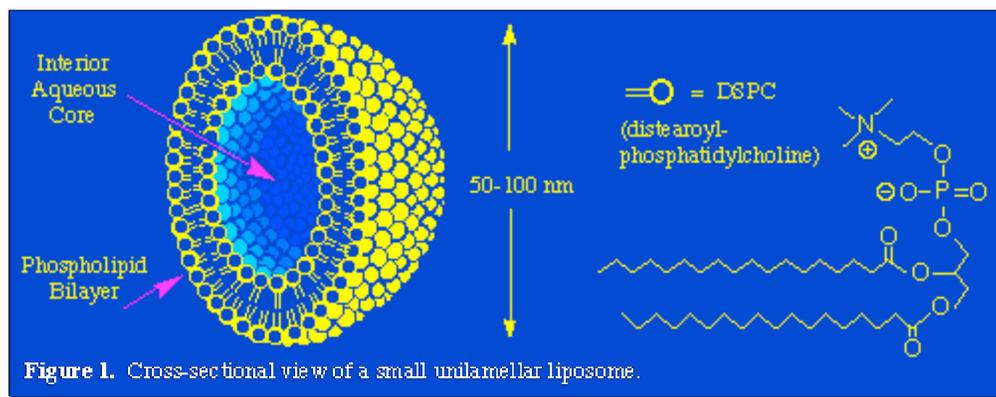


Liposomes

Background

The successful application of boron neutron capture therapy (BNCT) for cancer requires the preferential concentration of significant quantities of the stable ^{10}B isotope within tumors over normal healthy tissues. The slow evolution of effective drug-targeting methodologies for the selective delivery of sufficient amounts of boron to cancer cells remains as an important obstacle to the development of this therapy.

The goal of the liposome project is to develop drug delivery modalities consisting of liposomes (or other carriers such as micelles) which contain, or are constructed from, boron-containing compounds. This is accomplished through a) the identification and synthesis of appropriate hydrophilic and lipophilic boron agents; b) the incorporation of the candidate compounds within small unilamellar vesicles (SUVs) or similar delivery vehicles; and c) the evaluation of these preparations in boron biodistribution experiments using animal models.

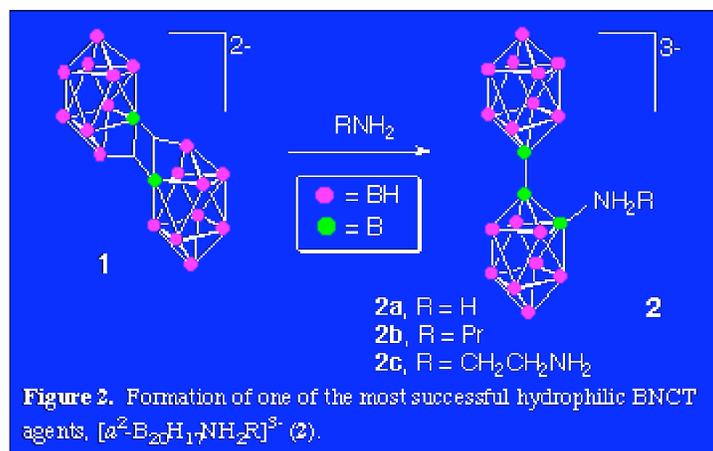


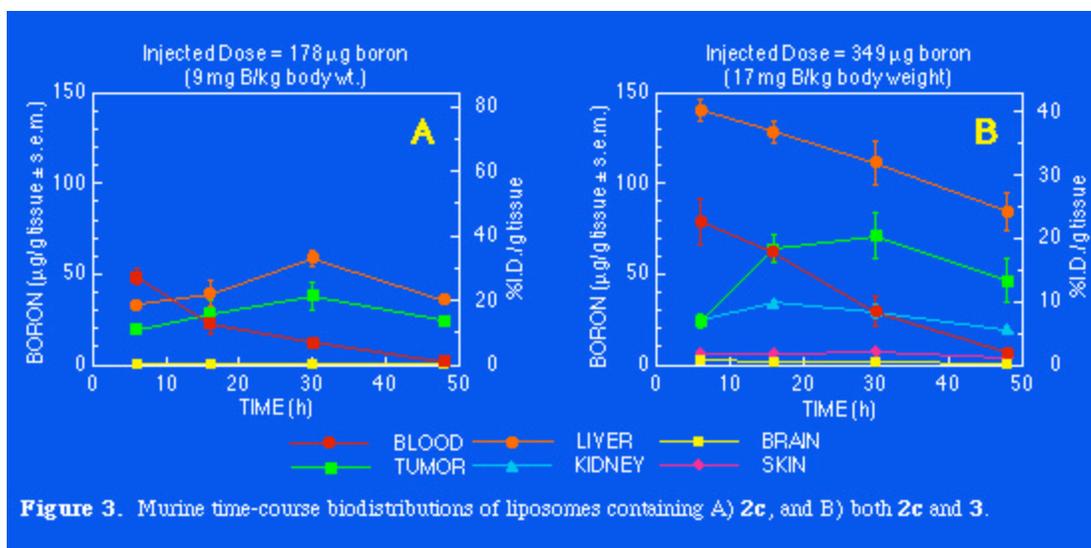
Small unilamellar liposomes (Figure 1) composed of the phospholipid DSPC and cholesterol have been shown to be a viable transport modality for the selective, intracellular delivery of boron-containing compounds to neoplastic tissues. This was demonstrated through boron biodistribution experiments which typically employed BALB/c mice bearing subcutaneous EMT6 (mammary adenocarcinoma) tumors. There are two ways that SUVs may be employed as boron carriers: water-soluble boron compounds are encapsulated within the aqueous core of the liposome, or lipophilic boron compounds are embedded within the phospholipid bilayer which defines the liposome structure. In addition, both types of boron compounds may be combined within the same liposomal formulation.

Current research is focused primarily on the identification of superior boron compound candidates for the liposome-mediated delivery of boron to tumors for application in BNCT. In addition, the development of new boron-containing materials for the construction of liposomes, micelles, and dendrimers is being investigated as a means to increase the quantity and selectivity of boron delivery. Finally, the utility of these delivery systems is being investigated for application to a variety of therapeutic targets including head and neck tumors, non-small cell lung cancer, and rheumatoid arthritis.

Hydrophilic Species for Liposome Encapsulation

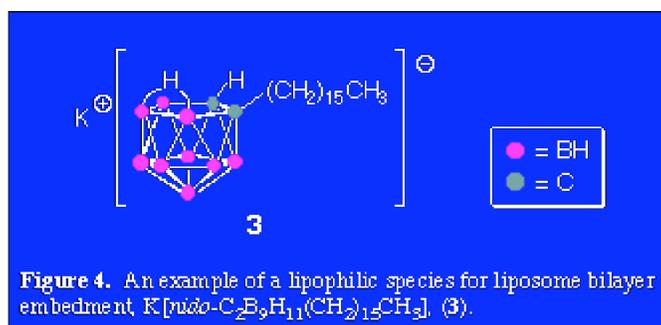
Hydrophilic boron compounds for encapsulation within liposomes must be highly water-soluble, hydrolytically stable, and have a high boron content. Furthermore, good results are obtained only when the boron-containing species includes a functional group or reactive polyhedral borane structure with the potential to combine with intracellular components, thus providing an anchor for the tumor retention of boron after initial liposomal delivery. Most investigations have employed species derived from the *closo*-B₁₂H₁₂²⁻, *closo*-B₁₀H₁₀²⁻, and *trans*-B₂₀H₁₈²⁻ (**1**) cage systems. The most successful hydrophilic boron compounds investigated have been derivatives of *a*²-B₂₀H₁₈⁴⁻, which are easily prepared from **1** (Figure 2), have a high boron content, and often exhibit favorable tumor retention due to the reactivity provided by the polyhedral structure. The reactivity of **1** permits the production of a wide variety of such derivatives. In particular, the amine derivatives *a*²-B₂₀H₁₇NH₂R³⁻ (**2**, R = H, Pr, CH₂CH₂NH₂) have demonstrated excellent biodistribution results. When injected in tumor-bearing mice (Figure 3A), liposomes containing *a*²-B₂₀H₁₇NH₂CH₂CH₂NH₂³⁻ (**2c**) produced therapeutically useful boron concentrations in tumor (38 ppm at 30 h) with good selectivity (tumor/blood boron ratio >3).



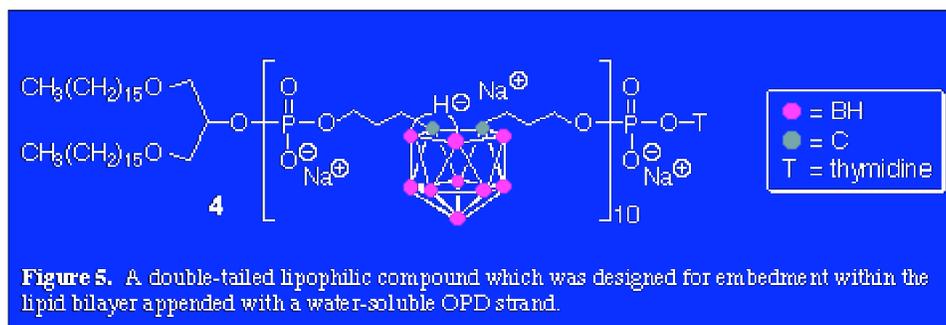


Lipophilic Species for Liposome Bilayer Embedment

The incorporation of boron-containing species within the liposome bilayer offers the potential for greater efficiency of boron compound incorporation and the production of liposome formulations with much higher boron concentrations. Structurally, these species usually should contain a polar head group and one or two long lipophilic tails. This allows the boron-containing compound to be stably incorporated within the phospholipid membrane at reasonable concentrations without adversely affecting the serum stability of the liposome. The most useful species we have found for this purpose so far is *K nido-C₂B₁₁H₁₁(CH₂)₁₅CH₃* (**3**), whose structure is shown in Figure 4. This carborane derivative can be incorporated into the lipid bilayer at concentrations of 15 mol % without affecting liposome stability or tumor selectivity. Liposomes containing **3** produce a favorable biodistribution when injected in mice, but even better results are obtained with liposomes containing both **3** and **2c** (Figure 3B). While both formulations shown in Figure 3 exhibit approximately the same tumor delivery (21% injected dose/g tumor), the additional boron dose provided by the presence of **3** results in an exceptionally high tumor boron concentration of 71 ppm.



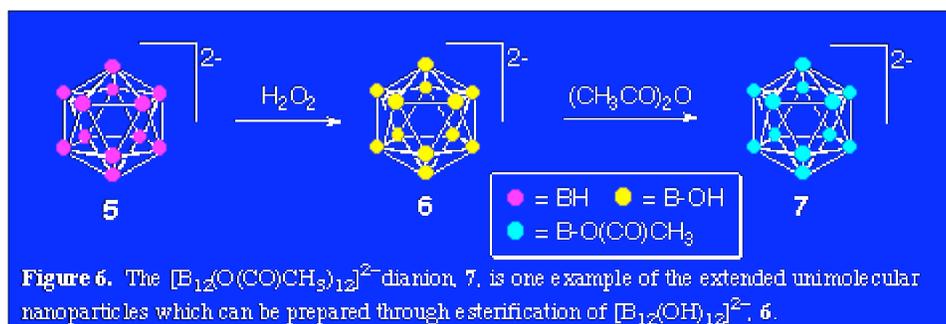
Current investigations are directed towards the development of new lipophilic species which may be incorporated within the bilayer at higher concentrations or incorporate much larger boron contents within their structures. One example is the species shown in Figure 5, which consists of a double-tailed lipophilic portion for embedment within the lipid membrane appended to a boron-rich oligophosphate diester (OPD).



New Compounds and Delivery Modalities

Ongoing and future avenues of research seek to identify new compounds and delivery systems with the potential to contain and deliver greater quantities of boron. For example, new types of liposomes and micelles are being developed which are constructed from boron-containing materials instead of the boron-free phospholipids currently employed. These vesicles, if properly sized, should retain the selectivity and delivery characteristics of standard phospholipid vesicles and carry a much larger quantity of boron to tumor cells. Stable liposomes with suitable dimensions have been produced by the self-assembly of carborane containing ionic surfactants.

Another methodology under investigation is the use of boron-rich micelles or dendrimers as boron carriers. The *closo*- $B_{12}H_{12}^{2-}$ ion (**5**) has been hydroxylated to produce derivatives with 1-4 substituents as well as the perhydroxy derivative **7** (*closo*- $B_{12}(OH)_{12}^{2-}$). As shown in Figure 6, these hydroxyl groups may be converted to a wide variety of useful derivatives through common organic reactions. The resultant species may form the bases for boron-rich 12-fold dendrimers (or molecular micelles) for the delivery of boron.



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