Supplemental Materials for:

Spectroscopic and Computational Studies of Reduction of the Metal versus the Tetrapyrrole Ring of Coenzyme F_{430} from Methyl-Coenzyme M Reductase

Mishtu Dey[†], Ryan Kunz[†], Katherine M. Van Heuvelen[‡], Jennifer L. Craft[‡], Yih-Chern Horng[†], Qun Tang[§], David F. Bocian[§], Simon J. George[¶], Thomas C. Brunold[‡], Stephen W. Ragsdale[†*]

[†]Department of Biochemistry, University of Nebraska, Lincoln, NE 68588

[‡]Department of Chemistry, University of Wisconsin-Madison, Madison, WI 53706

[§]Department of Chemistry, University of California, Riverside 92521

[¶]Physical Biosciences Division, Lawrence Berkeley National Lab, Berkeley, California 94720

AUTHOR EMAIL ADDRESS: sragsdale1@unl.edu

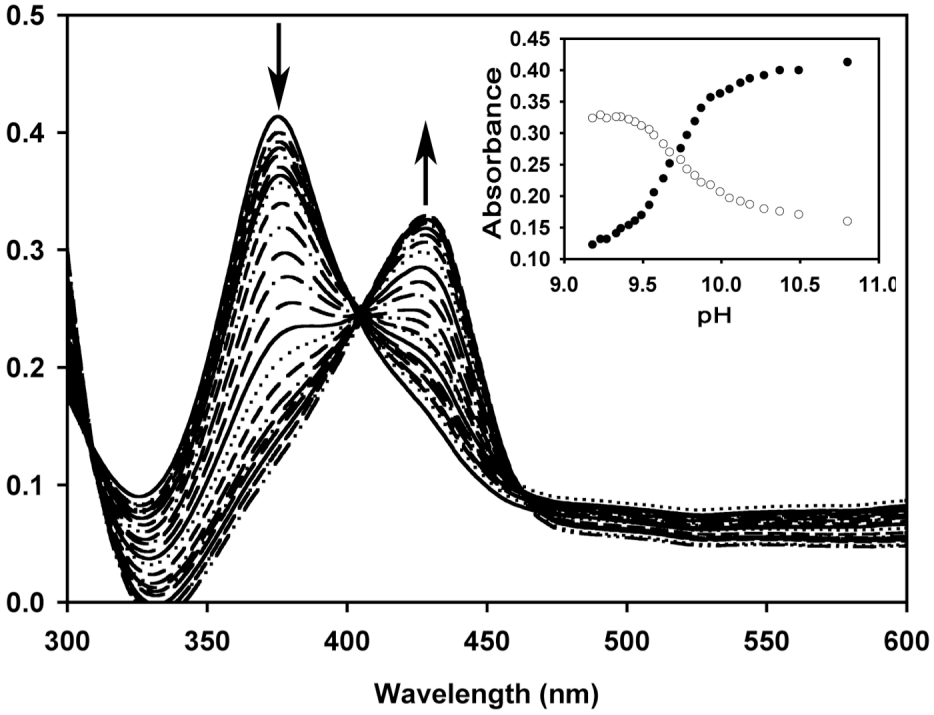

Figure S1. Reversion of F_{380} to F_{430} as a function of pH. Nearly 100% initial conversion of F_{430} to F_{380} was achieved by reacting 20 µM of F_{430} with 130 µM Ti(II) citrate in 60 mM NH_4OH, final pH of 10.8. UV-visible spectrum of F_{380} reversion to F_{430}, arrows indicate the decrease in the 376 nm band and an increase in the 432 nm band. Inset: Following the absorption peak at 376nm (○) and 428nm (●), points were taken at each 2.5 µL addition of 0.5 M formic acid .

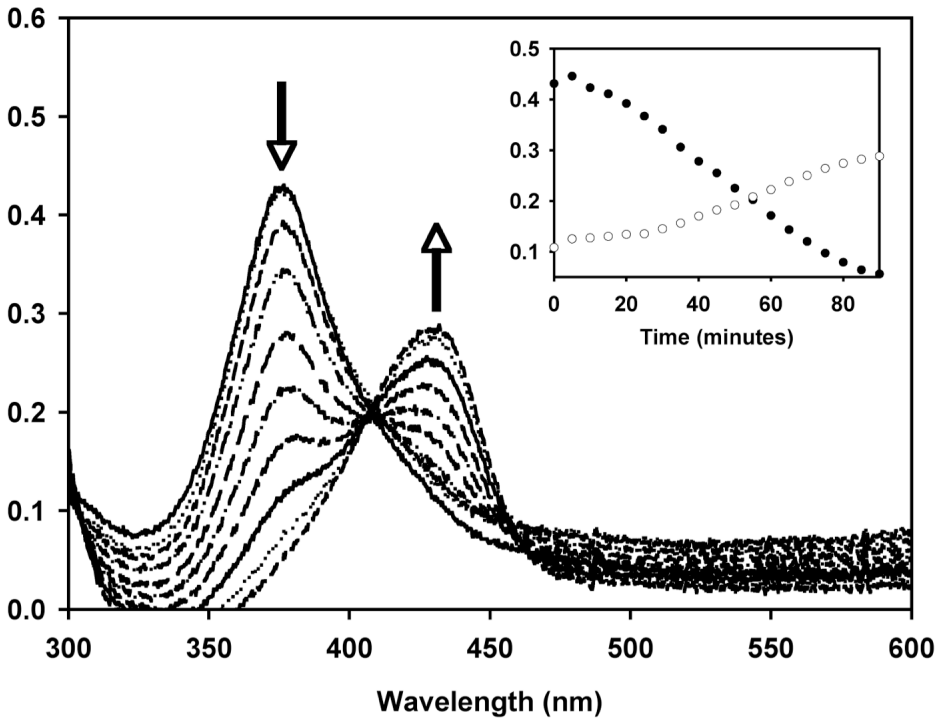

Figure S2. Reversion of F_{380} to F_{430} as a function of time. Nearly 100% initial conversion of F_{430} to F_{380} was achieved by reacting 20 μM of F_{430} with 130 μM Ti(II) citrate in 60 mM NH_4OH, final pH of 10.8. UV-visible spectrum of F_{380} reversion to F_{430}, arrows indicate the decrease in the 376 nm band and an increase in the 432 nm band. Inset: Following the absorption peak at 376nm (●) and 432nm (○), time points were taken every 5 minutes for 90 minutes.

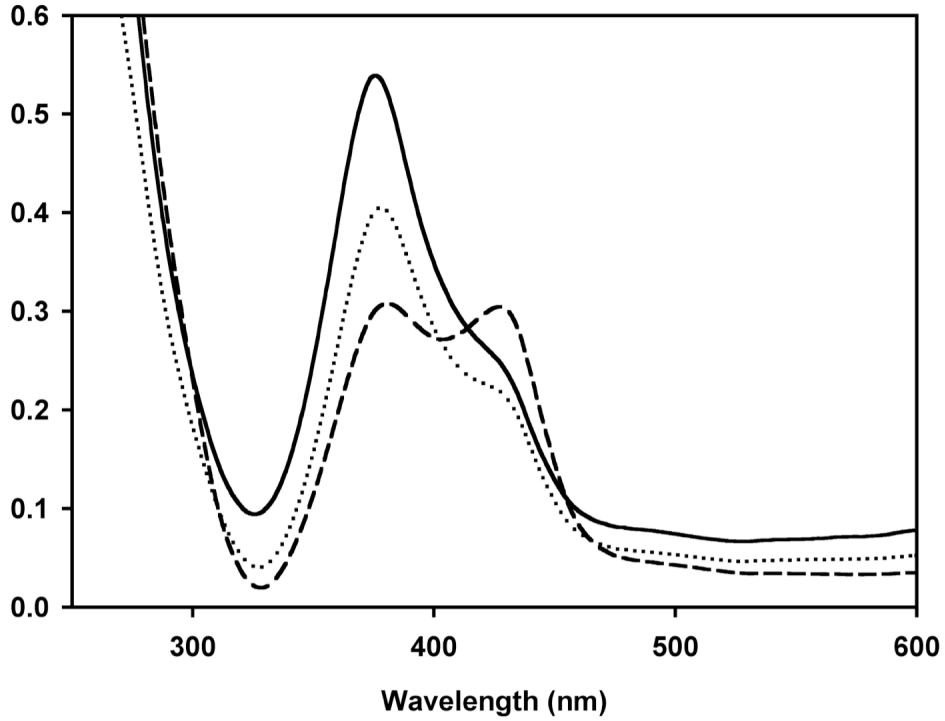

Figure S3 UV-Visible spectra used to verify the presence of F_{430} before and after MS analysis. (Solid line) 25 uM F_{430} was converted to F_{380} in the presence of 125 uM Ti(III) citrate and 63 uM NH_4OH at pH 10.5. (Dashed line) Following conversion of F_{430} to F_{380} (before MS analysis) the volume of the reaction was raised by 20% by the addition of anaerobic ACN (100%), final concentration of reaction components: 20 uM F_{430}/$F\text{-}_{380}$, 50 uM NH_4OH, 100 uM Ti(III), and 20% ACN. (Dotted line) Spectrum after MS analysis, concentrations of reaction components are the same as before analysis.

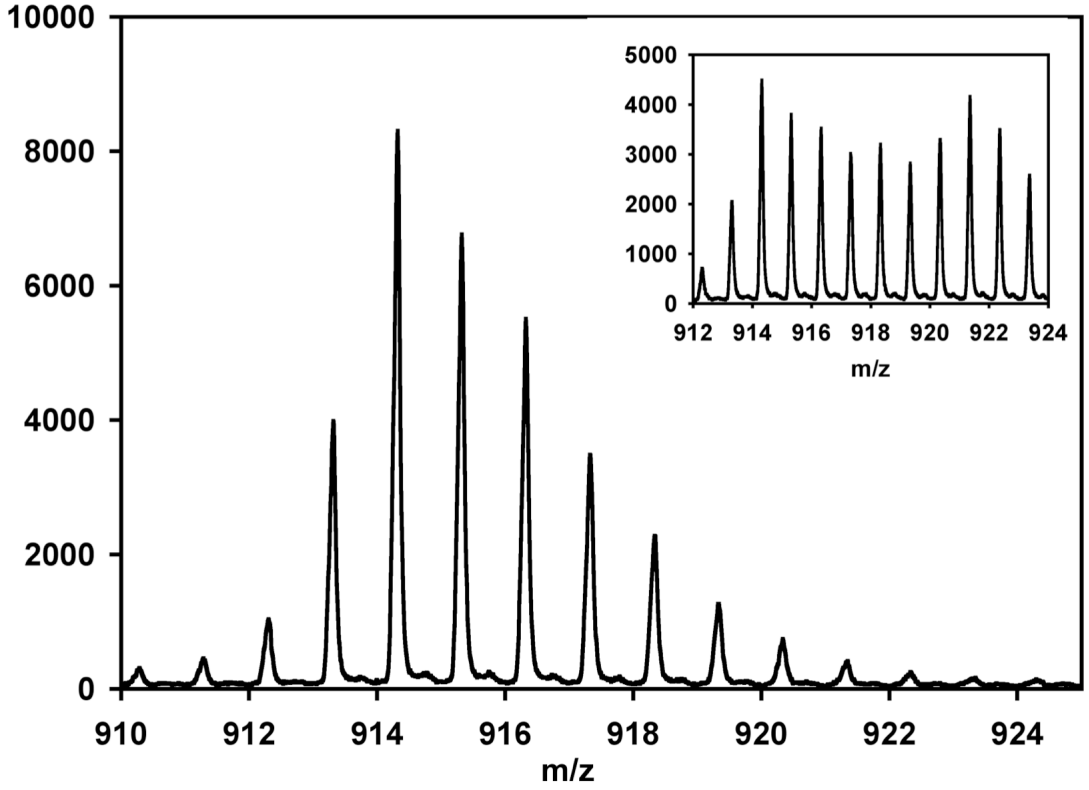

Figure S4. PIM MS spectrum of the single charged region of F_{430} in D_2O. A 20 μM solution (final concentration) of F_{430} in D_2O was prepared for analysis. Inset: MS spectrum of the single charged region of F_{330} in D_2O. 80% conversion of F_{430} to F_{330D} was achieved by reacting 42 μM F_{430} with 26 mM $NaBD_4$ in 60 mM NH_4OH.

Assignment of the one- and two-dimensional NMR spectra of F_{330}

^{1}H-^{13}C HMQC assignments: The ^{1}H-^{13}C HMQC spectrum of F_{330} is shown in Figure 7, with the assignments also included in Table 1 of the main paper. The resonances associated with the methyl group protons at C7 and C2 correlate with the carbon signals at 14.0 and 19.0 ppm respectively. The carbon signal at 38.0 ppm that correlates with two geminal proton resonances at 1.33 and 1.85 ppm is assigned to the bridging methylene carbon C5. Whereas, the 1.35 and 1.65 ppm proton signals that result from two geminal protons and correlating with the carbon signal at 21.5 ppm, has been assigned to C3a. The geminal proton signals at 1.37 and 2.43 ppm show strong cross peaks for carbon C8a at 22.5 ppm, while the proton signals at 1.75 and 2.20 ppm for C17a correlate with the carbon signal at 25.0 ppm. Similarly, the geminal proton signals at 1.68 and 2.10 ppm exhibit strong cross peaks for carbon C13a at 28.0 ppm. The remaining geminal protons with their carbon atoms attached to electron-withdrawing functional groups exhibit ^{1}H signals (and correlated carbon signals in the ^{1}H-^{13}C HMQC spectra) at 1.95, 2.18 ppm (C13b; 35.5 ppm), 2.26, 2.42 ppm (C3b; 35.0 ppm), 2.30, 2.51 ppm (C8b; 34.5 ppm), and 2.25 ppm (C12a; 40.0 ppm). The geminal methylene protons attached to a carbon atom adjacent to an amide group are most unambiguously assigned on the basis of the HMQC spectra (Figures 6 and 7). Protons attached to C7a (proton resonances at 2.25, 2.42 ppm) and C2a (proton resonances at 2.46 and 2.62 ppm) show cross peaks for their corresponding carbons near 44 ppm. The methine proton signals at 2.52, 2.63, 2.92 and 3.38 ppm correlate with the corresponding carbon signals at 49.5, 52, 50 and 49 ppm arising from C17, C8, C12 and C13 respectively. The ^{1}H NMR signal for the methine protons attached to carbons C19 and C4 that are adjacent to ring nitrogen

atoms appear at 3.55, and 4.62 ppm and correlate with carbon signals at 63 and 65 ppm, respectively.

^{1}H-^{1}H-COSY data: Direct scalar connectivities are established from 2D COSY spectra shown in Figures 8 and S5. The signal at 4.65 ppm correlates directly with protons appearing at 1.30, 2.20 and 2.52 ppm, where the later signals are due to the protons at C17b, C17a and C17 respectively. These cross coupling peaks are not observed in the COSY spectrum of F_{330D} as shown in Figure S5.

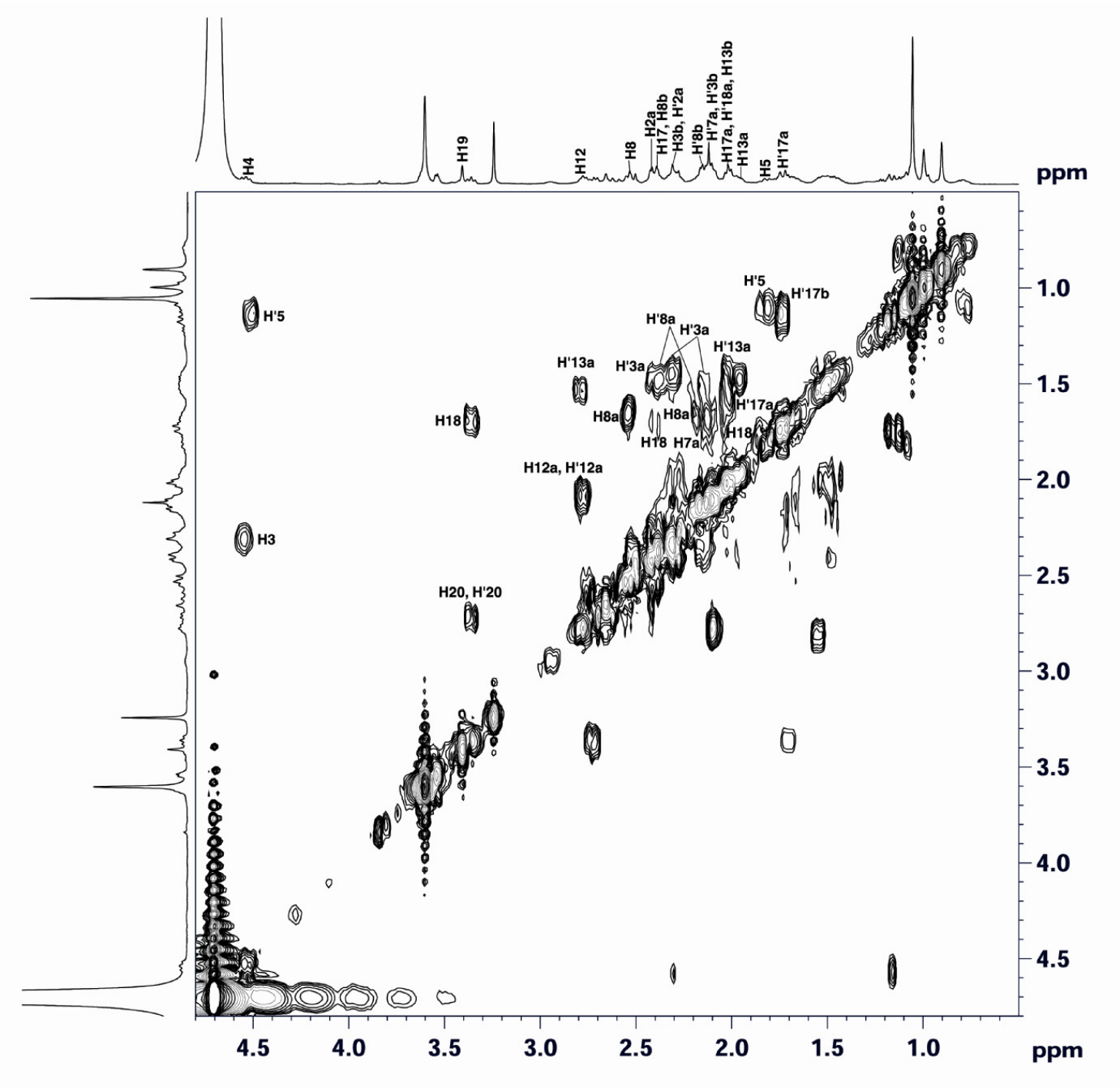

Figure S5. Two-dimensional ^{1}H-^{1}H COSY spectrum of F_{330D} recorded in D_2O. D_2O used in this experiment does not contain TSP as internal standard, therefore chemical shifts may vary slightly from the experiments reported in the main paper. (Imp= impurity)

^{1}H NMR assignments: The ^{1}H NMR spectrum of F_{330} and F_{330D} are shown in Figures S6 and S7, with the assignments shown in Table 1 of the main paper. Unlike the HMQC and the COSY spectra, the one-dimensional ^{1}H NMR spectra do not give a clear picture of the disappearance of the alcoholic (-***H***C-OH) proton at C17c upon reduction with $NaBD_4$. However, the remaining protons in both the spectra could be assigned unambiguously. The ^{1}H NMR spectra of both F330 and F330D undoubtably reveal the upfield shift of the protons attached to C17b (-***H***$_2$C-HC-OH) that are no longer under the dishielding influence of the carbonyl group (-***H***$_2$C-C=O-) in F_{430}. The ^{1}H NMR spectrum of F_{330} exhibits two singlets arising from methyl groups at around 1.02 ppm (CH_3-C(2), Figure 1) and 1.1 ppm (CH_3-C(7)). The bridged methylene group from H_2C(5) appears as an AB system with the peaks observed at 1.33 ppm and 1.85 ppm having a coupling constant (J) of about 13.0 Hz. The signal due to the methylene group at ***H***$_2$C(3a) shows an AB pattern at 1.35 and 1.65 ppm with a J value of 7.0 Hz. The methylene protons at C13a, C17a, C8a (-C***H***$_2$-CH_2-COOH), are nearly equivalent and each of these appear as pairs of doublets in the 1.37-2.2 ppm range. The chemical shifts of methylene protons directly adjacent to the carboxyl group (-CH_2-C***H***$_2$-COOH), i.e., those of C13b, C3b, C8b, and C18a, resonate as pairs of doublets between 1.95-2.51 ppm. The signals arising from the methylene protons at C7a, C2a (-***H***$_2$C-CO-NH),and C12a (-***H***$_2$C-CO-) appear as a set of doublets in the range of 2.25-2.62 ppm. The bridged methylene group at C20 exhibits an AB system with the peaks appearing at 2.75 and 2.85 ppm with a large J value of about 22 Hz, indicating that these protons are strongly coupled. The resonance due to the methine proton C18 appears relatively upfield near1.88 ppm. The angular methine (-***H***C) protons at C17, C8, C3,

C12, and C13 that constitute part of the heterocyclic ring appear as singlets in the range of 2.52-3.38 ppm. The methine protons at C19 and C4 attached to the carbon atoms that are directly attached to the heterocyclic nitrogen atoms, resonate relatively downfield at 3.55 and 4.62 ppm respectively.

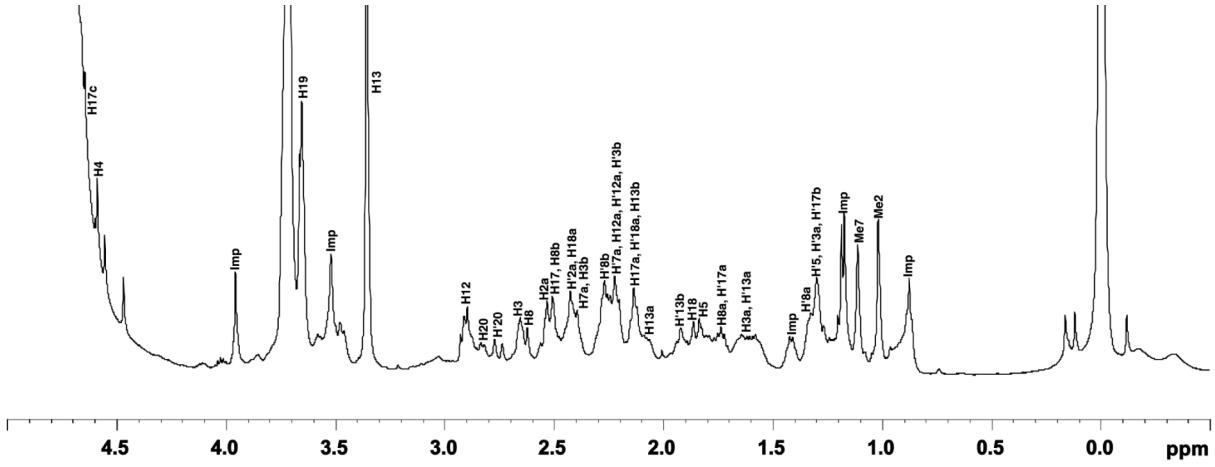

Figure S6. ^{1}H NMR spectrum of F_{330} recorded in D_2O at 25°C. (Imp= impurity).

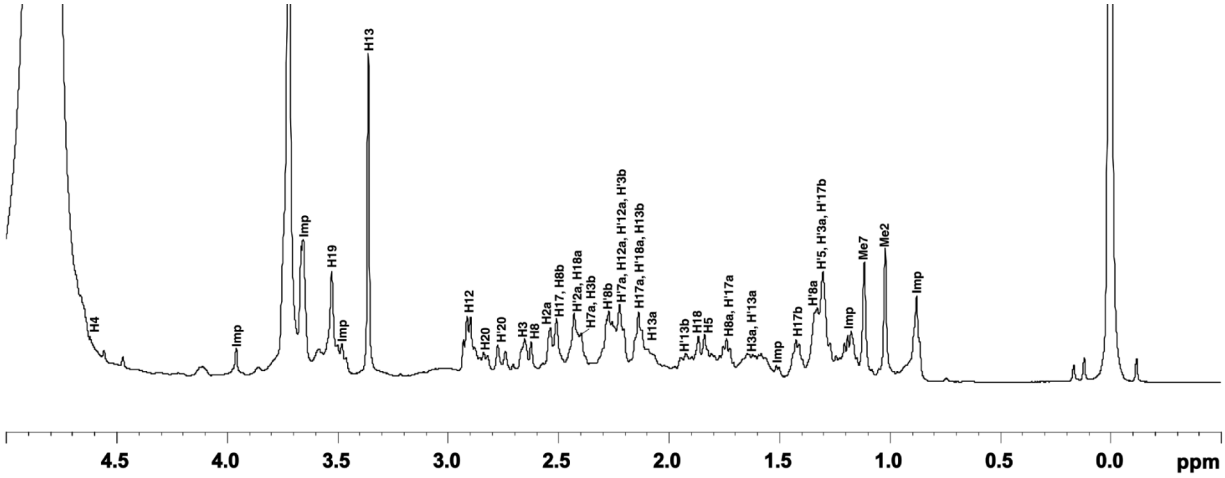

Figure S7. ^{1}H NMR spectrum of F_{330D} recorded in D_20 at 25°C. (Imp= impurity)