The bis-*p*-nitrophenyl ester of iron(iii) mesoporphyrin II acetate: preparation and incorporation into a model hemoprotein

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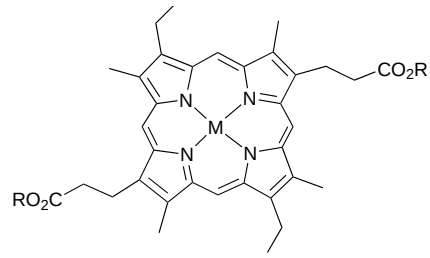
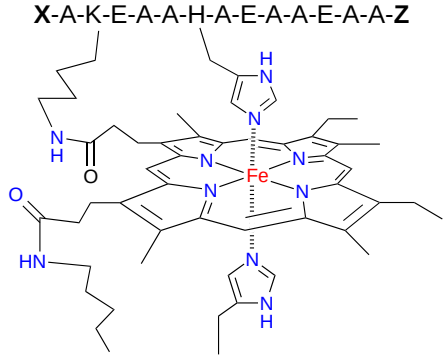
A method for cleanly inserting iron into the bis-*p*-nitrophenyl ester of mesoporphyrin II, and use of the highly soluble product in the synthesis of a model hemoprotein, is reported.

We have recently developed a new class of hemoprotein model compounds called peptide sandwiched mesohemes (PSMs; *e.g.* **1** and **2**), which consist of two peptides covalently attached to Fe^{III} mesoporphyrin IX (MPIX).[1] Histidine (His) residues within each peptide coordinate to the mesohemin iron. In **1**[1] and **2**,[2] Fe–His coordination leads the initially random coil peptides to adopt conformations in which peptide helix content approaches 50 and 60%, respectively. A problem that arises with use of MPIX, a commercially available porphyrin, is that each resulting PSM exists as a pair of interconvertible diastereoisomers. Any physical studies done with PSMs assembled from MPIX will be complicated by this equilibrium. Our interest in developing a new generation of PSMs in which only a single stereoisomer is possible (*e.g.* **3**) prompted us to pursue the synthesis of mesoporphyrin II **4**,[3] a C_{2h}-symmetric isomer of MPIX. The symmetry properties of **4** have led to its incorporation in several designed receptors,[4–6] systems for the study of electron transfer,[7] and cofacial porphyrin dimers for catalysis of oxygen reduction.[8] The high symmetry of **4** leads to an unfortunate situation: the porphyrin is only soluble in a few, mostly uncommon, solvents including TFA and pyridine. Functionalization of the molecule is limited to reactions which are compatible with such solvents. One reaction which is complicated by lack of solubility is iron insertion. A literature search revealed no references for iron complexes of **4**, nor for any simple ester derivatives of an iron complex. Iron has been inserted into a cofacial porphyrin dimer constructed from **4**.[9] However, this approach may not work in all cases, for example in systems containing more than one metal binding site, or when iron insertion chemistry is not compatible. Because **4** is such an excellent scaffold upon which to build hemoprotein models, including PSMs, we felt it would be useful to explore ways of generating an activated ester derivative of an iron(iii) complex of **4**. Our approach was to insert iron into the previously reported bis-*p*-nitrophenyl ester **5** to produce **6**.

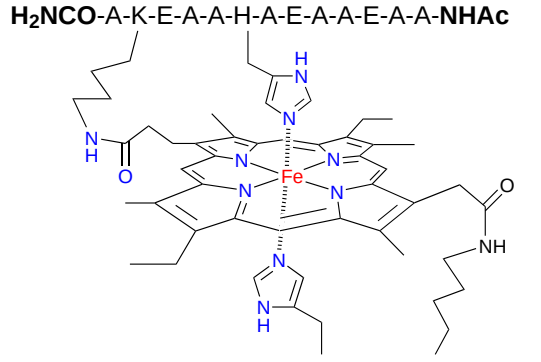
Synthesis of **4** followed Dolphin's method,[3] with subsequent conversion to **5** using the procedure of Collman *et al.*[8] Insertion of iron into porphyrins is commonly achieved using Fe^{II} salts in acetic acid (AcOH) at 100 °C, conditions under which **4** and its bis-methyl ester are completely insoluble. Although solubility of **5** in AcOH is slightly higher, no evidence for iron insertion was observed even after refluxing for several days in the presence of $Fe(OAc)_2$. Halogenated solvents, including dichloromethane (DCM) and chloroform, increase the solubility of **5** in AcOH. However, the low boiling points of these solvents leads to unacceptably long reaction times for iron insertion. In contrast, 1,1,2,2-tetrachloroethane (TCE; bp 147 °C) as cosolvent dramatically improved both solubility and rate of metal insertion. Thus, porphyrin **5** (43 mg) was dissolved in 6 ml of a 1 : 1 mixture of dry TCE and glacial acetic acid at 100 °C under N_2. Acetic anhydride (Ac_2O; 5 vol%) was added to eliminate any adventitious water. A saturated solution of $Fe(OAc)_2$ in AcOH[10] (3 ml; also containing 5% Ac_2O) was added to the porphyrin solution *via* syringe and the mixture was stirred at 100 °C until iron insertion was complete as determined by UV–VIS spectroscopy (*ca.* 1 h). The cooled mixture was filtered to



4 R = H; M = 2H
5 R = $C_6H_4NO_2$-*p*; M = 2H
6 R = $C_6H_4NO_2$-*p*; M = Fe^{III}-OAc



1 X = NHAc; Y = $CONH_2$
2 X = $CONH_2$; Y = NHAc



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remove excess $Fe(OAc)_2$ and the solvent was evaporated *in vacuo*. The dark purple residue was taken up in dry TCE, refiltered, and the solvent was again evaporated. No hydrolysis products were observed by FAB mass spectrometry.† However, a signal corresponding to TCE was present even after the sample had been heated for 12 h at 100 °C *in vacuo*. Elemental analysis† of the recovered material (49 mg; 90%) is consistent with the presence of one equivalent of TCE and an acetate ligand to the iron. The product is highly soluble in AcOH, TCE, DCM, THF, DMF, Me_2SO, dioxane, ethyl acetate, acetonitrile and pyridine.

Conversion of **6** to PSM **3** was achieved using our previously reported method for PSMs constructed from Fe^{III}–MPIX.[1] The Fe^{III} in **3** is low spin (Soret λ_{max} = 403 nm; not shown), demonstrating that it is bis-His coordinated. Helix content of the new compound, as determined by circular dichroism[1] (Fig. 1), is similar to that measured for **2**[2] (*ca.* 60%) at 8 °C in 2 mm potassium phosphate (pH 7). A monopeptide analogue appended on the lysine ε-amine by indoleacetic acid (**7**) exhibits a

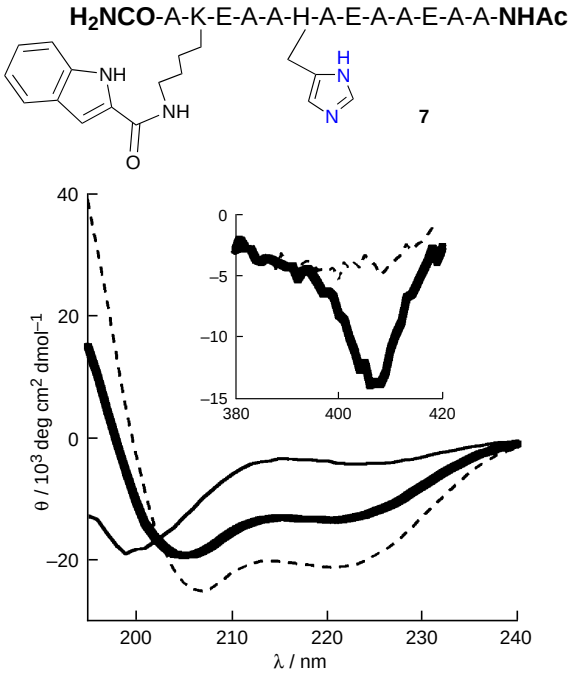

Fig. 1 CD spectra at 8 °C: 21 μm **7** (thin line) and 8.1 μm **3** (bold line) in water; 8.1 μm **3** (dashed line) in 7 : 3 H_2O–PrOH. Each sample is buffered to pH 7 with 2 mm potassium phosphate.

random coil conformation under the same conditions (Fig. 1), confirming that Fe–His coordination in **3** is responsible for helix induction. A Soret induced CD (ICD) signal is recorded as well (Fig. 1), commensurate with the location of the porphyrin within an asymmetric environment. Interestingly, the intensity of this Soret ICD band is only about 25% that of the corresponding band for the two diastereoisomers of **2** (not shown) in aqueous solution. As helix content is increased by addition of propan-1-ol (PrOH), the Soret ICD signal intensity diminishes (Fig. 1) whereas in **2** it increases (not shown).[2] Maximal helix content (*ca.* 95%) is recorded for both **2** and **3** in the presence of 30% (v/v) PrOH (spectrum for **3** is shown in Fig. 1). Additional studies on this and related PSMs will be reported in due course.

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Footnotes

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† FAB MS *m/z* (relative intensity): 865 (M^+ + 3, 10%), 864 (M^+ + 2, 31), 863 (M^+ + 1, 83), 862 (M^+, 100), 860 (M^+ −2, 13). Anal. calc. for $C_{46}H_{42}N_6O_8Fe{\cdot}C_2H_3O_2{\cdot}C_2H_2Cl_4$: C, 55.12; H, 4.35; N, 7.71. Found: C, 55.28; H, 4.09; N, 8.04.

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