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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1665–1668
Bioorganic & Medicinal Chemistry Letters

# Synthesis and characterization of a carboranyl-tetrabenzoporphyrin

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Received 16 December 2004; revised 14 January 2005; accepted 19 January 2005

**Abstract**—An expeditious synthetic route to a carboranyl-substituted tetrabenzoporphyrin is reported. The absorption and emission spectra of water-soluble tetrabenzoporphyrin **4** are distinct from those of a known carboranylporphyrin (**5**). Both tetrabenzoporphyrin **4** and porphyrin **5** were found to be non-toxic toward V79 hamster lung fibroblast cells at 300 μM, using an MTT assay. The X-ray structure of a Cu(II)–carboranyl-tetrabenzoporphyrin is presented and discussed.


Tetrabenzoporphyrins, as the name indicates, contain four β,β′-fused benzene rings onto a porphyrin macrocycle, and thus belong to the family of π-extended porphyrins. Due to the extension of the π-conjugated system, tetrabenzoporphyrins display unique optical, redox, chemical and physical properties, and have found numerous applications, for example, as non-linear optical materials, near-infrared dyes, gas sensors, photosynthetic model compounds, and as photosensitizers for the photodynamic therapy (PDT) of tumors.[1] PDT involves the activation of a tumor-localized porphyrin drug with red light with concomitant production of singlet oxygen and other cytotoxic species that cause cell death.[2,3] Tetrabenzoporphyrins have advantage over porphyrins as PDT sensitizers in that they typically absorb strongly in the red region of the optical spectrum, where light penetration through tissue is considerably deeper. Thus the discovery of new synthetic routes to functionalized tetrabenzoporphyrins has been the focus of research in recent years, particularly of water-soluble derivatives bearing sulfonic acid[4] or carboxylic acid[5] groups. We report herein the synthesis of a new water-soluble carboranyl-tetrabenzoporphyrin, the first boron-containing macrocycle of this type, for dual application as sensitizer in the PDT and the boron neutron capture therapy (BNCT) of tumors.

BNCT,[6] like PDT, is a binary modality for cancer treatment based on the capture of low energy neutrons by $^{10}B$ nuclei, selectively accumulated in tumor tissue. This neutron capture reaction produces a γ-ray and two highly cytotoxic particles, $^{4}He^{+2}$ and $^{7}Li^{+3}$, that cause tumor destruction via ionization processes. Boron-containing porphyrins are among the most promising BNCT agents because of their demonstrated selectivity for tumor tissues, and their persistence within tumors during irradiation.[7,8] The *closo*- and *nido*-carborane cages are normally the source of boron in these agents, due to their remarkable stabilities, high boron content, and easy linkage to organic platforms via the carbon atoms. Since the carborane groups do not significantly alter the photosensitizing properties of porphyrin macrocycles, carboranyl-substituted porphyrins have been proposed for dual application in PDT and BNCT.[9–11] The combination of these two localized cancer treatment modalities could potentially lead to a greater therapeutic efficacy with minimal side effects. With the aim to increase the effectiveness of dual sensitizers, we have recently synthesized a carboranylchlorin, with a significantly stronger absorption in the red region of the spectrum compared with carboranylporphyrins.[12] We now report the synthesis and characterization of a carboranyl-tetrabenzoporphyrin, and compare its absorption, emission, and cytotoxic properties with those of a known carboranylporphyrin.

The water-soluble tetrabenzoporphyrin **4** was prepared in 43% overall yield as shown in Scheme 1. Condensation of butanopyrrole **1**[13] and carboranyl-benzaldehyde **2**[10] under Lindsey-type conditions (using $BF_3{\cdot}OEt_2$ as the catalyst and excess DDQ as the oxidizing agent),[14] gave the corresponding carboranylporphyrin in 60% yield. Insertion of Cu(II) was achieved quantitatively with copper(II) chloride in refluxing toluene for 4 h. The choice of metal was based on the higher stability

*Keywords*: PDT; BNCT; Carborane; Tetrabenzoporphyrin.
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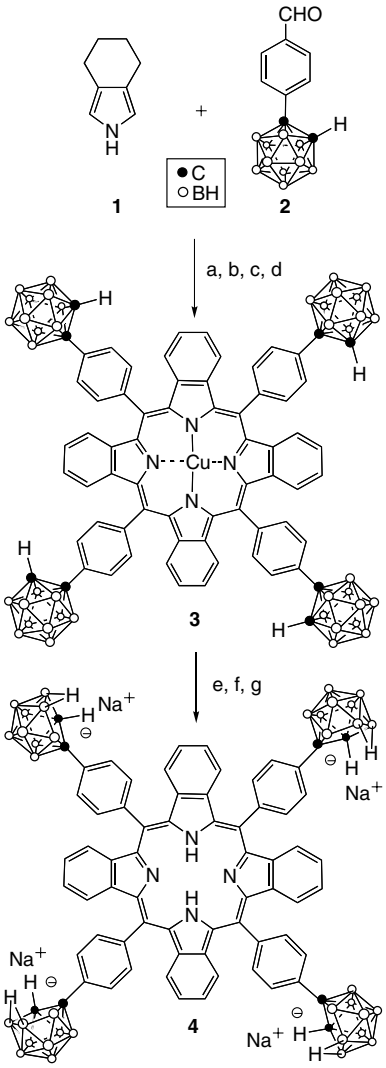
doi:10.1016/j.bmcl.2005.01.043




**Scheme 1.** Reagents and conditions: (a) $BF_3{\cdot}OEt_2$, $CH_2Cl_2$, rt, 1 h; (b) DDQ (2 equiv), $CH_2Cl_2$ reflux, 1 h (60% from **2**); (c) $CuCl_2$, toluene reflux, 4 h (quantitative); (d) DDQ (8 equiv), toluene reflux, 10 min (75%); (e) concd $H_2SO_4$, 5 min (quantitative); (f) $Bu_4NF$, THF reflux, 12 h; (g) Dowex 50WX2-100 in $Na^+$ form (95% from **3**).

of the Cu(II) versus the Zn(II) porphyrin complex, and the ease of demetallation after aromatization to tetrabenzoporphyrin. Oxidation of the Cu(II)–porphyrin to tetrabenzoporphyrin **3** was accomplished in 75% yield using 8 equiv of DDQ in refluxing toluene for 10 min.[15–17] The X-ray structure of Cu(II)–tetrabenzoporphyrin **3** is shown in Figure 1.[†] The tetra-

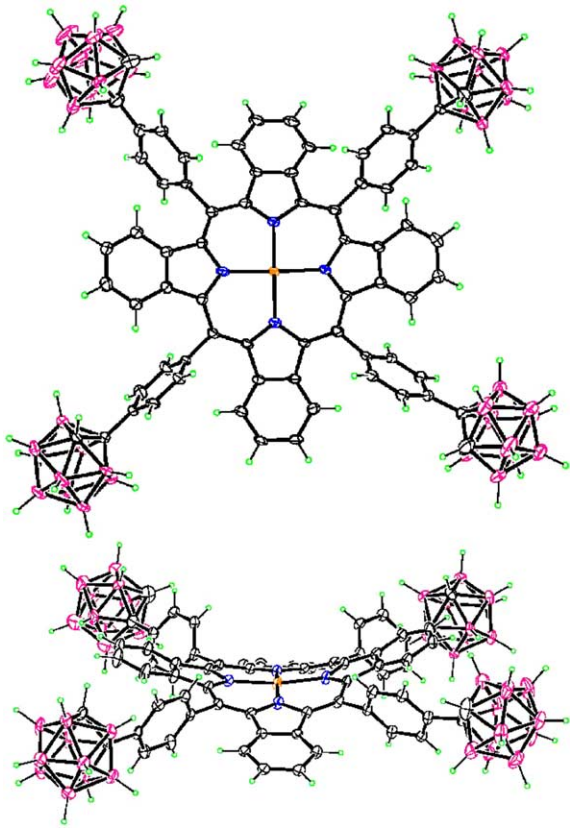


**Figure 1.** Molecular structure of Cu(II)–tetrabenzoporphyrin **3** (top and side views).

benzoporphyrin core has a saddle shape, with the benzopyrrole groups tilting alternately above and below its mean plane. The carbon atoms on the periphery of the benzo rings deviate from that plane by maximum values of +1.710(7) and −1.532(7) Å. This bending causes a slight tetrahedral distortion of the square-planar coordination plane itself, with N atoms lying alternately ±0.16 Å out of plane. The 4-carboranylphenyl groups are relatively unaffected by the saddle distortion, remaining roughly in the central plane of the molecule. The Cu–N distances are in the range 2.005(5)–2.024(5) Å.

Demetallation of Cu(II)–tetrabenzoporphyrin **3** occurred smoothly and rapidly in quantitative yield, upon treatment with concentrated $H_2SO_4$ at room temperature for 5 min. The resulting metal-free tetrabenzoporphyrin was converted into the water-soluble *nido*-carboranyl derivative **4** in 95% overall yield, by deboronation of the carborane cages in the presence of tetra-butylammonium fluoride,[18] followed by ion exchange using a Dowex 50WX2-100 resin in the sodium form.[10] Tetrabenzoporphyrin **4** was characterized by MS-MALDI, $^1$H NMR and UV–vis spectroscopy.[19] The $^1$H NMR spectrum of **4** characteristically shows the *meso*-phenyl protons as two doublets at 7.82 and 8.04 ppm, the benzo ring protons as a broad singlet centered at 7.3 ppm, and the CH protons of the *nido*-carborane cages at 2.69 ppm. The UV–vis spectra of tetrabenzoporphyrin **4** and of the known porphyrin **5**[10]

[†] The crystal structure of a $CHCl_3$ solvate of Cu(II)–tetrabenzoporphyrin **3** was determined, using data collected at $T = 105$ K to $\theta = 25°$ with Mo Kα radiation on a Nonius KappaCCD diffractometer. Crystal data: $C_{68}H_{76}B_{40}CuN_4{\cdot}4CHCl_3{\cdot}H_2O$, monoclinic space group $P2_1/c$, $a = 19.653(3)$, $b = 21.183(4)$, $c = 23.004(5)$ Å, $\beta = 104.804(13)°$, $V = 9259(3)$ Å$^3$, $Z = 4$, $R = 0.105$ ($F^2 > 2\sigma$), $Rw = 0.297$ (all $F^2$) for 162,208 unique data and 1165 refined parameters. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 258546. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

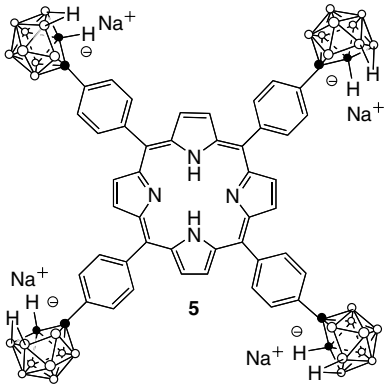



**Figure 2.** Structure of tetra(*nido*-carboranylphenyl)porphyrin **5**.[10]

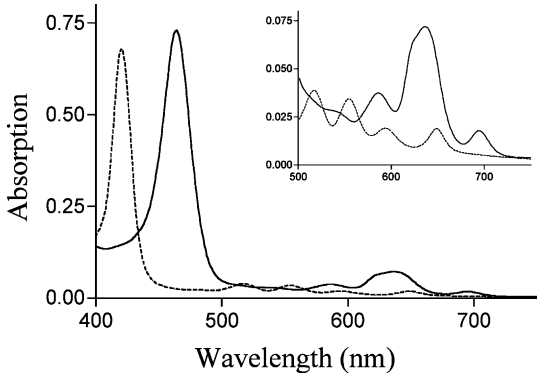



**Figure 3.** Optical spectra of tetrabenzoporphyrin **4** (full line) and porphyrin **5** (dashed line) at 5 μM concentration in methanol.

(Fig. 2) are shown in Figure 3. The Soret band of tetrabenzoporphyrin **4**, as it is characteristic of this type of compound, is red-shifted by 44 nm compared with that of porphyrin **5**. Furthermore, the molar extinction coefficient for compound **4** is significantly larger than that of porphyrin **5** in the red region of the spectrum, namely, at 641 and 693 nm.

The fluorescence emission of compounds **4** (excitation at 532 nm) and **5** (excitation at 511 nm) are shown in Figure 4. As previously observed,[11] the presence of the *nido*-carborane cages at the macrocycle periphery does not quench the fluorescence characteristic of porphyrin-type compounds.

The dark toxicity of tetrabenzoporphyrin **4** and of porphyrin **5** was evaluated toward V79 hamster fibroblast cells using an MTT-based cell viability assay.[20] The results obtained are shown in Figure 5. Concentrations of both compounds up to 300 μM had no effect on cell viability after 24 h of incubation. Our results show, in agreement with previous studies,[10,11,21] that *nido*-carboranylporphyrins and their derivatives have low dark cytotoxicity.

In summary, we developed a synthetic route to a novel water-soluble carboranyl-tetrabenzoporphyrin. This compound shows absorption and emission spectra characteristic of tetrabenzoporphyrin macrocycles, with stronger absorptions in the red region of the spectrum than carboranylporphyrin **5**. Similarly to porphyrin **5**, tetrabenzoporphyrin **4** shows low dark toxicity toward V79 cells, even at 300 μM concentration. Our studies suggest that tetrabenzoporphyrin **4** could have dual application as sensitizer for the PDT and BNCT treatment of tumors. We are currently investigating the in vivo toxicity and biodistribution of tetrabenzoporphyrin **4** and these results will be reported in due course.

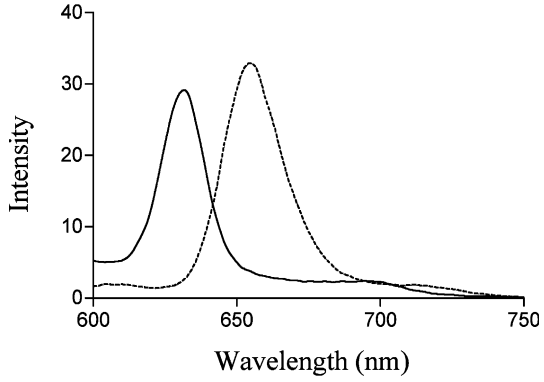



**Figure 4.** Fluorescence emission spectra of tetrabenzoporphyrin **4** (full line) and porphyrin **5** (dashed line) at 1 μM concentration in methanol (excitation at 532 and 511 nm, respectively).

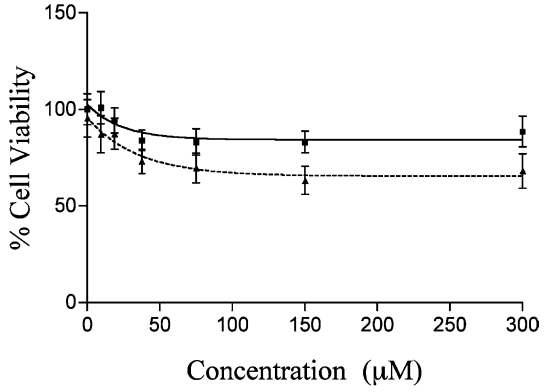



**Figure 5.** Cytotoxicity of tetrabenzoporphyrin **4** (full line) and porphyrin **5** (dashed line) toward V79 cells using an MTT-based assay.

## Acknowledgements

The work described was supported by the National Science Foundation (CHE-304833) and by the National Institutes of Health (CA098902).

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