ELSEVIER

Journal of Molecular Catalysis A: Chemical 113 (1996) 393–402

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

The synthesis of a new active-site analogue of cytochrome P450 carrying substrate recognition sites and athiolate ligand

Hamed Aissaoui, Sandro Ghirlanda, Christoph Gmür, Wolf-Dietrich Woggon *

Institut für Organische Chemie der Universität Basel, St. Johanns-Ring 19, CH-4056 Basel, Switzerland

Received 29 February 1996; accepted 29 April 1996

Abstract

The synthesis of a new mono-bridged iron(III)porphyrin **2** is described which contains all structural elements significant to an active-site analogue of cytochrome P450: (i) a thiolate ligand coordinating to the iron, and (ii) a substrate-recognition site provided by two substructures derived from Kemp's acid which are positioned perpendicular to the porphyrin plane. ^{1}H-NMR experiments show that esters like dimethyl glutarate bind with $K = 137\ M^{-1}$.

Keywords: Cytochrome P450; Enzyme Models; Heme-thiolate proteins; Iron(III)porphyrins; Kemp's acid; Porphyrins; Substrate recognition; Thiolate ligand

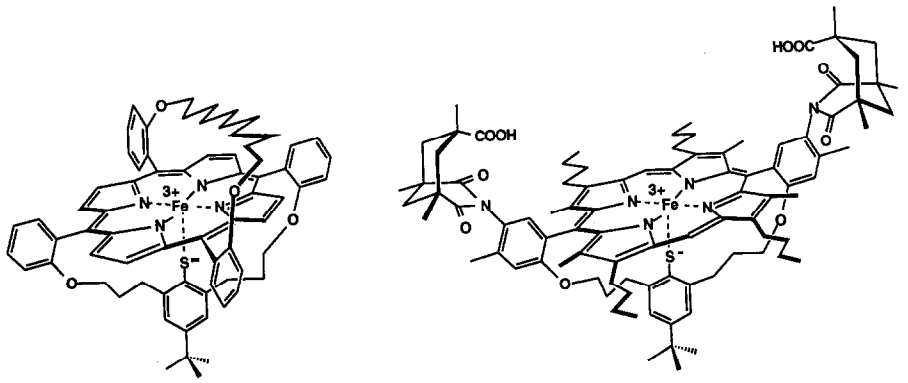
1. Introduction

The catalytic domain common to all heme-thiolate proteins contains an iron(III)proto-porphyrin(IX) complex with a thiolate ligand coordinating to the iron. These proteins are abundant in nature comprising important enzymes such as NO-synthase, chloroperoxidase, and cytochrome P450 which are significant to regulatory functions, and to the metabolism of endogenous compounds and xenobiotics [1]. Due to the fact that X-ray structures are known of four different cytochromes P450 [2], and since their mechanisms have been studied in great detail, our knowledge of this enzyme family is relatively advanced [3]. Nevertheless there is an ongoing debate on the significance of the quite unusual thiolate ligand with respect to spin state equilibria of the resting state, concerning the cleavage of molecular oxygen, and the redoxpotential of the iron porphyrin, and, last but not least the electronic nature of the high-valent iron(IV)oxo intermediate which is capable of e.g. O-insertion into nonactivated C–H bonds [4]. It can be expected that answers to these questions can be provided by means of synthetic active site analogues. Moreover such enzyme models may satisfy the curiosity of organic chemists concerning the unparalleled reactivity of this porphyrin complex.

In order to mimic P450 reactivity many interesting model compounds were synthesized in the last twenty years. However, most porphyrins were rather remote analogues of the active site because the capricious iron was replaced by

* Corresponding author.

PII S1381-1169(96)00166-5



1 2

Fig. 1. Synthetic active-site analogues of cytochrome P450.

manganese, the thiolate ligand was absent, and the difficult reductive cleavage of O_2 was circumvented using oxen donors like PhIO which in a shunt-pathway directly yielded the high-valent metaloxo porphyrin [1].

In contrast we have focused our efforts on the synthesis of iron porphyrin thiolate complexes, and we have recently prepared the doubly-bridged porphyrin **1** [5], Fig. 1, which is well designed for O_2 cleavage and O-insertion into nonactivated C–H bonds; in this instance hydroxylating one of the CH_2 groups of the substrate bridge which spans the porphyrin on the face opposite to the thiophenolate ligand [6]. In order to establish catalytic assemblies we decided to attach Kemp acids to a porphyrin to provide substrate recognition sites. The synthesis of **2** is described in this paper.

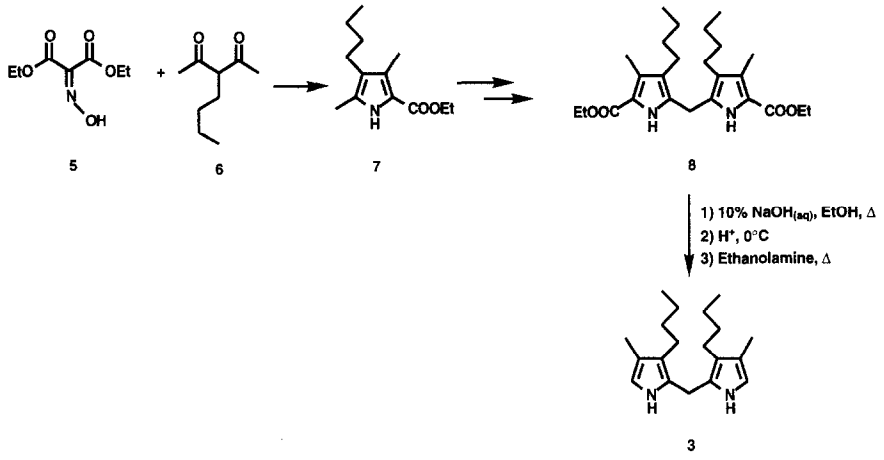
2. Results and discussion

Key intermediates of the synthesis of the iron porphyrin **2** are the pyrromethane **3** [7], and the aldehyde **4**. The former was prepared in a straightforward manner from diethyloximinomalonate **5** and 3-butyl-2,4-pentanedione **6** via the pyrrol **7** and the bis-ethoxycarbonyl pyrromethane **8**, Scheme 1. In order to furnish pure crystalline, alpha-free **3** treatment of **8** with ethanolamine at reflux proved to be the best method [8]. The aldehyde **4** was prepared from the commercially available 4-amino-3-methyl phenol **9** which after protection of the amino group was formylated according to Duff [9]. The resulting *o*-hydroxy aldehyde **10** was subsequently protected to yield the pivaloyl ester **4**, Scheme 2.

Condensation of **3** and **4** in the presence of catalytic amounts of *p*-TsOH in CH_3CN [10] furnished the porphyrin **11** in 73% yield as a mixture of $\alpha\alpha$- and $\alpha\beta$-atropisomers, Scheme 3. After deprotection of the phenol group $\alpha\alpha$-**12** was coupled with the dimesylate **13** that carries the protected thiophenolate ligand [5]. The mono-bridged porphyrin **14** was obtained in 68% yield.

After consecutive treatment of **14** with HCl and KOMe the diaminophenyl porphyrin **15** was isolated, ready for the attachment of Kemp's acid chloride **16** [11]. The latter was obtained pure from Kemp's triacid **17** on reaction of the corresponding Kemp's acid anhydride **18** [12] with thionyl chloride, Scheme 4.

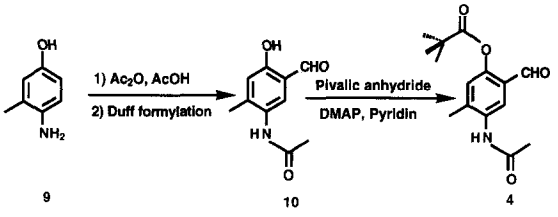
The diamino porphyrin **15** was then acylated with 2.5 equivalents of **16** to yield the di-Kemp porphyrin **19a** in 70% and 20% of the mono-Kemp porphyrin **19b**. The latter ligand is of particular interest because chiral recognition-sites can be connected to the free amino group. Insertion of iron into **19a** with $FeBr_2$/lutidine furnished the HPLC-pure iron(III)complex **2** in



Scheme 1. Synthesis of the α-free dipyrromethane **3**.

83% yield. This active-site analogue of cytochrome P450 has been characterized by UV (Soret-band 402 nm), MS (Maldi-Tof: 1579), ESR ($g = 5.245$, $g = 1.955$, toluene, 5 K), ^{1}H-NMR (*meso*-protons at 60 and 40 ppm), and cyclic voltammetry ($E_0 = -675$ mV vs. SCE in DMF/0.1 M $LiClO_4$; for **1** $E_0 = -607$ mV under the same conditions) Accordingly **2** is a high-spin iron(III)porphyrin suitable to mimic different features of heme-thiolate proteins, namely the catalytic hydroxylation of nonactivated C–H bonds. Investigating the binding of substrates by ^{1}H-NMR spectroscopy it was shown that esters like dimethyl glutarate typically bind to **2** with $K = 137$ M^{-1} (C_6D_6). Oxidation experiments will be reported in due course [4].

Another interesting aspect of **2** is its ability to bind solvent molecules via H-bridges in the cavity provided by the Kemp acids. Thus it will be possible to investigate the question whether H_2O binding to an iron(III)porphyrin with thiolate coordination establishes a low-spin or a high-spin complex. In this context it is important to note that the resting state of $P450_{cam}$, which, according to the X-ray structure [2] harbours a cluster of six water molecules in the substrate binding domain, is predominantly low-spin [3]. However, in view of the fact that both axial ligands in question are weak these results are quite surprising and still a matter of debate [13]. It seems more likely that in $P450_{cam}$ the water cluster is polarized such that the iron(III) sees an HO^- rather than H_2O. The complex **2** is a suitable model system to investigate this hypothesis notably due to the presence of the carboxylic acids of the Kemp groups which can be readily manipulated to polarize solvent molecules in the cavity [14].

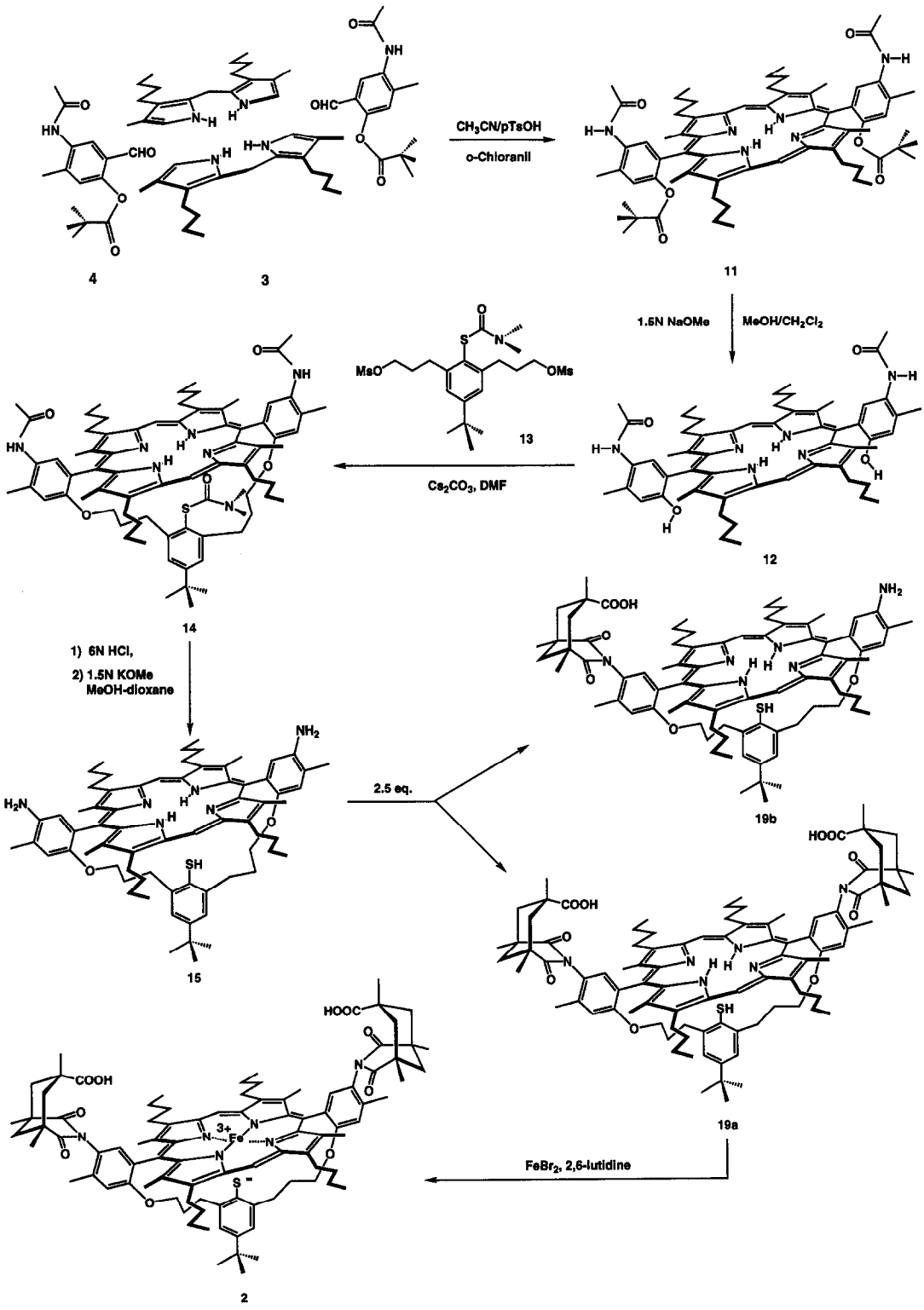


Scheme 2. Preparation of the protected aldehyde **4**.

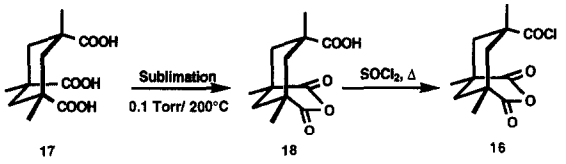
3. Experimental

3.1. General methods

All reactions were performed in freshly distilled solvents in an inert atmosphere. The insertion of the iron (III) into the porphyrin was carried out in a 'Glove Box' with distilled solvents which had been degassed just prior to use. Analytical thin layer chromatography (TLC) was conducted on 0.2 mm Merck silica gel 60



Scheme 3. Synthesis of the enzyme model **2**.



Scheme 4. Preparation of Kemp's acid chloride **16**.

F254 plates. ^{1}H-NMR spectra were recorded on a Brucker AC-300, ^{13}C-NMR spectra were measured on a Varian XL-50 (50 MHz) and a Varian XL-100 (100 MHz), chemical shifts (δ) are given in ppm with tetramethylsilane as an internal standard; coupling constants are reported in Hz. UV–VIS spectra were recorded on a Hewlett Packard 8452A Diode Array. IR spectra were measured on a Perkin–Elmer 1600. Melting points were determined on a Mettler FP52 melting point apparatus and are uncorrected. Mass spectra were recorded on a Varian MAT 112S at an ionization potential of 70 eV for CI and EI, and a Finnigan TSQ-700 (ESI) spectrometers. MALDI–TOF spectra were measured on a Vestec Benchtop II. EPR spectrum was measured on a Brucker ESP-300 system with a TE_{102} cavity.

3.2. 3,3'-Dibutyl-4-4'-dimethyl-2,2'-dipyrrylmethane (3) [7]

The diester dipyrrylmethane **8** (7.58 g, 17.61 mmol) was dissolved in 133 ml of refluxing EtOH. 35.5 ml of 10% $NaOH_{(aq)}$ was added and the reaction was stirred at reflux for 5 h. Solvents were then removed under vacuo and to the residue was added 100 ml of water. The resulting suspension was then extracted 3 times with CH_2Cl_2 to remove the remaining diester. The aqueous phase was cooled at 0°C and neutralized with glacial acetic acid to give a white precipitate which was then extracted into ether (3×50 ml).

Removal of ether under vacuo gave the crude diacid. This was dissolved in 45 ml of ethanolamine under argon, in the dark, and refluxed for 6 h. The dark brown solution was poured into ice-water, extracted 3 times with CH_2Cl_2. The combined organic phases were taken together and solvent was removed under vacuo. Purification by flash chromatography (silica gel, CH_2Cl_2, argon, dark) gave the product which was then recrystallised from degassed hexane to give 2.8 g (55%) of the title compound as white crystals.

mp: 97.5–98.5°C

TLC: (CH_2Cl_2) $R_f = 0.85$

IR ($CHCl_3$): 3460s, 3360br, 2990m, 2960s, 2920s, 2860m, 1590w, 1470m, 1460m, 1380w, 1310w, 1240w, 1120br, 1080m, 1040w, 980w.

^{1}H-NMR (300 MHz, $CDCl_3$): 7.37 (br.s, H–N); 6.34 (s, H–C(5), H–C(5')); 3.79 (s, H–C(6)); 2.41 (t, H–C(7), H–C(7'), $^{3}J_{(7,8)} = {}^{3}J_{(7',8')} = 7.5$); 2.17 (s, H_3C–C(4), H_3C–C(4'); 1.55–1.45 (m, H–C(8), H–C(8')); 1.45–1.38 (m, H–C(9), H–C(9')); 0.93 (t, $H_3C(10)$, $H_3C(10')$, $^{3}J_{(10,9)} = {}^{3}J_{(10',9')} = 7.10$).

^{13}C-NMR (100 MHz, $CDCl_3$): 124.96; 119.74; 118.10; 113.68 (C(5), C(5')); 33.70 (C(8), C(8')); 24.04 (C(7), C(7')); 22.80 (C(9), C(9'), C(6)); 14.02 (C(10), C(10')); 10.36.

MS (CI, NH_3): 288 (16), 287 (100, $[M+1]^+$), 150 (25), 149 (14).

Anal. calc. for $C_{19}H_{30}N_2$ (286.45): C 79.13, H 10.48, N 9.78; found: C 79.94, H 10.46, N 10.00.

3.3. 5-Acetylamino-2-hydroxy-4-methylbenzaldehyde (10)

To a suspension of 10.42 g (53.9 mmol) of fresh sublimed 4-acetylamino-3-methyl-phenol prepared (from 4-amino-3-methyl-phenol **9**) in 100 ml of trifluoroacetic acid, was added 13.34 g (80.9 mmol) of urotropin. The solution was stirred at 100°C for 1 h under argon. After cooling, the mixture was concentrated under vacuo. The orange residue was poured into ice water and treated with 10% $NaOH_{(aq)}$ until pH 3–4, extracted 3 times with CH_2Cl_2, washed with brine, dried over Na_2SO_4, to give a crude yellow solid. Recrystallisation from CH_3CN/AcOEt: 1/1 gave 6.48 g (62%) of **10** as yellow solid.

mp: 179.2–181.4°C

IR (KBr): 3400s, 3270s, 1660s, 1535m.

^{1}H-NMR (300 MHz, $CDCl_3$): 10.86 (s, 1 H, OH); 9.75 (s, 1 H, CHO); 7.79 (s, 1 H, H–C(6)); 7.19 (s, 1 H, HN); 6.78 (s, 1 H, H–C(3)); 2.24 (s, 3 H, CO–CH_3)); 2.18 (s, 3 H, Ar–CH_3).

^{13}C-NMR (50 MHz, DMSO-d_6): 191.12 (d, CHO); 168.80 (CO), 158.50 (s, C(2)); 142.90 (s, C(5)); 129.00 (s, C(4)); 126.00 (d, C(6)); 120.40 (s, C(1)); 118.70 (d, C(3)); 23.20 (q); 18.70 (q).

MS (EI): 193 (88, $[M]^+$, 151 (100), 43 (30).

Anal. calc. for $C_{10}H_{12}NO_3$ (194.22): C62.17, H5.74, N7.25; found: C61.98, H5.80, N7.44

*3.4. 5-Acetylamino-2-pivanoyl-4-methyl-benzaldehyde (**4**)*

To a solution of 3.64 g (18.91 mmol) of 4-methoxybenzaldehyde **10** in 35 ml of pyridine was added 4.32 g (22.61 mmol) of pivalic anhydride and 0.11 g (0.94 mmol) of DMAP.

After stirring at RT for 16 h, the solution was concentrated under vacuo, the residue was poured into 100 ml of 10% $HCl_{(aq)}$, extracted 3 times with CH_2Cl_2. The combined organic phases were washed with brine, dried over Na_2SO_4. Recrystallisation from Et_2O/pentane:1/1 gave 3.48 g (66.5%) of **4** as white solid.

mp: 140–141°C

IR ($CHCl_3$): 3440m, 3360br, 2980m, 2940w, 2910w, 2880w, 1750s, 1700s, 1620m, 1580w, 1540m, 1480m, 1460w, 1450w, 1410w, 1400w, 1380w, 1270m, 1250m, 1180m, 1110s, 1030m, 910m.

^{1}H-NMR (300 MHz, $CDCl_3$): 9.98 (s, 1 H, CHO); 8.11 (s, 1 H, H–C(3)); 7.28 (br.s, HN); 6.91 (s, 1 H, H–C(6)); 2.24 (s, 3 H, CH_3–C(5)), 2.18 (s, 3 H, $NCOCH_3$); 1.40 (s, 9 H, tert-Butyl).

^{13}C-NMR (50 MHz, $CDCl_3$): 187.75 (CHO); 177.30; 169.18; 148.47; 139.73; 134.02; 126.51; 126.31; 124.84, 39.20, 27.00; 23.43; 18.06.

MS (CI, NH_3): 296 (21), 295 (100), $[M + NH_4]^+$, 211 (21).

Anal. calc. for $C_{15}H_{19}NO_4$ (277.32): C 65.00, H 6.85, N 5.05; found: C 64.84, H 6.94, N 4.82.

*3.5. 10,20-Bis(5-acetylamino-2-pivaloyloxy-4-methyl-phenyl)-3,7,13,17-tetrabutyl-2,8,12,18-tetramethyl-porphyrin (αα-**11**) and 10,20-bis(5-acetylamino-2-pivaloyloxy-4-methyl-phenyl)-3,7,13,17-tetrabutyl-2,8,12,18-tetramethyl-porphyrin (αβ-**11**)*

To a solution of 1.01 g (3.66 mmol) of aldehyde **4** in 50 ml of degassed CH_3CN is added in the dark 1 g (3.5 mmol) of dipyrrylmethane **3** and 6 mg (0.35 mmol) of *p*-toluenesulfonic acid. The dark red solution was stirred at RT for 20 h in the dark after which time 1.3 g (5.25 mmol) of *o*-chloranil in 10 ml of THF was added. The mixture was stirred at RT for 1 h. When the reaction was deemed complete by UV, the solvents were removed under vacuo and the crude residue was purified by flash chromatography (silica gel, CH_2Cl_2/ MEOH: 99/1 to 98/2) to give 1.25 g (33%) of αα-**11** and 1.5 g (40%) of αβ-**11** atropoisomers.

αα-**11**:

TLC: (CH_2Cl_2/MEOH:95/5) $R_f = 0.49$

mp: > 250°C

UV–VIS (CH_2Cl_2): 408 (232), 506 (26), 540 (8), 574 (8).

^{1}H-NMR (300 MHz, $CDCl_3$): 10.16 (s, 2 H, *meso*-H), 8.42 (s, 2 H, N–H); 7.43 (s, 2 H, Ar–H); 7.20 (s, 2 H, Ar–H); 4.05–3.95 (m, 8 H, Pyrrol–CH_2–); 2.67 (s, 12 H, Pyrrol–CH_3); 2.62 (s, 6 H, Ar–CH_3); 2.25 (s, 6 H, CO–CH_3); 2.23–2.16 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.79 –1.71 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_2–); 1.10 (t, $^3J = 7.35$; 12 H, Pyrrol–$(CH_2)_3$–CH_3); −0.37 (s, 9 H; tert-Bu); −2.41 (br.s, 2 H, Porphyrin N–H).

MS (CI, NH_3): 1089 (9), 1088 (34), 1087 (77), 1086 (100, $[M + 1]^+$).

$C_{68}H_{88}N_6O_6$ (1084.68).

αβ-11:

TLC (CH_2Cl_2/MeOH: 95/5): $R_f = 0.54$

UV–VIS (CH_2Cl_2): 408 (330), 506 (28), 540 (12), 574 (12).

^{1}H-NMR (300 MHz, $CDCl_3$): 10.20 (s, 2 H, *meso*-H); 8.44 (s, 2 H, N–H); 7.48 (s, 2 H, Ar–H); 4.05–3.92 (m, 8 H, Pyrrol–CH_2–); 2.67 (s, 12 H, Pyrrol–CH_3); 2.63 (s, 6 H, Ar–CH_3); 2.24 (s, 6 H, $COCH_3$); 2.18–2.13 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.77–1.69 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_2–); 1.08 (t, $^3J = 7.34$, 12 H, Pyrrol–(CH_2)–CH_3); −0.28 (t, 9 H, tert.-Bu); −2.41 (s, 2 H, Porphyrin N–H).

MS (CI, NH_3): 1089 (9), 1088 (34), 1087 (77), 1086 (100, $[M+1]^+$).

$C_{68}H_{88}N_6O_6$ (1084.68).

*3.6. 10,20-Bis(5-acetylamino-2-hydroxy-4-methyl-phenyl-)-3,7,13,17-tetrabutyl-2,8,12,18-tetramethyl-porphyrin (**12**)*

To a solution of 1.17 g (1.07 mmol) of porphyrin αα-**11** in 40 ml of CH_2Cl_2 was added dropwise 3.6 ml (5.38 mmol) of 1.5 M sodium methylate solution in MeOH. The reaction was stirred at RT for 16 h and then poured into a mixture of 250 ml of CH_2Cl_2, 10 ml of MeOH, 250 ml H_2O. The aqueous phase was extracted 3 times with CH_2Cl_2.

The combined organic phases were washed with saturated NH_4Cl, brine, dried over Na_2SO_4 and concentrated under vacuo. Purification by flash chromatography (silica gel, CH_2Cl_2/MeOH:95/5) gave 0.94 g (95%) of the title compound αα-**12** as a dark red solid.

TLC (CH_2Cl_2/MeOH:95/5): $R_f = 0.37$

UV–VIS (CH_2Cl_2): 408 (194), 508 (15), 542 (7), 574 (8), 626 (4).

^{1}H-NMR (300 MHz, $CDCl_3$): 10.24 (s, 2 H, *meso*-H); 7.97 (s, 2H, N–H); 7.21 (s, 2 H, Ar–H); 7.10 (s, 2 H, Ar–H); 4.90 (br.s, 2 H, O–H); 4.04–3.95 (m, 8 H, Pyrrol) CH_2–); 2.72 (s, 12 H, Pyrrol–CH_3); 2.58 (s, 6 H, Ar–CH_3); 2.20 (s, 6 H, $COCH_3$); 2.20–2.12 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.80–1.71 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_3); 1.10 (t, $^3J = 7.4$, 12 H, Pyrrol–$(CH_2)_3$–CH_3); −2.20 (br.s, 2 H, Porphyrin N–H).

MS (ESI, $CHCl_3$): 918 ($[M+1]^+$)

$C_{58}H_{72}N_6O_4$ (917.26).

*3.7. 10,20-((5-Acetylamino-4-methyl-)-((4-(tert-butyl)-2-N,N-dimethylcarbamoyl)thio-1,3-phenylene)bis(trimethylene-oxy))di-2,1-phenylene)-2,8,12,18-tetrabutyl-3,7,13,17-tetramethyl-porphyrin (**14**)*

To a solution of 340 mg (0.371 mmol) of the dihydroxyporphyrin αα-**12** in 150 ml of DMF was added 3.65 g (12 mmol) of caesium carbonate. The dark red mixture was stirred for 3 h at RT. A solution of 210 mg (0.412 mmol) of dimesylate **13** [5] in 5 ml of DMF was then added dropwise, after which 4.5 mg (0.0371 mmol) of DMAP was added. The reaction was stirred for 16 h at 90°C after which DMF was removed under vacuo. The crude residue was taken up in a mixture of 50 ml CH_2Cl_2, 10 ml MeOH, and washed with H_2O, 1 M HCl, a portion of sat $NaHCO_3$, and brine. The organic phase was dried over Na_2SO_4 and concentred under vacuo. Purification by flash chromatography (silica gel CH_2Cl_2/MeOH:95/5) gave 312 mg (68%) of the title compound **14** as a red solid.

TLC (CH_2Cl_2/MeOH:95/5): $R_f = 0.75$

mp: > 250°C

UV–VIS (CH_2Cl_2): 412 (173), 508 (23), 576 (12), 628 (6), 658 (5).

^{1}H-NMR (300 MHz, $CDCl_3$ + CD_3OD:4/1): 10.2 (s, 2 H, *meso*-H); 8.51 (s, 2 H, H–NAc); 7.61 (s, 2 H, Ar–H); 7.12 (s, 2 H, Ar–H); 6.39 (s, 2 H, C(3′), H–C(5′)); 4.1–3.9 (m, 8 H, Pyrrol–CH_2–); 3.8–3.4 (m, 4 H, O–CH_2–); 2.9, and 2.8 (2s, 6 H, $CON(Me)_2$); 2.7 (s, 2 H, Ar–CH_3); 2.55, and 2.6 (2s, 6 H, Pyrrol–CH_3); 2.25 (s, 6 H, HNCO–CH_3); 2.15–1.6 (m, 16 H, Pyrrol–CH_2–CH_2–CH_2–); 1.05–1.2 (m, 12 H, Pyrrol–$(CH_2)_3$–CH_3); 0.97 (s, 9 H, tert.-Bu); 0.8 0.6 (m, 8 H, O–CH_2–CH_2–); 0.42–0.35 (m, 4 H, O–$(CH_2)_2$–CH_2–); 2.25 (s, 2 H, Porphyrin N–H).

MS (ESI, CH_2Cl_2/MeOH: 9/1): 1273.5

($[M + K]^+$), 1257.5 ($[M + Na]^+$), 1235 ($[M + H]^+$),

$C_{77}H_{101}N_7O_5S$ (1235.52).

3.8. 10,20-((5-Amino-4-methyl-)-((4-tert-butyl)-2-mercapto-1,3-phenylene)bis(trimethylenoxy))-di-2,1-phenylene)-2,8,12,18-tetrabutyl-3,7,13,17-tetramethyl-porphyrin (15)

To a solution of 160 mg (0.13 mmol) of thiocarbamate porphyrin **14** in 1 ml of CH_2Cl_2/MeOH(1/1), 120 ml of 6 M $HCl_{(aq)}$ was added. The green solution was stirred at 130°C overnight. After cooling, the reaction was brought to pH 10 with 50% $NaOH_{(aq)}$. The reaction was then extracted twice with CH_2Cl_2/MeOH (4/1). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentred gave 125 mg (83%) of the intermediate diaminothiocarbamate. To a solution of 125 mg (0.108 mmol) of this intermediate in 20 ml of dioxane, was added dropwise 9.7 ml (14.55 mmol) of 1.5 M potassium methylate solution in MeOH. The solution was concentrated by removal of MeOH with a flow of argon until it became green. The reaction was then stirred at reflux for 2 h. After cooling, 4 ml of saturated NH_4Cl, 40 ml of H_2O, and 40 ml of CH_2Cl_2 was added. The aqueous phase was extracted 3 times with CH_2Cl_2.

The combined organic phases were washed with brine, dried over Na_2SO_4. Purification by flash chromatography (silica gel, CH_2Cl_2/MeOH:9/1) gave 95 mg (82%) of **15** as a red–brown solid.

TLC (CH_2Cl_2/MeOH: 9/1): $R_f = 0.85$

mp: > 250°C

UV–VIS (CH_2Cl_2): 410 (100), 506 (8), 542 (5), 576 (7), 628 (3).

^{1}H-NMR (300 MHz, $CDCl_3$): 10.21 (s, 2 H, *meso*-H); 7.89 (s, 2 H, Ar–H); 6.97 (s, 2 H, Ar–H); 6.15 (s, 2 H, H–C(5′), H–C(3′)); 4.02–3.45 (m, 8 H, Pyrrol–CH_2–, and O–CH_2–); 2.78 (s, 12 H, Pyrrol–CH_3); 2.60 (s, 6 H, Ar–CH_3); 2.40–2.15 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.75–1.65 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_2–); 1.21–1.12 (m, 12 H, Pyrrol–$(CH_2)_3$–CH_3); 0.96 (s, 9 H, tert-Bu); 0.61–0.45 (m, 4 H, O–CH_2–CH_2–, O–$(CH_2)_2$–CH_2–); −2.12 (br.s, 2 H, Porphyrin–NH); −3.25 (br.s, 1 H, SH).

MS (ESI): 1080 (100, $[M + H]^+$).

$C_{70}H_{90}N_6O_2S$ (1079.60).

3.9. 1,5,7-Trimethyl-2,4-dioxo-3-oxa-bicyclo[3.3.1]nonane-7-carboxylacid(18) [11]

500 mg of Kemp's triacid **17** was sublimed at 0.1 mm Hg/200°C for 4 h to give 435 mg (92%) of the title compound as colourless crystals.

mp: 251–252.5°C

IR (KBr): 3250–2200br, 1793s, 1771s, 1702s, 1461s, 1388w, 1328w, 1284s, 1219s, 1183m, 1129s, 1093s, 1004s.

^{1}H-NMR (300 MHz, pyridine-d_5): 2.95 (d, 2 H, $^2J = 13.85$, H_{eq}); 2.05 (d, 1 H, $^2J = 12.97$, H_{eq}); 1.37–1.20 (m, 12 H, including 1.34 [s, 6 H, 2Me], 1.28 [s, 3 H, 1Me]).

MS (CI, NH_3): 258.56 (100, $[M + NH_4]^+$).

Anal. calc. for $C_{12}H_{16}O_5$ (240.26): C 59.99, H 6.71, O 33.30; found: C 60.16, H 6.78 O 33.21.

3.10. 1,5,7-Trimethyl-2,4-dioxo-3-oxa-bicyclo[3.3.1]nonane-7-carbonylchlorid (Kemp's chloride: 16) [12]

200 mg (0.833 mmol) of anhydride acid **10** was refluxed in 3 ml of thionyl chloride for 3 h under argon. After cooling, all volatile components were removed under vacuo to give 189 mg (88%) of the title compound as a pale yellow solid.

mp: 265–268°C

IR (KBr): 1771s, 1452s, 1390m, 1326m, 1224m, 1126s, 1091s, 1004s, 927s, 901s, 840s, 824s.

^{1}H-NMR (300 MHz, pyridine-d_5): 2.85 (d, 2 H, $^2J = 14$, H_{eq}); 2.01 (d, 1 H, $^2J = 13.2$, H_{eq}); 1.47–1.23 (m, 12 H, including 1.34 [s, 6 H, 2 Me], 1.31 [s, 3 H, 1 Me]).

MS (CI, NH_3): 276.1 (100, $[M + NH_4]^+$.

Anal. calc. for $C_{12}H_{15}O_4Cl$ (258.7): C 55.71, H 5.84, O 24.74; found: C 55.52, H 5.66, O 24.52.

*3.11. 10,20-((5-(7-Hydroxycarbonyl-1,5,7-trimethyl-2,4-dioxo-3-aza-bicyclo[3.3.1]non-3-yl)-4-methyl-)-((4-(tert-butyl)-2-mercapto-1,3-phenylene)bis(trimethyleneoxy))di-2,1-phenylene)-2,8,12,18-tetrabutyl-3,7,13,17-tetramethyl-porphyrin (**19a**) and 10,20-((5-(Amino)-5'-(7-hydroxycarbonyl-1,5,7-trimethyl-2,4-dioxo-3-aza-bicyclo[3.3.1]non-3-yl)-4-methyl)-((4-(tert-butyl)-2-mercapto-1,3-phenylene)-bis-(trimeth - ylenoxy))-di-2,1-phenylene)-2,8,12,18-tetrabutyl-3,7,13,17-tetramethyl-porphyrin (**19b**)*

To a solution of 88 mg (0.082 mmol) of the porphyrin **15** in 10 ml of pyridine was added 53 mg (0.205 mmol) of Kemp's chloride **16** and 5 mg (0.04 mmol) of DMAP.

The solution was stirred for 25 h at 90°C under argon. After cooling and removal of pyridine under vacuo, the crude product was purified by flash chromatography (silica gel, AcOEt/toluene:4/6) to give 85 mg (70%) of **19a** as a red solid and 21 mg (18%) of the mono-Kemp's acid porphyrin **19b**.

19a:

TLC (AcOEt/toluene: 4/6): $R_f = 0.65$

mp: > 250°C

UV–VIS (CH_2Cl_2): 416 (143), 486 (21), 510 (23), 622 (5), 648 (4).

^{1}H-NMR (300 MHz, acetone-d_6): 10.15 (s, 2 H, *meso*-H); 8.37 (s, 2 H, Ar–H); 7.11 (s, 2 H, Ar–H); 6.25 (s, 2 H, H–C(5'), H–C(3')); 4.03–4.00 (m, 8 H, Pyrrol–CH_2–); 3.64–3.66 (m, 4 H, O–CH_2–); 2.87–2.73 (m, 16 H, including [s, 12 H, Pyrrol–CH_3]); 2.49 (d, $^2J = 14.1$, 2 H, H_{eq}); 2.28 (s, 6 H, Ar–CH_3); 2.13–2.04 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.79–1.76 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_2–); 1.45 (s, 16 H, 4 Me); 1.29 (s, 6 H, 2 Me); 1.20 (d, $^2J = 12.5$, 4 H, H_{ax}); 1.06 (t, 12 H, Pyrrol–$(CH_2)_3$–CH_3); 0.86 (s, 9 H, tert-Bu); 0.71–0.62 (m, 4 H, O–CH_2–CH_2–); 0.47–0.39 (m, 4 H, O–$(CH_2)_2$–CH_3); −2.1 (s, 1 H, Porphyrin–NH); −2.8 (s, 1 H, Porphyrin–NH); −3.1 (s, 1 H, SH).

MALDI–TOF: 1524

$C_{94}H_{118}N_6O_{10}S$ (1524.28).

19b:

TLC (AcOEt/toluene: 4/6): $R_f = 0.65$

mp: > 250°C

UV–VIS (CH_2Cl_2): 414 (216), 472 (4), 510 (19), 558 (3), 576 (8), 616 (1).

^{1}H-NMR (300 MHz, acetone-d_6): 10.17 (s, 2 H, *meso*-H); 8.38 (s, 2 H, Ar–H); 7.98 (s, 2 H, NH_2); 7.15 (s, 2 H, Ar–H); 6.22 (s, 2 H, H–C(5'), H–C(3')); 4.15–3.98 (m, 8 H, Pyrrol–CH_2–); 3.75–3.59 (m, 4 H, O–CH_2–); 2.90–2.77 (m, 12 H, including 2s, Pyrrol–CH_3); 2.37 (d, $^2J = 14.10$, 1 H, H_{eq}); 2.30 and 2.28 (2s, 6 H, Ar–CH_3); 2.15–2.08 (m, 8 H, Pyrrol–CH_2–CH_2–); 1.90–1.77 (m, 8 H, Pyrrol–$(CH_2)_2$–CH_2–); 1.46 (s, 6 H, 2 Me); 1.28 (s, 3 H, 1 Me); 1.21–1.19 (m, 3 H, H_{ax}); 1.09–1.03 (m, 12 H, Pyrrol–$(CH_2)_3$–CH_3); 0.84 (s, 9 H, tert-Bu); 0.76–0.61 (m, 4 H, O–CH_2–CH_2–); 0.58–0.32 (m, 4 H, O–$(CH_2)_2$–CH_2–); −2.05 (s, 2 H, Porphyrin–NH); −3.10 (br.s, 1 H, SH).

MALDI–TOF (1303.8)

$C_{82}H_{104}N_6O_6S$ (1301.83).

*3.12. 10,20-((5-(7-Hydroxycarbonyl-1,5,7-trimethyl-2,4-dioxo-3-aza bicyclo[3.3.1]non-3-yl)-4-methyl-)-((4-(tert-butyl)-2-mercapto-1,3-phenylene)bis(trimethylenoxy))-di-2,1-phenylene)-2,8,12,18-tetrabutyl-3,7,13,17-tetramethyl-porphyrin Iron (III) (**20**)*

To a solution of 10 mg (0.066 mmol) of **19a** in 10 ml of refluxing toluene was added a spatula of $FeBr_2$ and 3 drops of 2,6-lutidine. The dark brown solution was stirred at reflux for 1 h. After cooling and removal of toluene under vacuo, the residue was purified by flash chromatography (silica gel, toluene/AcOEt:8/2) to give 7.7 mg (73%) of **20** as a brown solid.

TLC (toluene/AcOEt:8/2): $R_f = 0.85$

mp: > 250°C

UV–VIS (toluene): 402 (144), 512 (22), 576 (12), 622 (10), 662 (9).

^{1}H-NMR (300 MHz, $CDCl_3$): 56 (br.s, 9 H, *meso*-H, and Pyrrol–CH_2–); 45 (br.s, 2 H, H–C(5′), H–C(3′)); 40 (br.s, 1 H, *meso*-H); 14.85 (br.s, 2 H, Ar–H); 11.35 (br.s, 4 H, O–$(CH_2)_2$–CH_2–); 5.30 (br.s, 12 H, O–CH_2–CH_2–, and Pyrrol–CH_2–CH_2–); 3.79 (br.s, 16 H, Pyrrol–CH_3, and H_{eq}); 3.15–0.86 (48 H, 2 H_{eq}, Ar–CH_3, Pyrrol–$(CH_2)_2$–CH_2, O–CH_2–, 4 Me, 4 H_{ax}, Pyrrol–$(CH_2)_3$–CH_3); −0.64 (br.s, 9 H, tert-Bu)

MALDI–TOF: 1579.40

$C_{94}H_{115}N_6O_{10}FeS$ (1576.88)

References

[1] W.D. Woggon, Top. Curr. Chem., Bioorg. Chem. Frontiers (1996) in press.

[2] J.R. Cupp-Vickerey and T.L. Poulos, Struct. Biol. 2 (1995) 144; C.A. Hasemann, R.G. Kurumbail, S.S. Boddupalli, J.A. Peterson and J. Deisenhofer, Curr. Biol. 3 (1995) 41 and references cited therein.

[3] ed. P.R. Montellano, Cytochrome P450: Structure, Mechanism, and Biochemistry (Plenum Press, New York, 1986).

[4] W.D. Woggon, in preparation.

[5] B. Stäubli, H. Fretz, U. Piantini and W.D. Woggon, Helv. Chem. Acta 70 (1987) 1173.

[6] H. Patzelt and W.D. Woggon, Helv. Chem. Acta 75 (1992) 523.

[7] P.S. Clesy and G.A. Smythe, Aust. J. Chem. 22 (1969) 239.

[8] I. Abdalmuhdi and C.K. Chang, J. Org. Chem. 50 (1985) 411.

[9] J.C. Duff, J. Chem. Soc. (1941) 547.

[10] A. Osuka, B.L. Liu and K. Marayama, J. Org. Chem., 58 (1993) 3582.

[11] D.S. Kemp and K.S. Petrakis, J. Org. Chem. 46 (1981) 5140.

[12] B. Askew, P. Ballester, C. Buhr, K.S. Jeong, S. Jones, K. Parris, K. Williams and J. Rebek, Jr., J. Am. Chem. Soc. 111 (1989) 1082.

[13] D. Harris and G. Loew, J. Am. Chem. Soc. 115 (1993) 8775; H. Thomann, M. Bernardo, D. Goldfarb, P.M.H. Kroneck and V. Ullrich, J. Am. Chem. Soc. 117 (1995) 8243.

[14] H. Aissaoui, R. Baumann, A. Schweiger and W.D. Woggon, in preparation.