# Synthesis and Nuclease Activity of Some 'Porphyrin–Acridone' Hybrid Molecules

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A short synthesis of several 'porphyrin–acridone' hybrids **4a–e** based on 5-(4-hydroxyphenyl)-10,15,20-tris(*p*-tolyl)porphyrin **1** and observations on their photoinitiated nuclease activity are reported.

Synthesis of novel and structurally modified porphyrins for use as photosensitizers in the photodynamic therapy (PDT) of malignant cancers is an actively pursued area of research.[1] These synthetic efforts have been mainly directed towards fine-tuning of absorption maxima, enhancing aggregation and cellular recognition and increasing the singlet oxygen quantum efficiency of the porphyrin system. However, as the exact mechanism of PDT in cancer treatment is unknown, there is scope for exploring new variations to amplify the therapeutic efficiency of the sensitizer. It occurred to us that porphyrin based sensitizers in combination with an intracellular recognition element might acquire 'dual action' capabilities.[2] To this end, we sought to generate a hybrid of a suitable porphyrin with an acridone moiety as the latter is known to be a DNA intercalator and some acridone alkaloids have recently been shown to possess anti-cancer activity.[3] Herein, we describe the synthesis of several hybrid molecules employing 5-(4-hydroxyphenyl)-10,15,20-tris(*p*-tolyl)porphyrin **1**[2b,4] and acridone **3** linked through a $-(CH_2)_n-$spacer and also record observations on their singlet oxygen quantum efficiency and light induced DNA cleavage abilities.

Porphyrin derivative **1**[2b,4] appeared well suited for linkage to **3** and on treatment with an excess of α,ω-dibromoalkanes ($n$ = 2–6) in the presence of $K_2CO_3$–dimethylformamide (DMF)[5] led to the ω-bromoalkoxy derivatives **2a–e** in high yield (>90%). Under phase transfer conditions (PTC) (TEBACl), acridones are known[6] to undergo preferential *N*-alkylation and accordingly **2a–e** underwent smooth displacement of bromine with **3** to furnish the desired hybrid molecules **4a–e** (45–70%), which were characterized through their UV–VIS, $^1H$ NMR data and elemental analyses. A reference compound **2f** was also prepared from **1** with 1-amino-3-bromopropane under $K_2CO_3$–DMF conditions, Scheme 1.

The singlet oxygen quantum yields of **4a–e** and **2f** are presented in Table 1. These values are in the same range as the model **1** and indicate that appending an acridone moiety has no appreciable adverse effect on the photosensitizing ability. However, we have observed exceptionally efficient energy transfer from the acridone to the porphyrin moiety, which exhibits an interesting dependence on the spacer length (Table 1). More efficient energy transfer is observed for **4b–d** ($n$ = 3, 4, 5) compared to **4a** ($n$ = 2) and **4f** ($n$ = 6), possibly owing to the conformational flexibility in **4b–d** that enables the acridone moiety to fold-over. The efficient energy transfer observed here is a positive feature as it provides flexibility of irradiation anywhere inside the absorption envelope of **4a–e** and yet eventuates in the excited state of the sensitizer.

The nuclease activity of hybrid molecules **4a–e** was studied using the supercoiled plasmid DNA pBR322 in the presence and absence of light. While no nicking was observed in the absence of light, irradiation by visible light (see Experimental section) caused nicking and generation of relaxed circular DNA as shown in Fig. 1. However, in the model compound **2f**, without the acridone moiety and also in 9-propylacridone, nicking was not perceptible under similar experimental conditions. These observations demonstrate that intercalation and photosensitization can be coupled to enhance nuclease activity. We are presently extending this 'dual action' theme to prepare new hybrids for PDT and DNA cleavage.

**Table 1** Energy transfer efficiency (%$T$) and singlet oxygen quantum yield $\varphi(^1O_2)$ data of the acridone linked porphyrins[a]

| Compound | %$T$[b] | $\varphi(^1O_2)$[c] |
|---|---|---|
| **4a** | 74 | 0.56 |
| **4b** | 85 | 0.69 |
| **4c** | 98 | 0.66 |
| **4d** | 80 | 0.70 |
| **4e** | 77 | 0.60 |
| **2f** | — | 0.60 |

[a] Error limits for both %$T$ and $\varphi(^1O_2)$ are ± 10%. [b] Calculated from the overlap of the corrected and normalized (515 nm) fluorescence excitation ($\lambda_{em}$ 650 nm) spectra with the absorption spectra of each compound (solvent $CH_2Cl_2$). [c] Measured in DMF by the steady-state photolysis method using 1,3-diphenylisobenzofuran (DPBF) as the $^1O_2$ acceptor.[7] All the samples were irradiated at 555 nm using 150 W Xe lamp as the light source.

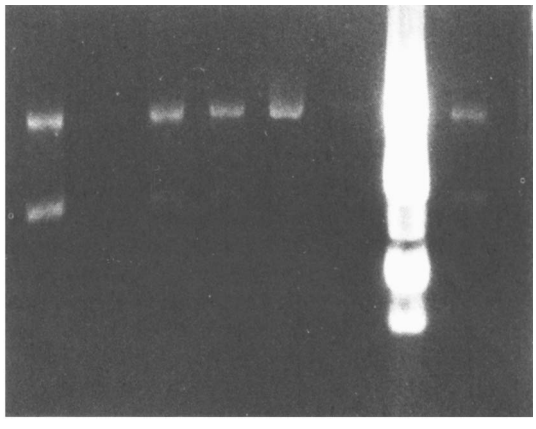
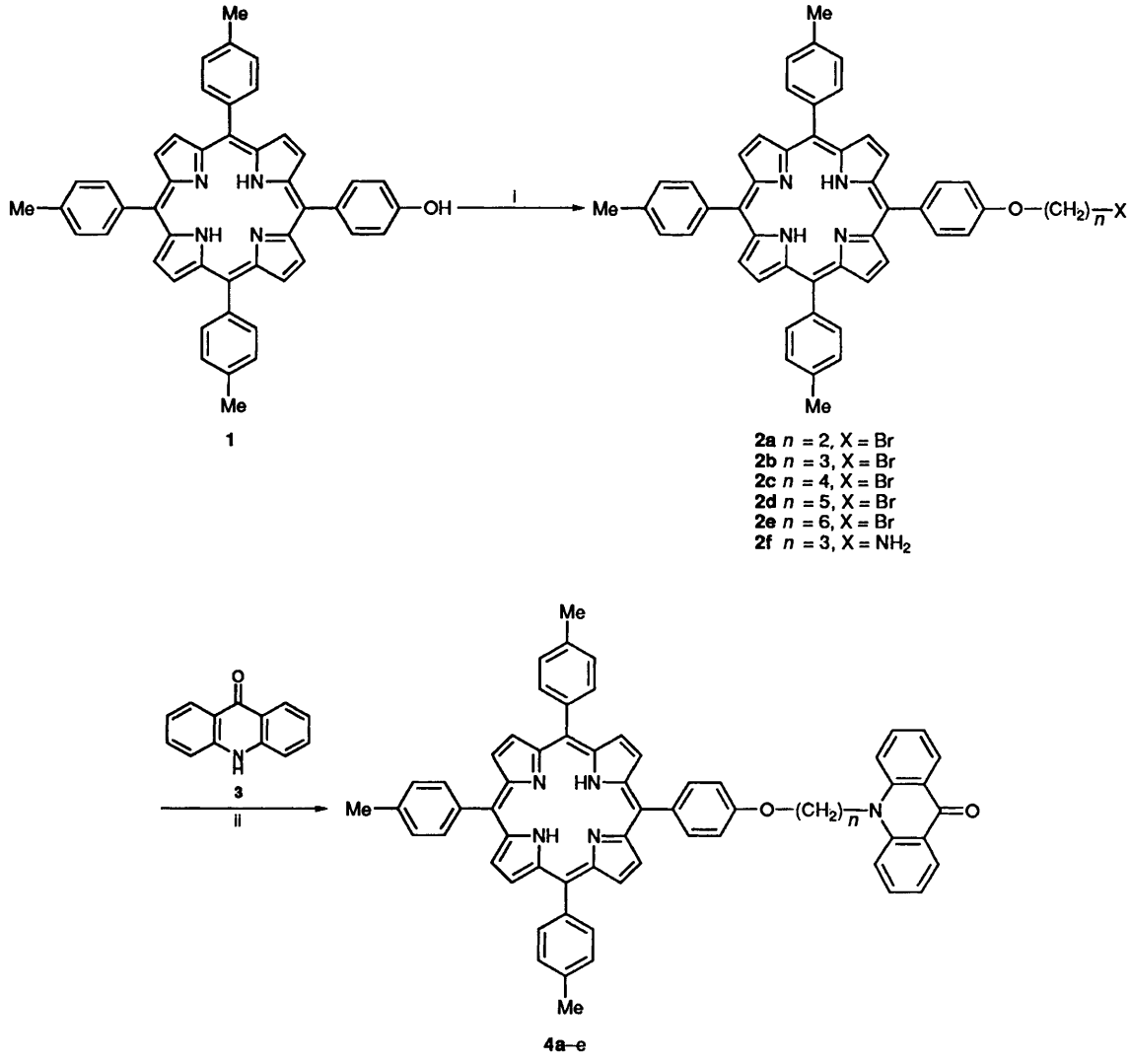


**Fig. 1** Lane 1: untreated pBR322; lanes 2, 3, 4 and 5: pBR322 + **4a**, **4b**, **4c** and **4f**, respectively. DNA migration is from top to bottom and the bright spot is the DNA-molecular weight marker.

## Experimental

*General Procedure for the Preparation of 'Porphyrin–Acridone' Hybrid Molecules* **4a–e**.—A mixture of **1** (0.15 mmol),




**Scheme 1** *Reagents:* i, $Br(CH_2)_nX$, $K_2CO_3$, DMF, >90%; ii, **3**, aq. KOH–toluene, TEBACl, 45–70%

α,ω-dibromoalkane (1.98 mmol) and anhydrous $K_2CO_3$ (300 mg) in DMF (15 $cm^3$) was stirred under $N_2$ for 24 h. The reaction mixture was poured into water (50 $cm^3$) and filtered. Chromatography of the solid on basic $Al_2O_3$ using $CH_2Cl_2$ as eluent furnished **2a–e** in >90% yield, which were characterized by $^1H$ NMR data. A mixture of **2a–e** (0.027 mmol), acridone (0.054 mmol), triethylbenzylammonium chloride (TEBACl) (20 mg), 50% aq. KOH (10 $cm^3$) and toluene (20 $cm^3$) was refluxed for 5 days. The organic layer was concentrated and chromatographed on $SiO_2$ gel to furnish the title compounds **4a–e** in 45–70% yield (based on starting material recovery). **4a–e** $\lambda_{max}$-($CH_2Cl_2$)/nm 647–650, 590–593, 549–553 and 514–517 (Q bands), 417–420 (Soret band) and 254–257 (acridone band). **4a**: $\delta_H$(200 MHz; $CDCl_3$) −2.77 (2 H, s), 2.69 (9 H, s), 4.73 (2 H, t), 5.00 (2 H, t), 7.20–7.38 (4 H, m), 7.52–7.56 (8 H, m), 7.81–7.85 (2 H, m), 8.05–8.11 (8 H, m), 8.65 (2 H, dd) and 8.85–8.77 (8 H, m) (Found: C, 83.35; H, 5.3; N, 7.9. $C_{62}H_{47}N_5O_2$ requires C, 83.29; H, 5.30; N, 7.83%). **4b**: $\delta_H$(200 MHz; $CDCl_3$) −2.76 (2 H, s), 2.62 (2 H, m), 2.70 (9 H, s), 4.41 (2 H, t), 4.81 (2 H, t), 7.26–7.36 (4 H, m), 7.53–7.57 (8 H, m), 7.77–7.80 (2 H, m), 8.08–8.17 (8 H, m), 8.65 (2 H, dd) and 8.85 (8 H, s) (Found: C, 83.15; H, 5.5; N, 7.7. $C_{63}H_{49}N_5O_2$ requires C, 83.32; H, 5.44; N, 7.71%). **4c**: $\delta_H$(200 MHz; $CDCl_3$) −2.76 (2 H, s), 2.41 (4 H, m), 2.69 (9 H, s), 4.31 (2 H, t), 4.50 (2 H, t), 7.23–7.33 (4 H, m), 7.51–7.62 (8 H, m), 7.69–7.80 (2 H, m), 8.06–8.13 (8 H, m), 8.61 (2 H, dd) and 8.85 (8 H, s) (Found: C, 83.45; H, 5.6; N, 7.6. $C_{64}H_{51}N_5O_2$ requires C, 83.36; H, 5.57; N, 7.59%). **4d**: $\delta_H$(200 MHz; $CDCl_3$) −2.75 (2 H, s), 1.96 (2 H, m), 2.14 (4 H, m), 2.71 (9 H, s), 4.32 (2 H, t), 4.48 (2 H, t), 7.26–7.38 (4 H, m), 7.54–7.62 (8 H, m), 7.75–7.84 (2 H, m), 8.08–8.15 (8 H, m), 8.63 (2 H, dd) and 8.86 (8 H, s) (Found: C, 83.5; H, 5.7; N, 7.5. $C_{65}H_{53}N_5O_2$ requires C, 83.39; H, 5.71; N, 7.48%). **4e**: $\delta_H$(200 MHz; $CDCl_3$) −2.76 (2 H, s), 1.81 (4 H, m), 2.06 (4 H, m), 2.70 (9 H, s), 4.30 (2 H, t), 4.45 (2 H, t), 7.26–7.37 (4 H, m), 7.54–7.60 (8 H, m), 7.73–7.80 (2 H, m), 8.63 (2 H, dd) and 8.85 (8 H, s) (Found: C, 83.4; H, 5.8; N, 7.35. $C_{66}H_{55}N_5O_2$ requires C, 83.42; H, 5.83; N, 7.37%).

*Relaxation of Supercoiled* pBR322 *DNA upon Photosensitization by* **4a–e**.—DNA (0.75 μmol $dm^{-3}$ DNA phosphate) in tris-HCl buffer (pH 8) was treated with an equimolar concentration of the porphyrin dissolved in dimethyl sulfoxide (DMSO) (minimum volume)[8] and the mixture was incubated for 1 h. Irradiation (1 h) was carried out in the sample compartment of a Perkin-Elmer 650-10S spectrofluorimeter at 425 nm by keeping the excitation slit wide open. The samples were analysed by 1% agarose gel electrophoresis [tris-borate-ethylenediaminetetraacetic acid (EDTA) buffer, pH 8.3] at a constant voltage.

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