Supporting Information

Model Studies for the Mechanism of Heme Oxygenase-Catalyzed Porphyrin Meso Hydroxylation

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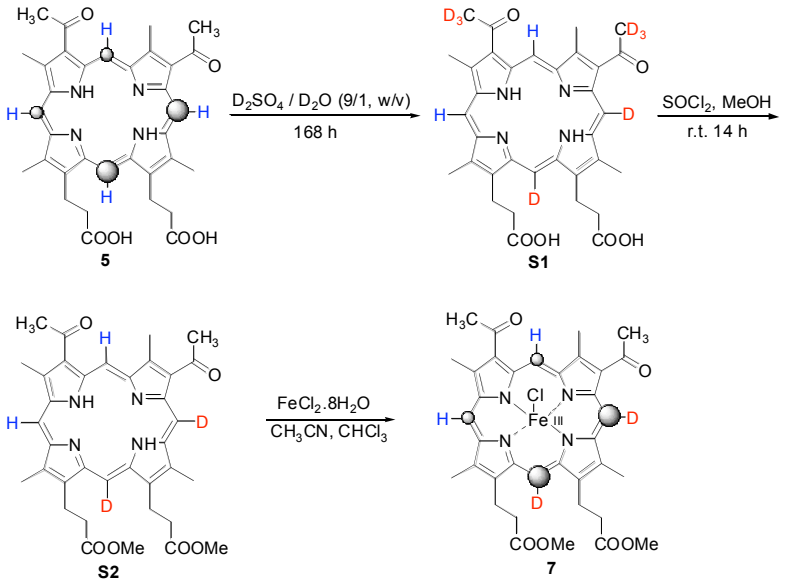
General Methods and Reagents. All NMR spectra were recorded on an Inova 500 spectrometer. Chemical shifts (δ) are reported downfield from tetramethylsilane (Me_4Si) in parts per million (ppm). Mass spectra were recorded on a Micromass Quattro II atmospheric pressure ionization (API) triple quadrupole mass spectrometer.

Flash chromatography was performed with Merck silica gel (230-400 mesh). TLC plates (silica gel 60-F254) were purchased from VWR Scientific. 2,4-Diacetyldeuteroporphyrin dimethyl ester was either synthesized or purchased from Frontier Scientific, Inc. Sulfuric acid-d_2 and trifluoroacetic acid-d were purchased from Acros Organic Co. D_2O was purchased from Sigma Chemical Co. Acids, bases, and conventional organic solvents were purchased from Fisher.



Scheme S1. Preparation of ferric d_2-2, 4-diacetyldeuteroporphyrin IX dimethyl ester (**6**).

***d_2*-2,4-Diacetyldeuteroporphyrin Dimethyl Ester (S2).** 2,4-Diacetyldeuteroporphyrin (**5**, 25 mg) was dissolved in 0.86 g of D_2SO_4/D_2O (9/1, w/v) mixture portionwise (Scheme S1). The deep-green mixture was stirred at room temperature for 7 days before being poured onto ice H_2O (20 mL) and extracted with 20 mL of

MeOH/$CHCl_3$ (1/4, v/v) three times. The extracts were combined and dried over anhydrous Na_2SO_4 before being evaporated to dryness. The green residue (**S1**) was redissolved in a methyl esterification mixture made by adding 20 drops of $SOCl_2$ (~ 0.5 mL) dropwise into a flask containing 20 mL of MeOH on an ice-H_2O bath. The purple mixture was stirred at room temperature for 42 h. The solvent was evaporated to afford a green residue, which was purified by silica gel column chromatography using MeOH/CH_2Cl_2 (1/100), giving d_2-2,4-diacetyldeuteroporphyrin dimethyl ester (**S2**, 17 mg) as a dark red solid. ^{1}H NMR ($CDCl_3$): δ 3.18-3.24 (m, 4H), 3.26, (s, 3H), 3.31 (s, 3H), 3.48 (s, 3H), 3.55 (s, 3H), 3.656 (s, 3H), 3.664 (s, 3H), 3.73 (s, 3H), 3.82 (s, 3H), 4.24-4.34 (m, 4H), 9.72 (s, 1H), 10.66 (s, 1H). ^{1}H NMR spectrum is shown in Figure S1.

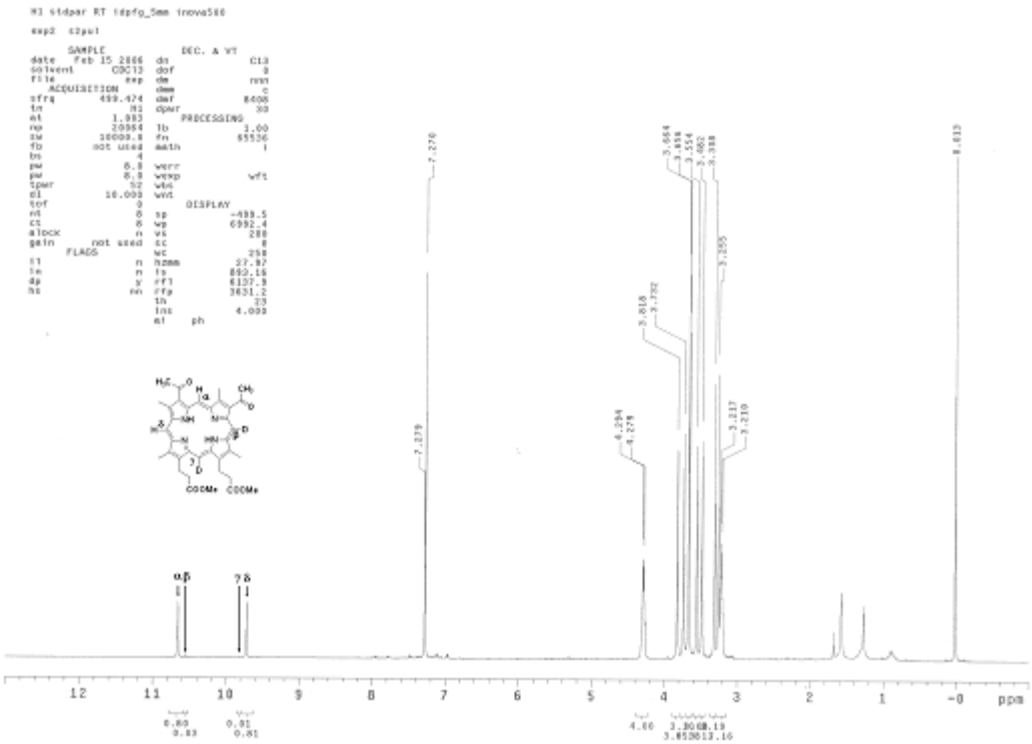
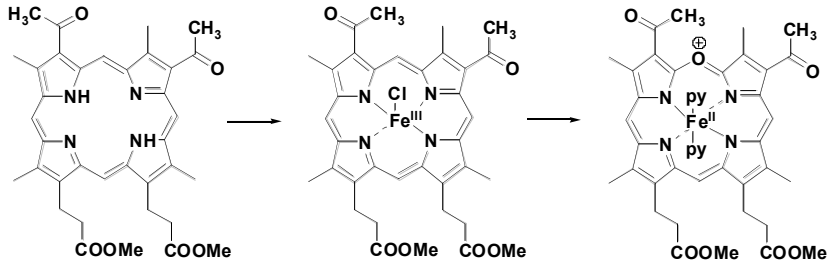


Figure S1. ^{1}H NMR of d_2-2,4-diacetyldeuteroporphyrin dimethyl ester (**S2**).

Typical Iron Insertion Reaction. Into a 50 mL three-necked round bottom flask equipped with an addition funnel and a condenser was added 25 mL of CH_3CN. The flask was capped, flushed with N_2, and heated to reflux for 30 min. $FeSO_4 \cdot 8H_2O$ (150 mg) was

added and dissolved. The flask was cooled to 50 ^{o}C. Porphyrin dimethyl ester (100 mg) was dissolved in 12 mL of $CHCl_3$ and was deaerated by N_2 bubbling for 15 min. The porphyrin solution was transferred to the addition funnel and was added dropwise to the reaction flask. The temperature of the reaction flask was kept at 50 ^{o}C during the reaction. After addition was complete, the mixture was stirred under N_2 for another 30 min before being diluted with 30 mL of CH_2Cl_2, washed with 0.2 M HCl, H_2O, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by silica gel chromatography (MeOH/ CH_2Cl_2, 1/30). The fractions containing only the product were combined and evaporated to dryness. The residue was dissolved by 20 mL of CH_2Cl_2 and washed with 0.2 M HCl and H_2O before being dried over anhydrous Na_2SO_4. After evaporation of the solvent, the ferric porphyrin dimethyl ester was obtained in 80-90% yield. Following this procedure, ferric 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**7**) and ferric d_2-2, 4-diacetyldeuteroporphyrin IX dimethyl ester (**6**) were obtained. MS analysis: **7**, $[C_{36}H_{36}N_4O_6Fe]^+$: caled 676.2, found 676.6; **6**, $[C_{36}H_{34}D_2O_8N_4Fe]^+$: caled 678.2, found 678.5.

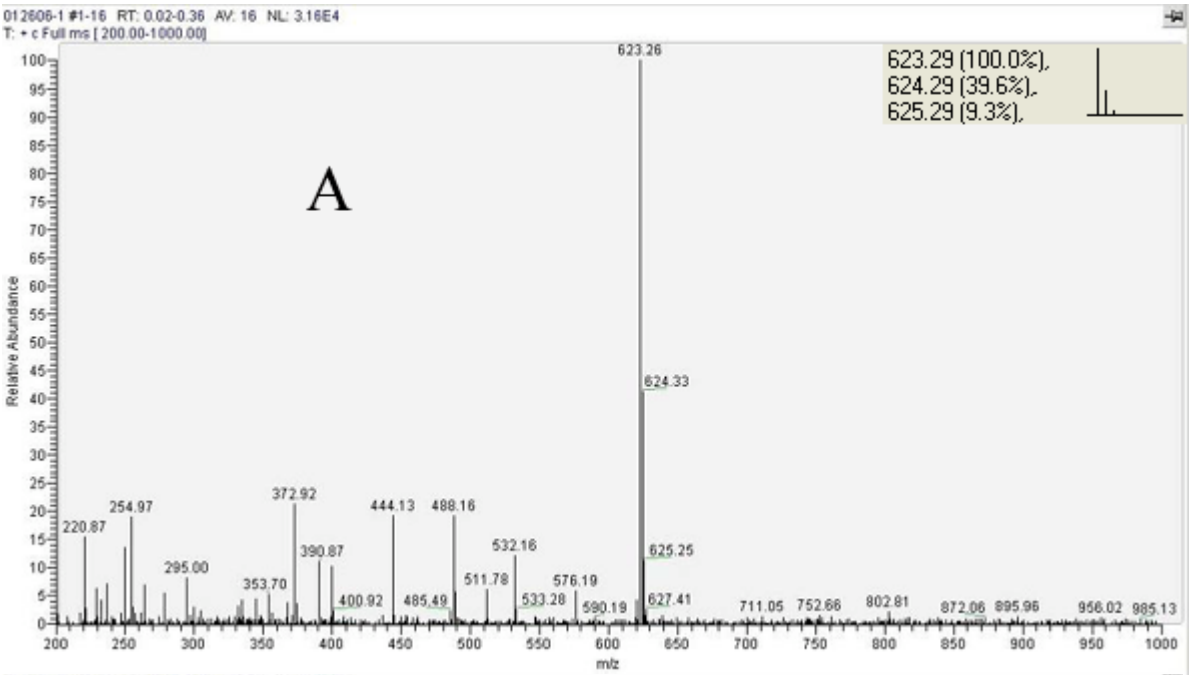
Coupled Oxidation of Ferric 2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester (7). Purified ferric 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**7**, 10 mg) was dissolved in 200 mL of pyridine and 50 mL of H_2O. The mixture was bubbled with oxygen vigorously for 10 min before a solution of 500 mg of ascorbate in 1 mL of H_2O was added. The flask was then stoppered and shaken for 5 min. The yellowish green solution was diluted with H_2O and extracted with CH_2Cl_2 (3 x 50 mL). After being dried over Na_2SO_4 and evaporation of most of the solvents, verdoheme **8** was diluted with CH_2Cl_2 and analyzed by mass spectroscopy. $[C_{35}H_{35}O_7N_4Fe]^+$: caled 679.2, found 679.6. The mass spectra in this control reaction are shown in Figure S2.

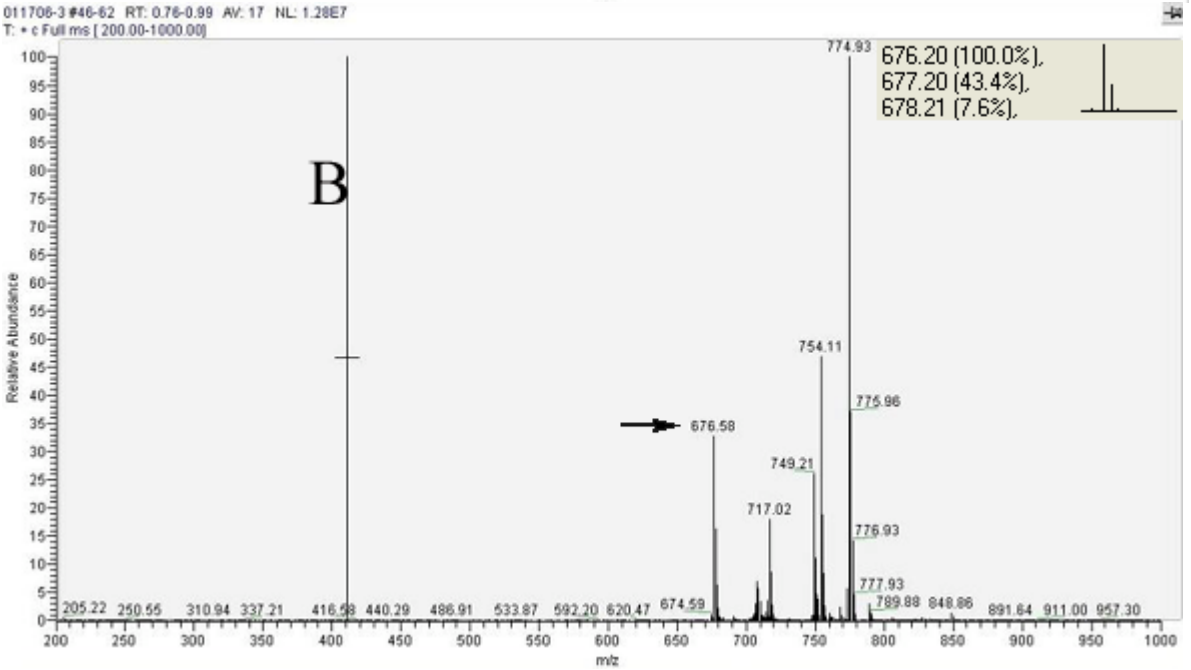


S3
$[M+H]^+$ = 623.3

7
M^+ = 676.2

8
M^+ = 679.2





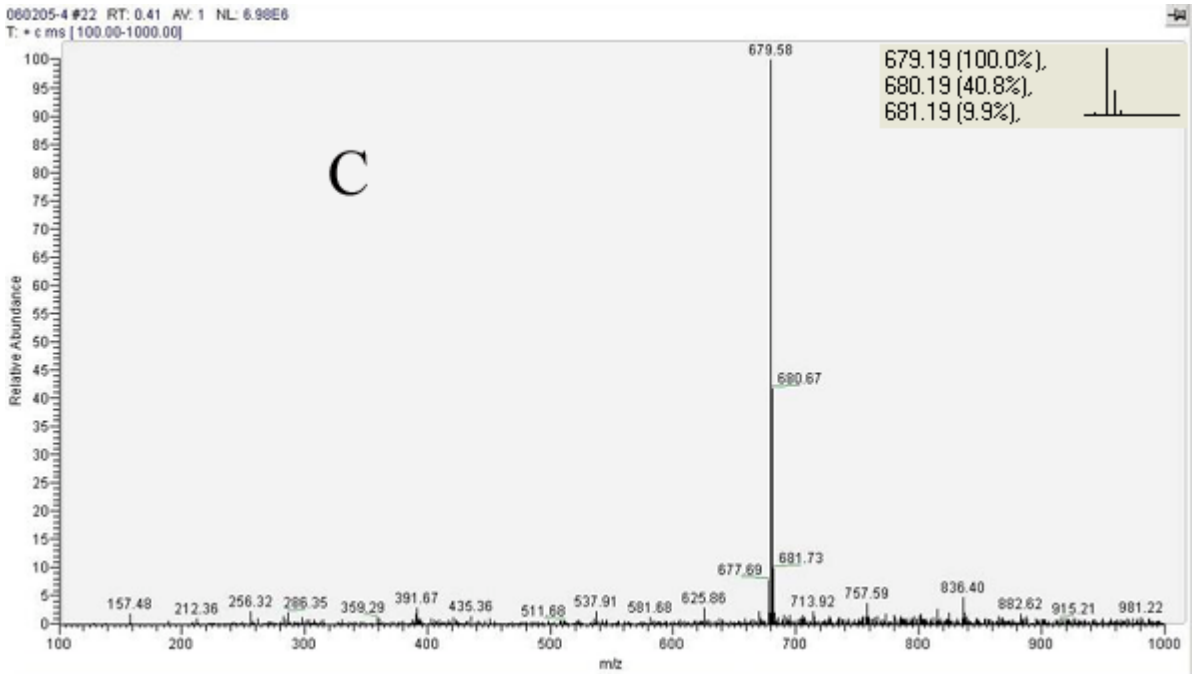
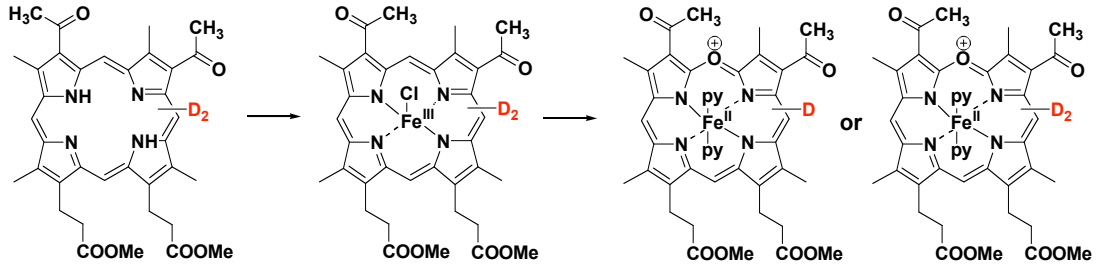
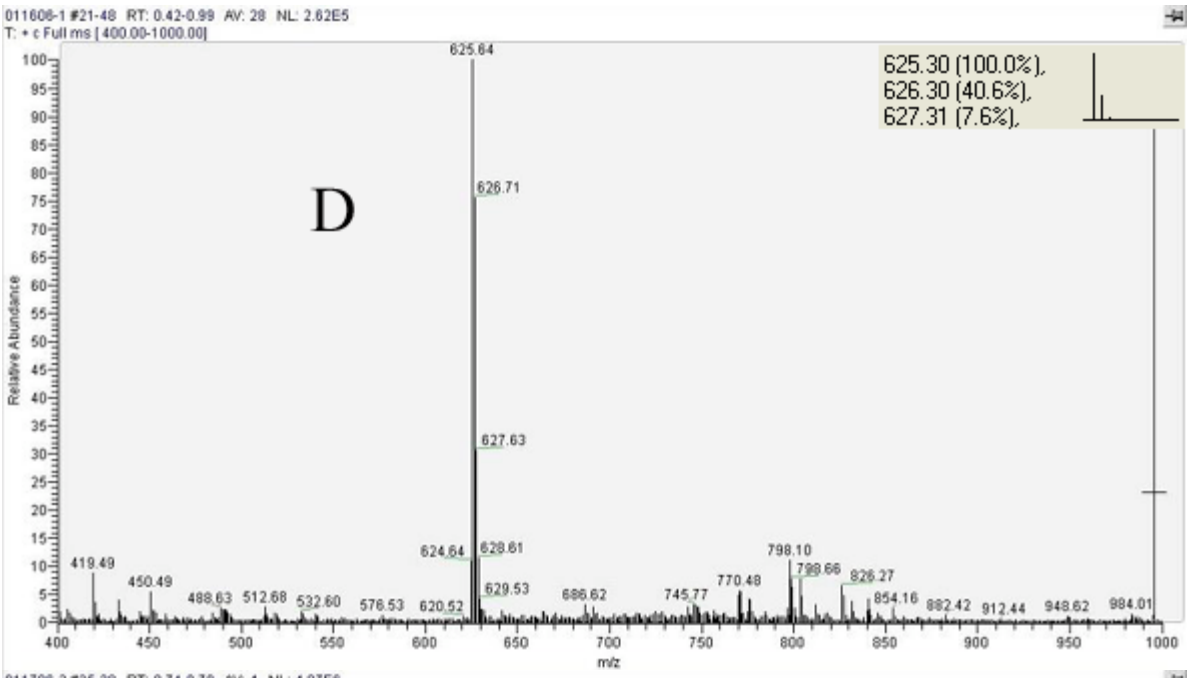


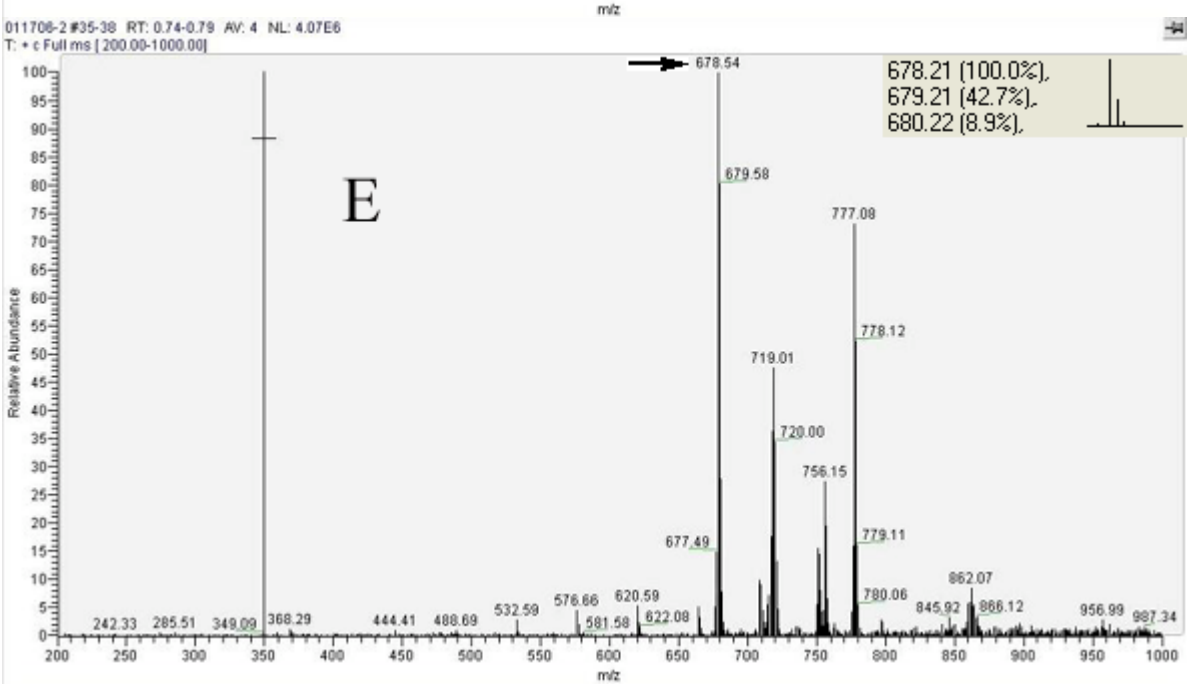
Figure S2. Mass spectrum analysis in the control reaction with non-deuterated porphyrin (A: S3; B: 7; C: 8). The inserted pictures are the simulation diagrams of the isotopic pattern as calculated by Chemdraw.

Coupled Oxidation of Ferric d_2-2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester (6). Purified ferric d_2-2,4-diacetyldeuteroporphyrin IX dimethyl ester (**6**, 10 mg) was subjected to the coupled oxidation conditions as described above. The verdoheme product was diluted with CH_2Cl_2 and analyzed by mass spectroscopy to be **9**: $[C_{35}H_{34}DO_7N_4Fe]^+$: caled 680.2, found 680.7.

The mass spectra in this experiment are shown in Figure S3. The relative intensity of the "$(M+H^+)+1$" peaks in Spectra D are ~80% of the "$M+H^+$" peak, which is higher than the calculated value based on di-deuteration (40%). This indicates that after the β- and γ-*meso* positions get fully deuterated after 7-day reaction, some other positions in compound S2 also get slightly deuterated. This deuteration pattern was kept very consistent in the spectra of compound **6** and compound **9**. One D-atom loss was clearly demonstrated in this experiment, which supports an electrophilic aromatic substitution mechanism.


S2
[M+H]+ = 625.3
6
M+ = 678.2
9
M+ = 680.2
or
10
M+ = 681.2


D
625.30 (100.0%),
626.30 (40.6%),
627.31 (7.6%),
Relative Abundance
m/z


E
678.21 (100.0%),
679.21 (42.7%),
680.22 (8.9%),
Relative Abundance
m/z

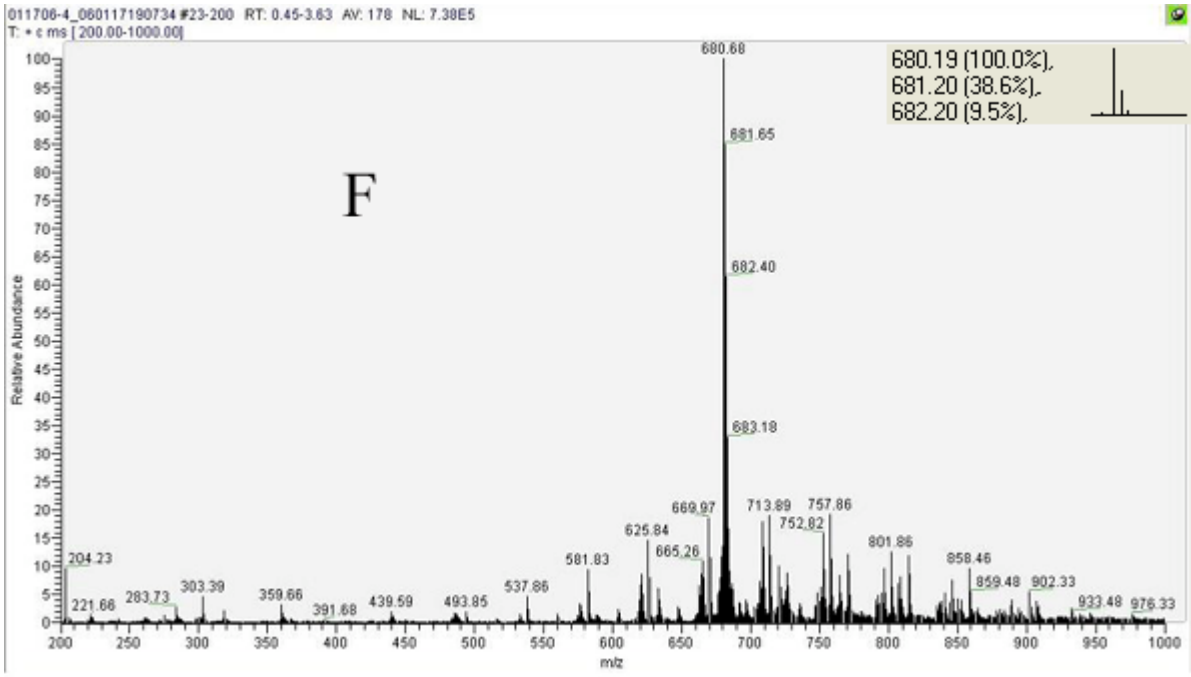


Figure S3. Mass spectrum analysis in the experiment with deuterated porphyrin (D: S2; E: 6; F: 9). The inserted pictures are the simulation diagrams of the isotopic pattern as calculated by Chemdraw.