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[Gallium(III) protoporphyrin IX]$_2$: A Soluble Diamagnetic Model for Malaria Pigment

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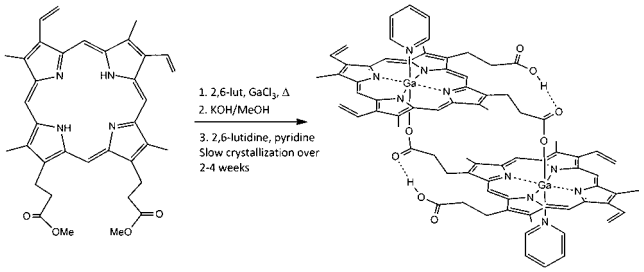
Supporting Information

ABSTRACT: Gallium(III) protoporphyrin IX forms a dimeric propionate-bridged dimer, **2**, that is a soluble diamagnetic analogue of hematin anhydride. The single-crystal structure of **2** corresponds to a nondisordered inversion-symmetric dimer similar to malaria pigment but, unlike it, has a six-coordinate metal and an intraporphyrin rather than an interporphyrin hydrogen bond. NMR NOE correlations demonstrate the presence of the propionate linkage in solutions with pyridine. Taken together, this is the first single-crystal X-ray diffraction study of a propionate-linked dimer as found in malaria pigment and the first evidence for its presence in solution.

Malaria continues to be a global problem affecting at least a quarter of the planet's population. As the need to develop new drugs becomes more and more pressing,[1,2] it is vital that we understand the chemistry of their drug targets. For quinoline antimalarials,[3,4] this is thought to be disruption of heme detoxification by biocrystallization of hemozoin. Hemozoin is a relatively inert form of the heme dimer in which the propionate group of one porphyrin unit coordinates to the Fe^{III} center of the other and vice versa, and it is proposed that drugs such as chloroquine inhibit hemozoin formation with subsequent disruption of heme detoxification. Hemozoin is isostructural with the synthetic phase hematin anhydride (β-hematin),[5,6] and both natural and synthetic materials are completely insoluble in aqueous and organic solvents, with which they do not react. This profound insolubility means that many useful solution-phase methods for characterizing drug/target interactions, such as NMR, fluorescence, and UV−vis spectroscopy, have to be modified and/or often fail when applied to the hemozoin/quinoline drug problem. We recently discovered that with suitable substitution of the protoporphyrin ring of hematin anhydride modest but useful solubilities can be obtained that enable simple solution spectrophotometric titrations to be measured.[7] Herein we describe a more spectacular improvement in solubility in a structurally related malaria pigment dimer analogue by replacing Fe^{III} with Ga^{III}. In particular, a new synthesis and a solid-state structure of this dimer by single-crystal X-ray diffraction are reported. The existence of the dimer in a methanol solution and its dependence on interactions with pyridine have been determined by 1D ^{1}H NOESY NMR.

When the dimethyl ester of protoporphyrin IX is treated with gallium trichloride in 2,6-lutidine at reflux followed by KOH in methanol, a metalated deesterified product, Ga(PPIX)(OH) (**1**), can be isolated.[8] Alternatively, a new dimeric product, [Ga(PPIX)(py)]$_2$·py (**2**), can be isolated by the slow crystallization of **1** from 2,6-lutidine in the presence of pyridine (eq 1). Both **1** and **2** are isolated as dark-purple-red



diamagnetic solids that have excellent solubilities in methanol. They are modestly light-sensitive singlet oxygen sensitizers, and care must be taken to avoid degradation.

Small crystals of **2** suitable for single-crystal X-ray diffraction grow as a pyridine solvate and diffract well at 100 K. These allow for the first single-crystal determination of a hemozoin-like reciprocal dimer structure (Figure 1). As with hemozoin, this new dimer has crystallographically imposed inversion symmetry, with the propionates at the 2 and 18 positions being engaged in metal coordination and propionic acid hydrogen bonding, respectively. The vinyl substituents are well ordered in **2**. Unlike hemozoin, gallium is six-coordinate and has a pyridine ligand bound trans to the propionate. Overall, the porphyrin is planar, with the largest mean plane deviation for the ring atoms being 0.175 Å by C7 and gallium being only 0.031 Å out of the porphyrin mean plane in the direction of oxygen. The Ga−O bond in **2** is also longer by 0.1 Å than both the Fe−O bond in hematin anhydride and the corresponding bond in known gallium(porphyrin)(acetate) compounds (Table S1 in the Supporting Information, SI). Another unique feature of **2** is that the free propionic acid forms an intradimer hydrogen bond with the gallium-bound propionate of the same porphyrin unit (Figure 2). Among the consequences of this intradimer hydrogen bonding is an alteration in the carboxylate stretching modes to give bands at 1725, 1628, and 1379 cm^{-1}. The latter band is markedly shifted from its position in hemozoin dimers (1208 cm^{-1}) and in known gallium(porphyrin)(acetate) compounds (in the range 1270−1295 cm^{-1})[9] and follows established trends in the C−O bond lengths.[10]

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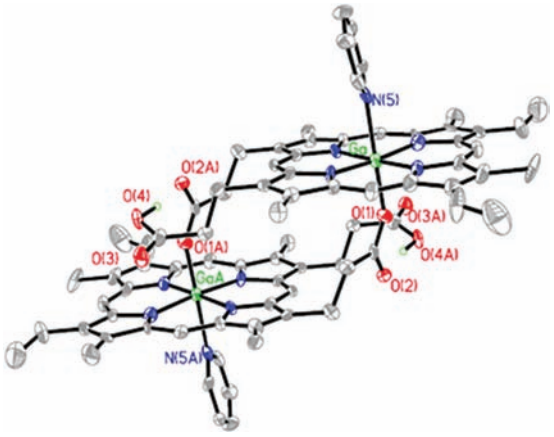


Figure 1. Thermal ellipsoid diagram of $[Ga(PPIX)(py)]_2$ with 40% thermal ellipsoids showing only slight disorder in the vinyl groups. Carbon-bound hydrogens and the pyridine solvate are omitted for clarity. Key metric parameters (Å): Ga–O(1A) 2.010(2), Ga–N(5) 2.230(3), Ga–N(1) 2.018(3), Ga–N(2) 2.025(3), Ga–N(3) 2.027(3), Ga–N(4) 2.030(2), O(1)–C(34) 1.263(4), O(2)–C(34) 1.251(4), O(3)–C(23) 1.214(4), O(4)–C(23) 1.320(4), O(2)–O(4) 2.607(4).

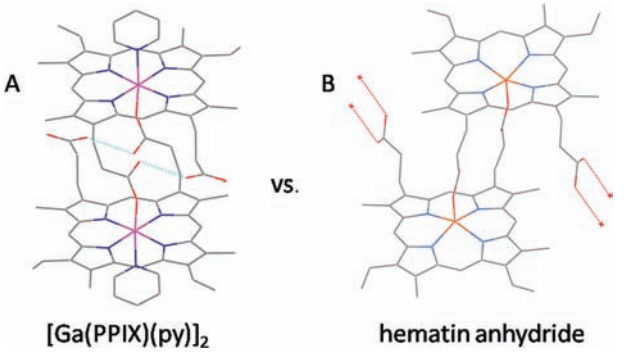


Figure 2. Contrast in hydrogen bonding in the gallium and iron protoporphyrin IX dimers. (a) Hydrogen-bonding interactions in $[Ga(PPIX)(py)]_2$ are intramolecular between propionic acid chains of the same molecule. (b) Hematin anhydride dimer units (for comparison) are linked by an extended hydrogen-bonded network utilizing the free propionates.

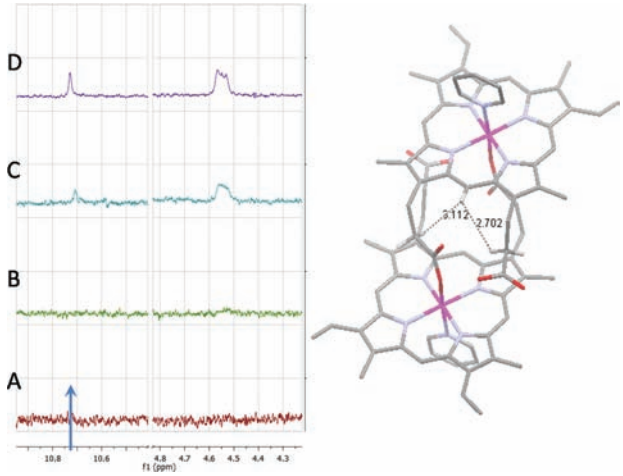
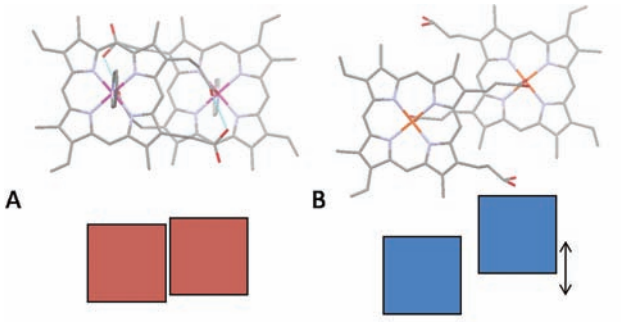


Figure 3. Stacked 1D NOESY of **1** with (a) 0 equiv, (b) 3 equiv, (c) 14 equiv, and (d) 27 equiv of pyridine added. Constrained propionate distances in the dimer are sufficient to observe NOE via NMR.



Intra-dimer parameters	$[Ga(PPIX)(py)]_2$	$[Fe(PPIX)]_2$
M-O distance	2.010(2)	1.886(2)
M out of plane	0.031	0.47
M-M distance	8.199	9.047
mean porphyrin plane separation	4.651	4.724
porphyrin offset	0.63	3.67

Figure 4. Contrast in porphyrin overlap between iron and gallium dimers, with colored squares representing porphyrin units and a vertical offset of the squares demonstrating a porphyrin offset: (a) porphyrin planes are minimally offset in $[Ga(PPIX)(py)]_2$; (b) porphyrin planes are maximally offset in hematin anhydride.

The presence of the coordinated pyridine, which disrupts π stacking in the crystal, and the lack of extensive intermolecular hydrogen bonding together account for the considerable solubility for **2** in methanol and other organic solvents. Solution ^{1}H NMR of **2** in methanol-d_4 by 1D NOESY indicates that pyridine promotes dimerization, as detected by the increase in the intensity of the methine-20–propionate-2β,18β NOE peak as pyridine is added (Figure 3; for the porphyrin numbering scheme, see Figure S3 in the SI).

This is consistent with findings by Kadish et al.,[9] in which pyridine was found to chelate to gallium porphyrins but not displace anionic ligands acetate, fluoride, or hydroxide.

The structure of **2** invites speculation on the possible structure of an as yet unknown six-coordinate iron(III) (protoporphyrin IX) propionate-bridged dimer. The structure contrast, shown in Figure 4, illustrates the consequences of the difference between inter- and extramolecular hydrogen bonds when the metal drops into the plane of the porphyrin and the free propionic acid group folds in on the structure. This transition is accompanied by a 1 Å decrease in the metal–metal separation as well as a decrease in the interporphyrin plane separation and a marked decrease in the offset of the porphyrins. One prediction of this model is that the binding of the pyridines would be pairwise and cooperative. Another prediction is that, as with **2**, a six-coordinate complex may induce disruption of the interdimer hydrogen-bonding characteristic of hemozoin, and the increased solubility that accompanies this change may be one of the keys for antimalarial drug action: any drug that promotes an increase in the coordination number may lead to increased solubility and increased heme toxicity.

Among the important constraints and consequences of the recognition of the structure of **2** and that of hematin anhydride is the difference in their solution chemistry with pyridine. Hematin anhydride reacts slowly in pyridine to give an evolving mixture of hemochrome and a μ-oxo-bridged dimer. For the gallium analogue, increasing pyridine leads to the formation of the dimer and, when pyridine is used as a cosolvent with 2,6-lutidine, crystallization. Despite numerous attempts in ours and other laboratories to make trivalent nonferric analogues to

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hematin anhydride, **2** is the first report of such a complex. In fact, ferrous protoporphyrin IX[11] and many noniron porphyrins[12] inhibit the formation of malaria pigment, possibly for these reasons. While this may reflect the large interplanar separation allowed by the out-of-plane five-coordinate iron, it may also reflect the use of conditions that avoid formation of the structure as in **2**. It is possible that, given the right synthetic condition, six-coordinate hematin anhydride analogues with transition-metal analogues to **2** may be accessible.

Finally, we note that a family of proposed pyridine-based antimalarials are excellent malaria pigment crystallization inhibitors but are not effective antimalarials in vivo. A possible interpretation of these results[13,14] is that these derivatives may promote an increase in the coordination number and a decrease in aggregation of the hemes and thus lead to their solubility. Their low apparent activity purportedly stems from them not being taken up in the digestive vacuole.

In conclusion, the first single-crystal diffraction structure of a propionate-bridged protoporphyrin dimer, **2**, reveals a new intradimer hydrogen bonding not seen in the structures of hematin anhydride and hemozoin as determined by powder diffraction.[5,6,15] The high solubilities of the diamagnetic **1** and **2** allow for their solution characterization by ^{1}H NMR, and a model for these structures suggests that axial coordination to malaria pigment might lead to an important transition in geometry.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data in CIF format, experimental procedures, crystallographic data, and analyses. This material is available free of charge via the Internet at http://pubs.acs.org. X-ray crystallographic data have also been deposited with the Cambridge Crystallographic Data Centre as CCDC 813456. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.

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Notes

The authors declare no competing financial interest.

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