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Synthesis of a new estradiol–iron metalloporphyrin conjugate used to build up a new hybrid biocatalyst for selective oxidations by the 'Trojan horse' strategy

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Abstract

The synthesis of a new cationic iron metalloporphyrin–estradiol conjugate is reported. After a study of its association with the anti-estradiol antibody 7A3 by UV–visible spectroscopy, the influence of the antibody on the sulfoxidation of thioanisole by H_2O_2 catalyzed by the iron–metalloporphyrin has been investigated.

Keywords: Artificial metalloenzyme; Metalloporphyrin; Antibody; Enantioselective catalysis; Sulfoxidation

Obtention of enantiopure compounds is a major issue in organic chemistry. Among the several strategies explored to induce stereoselectivity into chemical reactions, the construction of artificial metalloenzymes has appeared to be one of the most promising. Indeed, such hybrid biocatalysts combine the efficiency and wide scope of reactions of synthetic catalysts with the high selectivity and ability to work under mild conditions of enzymes. Moreover, they can be optimized by directed evolution, a very powerful way to enhance their performances.[1]

Artificial metalloenzymes can be obtained by insertion of a metal cofactor into the cavity of a protein. This cofactor can be linked either covalently[2] or non-covalently[3–5] to the protein. In particular, Ward and co-workers[6,7] have built artificial metalloenzymes by the association of biotinylated rhodium complexes with (strept)avidin, using the strong (strep)avidin/biotin affinity to anchor the cofactor into the cavity of the protein. They have obtained biocatalysts that are able to catalyze the reduction of acetamidoacrylic acid by H_2, with excellent enantiomeric excesses.

We present here the elaboration of a new artificial metalloenzyme by this so-called 'Trojan horse strategy' that is based on the supramolecular association of an iron metalloporphyrin–estradiol conjugate with an anti-estradiol antibody. The synthesis of the iron metalloporphyrin–estradiol conjugate will first be presented, and the activity and selectivity of the antibody–estradiol–iron(III)metalloporphyrin complex on the catalysis of the sulfoxidation of thioanisole by hydrogen peroxide will be subsequently examined.

The antibody that was used as apoprotein was a monoclonal IgG, 7A3, that has been generated by immunization of mice with an antigen obtained by covalent linkage of estradiol in 3-position to BSA.[8] The high affinity of 7A3 for estradiol ($K_d \sim 10^{-10}$ M) made it an excellent candidate for the elaboration of a new artificial metalloenzyme by this strategy, that requires a high affinity of the host protein for the estradiol–iron(III)metalloporphyrin cofactor.

The first step was the synthesis of 5,10,15-tris(4-pyridyl)-20-(4-nitrophenyl)porphyrin **1** by a method slightly modified

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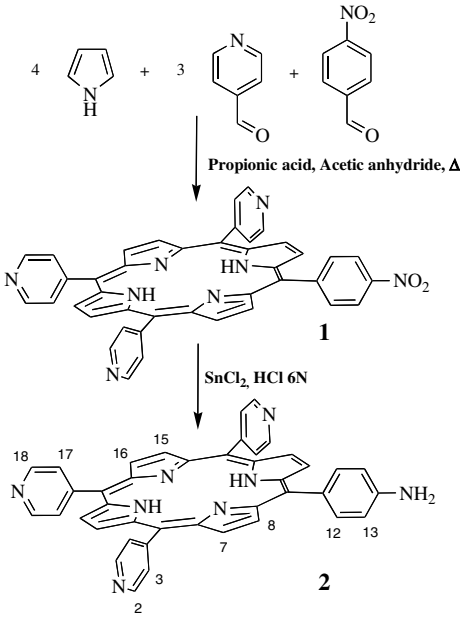
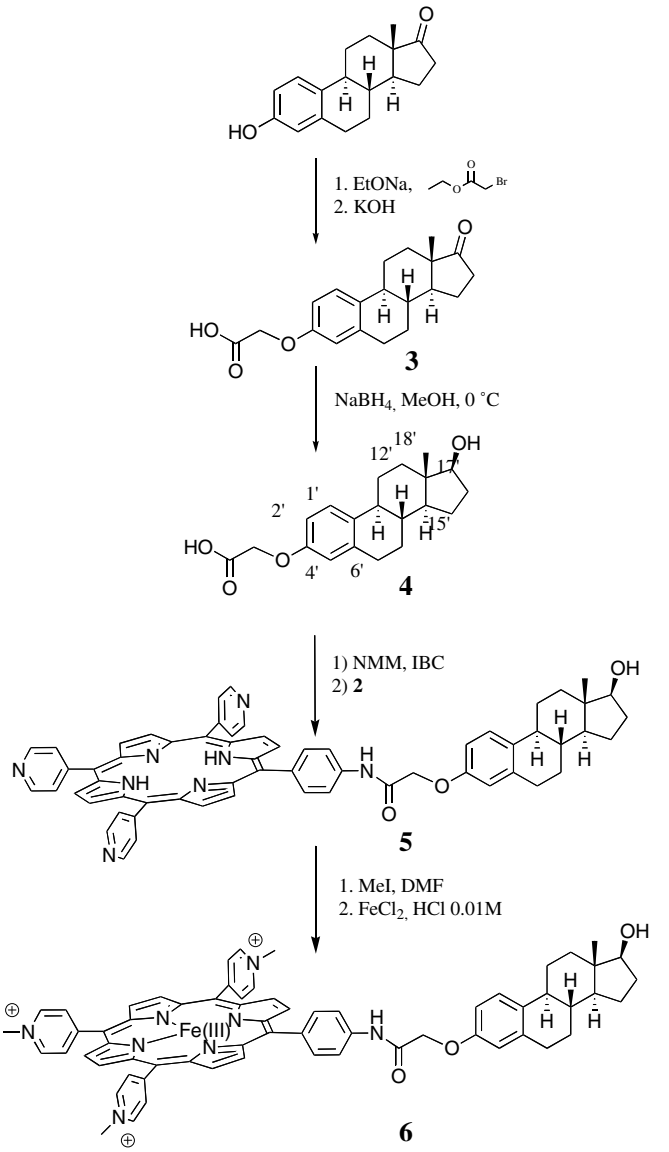
from the one reported by Adler[9,10] that consisted of a statistical reaction between stoichiometric amounts of pyrrole, 4-pyridyl and 4-nitrobenzaldehyde, which led to a mixture of six porphyrins (Scheme 1). Thus, 4 equiv of pyrrole (15 mL, 0.208 mol) were added to a refluxing solution of 3 equiv of 4-pyridinecarboxaldehyde (15 mL, 0.157 mol) and 1.6 equiv of 4-nitrobenzaldehyde (12.9 g, 0.085 mol) in a 10:1 mixture of propionic acid and acetic anhydride. After refluxing for 1.5 h, a catalytic amount (1.2 g) of tetrachlorobenzoquinone was added to oxidize the porphyrinogens formed. Porphyrin **1** was then separated from the other five porphyrins by silica gel MPLC (eluent CH_2Cl_2/MeOH 97:3, $R_f = 0.45$) and obtained in a 4% yield (1.329 g).

5,10,15-Tris(4-pyridyl)-20-(4-aminophenyl)porphyrin **2** was obtained by the reduction of the nitro group of **1** (50 mg, 7.6×10^{-5} mol) with an excess of stannous chloride (85 mg, 38×10^{-5} mol) in 6 N hydrochloric acid for 24 h at room temperature (Scheme 1). After neutralization with solid NaOH at 0 °C, and extraction with dichloromethane, porphyrin **2** was obtained in a 85% yield.

3-*O*-Carboxymethylestrone **3** was synthesized from estrone following the method described by Katzenellenbogen.[11] The ketone function of **3** (314 mg, 8.7×10^{-4} mol) was then selectively reduced by 5 equiv of $NaBH_4$ (165 mg, 44×10^{-4} mol) in methanol at 0 °C to obtain 3-*O*-carboxymethylestradiol **4** (273 mg, 86% yield, Scheme 2). The carboxy function of **4** (23 mg, 7×10^{-5} mol, 1.1 equiv) was activated by isobutyl chloroformate (10 μL, 8×10^{-5} mol, 1.2 equiv) in the presence of *N*-methyl morpholine (9 μL, 8×10^{-5} mol, 1.2 equiv), and condensed with porphyrin **2** (40 mg, 6×10^{-5} mol, 1 equiv). The solvent was evaporated, and the residue was redissolved in dichloromethane. The organic layer



Scheme 1.



Scheme 2.

was washed successively with water, 5% citric acid, a saturated $NaHCO_3$ solution and water again, and the solvent was evaporated. The residue was washed with methanol, and, after drying, the estradiol–porphyrin conjugate **5** was isolated (48 mg, 80% yield).

It is well known that the nitrogen atoms of the meso-pyridyl substituents of porphyrins are able to complex metal ions, which leads to the formation of polymers.[12,13] It was thus decided to methylate them prior to the metallation of the porphyrin macrocycle. Consequently, **5** was reacted with an excess of methyl iodide in anhydrous DMF for 3 h at 40 °C.[14] To make the porphyrin water-soluble, the iodide counter-ions were then replaced by chloride ions upon dropwise addition of a concentrated solution of tetra-butylammonium chloride (TBAC) in acetone (100 g/L) until precipitation of the methylated porphyrin was complete. The precipitated porphyrin (20 mg, 2×10^{-5} mol) was then dissolved in 0.01 M degassed

HCl, and 40 equiv of $FeCl_2{\cdot}4H_2O$ (145 mg, 7.3×10^{-4} mol) were added.[15] The solution was heated at 80 °C for 28 h, and the iron–porphyrin **6** was isolated by precipitation upon the addition of 10 equiv of $Bu_4N^+{\cdot}PF_6^-$. The precipitate was washed with a 1:1 mixture of isopropanol and diethyl ether, and the PF_6^- counter-ions were then substituted by chloride ions upon the addition of concentrated TBAC in acetone to yield the water-soluble metalloporphyrinic cofactor **6** (23 mg, 93%).

The association of **6** with the antibody was studied by UV–visible spectroscopy. The spectrum of the **6**-7A3 complex was very similar to the one of **6** alone with maximas at 427 nm instead of 428 nm, 523 nm and 659 nm (at pH 4.4), which showed that no amino acid side-chain of 7A3 acted directly as a fifth ligand of the iron (Fig. 1). However, an increase in the Soret band of the porphyrin at 428 nm was observed, which allowed to perform a titration of 7A3 by **6**. It then appeared that two metalloporphyrinic cofactors were bound per antibody, and a dissociation constant of the **6**–7A3 complex could be estimated to be 10^{-8} M.

The influence of the antibody on the kinetics and selectivity of oxidation reactions catalyzed by the iron(III)metalloporphyrin cofactor has been studied.

For this, thioanisole was chosen as a typical substrate, since it is well known to be oxidized into an asymmetric sulfoxide by naturally occurring peroxidases like horseradish peroxidase (HRP),[16–18] which makes it a perfect candidate for a study of the enantioselectivity of the catalysis.

The kinetic of the sulfoxidation reaction was performed under the following conditions. The antibody 7A3 (12.5 μM) was incubated for 2 h with **6** (5 μM) in a mixture of 250 μL of 0.1 M phosphate buffer pH 7.4 and 200 μL of phosphate–citrate buffer pH 3 (to reach a pH of 4.4). 25 μL of a 10 mM solution of thioanisole in acetonitrile were then added, followed by 25 μL of 5 mM H_2O_2. The same procedure was followed with **6** alone. The sulfoxide was extracted with ethyl acetate at different reaction times, and quantified by gas chromatography, using benzophenone as the internal standard.

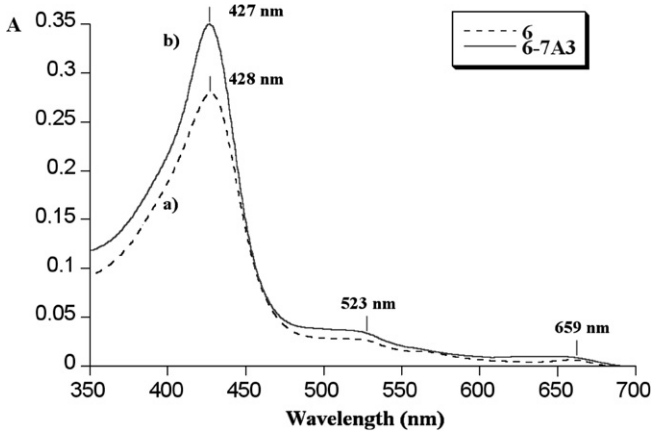


Fig. 1. Absorption spectra of 5 μM 6 in (a) the absence (b) the presence of 12.5 μM 7A3 antibody (phosphate–citrate buffer, pH 4.4).

Whereas the reaction was negligible in the absence of catalyst (data not shown), it was catalyzed by both **6** and 7A3–**6** (Fig. 2). Moreover, it proceeded about 1.5-fold faster with 7A3–**6** than with **6** as a catalyst. The quantity of sulfoxide formed reached a plateau after 80 min with 7A3–**6** as a catalyst, and only after 40 min with **6** alone as catalyst. In addition, the quantity of sulfoxide formed at the plateau was twice larger with 7A3–**6** than with **6** alone. This showed the protecting effect of the antibody towards the oxidative degradation of the porphyrin ring by H_2O_2. It is noteworthy that, in the presence of 250 μM H_2O_2, the final yield of sulfoxide increased linearly with the concentration of catalyst, from 0 to 59 μM, for concentrations of 7A3–**6** between 0 and 5 μM. Additionally, in the presence of 5 μM 7A3–**6** as a catalyst, the final yield of sulfoxide increased with the concentration of hydrogen peroxide to reach a plateau for hydrogen peroxide concentrations above 1 mM, which was consistent with an oxidative destruction of the catalyst.

To study the effect of the antibody on the enantioselectivity of the catalysis, a reaction was performed under similar conditions, but with the dropwise addition of hydrogen peroxide over 50 min. The enantiomeric excess was measured with a chiral HPLC column. Whereas the sulfoxide formed with **6** alone was found to be a racemic mixture, an enantiomeric excess of 8% in favour of the *S* enantiomer was measured for the reaction in presence of 7A3.

In conclusion, we have reported the synthesis of a new iron metalloporphyrin–estradiol conjugate that has been used to build an artificial metalloenzyme by association with an anti-estradiol antibody. The first results on the catalysis of the sulfoxidation of thioanisole by H_2O_2 are encouraging, as the antibody not only protects the cofactor against self-oxidation, but also induces a slight enantiomeric excess in the reaction. Further work is in progress to extend the use of this new biocatalyst to the selective oxidation of other substrates including phenol derivatives, alkenes and alkanes.

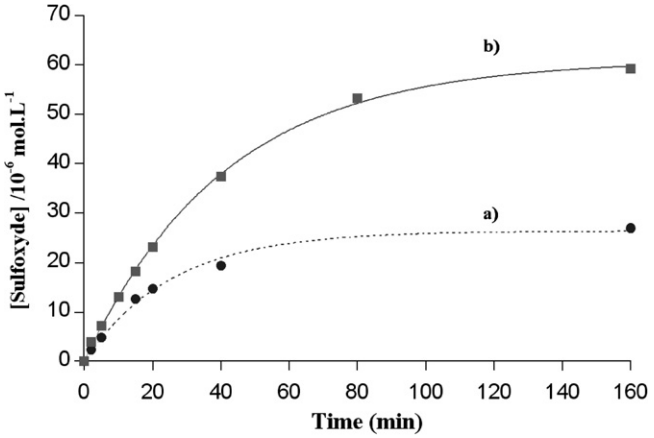


Fig. 2. Sulfoxidation of 0.5 mM of thioanisole by 0.25 mM H_2O_2; (a) in the presence of 5 μM **6**; (b) in the presence of 5 μM **6** and 12.5 μM 7A3 protein.

Notes

All the new compounds described in this work gave satisfactory spectrometric data (^{1}H NMR, ^{13}C NMR, MS) and consistent high-resolution mass spectrometry data. The chemical shifts are given in ppm versus TMS. The usual nomenclature of steroids has been used for carbon and proton numbering.

5,10,15-Tris(4-pyridyl)-20-(4-nitrophenyl)porphyrin 1

^{1}H NMR (250 MHz, $CDCl_3$, 300 K): δ 9.07 (dd, 6H, $J_1 = 4.5$ Hz, $J_2 = 1.6$ Hz, H2, H18); 8.88 (s, 4H, H15, H16); 8.87 (d, 2H, $J = 5.0$ Hz, H7); 8.82 (d, 2H, $J = 5.0$ Hz, H8); 8.67 (dd, 2H, $J_1 = 6.6$ Hz, $J_2 = 2.0$ Hz, H13); 8.40 (dd, 2H, $J_1 = 6.6$ Hz, $J_2 = 2.0$ Hz, H12); 8.14 (dd, 6H, $J_1 = 4.5$ Hz, $J_2 = 1.6$ Hz, H3, H17); −2.90 (s, 2H, NH pyrr.) UV–vis (CH_2Cl_2/MeOH 97:3): λ_{max} 419, 515, 549, 589, 643 nm. MS (MALDI-MS) m/z $[M+H]^+$ 663.22.

5,10,15-Tris(4-pyridyl)-20-(4-aminophenyl)porphyrin 2

^{1}H NMR (250 MHz, $CDCl_3$, 300 K): δ 9.04 (dd, 6H, $J_1 = 5.7$ Hz, $J_2 = 1.5$ Hz, H2, H18); 9.03 (d, 2H, $J = 4.9$ Hz, H8); 8.84 (s, 4H, H15, H16); 8.81 (d, 2H, $J = 4.9$ Hz, H7); 8.16 (dd, 6H, $J_1 = 5.7$ Hz, $J_2 = 1.5$ Hz, H3, H17); 7.98 (d, $J = 8.3$ Hz, 2H, H12); 7.08 (d, 2H, $J = 8.3$ Hz, H13); 4.09 (br s, 2H, NH_2); −2.85 (s, 2H, NH pyrr.) UV–vis (CH_2Cl_2/MeOH 97:3): λ_{max} 420, 517, 553, 591, 650 nm. HR-MS (MALDI-MS) m/z calcd: 633.25115 $[M+H]^+$ for $C_{41}H_{28}N_8$, found: 633.25097.

3-*O*-Carboxymethylestradiol 4

^{1}H NMR (300 MHz, MeOD, 300 K): δ 7.12 (d, 1H, $J = 8{,}6$ Hz, H1′); 6.64 (dd, 1H, $J_1 = 8.6$ Hz, $J_2 = 2.6$ Hz, H2′); 6.58 (d, 1H, $J = 2.6$ Hz, H4′); 4.52 (s, 2H, O–CH_2–); 3.62 (t, 1H, $J = 8{,}5$ Hz, H17′), 2.75 (m, 2H, H6′); 2.24 (br d, 1H, $J_{11'-11'} = 13.0$ Hz, H11′), 2.12–1.96 (m, 1H, H9′); 1.92 (d, 1H, $J_{16'-16'} = 12$ Hz, H16′); 1.81 (m, 1H, H7′); 1.64 (m, 1H, H12′); 1.57–1.00 (m, 8H, H15′, H7′, H8′, H11′, H12′, H14′, H16′); 0.73 (s, 3H, H18′). ^{13}C NMR (75 MHz, MeOD, 300 K) δ 172.8 (–COOH); 157.1 (C3′); 139.0 (C5′); 134.6 (C10′); 127.3 (C1′); 115.6 (C4′); 113.0 (C2′); 82.4 (C17′); 65.8 (O–CH_2–); 51.2 (C14′); 45.2 (C9′); 44.3 (C13′); 40.2 (C8′); 37.9 (C16′); 30.73 (C12′); 30.67 (C6′); 28.4 (C7′); 27.5 (C11′); 24.0 (C15′); 11.8 (C18′). HR-MS (ESI-MS) m/z calcd: 353.1729 $[M+H]^+$ for $C_{20}H_{26}O_4$ found: 353.1729. Anal. Calcd for $C_{20}H_{26}O_4 \cdot 1/3H_2O$: C, 71.40; H, 7.99. Found: C, 71.37; H, 7.83.

5,10,15-Tris(4-pyridyl)-20-(4-(3-*O*-amidoxymethylestradiol))porphyrin 5

^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ 9.06 (d, 6H, $J = 4{,}5$ Hz, H18); 8.95 (d, 2H, $J = 4.8$ Hz, H8); 8.86 (s, 2H, H15–H16); 8.83 (d, 2H, $J = 4{,}8$ Hz, H7); 8.74 (s, 1H, NH amide); 8.21 (d, 2H, $J = 8.4$ Hz, H13); 8.16 (d, 6H, $J = 4.5$ Hz, H17); 8.05 (d, 2H, $J = 8.4$ Hz, H12); 7.31 (d, 1H, $J = 8.6$ Hz, H1′); 6.91 (dd, 1H, $J_1 = 8.6$ Hz, $J_2 = 2.4$ Hz, H2′); 6.84 (d, 1H, $J = 2.4$ Hz, H4′); 4.77 (s, 2H, O–CH_2–); 3.69 (t, 1H, $J = 8.4$ Hz, H17′); 2.91 (br t, 2H, $J = 3.5$ Hz, H6′); 2.32 (br dd, 1H, $J_1 = 13.3$ Hz, $J_2 = 2.7$ Hz, H11′); 2.2–2.0 (m, 2H, H9′, H16′); 2.0–1.78 (m, 2H, H15′, H7′); 1.75–1.60 (m, 1H, H12′); 1.59–0.97 (m, 7H, H16′, H15′, H11′, H7′, H12′, H8′, H14′); 0.80 (s, 3H, H18′); −2.87 (s, 2H, NH pyrr.). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K) δ 167.1 (NHC═O); 155.0 (C3′); 150.0 (Cq); 149.9 (Cq); 148.3 (C2, C18); 138.7 (Cq); 137.9 (Cq); 137.0 (Cq); 135.1 (C13); 134.8 (C10′); 131.0 (br, C pyrr.); 129.3 (C3, C17); 126.9 (C1′); 120.9 (Cq); 118.5 (C12); 117.4 (Cq); 117.1 (Cq); 114.9 (C4′); 112.3 (C2′); 81.7 (C17′); 67.9 (O–CH_2–); 49.9 (C14′); 43.9 (C9′); 43.2 (C13′); 38.6 (C8′); 36.6 (C16′); 30.5 (C12′); 29.8 (C6′); 27.0 (C7′); 26.3 (C11′); 23.1 (C15′); 11.0 (C18′). UV–vis (CH_2Cl_2): λ_{max} 418, 514, 549, 590, 651 nm. Anal. Calcd for $C_{61}H_{52}N_8O_3 \cdot 3$MeOH: C, 73.82; H, 6.20; N, 10.76. Found: C, 73.95; H, 6.06; N 10.24. HR-MS (MALDI-MS) m/z calcd: 945.42705 $[M+H]^+$ for $C_{61}H_{52}O_3$, found: 945.42351.

5,10,15-Tris(4-*N*-methylpyridiniumyl)-20-(4-(3-*O*-amidoxymethylestradiol))porphyrin

^{1}H NMR (360 MHz, MeOD, 300 K): δ 9.39 (d, 6H, $J = 6.1$ Hz, H2, H18); 9.26–9.00 (m, 8H, H7, H8, H15, H16); 8.96 (d, 6H, $J = 6.1$ Hz, H3, H17); 8.22 (d, 2H, $J = 8.5$ Hz, H13); 8.16 (d, 2H, H12); 7.23 (d, 1H, $J = 8.3$ Hz, H1′); 6.92 (dd, 1H, $J_1 = 8.3$ Hz, $J_2 = 2.4$ Hz, H2′); 6.86 (d, 1H, $J = 2.4$ Hz, H4′); 3.54 (t, 1H, $J = 9.0$ Hz, H17′); 2.87 (m, 2H, H6′); 2.24 (br d, 1H,H11′); 2.08–1.78 (m, 5H, H9′, H16′, H7′, H11′); 0.74 (s, 3H, H18′). ^{1}H NMR (360 MHz, DMSO, 300 K): δ 4.84 (s, 2H, O–CH_2–); 4.71 (s, 9H, N^+–CH_3); 0.70 (s, 3H, H18′). UV–vis (MeOH): λ_{max} 429.5, 522, 561, 591, 657 nm. MS (MALDI-MS) m/z $[M+H]^+$ 990.45; HR-MS (ESI-MS) m/z calcd: 329.82812 for $[M/3]^+$; found: 329.8283.

Iron(III) 5,10,15-tris(4-*N*-methylpyridiniumyl)-20-(4-(3-*O*-amidoxymethylestradiol))porphyrin 6

UV–vis (citrate–phosphate buffer, pH 4.4): λ_{max} (log(ε)) 428 (4.7), 523 (3.7), 572 (3.4), 659 (3.1) nm. HR-MS (MALDI-MS) m/z calcd: 1043.40540 for $C_{64}H_{59}FeN_8O_3$; found: 1043.40503.

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