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New conjugates of cobalt bis(dicarbollide) with chlorophyll *a* derivatives

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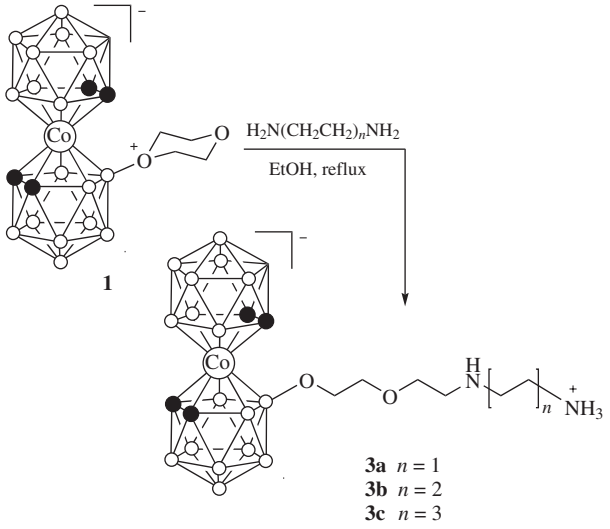
New boronated conjugates of chlorophyll *a* derivatives were prepared by reactions of the 1,4-dioxane derivative of cobalt bis(dicarbollide) with amino derivatives of pheophorbide *a* methyl ester and purpurinimide.

Boron neutron capture therapy (BNCT)[1] and photodynamic therapy (PDT)[2] are two bimodal new binary modalities for the local destruction of cancer cells. Both rely on the selective uptake and retention of a sensitizer molecule by tumor cells followed by an activation of sensitizer using an external radiation source. BNCT is based on the nuclear reaction of two essentially non-toxic species, non-radioactive ^{10}B and low energy thermal neutrons producing high-linear-energy transfer ions $^{4}He^{2+}$ and $^{7}Li^{3+}$ that dissipate their kinetic energy during traveling one cell diameter (5–9 μm). PDT involves photosensitization of porphyrins by red laser light, which generates cytotoxic reactive oxygen species (primarily singlet oxygen) at a selected treatment site. The average free path of singlet oxygen is very short (< 0.5 μm), the resultant apoptosis and necrosis occur only in tumor cells that have accumulated the sensitizer and not in surrounding normal cells. As a result, both BNCT and PDT are highly selective methods of cancer treatment producing minimum side effects in comparison to conventional radio- and chemo- therapies.

The family of dyes most extensively studied with respect to PDT and BNCT are the porphyrins and related macrocycles (*e.g.*, chlorins, phthalocyanines, porphyrazines, *etc.*).[3] Chlorins are photosensitizers that have considerable absorption in the so-called 'phototherapeutic window' (630–900 nm), where light absorption and scattering in human tissues are minimized and they are relatively transparent for activating light and high quantum yields of singlet oxygen production.[4] Syntheses of several carborane-containing derivatives of chlorin e_6 have been reported and their efficiency in the *in vivo* PDT tests was demonstrated.[5]

The cobalt bis(dicarbollide) anion[6] contains a larger amount of boron atoms than carboranes and demonstrates low toxicity[7] and good water solubility in the form of sodium or potassium salts. These properties, along with the remarkable chemical stability of cobalt bis(dicarbollide), has led to synthesis of a series of boron-rich dendrimers,[8,9] nucleosides,[10] phthalocyanines[11] and synthetic porphyrins[12] on its basis. All these syntheses exploit nucleophilic ring-opening of the 1,4-dioxane derivative of cobalt bis(dicarbollide) **1**[13] (Scheme 1). Recently, we described preparation of conjugates of chlorin e_6 and purpurinamide with cobalt bis(dicarbollide) using 'click chemistry'[14] and Sonogashira[15] methodologies. In this contribution we describe synthesis of new conjugates of chlorin e_6 and purpurinamide with cobalt bis(dicarbollide) with different spacer length between the tetrapyrrole macrocycle and the metallacarborane cluster.

The cleavage of the *E* ring in pheophorbide *a* methyl ester **2** by the action of amines (Scheme 2) is known as useful route for synthesis of various functional derivatives of chlorophyll *a*.[5(*a*),16–19]

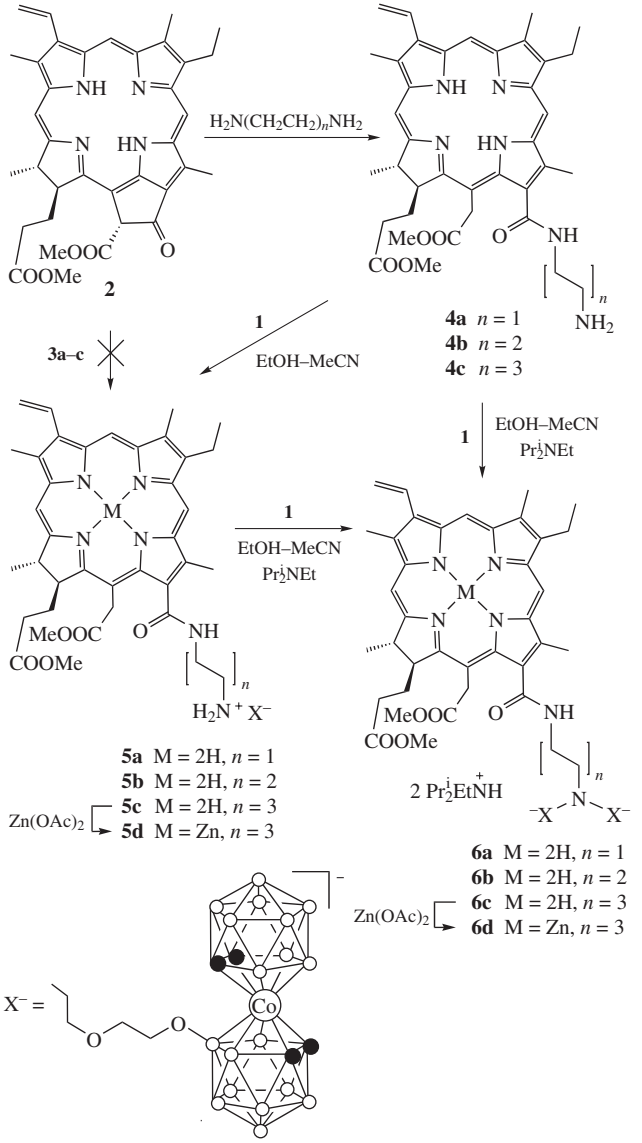


Scheme 1

In this work we combined known ring opening reactions of the oxonium cycle in **1** and the *E* ring in **2** to prepare new boron-containing conjugates of chlorin e_6 using 1,2-diaminoethane, 1,4-diaminobutane and 1,6-diaminohexane as bifunctional nucleophilic agents. Two different synthetic schemes were tested. The first one involves the cleavage of the oxonium ring in **1** with diamines followed by the *E* ring opening in **2** with terminal amino group of cobaltacarboranes **3**. Another strategy consists in the reverse reaction sequence: the *E* ring cleavage in **2** followed by the oxonium ring opening in **1**. The reaction of **1** with α,ω-diaminoalkanes in refluxing ethanol proceeded smoothly giving the corresponding amino derivatives **3a–c** as N-protonated inner salts (Scheme 1).† The ^1H NMR spectra of these compounds suggest the existence of an equilibrium mixture of two

† All reactions were carried out under light protection. All solvents were carefully dried and purified by standard techniques. Compounds **1**,[24] **4a**,[5a] **4b**,[19] **4c**,[20] and **8**[22] were prepared according to the previously described procedures.

Synthesis of **3a–c**. 1.05 mmol of diamine was added to a solution of 0.41 g (1.00 mmol) of **2** in 40 ml of ethanol. The reaction mixture was heated at reflux for 1 h, allowed to cool to room temperature and concentrated to dryness under reduced pressure. The crude product was subjected to column chromatography on silica and eluted with CH_2Cl_2 and CH_2Cl_2–MeCN (3:1) to obtain the product as an orange solid. Yield was 0.34 g (77%), 0.39 g (82%) and 0.39 g (78%) for **3a**, **3b** and **3c**, respectively.



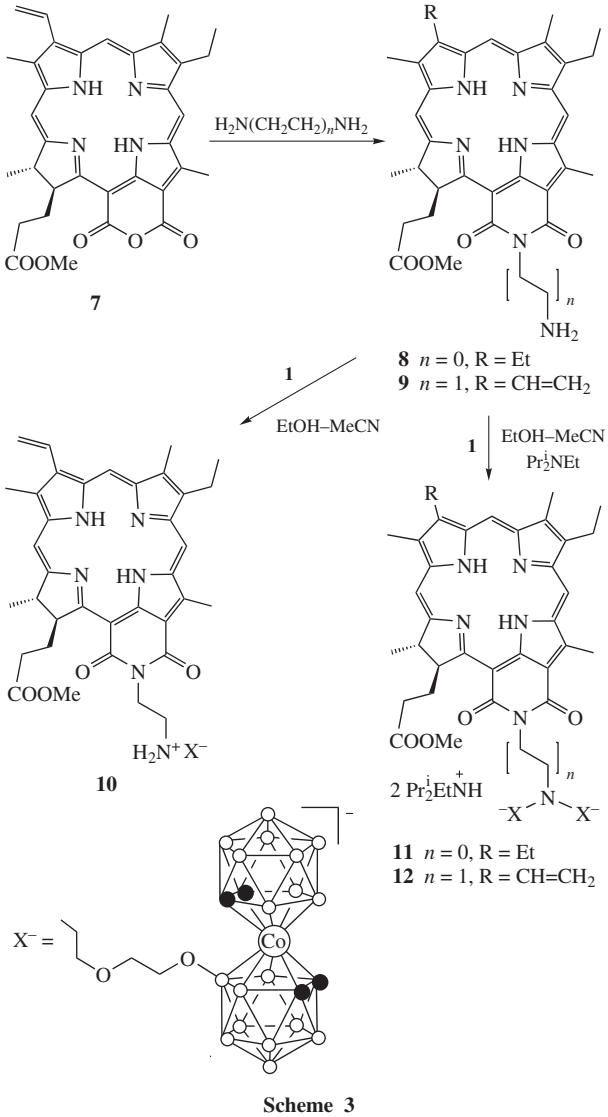
Scheme 2

isomers differing in the protonation site as well as chelation of this ammonium centre with oxygen atoms of the podand chain. The proton migration could be suppressed by addition of excess pyridine or trifluoroacetic acid. The high stability of NH-protonated forms results in low reactivity of **3a–c** in the reaction with pheophorbide **2**.

To accomplish the second reaction scheme we used earlier described amino derivatives of chlorin e_6 **4a–c** with various spacer lengths between the tetrapyrrole macrocycle and terminal amino group.[5(a),19,20] The reaction of **4a–c** with **1** results in the desired conjugates **5a–c** in moderate yield (Scheme 2).‡ As in the case of **3a–c**, the amino group in conjugates **5a–c** is protonated. It was shown earlier[21] that deprotonation of inner ammonium salts of cobalt bis(dicarbollide) with strong bases followed by treatment with second equivalent of **1** produces compounds with two cobaltacarborane fragments. We found that in the presence of *N*,*N*-diisopropyl-*N*-ethylamine (DIPEA) as sterically hindered base, amines **4a–c** react with two equivalents of oxonium derivative **1** to afford the corresponding conjugates **6a–c**§ containing two cobaltcarborane clusters (36 boron atoms) per molecule (Scheme 2). The same compounds can be obtained by the reaction of **5a–c** with **1** in the presence of DIPEA.

Another type of chlorine derivatives that was used here for synthesis of boron-containing conjugates is purpurin imide derivatives with terminal amino group. The reactions of the amino derivative **9** with **1** proceed similarly to amines **4a–c** resulting in conjugates containing one (**10**) and two (**12**) boron fragments depending on the reaction conditions. In the case of purpurin imide **8** no reaction occurred in the absence of a base. Reaction of **8**[22] with **1** in the presence of DIPEA produces the corresponding conjugate **11** containing two boron fragments (Scheme 3).¶ The revealed difference in reactivity of cycloimides **8** and **9** can be explained by low nucleophilicity of amino group connected directly to imide nitrogen in compound **8**. Low reactivity of the amino group in cycloimide **8** in the quaternization reaction was reported earlier.[22]

In conclusion, a series of the conjugates of chlorin e_6 and purpurin imides with cobalt bis(dicarbollide) anions was prepared using reaction of the 1,4-dioxane derivative of cobalt bis(dicarbollide) with amino derivatives of chlorins. The preliminary *in vitro* studies revealed high level of accumulation of conjugates **5a–c** in A549 human lung adenocarcinoma cells with the accumulation coefficient reaching 80 that is at least an order of magnitude more than those found for earlier synthesized conjugates of chlorin series.[23] Taking into account their straightforward



Scheme 3

‡ *Synthesis of conjugates* **5a–c**. 37 mg (0.09 mmol) of **1** was added to a solution of 0.06 mmol of **4a–c** in 5 ml of acetonitrile–ethanol (1:1) mixture. The reaction mixture was heated at reflux under argon atmosphere protected from light for 3 h, allowed to cool to room temperature and evaporated to dryness *in vacuo*. The crude product was purified by preparative TLC on silica using $CHCl_3$–MeOH (25:1) as an eluent. Yield was 28 mg (43 %), 30 mg (45%) and 33 mg (48%) for **5a**, **5b** and **5c**, respectively.

two-step synthesis from easily available pheophorbide *a* methyl ester and 1,4-dioxane derivative of cobalt bis(dicarbollide) these compounds can be considered as promising ones for photodynamic and boron neutron capture therapies of cancer.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2011.03.008.

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§ *Synthesis of conjugates* **6a–c**. 37 mg (0.09 mmol) of **1** and 300 μl (1.70 mmol) of DIPEA were added to solution of 0.06 mmol of **4a–c** in 5 ml of acetonitrile–ethanol (1:1) mixture. The reaction mixture was heated at reflux under argon atmosphere protected from light for 3 h, and after cooling to room temperature evaporated to dryness *in vacuo*. The crude product was purified by preparative TLC on silica using $CHCl_3$–MeOH (25:1) as an eluent. Yield was 61 mg (61%), 65 mg (64%) and 68 mg (66%) for **6a**, **6b** and **6c**, respectively.

Synthesis of Zn complexes **5d** *and* **6d**. 2.5 mg (0.02 mmol) of $Zn(OAc)_2$ in methanol was added to a solution of 0.017 mmol of **5c** or **6c** in dichloromethane and the mixture was stirred for 30 min. The reaction course was monitored by UV spectroscopy. The reaction mixture was washed with water and extracted with chloroform. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness *in vacuo*. Yield was 18 mg (90%) and 28 mg (90%) for **5d** and **6d**, respectively.

¶ *Synthesis of 2′-aminoethylpurpurinimide methyl ester* **9**. 0.5 ml (7.50 mmol) of 1,2-diaminoethane in 2 ml of pyridine was added to a solution of 26 mg (0.05 mmol) of **7** in 5 ml of pyridine. The reaction course was monitored using UV spectroscopy. After hypsochromic shift of the Q band to 665 nm the solvent was removed *in vacuo*. The residue was dissolved in 10 ml of pyridine and heated at 80 °C with removal of water. After hypsochromic shift of the Q band to 707 nm the solvent was removed *in vacuo*. The residue was recrystallized from a mixture of chloroform and hexane. Yield, 22 mg (73%).

Conjugate **10** (yield 42%) was synthesised by the procedure similar to that for obtaining **5a–c**. Conjugates **11** and **12** were prepared with the yields 63 and 62%, respectively, using the procedure analogous to that for synthesizing **6a–c**.

For characteristics of compounds **3a–c**, **5a–d**, **6a–d**, **9**–**12**, see Online Supplementary Materials.