62 mmol) of diphenyl disulfide was dissolved in 2 mL of toluene and added to the iron porphyrin solution. The solution was heated for ~4 hours at 70°C under an argon atmosphere. The reaction was monitored by UV-visible spectroscopy and once complete, the product was precipitated by layering the solution with 40 mL of hexanes and

stored at -33°C in the glovebox freezer for 24 hours. The next day, the reaction mixture was vacuum filtered in the glovebox through a medium frit and washed with cold hexanes to give a dark purple powder. Yield: 87 mg (51%) UV-vis (toluene): 408, 514, 567, 613, 700 nm.

Synthesis of $[\text{Fe}(\text{TPP})(^{34}\text{SPh})]$: A 76 mg (0.11 mmol) portion of $[\text{Fe}(\text{TPP})]$ was dissolved in 10 mL of dry, degassed toluene. Then, 113 mg (0.51 mmol) of ^{34}S -diphenyl disulfide was added to the iron porphyrin solution. The solution was heated for ~6 hours at $\sim 70^{\circ}\text{C}$ under an argon atmosphere and monitored by UV-vis spectroscopy. The solution was stirred in the glove box overnight and the following day the solution was evaporated under reduced pressure to give a dark purple solid. The crude iron complex was washed with 50 mL of cold hexanes and used without further purification. Yield: 55 mg (0.071 mmol, 65%) The complex exhibits the same UV-vis features as the natural abundance isotope compound described above.

Synthesis of the CO complexes: CO gas was generated by dehydrating ammonium formate with conc. H_2SO_4 in Ar atmosphere. The gas was bubbled through 4N KOH solution, water and CaCl_2 drying tube. The samples were placed in a CO atmosphere with the CO gas in a pressurized balloon. The entire set up was placed in a hood connected with a high speed exhaust. The color of the sample slowly changed from brown to bright red and depending on the thiolate ligand this reaction takes 12-48 hrs and can be followed with UV-Vis. These complexes are extremely sensitive to both O_2 and H_2O . Hence they have to be carefully handled.

Instrumentation

For the characterization of the $[\text{Fe}(\text{TPP})(\text{SPh})]$ complex the following instrumentation was utilized. The EPR spectra were recorded on a Bruker X-band EMX spectrometer equipped with Oxford Instruments liquid nitrogen and helium cryostats. Electronic absorption spectra were

recorded using an Analytical Jena Specord 600 instrument at room temperature. Proton and carbon magnetic resonance spectra were recorded on Varian Inova 400 and 500 MHz instruments.

Resonance Raman Spectroscopy

The data were collected using 413.1 nm laser excitation by irradiating the sample with <10 mW power. Normally the data are collected using a 45° back scattering configuration. The stray light is rejected using the first two stages of the Trivista monochromator as a tunable band pass. The variable temperature data are collected using a home built set-up where the temperature of a sample is maintained by controlling the flow of He/N₂ gas cooled by passing it through a Cu tube immersed in liq. N₂. The temperature is adjusted by controlling the flow of the cold N₂ gas and measured in-situ using an alcohol thermometer. The typical sample concentration is 1 mM. The samples must be cooled gradually to get full conversion between the ferric and ferrous states. It is important to maintain strictly anaerobic conditions during the freezing and thawing of the samples. Introduction of air in the headspace of sample holders leads to degradation of the complexes to a 5C high spin ferric porphyrin samples which no longer shows the Fe-S or the C-S vibrations. The frozen samples inside a sample tube sealed tightly with a rubber septum and paraffin tape were thawed under vacuum in the small transfer chamber of the glove box.

EPR Spectroscopy

In general data were acquired using 10 mW power and by scanning for 10-20 minutes. The variable temperature data were obtained using a digitally controlled variable temperature X-band cavity from Jeol. The typical sample concentration is 1 mM.

The EPR spectrum for the aromatic complex at 10 K (in Figure 2) was obtained on a frozen solution using 20 mW microwave power and 100 kHz field modulation with the amplitude set to 1G. The sample concentration was ~2.5 mM.

Spin quantification (double integration of the EPR signal) of the samples were carried out at RT against a 1mM solution of TMPO. The TMPO solution in turn was calibrated against a 1mM solution of Cu²⁺ standard solution (1mM CuSO₄ in 1N HClO₄) at 77K. The spin quantification of the CO complexes were carried out against 1mM Cu²⁺ standard solution at 77K.

Table S1: Oxidation and SpinState Sensitive rR Bands of Iron Porphyrin Complexes (in bold) and the Bands obtained in the Aliphatic Thiolate Complex			v₄	v₂
Fe(III) HS			1361-1364	1553-1555
Fe(III) LS			1368-1370	1565-1568
Fe(II) HS			1340-1345	1537-1543
Fe(II) LS			1352-1354	1555-1358
Aliphatic complex in THF	RT	Fe(III) HS	1361	1554
		Fe(II) HS	1346	1542
	LT	Fe(III) HS	1361	1554
Aliphatic complex in MeOH	RT	Fe(III) HS	1361	1554
		Fe(II) HS	1346	1542
	LT	Fe(III) LS	1369	1567

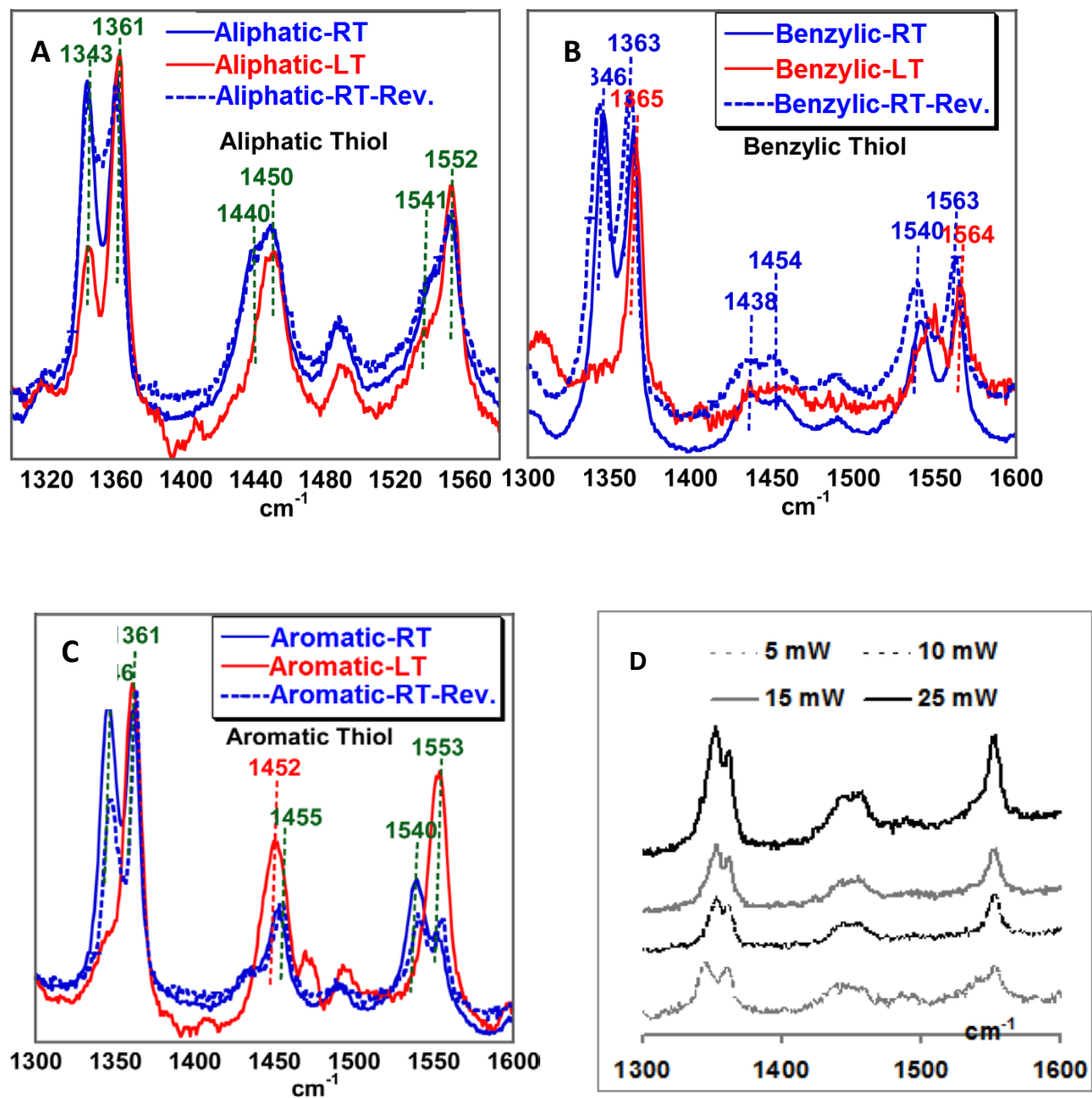


Figure S1: rR data of the A) aliphatic, B) benzylic and C) aromatic complexes in THF at RT (blue line), LT (red line) and reversibility at RT (dashed line). D) Power dependence of PPSR.

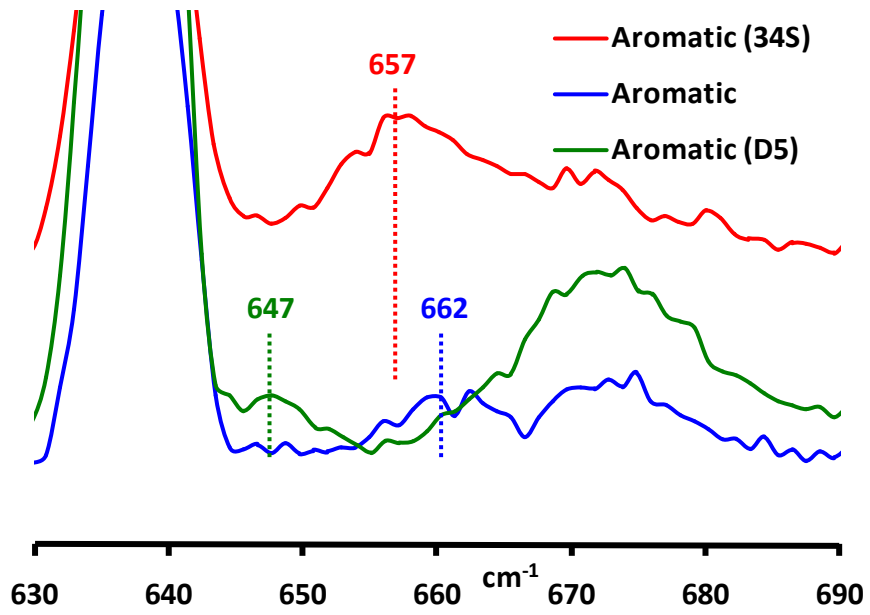


Figure S2: rR data of the aromatic complexes in THF at LT (77 K).

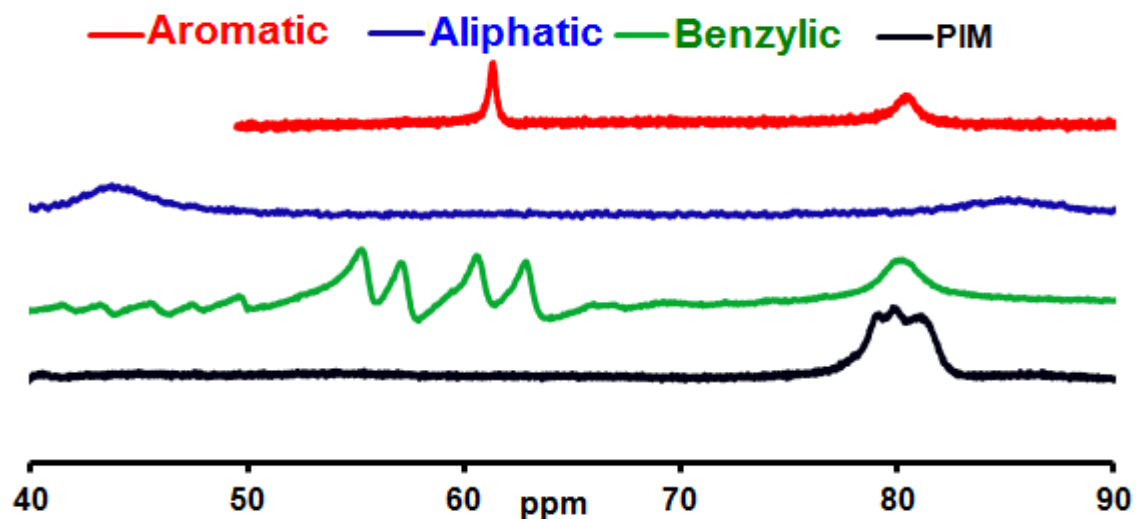


Figure S3: ^1H NMR data of the aromatic (red) in CD_2Cl_2 , aliphatic (blue), benzylic (green) and the imidazole bound complex PIM (black) in CDCl_3 at 25°C (the aliphatic complex data is recorded at 0°C). The line width is 3 Hz. The pyrrole resonances characteristic of ferric porphyrin are indicated. Note that the ferrous porphyrin also contains a thiyl radical. Thus unambiguous assignment of the resonances from this species is not possible at this time.

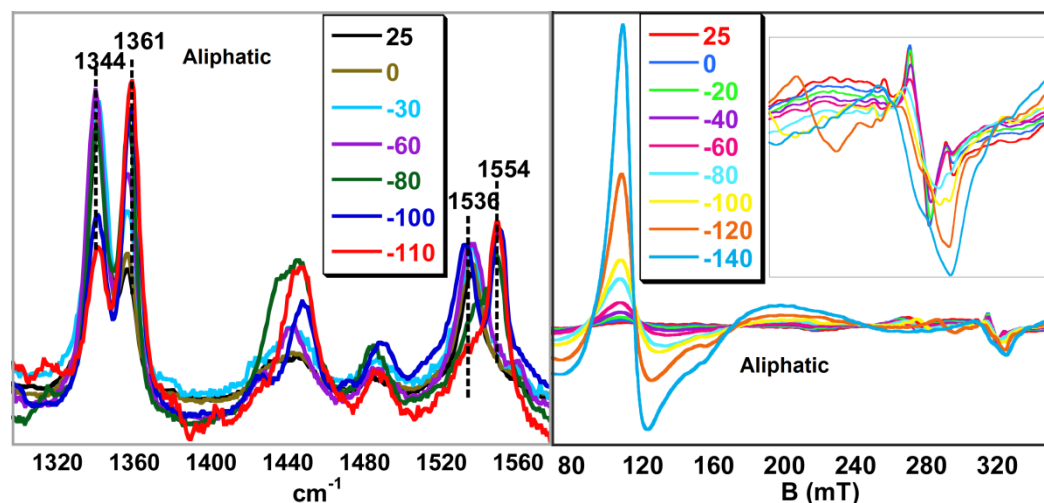


Figure S4: (left) VT rR of the aliphatic complex in THF and (right) VT EPR of aliphatic complex in toluene. Temperatures are indicated in the legend in $^\circ\text{C}$.

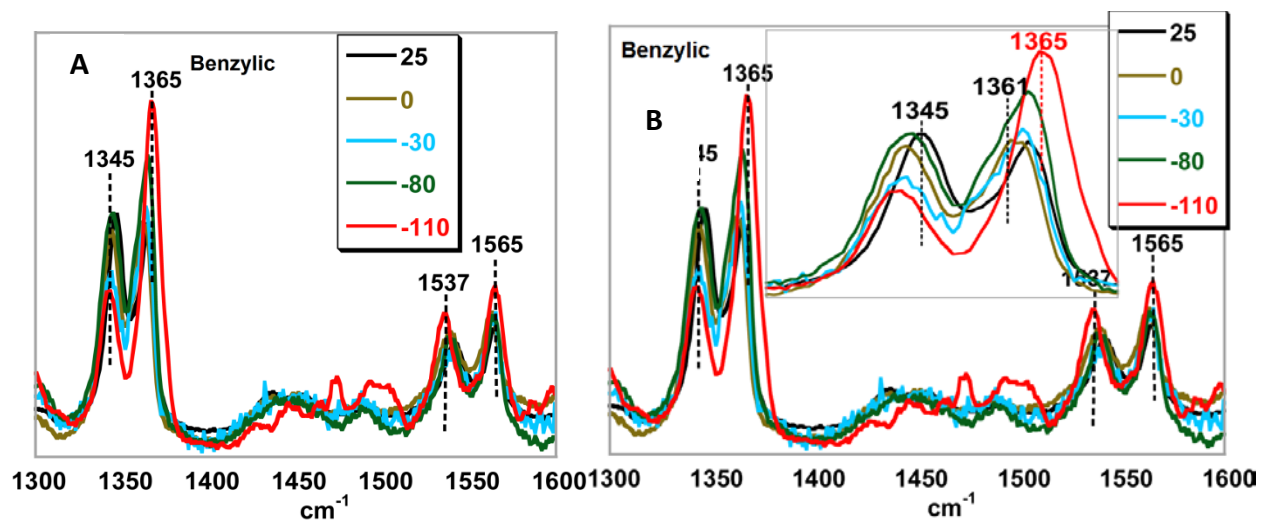


Figure S5: VT rR of the benzylic complex in THF, showing a transition from high-spin to low-spin (inset, right).

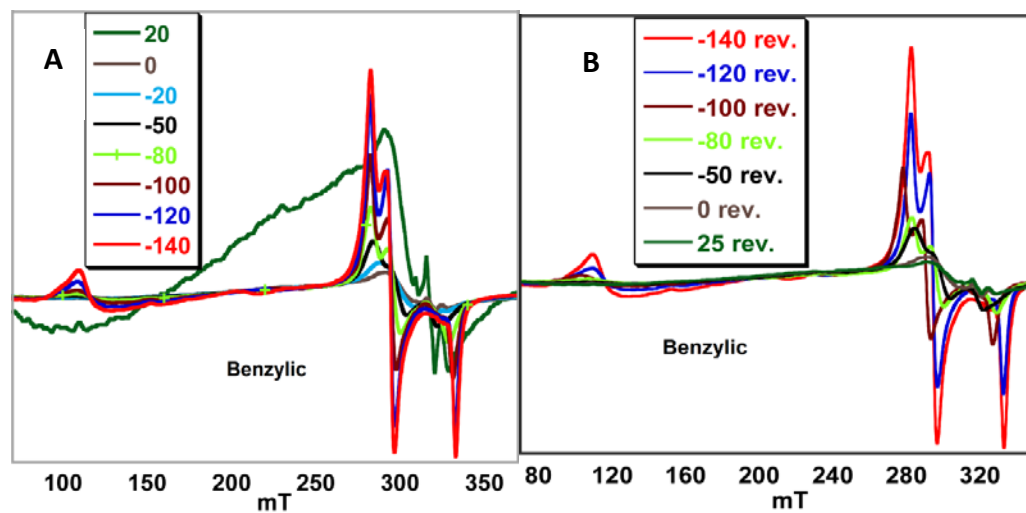


Figure S6: VT EPR of the benzylic complex (A and B) in toluene. The temperatures are indicated in the legend in °C.

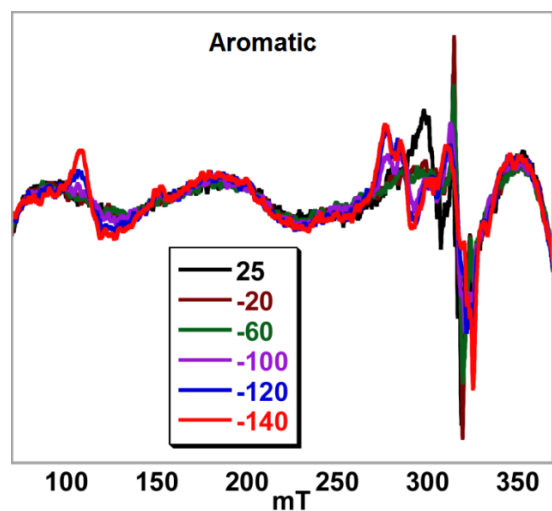


Figure S7: VT EPR of the aromatic complex in toluene. The temperatures are indicated in the legend in °C.

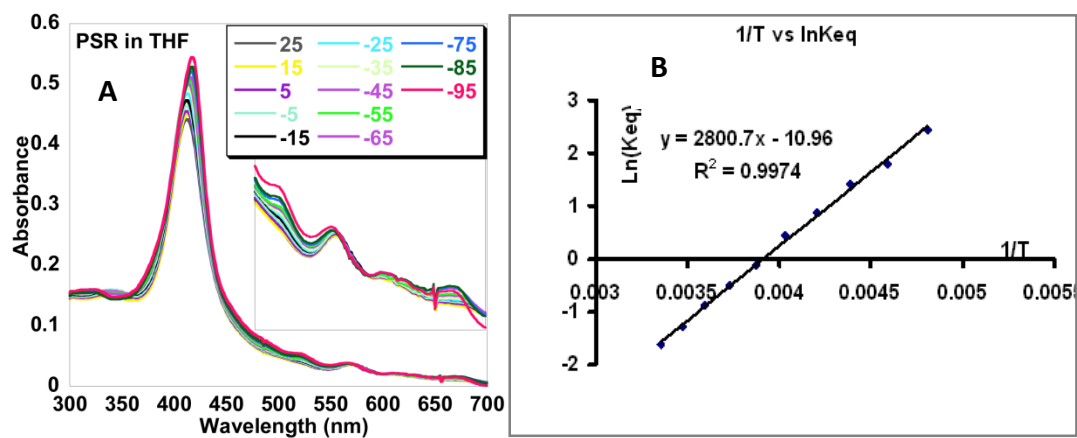


Figure S8: A) VT absorption data of the aliphatic complex in THF and B) Eyring plot for the corresponding data.

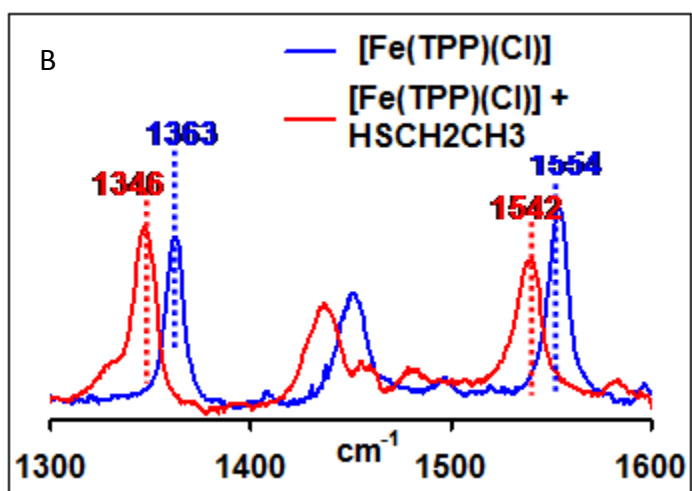
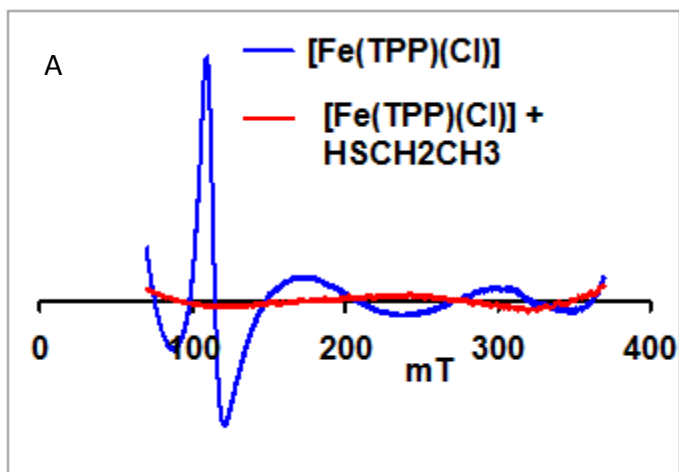


Figure S9: **A)** EPR data of the [Fe(TPP)(Cl)] and [Fe(TPP)(Cl)]+HSCH₂CH₃ complex in THF at 77 K, **B)** rR of the above complex in THF at RT.

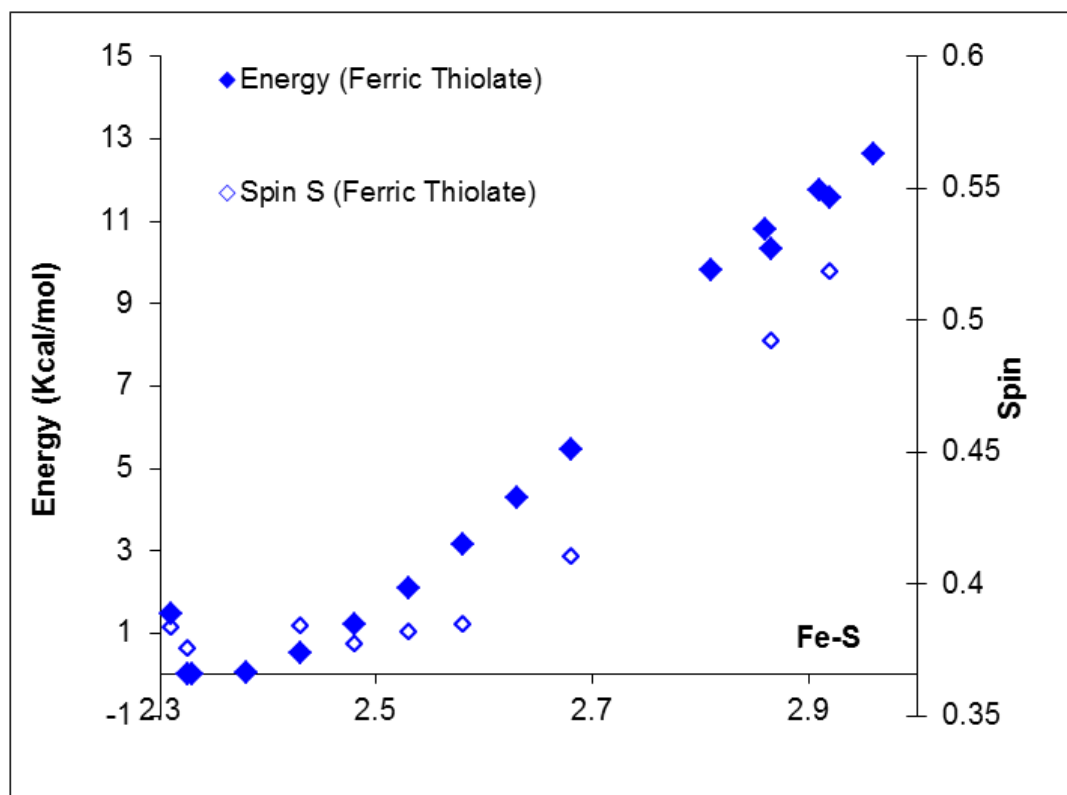


Figure S10:A potential energy scan of the Fe-S bond of the aliphatic thiol complex.

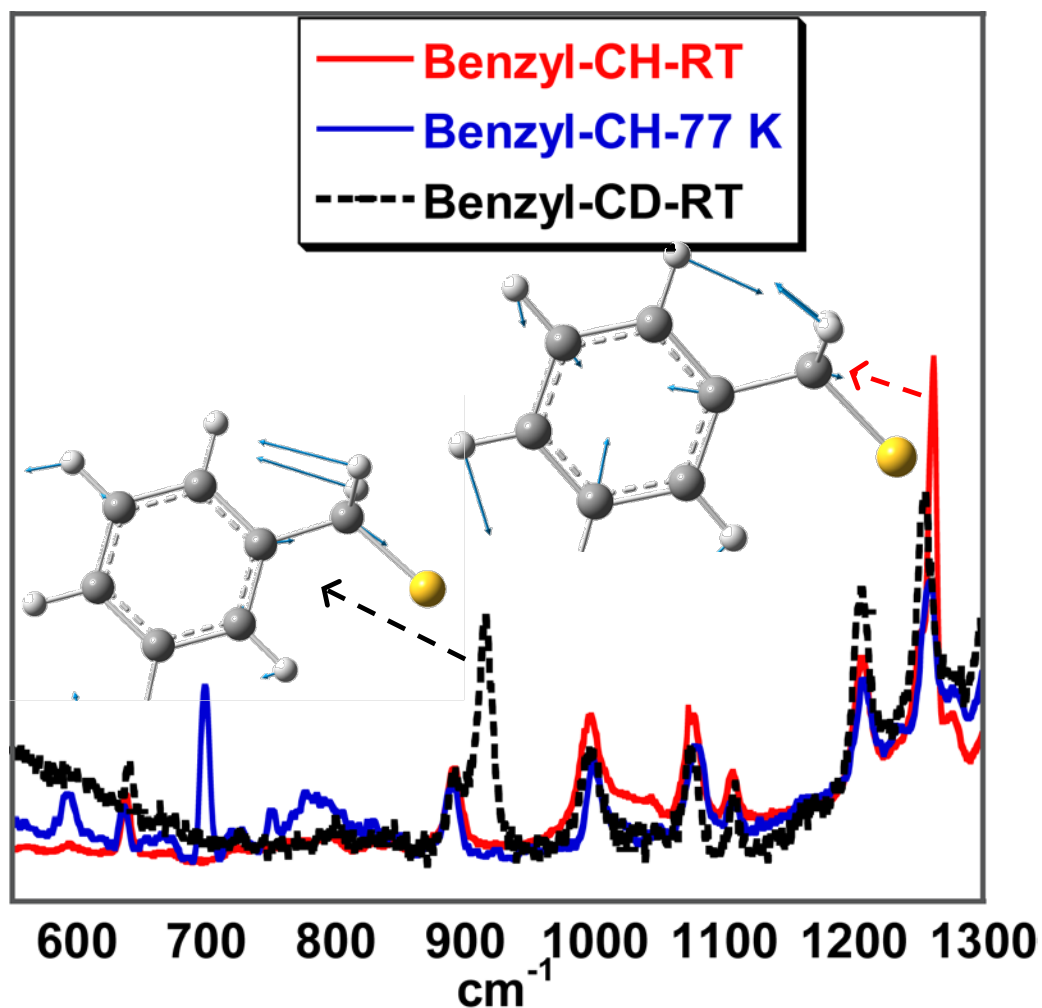


Figure S11: rR of the deuterated complexes in the low frequency region. A vibration at 1260 cm^{-1} shifts to 914 cm^{-1} on deuteration of the benzyl thiolate complex. The peak at 700 cm^{-1} is from CH_2Cl_2 solvent. The calculated frequencies for the CH_2 and CD_2 wagging mode of a protonated and deuterated *m*-hydroxy benzyl thiyl radical are 1271 cm^{-1} and 914 cm^{-1} , respectively. The computed (Gaussian 03 Ver. C02, BP86/6-311g*) vibrational modes are depicted in the inset. It is likely that this mode, characteristic of thiyl radical, gets enhanced in the resonance Raman experiments.

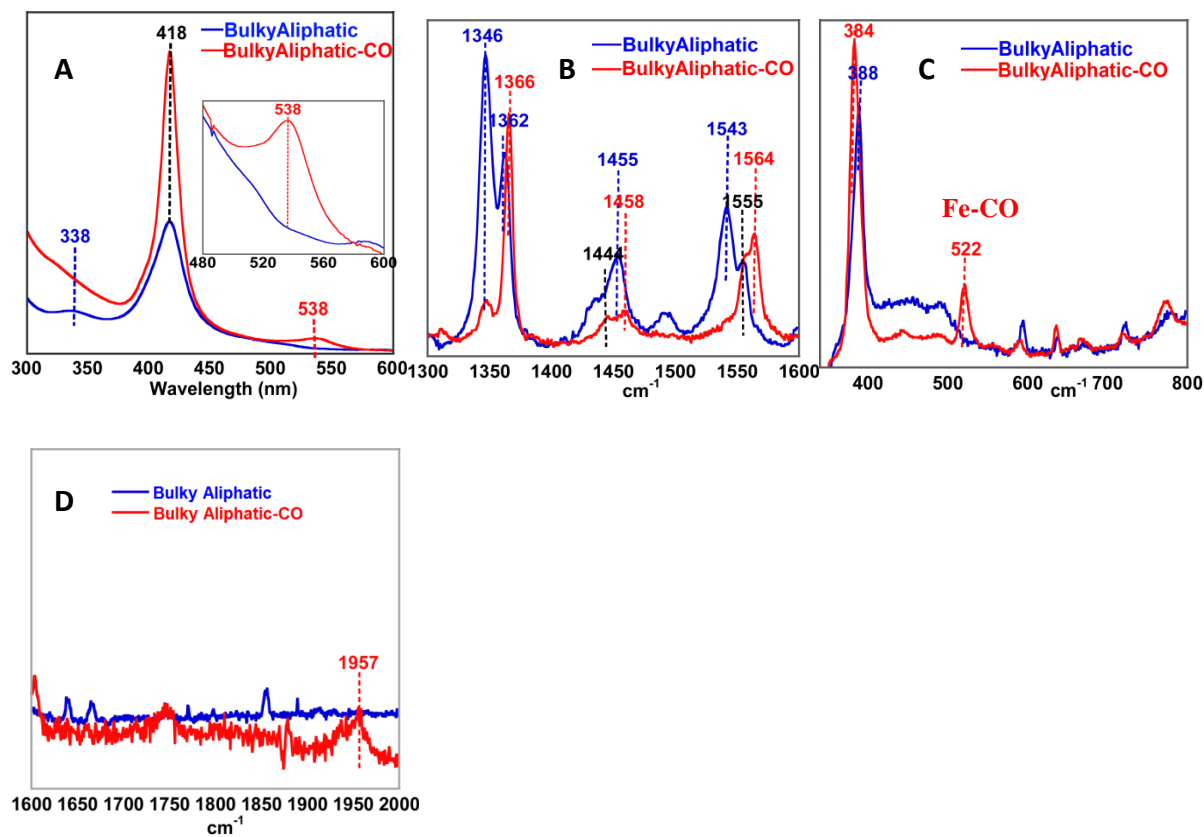


Figure S12. A) Absorption, B) and C) rR data of the bulky aliphatic complex (blue) and bulky aliphatic complex + CO (red) at RT, D) rR in the high frequency region.

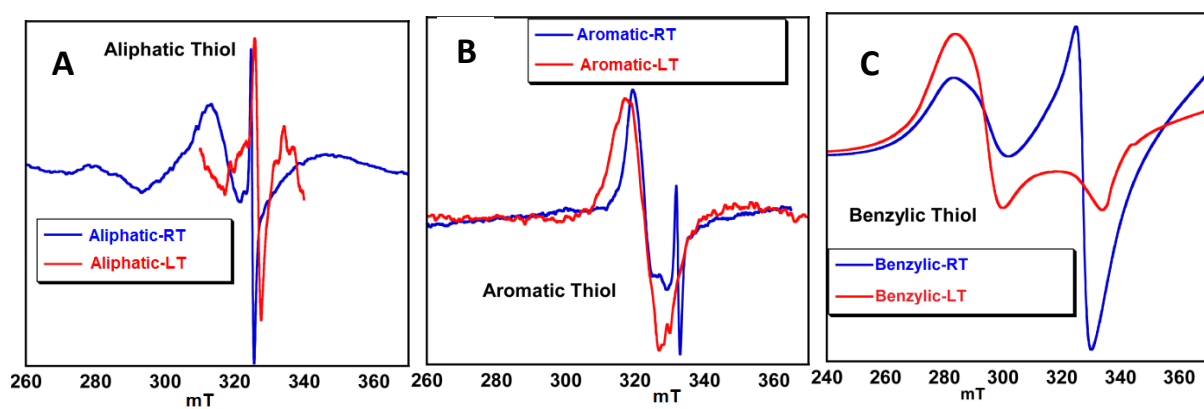


Figure S13: EPR data of the A) aliphatic, B) aromatic and C) benzylic complexes in the solid state at RT (blue) and LT (red).

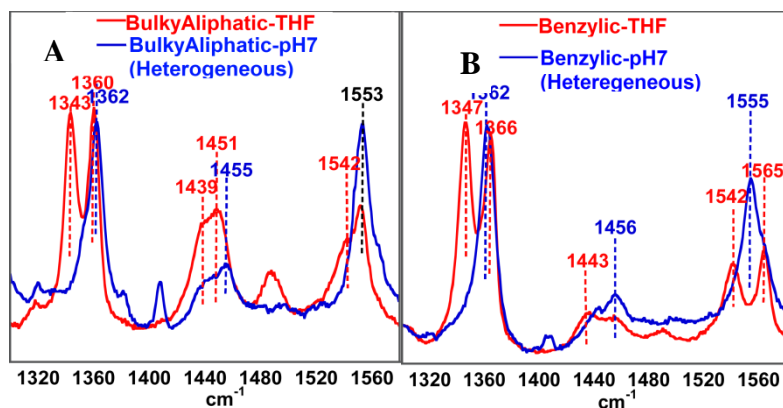


Figure S14: rR data of the air stable **A)** bulky aliphatic and **B)** benzylic complex in THF (red) and in an aqueous environment (blue) at RT.

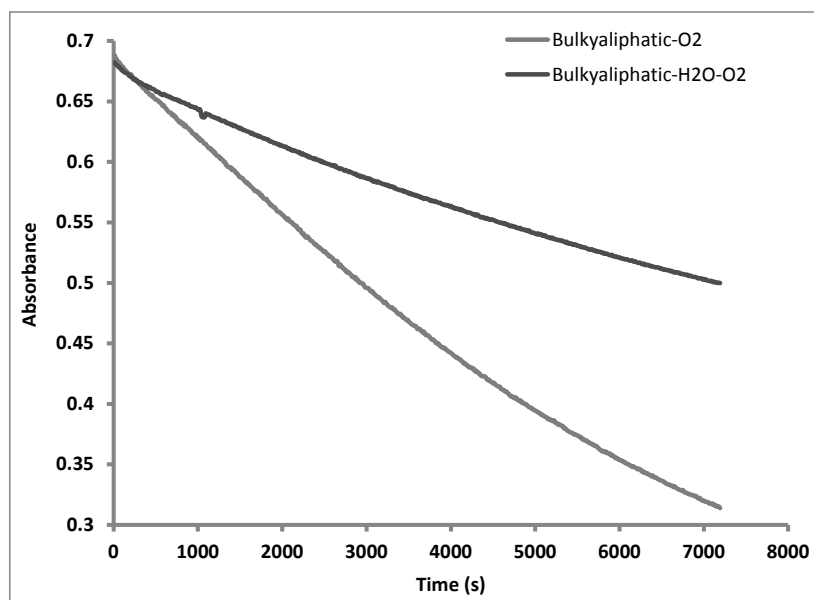


Figure S15: O₂ reaction of the bulky aliphatic thiolate complex with O₂ in the presence and absence of 0.5% H₂O in THF. The trace represents the absorption at the λ_{max} of the Soret band and shows clear degradation of the porphyrin with time. The rate is much greater in dry THF.

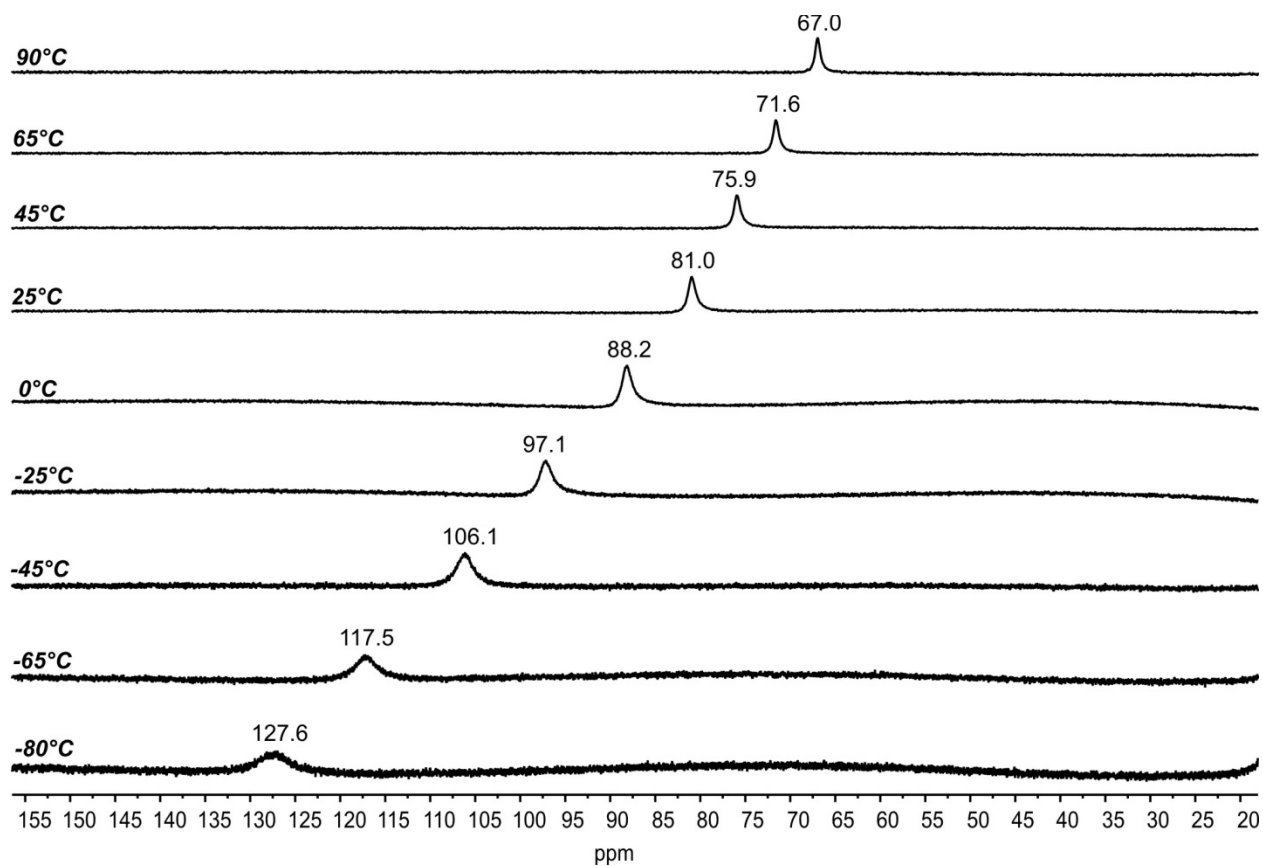


Figure S16: Variable temperature ¹H NMR spectra of [Fe(TPP)(Cl)] in toluene-d₈. The line broadening of the spectra is set to 3 Hz.

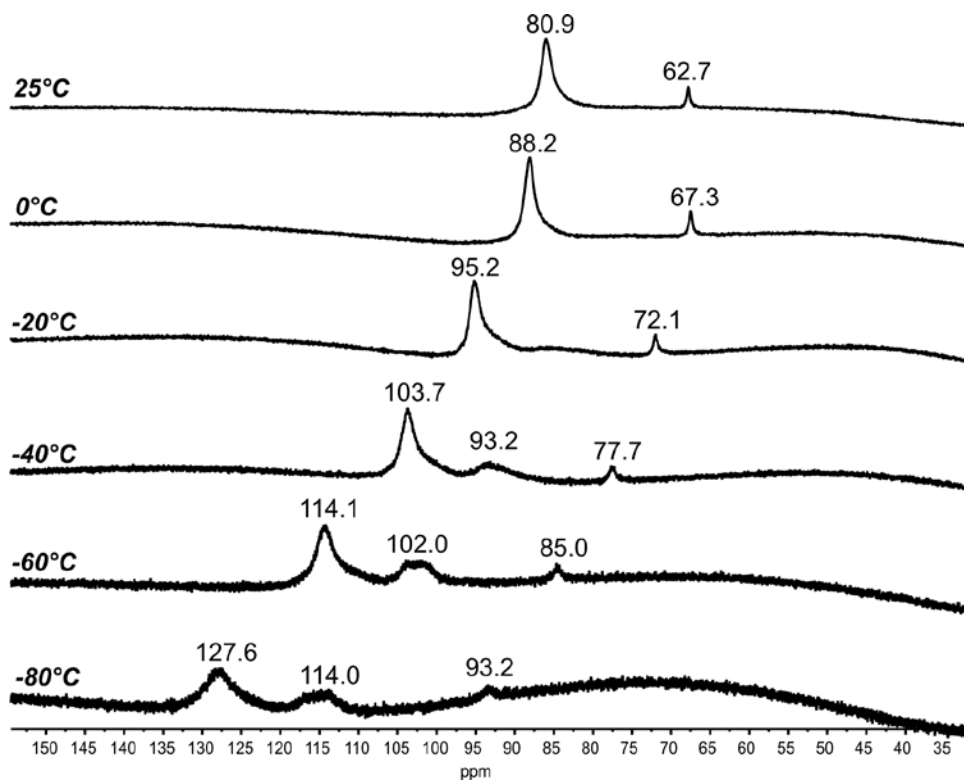


Figure S17: Variable temperature ^1H -NMR spectra of the aromatic complex in toluene- d_8 . The line broadening of the spectra is set to 3 Hz.

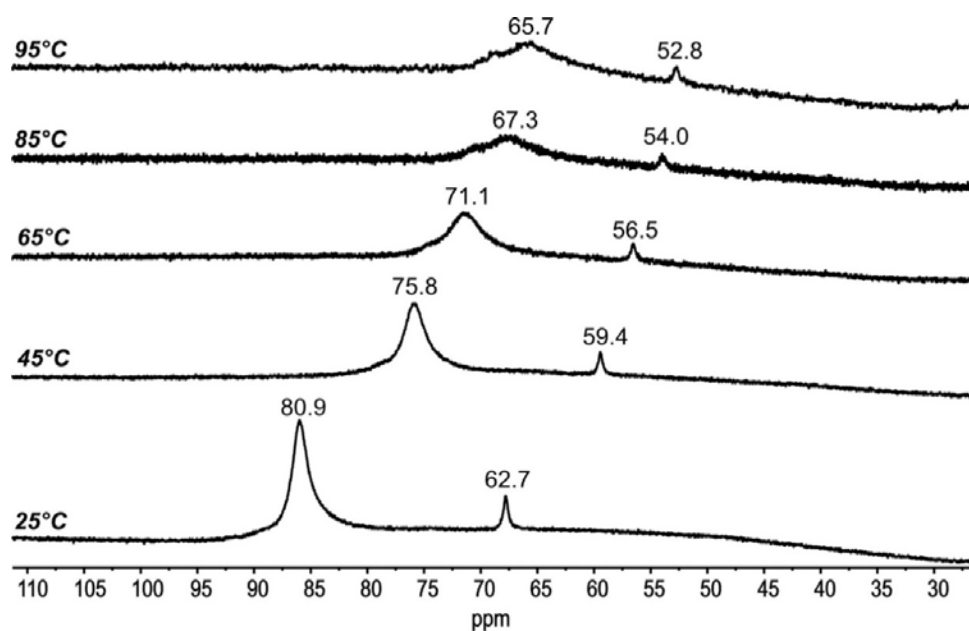


Figure S18: Variable ^1H -NMR spectra of the aromatic complex in toluene- d_8 (cont.). The line broadening of the spectra is set to 3 Hz.

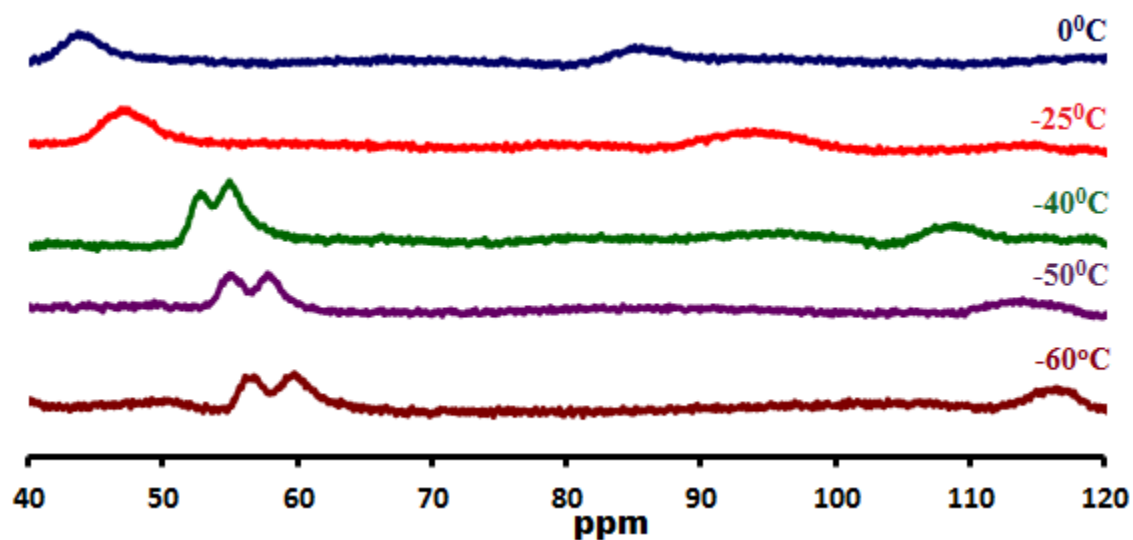


Figure S19: Variable temperature ^1H -NMR spectra of the aliphatic complex in CDCl_3 . The line broadening is set to 3 Hz.

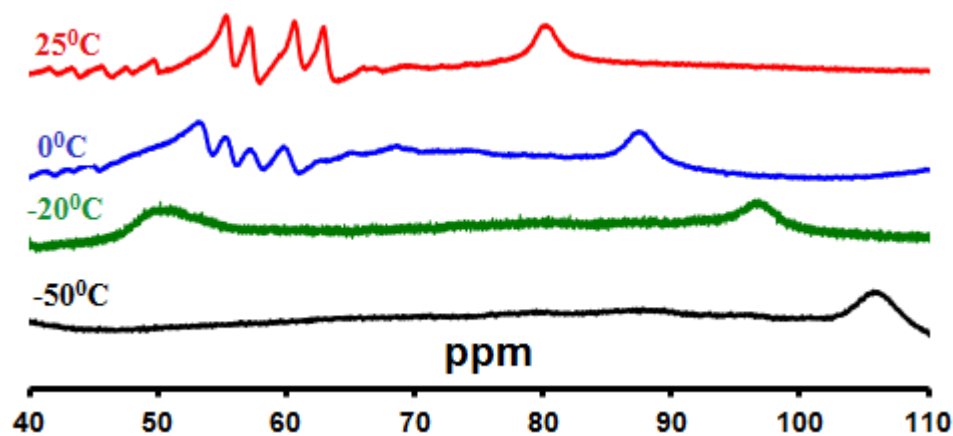


Figure S20: Variable temperature ^1H -NMR spectra of the benzylic complex in CDCl_3 . The line broadening is set to 3 Hz.

References

1. Adler, A. D., Longo, F. R., Kampas, F., & Kim, J. (1970) On the preparation of metalloporphyrins. *J. Inorg. Nucl. Chem.* 32(7):2443-2445.
2. Rai, B. K., Durbin, S. M., Prohofskey, E. W., Sage, J. T., Ellison, M. K., Roth, A., Scheidt, W. R., Sturhahn, W., & Alp, E. E. (2003) Direct Determination of the Complete Set of Iron Normal Modes in a Porphyrin-Imidazole Model for Carbonmonoxy-heme Proteins: [Fe(TPP)(CO)(1-MeIm)]. *J. Am. Chem. Soc.* 125(23):6927-6936.
3. Goodrich, L. E., Roy, S., Alp, E. E., Zhao, J., Hu, M. Y., & Lehnert, N. (2013) Electronic Structure and Biologically Relevant Reactivity of Low-Spin {FeNO}8 Porphyrin Model Complexes: New Insight from a Bis-Picket Fence Porphyrin. *Inorg. Chem.* 52(13):7766-7780.
4. Jarboe, S. G., Terrazas, M. S., & Beak, P. (2008) The Endocyclic Restriction Test: The Geometries of Nucleophilic Substitutions at Sulfur(VI) and Sulfur(II). *J. Org. Chem.* 73(24):9627-9632.
5. Abaee, M. S., Mojtahedi, M. M., & Navidipoor, S. (2010) Diethylamine-Catalyzed Dimerization of Thiols: An Inexpensive and Green Method for the Synthesis of Homodisulfides Under Aqueous Conditions. *Synth. Commun.* 41(2):170-176.