COMMUNICATION

Porphyrin–cholic acid–chlorambucil triads: synthesis and light-induced nuclease activity

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A new module composed of a porphyrin, cholic acid and chlorambucil has been designed for use in photodynamic therapy. Light-induced nuclease activity of the new sensitizers has been evaluated.

Photodynamic therapy (PDT), involving the action of light on a photosensitizing drug to generate an activated oxygen species that causes tumour necrosis, is being widely explored as an alternative modality to radio- and chemo-therapy for the treatment of cancer.[1] With the first generation Photofrin®, a haematoporphyrin derivative, already in clinical use, efforts are currently underway to develop new porphyrin-based sensitizers to achieve greater therapeutic efficacy through enhanced tumour selectivity, tissue penetration capability and improved quantum efficiency of singlet oxygen generation.[1] In this context, structurally and functionally modified porphyrins incorporating some of these desirable characteristics are being synthesized and evaluated. However, an alternative strategy involving 'hybrids' or 'conjugates' of porphyrins with other biologically potent molecular entities, to 'add-on' attributes like intracellular recognition, tissue and organ selectivity, has received only limited attention.[2,3] We have recently reported the synthesis of conjugates of porphyrin with DNA intercalators (acridone, phenothiazine, *etc.*)[3a,c] and cross-linking agents (the cytotoxic drug chlorambucil)[3b] and shown that these 'hybrids' exhibit impressive light-induced nuclease activity. In view of these promising observations, we considered the design of the next generation of 'porphyrin hybrids' for use in PDT, in which intra- and inter-cellular and organ specific recognition elements are concurrently present. Towards this objective, cholic acid **1a**, a cheap, readily available bile acid with rigid, well-defined stereostructure and plentiful, dispersed functionalization, and known for its membrane affinity and liver cell selectivity, appeared to be an advantageous platform to build new 'porphyrin hybrids'.[4] Here, we report on the preparation, characterization and light-induced nuclease activity of porphyrin–cholic acid–chlorambucil triads.

Initially, we planned to append the porphyrin (P) and chlorambucil (C) moieties on cholic acid as far apart as possible, so as to additionally exploit the rigid steroidal framework of **1a** as a well defined spacer unit. Thus, **1a** was esterified with 5-(4-hydroxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin **2a** employing the classical 1,3-dicyclohexylcarbodiimide (DCC)–4-dimethylaminopyridine (DMAP) (0 °C, THF, 93%) procedure to introduce the porphyrin moiety (P) at the carboxy terminus. The resulting cholic acid–porphyrin conjugate **4**, on further reaction (DCC, DMAP, 0 °C, CH_2Cl_2) with chlorambucil **3a**, furnished **5**, **6** and **7** (2:3:1) in 79% yield, which could be separated *via* extensive column chromatography (silica gel). The regioisomers **5** and **6** were characterized on the basis of the relative deshielding of C-3 and C-7 proton resonances of the cholic acid moiety which is well precedented. Formation of **7** was particularly noteworthy as it was possible to 'load' the cholic acid platform with two units of the clinically used cytotoxic drug chlorambucil in addition to the porphyrin moiety. For comparison purposes, we also prepared the methyl cholate **1b**, chlorambucil conjugates **8**, **9** and **10** (2:3:1, 84%) and the diporphyrin derivative **11** (obtained by reacting **4** with **2b**, 64%). Interestingly, under our reaction conditions (DCC–DMAP), chlorambucil **3a** was non-discriminating in engaging the C_3- and C_7-hydroxy groups of cholic acid and as per our requirement, all the three triads **5**, **6** and **7** were formed in the reaction. However, when **4** or **1b** was reacted (a few drops of pyridine, 0 °C, CH_2Cl_2) with the acid chloride **3b**, only **5** (63%) and **8** (67%) were formed in a regioselective manner.

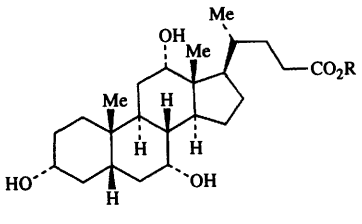
Table 1 Fluorescence [ϕ(f)] and singlet oxygen [$\phi(^1O_2)$] quantum yield data

Compounds	ϕ(f)[a]	$\phi(^1O_2)$[b]
2a	0.13	0.60
4	0.10	0.66
5	0.10	0.60
6	0.11	0.75
7	0.11	0.81
11	0.13	0.80

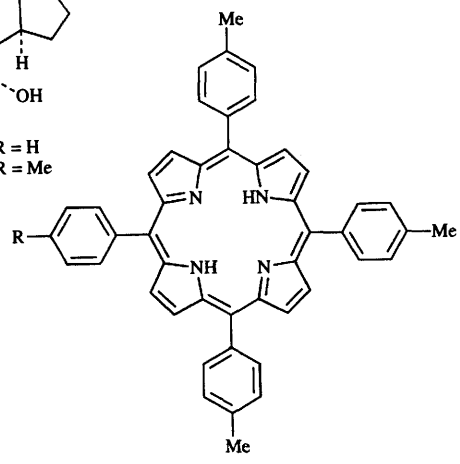
[a] Measured in CH_2Cl_2 (λ_{ex} = 420 nm) with H_2TPP [ϕ(f) = 0.11] as the standard. Error limits, ± 10%. [b] Measured in DMF by the steady state photolysis method using diphenylisobenzofuran as the singlet oxygen scavenger. All the samples were irradiated at 555 nm using a 150 W Xe arc lamp. Error limits, ± 15%.

The electronic absorption and emission spectral data and the redox potential data of these new porphyrin–chlorambucil triads were found to be in the same range as those of control porphyrins **2a**, **2b** and the porphyrin–chlorambucil 'diads' reported earlier.[3b] In addition, and more importantly, the singlet oxygen generation efficiency of **5**, **6** and **7** was essentially the same as that of the unlinked porphyrins **2a** or **2b**, as seen by the $\phi(^1O_2)$ data (Table 1). Collectively, these observations suggest that in the new sensitizers **5**–**7**, wherein a porphyrin is appended to cholic acid and chlorambucil sub-units, the desirable photophysical attributes are retained.

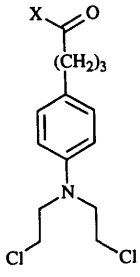
As a prelude to more detailed biological evaluation of the novel 'triads' **5**–**7**, we studied their nuclease activities and compared them with those of **8**–**11**, using the supercoiled plasmid DNA pBR 322. In the absence of light, **5**–**11** exhibited no perceptible nicking. However, in the presence of light (Fig. 1) and under the experimental conditions employed, the results appeared to be quite promising. While the 'triads' **5**–**7** almost fully relaxed the supercoiled form I to II, with the 'loaded' **7** exhibiting highest efficacy, the cholic acid–chlorambucil conjugates **8**–**10** without the porphyrin moiety displayed little photocleavage activity. The reference porphyrin **2a** and the diporphyrin **11** also showed only marginal nicking. These results indicate that incorporation of a cellular level recognition element like cholic acid does not adversely effect the photonuclease activity of triads **5**–**7**. We are presently studying the efficacy of these new hybrids against human cancer cell lines.



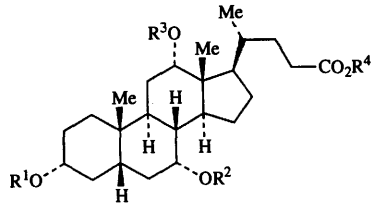
1a R = H
b R = Me



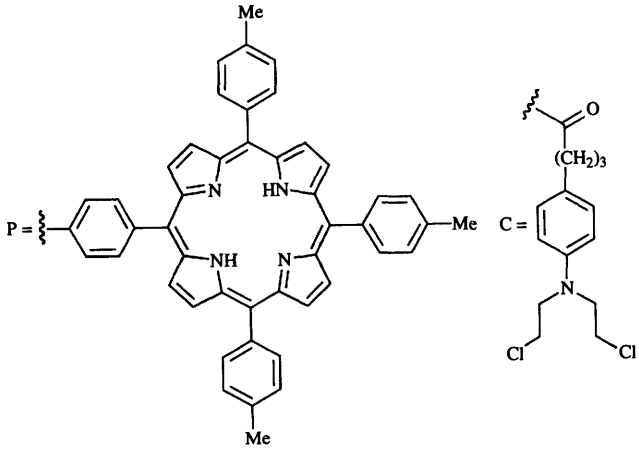
2a R = OH
b R = CO_2H



3a X = OH
b X = Cl



4 R^1 = H, R^2 = H, R^3 = H, R^4 = P
5 R^1 = C, R^2 = H, R^3 = H, R^4 = P
6 R^1 = H, R^2 = C, R^3 = H, R^4 = P
7 R^1 = C, R^2 = C, R^3 = H, R^4 = P
8 R^1 = C, R^2 = H, R^3 = H, R^4 = Me
9 R^1 = H, R^2 = C, R^3 = H, R^4 = Me
10 R^1 = C, R^2 = C, R^3 = H, R^4 = Me
11 R^1 = C(O)P, R^2 = H, R^3 = H, R^4 = P



In summary, we have shown that triads composed of porphyrin, cholic acid and chlorambucil moieties exhibit significant photo-induced nuclease activity.

Experimental

Preparation of the triad molecules 5–7

DCC (0.43 g, 2 mmol) in THF (15 cm^3) was added to a stirred mixture of 5-(4-hydroxyphenyl)-10,15,20-tris(4-methylphenyl)-porphyrin **2a** (1 g, 1.5 mmol), cholic acid (0.61 g, 1.5 mmol) and

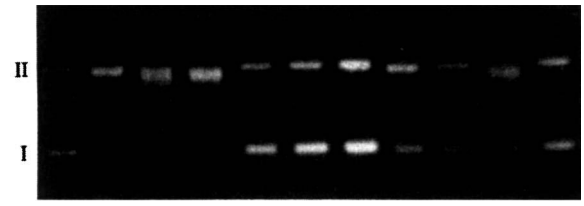


Fig. 1 Light-induced nuclease activity of the porphyrin–cholic acid–chlorambucil triads with the percentage relaxation to Form II measured using UVP gel documentation system GDS 2000. (Left to right) Lane 1: untreated pBR 322 (35% II); Lanes 2–11: pBR 322 + **5** (89), **6** (90), **7** (100), **8** (41), **9** (35), **10** (39), **4** (65), **11** (56), **2a** (66), **3a** (52), respectively. In each case, the proportion DNA/Drug = 1 and samples were incubated for 1 h before being irradiated with visible light (λ > 400 nm) for 2.5 h. Electrophoresis experiments and analysis of the data were carried out as described in ref. 3(*b*).

DMAP (18 mg, 0.15 mmol) in dry THF (50 cm^3) under N_2 at 0 °C. Stirring was continued for 25 h at room temperature. The reaction mixture was filtered to remove the precipitated dicyclohexylurea and the filtrate was washed with water, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate–chloroform, 3 : 1) to furnish the porphyrin–cholic acid conjugate **4** in 93% yield.

DCC (70 mg, 0.3 mmol) in CH_2Cl_2 (2 cm^3) was added dropwise to a stirred mixture of conjugate **4** (0.24 g, 0.2 mmol), chlorambucil (0.07 g, 0.2 mmol) and DMAP (2 mg, 0.02 mmol) in dry CH_2Cl_2 (15 cm^3) at room temperature under N_2. The reaction mixture was stirred for 12 h and worked up as described above. The chromatographic purification (ethyl acetate–hexane eluent) of the residue on silica gel furnished the products **5** (71 mg, 28%), **6** (97 mg, 39%) and **7** (37 mg, 12%). Selected data for **5**: ν_{max}(KBr)/cm^{-1} 3447, 2924, 1752, 1725, 1615, 1518, 1470, 1349, 1202, 801; δ_H(200 MHz; $CDCl_3$) −2.76 (2 H, br s), 0.78 (3 H, s), 0.93 (3 H, s), 1.18 (3 H, d, *J* 5.0†), 1.42–2.21 (br m), 2.30 (2 H, t), 2.57 (2 H, t), 2.72 (9 H, s), 3.67 (8 H, q), 3.89 (1 H, s), 4.08 (1 H, s), 4.63 (1 H, br s), 6.64 (2 H, d, *J* 8.6), 7.09 (2 H, d, *J* 8.6), 7.50 (2 H, d, *J* 8.6), 7.57 (6 H, d, *J* 7.8), 8.12 (6 H, d, *J* 7.8), 8.24 (2 H, d, *J* 8.6), 8.89 (8 H, s); *m*/*z* (FAB) 1348 (M^+). For **6**: ν_{max}(KBr)/cm^{-1} 3441, 2930, 1755, 1726, 1615, 1518, 1472, 1202, 1165, 966, 801, 735; δ_H(200 MHz; $CDCl_3$) −2.78 (2 H, br s), 0.79 (3 H, s), 0.95 (3 H, s), 1.17 (3 H, d, *J* 5.0), 1.27–2.10 (br m), 2.36 (2 H, t), 2.61 (2 H, t), 2.72 (9 H, s), 3.53 (1 H, br s), 3.67 (8 H, q), 4.10 (1 H, s), 4.98 (1 H, s), 6.65 (2 H, d, *J* 8.6), 7.11 (2 H, d, *J* 8.6), 7.48 (2 H, d, *J* 8.0),7.57 (6 H, d, *J* 7.7), 8.10 (6 H, d, *J* 7.7), 8.22 (2 H, d, *J* 8.0), 8.87 (8 H, s); *m*/*z* (FAB) 1348 (M^+). For **7**: ν_{max}(KBr)/cm^{-1} 3420, 2926, 1753, 1728, 1615, 1519, 1468, 1352, 1182, 862, 737; δ_H(200 MHz; $CDCl_3$) −2.78 (2 H, br s), 0.79 (3 H, s), 0.97 (3 H, s), 1.17 (3 H, d, *J* 5.0), 1.27–2.38 (br m), 2.55 (2 H, t), 2.72 (9 H, s), 3.56–3.69 (16 H, m), 4.10 (1 H, s), 4.63 (1 H, br s), 4.97 (1 H, s), 6.63 (4 H, d, *J* 8.0), 7.08 (4 H, d, *J* 8.0), 7.47 (2 H, d, 8.6), 7.57 (6 H, d, *J* 8.0), 8.1 (6 H, d, *J* 8.0), 8.22 (2 H, d, *J* 8.6), 8.87 (8 H, s); *m*/*z* (FAB) 1633 (M^+).

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† *J* Values given in Hz.

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