**Oligomeric Phosphate Diesters (OPDs)**

Boron-rich oligomeric phosphate diesters (OPDs) are attractive boron delivery vehicles for use in BNCT. They are readily obtained from selected carborane-containing dihydroxy precursors and assembled using well-established phosphoramidite coupling chemistry. In addition, boron-rich OPDs are inherently hydrophilic or amphiphilic by virtue of their anionic phosphate backbone. Hawthorne and co-workers first examined the automated synthesis of selected OPDs using standard DNA synthesizer techniques. Carboranyl phosphoramidites, such as 1, are routinely prepared in good yield and efficiently coupled to form boron-rich OPDs such as 2 without modification of the standard conditions for the automated synthesis of DNA.

As an example, the oligophosphate chain 2 contains 100 boron atoms with the sequence beginning with a commercially available thymine residue, and ending with an amino group incorporated at the conclusion of the automated synthesis. Compound 2 is water-soluble (> 75 mg/ml) and its hydrophilicity is enhanced through the conversion of the closo-carborane cages to their corresponding anionic nido-derivatives by heating with ammonium hydroxide. The fast (< 5 min per coupling cycle), efficient (> 98% coupling efficiency per step) and flexible methodology for the automated synthesis of species such as 2 lends itself to the synthesis of a vast library of designed boron-rich oligomers such as those shown in Figure 2 and others in which single strands of DNA or other biomolecules may be attached.
Oligomers such as those described above were labeled with fluorescein and investigated in vitro by both microinjection of living cells (10^9 boron atoms ~ nominal BNCT dose) and in other experiments with permeabilized cells. TC7 cells, a subline of African green monkey kidney cells, were chosen because of their apparent resilience to the process of microinjection and the need to use a mammalian cell line. The objective of this study was to explore possible subcellular boron localization and distribution by both closo- and nido-OPDs in living cells using microscopy and a fluorescent marker. The study revealed that nido-(CB)_5 (3) accumulated in the nucleus within 10 min after injection (Figure 3a) and was retained in the cell nucleus for at least 24 h (Figure 3c). Injected cells remained motile and reproduced during the observation period. When the plasma membrane of the cells was selectively permeabilized with digitonin followed by incubation with nido-(CB)_5 (3), its accumulation in the nucleus was observed (not shown). Nuclear accumulation of nido-(G1)_5 (4a) after its microinjection into the cytoplasm (Figure 3c, 3d) and after its incubation with permeabilized cells (not shown) was similar to that observed with nido-(CB)_5 (3) for up to 2 h (Figure 3b). However, unlike nido-(CB)_5 (3), at 24 h post-injection nido-(G1)_5 (4a)
was redistributed in the cytoplasm and nucleus (Figure 3d). In contrast to the distinct nuclear accumulation pattern of nido-(CB)$_3$ (3) and nido-(G1)$_3$ (4a), the less hydrophilic closo-(G1)$_3$ (4b) remained in the cytoplasm. The differences in cellular localization properties of oligomers 3, 4, and 5 suggest the existence of a structure-activity relationship which can be further elucidated by the study of a diverse family of nido-OPDs. Encapsulation of these compounds in tumor cell-targeted liposomes should enhance the compound's ability to selectively penetrate the plasma membrane. In addition, nido-OPDs bearing hydrophobic side chains, such as compounds 5 and 6, can be prepared. Such amphiphilic species should demonstrate increased cellular uptake after incubation (amphiphilic plasma membrane penetrators). Recent data suggests that selected boron-rich nido-OPDs could play a decisive role in BNCT as well as drug delivery to cell nuclei.

**Selected References**


