Cholate-interspersed porphyrin–anthraquinone conjugates: Photonuclease activity of large sized, 'tweezer-like' molecules

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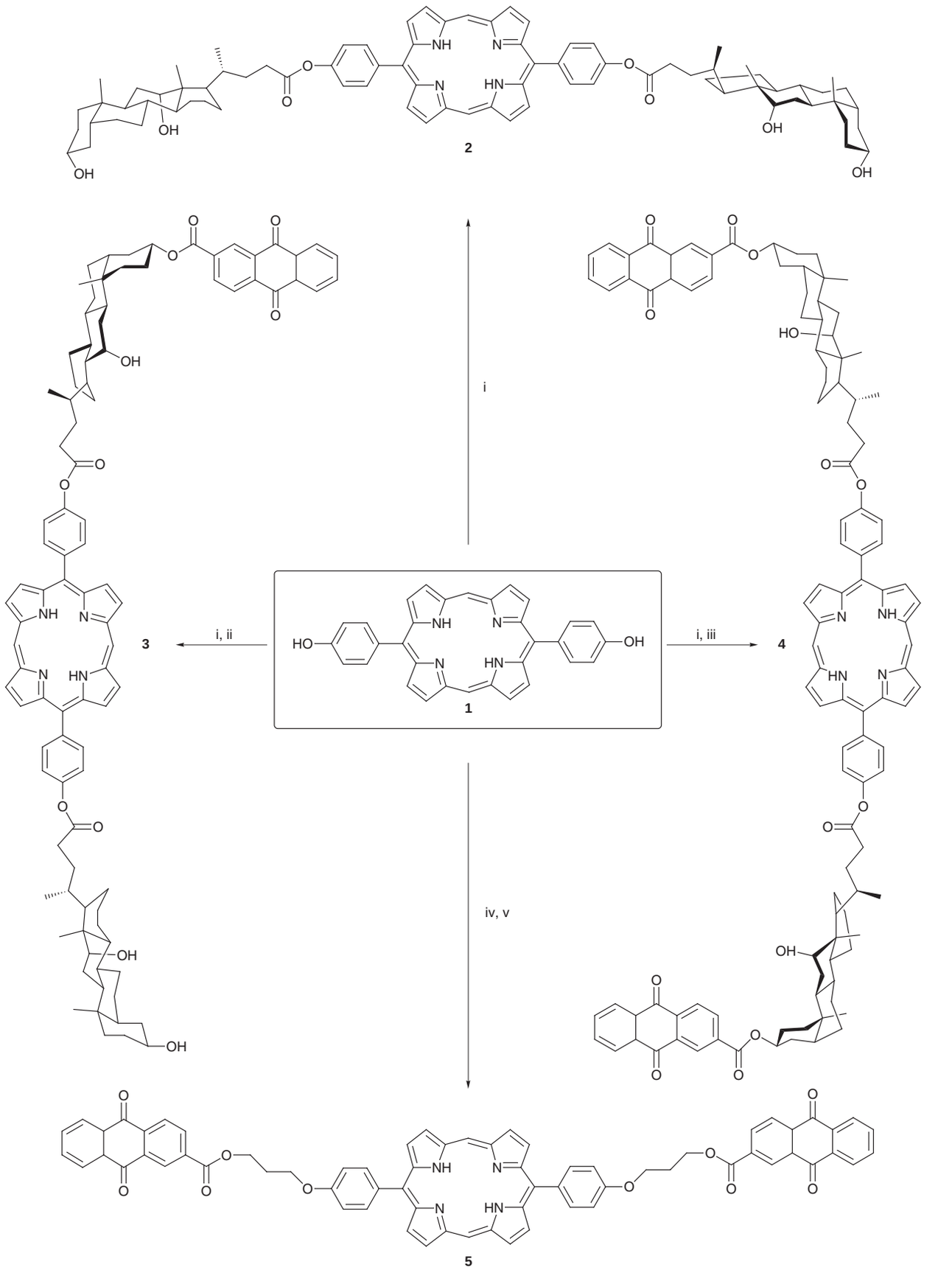
In a new approach towards the development of a 'dual-wavelength dual-mechanism' type of photosensitizer for use in photodynamic therapy (PDT), covalently linked bichromophoric systems comprising of porphyrin (P) and anthraquinone (AnQ) subunits have been synthesized and fully characterized by FAB-MS, IR, UV–Visible and ^{1}H NMR methods. The porphyrin donor and the anthraquinone acceptor subunits of these mono- or bis-intercalating hybrid molecules are interspersed with either cholate or polymethylene spacers. There exists minimal ground- and singlet-state interaction between the porphyrin and anthraquinone subunits in the giant-sized, cholate-interspersed P–AnQ systems as revealed by a comparison of their spectroscopic and electrochemical properties with those of the corresponding individual reference compounds. On the other hand, quenching of fluorescence observed for the P–AnQ systems endowed with polymethylene spacers has been interpreted in terms of a possible intramolecular electron transfer between the singlet porphyrin and the anthraquinone acceptor. When excited into their porphyrin absorption band maxima, each new P–AnQ system could generate singlet molecular oxygen in good-to-moderate yield. Wavelength-dependent photonuclease activity of these new bis-intercalating species has been examined.

Treatment of tumors by photodynamic therapy (PDT) of cancer requires a photosensitizer which is a single, nontoxic, stable compound of known chemical structure that can be retained with a high degree of selectivity in malignant tumors.[1] In addition, from the photochemical point of view, an ideal photosensitizer should be able to absorb light in the tissue-transparent region (≈600–800 nm) and photogenerate high yields of $^1O_2$ and/or other cytotoxic species. Hematoporphyrin derivative (HpD or Photofrin II®) is currently being marketed as an anticancer drug, and second-generation PDT agents based on porphyrin and related macrocycles are under development.[1,2] Most of the synthetic efforts towards the second-generation photosensitizers have relied upon the appropriate modification of the porphyrin system in such a way that the derived macrocycles either absorb in the far-red spectral region and/or photogenerate high yields of $^1O_2$.[2] Simultaneously, in a new approach to the design of PDT agents, we and others have endeavored to impart molecular (and cellular) recognition abilities to porphyrin sensitizers with a view to localizing the sensitizer in the desired subcellular components of the tumor.[3,4] Thus, we have recently reported the synthesis and photonuclease activity of several porphyrin–DNA intercalator and porphyrin–chemotherapeutic drug conjugates.[3] More recently, design and synthesis of a new 'dual-wavelength dual-mechanism' series of porphyrin–anthraquinone (P–AnQ) hybrids and advantageous utilization of the photoactivities of both the chromophores present on these hybrids in accentuating the DNA photocleavage has been reported by us.[5] Herein, we demonstrate that our new approach can be extended to the design of more elaborate, bis-intercalating, P–AnQ systems having an additional cellular-level recognition element (*viz*: cholic acid[6]) in their 'tweezer-like' architecture. As a first step towards evaluating their efficacy in PDT, the photonuclease activity of these new hybrid molecules has been examined.

Results and discussion

The scheme leading to the synthesis of the new bis-intercalators is illustrated in Scheme 1. The 5,15-bis(4-hydroxyphenyl)-porphyrin **1**[7] was treated with 7-deoxycholic acid in the presence of 1,3-dicyclohexylcarbodimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to obtain the key precursor **2**. Porphyrin **2** was esterified with anthraquinone-2-carboxylic acid (AnQ2-CO_2H) employing the same classical DCC–DMAP procedure to yield the cholate-interspersed mono- and bis-intercalators **3** and **4**. Reaction of **1** with 3-bromopropan-1-ol in K_2CO_3–DMF furnished the corresponding porphyrin diols, which upon further reaction with AnQ2-CO_2H under the standard ester-forming conditions gave **5**. The steps involved in the preparation of conjugates **2**–**5** are quite straightforward and the desired compounds are readily obtained in pure form and good yield.

The structural identity of hybrid molecules **2**–**5** was firmly established on the basis of elemental analyses, ^{1}H and ^{13}C NMR, IR, UV–Visible and FAB-MS data (see Experimental section). The ^{1}H NMR spectra of conjugates **3** and **4** exhibit clearly discernible peaks due to the three constituent partners (*i.e.* porphyrin, deoxycholic acid and anthraquinone) present in these molecules. For example, in the symmetrical compound **4**, resonances due to the two outermost *meso*- and the two inner imino protons on the porphyrin ring appear at the two ends (δ 10.29 and −3.22) of the spectrum. In between, the pyrrole protons resonate as two doublets, at δ 9.40 and 9.11 (J 4.5 Hz), the *ortho* and *meta* aryl protons are located at δ 7.79 and 7.58 (both doublets, J 8.5 Hz), and the anthraquinone protons appear as a singlet (δ 8.86, 2H) and a complex multiplet (δ 8.23–8.42, 12H). The δ 5.08–0.83 region of the spectrum is characterized by the peaks due to the protons on the cholate spacer moieties with the 3-H resonance appearing as a characteristic broad signal at δ 5.08. In the UV–Visible spectra, each new porphyrin conjugate investigated in this study shows one Soret band and four Q-bands in the visible region. The band due to the anthraquinone moiety is located at 253–258 nm for compounds **3**–**5**. Further analysis of the UV–Visible spectra suggests that the AnQ part of these hybrids strongly absorbs between 200–375 nm, a region in which the porphyrin part shows minimum absorbance. Analogously, the porphyrin part of each hybrid shows bands in the wavelength region (400–700 nm) where the AnQ part of the molecule does not absorb. Thus, the two chromophores on these hybrid molecules are individu-



Scheme 1 *Reagents and conditions*: (i) 7-deoxycholic acid, THF, DCC, DMAP, 20 h; (ii) anthraquinone-2-carboxylic acid, CH_2Cl_2, DCC, DMAP, 2 h; (iii) anthraquinone-2-carboxylic acid, CH_2Cl_2, DCC, DMAP, 3 h; (iv) $Br(CH_2)_3OH$, K_2CO_3–DMF, 36 h; (v) anthraquinone-2-carboxylic acid, CH_2Cl_2, DCC–DMAP.

ally addressable. Comparison of the UV–Visible data of **3**–**5** with the spectra of **1** and AnQ (or AnQ2-CO_2H) further suggests that the λ_{max} and the log ε (ε = molar extinction coefficient)-values of these porphyrin–intercalator conjugates are in the same range as those of the reference compounds. Similarly, the redox potential data of **3**–**5** were also found to be

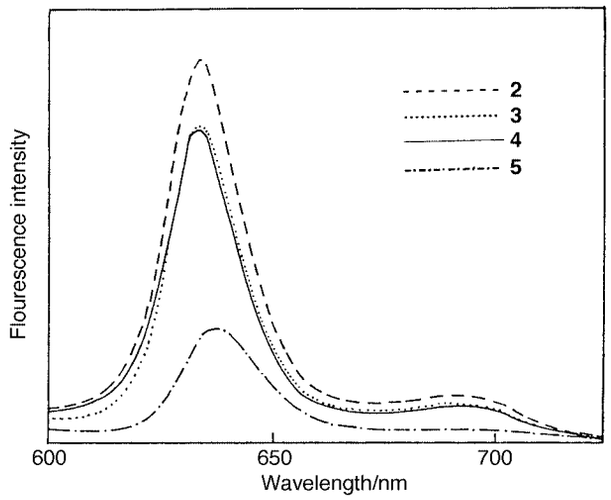


Fig. 1 Fluorescence spectra of equi-absorbing solutions (CH_2Cl_2; A at λ_{exc} 550 nm is 0.19) of compounds **2**–**5**.

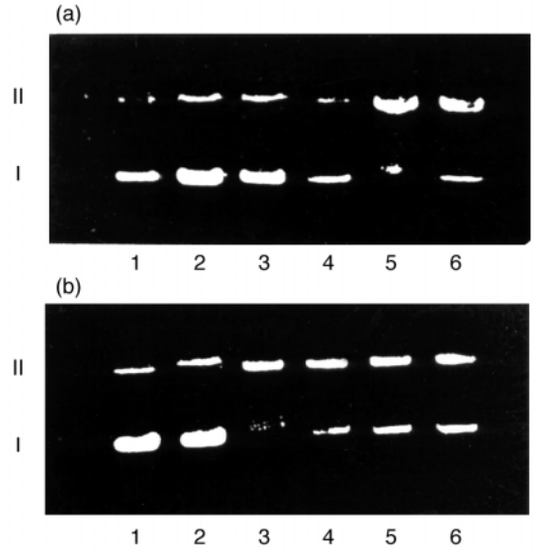


Fig. 2 Light-induced nuclease activity of porphyrin–anthraquinone hybrids. (a) λ_{exc} = 350 nm (0.5 mW cm^{-2}; 380 mJ); Lane 1: untreated pBR 322, Lanes 2–6: pBR 322+ **1**, **2**, **3**, **4** and **5** respectively. (b) λ_{exc} = 550 nm (0.5 mW cm^{-2}; 380 mJ); Lane 1: untreated pBR 322, Lanes 2–6: pBR 322+ **1**, **2**, **3**, **4** and **5**, respectively. In each case, the proportion DNA/Drug = 1:1 and the samples were incubated for 1 h before being irradiated using a 150 W Xe-arc lamp/monochromator assembly. Electrophoresis experiments were carried out as described in ref. 5.

in the same range as those of **1** and AnQ (or AnQ2-CO_2H). While the one-electron oxidation and reduction of the porphyrin part of the conjugates was observed at +(0.96 ± 0.02) and −(1.25 ± 0.02) V respectively, the anthraquinone part could be reduced at −(0.78 ± 0.02) V. Collectively, ^{1}H NMR, UV–Visible and redox potential data of these new P–AnQ systems suggest that there exists no ground-state interaction between the porphyrin and anthraquinone chromophores in these hybrids, the cholate spacer being seen to be both photo- and electro-inactive.

On the other hand, fluorescence due to the porphyrin moiety in bis-intercalator **5** was found to be quenched (Φ_f 0.03) in comparison with the unlinked porphyrin **2** (Φ_f 0.095) or *meso*-5,10,15,20-tetra(4-tolyl)porphyrin (H_2TTP, Φ_f 0.13), see Fig. 1. We reason that this quenching is due to a photoinduced electron-transfer (PET) reaction from the porphyrin singlet state to the appended quinone, as is true for the covalently linked, porphyrin–quinone systems reported earlier.[8] Interestingly, the fluorescence quantum yields of **3** and **4** (Φ_f 0.085) were found to be close to that of their common precursor **2**. Probably, the distance between the donor (D; porphyrin) and the acceptor (A; quinone) is too large in these D–A systems owing to the rigid framework provided by the cholic acid spacer. In contrast, the polymethylene spacers in **5** are expected to provide sufficient flexibility such that the P–AnQ distance is short enough to promote an electron-transfer quenching process in this bichromophoric system. Finally, the singlet oxygen ($^1O_2$) quantum yields of **1**–**5**, as estimated from the standard method involving the bleaching of 1,3-diphenylisobenzofuran (DPBF)[3,5] in DMF, were found to range from 0.62 (**1**), 0.68 (**2**), 0.58 (**3**, **4**) to 0.43 (**5**) (±15%), with **5** showing the least ability for $^1O_2$ generation, as expected.

The nuclease activity of the new-generation PDT agents **3**–**5** and the corresponding unlinked, reference compounds was investigated using the supercoiled plasmid DNA pBR 322 in the presence and absence of light. While no nicking was observed in the absence of light, irradiation of DNA in the presence of **3**–**5** at either 550 nm (porphyrin absorption) or 350 nm (AnQ absorption) caused nicking and generation of relaxed circular DNA, Fig. 2. Similarly, the reference porphyrin **1** showed only marginal nicking when irradiated at 550 nm (and also 350 nm) but, AnQ2-CO_2H was seen to efficiently photocleave the DNA upon excitation by a 350 nm light source. In no case, however, was linearization of DNA evident even upon prolonged irradiation of the plasmid in the presence of any of the compounds investigated in this study. Based on the data obtained during our previous studies on similar porphyrin–intercalator conjugates[3,5] and also on the basis of reported literature on the photochemistry of anthraquinone-based intercalators in the presence of DNA,[9] it is surmised that while the porphyrin-based DNA photocleavage (550 nm excitation) is mediated by $^1O_2$, that based on the quinone (350 nm excitation) is due to the reactions of quinone triplet/anion radical with the biomolecule. In this regard, it is interesting to note that the quinone anion radical can also be generated *via* a PET-based pathway upon excitation of hybrid **5** at 550 nm.

The photonicking efficiency follows the order **4** > **5** > **3** when these hybrids are irradiated at 350 nm under the identical experimental conditions of concentration and light-dose. While the superior photonicking ability of **4** with respect to **3** can be understood in terms of the bis-intercalating ability of the former hybrid, the relative inability of the bis-intercalator **5** to photocleave DNA is surprising. Probably, the spacer cholic acid moieties play an important role in the binding of these PDT agents with DNA. This supposition is consistent with the better DNA-nicking ability (λ_{exc} 550 nm) of the bis-cholate porphyrin **2** in comparison with the rest of the compounds investigated in this study. Finally, complete conversion of form I to form II DNA could be achieved with hybrids **3**–**5** under the conditions of white light (λ > 350 nm) irradiation, as was the case with the porphyrin–quinone diads reported by us earlier.[5]

In summary, the results described here suggest that our P–AnQ based 'dual-wavelength dual-mechanism' hybrid molecules provide new possibilities towards the design of potent photonucleases for use in PDT.

Experimental

Materials and methods

The chemicals and solvents utilized in this study were purchased from either Aldrich Chemical Co. (USA) or BDH (Mumbai, India). The solvents utilized for spectroscopic and electrochemical experiments were further purified using standard procedures.[10] The supercoiled pBR 322 DNA (Bangalore Genie, India) was used as received. Agarose (molecular biology grade) and ethidium bromide were purchased from Bio-Rad (USA). *meso*-5,15-Bis(4-hydroxyphenyl)porphyrin **1** was synthesized as *per* a reported method.[7]

Elemental analyses were performed on a Perkin-Elmer model 2400 analyzer. UV-Visible spectra were recorded with a JASCO Model 7800 UV–Visible spectrophotometer. Concentration of the samples used for these measurements ranged from about 2×10^{-6} M (porphyrin Soret and AnQ bands) to 5×10^{-5} M (porphyrin Q-bands). Extinction coefficients are measured in $dm^3\ mol^{-1}\ cm^{-1}$. While the ^1H NMR spectra were recorded with a Bruker NR-200 AT-FT NMR spectrometer, ^{13}C spectra were recorded on a JEOL 300 MHz spectrometer at an operating frequency of 75 MHz. In both cases, $CDCl_3$ and tetramethylsilane (TMS) were employed as the solvent and internal standard, respectively. The FAB-mass spectra were recorded with a JEOL SX 102/DA-6000 mass spectrometer/data system. Cyclic voltammetric experiments (CH_2Cl_2 and 0.1 M tetrabutylammonium perchlorate, TBAP) were performed on a Princeton Applied Research (PAR) 174A polarographic analyzer coupled with a PAR 175 universal programmer and a PAR RE 0074 *x–y* recorder, as detailed in our previous studies.[11] Steady-state fluorescence spectra were recorded using a JASCO Model 777 spectrofluorimeter. The emitted quanta were detected at right angles to the incident beam. The utilized concentrations of the fluorophores were such that the optical densities at the excitation wavelength (540 nm) were always less than 0.2. The fluorescence quantum yields (Φ_f, ±10%) were estimated by integrating the areas under the fluorescence curves and by using *meso*-5,10,15,20-tetraphenylporphyrin (H_2TPP) (Φ_f 0.13 in CH_2Cl_2 for excitation into the porphyrin band, 540 nm) as the standard.[12] Refractive-index corrections have been incorporated while reporting the fluorescence data in various solvents.[13]

For the singlet oxygen ($^1O_2$)-yield measurements, 2.5 ml of a DMF (saturated with AR-grade molecular oxygen) solution containing 1.0×10^{-6} M concentration of a given sensitizer and $0.5–1.0 \times 10^{-4}$ M DPBF was placed in a 1 cm path-length quartz cuvette and irradiated at 550 ± 10 nm (a PTI Model A1010 150 W Xe arc lamp coupled with a PTI Model S/N 1366 monochromator with 10 nm band-pass). The solution was stirred during the irradiation period. The depletion of DPBF due to its reactions with $^1O_2$ was monitored at 410 nm spectrophotometrically. The total depletion of DPBF was restricted to 10–15% of its original concentration and an average of five or six depletion-rate runs were used for quantum-yield measurements.[14]

For the gel electrophoresis experiments, supercoiled pBR 322 DNA (100 μM in nucleotide phosphate; buffer B: 10 mmol Tris, pH 8.0) was treated with a 100 μM solution (buffer B + 2–3% DMF) of the P–AnQ conjugates and the mixture was incubated for 1 h in the dark. The samples were then analyzed by 0.8% agarose gel electrophoresis (Tris–acetic acid–EDTA buffer, pH 8.0) at 40 V for 5 h. The gel was stained with 1 μg ml^{-1} ethidium bromide for 0.5 h, after which it was analyzed using the UVP gel documentation system GDS 2000 and was also directly photographed and developed as described previously.[3,5] Irradiation experiments were carried out by keeping the pre-incubated (dark, 1 h) samples inside the sample chamber of a JASCO Model FP-777 spectrofluorimeter (λ 350 ± 10 nm or 550 ± 10 nm).

Porphyrin–deoxycholic acid hybrid molecule 2

To a stirred mixture of **1** (30 mg, 0.06 mmol), 7-deoxycholic acid (50 mg, 0.13 mmol) and DMAP (1 mg) in dry THF (15 ml) was added a solution of DCC (30 mg, 0.15 mmol) in dry THF (5 ml) dropwise under N_2 at ambient temp. The reaction was monitored by TLC and was terminated after 20 h. The reaction mixture was filtered to remove the precipitated dicyclohexylurea (DCU) and the filtrate was washed with water, dried (anhydrous Na_2SO_4) and concentrated. Purification of the residue on silica gel chromatography (ethyl acetate–hexane, 2:1, v/v) furnished **2** (62 mg) in 82% yield, λ_{max} [CH_2Cl_2 (log ε)]/nm 406 (6.04), 503 (4.98), 536 (4.57), 576 (4.45), 630 (3.91); ^1H NMR (200 MHz; $CDCl_3$) δ −3.16 (2H, br s), 0.79 (6H, s), 0.94 (6H, s), 1.17 (6H, d), 1.27–2.20 (m, H), 2.78 (2H, m), 3.58 (1H, br s), 4.08 (1H, s), 7.55 (4H, d), 8.28 (4H, d), 9.11 (4H, d), 9.40 (4H, d), 10.31 (2H, s); $E_{1/2}$ (CH_2Cl_2 0.1 M TBAP; V *vs.* SCE) +0.98, −1.26.

Porphyrin–deoxycholic acid–anthraquinone triad 3

DCC (5 mg, 0.02 mmol) in dry CH_2Cl_2 (1 ml) was added dropwise to a stirred mixture of **2** (25 mg, 0.02 mmol), anthraquinone-2-carboxylic acid (AnQ2-CO_2H, 4 mg, 0.02 mmol) and DMAP (1 mg) in dry CH_2Cl_2 (8 ml), under N_2 at room temp. The reaction was complete in 2 h (TLC). The reaction mixture was filtered to remove the precipitated DCU and the filtrate was washed with water, dried (anhydrous Na_2SO_4) and concentrated. Purification of the residue by column chromatography on silica gel (ethyl acetate–hexane, 1.5:1, v/v) furnished the *desired product* **3** (28 mg) as a purple amorphous solid in 82% yield, mp 195–197 °C (Found: C, 77.40; H, 6.91; N, 3.75. $C_{95}H_{102}N_4O_{11}$ requires C, 77.31; H, 6.97; N, 3.80%). FAB-MS (*m/z*) 1477 $[M + H]^+$. Calc. for $C_{95}H_{102}N_4O_{11}$: *M*. 1476; ν_{max} (KBr pellet)/cm^{-1} 3447br, 2936, 2870, 1746, 1717, 1676, 1503, 1451, 1418, 1271, 1244, 1202, 1165, 793, 737, 708; λ_{max}[CH_2Cl_2 (log ε)]/nm 258 (5.44), 406 (6.01), 502 (4.91), 536 (4.57), 575 (4.41), 630 (3.93); ^1H NMR (200 MHz, $CDCl_3$) δ −3.18 (2H, br s), 0.80 (3H, s), 0.83 (3H, s), 0.89 (3H, s), 0.95 (3H, s), 1.02–2.18 (58H, m), 2.85 (2H, m), 3.64 (1H, br s), 4.10 (1H, br s), 5.08 (1H, br s), 7.58 (4H, m), 7.81 (4H, m), 8.34 (6H, m), 8.87 (1H, br s), 9.12 (4H, d), 9.42 (4H, d), 10.32 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8 (2C), 12.9, 17.4, 17.5, 23.2 (2C), 23.7, 26.0, 26.1, 26.6, 27.0, 27.1, 27.6, 27.7, 28.8, 28.9, 29.7, 30.5, 31.0, 31.5, 31.6, 31.9, 32.3, 33.7, 33.8, 34.1, 34.2, 34.9, 35.2, 36.1, 36.4, 42.0, 42.1, 46.6, 46.7, 47.4, 48.3, 71.8, 73.2, 73.3, 76.0, 105.4, 118.0, 120.2, 127.0, 127.2, 128.3, 131.0, 131.7, 133.0, 133.0, 133.1, 134.0, 134.1, 134.3, 135.6, 138.8, 145.2, 147.1, 150.8, 164.5, 172.9, 181.9, 182.2; $E_{1/2}$ (CH_2Cl_2, 0.1 M TBAP; V *vs.* SCE) +0.95, −0.77, −1.23.

Porphyrin–deoxycholic acid–anthraquinone triad 4

DCC (10 mg, 0.05 mmol) in dry CH_2Cl_2 (1 ml) was added dropwise to a stirred mixture of **2** (25 mg, 0.02 mmol), AnQ2-CO_2H (12 mg, 0.05 mmol) and DMAP (1 mg) in dry CH_2Cl_2 (10 ml) under N_2 at room temp. The reaction was complete in 3 h (TLC). Work-up and purification of the reaction mixture, as described above for **3**, furnished **4** (24 mg) as a *purple amorphous solid* in 77% yield, mp 228–230 °C (Found: C, 77.19; H, 6.34; N, 3.24. $C_{110}H_{108}N_4O_{14}$ requires C, 77.26; H, 6.37; N, 3.28%). FAB-MS (*m/z*) 1711 $[M + H]^+$. Calc. for $C_{110}H_{108}N_4O_{14}$: *M* 1710; ν_{max} (KBr pellets)/cm^{-1} 2932, 1755, 1721, 1678, 1593, 1271, 1202, 1167, 930, 793, 737, 706; λ_{max}[CH_2Cl_2 (log ε)]/nm 258 (5.72), 407 (6.21), 502 (4.92), 538 (4.51), 575 (4.41), 630 (3.90); ^1H NMR (200 MHz; $CDCl_3$) δ −3.22 (2H, br s), 0.83 (6H, s), 1.02 (6H, s), 1.20–2.18 (58H, m), 2.85 (2H, m), 4.13 (1H, s), 5.08 (1H, br s), 7.58 (4H, d), 7.79 (4H, m), 8.23–8.42 (12H, m), 8.86 (2H, br s), 9.11 (4H, d), 9.40 (4H, d), 10.29 (2H, br s); ^{13}C NMR (75 MHz; $CDCl_3$) δ 12.8, 17.5, 23.1, 23.7, 26.0, 26.6, 26.9, 27.6, 28.8, 31.0, 31.5, 32.3, 33.8, 34.2, 34.9, 35.2, 36.0, 42.0, 46.6, 47.4, 48.4, 73.2, 76.0, 105.3, 118.0, 120.2, 127.0, 127.1, 128.2, 131.0, 131.7, 132.93, 132.95, 132.97, 133.9, 134.0, 134.1, 135.5, 135.6, 138.8, 145.1, 147.0, 150.7, 164.4, 172.9, 181.7, 182.0; $E_{1/2}$ (CH_2Cl_2, 0.1 M TBAP; V *vs.* SCE): +0.98, −0.79, −1.23.

Porphyrin–polymethylene–anthraquinone triad 5

A mixture of porphyrin **1** (60 mg, 0.12 mmol) and 1-bromo-3-hydroxypropane (67 mg, 0.48 mmol) in dry DMF (5 ml) containing anhydrous K_2CO_3 was stirred at room temp. for 36 h, after which the reaction mixture was poured into 20 ml water and extracted thrice with $CHCl_3$. The organic layer was washed

with water, dried (anhydrous Na_2SO_4) and concentrated. The resulting residue, after chromatographic purification (alumina; $CHCl_3$–CH_3OH, 10:1, v/v), furnished a pure sample of *meso-5,15-bis[4-(3-hydroxypropyl)phenyl]porphyrin* (45 mg) as a purple amorphous solid in 60% yield, mp >300 °C (Found: C, 74.68; H, 5.57; N, 9.22. $C_{38}H_{34}N_4O_4$ requires C, 74.74; H, 5.61; N, 9.17%). λ_{max} (CH_2Cl_2)/nm 408, 505, 540, 576, 631; ^{1}H NMR (200 MHz; $CDCl_3$) δ −3.10 (2H, br s), 2.28 (4H, t), 3.94 (4H, m), 4.45 (4H, t), 7.36 (4H, d), 8.16 (4H, d), 9.10 (4H, d), 9.45 (4H, d), 10.40 (2H, s).

DCC (10 mg, 0.05 mmol) in dry CH_2Cl_2 (1 ml) was added dropwise to a stirred mixture of *meso*-5,15-bis[4-(3-hydroxypropyl)phenyl]porphyrin (12 mg, 0.02 mmol), AnQ2-CO_2H (12 mg, 0.05 mmol) and DMAP (1 mg) in dry CH_2Cl_2 (10 ml) under N_2 at room temp. Work-up and purification of the reaction mixture, as described above for **4**, furnished **5** (15 mg) as a *purple solid* in 70% yield, mp >300 °C (Found: C, 75.60; H, 4.25; N, 5.22. $C_{68}H_{46}N_4O_{10}$ requires C, 75.69; H, 4.30; N, 5.19%). λ_{max} [CH_2Cl_2 (log ε)] 253 (5.93), 409 (6.13), 505 (4.75), 539 (4.57), 576 (4.32), 632 (3.97); ^{1}H NMR (200 MHz; $CDCl_3$) δ −3.09 (2H, br s), 2.56 (4H, t), 4.52 (4H, m), 4.85 (4H, t), 7.38 (4H, d), 7.82 (4H, d), 8.18–8.54 (12H, m), 9.09 (6H, m), 9.39 (4H, d), 10.29 (2H, br s); $E_{1/2}$ (CH_2Cl_2, 0.1 M TBAP; V *vs.* SCE) +0.94, −0.80, −1.28.

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