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Synthesis and Characterization of a New Family of Iron Porphyrins

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Abstract: A significant tool for better understanding the complex nature of the cofactor of heme thiolate proteins such as Cytochromes P450 is the investigation of model compounds. In this context a new family of iron porphyrins has been synthesized by replacing the native thiolate ligand for a SO_3^- group coordinating the heme iron.

Keywords: Cytochrome P450 · Enzyme models · Iron porphyrins · Monooxygenases · Redox potential

1. Introduction

Cytochromes P450 are heme-thiolate proteins abundant in nature. These mono-oxygenases catalyze diverse reactions significant to the metabolism of xenobiotics as well as to the biosynthesis of important biomolecules [1].

Earlier investigations on iron porphyrin active site analogues carrying a thiolate as the fifth ligand (Fe(III)···S^-) [2] revealed a rather negative $E_o < -600$ mV (*vs.* SCE) in contrast to *e.g.* $P450_{cam}$, one of the best-known P450s, displaying $E_o = -280$ mV for the resting state. From more recent X-ray studies of $P450_{cam}$ it could be deduced that this difference is due to H-bonding of the thiolate ligand to amino acid residues of the protein backbone. Taken into account this obviously reduced charge density at sulfur a set of new enzyme models was conceived carrying a SO_3^- group as the fifth ligand.

2. Strategy

DFT calculations on SO_3^- coordinated iron porphyrins [3] supported our idea that one of the oxygens of the SO_3^- indeed coordinates to iron donating a charge of 0.3 instead of 1.0 for S^-. Energy-profile calculations further assigned the reactivity of the SO_3^- system to be very similar to the Fe···S^-- coordination. To improve the stability of the model compounds aromatic substituents were introduced at the oxygen-sensitive *meso*-positions to prevent μ-oxo dimer formation through steric congestion.

3. Synthesis

The synthetic pathway is outlined in the Scheme. From mesitylaldehyde (**1**) on reaction with pyrrol (**2**) a light sensitive dipyrromethane **3** was obtained, which underwent cyclization with 2-methoxy-benzaldehyde (**4**) to form the properly substituted porphyrin ring structure **5** following standard procedures [4]. The latter was deprotected to obtain the free phenol **6** as a mixture of atropisomers (α,α-**6** and α,β-**6**) that interconvert at room temperature. Condensation under diluted conditions with the S-protected 'bridge' **7**, which had been prepared according to our own protocol, gave product **8**. On treatment with a strong base under oxygen-saturated conditions **8** was converted to **9**. Intermediates that were not oxidized completely to SO_3^- under these conditions were collected and separately converted to **9** to increase the yield (**F**). Final iron insertion gave model compound **10**. A 2,6-dichlorophenyl-*meso*-substituted model **11** was synthesized in a similar fashion starting from 2,6-dichlorobenzaldehyde (**12**) instead of mesitylaldehyde (**1**).

4. Characterization

The X-ray structure of **10** (Fig. 1) validates the synthetic procedures and the assumption of one of the oxygens of the SO_3^- group coordinating to iron. The analysis further shows a slightly strained system with the iron out of plane towards the fifth ligand in agreement with the EPR spectrum displaying g-values characteristic of a high-spin Fe^{III} system (toluene, 94 K, g-factor: 5.7).

The UV-Vis spectrum of **10** exhibits typical iron porphyrin absorptions (CH_2Cl_2 λ_{max} : 415 nm (100, Soret) and 511 nm (12), 580 nm (3) , 691 nm (2) (Q-bands)).

Cyclovoltammetry (Fig. 2) reveals redox potentials of **10** and **11** similar to the resting state of P450 enzymes (Table). This underlines their value as model compounds in this field. A further advantage of iron complexes with SO_3^- is the stability relative to S^--coordination under aerobic conditions, which makes their handling much more convenient.

5. Reactivity

From spectroscopic data [1] the dominant reactive oxidant in the natural system is claimed to be a Fe^{IV}-porphyrin radical cation (Cpd I) which is formed from the

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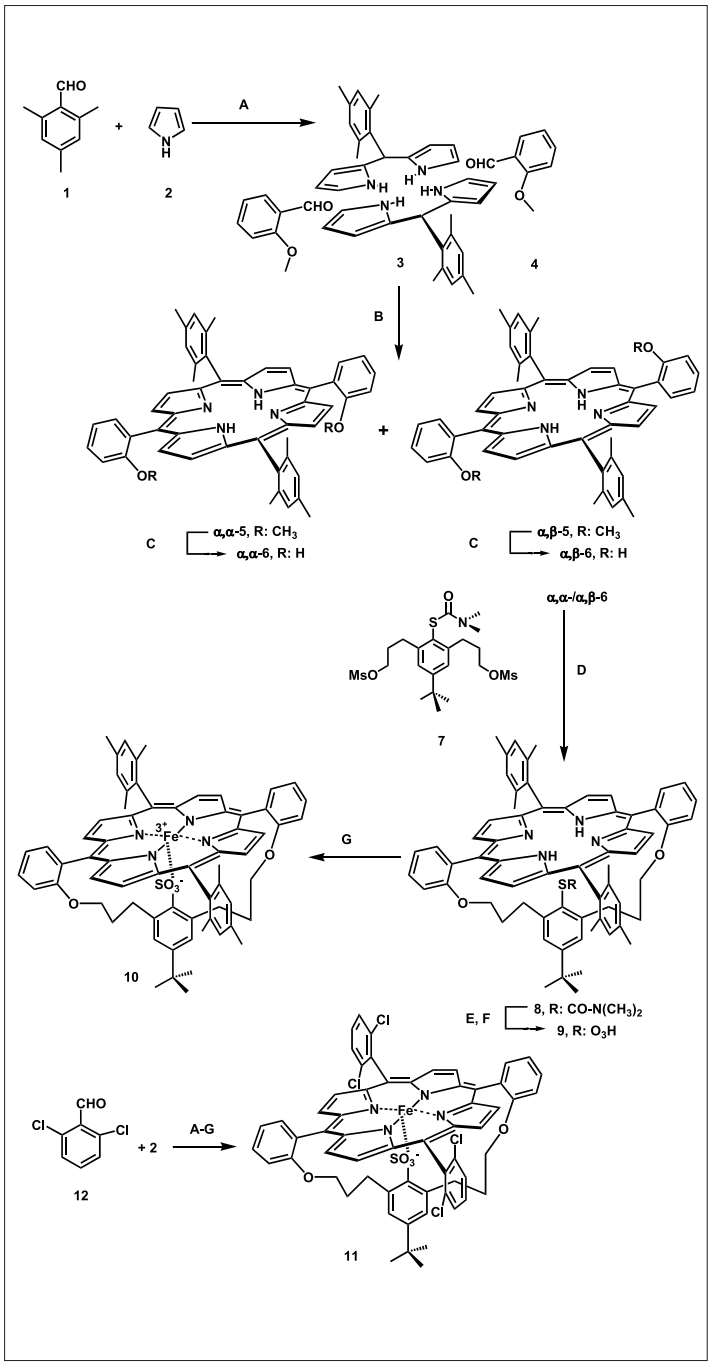

Scheme. Key: **A**: 0.3 equiv. BF_3OEt_2, 1 h, RT, 30%; **B**: 1.8 equiv. TFA, CH_2Cl_2, 0.5 h, RT, then 2 equiv. DDQ, 1 h, reflux, 27%; **C**: 32 equiv. BBr_3, CH_2Cl_2, 16 h, RT, 79%; **D**: 30 equiv. Cs_2CO_3, DMF, 0.5 h, 80 °C then 1.5 equiv. **7**, 4 h, 80 °C, 75%; **E**: 60 equiv. KOMe, dioxane, O_2, 16 h, reflux; **F**: 2 equiv. nBu_4NHSO_5, CH_2Cl_2, 2d, RT, **E&F**: 55%; **G**: 10 equiv. $FeBr_2$ and 2,6-lutidine, toluene, 1 h, reflux, 86%

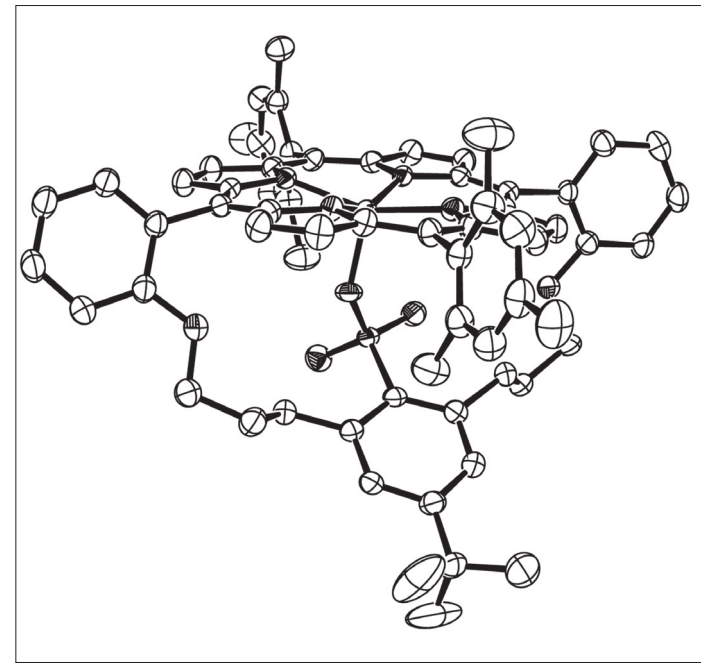

Fig. 1. ORTEP representation of model compound **10**

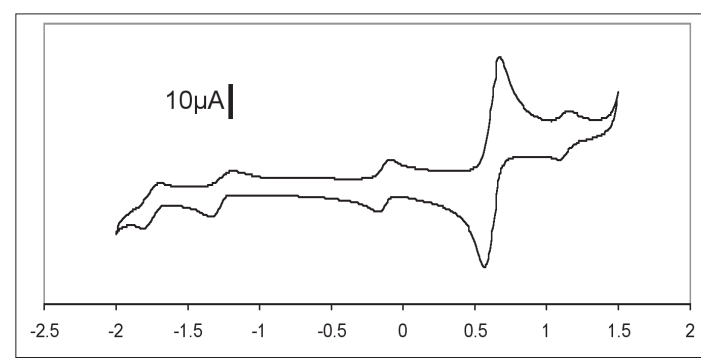

Fig. 2. Cyclovoltammogram of **10** (0.6 mM) in 0.1M $LiClO_4$ soln. in DMF with ferrocene as an internal standard. Scan rate: 100 mVs^{-1}

Table. Redox potentials of two model compounds measured by cyclovoltammetry. The given values (*vs.* SCE) are calculated from the relative potential to ferrocene used as an internal standard.

	1st ox	1st red	2nd red	3rd red
10	920 mV	–340 mV	–1480 mV	–1970 mV
11	1010 mV	–280 mV	–1420 mV	–1900 mV

resting state after substrate binding, reduction, oxygen binding and reductive oxygen cleavage [5].

The O=Fe(IV) porph.+ species (Cpd I) can be obtained from **10** or **11** on reaction with oxidants such as mCPBA, PhIO, H_2O_2 or certain N-oxides [6]. In that way we obtained UV-Vis spectra (Fig. 3) in agreement with published data for simpler porphyrin systems in the Cpd I – state [6][7].

6. Outlook

The synthesis and characterization of a new family of iron porphyrins has been accomplished and assigns them promising capacity as P450 enzyme models. These results are a prerequisite to employ our model compounds for further enzyme-mimetic studies which are currently under investigation. Therein our interest focuses on enzymatic reactions such as epoxidation [3], oxidation of non-activated positions and carbon–carbon bond cleavage. Preliminary results indicate the capability of our model compounds to cleave vicinal diols to the corresponding aldehydes. This reaction represents the last step of the C–C bond cleavage in the biotransformation of cholesterol to pregnenolone by P450scc (CYP 11A1) in the mammalian steroid hor-

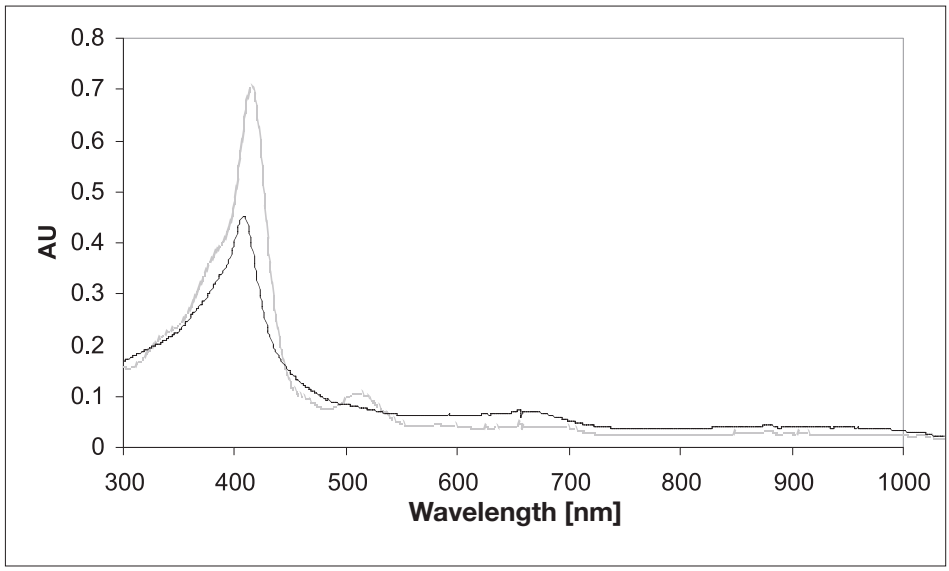

Fig. 3. UV-Vis change after addition of 1.5 equiv. of mCPBA (10 µM, CH_2Cl_2, –50 °C), dotted line: spectrum 30 sec after addition, full line: 25 min after addition

mone biosynthesis [8]. The same reaction sequence is also claimed to be part of other biotransformation processes *e.g.* in the biotin biosynthesis in *bacillus subtilis* [9].

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