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Octaalkynyltetra[6,7]quinoxalinoporphyrazines: a new class of photosensitisers with potential for photodynamic therapy

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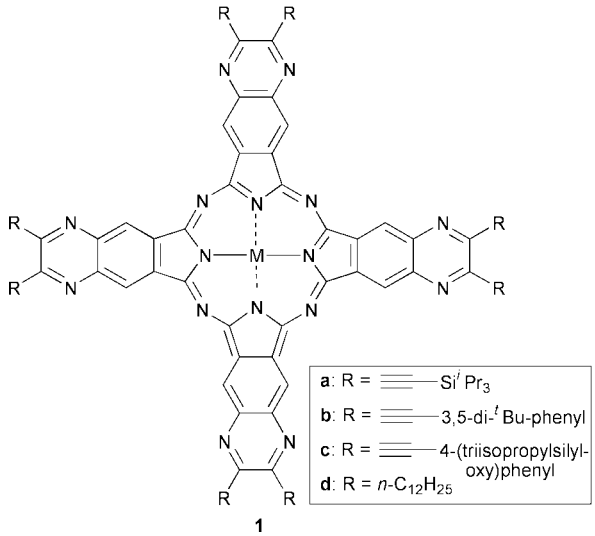
Acetylene-substituted tetra[6,7]quinoxalinoporphyrazines, obtained in two steps from dialkynyl-1,2-diones and 1,2-diamino-4,5-dicyanobenzene, show intense absorptions in the near infrared and have photooxidising properties that make them promising candidates as agents active in photodynamic therapy.

Photodynamic therapy (PDT) is an increasingly valuable method for the treatment of a variety of conditions, among them various forms of cancer, age-related macular degeneration, and, most recently, atherosclerosis.[1–4] At the core of PDT lies the generation of reactive oxygen species by energy transfer from a photosensitiser embedded in the malignant tissue which is irradiated with external light of appropriate wavelength. The reactive oxygen species in turn cause significant cellular damage and thereby ultimately lead to the eradication of the pathogenic tissue. Sensitisers suitable for biomedical applications of this type must meet the minimum requirements of accessibility by light in a biological matrix and of efficient induction of photooxidation. These requirements lead to the definition of a therapeutic window of *ca.* 650–800 nm in the absorption of practical PDT agents. Current applications largely exploit the photosensitising action of functionalised porphyrins such as a mixture of haematoporphyrin derivatives HpD, which is marketed under the tradename Photofrin®.[5] Despite the success of this drug in PDT, efforts are continuing to devise photosensitisers with an improved photochemical profile with respect to that of Photofrin®,[6] whose role in PDT is somewhat limited, among other things, by its low-intensity absorption around 630 nm.

We wish to present here octaalkynyltetra[6,7]quinoxalinoporphyrazines **1a–c** as a new class of photosensitisers which, by virtue of their concise and flexible synthesis and their (photo)chemical properties, are promising candidates as effective PDT agents. As such, **1a–c** represent viable alternatives to some of the phthalo- and naphthalocyanines that are currently being investigated as second generation sensitisers for PDT[6,7] and are a significant advancement over the corresponding octaalkynylphthalocyanines[8,9] and tetrapyrazinoporphyrazines previously reported.[10]

The syntheses of the quinoxalinoporphyrazines **1** are summarised in Scheme 1 and follow a route similar to the one outlined for the related octaalkynyltetrapyrazinoporphyrazines.[10] Key step in the preparation of the new acetylenic chromophores is the condensation of the dialkynyl-1,2-diones **4a–c**[11] with 1,2-diamino-4,5-dicyanobenzene **3** followed by a base-induced cyclotetramerisation of the intermediate quinoxalines to the porphyrazine framework. The alkyl-substituted derivative was prepared for comparison.† The new chromophores are deep blue (**1a**, **1d**) or green (**1b**, **1c**) solids that show good solubility in common organic solvents (CH_2Cl_2, THF).

The electron absorption spectra of compounds **1a–d** in THF are dominated by two transitions, namely the higher-energy *B*-band and the lower-energy *Q*-band, that in case of **1a–c** stretches into the near infrared (Fig. 1). The influence of peripheral substituents on the appearance of the UV–Vis–NIR



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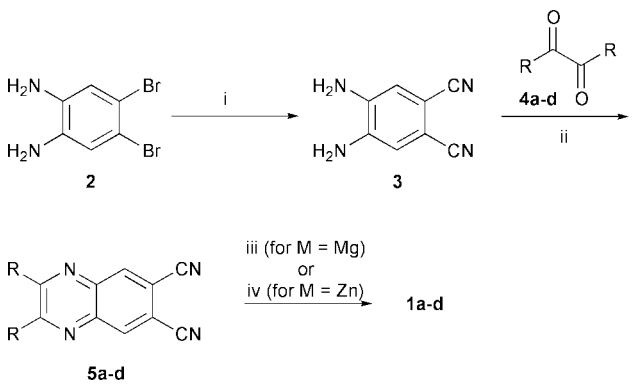
spectra of the tetra[6,7]quinoxalinoporphyrazines is quite remarkable. Whereas non-acetylenic **1d** shows a *B*-band at 364 nm (ε = 132 000 M^{-1} cm^{-1}) and a *Q*-band at 735 nm (ε = 431 000 M^{-1} cm^{-1}), the corresponding maxima of acetylenic compound **1a** (M = Mg, *B*-band: 388 nm, ε = 288 000 M^{-1} cm^{-1}; *Q*-band at 770 nm, ε = 512 000 M^{-1} cm^{-1}) are significantly batho- and hyperchromically shifted. This indicates that the π-system of the main chromophore of **1a** extends beyond the periphery of the polyazamacrocycle and includes the acetylene substituents. However, an extension of the π-system of **1a** by the appendage of terminal aryl groups to the acetylene units as, for example, in **1b** (*B*-band: 394 nm, ε = 306 000 M^{-1} cm^{-1}; *Q*-band: 770 nm, ε = 517 000 M^{-1} cm^{-1}, THF) does not result in a further bathochromic shift of the *Q*-band. In comparison to octaalkynyltetrapyrazinoporphyrazines[10] and −phthalocyanines,[8,9] whose *Q*-band absorption



2 **3** **5a-d**

Scheme 1 *Reagents and conditions*: i, CuCN, DMF, 140 °C, 15 h, 25%; ii, AcOH, rt., 20 min, 66–80%; iii, $Mg(OBu)_2$, BuOH, reflux, 1 h, 22–41%; iv, LiOPent, PentOH, reflux, 1 h, then $Zn(OAc)_2$, PentOH, reflux, 3 h, 44%.

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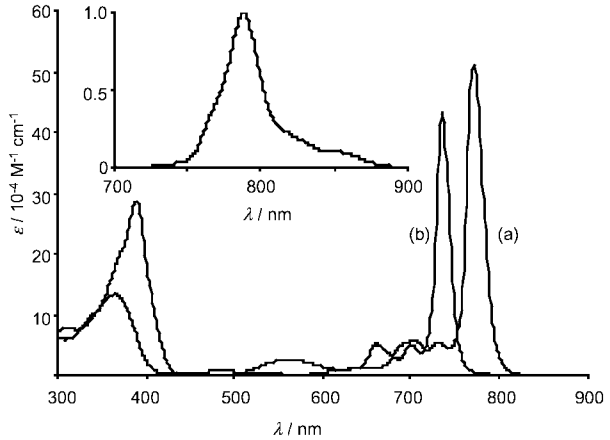

Fig. 1 Electronic absorption spectra of **1a** (a) and **1d** (b) in THF at 298 K. The inset shows the fluorescence spectrum of **1a** in THF at 298K upon irradiation at 388 nm.

maxima centre around 670 nm and 720 nm, respectively, the significantly red-shifted absorption of **1a–c** will allow the use of more deeply penetrating light for the excitation of **1a–c** in biological tissue. In addition, the high molar extinction coefficients associated with their NIR absorptions suggest a more detailed exploration of their suitability for PDT applications. Furthermore, the photostability of the compounds is satisfactory. Their aerated solutions in hexanol show very little degradation upon irradiation with light at a fluence rate of 50 mW cm^{-2} for several hours (*e.g.* 92% of the initial absorption of **1a** (M = Zn) at longest wavelength maximum still present after 4h irradiation). In light of the fluorescence at 790 nm that the alkynyl-substituted compounds **1a–c** exhibit upon irradiation at either their respective *B*- or *Q*-bands (Fig. 1), usage of this class of compounds as fluorescent probes in biological tissue or diagnostic tools in PDT can also be envisioned.

A qualitative evaluation of the photooxidising ability of **1a** (M = Zn) was performed using the singlet oxygen quencher 1,3-diphenylisobenzofuran (DPBF).[12] Hence, an aerated solution of **1a** (M = Zn) and DPBF (120-fold molar excess) in hexan-1-ol was exposed to filtered light (cut-off < 550 nm) of a slide projector lamp while monitoring the 413 nm absorption of DPBF (Fig. 2). No or little photooxidation of DPBF is observed in the absence of either light, **1a** (M = Zn) or oxygen (traces a–c, Fig. 2). However, significant photodegradation of DPBF occured in the presence of **1a** (M = Zn) and oxygen

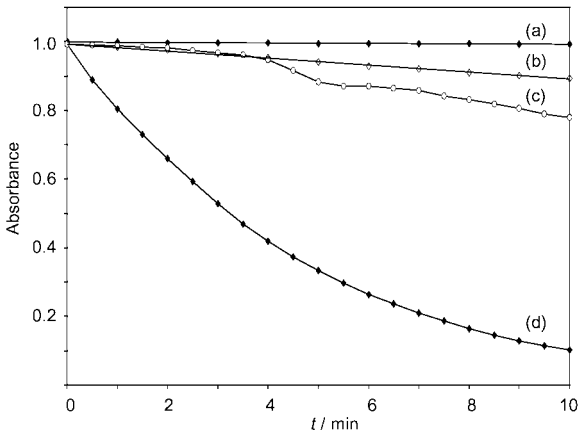

Fig. 2 Photooxidation of 1,3-diphenylisobenzofuran (DPBF) with **1a** (M = Zn) in hexanol at 298 K; c_o(DPBF) = 4.5×10^{-5} mol l^{-1}, c_o (**1a**) = 4.0×10^{-7} mol l^{-1}, light source: slide projector lamp (24 V, 250 W), cut-off filter <550 nm. The absorption at 413 nm was monitored in an aerated solution of **1a** (M = Zn) and DPBF upon irradiation (trace d); trace a, in the absence of light; trace b, in the absence of **1a** (M = Zn); trace c, in the absence of oxygen.

Table 1 Fluorescence lifetimes τ_f, fluorescence quantum yields Φ_f and singlet oxygen quantum yields Φ_Δ for selected tetra[6,7]quinoxalinoporphyrazines[a]

Compound	τ_f/ns	Φ_f[b]	Φ_Δ[c]
1a (M = Mg)	4.3 ± 0.1	0.46	0.19
1a (M = Zn)	2.4 ± 0.1	0.25	0.56
1d (M = Mg)	5.3 ± 0.1	0.59	0.15

[a] All measurements were performed in aerated THF at 298 K. [b] Absolute values (±10%) relative to cresyl violet in MeOH (Φ_f = 0.54) and disulfonated aluminium phthalocyanine in H_2O (Φ_f = 0.40) standards. [c] Obtained by time-resolved phosphorescence measurements using excitation at λ_{ex} = 355 nm. Values are relative to perinaphthenone (Φ_Δ = 0.97) and have an error of ±10%.

(trace d, Fig. 2), clearly demonstrating the photooxidising ability of the zinc quinoxalinoporphyrazine. The corresponding magnesium derivative **1a** (M = Mg) shows a markedly reduced photosensitising effect.

As shown in Table 1, these qualitative findings are further substantiated by the photophysical data obtained for compounds **1a** (M = Mg, Zn) and **1d** (M = Mg). Whereas the magnesium porphyrazinato complexes are strong fluorophores with only limited singlet oxygen producing capacity, the corresponding zinc complex **1a** (M = Zn) is a very efficient singlet oxygen sensitiser with a singlet oxygen quantum yield of Φ_Δ = 0.56. In fact, this value is comparable with that determined for photofrin (Φ_Δ = 0.57 in benzene)[1] and significantly exceeds that determined for silicon naphthalocyanine (Φ_Δ = 0.35 in benzene),[1] an analogue of **1** that has been investigated in the context of PDT applications.

The short and flexible synthesis of alkynyl-substituted quinoxalinoporphyrazines coupled with their high intensity absorption and their emission in the near infrared make them interesting candidates for future PDT applications. While the lipophilicity of the prototypical compounds presented here will require an administration *via*, for example, liposomal formulations, further adaptations of the chromophores to the requirements set by biological environments can be easily achieved by exploiting, for example, the chemistry of the protected phenol functionality in **1c**. Work along these lines is currently under way.

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Notes and references

† All new compounds are fully characterised by spectroscopic and analytical data. Detailed procedures for their syntheses will be reported elsewhere.

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