Supporting Information for

Stereoselective olefin cyclopropanation under aerobic conditions with an artificial enzyme incorporating an iron-chlorin e6 cofactor.

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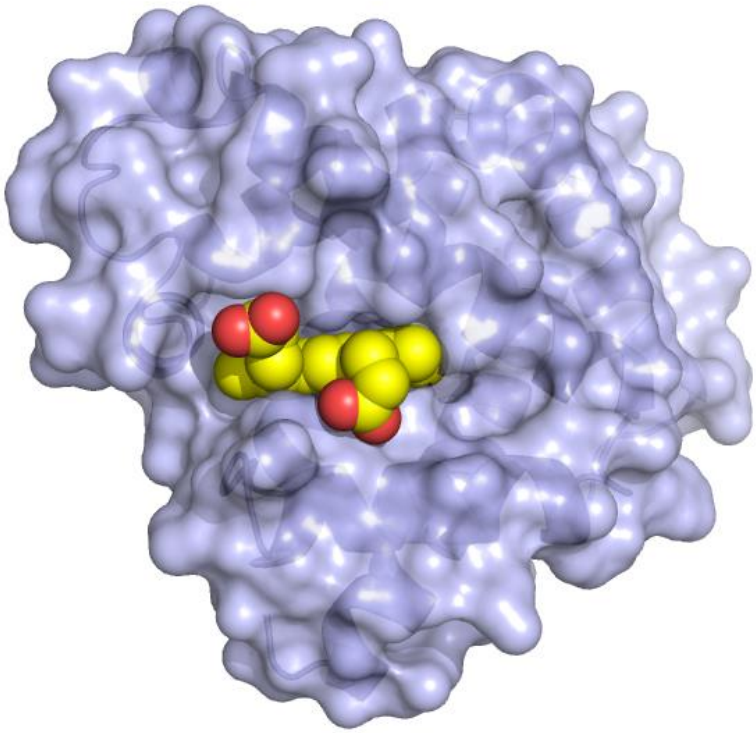
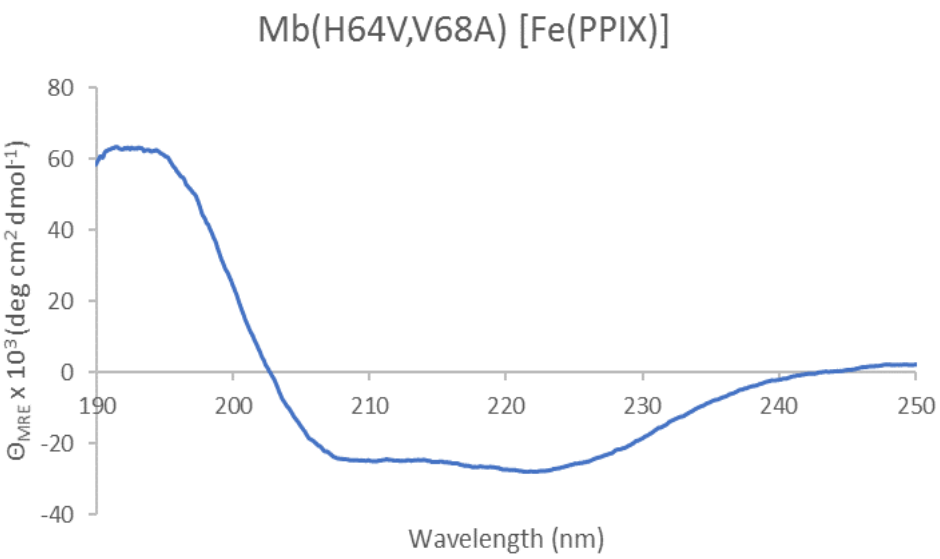


Figure S1. Surface representation of the crystal structure of sperm whale myoglobin (PDB 1A6K). The heme cofactor is shown as sphere models (yellow). In the Mb[Fe(Ce6)] complex, the Fe(Ce6) cofactor is expected to adopt an orientation along the C10-C20 axis analogous to that of the heme cofactor in Mb, whereby the propionic, acetyl, and carboxylic group appended to the tetrapyrrole ring are projected toward the solvent.

(a)



(b)

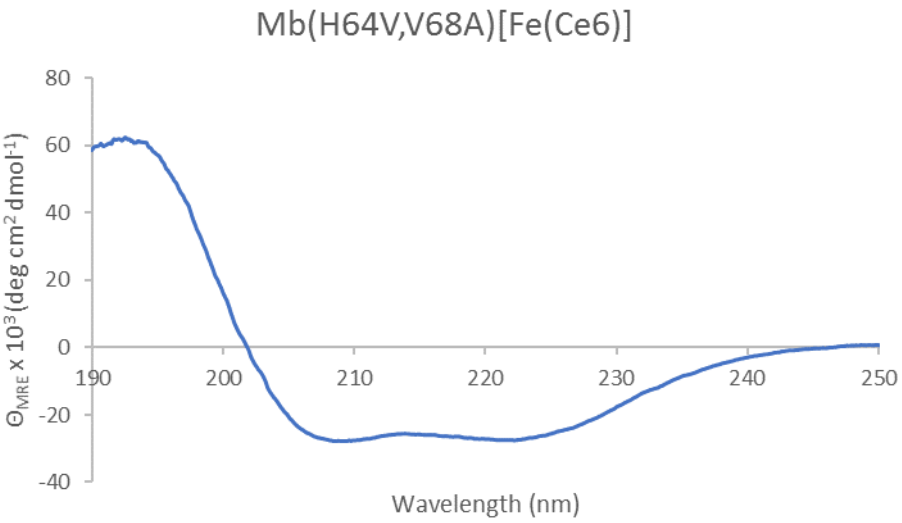
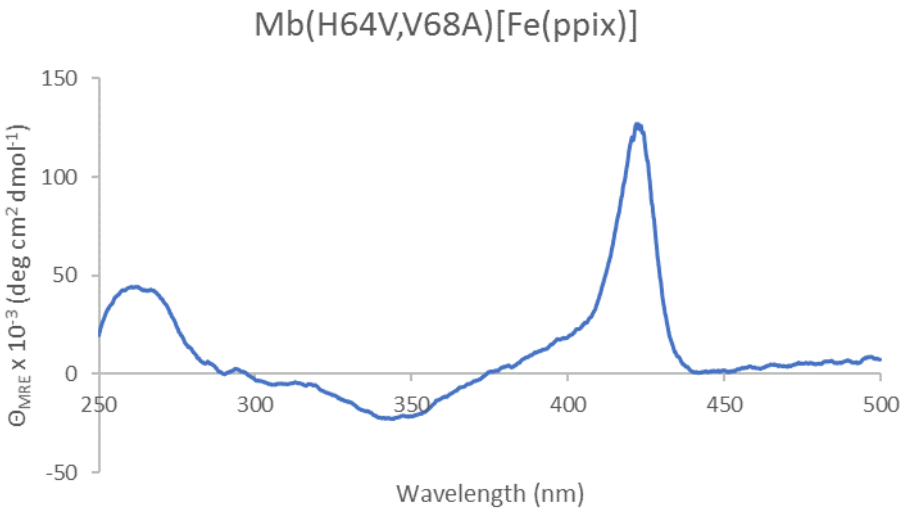


Figure S2. UV-range circular dichroism (CD) spectra corresponding to (a) Mb(H64V,V68A) and (b) Mb(H64V,V68A)[Fe(Ce6)].

(a)



(b)

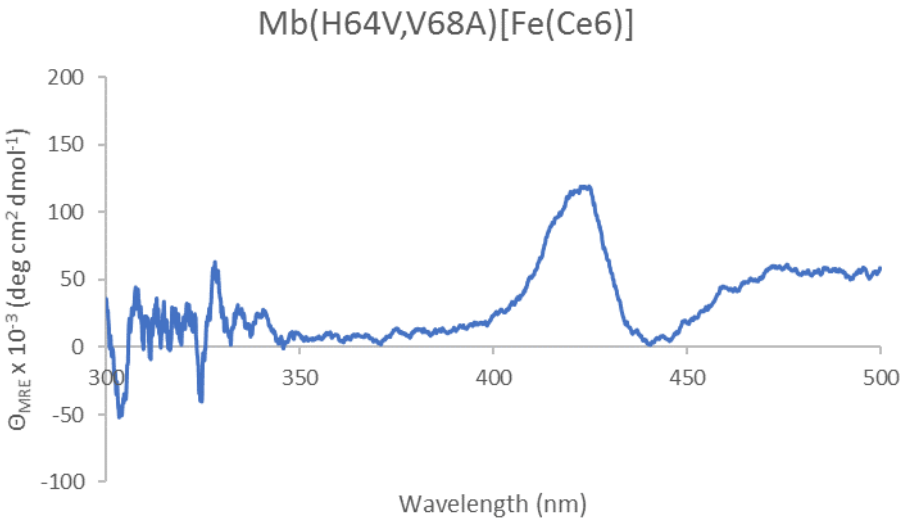
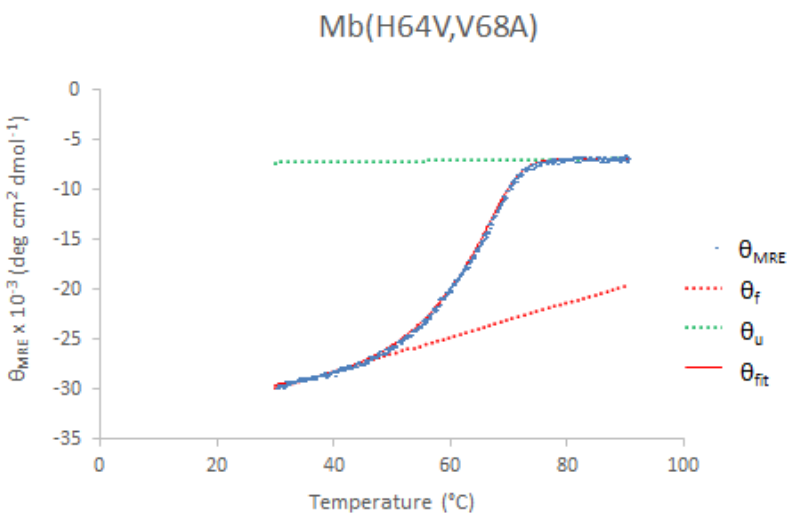


Figure S3. Visible-range circular dichroism (CD) spectra corresponding to (a) Mb(H64V,V68A) and (b) Mb(H64V,V68A)[Fe(Ce6)].

(a)



(b)

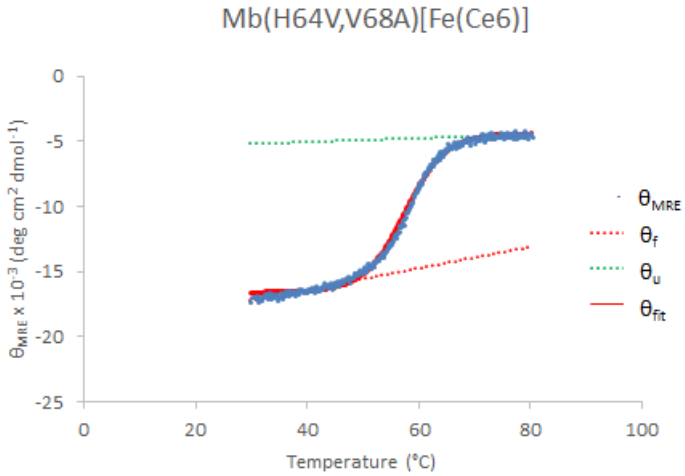
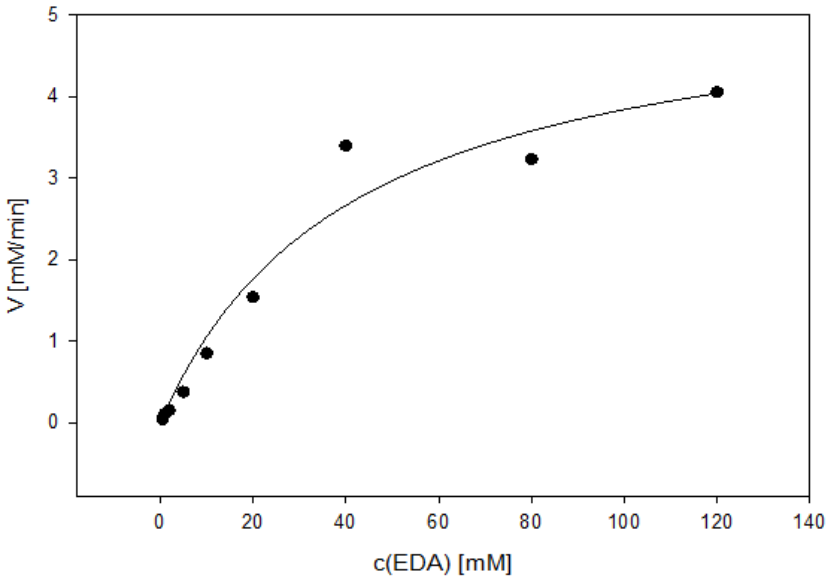


Figure S4. Thermal denaturation curves for (a) Mb(H64V,V68A) and (b) Mb(H64V,V68A)[Fe(Ce6)]. For each protein, a single set of raw data (θ_{MRE}) is shown along with extrapolated signals for folded (θ_f) and unfolded (θ_u) protein and the fitting curve (θ_{fit}).

(a) Mb(H64V,V68A):



(b) Mb(H64V,V68A)[Fe(Ce6]:

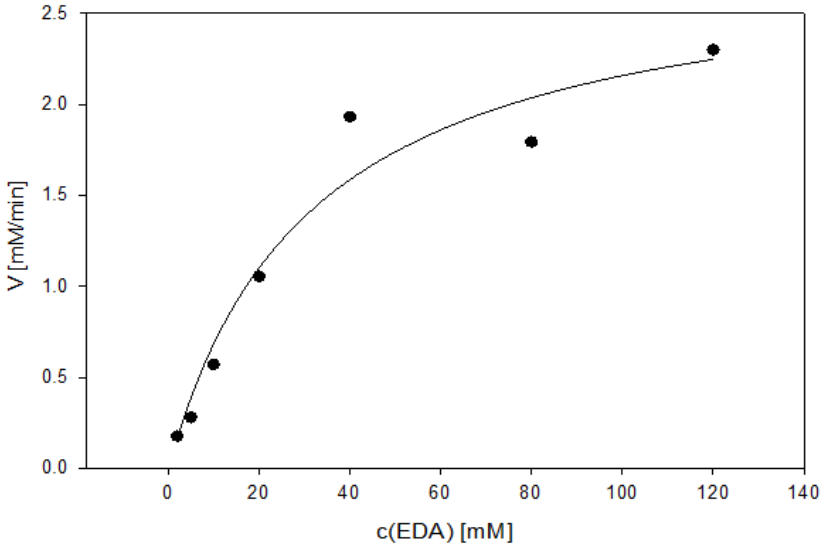


Figure S5. Michaelis-Menten curves for cyclopropanation of styrene with EDA in the presence of (a) Mb(H64V,V68A), and (b) Mb(H64V,V68A)[Fe(Ce6] as the catalyst. Reaction conditions: 1 μM Mb(H64V,V68A)[Fe(Ce6)] or 10 μM Mb(H64V,V68A), 10 mM sodium dithionite, 20 mM styrene, and EDA at varying concentrations (0.5-120 mM) in KPi buffer (50 mM, pH 7.0).

Experimental Procedures

Reagents. All the chemicals and reagents were purchased from commercial suppliers (Sigma-Aldrich, Alfa Aesar) and used without any further purification, unless otherwise stated. Chlorin e6 was purchased from Frontier Scientific. EDA was purchased from Sigma-Aldrich as 87% m/v solution in dichloromethane. All dry reactions were carried out under argon in oven-dried glassware with magnetic stirring using standard gas-tight syringes, cannulae and septa. Silica gel chromatography purifications were carried out using AMD Silica Gel 60 230-400 mesh. Thin Layer Chromatography (TLC) and preparative TLC were carried out using Merck Millipore TLC silica gel 60 F254 glass plates.

Growth Media. Cell cultures were grown in enriched M9 medium which was prepared as follows. For 1 L, 770 mL deionized H_2O was added with 200 mL M9 salts (5x) solution, 20 mL glucose (20% v/v), 10 mL casamino acids (20% m/v), 1 mL $MgSO_4$ (2 M), and 100 μL $CaCl_2$ (1 M). The M9 salts (5x) solution was prepared by dissolving 15 g Na_2HPO_4, 7.5 g K_2HPO_4, 0.3 g NaH_2PO_4, 0.3 g KH_2PO_4, 1.5 g NaCl, 5 g NH_4Cl in 2 L deionized H_2O and sterilized by autoclaving. The casamino acids and $MgSO_4$ solutions were autoclaved separately. The $CaCl_2$ and glucose stock solutions were sterilized by filtration. Enriched M9 agar plates were prepared by adding 17 g agar to 1 L of enriched M9 media containing all of the aforementioned components at the specified concentrations with the exception of glucose and $CaCl_2$, which were added immediately prior to plating. To media and plates, ampicillin was added to a final concentration of 100 mg/L and chloramphenicol was added to a final concentration of 34 mg/L.

In vitro reconstitution and recombinant expression of [Fe(Ce6)]-containing myoglobins. Wild-type Mb, Mb(H64V,V68A), Mb(H64V,V68A,H93A), and Mb(H64V,V68A,H93F) were expressed from pET22-based vectors, whose preparation was described previously.[1] All

proteins contained a C-terminal polyhistidine tag. BL21(DE3) cells were transformed with the pET22-based vector encoding for the appropriate Mb variant and the transformed cells were selected on enriched M9 agar plates containing ampicillin (100 mg L^{-1}). Single colonies were used to inoculate 5 mL of enriched M9 media supplemented with ampicillin (100 mg L^{-1}), followed by incubation at 37°C with shaking (180 rpm) for 10 to 15 hours. For in vitro reconstitution of the [Fe(Ce6)]-containing myoglobins, the overnight cultures were transferred to 1 L enriched M9 medium containing ampicillin, followed by incubation at 37°C with shaking (180 rpm). At an OD_{600} of 1.4, cells were induced with IPTG (final conc.: 0.5 mM) and incubated at 20°C with shaking (180 rpm) for 20 to 24 hours. After harvesting, the cell pellets were resuspended in 20 mL of Ni NTA Lysis Buffer (50 mM KPi, 250 mM NaCl, 10 mM histidine, pH = 8.0) and iron(Cl)-chlorin e6 was added to the cell suspension to a final concentration of 0.05 mM. Cells were lysed by sonication and the cell lysate was clarified by centrifugation (14,000 rpm, 4°C, 30 min).

For the recombinant expression of the [Fe(Ce6)]-containing myoglobins, overnight cultures of C41(DE3) cells containing the appropriate pET22-based expression vector were transferred to 1 L enriched M9 medium containing ampicillin, followed by incubation at 37°C with shaking (180 rpm). At an OD_{600} of 1.4, cells were condensed (5x) by centrifugation and resuspension in 200 mL of enriched M9 medium. The resulting cell culture was incubated with iron(Cl)-chlorin e6 (final concentration: 30 mg/L) and then induced with IPTG (final conc.: 0.5 mM). Cells were incubated at 20°C with shaking (180 rpm) for 20 to 24 hours. After harvesting, the cell pellets were resuspended in 20 mL of Ni NTA Lysis Buffer (50 mM KPi, 250 mM NaCl, 10 mM histidine, pH = 8.0) and lysed by sonication, and the cell lysate was clarified by centrifugation (14,000 rpm, 4°C, 30 min).

Protein Purification. The clarified lysate was transferred to a Ni-NTA column equilibrated with Ni-NTA Lysis Buffer. The resin was washed with 50 mL of Ni-NTA Lysis Buffer and

then 50 mL of Ni-NTA Wash Buffer (50 mM KPi, 250 mM NaCl, 20 mM Histidine, pH = 8.0). Proteins were eluted with Ni-NTA Elution Buffer (50 mM KPi, 250 mM NaCl, 250 mM Histidine, pH = 7.0). After elution from the Ni-NTA column, the protein was buffer exchanged against 50 mM KPi buffer (pH 7.0) using 10 kDa Centricon filters. The concentration of the Mb variants (ferric form) was calculated using an extinction coefficient of ε_{414} = 188 mM^{-1} cm^{-1}, which was determined using the pyridine hemochrome assay[2] with Mb[Fe(Ce6)] and [Fe(Ce6)] as reference.

CD analyses and T_m determination. Far UV CD spectra (250-190 nm) were obtained using 3 μM solutions of purified Mb variant in 50 mM potassium phosphate buffer (pH 7.0) and recorded at 20°C at a scan rate of 50 nm/min with a bandwidth of 1 nm and an averaging time of 10 seconds per measurement. Visible range CD spectra (500-300 nm) were obtained using a 100 μM solution of purified Mb(H64V,V68A) and a 25 μM solution of purified Mb(H64V,V68A)[Fe(Ce6)] in 50 mM potassium phosphate buffer (pH 7.0). For these experiments, the proteins were reduced with dithionite and exposed to CO for 1 minute prior to recording the CD spectra. Thermal denaturation experiments were carried out using a JASCO J-1100 CD spectrophotometer equipped with variable temperature/wavelength denaturation analysis software and samples of purified Mb variant at 3 μM in 50 mM potassium phosphate buffer (pH 7.0). Thermal denaturation curves were measured by monitoring the change in molar ellipticity at 222 nm (θ_{222}) over a temperature range from 20°C to 100°C. The temperature increase was set to 0.5°C per minute with an equilibration time of 10 seconds. Data integration time for the melt curve was set to 4 seconds with a bandwidth of 1 nm. Linear baselines for the folded (θ_f) and unfolded state (θ_u) were generated using the low temperature ($\theta_f = m_f T + b_f$) and high temperature ($\theta_u = m_u T + b_u$) equations fitted to the experimental data before and after global unfolding, respectively (**Figure S2**).

Using these equations, the melt data were converted to fraction of folded protein (F_f) vs. temperature plots and the resulting curve was fitted to a sigmoidal equation (θ_{fit}) via non-linear regression analysis in SigmaPlot (**Figure 1c**), from which apparent melting temperatures were derived. The reported mean values and standard errors were derived from experiments performed at least in duplicate.

Anaerobic and Aerobic Cyclopropanation Reactions. Reactions with styrene and EDA were carried out as described previously.[1a] Briefly, under standard reaction conditions, 400 μL-scale reactions were carried out using 10 μM or 20 μM Mb variant, 10 mM styrene, 20 mM EDA and 10 mM sodium dithionite. In a typical procedure, a solution containing sodium dithionite (100 mM stock solution) in potassium phosphate buffer (50 mM, pH 7.0) was degassed by bubbling argon into the mixture for 3 min in a sealed vial. A buffered solution containing the myoglobin variant was carefully degassed in a similar manner in a separate vial. The two solutions were then mixed together via cannula. Reactions were initiated by addition of 10 μL of styrene (from a 0.4 M stock solution in ethanol), followed by the addition of 10 μL of EDA (from a 0.8 M stock solution in ethanol) with a syringe, and the reaction mixture was stirred for 12 hours at room temperature, under positive argon pressure. Aerobic reactions were carried out without degassing the solution with argon and in open reaction vessels. Reactions without reductant were performed in a similar manner without the addition of sodium dithionite. Reactions with the Fe(Ce6) cofactor were performed in a similar manner as mentioned above.

Whole-cell cyclopropanation reaction. *E. coli* C41(DE3) cells expressing the Mb(H64V,V68A)[Fe(Ce6)] variant were prepared according to the protocol described above. After harvesting, the cells were suspended in phosphate buffer (KPi pH 7.2) and diluted to an OD_{600} of 30. The cell suspension was transferred to an open Erlenmeyer flask equipped with a

stir bar and supplemented with 2 mL of a 50 mM $_{D}$-glucose solution (from a 2 M stock solution). Reaction was initiated by addition of 100 mg of styrene (indicated amount dissolved in 1/100 of total reaction volume of ethanol) in one portion, followed by the addition of 120 μL of EDA (indicated amount dissolved in 1/100 of total reaction volume of ethanol) with a syringe pump over a period of 2 hours at room temperature. Reaction mixtures were stirred at room temperature for 14-16 hours and extracted with ethyl acetate (100 mL x 3). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel) with 5-10% diethyl ether/hexanes as the eluent to afford 170 mg of (1*S*,2*S*)-ethyl 2-phenylcyclopropanecarboxylate (**5**) (93% isolated yield) in 96% *de* and 90% *ee*.

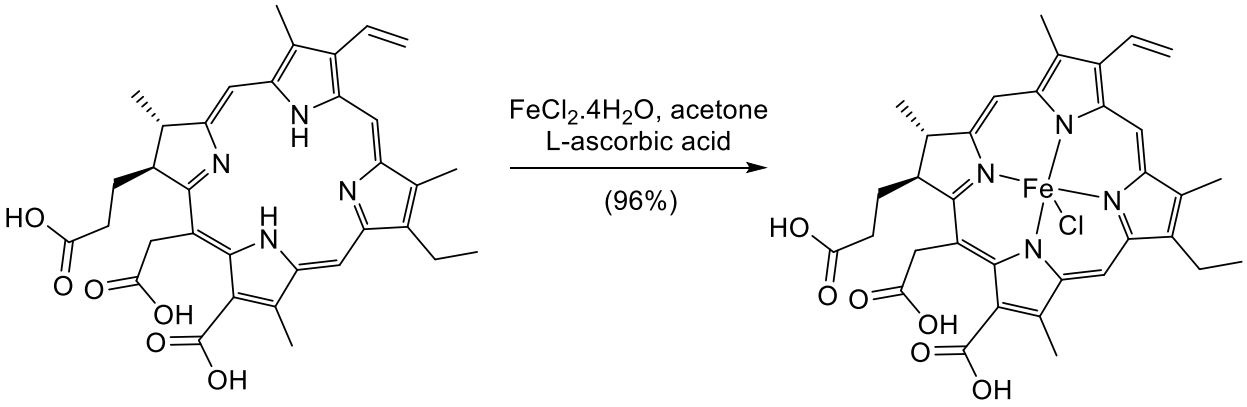
Product analysis: Cyclopropanation reactions were analyzed by adding 20 μL of internal standard (benzodioxole, 50 mM in ethanol) to the reaction mixture, followed by extraction with 400 μL of dichloromethane and analysis by gas chromatography (GC). Gas chromatography (GC) analyses were carried out using a Shimadzu GC-2010 gas chromatograph equipped with a FID detector and a Chiral Cyclosil-B column (30 m x 0.25 mm x 0.25 μm film). Separation method for cyclopropanation reaction: 1 μL injection, injector temp.: 200 ºC, detector temp: 300 ºC. Gradient: column temperature set at 120 ºC for 3 min, then to 150 ºC at 0.8 ºC/min, then to 245^{o}C at 25 ºC/min. Total run time was 46.30 min. Calibration curves for quantification of the cyclopropanation products were constructed using authentic (racemic) standards prepared synthetically or enzymatically as described previously.[3] All measurements were performed at least in duplicate.

Kinetic (Michaelis-Menten) analyses. Reactions were carried out on a 400 μL scale using the enzyme at a fixed concentration (Mb(H64V,V68A)[Fe(Ce6)]: 1 μM;

Mb(H64V,V68A)[Fe(ppIX)]: 10 μM), 10 mM sodium dithionite, 20 mM styrene, and EDA at varying concentrations (0.5, 1, 2, 5, 10, 20, 40, 80, 120 mM) in KPi buffer (50 mM, pH 7.0). Initial velocity (V) was measured based on the amount of Ethyl 2-phenylcyclopropane-1-carboxylate (**5**) formed after 30 seconds, at which point the reaction was quenched by the addition of 100 μL of 6 M guanidinium hydrochloride in 2 M HCl and immediately extracted with 400 μL of dichloromethane containing 20 μL of benzodioxole as internal standard. The kinetic parameters V_{max}, k_{cat}, and K_M were obtained by fitting the resulting plot of initial velocity (V) vs. substrate concentrations (c(EDA)) to the Michaelis-Menten equation using SigmaPlot software. Experiments were performed in triplicate and a representative curve is shown in **Figure S5**.

Synthetic Procedures:

Synthesis of Fe(Cl)-chlorin e6 (2).

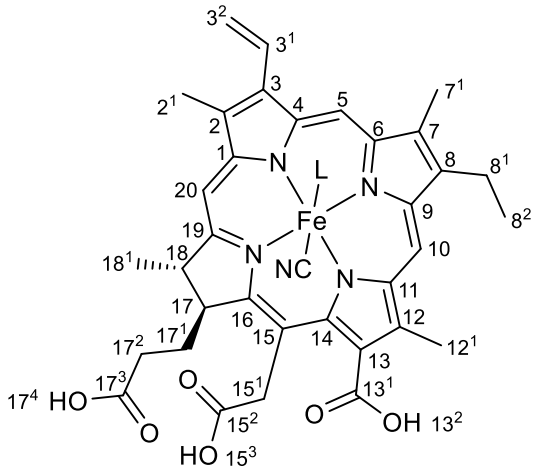


Chlorin e6 (**1**) Fe(Cl)-chlorin e6 (**2**)

Chlorin e6 (100 mg) was dissolved in acetone (100 mL) and to the solution (+)-ascorbic acid (360 mg) in acetone (100 mL) and Fe(II)$Cl_2.4H_2O$ were added. The reaction mixture was stirred at 60°C for 4 hrs. After cooling to room temperature, the reaction mixture was added with 100 mL dichloromethane and washed with saturated brine solution (3 x 100 mL), then with 100 mL of 0.01 M HCl solution, and then with water (200 mL). The organic layer was dried and evaporated to yield Fe(Cl) chlorin e6 as a dark green powder (113 mg, 96%). All the processes were done in the dark and under argon atmosphere. LC-MS: *m/z* ($[M - Cl]^+$) calc. 650.5; obs. 650.0. UV-Vis (ε M^{-1} cm^{-1}): 395 nm (33,840), 603 nm (5,900), 684 nm (7,700).

For NMR characterization, 20 mg of the complex were dissolved in a minimal amount of MeOH (0.5-1 mL) and diluted with 3 mL 0.1 M sodium phosphate buffer (pH 5). Slow acidification with 0.5 M HCl resulted in precipitation of a dark green solid, which was centrifuged and the light green supernatant decanted. The precipitate was washed twice with 6 mL water and dried *in vacuo*. Fe(Cl)-chlorin e6 was converted into the corresponding biscyano complex according to a modified procedure for porphyrin iron(III) chlorides.[4] The complex was dissolved in MeOH-d_4 (0.05 M solution) and subsequently treated with 5

equivalents of potassium cyanide. The mixture was incubated for 30 min, sonicated and centrifuged prior to NMR analysis. The NMR spectrum showed the occurrence of ligand exchange with the solvent to give two Fe(CN)(MeOH)-chlorin e6 complexes, i.e., with the methanol ligand residing on either side of the chlorin e6 plane, in addition to the *bis*-cyano complex ($Fe(CN)_2$-chlorin e6). Indicative of the mixed ligand complexes were two sets of downfield signals (-0.33/-0.12 ppm and -4.07/-4.20 ppm, each with 3:1 rel. int.), which were attributed to the metal-bound methanol from the two cyano/methanol complexes. This assignment was confirmed by H/D exchange experiments, which showed disappearance of the signals at -0.33 and -4.20 ppm upon addition of D_2O (see insert in ^{1}H NMR spectrum). The ligand-exchanged complexes likely account for the downfield signals (>180 ppm) observed in the ^{13}C NMR spectrum.

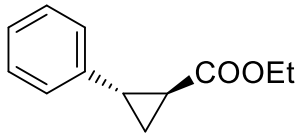


^{1}H NMR (500 MHz, MeOH-*d4*) δ 11.71 (s, 2H), 9.71 (s, 1H, H-10), 9.60 (s, 1H, H-5), 9.04 (s, 1H, H-20), 8.85 (dd, *J* = 17.5, 11.5 Hz, 1H, H-3^1), 6.29 (d, *J* = 17.5 Hz, 1H, H-3^2), 6.06 (d, *J* = 11.5 Hz, H-3^2, 1H,), 5.57 (d, *J* = 18.7 Hz, 1H, H-15^1), 5.27 (d, *J* = 18.5 Hz, 1H, H-15^1), 4.65 (q, *J* = 7.0 Hz, 1H, H-18), 4.55 (d, *J* = 10.0 Hz, 1H, H-17), 3.80 (q, *J* = 7.0 Hz, 2H, H-8^1), 3.58 (s, 3H, H-12^1), 3. 79 (s, 3H, H-2^1), 3.24 (s, 3H, H-7^1), 2.57 (m, 1H, H-17^1), 2.36 (m, 2H, H-17^2), 2.22 (m, 1H, H-17^1), 1.76 (t, *J* = 7.0 Hz, 3H, H-8^2), 1.74 (s, 3H, H-18^1), -0.12 (s,

3H, C*H*$_3$OH), -0.33 (br, 1H, CH$_3$O*H*), -4.07 (s, 3H, C*H*$_3$OH), -4.20 (br, 1H, CH$_3$O*H*). ^{13}C NMR (125 MHz, MeOH-*d4*) δ 182.2, 181.6, 180.4, 177.7, 170.9, 169.6, 159.8, 153.6, 150.4, 144.9, 138.3, 137.1, 136.9, 134.6, 134.2, 133.5, 132.5, 130.8, 130.1, 121.4, 108.9, 100.0, 99.0, 95.0, 55.6, 42.6, 36.5, 32.9, 24.0, 20.6, 20.4, 18.1, 13.4, 12.2, 12.1, 11.3, 4.9.

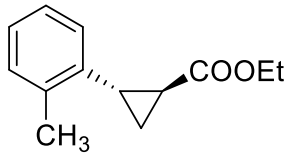
Synthesis of olefin substrates and cyclopropanation products. Compound **16a** and **17a** were synthesized as described in Bajaj *et al.*.[3] Authentic standards for **5** and **6b**-**17b** were prepared as described previously.[1a,3]

Ethyl 2-phenylcyclopropane-1-carboxylate (5):



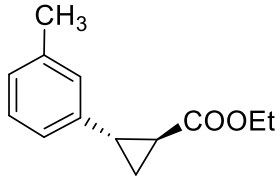
GC-MS m/z (% relative intensity): 190(29.5), 144(28.3), 135(20.7), 117(100), 107(7.3); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.34-7.28 (m, 2H), 7.23-7.20 (m, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 2.56-2.51 (m, 1H), 1.94-1.90 (m, 1H), 1.64-1.59 (m, 1H), 1.35-1.30 (4H) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz): δ 173.2, 140.0, 128.3, 126.3, 126.0, 60.6, 26.1, 24.1, 17.0, 14.2 ppm.

Ethyl 2-(o-tolyl)cyclopropane-1-carboxylate (6b):



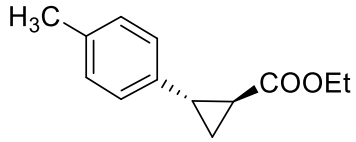
GC-MS m/z (% relative intensity): 204(29.13), 158(16.7), 147(16.7), 131(100), 91(28.30). ^{1}H NMR (CDCl$_3$, 400 MHz): δ 7.16 (m, 3H), 7-01-6.99 (m, 1H), 4.23 (q, *J* = 7.6 Hz, 2H), 2.55-2.49 (m, 1H), 2.38 (s, 3H), 1.82-1.76 (m, 1H), 1.60-1.55 (m, 1H), 1.31-1.28 (m, 4H) ppm, ^{13}C NMR (CDCl$_3$, 100 MHz): δ 173.8, 138.0, 137.8, 129.8, 126.7, 125.8, 60.6, 24.6, 22.3, 19.5, 15.3, 14.3 ppm.

Ethyl 2-(m-tolyl)cyclopropane-1-carboxylate (7b):



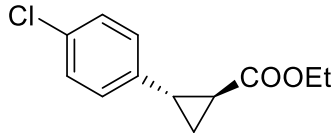
GC-MS m/z (% relative intensity): 204(20.9), 158(16.1), 147(13.7), 131(100), 115(21.2). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.19-7.16 (m, 1H), 7.03-7.01 (m, 1H), 6.93-6.89 (m, 2H), 4.20 (q, *J* = 6.8 Hz, 2H), 2.50-2.49 (m, 1H), 2.33 (s, 3H), 1.91-1.88 (m, 1H), 1.60-1.57 (m, 1H), 1.31-1.27 (m, 4H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.4, 140.0, 138.0, 128.3, 127.2, 127.0, 123.1 60.6, 26.1, 24.1, 21.3, 16.9, 14.3 ppm.

Ethyl 2-(*p*-tolyl)cyclopropane-1-carboxylate (8b):



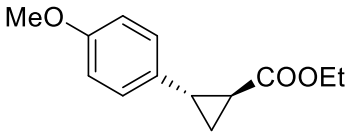
GC-MS m/z (% relative intensity): 204(28.4), 158(19.7), 147(21.1), 131(100), 91(28.1); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.11 (d, *J* = 7.6 Hz, 2H), 7.01 (d, *J* = 7.6 Hz, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 2.53-2.48 (m, 1H), 2.32 (s, 3H), 1.90-1.85 (m, 1H), 1.61-1.56 (m, 1H), 1.31-1.27 (m, 4H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.5, 137.0, 136.0, 129.1, 126.1, 60.6, 25.9, 24.0, 20.9, 16.9, 14.2 ppm.

Ethyl 2-(4-chlorophenyl)cyclopropane-1-carboxylate (9b):



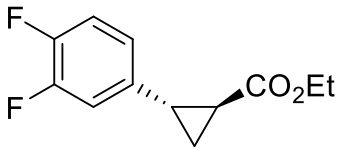
Following the standard procedure, yield = 82%, GC-MS m/z (% relative intensity): 224(38.9), 178(20.9), 151(100), 115(98.9), 89(14.9), *E-isomers*: colorless liquid, ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.24 (d, *J* = 7.6 Hz, 2H), 7.02 (d, *J* = 7.2 Hz, 2H), 4.19 (q, *J* = 6.8 Hz, 2H), 2.50-2.45 (m, 1H), 1.87-1.83 (m, 1H), 1.61-1.56 (m, 1H), 1.29-1.23 (m, 4H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.1, 138.6, 132.1, 128.5, 127.5, 60.8, 25.4, 24.1, 16.9, 14.2 ppm.

Ethyl 2-(4-methoxyphenyl)cyclopropane-1-carboxylate (10b):



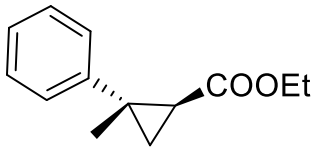
GC-MS m/z (% relative intensity): 220(41.8), 191(14.5), 147(100), 91(32.6). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.03(d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 8.4 Hz, 2H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 2.49-2.45 (m, 1H), 1.83-1.79 (m, 1H), 1.56-1.52 (m, 1H), 1.32-1.21 (m, 4H), ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.5, 158.3, 132.1, 127.5, 113.9, 60.6, 55.2, 25.6, 23.8, 16.7, 14.2 ppm.

Ethyl 2-(3,4-difluorophenyl)cyclopropane-1-carboxylate (11b):



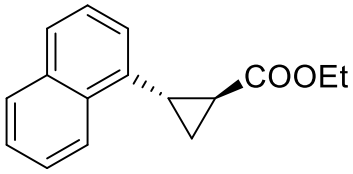
GC-MS m/z (% relative intensity): 226(56.1), 198(18.3), 181(25.7), 153(100), 133(53.3), 125(39.1), 101(6.8); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.04-6.98 (m, 1H), 6.87-6.78 (m, 2H), 4.13 (q, *J* = 7.2 Hz, 2H), 2.46-2.41 (m, 1 H), 1.82-1.78 (m, 1H), 1.57-1.52 (m, 1H), 1.24 (t, *J* = 6.8 Hz, 3H), 1.21-1.17 (m, 1H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.7, 151.4, 151.3, 150.2, 150.1, 148.9, 148.8, 147.8, 147.7, 137.0, 122.2, 177.1, 116.9, 115.1, 114.9, 60.7, 24.9, 23.9, 16.7, 14.1 ppm.

Ethyl 2-methyl-2-phenylcyclopropane-1-carboxylate (12b):



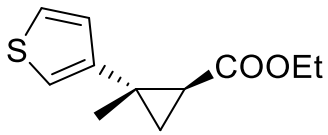
GC-MS m/z (% relative intensity): 204(4.18), 175(9.7), 159(15.19), 147(13.9), 131(100), 91(41.1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30 (m, 4H), 7.22-7.20 (m, 1H), 4.23 (q, *J* = 6.2 Hz, 2H), 1.99-1.96 (m, 1H), 1.54 (s, 3H), 1.46-1.40 (m, 2H), 1.32-1.29 (m, 3H) ppm, ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.1, 145.9, 128.4, 127.3, 126.4, 60.4, 30.5, 27.8, 20.7, 19.6, 14.4 ppm.

Ethyl 2-(naphthalen-1-yl) cyclopropane-1-carboxylate (13b):



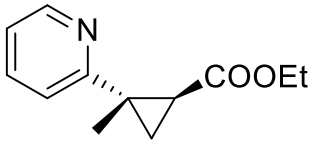
GC-MS m/z (% relative intensity): 240(34.3), 167(100), 152(54.1), 115(7.2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.22 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.61-7.52 (m, 2H), 7.41 (t, *J* = 8.0 Hz, 1H), 7.29 (d, *J* = 8 Hz, 1H), 4.32-4.26 (m, 2H), 3.06-3.01 (m, 1H), 1.99-1.95 (m, 1H), 1.77-1.72 (m, 1H), 1.48-1.43 (m, 1H), 1.37 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.0, 138.1, 135.9, 133.7, 137.7, 133.1, 128.7, 127.7, 126.4, 126.0, 125.5, 124.2, 60.9, 24.2, 22.4, 15.6, 14.5 ppm.

Ethyl 2-methyl-2-(thiophen-3-yl)cyclopropane-1-carboxylate (14b):



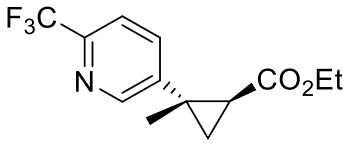
GC-MS m/z (% relative intensity): 210(24.6), 165(14.6), 137(100), 103(6.9); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.22-7.20 (m, 1H), 6.98-6.97 (m, 1H), 6.84 (d, *J* = 4.8 Hz, 1H), 1.94 (dd, *J* = 8.4, 6.4 Hz, 1H), 1.54 (s, 3H), 1.46-1.44 (m, 1H), 1.39-1.36 (m, 1H), 1.24 (t, *J* = 7.2 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.5, 146.9, 125.7, 125.3, 119.3, 60.4, 29.1, 25.9, 22.1, 17.8, 14.2 ppm.

Ethyl 2-methyl-2-(pyridin-2-yl)cyclopropane-1-carboxylate (15b):



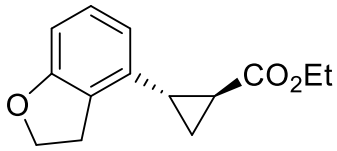
GC-MS m/z (% relative intensity): 205(25.1), 176(43.4), 132(100), 117(68.7), 104(9.2); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 68.48 (d, *J* = 4.0 Hz, 1H), 7.62-7.59 (m, 1H), 7.37 (d, *J* = 8.0 Hz, 1H), 7.08 (dd, *J* = 8.0, 4.0 Hz 1H), 4.19-4.13 (m, 2H), 2.39-2.37(m, 1H), 1.80-1.78 (m, 1H), 1.63 (s, 3H), 1.49-1.47 (m, 1H), 1.26 (t, *J* = 8.0 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.9, 162.4, 149.0, 136.1, 120.9, 120.2, 60.5, 29.9, 29.3, 22.5, 15.7, 14.4 ppm.

Ethyl 2-methyl-2-(6-(trifluoromethyl)pyridin-3-yl)cyclopropane-1-carboxylate (16b):



GC-MS m/z (% relative intensity): 273(20.8), 244(76.4), 228(43.3), 200(100), 180(68.3), 130(20.7); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.63 (d, *J* =1, Hz, 1H), 7.74 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 4.21-4.15 (m, 2H), 1.96 (dd, *J* = 8.5, 6.0 Hz, 1H), 1.52 (s, 3H), 1.44 (dd, *J* = 8.5, 5.5 Hz, 3H), 1.27 (t, *J* = 7.5 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.1, 149.3, 146.7, 146.4, 146.1, 145.9, 144.4, 136.1, 124.8, 122.6, 120.4, 120.2, 120.1, 60.9, 27.7, 20.3, 19.1, 14.3 ppm.

Ethyl 2-(2,3-dihydrobenzofuran-4-yl)cyclopropane-1-carboxylate (17b):



GC-MS m/z (% relative intensity): 232(100), 159(90.1), 144(68.9), 132(70.2), 115(43.5), 101(16.7); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.04 (t, *J* = 8.0 Hz, 1H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.41 (d, *J* = 8.0 Hz, 1H), 4.59 (t, *J* = 8.4 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.24 (t, *J* = 8.4 Hz, 2H), 32.44-2.39 (m, 1H), 1.93-1.88 (m, 1H), 1.60-1.56 (m, 1H), 1.32 (t, *J* = 4.4 Hz, 3H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.4, 159.7, 136.5, 128.1, 126.4, 116.0, 107.4, 70.9, 60.6, 28.4, 23.8, 22.7, 15.9, 14.1 ppm.

References

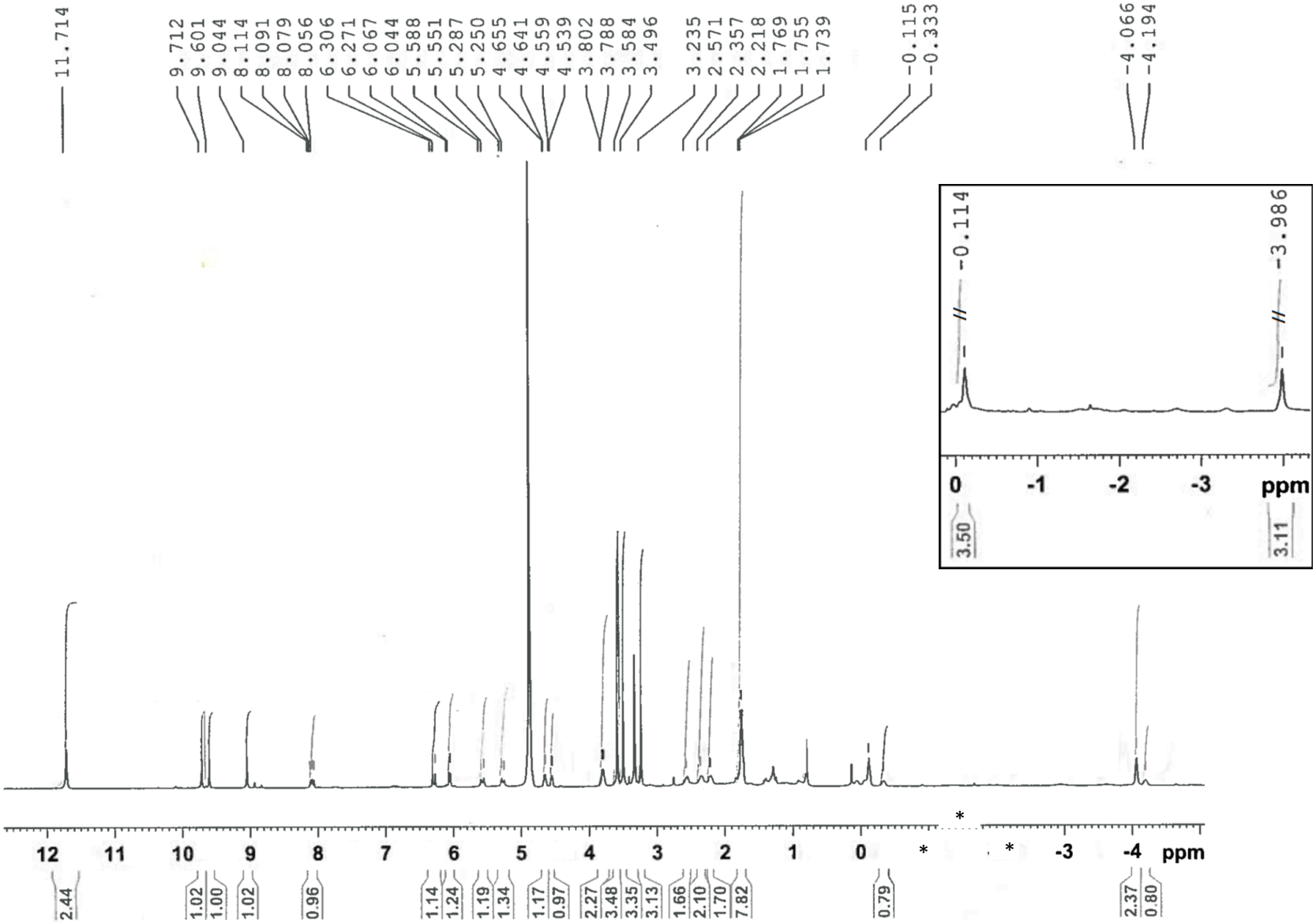
(1) (a) Bordeaux, M.; Tyagi, V.; Fasan, R. *Angew. Chem. Int. Ed.* **2015**, *54*, 1744; (b) Sreenilayam, G.; Moore, E. J.; Steck, V.; Fasan, R. *Adv. Synth. Cat.* **2017**, *359*, 2076.

(2) Berry, E. A.; Trumpower, B. L. *Analyt. Biochem.* **1987**, *161*, 1.

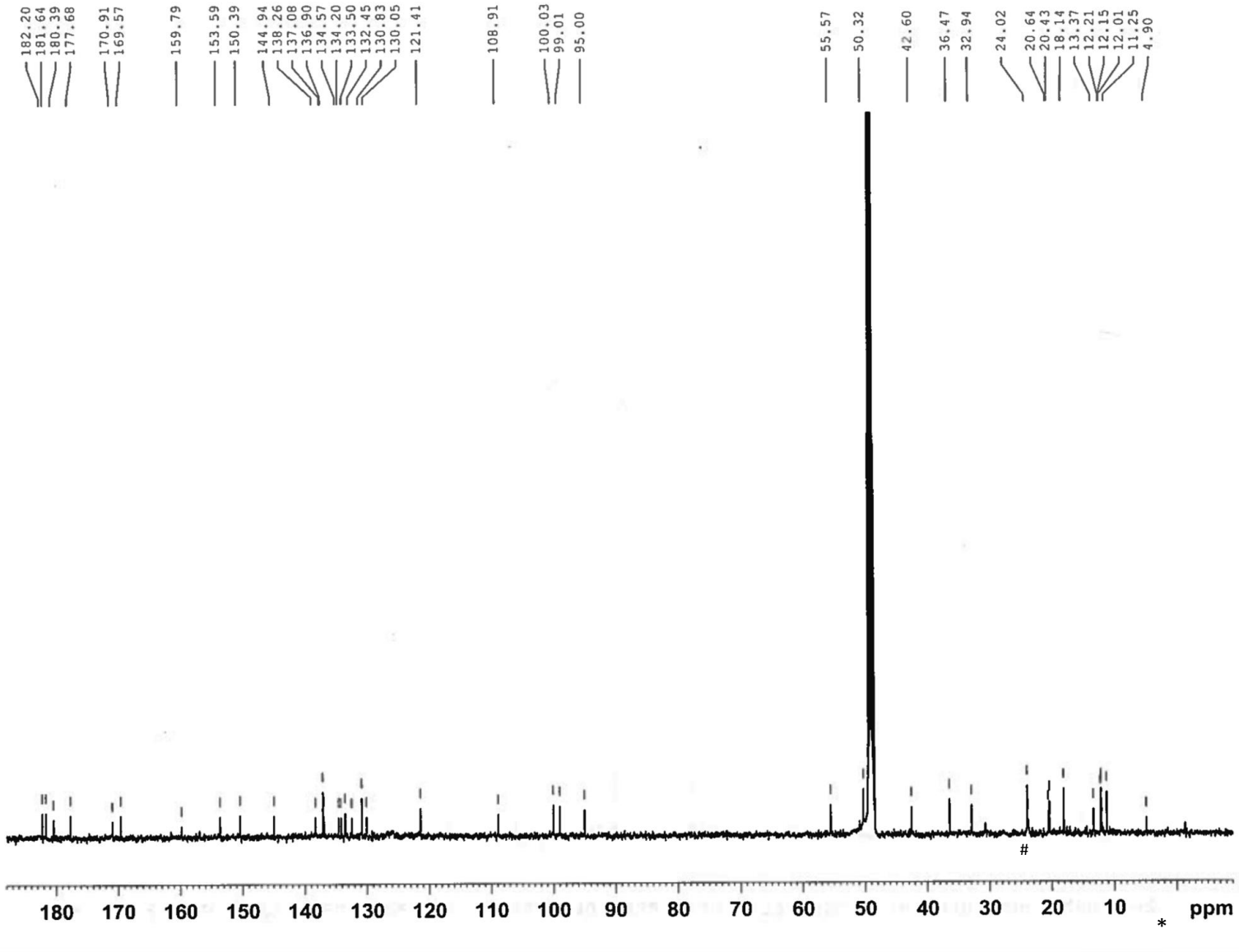
(3) Bajaj, P.; Sreenilayam, G.; Tyagi, V.; Fasan, R. *Angew. Chem. Int. Ed.* **2016**, *55*, 16110.

(4) La Mar, G. N.; Viscio, D. B.; Smith, K. M.; Caughey, W.; Smith, M. L. *J. Am. Chem. Soc.* **1978**, *100*, 8085.

^{1}H NMR of $Fe(CN)_2$-chlorin e6 complex in MeOH-d_4. Insert: Upfield region after addition of D_2O (30 μL). * = grease



^{13}C NMR of Fe(CN)$_2$-chlorin e6 complex in MeOH-d_4. * = grease; # = MeOH.



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