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Electronic Effects of Peripheral Substituents at Porphyrin Meso Positions

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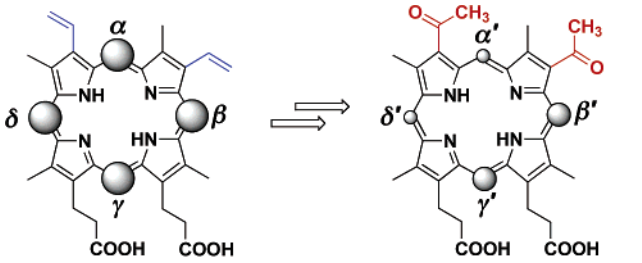

protoporphyrin IX — 2, 4-diacetyldeuteroporphyrin

Porphyrins are stable molecules with a macrocyclic conjugated system and often peripheral substituents. This unique structure makes the electronic properties of the four meso-carbons (the methine bridges) nearly identical. Replacement of the weakly electron-polarizing 2,4-vinyl groups of protoporphyrin IX with strongly electron-polarizing acetyl groups not only leads to much lower meso-carbon reactivities toward electrophilic aromatic substitution but also results in a significant meso-selectivity (the β- and γ-meso-positions become much more nucleophilic (basic) than the α- and δ-meso-positions). To further investigate the relationship between the porphyrin meso-carbon reactivities and the peripheral substituents, two monoacetylporphyrin analogues also were synthesized. This investigation not only leads to empirical rules for predicting porphyrin meso-carbon selectivities but also provides important models for theoretical calculations of porphyrin aromaticity.

Porphyrins and other closely related tetrapyrrolic pigments occur widely in nature, and they play very important roles in various biological processes.[1] A porphyrin is a heterocyclic macrocycle made from four pyrrole subunits linked on opposite sides (α positions) through four methine bridges. The interior of porphyrins complexes readily with many metal atoms such as iron, zinc, copper, magnesium, cobalt, and nickel.[2] Iron-containing protoporphyrin IX, known as heme (**1**), is one of the most important cofactors in biochemistry. Besides its important biological functions of being an oxygen transport agent in proteins like hemoglobin and as an oxygenation cofactor in enzymes such as cytochrome P450s,[3] iron-containing porphyrin-dependent enzymes are under extensive study for their important role in human xenobiotic metabolism.[4] The porphyrin structure is characterized by high stability, which derives from its conjugated macrocyclic ring system. The most reactive sites on porphyrins are the four meso-positions. A variety of reactions including nitration,[5] halogenation,[6] formylation,[7] and oxidative cleavage[8] occur at the meso-carbons. Although reactions at the porphyrin meso-carbons have been extensively studied, the effects of the peripheral substituents have rarely been discussed as they relate to the meso-position reactivities, mainly because of the unclear understanding of porphyrin aromaticity.

The porphyrin structure can be viewed as four pyrrole rings

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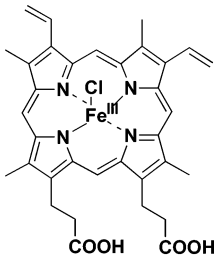

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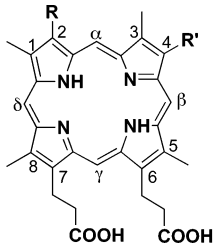
connected to each other by four meso-carbons, and several models for electron delocalization in porphyrins have been advanced (Figure 1). The representation of two tautomeric structures (**a** and **b**) focuses on the inside−outside 18-atom pathway for π-electron delocalization (shown in red type), which suggests that porphyrins are bridged [18]annulenes in Nature.[9] Free-base porphyrins show a preference for placing the inner two protons on opposite nitrogen atoms, but at room temperature, tautomerization between two equivalent structures **a** and **b** occurs rapidly.[10] This delocalization pathway leaves out the peripheral carbon atoms of the two diagonal pyrrole rings (shown in black type), suggesting that these carbon−carbon bonds have greater double-bond character, and this has been confirmed by NMR spectroscopy[11] and X-ray crystallography.[12]

The versatility of porphyrins can be reflected by other alternative proposals regarding their aromaticity. Representation **c** is used to describe some metalloporphyrins, which have a large degree of symmetry.[13] This delocalization pathway might not be applicable to the iron-containing heme porphyrin because the hydrogen atoms at the 2,4-positions of deuteroheme (same structure as heme except the 2- and 4-vinyl groups are replaced by hydrogen atoms) can be substituted by acetyl groups (a typical Friedel−Crafts acylation reaction), which become part of the aromatic system, although these carbons are left out of conjugation in representation **c**.[14] To explain some features of porphyrin reactivity Woodward proposed that the individual pyrrole rings retain some of their aromatic character through dipolar resonance interactions of the type shown in canonical form **d**.[15] Representation **e** features a 22-π electron system, which includes the lone pair electrons of two pyrrole rings.[16]

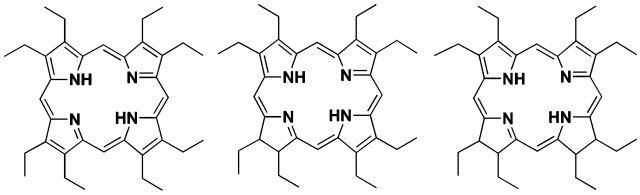
Protoporphyrin IX (**2**, R = R′ = vinyl) is the most common porphyrin in Nature mainly because its iron-containing form, heme (**1**), is so important. Protoporphyrin IX has eight peripheral substituents (two vinyl, four methyl, and two propionate groups) on the porphyrin system, which are often represented by two



2, R = R' = vinyl
8, R = R' = Ac
9, R = R' = Et
10, R = Ac, R' = Et
11, R = Et, R' = Ac

classical resonance structures (**3** and **4**, Figure 2). In each resonance structure, the peripheral substituents of the conjugated systems are at counteractive positions, which result in no net polarizing effect on the meso-positions. Consistent with that fact, the four meso-positions are found to be very similar in their reactivities under nonenzymatic conditions.[8] The well-balanced electronic nature of protoporphyrin IX limits an understanding of the relationship between the peripheral substituents and the meso-position reactivities.

Driven by considerations of their great biological importance, extensive coordination chemistry, and a number of new practical applications, the molecular structures of porphyrins have been under extensive study.[17] Researchers have been interested in identifying different porphyrin analogues having meso-position selectivities in order to apply theoretical approaches for structure elucidation. Partial reduction of octaethylporphine (**5**) affords octaethylchlorin (**6**) and octaethyltetrahydroporphyrin (**7**).[18] The



5 **6** **7**

meso-positions within these incomplete porphyrin systems were found to be quite different in deuteration reactions as a result of the disruption of the macrocyclic conjugation balance.[19] By substituting one of the four meso-positions of the porphyrin ring with either a nitro or a formyl group, selectivities also occurred in the other three meso-positions because of the disruption of the electronic peripheral substituent balance.[5,20] Calculated π-electron densities of these porphyrin analogues are in good accord with the observed meso-position selectivities; however, the relationships among porphyrin aromaticity, peripheral ring substituents, and meso-position reactivities are still unclear because these porphyrin analogues deviate dramatically from natural porphyrins.[21] So the conundrum is that support for theoretical calculations on porphyrin aromaticity needs analogues with significant meso-position selectivities, but meso-position selectivities have only been created with major structural modifications. To explore porphyrin aromaticity and the relationship between peripheral substituents and meso-position reactivity, theoretical calculations need the support of

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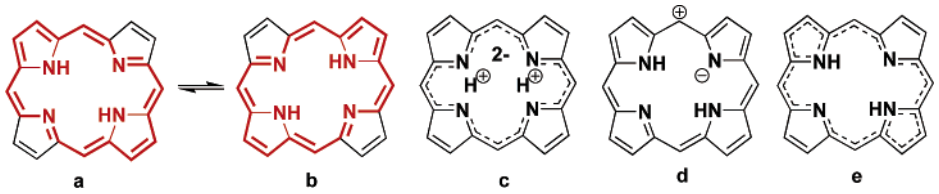


FIGURE 1. Different representations of π-electron delocalization of porphyrin.

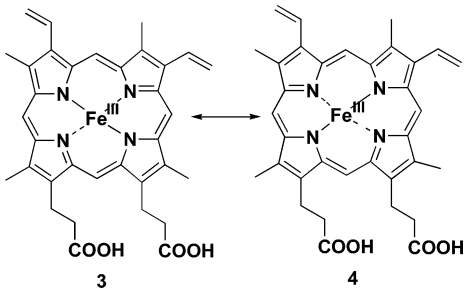


FIGURE 2. Two resonance structures of heme.

new porphyrin analogues having subtle structural changes, which lead to significant meso-position selectivities.

Replacement of the two vinyl groups of protoporphyrin IX with two acetyl groups leads to 2,4-diacetyldeuteroporphyrin (**8**, R = R′ = Ac). (The nomenclature and numbering system chosen for this paper is based on the common usage of protoporphyrin IX and deuteroporphyrin. For IUPAC nomenclature and numbering of porphyin analogues, see http://www.chem.qmul.ac.uk/iupac/tetrapyrrole/TP1.html.) The substitution of peripheral alkyl groups by acetal groups was made to disrupt the porphyrin aromaticity without making a major modification to the ring structure. The four meso-positions of this porphyrin analogue should have different reactivities because of the strong polarizing effects of the two acetyl groups.

The meso-position π-electron densities of porphyrins have been explored by deuteration, which involves an electrophilic aromatic substitution reaction.[19] The rate of meso-position H−D exchange in [^{2}H]trifluoroacetic acid (*d*-TFA) or in concentrated dideuteriosulfuric acid (D_2SO_4) is a function of the meso-position π-electron density.

Here, we present experiments to demonstrate that replacement of the porphyrin vinyl substituents with acetyl substituents not only leads to lower meso-position π-electron densities but also creates significant meso-position selectivities. Further investigations with monoacetylporphyrins (**10**, R = Ac, R′ = Et; **11**, R = Et, R′ = Ac) provide important models for theoretical calculations on porphyrin aromaticity and empirical rules for meso-position selectivity predictions.

Results and Discussion

Replacement of the two vinyl groups of protoporphyrin IX with two acetyl groups leads to 2,4-diacetyldeuteroporphyrin (DAP). Unlike protoporphyrin IX, the conjugated macrocyclic system of DAP will be strongly polarized by the acetyl groups, which should translate into different electronic properties of the porphyrin meso-positions. To test the relative π-electron densities of the meso-position carbons in porphyrins with different substituents at the 2- and 4-positions, mesoporphyrin (2,4-diethyldeuteroporphyrin; **9**, R = R′ = Et) and DAP (**8**, R = R′ = Ac) were subjected to the deuteration model for electrophilic aromatic substitution in parallel.[19] Mesoporphyrin was used instead of protoporphyrin IX (**2**, R = R′ = vinyl) for this study because the vinyl groups of protoporphyrin IX are not stable to the deuteration reagents (strong deuterated acids), and decomposition may be induced by electrophilic attack on the two vinyl groups of protoporphyrin IX.

TABLE 1. Half-Lives and Pseudo-First-Order Rate Constants for Meso-Position Deuteration of 9 (R = R′ = Et) and 8 (R = R′ = Ac)

	$t_{1/2}$ (min)	k (min^{-1})
9 (R = R′ = Et)	107.4	0.0093
8 (R = R′ = Ac)	2562	0.00039

[^{2}H]Trifluoroacetic acid (*d*-TFA) was used first as the deuteration reagent to explore the meso-position π-electron density maps of the porphyrins. The reaction was monitored by ^{1}H NMR spectroscopy. The four meso positions of **9** were found to be deuterated at the same rate at 100 °C with a $t_{1/2}$ = 125.1 h, but the meso-positions of **8** were unchanged after 5 days. This indicates that the π-electron densities of the meso-position carbons in **8** are much lower than those in **9**. To deuterate the meso-positions of **8**, a stronger acid was needed.

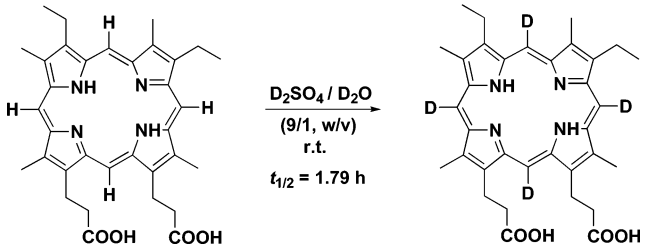
A concentrated sulfuric acid-d_2 solution (D_2SO_4, 87 wt % in D_2O) was used in place of *d*-TFA. After 42 h of reaction at room temperature, both mass spectra and proton NMR spectra confirmed that all four of the meso-positions of **9** had been deuterated, but only two of the four meso-positions of **8** had undergone some H−D exchange; the other two meso-positions remained unchanged. After 10 days, two of the four meso-protons were fully deuterated, and the other two meso-protons were unchanged.

Kinetic studies were carried out for the meso-position H−D exchange reaction of **9** and **8** in D_2SO_4. Samples were withdrawn from the deuteration reaction mixtures containing porphyrin and concentrated D_2SO_4, worked up, and analyzed by ^{1}H NMR spectroscopy. The four meso-positions of **9** have the same deuteration rates; likewise, two of the meso-positions of **8** also are the same, but different from those of **9**. The half-life times and the pseudo first-order rate constants for deuteration are listed in Table 1.

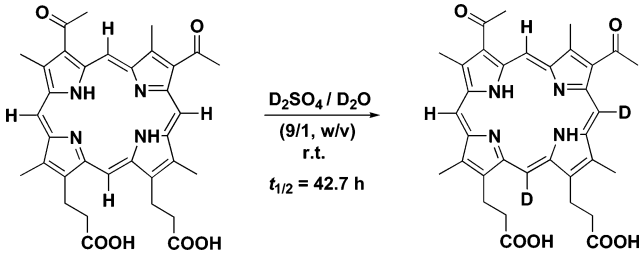
As reflected by the difference in the half-lives and pseudo-first-order rate constants shown in Table 1, the electronic properties of the peripheral substituents of these porphyrins significantly affect the π-electron densities of the meso-position carbon atoms. The electron-withdrawing acetyl groups reduce the electron densities of the porphyrin aromatic systems, which leads to lower meso-position reactivities toward electrophilic aromatic substitution (in this case, protonation).

To identify the meso-position selectivities of **8** during electrophilic aromatic substitution, 2D NOESY spectroscopy was used to analyze a partially deuterated **8** sample, which was prepared by deuteration of **8** for 102 h before it was converted to its dimethyl ester form and dissolved in $CDCl_3$ for NMR analysis. NOESY spectra revealed that the two deuterated positions are the β- and γ-meso-positions (Supporting Informa-

SCHEME 1. Meso-Position Deuteration of 9 (R = R′ = Et) in Concentrated D_2SO_4



SCHEME 2. Meso-Position Deuteration of 8 (R = R′ = Ac) in Concentrated D_2SO_4



tion). Therefore, the order of decreasing π-electron densities at the meso-positions in **8** is $\beta = \gamma >> \alpha, \delta$.

The four meso-positions in **9** have the same H−D exchange rates under these electrophilic aromatic substitution conditions (Scheme 1). This is because there are eight weak electron-polarizing alkyl substituents, which leads to no net polarizing effect on the meso-positions. When the alkyl groups are replaced by acetyl groups at the 2,4-positions, the balance among the peripheral substituents is disturbed, and the polarizing effects of the two acetyl groups not only reduce the electron density of the porphyrin aromatic ring system but also create a significant selectivity among the four meso-positions (Scheme 2).

It may be possible to extend these findings of the relationship between peripheral substituent electronic effects and the porphyrin meso-position reactivity to include their effect on porphyrin aromaticity, whose elusive character has drawn much attention from both organic chemists and theoretical chemists.[22] Many representations of porphyrin aromaticity have been proposed to accommodate the versatility of porphyrins and metal-containing porphyrins. Theoretical calculations of porphyrin aromaticity, however, are based on partially reduced porphyrins,[23] *N*-fused porphyrins,[24] and carboporphyrins.[25] Although results from these theoretical calculations are in good accord with the experimental data, the analogues on which these calculations were made were too far removed from biologically relevant porphyrins so the conclusions about porphyrin aromaticity are not general. Our studies show that the π-electron densities of the four porphyrin meso-positions are sharply divided into two groups ($\beta = \gamma >> \alpha,\delta$). This difference in meso-position π-electron density is achieved by the polarizing effects of the 2,4-diacetyl groups via the porphyrin aromatic system. This analogue could be an important model to which theoretical calculations could be applied to investigate porphyrin aromaticity more effectively.

It was thought that further dissection of the electronic effects of the 2,4-diacetyl groups of **8** could be accomplished by a study of the relationship between the meso-position reactivity and porphyrin peripheral substituents using the two monoacetylated porphyrins, 2-acetyl-4-ethyldeuteroporphyrin (**10**, R = Ac, R′ = Et) and 2-ethyl-4-acetyldeuteroporphyrin (**11**, R = Et, R′ = Ac), which were prepared from hematoporphyrin.[26] After separation, each isomer was subjected to deuteration and 2D NOESY spectral analysis (Supporting Information). The meso-position π-electron density maps for these two analogues, as expected, were different: $\beta,\gamma > \alpha >> \delta$ for **10** and $\gamma > \beta,\delta >> \alpha$ for **11**. The presence of the acetyl group at the 2-position (**10**) significantly reduces the π-electron density of the δ-meso position, and the acetyl group at the 4-position (**11**) significantly reduces the π-electron density of the α-meso position. In the case of the 2,4-diacetyldeuteroporphyrin analogue (**8**, R = R′ = Ac), these were the two positions with the lowest π-electron densities (the meso positions that were *not* deuterated). This suggests that the polarizing effects of the peripheral substituents are additive on the porphyrin meso-position reactivities.

To determine why the π-electron densities of the δ-meso position in **10** and the α-meso position in **11** are lowered most by acetyl substitution at the 2- and 4-positions, respectively, we considered the tautomeric and resonance structures for these compounds. Each monoacetylporphyrin has two different tautomeric structures, each of which having a variety of resonance structures; two of each are shown in Schemes 3 and 4.

In **10**, the acetyl group is attached directly to the porphyrin aromatic macrocyclic system in structures **10a** (in red) but not in structures **10b** (it is attached to the double bond outside of the aromatic system). Thus, these two tautomeric structures are different, and they do not contribute equally to the reactivity of the meso-positions of the porphyrin ring. Each tautomeric structure has two important resonance structures (**10a-1** and **10a-2**; **10b-1** and **10b-2**). Among the four structures of **10**, the **10a-1** structure reflects the most effective polarization of the δ-meso position because the acetyl group has a direct effect on attracting electron density from that position, which is consistent with the experimental results. This suggests that **10a-1** is closest to representing the real electronic structure of **10**, and the equilibrium should favor the left side. The same analysis can be applied to **11**; structure **11b-1** reflects the most significant effect of acetyl polarization on the α-meso position, and the tautomeric equilibrium in Scheme 4 should favor the right side. When the acetyl group is attached directly to the aromatic π-system, conjugation is expected to be more effective at lowering the energy of the structure.

On the assumption that the substituents (except for the acetyl group) all have comparable electronic effects, structures **10a-1** and **11b-1** can be effectively superimposed on each other by a 90° counterclockwise rotation (Figure 3). When that rotation is carried out, the α-meso position of **11b-1** corresponds to the δ-meso position of **10a-1**, thereby reinforcing the observed effects on the α- and δ-meso positions and suggesting why the α-meso position of **10** and the δ-meso position of **11** are correspondingly affected.

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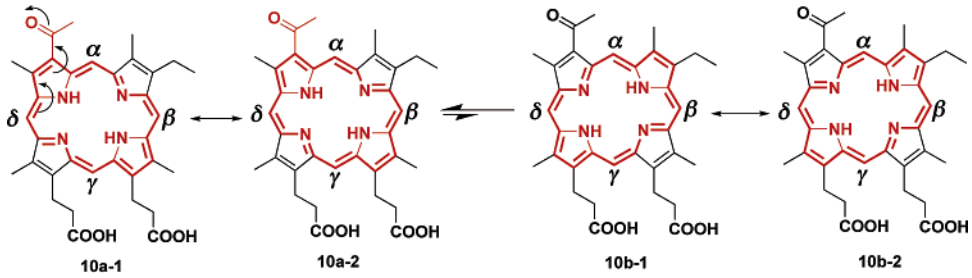
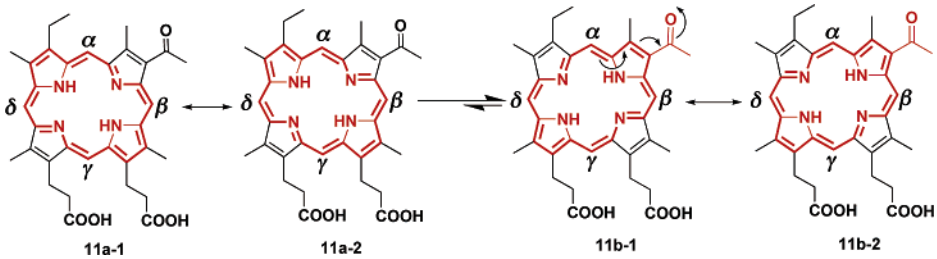
SCHEME 3. Tautomer and Resonance Structures of 10



SCHEME 4. Tautomer and Resonance Structures of 11



Based on this correspondence, the porphyrin meso-position selectivities could be predicted for peripheral substituents at other positions as well. For example (Figure 4), to determine how an electron-withdrawing group at the 1-position should compare to the effect of the 2-position group, various rotations can be made. First, the electron-withdrawing group at the 1-position should exhibit its strongest effect at the α-meso position. Rotation about a C_2-axis in the vertical direction followed by a counterclockwise 90° rotation corresponds to a porphyrin with an electron-withdrawing group at the 2-position (δ-meso-position effect).

Although this study focuses on metal-free porphyrin analogues, this could also be extended to metal-containing porphyrins because the binding of the central metal ion should not impact significantly on the porphyrin meso-position selectivities. An understanding of meso-position selectivities in metal-containing porphyrins should be useful in predicting its reactions toward electrophilic and nucleophilic species.

These studies demonstrate the effect of peripheral substituents on the reactivity of the meso-positions of porphyrins and provide a basis for predicting which positions will be most affected by substitution. They also allow predictions of where peripheral substitution will be most suitable for reactivity at specific meso-positions.[27]

Experimental Section

2,4-Diacetyldeuteroporphyrin (8).[28] 2,4-Diacetyldeuteroporphyrin dimethyl ester (120 mg) was dissolved in 15 mL of a solution containing 1 g of KOH in 95 mL of MeOH and 5 mL of H_2O. The mixture was refluxed at 60−70 °C for 4 h under N_2. The warm solution was diluted with 15 mL of MeOH/CH_2Cl_2 (1/50, v/v), and the product was extracted with 50 mL of MeOH /CH_2Cl_2 (1/4, v/v) from 20 mL of 0.2 M HCl saturated with NaCl. The MeOH/CH_2Cl_2 extracts were combined and dried over anhydrous Na_2SO_4 before evaporation of the solvents to dryness. The deep-red residue (104 mg, 91%) was not very soluble in pure MeOH or pure CH_2Cl_2 but was soluble in a mixture of the two (MeOH/CH_2Cl_2 = 1:4) or in TFA. ^{1}H NMR (CF_3COOD): δ 3.18−3.22 (m, 4H), 3.46 (s, 3H), 3.47 (s, 3H), 3.63 (s, 3H), 3.68 (s, 3H), 3.69 (s, 3H), 3.98 (s, 3H), 4.50−4.54 (m, 4H), 10.94 (s, 1H), 10.96 (s, 1H), 11.29 (s, 1H), 11.60 (s, 1H). MS (ESI): $C_{33}H_{34}N_4O_6$ $[M + H]^+_{calcd}$ = 595.3, $[M + H]^+_{obs}$ = 595.6.

Deuteration of Mesoporphyrin in *d*-TFA. Mesoporphyrin (**9**, 10 mg) was dissolved in 0.4 mL of *d*-TFA in a NMR tube. The NMR tube was capped and wrapped with aluminum foil before being inserted into an oil bath at 100 °C. Every 24 h, the NMR tube was removed from the oil bath and a ^{1}H NMR spectrum was taken. From these spectra, the deuteration percentage of the four meso-position hydrogens was calculated.

Deuteration of 2,4-Diacetyldeuteroporphyrin in *d*-TFA. 2,4-Diacetyldeuteroporphyrin (**8**, 10 mg) was dissolved in 0.4 mL of *d*-TFA in an NMR tube. The NMR tube was capped and wrapped with aluminum foil before being placed in an oil bath at 100 °C. After 5 days, no significant H−D exchange was observed in the ^{1}H NMR spectrum. The NMR tube was cooled, and the solution was transferred to a flask before being evaporated to dryness. The residue was then dissolved in a mixture made by adding 20 drops of $SOCl_2$ (~0.5 mL) dropwise into a flask containing MeOH (20 mL) in an ice−H_2O bath to exchange the deuterium of the deuterated acetyl groups and to re-esterify the carboxylic acids. The purple mixture was stirred at room temperature overnight. The solvent was evaporated to afford a green residue, which was then purified by silica gel chromatography using MeOH/CH_2Cl_2 (1/100, v/v) as eluent. Both the ^{1}H NMR spectrum and mass spectrum of the diacetylporphyrin dimethyl ester showed that there was no meso-position H−D exchange. ^{1}H NMR ($CDCl_3$): δ 3.05 (s, 3H), 3.12 (t, 2H), 3.16 (t, 2H), 3.21 (s, 3H), 3.27 (s, 3H), 3.39 (s, 3H), 3.48 (s, 3H), 3.58 (s, 3H), 3.65 (s, 3H), 3.67 (s, 3H), 4.14 (t, 2H), 4.20

(27) After the manuscript was submitted for publication, we became aware that iron 2,4-diacetyldeuteroporphyrin had been previously shown to be a substrate for heme oxygenase with a lower rate of reaction (Frydman, R. B.; Tomaro, M. L.; Buldain, G.; Awruch, J.; Diaz, L.; Frydman, B. *Biochemistry* **1981**, *20*, 5177−5182). Also, another electron-withdrawing monosubstituted porphyrin, 3-demethyl-3-(trifluoromethyl)mesohemin IX, was previously reported and shown to produce regioselectivity in the reaction of oxygen and ascorbic acid in aqueous pyridine that leads to meso-oxidation (Crusats, J.; Suzuki, Z.; Mizutani, T.; Ogoshi, H. *J. Org. Chem.* **1998**, *63*, 602−607). Their conclusion, as is ours, was that the electron-withdrawing trifluoromethyl substituent played an important role in controlling regioselectivity.

(28) Smith, K. M.; Fujinari, E. M.; Langry, K. C.; Parish, D. W.; Tabba, H. D. *J. Am. Chem. Soc.* **1983**, *105*, 6638−6646.

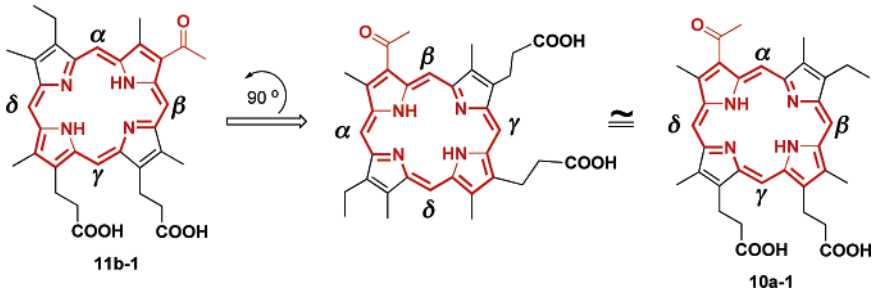


FIGURE 3. Overlap of **10a-1** and **11b-1** by a 90° rotation.

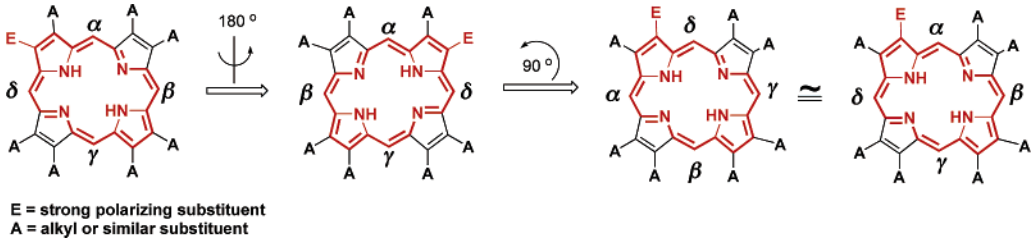


FIGURE 4. Correspondence of a group at the 1-position and the 2-position

(t, 2H), 9.18 (s, 1H), 9.42 (s, 1H), 10.21 (s, 1H), 10.31 (s, 1H). MS: $C_{36}H_{38}N_4O_6$ $[M + H]^+_{calcd} = 623.3$, $[M + H]^+_{obs} = 623.6$.

Deuteration of Mesoporphyrin in D_2SO_4. Mesoporphyrin (**9**, 25 mg) was dissolved in 0.86 g of a D_2SO_4/D_2O (9/1, w/v) mixture portionwise. The deep-red mixture was stirred at room temperature for 42 h before being poured onto an ice/H_2O mixture (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 evaporation to dryness. The deep-red residue was dissolved in CF_3COOD for the NMR experiment. ^{1}H NMR (CF_3COOD): δ 1.77−1.82 (m, 6H), 3.26−3.38 (m, 4H), 3.62−3.78 (m, 12H), 4.18−4.23 (m, 4H), 4.58−4.62 (m, 4H). The meso-position protons at 10.93 (3H) and 11.17 (s, 1H) were completely exchanged.

Deuteration of 2,4-Diacetyldeuteroporphyrin in D_2SO_4. The deuteration reaction described above was repeated with 2,4-diacetyldeuteroporphyrin (**8**). The deep-green mixture was worked up similarly after 102 h of stirring. The ^{1}H NMR spectrum was first taken in CF_3COOD to confirm that the methyl peaks of the two acetyl groups at 3.46 (s, 3H) and 3.47 (s, 3H) had disappeared as a result of fast H−D exchange. Since the solvent peak of CF_3COOD (11.50 ppm) interferes with the meso-position proton peaks (10.92−11.73 ppm), it is difficult to see if the meso-position proton peaks have decreased or not as a result of H−D exchange. Because porphyrin (the free acid form) is not very soluble in CD_3OD, $CDCl_3$, or CD_3COOD, it has to be converted to its methyl ester form for ^{1}H NMR analysis of possible meso-position H−D exchange.

Methyl Esterification of 2,4-Diacetyldeuteroporphyrin. The deep-red residue of 2,4-diacetyldeuteroporphyrin after the deuteration reaction 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 in an ice−H_2O bath. The purple mixture was stirred at room temperature overnight. The solvent was rotary evaporated off to afford a green residue, which was then purified by silica gel column chromatography using MeOH/CH_2Cl_2 (1/100, v/v) as the elution solvent. The purified 2,4-diacetyldeuteroporphyrin dimethyl ester was dissolved in $CDCl_3$ for the ^{1}H NMR experiment: δ 2.96 (s, 3H), 3.04−3.14 (m, 4H), 3.14 (s, 3H), 3.19 (s, 3H), 3.27 (s. 3H), 3.42 (s, 3H), 3.47 (s, 3H), 3.62 (s, 3H), 3.65 (s, 3H), 4.06 (t, 2H), 4.13 (t, 2H), 9.01 (s, 1H), 9.31 (s, 0.2H), 10.04 (s, 1H), 10.21 (s, 0.2 H). The ^{1}H NMR spectra show that two of the four meso positions were partially deuterated (deuteration reached about 80% after 102 h), and the two deuterated acetyl groups had been exchanged for acetyl groups.

2D NOESY Experiment with Partially Deuterated Diacetylporphyrin for Meso-Position Proton Identification. The methyl ester form of the partially deuterated diacetylporphyrin was dissolved in $CDCl_3$ and subjected to 2D-NOESY analysis. The meso-position proton peaks in the ^{1}H NMR spectrum were assigned as follows: δ 9.01 ppm, γ 9.31 ppm, α 10.04 ppm, β 10.21 ppm (see the Supporting Information for the 2D NOESY analysis).

Preparation of Monoacetylporphyrins. The mixture of monoacetylporphyrin (2-acetyl-4-ethyldeuteroporphyrin (**10**) and 2-ethyl-4-acetyldeuteroporphyrin (**11**)) dimethyl esters was synthesized from hematoporphyrin according to a known procedure.[26]

2-Acetyl-4-ethyldeuteroporphyrin Dimethyl Ester. ^{1}H NMR ($CDCl_3$): δ 1.86 (t, 3H), 3.20−3.30 (m, 7H), 3.55 (s, 3H), 3.61 (s, 3H), 3.65 (s, 3H), 3.66 (s, 3H), 3.69 (s, 3H), 3.80 (s, 3H), 4.19 (t, 2H), 4.31 (t, 2H), 4.41 (t, 2H), 9.94 (s, 1H), 9.95 (S, 1H), 10.01 (s, 1H), 10.77 (s, 1H). MS (ESI): $C_{36}H_{40}N_4O_5$ $[M + H]^+_{calcd} = 609.3$, $[M + H]^+_{obs} = 609.5$.

2-Ethyl-4-acetyldeuteroporphyrin Dimethyl Ester. ^{1}H NMR ($CDCl_3$): δ 1.86 (t, 3H), 3.22−3.34 (m, 7H), 3.55 (s, 3H), 3.63 (s, 3H), 3.67 (s, 3H), 3.69 (s, 3H), 3.70 (s, 3H), 3.87 (s, 3H), 4.19 (t, 2H), 4.31 (t, 2H), 4.45 (t, 2H), 9.95 (s, 1H), 9.97 (s, 1H), 10.08 (s, 1H), 10.80 (s, 1H). MS (ESI): $C_{36}H_{40}N_4O_5$ $[M + H]^+_{calcd} = 609.3$, $[M + H]^+_{obs} = 609.5$.

Deuteration of Monoacetylporphyrins in D_2SO_4. The monoacetylporphyrin dimethyl ester isomers (10 mg) were dissolved in 0.86 g of D_2SO_4/D_2O (9/1, w/v) portionwise. The deep-green solution was stirred for 21 h before being poured over ice−water (20 mL). The mixture was worked up and re-esterified as described above. The deuterated monoacetylporphyrin dimethyl ester isomers were purified by preparative TLC (MeOH/$CHCl_3$ = 1/60, v/v). ^{1}H NMR spectra were taken in $CDCl_3$ solvent to identify the meso-position H−D exchange. **10** dimethyl ester: δ 9.94 (s, 0.25H), 9.95 (s, 0.25H), 10.01 (s, 1H), 10.77 (s, 0.5H); **11**, dimethyl ester: δ 9.95 (s, 0H), 9.97 (s, 0.5H), 10.08 (1H), 10.80 (s, 0.4H).

2D NOESY Experiment with Deuterated Monoacetylporphyrins. Each of the two deuterated monoacetylporphyrin dimethyl ester isomers (4 mg each) was dissolved in $CDCl_3$ and subjected to 2D NOESY analysis. The percentage of deuteration of the meso-position protons in each isomer was identified after 21 h. **10**, dimethyl ester: γ 9.94 ppm (75%), β 9.95 ppm (75%), δ 10.01 ppm (0%), α 10.77 ppm (50%); **11**, dimethyl ester: γ 9.95 ppm (100%), β 9.97 (50%), α 10.08 ppm (100%), δ 10.80 ppm (40%).

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Supporting Information Available: Synthesis of 2,4-diacetyldeuteroporphyrin dimethyl ester (**14**); synthesis of 2-acetyl-4-ethyldeuteroporphyrin (**10**) dimethyl ester and 2-ethyl-4-acetyldeuteroporphyrin (**11**) dimethyl ester; kinetic studies of *meso*-H−D exchange in mesoporphyrin (**9**) and 2,4-diacetyldeuteroporphyrin (**8**); 2D NOESY analysis of partially deuterated 2,4-diacetyldeuteroporphyrin dimethyl ester; 2D NOESY analysis of partially deuterated monoacetylporphyrin dimethyl ester. This material is available free of charge via the Internet at http://pubs.acs.org.

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