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Synthesis of tetrasubstituted Zn(II)-phthalocyanines carrying four carboranyl-units as potential BNCT and PDT agents

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Abstract—The synthesis of two tetrasubstituted zinc(II)phthalocyanines carrying four carbon–carbon linked *o*-carboranyl-units (40 boron atoms, 27.5% boron by weight) is presented. In an in vitro model test the new compounds showed a good photosensitizing efficiency, that encourages further studies for their evaluation as possible BNCT–PDT sensitizers.

Photodynamic therapy (PDT) and boron neutron capture therapy (BNCT) are two binary therapeutic modalities, which are currently under investigation for the treatment of several kinds of malignancies. Both of them rely on the interaction of two relatively harmless factors: a photo- or radiosensitizing compound and an external radiation. PDT treatment[1,2] consists in loading the target cells with a photosensitizer that is able to generate highly reactive species (mainly singlet oxygen) upon irradiation with light of the appropriate frequency. The consequent oxidative modification of several subcellular targets, including proteins and unsaturated lipids, in the microenvironment of the photosensitizer causes cell death, via either random necrosis or apoptosis. Similarly, BNCT[3–5] is based on the interaction of the non radioactive ^{10}B nucleus and a thermal neutron. Thus, a ^{10}B-containing sensitizer, that can be selectively delivered to the target cell, is administered; the subsequent irradiation of the area with thermal neutrons causes the ^{10}B nucleus to split into high linear energy transfer (LET) particles, namely an α particle and a lithium ion; such particles deliver a relatively large amount of energy (~2.3 MeV) in a mean free path of about 10 μm, which is equivalent to the average diameter of a normal cell. The cytotoxic effect exerted by LET particles through ionization processes is thus confined to the cell they are generated in. The success of BNCT treatment depends both on the selective delivery of the sensitizers to the tumor tissue and on the possibility of achieving a sufficiently high endocellular concentration of ^{10}B. It has been calculated that the minimum effective concentration of ^{10}B in the tumor is 20–30 μg per gram of tissue.[6] Due to their selectivity of accumulation in tumor over many normal tissues, porphyrins, and phthalocyanines have recently been proposed as boron carriers to target tumoral tissue in BNCT treatment, and indeed a number of papers reported the synthesis of natural abundance carboranyl-containing porphyrins and phthalocyanines as models to develop novel radiosensitizers.[7] As a part of our current interest in the synthesis of phthalocyanines for biomedical applications,[8–12] we undertook the synthesis of two tetrasubstituted Zn(II)-phthalocyanines containing four carborane cages, with the purpose of developing a radio/photosensitizing agent for the treatment of tumors by means of a combination of BNCT and PDT. The photosensitizing efficiency of the compounds obtained was evaluated in vitro.

As it is shown in Scheme 1, the benzylic alcohols **3** and **4** were prepared according the to literature procedures,[13] starting from commercially available 3- and 4-nitrophthalonitriles **1** and **2**, by base-catalyzed aromatic nucleophilic substitution in anhydrous DMSO.[14] The synthesis of benzylic bromides **5** and **6** was accomplished by converting **3** and **4** into the corresponding mesylates

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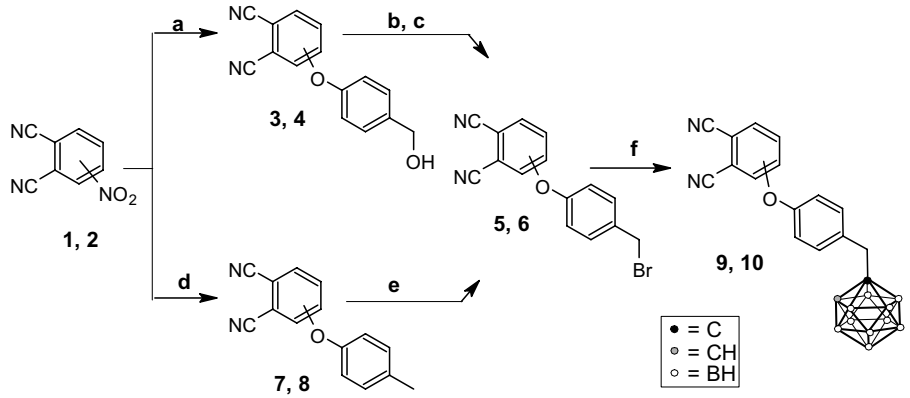
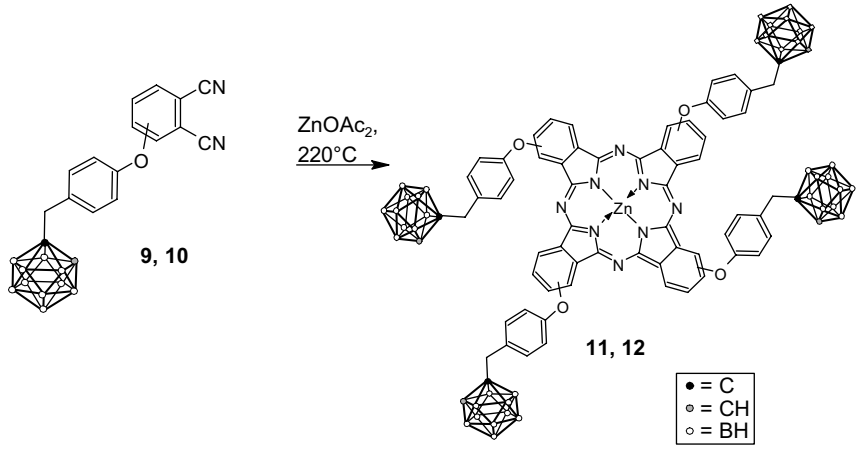


Scheme 1. Reagents and conditions: (a) 4-hydroxybenzylic alcohol, K_2CO_3, DMSO, 40 °C; (b) MsCl, TEA, CH_2Cl_2, 0 °C; (c) LiBr, THF, reflux; (d) 4-methylphenol, K_2CO_3, DMSO, 40 °C; (e) NBS, BPO, $ClCH_2CH_2Cl$, reflux; (f) Li-*o*-carborane, THF, −78 °C.

and by subsequent bromine substitution of the mesylate group upon treatment with lithium bromide in refluxing THF.[15] An alternative route to obtain the benzylic bromides **5** and **6** was also developed. According to this approach, base-catalyzed aromatic nucleophilic substitution of *p*-cresol on 3- and 4-nitrophthalonitriles **1** and **2** yielded 3- and 4-(4-methylphenoxy)-phthalonitriles **7** (93%) and **8** (92%), respectively. Conversion to the corresponding bromides **5** and **6** was achieved in excellent yields (95% and 94%, respectively), by bromination with *N*-bromosuccinimide in presence of benzoyl peroxide.[16] Reaction of the lithium salt of *o*-carborane[17–19] with benzyl bromides **5** and **6** in anhydrous THF led to 3- and 4-substituted phthalonitriles **9** and **10** in satisfactory yields (55% and 48%, respectively). The condensations of the phthalonitriles **9** and **10** to the corresponding Zn(II)-phthalocyanines **11** and **12** (1,8(11),15(18),22(25)- and 2,9(10),16(17),23(24)-tetrasubstituted, respectively), were carried out by heating a finely ground mixture of **9** or **10** and zinc acetate at 220 °C for several hours[20] (Scheme 2). Compounds **11** and **12** were obtained as a statistical mixture of regioisomers, in 43% and 40% yield, respectively, such results are in agreement with the typical yields reported for this kind of reactions,[21] confirming that the presence of the carborane cage does not impair the formation of the phthalocyanine. Any attempt to obtain phthalocyanines **11** and **12** by different methods,[21–23] such as DBU-mediated cyclization in DMF or lithium/alcoholate method, led to the extensive decomposition of the starting material supposedly because of the low stability of the carborane cage towards strong basic conditions.[24] Phthalocyanines **11** and **12** were characterized by means of ^1H and ^{13}C NMR spectroscopy, UV–vis spectroscopy, and mass spectrometry. ^1H NMR spectra of compounds **11** and **12** displayed the typical pattern of signals of 1,8(11), 15(18),22(25)- and 2,9(10),16(17),23(24)-tetrasubstituted phthalocyanines, respectively.[25] The absorption spectra of compounds **11** and **12** in DMF are presented in Figure 1, showing the expected absorption bands of phthalocyanines, with the Soret band centered at 329 nm and 357 nm, respectively, and Q-bands, respectively, at 690 nm and 677 nm; both of the values concerning the Q-bands are in agreement with the literature data relative to the absorption properties of 1,8(11),15(18), 22(25)- and 2,9(10),16(17),23(24)-tetrasubstituted phthalocyanines.[26] Compounds containing a large number of boron atoms usually display complex MS spectra.[27] Indeed, in the ESI-MS spectra of compounds **11** and **12** the molecular ion gave very large isotopic patterns, because of the presence of a zinc atom and 40 boron atoms, both existing as several stable isotopes. However, the experimental spectra were in agreement with the calculated ones both for position



Scheme 2.

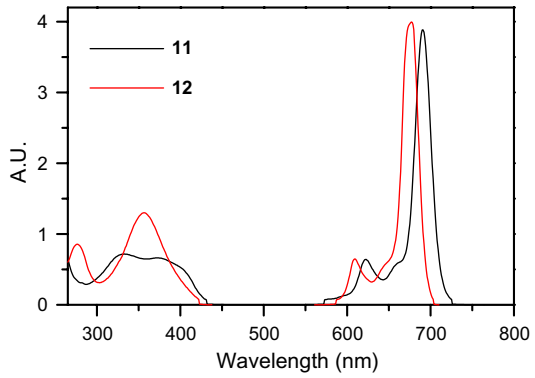


Figure 1. UV–vis absorption spectra of compounds **11** and **12** in DMF.

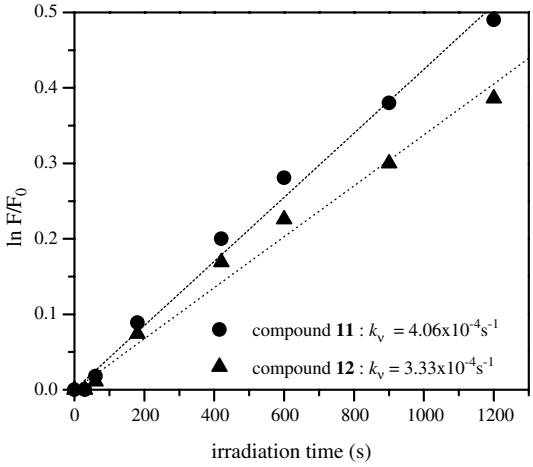


Figure 2. Plot of the decay of NATA fluorescence after predetermined irradiation times.

of the peaks (*m*/*z* values) and relative intensities (isotopic pattern). (See Supplementary data.) Phthalocyanines **11** and **12** proved to be soluble in several organic solvents, such as dichloromethane, chloroform, DMSO, THF, methanol, and DMF. The lack of solubility in water exhibited both by **11** and **12**, which might represent a severe drawback for future in vivo application, can be overcome by incorporating the molecules in liposomes (unpublished results).

The photodynamic efficiency of compounds **11** and **12** was determined by measuring the degradation of NATA (*N*-acetyl-L-tryptophanamide). The indolic moiety of tryptophan is known to react with a wide range of electrophiles,[28] and NATA is a derivative of tryptophan that can be regarded as a suitable model substrate for in vitro studies of photosensitized oxidations,[29] because it readily reacts with the electrophiles generated during type I (radical involving) and type II (singlet oxygen involving) photodynamic processes. Typically, such photoprocesses follow first-order kinetics with respect to the substrate concentration.[30] The rate of photodegradation of the indolic ring of NATA can be followed by measuring the decrease of its fluorescence emission intensity (λ_{em} = 360 nm) as a function of the irradiation time in the presence of the photosensitizer. In a typical experiment, a solution of compound **11** or **12** (2 μM) and NATA (10 μM) in DMF is irradiated with red light (650–750 nm, fluence rate: 100 mW/cm^2) and the decrease of the concentration of NATA is followed by measuring the decrease of the intensity of its fluorescence emission in 300–400 nm range (λ_{exc} = 290 nm). The rate constant of the photoprocess is obtained from the slope of the semilogarithmic plot (Fig. 2). The values thus found for compounds **11** and **12** are 4.06×10^{-4} s^{-1} and 3.33×10^{-4} s^{-1}, respectively. In the same conditions, the rate of the photoprocess we found using unsubstituted Zn(II)-phthalocyanine as the photosensitizer is 2.70×10^{-4} s^{-1} (data not shown). Such values are comparable with those found for the same substrate using other photodynamically active sensitizers, namely hematoporphyrin[31] and tetrasulfonated-Zn(II)-phthalocyanine.[32]

In summary, the synthesis of two Zn(II)-phthalocyanines containing four carborane cages has been described. These phthalocyanines contain 28% boron by weight and may be regarded both as models for sensitizers to be used in boron neutron capture therapy and as photosensitizers for PDT, as well as for the BNCT–PDT combined treatment of tumors. The photodynamic efficiency of the compounds obtained was evaluated by measuring the rate of the photosensitized oxidation of a model substrate (NATA). The present results show that the presence of four carborane cages does not impair the ability of the phthalocyanine molecule to act as a photosensitizer. Studies concerning the localization in vitro and in vivo of these compounds, as well as their photosensitizing activity on human fibroblasts are currently ongoing in our laboratories.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.03.030.

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25. Selected data for compound **11**: ^{1}H NMR (300 MHz, DMSO-d_6): 9.07 (d, J = 7.2 Hz), 8.90–8.79 (m), 8.68–8.56 (m), 8.45 (d, J = 7.2 Hz), 8.11–7.77 (m), 7.65 (d, J = 7.8 Hz), 7.52–7.40 (m), 7.43–7.10 (m), 5.21–5.17 (m), 4.89 (br s), 3.68–3.59 (m), 3.48 (br s), 2.71–1.18 (br m) δ; ^{13}C NMR (75 MHz, DMSO-d_6): 159.80, 159.53, 159.40, 157.50, 157.43, 157.13, 156.97, 154.66, 154.48, 154.11, 153.97, 153.55, 153.36, 153.05, 152.82, 152.63, 151.75, 151.60, 151.42, 151.25, 150.42, 150.26, 141.47, 141.41, 141.34, 141.10, 140.93, 132.95, 132.24, 132.13, 132.07, 132.00, 131.65, 131.47, 131.25, 129.93, 129.84, 129.76, 129.10, 129.02, 128.79, 127.64, 127.53, 127.33, 123.79, 123.43, 123.21, 121.03, 120.67, 120.52, 120.21, 119.88, 119.66, 119.37, 119.11, 118.67, 118.09, 116.77, 116.69, 116.55, 77.45, 77.33, 63.59, 63.08, 42.18, 41.94 δ (selected data); FAB^{+}-MS: *m/z* 1571 [$C_{68}H_{80}N_8O_4B_{40}Zn$]$^{+}$; UV–vis (DMF): nm (%) 690 (100), 622 (16), 329 (18), ε_{690} = 230,000 M^{-1} cm^{-1}. Selected data for compound **12**: ^{1}H NMR (300 MHz, DMSO-d_6) 8.98–8.91 (2H, m), 8.68–8.69 (2H, m), 8.45–8.41 (2H, m), 8.27–8.23 (2H, m), 7.79–7.40 (2H, m), 5.31 and 5.18 (4H, 2 br s), 3.76 and 3.68 (8H, 2 br s), 2.90–1.18 (40H, br m) δ; ^{13}C NMR (75 MHz, DMSO-d_6) 159.70, 158.71, 158.57, 157.34, 156.53, 156.41, 151.80, 140.03, 139.92, 132.99, 132.90, 132.68, 132.11, 124.40, 121.17, 119.98, 111.73, 77.45, 77.26, 63.76, 63.51, 42.15 δ (selected data); FAB^{+}-MS: *m/z* 1571 [$C_{68}H_{80}N_8O_4B_{40}Zn$]$^{+}$; UV–vis (DMF): nm (%) 677 (100), 609 (17), 357 (34), ε_{677} = 240,000 M^{-1} cm^{-1}.
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