# Solution-Phase Synthesis of Boron-Rich Phosphates

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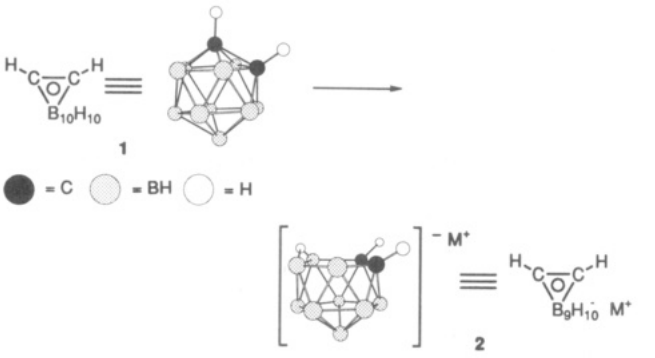
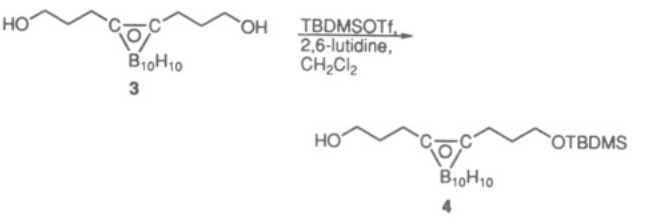
*Summary:* The stepwise synthesis in solution of small oligophosphates derived from a carboranyl diol has been demonstrated.

Boron neutron capture therapy (BNCT) is a binary approach to cancer therapy based on the capture of low-energy neutrons by $^{10}B$, which results in the emission of extremely cytotoxic $^{7}Li^{+}$ nuclei and $\alpha$-particles ($^{10}B(n,\alpha)^{7}Li$).[1] The major obstacle to clinically viable BNCT is that effective therapy requires the selective localization of 5–30 ppm $^{10}B$ in tumor.[2] Tumor-directed antibodies or their immunoreactive fragments are attractive candidates for the selective delivery of $^{10}B$, provided that ~1000 $^{10}B$ atoms can be attached to each immunoreactive protein without significantly altering its biological properties.[3] Previous studies have revealed problems associated with randomly conjugating whole monoclonal antibodies (Mabs) with large numbers of small boron-containing compounds[4] or with limited numbers of heterogeneous[5] or homogeneous boron-rich polymers.[6] Recently, we have described an approach to this problem based upon the synthesis of a homogeneous boron-rich "trailer" compound and its conjugation to a specific site of a tumor-directed antibody fragment (Fab-SH).[7] The success of this approach rests upon the synthesis of a hydrophilic "trailer" molecule containing ~1000 $^{10}B$ atoms. An oligophosphate-based boronated "trailer" is an attractive target, since the requisite coupling chemistry is well developed (in the context of DNA synthesis[8]) and such polyanionic oligomers are expected to be inherently hydrophilic. We report herein preliminary results concerning the stepwise solution-phase synthesis of short boron-rich oligophosphates, novel compounds that may find utility as intermediates in the assembly of tumor-localizing boron-rich compounds for BNCT.[9]

**Scheme I**




**Scheme II**




The use of derivatives of *o*-carborane[10] (**1**) is pervasive in BNCT research, since these relatively stable boron-rich compounds can be readily functionalized.[11] Additionally, lipophilic *closo*-carborane derivatives can be converted under mild conditions to stable anionic *nido* carborane derivatives[10] (**2**, Scheme I) which exhibit enhanced hydrophilicity.[12] The oligophosphates described herein are derived from the *o*-carborane diol **3**, which is prepared by the condensation of dilithio-*o*-carborane with an excess of trimethylene oxide (90%).[13] Treatment of diol **3** with 1 equiv of TBDMSOTf affords **4** (48%, Scheme II) after chromatographic separation of the mixture of mono- and diprotected products and unreacted diol. Attempts to synthesize several monoprotected diols analogous to **4** have proven to be less successful. For example, we were unable to efficiently monoacetylate diol **3** utilizing sulfate catalysts supported on silica gel.[14] We were also surprised to encounter difficulties in the alkylation of carbanions **6** derived from monosubstituted carboranes **5** (Scheme III).

We examined monoprotected *o*-carboranyl diol **4** in its coupling with isobutyl alcohol under a variety of conditions, which afforded phosphotriesters **7** (Scheme IV). Results

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(1) For a recent review, see: Barth, R. F.; Soloway, A. H.; Fairchild, R. G. *Cancer Res.* **1990**, *50*, 1061–1070.

(2) Fairchild, R. G.; Bond, V. P. *Int. J. Radiat. Oncol. Biol. Phys.* **1985**, *11*, 831.

(3) It has be calculated that antibodies bearing this quantity of $^{10}B$ could deliver therapeutic amounts of $^{10}B$ to tumor.

(4) Mizusawa, E.; Dahlman, H. L.; Bennett, S. J.; Goldenberg, D. M.; Hawthorne, M. F. *Proc. Nat. Acad. Sci. U.S.A.* **1982**, *79*, 3011–3014. Goldenberg, D. M.; Sharkey, R. M.; Primus, F. J.; Mizusawa, E.; Hawthorne, M. F. *Proc. Nat. Acad. Sci. U.S.A.* **1984**, *81*, 560–563. Mizusawa, E.; Thompson, M. R.; Hawthorne, M. F. *Inorg. Chem.* **1985**, *24*, 1911–1916.

(5) For example, see: Alam, F.; Soloway, A. H.; Barth, R. F.; Mafune, N.; Adams, D. M.; Knoth, W. H. *J. Med. Chem.* **1989**, *32*, 2326–2330. Pettersson, M. L.; Courel, M.-N.; Girard, N.; Gabel, D.; Delpech, B. *Strahlenther Oncol.* **1989**, *163*(213), 151–152.

(6) Varadarajan, A.; Hawthorne, M. F. *Bioconjugate Chem.* **1991**, *2*(4), 242–253. Paxton, R. J.; Beatty, B. G.; Varadarajan, A.; Hawthorne, M. F. *Bioconjugate Chem.* **1992**, *3*(3), 241–247.

(7) Hawthorne, M. F. *Pure Appl. Chem.* **1991**, *24*, 327–334.

(8) Gait, M. J., Ed. *Oligonucleotide Synthesis: A Practical Approach*; IRL, Ltd.: Oxford, 1984.

(9) Some of these results were presented at the 203rd National Meeting of the American Chemical Society (ORGN #242).

(10) Throughout this paper *closo*-carborane, *o*-carborane, or carboranyl refer to derivatives of the *closo*-1,2-$C_2B_{10}H_{12}$ cage, while *nido*-carborane refers to derivatives of the [*nido*-7,8-$C_2B_9H_{11}$]$^-$ cage fragment.

(11) For example, see: Grimes, R. N. *Carboranes*; Academic Press: New York, 1970.

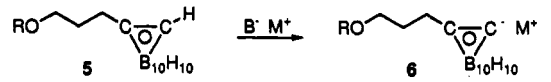
(12) Wiesboeck, R. A.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1964**, *86*, 1643–1644. Hawthorne, M. F.; Wegner, P. A; Stafford, R. C. *Inorg. Chem.* **1965**, *4*, 1675.

(13) Yields refer to material that is homogeneous by NMR, TLC, and/or HPLC. All new compounds reported in this paper have been appropriately characterized (HRMS, HPLC, multinuclear NMR, etc.).

(14) Nishiguchi, T., Kawamine, K., and Ohtsuka, T. *J. Org. Chem.* **1992**, *57*(1), 312–316.

0022-3263/93/1958-3227$04.00/0 

**Scheme III**



R = Dimethoxytrityl, TBDMS
$M^+$ = $Na^+$, $K^+$, $Li^+$

**Scheme IV**

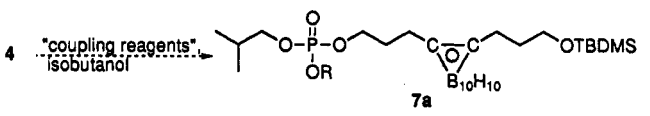
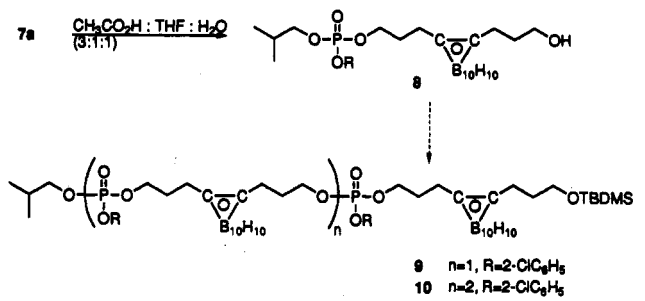


**Table I**

| entry | coupling reagent | product | yield (%) |
|---|---|---|---|
| 1 | $Cl_2P(O)OR$ | **7a** | 25 |
| 2 | $(BTO)_2P(O)OR^a$ | **7a** | 47 |
| 3 | $Cl_2POR^b$ | **7a** | 68 |
| 4 | $Cl_2POR^c$ | **7a** | 88 |
| 5 | $ClP(OR)N(i\text{-}Pr)_2{}^{d,c}$ | **7b** | 54[e] |

[a] BT = benzotriazole. [b] The initially formed phosphite triester was oxidized *in situ* with aqueous iodine (0.1 M). [c] The initially formed phosphite triester was oxidized *in situ* with 0.1 M iodine in THF/$H_2O$/2,6-lutidine (40/1/10). [d] The intermediate phosphoramidite was isolated in 90% yield and was coupled with isobutyl alcohol in the presence of tetrazole. [e] Yield is from two steps.

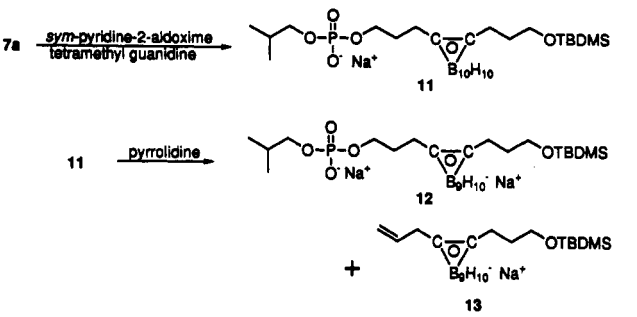
**Scheme V**



from these studies are summarized in Table I. The simplicity, speed, economy, and efficacy of the dichloro phosphite coupling reaction (entry 4, Table I) prompted us to adopt this method for subsequent coupling reactions. Accordingly, phosphotriester **7a** was converted under acidolytic conditions[15] to alcohol **8**, which could then be condensed with monoprotected diol **4** to afford the diphosphate derivative **9** in moderate yield (35% from two steps, Scheme V). Repetition of the deprotection and coupling steps provided triphosphate **10** in a low but reproducible yield (18% from two steps). Work in our laboratories is currently aimed at improving these yields, especially in the hydroxyl group deprotection step. It is likely that the substitution of a more labile protecting group for the resilient *tert*-butyldimethylsilyl ether will prove to be advantageous.

(15) Corey, E. J. *J. Am. Chem. Soc.* **1972**, *94*, 6190–6192. Attempted deprotection of **4** with TBAF resulted in the formation of an intractable mixture of products.

**Scheme VI**



At this point we decided to explore the conversion of the hydrophobic phosphotriester **7a** to anionic derivatives expected to be much more hydrophilic. We removed the 2-chlorophenyl phosphate protecting group under standard conditions (*syn*-pyridine-2-aldoxime and tetramethylguanidine in THF at room temperature).[16] Workup followed by cation exchange ($Na^+$ form resin) afforded sodium salt **11** in 87% yield (Scheme VI). Suspension of this anion in neat pyrrolidine at room temperature (1 h) resulted in the isolation (71% crude yield) of the desired anionic *nido*-carboranyl phosphate **12** contaminated with a small amount of an alkene (presumably elimination product **13**). Alternate procedures for this critical transformation are being explored.

In summary, we have demonstrated the viability of synthesizing small boron-rich oligophosphates in solution. However, the combination of steadily decreasing yields and increasingly difficult purifications seems to preclude the use of this methodology for the synthesis of lengthy oligomers. We are presently exploring the synthesis of boron-rich oligophosphate "trailers" using phosphoramidite coupling chemistry on an automated DNA synthesizer; the preliminary results are promising and will be reported shortly. In any event, the solution-phase strategy described herein will be useful for the synthesis of large quantities of short boron-rich oligophosphates that can be further derivatized and as such describes a noteworthy method for the production of extremely hydrophilic boron-rich compounds for use in the boron neutron capture therapy of cancer.

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**Supplementary Material Available:** Full experimental details and compound characterization data for compounds **4**, **7a**, and **8–12** (18 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(16) Reese, C. B.; Titmas, R. C.; Yau, L. *Tetrahedron Lett.* **1978**, *30*, 2727–2730.