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Synthesis of a porphyrin-labelled carboranyl phosphate diester: a potential new drug for boron neutron capture therapy of cancer

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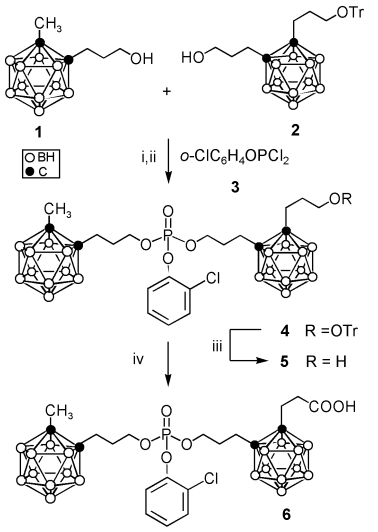
A boron-rich, water-soluble porphyrin conjugate was synthesized by coupling of two carboranyl alcohols with 2-chlorophenoxyphosphorus dichloride, followed by conjugation to an amine-functionalized tetraphenyl-porphyrin *via* an amide linkage.

Boron neutron capture therapy (BNCT) of cancer[1,2] is a binary radiation therapy that entails the capture of thermal neutrons by boron-10 (^{10}B) nuclei, which have been selectively delivered to tumor cells. The neutron capture event results in the formation of excited, highly cytotoxic $^{4}He^{2+}$ and $^{7}Li^{3+}$ ions. These high linear energy transfer particles create ionization tracks along their trajectories, which result in irreversible cellular damage and effective cell death. One of the most challenging requirements for effective BNCT is the selective transport of therapeutic amounts of boron to tumor cells. It has been estimated that 15–35 μg of ^{10}B per gram of tumor (~ 10^9 atoms of ^{10}B/tumor cell) are necessary for effective BNCT, depending upon the precise location of the ^{10}B atoms; less boron is needed if localized intracellularly, in close proximity to the cell nucleus.[3,4] Over the past decades many different substituted boron clusters, predominantly derivatives of *closo*-$B_{12}H_{12}^{2-}$, *closo*-$B_{10}H_{10}^{2-}$, and the three isomeric dicarba-*closo*-dodecaborane(12) species,[5] including their anionic deboronated counterparts, were synthesized and incorporated into a variety of delivery systems with varying degrees of tumor specificity.[6,7] Of particular promise as fluorescent tumor targeting moieties are porphyrin derivatives, which have been repeatedly shown to be selectively taken up and retained by tumors.[8] In the past, several boron-containing porphyrins were synthesized and evaluated as potential agents for BNCT.[6–8]

Herein we describe the synthesis of the water-soluble trianionic porphyrin conjugate **12** featuring a carboranyl-containing phosphate diester substituent attached *via* an amide linkage. The dimeric carboranyl substituent is of particular interest because it represents the shortest member of the family of the oligomeric carboranyl phosphate diesters (OPD's);[9] these compounds have been shown to be taken up and persistently held by the nuclei of TC7 cells, after delivery to their cytoplasm by microinjection.[10] By attaching a structural unit of these OPD's to a porphyrin macrocycle we were hoping to create a targeting device for cell nuclei, combining the promising biological properties of porphyrins with those of carboranyl phosphate diesters.

Scheme 1 shows the assembly of the carboranyl precursor **6** exhibiting a carboxylic acid function available for subsequent conjugation. Coupling of the carboranyl mono alcohol **1**[11] and the monoprotected carboranyl diol **2**[12] to 2-chlorophenoxyphosphorus dichloride, **3**,[13] led, after oxidation of the phosphorus atom with iodine, to the phosphate diester **4** in 53% yield. Phosphorus reagents like **3** have been previously employed in asymmetric coupling reactions of ribonucleotides, under various temperature conditions.[14] The synthesis of **4** was conducted in two steps; in the first step one equivalent of alcohol **1** was added at 0 °C to a solution of the phosphorus dichloride **3** in THF, in the presence of 2,6-lutidine as the base. After keeping the mixture for five hours at this temperature, one equivalent of alcohol **2** was then added in the second step. Chromatographic purification of the reaction mixture gave, along with the desired phosphate diester **4**, a symmetrical coupling product of **1** and unreacted **2**, which could be recovered and re-used. The optimized coupling conditions for the synthesis of **4** and related species are currently being developed in our laboratories and will be reported elsewhere. Deprotection of the trityl group of **4** and oxidation of the resulting alcohol **5** with periodic acid in wet acetonitrile[15] afforded the carboxylic acid **6**. Coupling of **6** with the mono-aminoporphyrin **8** was accomplished using carbonyldimidazole as the coupling reagent (Scheme 2). The mono-functionalized porphyrin **8** was obtained from nitration of *meso*-tetraphenylporphyrin (TPP) **7** with sodium nitrite in trifluoroacetic acid (TFA), followed by reduction of the nitro group with tin(II) chloride in concentrated HCl,[16,17] in 54% overall yield. The use of excess $NaNO_2$–TFA for regiospecific *para*-phenyl nitration of TPP is a convenient methodology, affording the mono-nitrated product in high yield, after a 3 minute reaction time. The coupling reaction of **6** and **8** took five days for completion (monitored by TLC) and produced conjugate **9** in 68% yield. Compound **9** was purified by HPLC using normal phase silica as a stationary phase and a mixture of 62% hexane and 38% ethyl acetate as the mobile phase. The subsequent cleavage of the phosphate protection group was acomplished in quantitative yield by using aqueous sodium hydroxide in dioxane. NMR and mass spectrometric analysis of the resulting deprotected conjugate **10** revealed that the *closo*-carborane cages had not been deboronated under the conditions used for the deprotection procedure. Attempts to deboronate the *closo*-cages in **10** using caesium fluoride[18] led to significant cleavage of the amide bond as well as the phosphate diester



Scheme 1 Reaction conditions: (i) THF, 2,6-lutidine (4 equiv. ref. to **3**), 0 °C, (ii) I_2, 53%, (iii) $BF_3{\cdot}Et_2O$–MeOH–CH_3CN, 78%, (iv) H_5IO_6–CrO_3, CH_3CN–H_2O, 90%.

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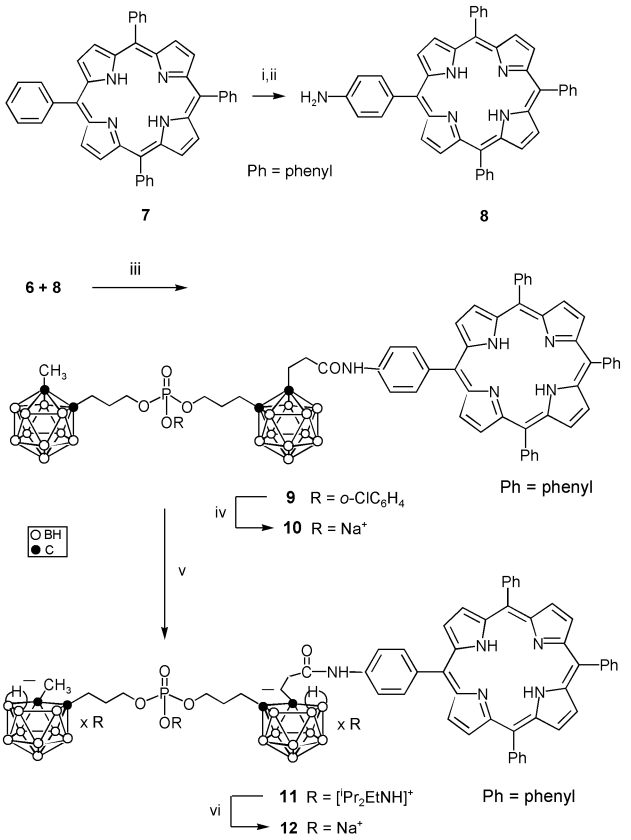
bonds. To overcome this problem, a new and selective deboronation procedure was developed by heating a solution of **10** in a mixture of methanol and *N*,*N*-diisopropylethylamine (2:1) at 80 °C. The reaction, monitored by electrospray mass spectrometry, proceeded smoothly and was completed after nine days without significant amide or ester bond cleavage. Interestingly, the deboronation did not take place if methanol was replaced by acetonitrile. We surmise that the active base during the deboronation reaction is *N*,*N*-diisopropylethylammonium methoxide, formed by protonation of *N*,*N*-diisopropylamine with methanol, which due to its steric requirements is not capable of cleaving the amide and ester bonds present in **10**. After deboronation the conjugate **11** was obtained as a water-insoluble tris-*N*,*N*-diisopropylethylammonium salt. To obtain the water-soluble tris-sodium salt **12**, conjugate **11** was dissolved in acetonitrile followed by the addition of aqueous sodium chloride (excess). Purification and separation from inorganic salts using reversed phase silica (C2) gave the title compound **12** in 82% yield. The electrospray mass spectrum (negative mode) shows the molecular ion peak for **12** at m/z = 571 $[M+H]^{2-}$ (100%) and m/z = 380 $[M]^{3-}$ (23%). The proton- and boron-NMR spectra display the signals expected for **12**. The absorption spectrum of **12** in methanol shows an etio-type spectrum with an intense Soret band at 415 nm and four Q bands at 513, 548, 590 and 645 nm, characteristic of TPP-like macrocycles. The fluorescence spectra displays a λ_{max} (emission) at 649 nm in methanol (excitation λ_{max} = 469 nm). Therefore, the absorption and fluorescent properties of the porphyrin macrocycle are retained in conjugate **12**, and future investigations of specific sites of boron-10 localization within cells may be assessed by fluorescence microscopy.



Scheme 2 Reaction conditions: (i) $NaNO_2$–TFA, (ii) $SnCl_2$–HCl, 54%, (iii) CDI, THF, 5 d, 68%, (iv) 0.2 M aq. NaOH–dioxane (1:3), 4 h, 99%, (v) MeOH–$^{i}Pr_2Net$ (2:1), 80 °C, 9 d, (vi) CH_3CN–H_2O, NaCl, C2-reversed phase silica, 82%.

In summary, we have disclosed the eight-step synthesis of the fluorescent porphyrin conjugate **12** featuring a carboranyl phosphate diester attached *via* an amide linkage. We expect that the amphiphilic nature of conjugate **12** will confer enhanced affinity for lipid–aqueous interfaces, thus favoring tumor uptake and effective delivery of ^{10}B to the nuclei of tumor cells. Biological evaluation studies in this regard are under way and will be reported elsewhere.

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