Automated Syntheses of Carborane-Derived Homogeneous Oligophosphates: Reagents for Use in the Immunoprotein-Mediated Boron Neutron Capture Therapy (BNCT) of Cancer

Robert R. Kane, Karin Drechsel,† and M. Frederick Hawthorne*

Department of Chemistry and Biochemistry
University of California, Los Angeles
Los Angeles, California 90024-1569

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Boron neutron capture therapy (BNCT) is a binary approach to cancer therapy based upon the *in situ* production of extremely cytoxic particles ($^7Li^+$ and α, sharing ~2.3 MeV of kinetic energy) subsequent to capture of a low-energy neutron by a ^{10}B nucleus ($^{10}B(n,\alpha)^7Li$).[1] The vast potential of this therapeutic modality has resulted in worldwide efforts aimed at the development of agents capable of the selective localization of ^{10}B in tumor cells at concentrations of 10–30 ppm—concentrations which have been predicted to afford a significant therapeutic advantage.[2] One approach to ^{10}B localization is based upon tumor-selective antibodies conjugated to boron-rich macromolecules (trailers).[3] Boron-rich trailer molecules could also be used in conjunction with other tumor-targeting agents such as bioregulatory peptides or sex hormones or as boron-rich reagents in a bispecific antibody-mediated approach to localization.[3] Although structurally heterogeneous boron-rich trailers have been utilized in several investigations,[4] our efforts have been concentrated upon the synthesis and conjugation of functionalized homogeneous trailers of precisely defined structure.[5] This communication describes the use of a conventional automated DNA synthesizer for the rapid and efficient synthesis of large functionalized boron-rich oligophosphate trailers that are homogeneous and extremely hydrophilic.[6]

The most common method for automated DNA synthesis involves the stepwise coupling of 5′-*O*-(dimethoxytrityl)-3′-(*N*,*N*-diisopropylamino)–(β-cyanoethyl)phosphoramidite nucleoside derivatives **1**.[7] We have found that the boron-rich phosphoramidite **2**, which is readily synthesized in three steps[8] starting from *o*-carborane,[9] is functionally equivalent to the nucleoside monomers in supporting oligophosphate synthesis. Boron-rich oligophosphates can be constructed using compound **2** on automated DNA synthesis instruments *with no modification to the standard reagents or procedures*.[10] Table I lists the sequences of a number of the oligophosphates that we have synthesized using standard DNA synthesis techniques and the average stepwise coupling yields observed during their construction.[11] The structures of the subunits are shown in Figure 1.[12] The flexibility of this approach to the synthesis of homogeneous functionalized boron-rich oligomers is evident in the varied compositions and sequences of oligomers **3–15**. It should be noted that other than the carborane-containing units CB and CBX, all of the building blocks are available commercially.

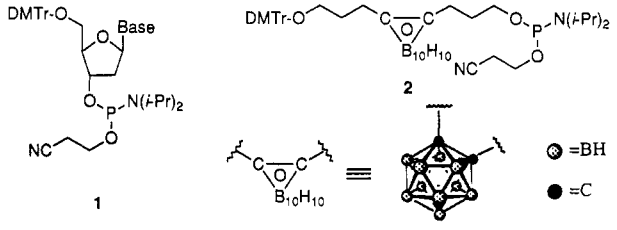

Table I

compound	sequence (3′→5′)[a]	efficiency (%)[b]
3	HO-T-$(CB)_{10}$-H	97.1
4	HO-T-$(CB)_{20}$-N	99.3
5	HO-T-$(CB)_{20}$-Fl-N	99.0
6	HO-T-Fl-$(CB)_{10}$-N	99.2
7	HO-T-Fl-$(CB)_{10}$-T_5-N	98.9
8	HO-T-$(CB)_{20}$-CBX	97.0
9	HO-T-$(CB)_{20}$-B_A	97.5
10	HO-T-Fl-$(CB)_2$-S	97.8
11	HO-T-Fl$(CB)_{10}$-S	97.2
12	B_B-$(CB)_{10}$-N	98.2
13	B_B-$(CB)_{20}$-N	98.5
14	B_B-$(CB)_{40}$-N	97.3
15	B_B-$(CB)_{20}$-Fl-N	98.0

[a] Sequence is written in the order of assembly (*pseudo* 3′→*pseudo* 5′). See Figure 1 for the structures corresponding to the abbreviations. [b] Average coupling efficiencies per step.[11]

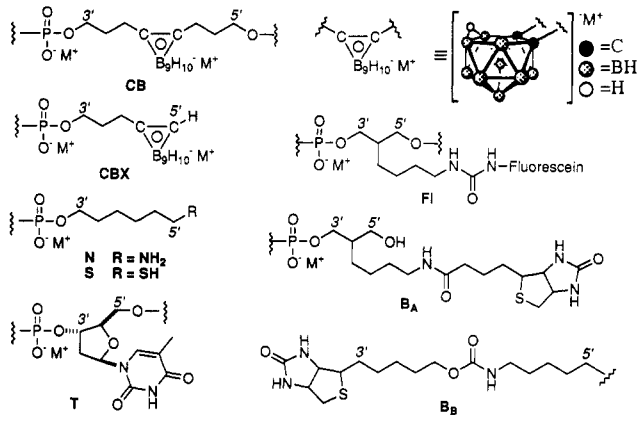

Figure 1.

After the completion of their synthesis, the boron-rich oligophosphates were removed from the CPG (controlled pore glass)

† On leave from the Technische Hochschule Aachen, Templergraben 55, D-5100 Aachen, Germany.

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

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(4) 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**, *165*(2/3), 151–152.

(5) 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. Kane, R. R.; Pak, R. H.; Hawthorne, M. F. *J. Org. Chem.* **1993**, *58*(5), 991–992.

(6) We have recently reported the solution-phase synthesis of oligophosphates containing up to 30 boron atoms: Kane, R. R.; Lee, C. S.; Drechsel, K.; Hawthorne, M. F. *J. Org. Chem.* **1993**, *58*(12), 3227–3228.

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(8) (1) (a) *n*-BuLi (2.2. equiv), THF, 0 °C to room temperature, 30 min; (b) oxetane (2.5 equiv), reflux, 12 h; (c) H^+ (85% overall). (2) Dimethoxytrityl chloride (1 equiv), pyridine, DMAP (5%), Et_3N (1.4 equiv), room temperature, 30 h (49%). (3) Chloro-(2-cyanoethyl)-(*N*,*N*-diisopropylamino)phosphoramidite (1.5 equiv), CH_2Cl_2, $EtN(i\text{-}Pr)_2$ (3 equiv), 0 °C to room temperature; 1 h (92%). Experimental details for these transformations, including spectral data, may be found in the supplemental material.

(9) 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 anionic *nido*-7,8-$C_2B_9H_{11}^-$ cage fragment.

(10) Oligomers were synthesized on a 1 μmol scale at the Midland Certified Reagent Company, Midland, TX. In general, a 0.1 mmol solution of **2** in acetonitrile is loaded onto an unused monomer port of an ABI Model 391 DNA synthesizer, and the standard coupling program is used. These boron-rich oligomers have also been successfully synthesized on other automated DNA synthesizers. We wish to thank Dr. Lynn Myers of the Midland Certified Reagent Company for his assistance and helpful suggestions.

(11) Determined from recovery of dimethoxytrityl cation, and averaged over a complete oligomer synthesis.

(12) Oligomers were characterized after conversion of the carboranes to the anionic *nido* structures shown for **CB** and **CBX**. See text for details.

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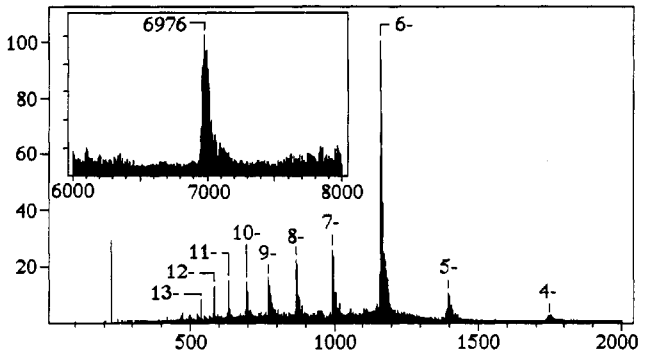

Figure 2. Averaged negative ion mass spectrum of oligomer **9** (NH_4^+ form) obtained on a Finnigan TSQ-700 fitted with electrospray source under LC conditions (C_{18} capillary, eluted with a solvent gradient from 98% A/2% B to 100% B. Solvent A is 0.01% aqueous TFA; solvent B is 90% CH_3CN/10% A). The inset is the computer deconvoluted molecular mass, which corresponds to oligomer **9** associated with a mixture of ammonium ions and protons as counterions.

support by treatment with concentrated NH_4OH (~5 min at room temperature), which also removes the β-cyanoethyl phosphate protecting groups, affording free water-soluble boron-rich oligophosphates. ^{11}B NMR analysis of these oligomers revealed that the carborane residues retained their *closo* structure. Extended NH_4OH treatment (30 min at 80 °C or 2.5 h at 66 °C, conditions routinely employed for removing the base protecting groups in DNA synthesis), cleanly and quantitatively converted the oligophosphates containing *closo*-carborane cages to their anionic *nido* derivatives.[8] Thus, water-soluble oligophosphates containing either neutral *closo*-carboranes or anionic *nido*-carboranes are readily available, depending on the deprotection conditions used subsequent to oligomer synthesis. The oligomers listed in Table I were characterized in the *nido* form[13] after ion exchange using Dowex 50X2-150 (Na^+ form). Although the conversion of *closo*-carboranes to the anionic *nido* forms upon treatment with base in the presence of a proton source is well known,[14] the use of NH_4OH to effect this transformation has not been previously reported.

Another useful method for oligomer characterization is polyacrylamide gel electrophoresis.[15] In general, the boron-rich oligophosphates migrate slightly faster than single-stranded DNA containing the same number of phosphate bonds, presumably due to the presence of anionic *nido*-carborane residues. Several methods can be used to visualize these gels, including treatment with silver(I) ions (10% silver nitrate in concentrated NH_4OH), direct visualization under UV irradiation of oligomers incorporating a fluorescent monomer (e.g., compounds **5**, **6**, **7**, **10**, **11**, and **15**), and avidin peroxidase treatment of biotin-labeled oligomers (e.g., compounds **9** and **12–15**) after transfer to a nitrocellulose membrane. In all cases, the oligomers appear as homogeneous bands with occasional minor bands arising from chain-truncated products.

Several of these oligomers have been characterized by negative-ion electrospray mass spectroscopy.[16] As expected, the mass spectrum consists of a series of charge states, which differ from each other by the degree of protonation and which deconvolute to a mass which approximates the expected mass (Figure 2).

In conclusion, we have demonstrated a general method for the rapid and efficient synthesis of homogeneous boron-rich oligophosphates containing up to 400 boron atoms. These oligomers have been equipped with diverse and useful functionality. Recent work has demonstrated the coupling of both amino and thiol functionalized oligomers to a free thiol group present on an antibody fragment (F(ab')) using appropriate bifunctional cross-linking reagents.[17] Biotin-functionalized oligomers have been demonstrated to bind to avidin *in vitro*[18] and oligomer **16**, which is fitted with a monosubstituted *nido*-carborane, is recognized by a *nido*-carborane specific antibody which we have developed for use in a bispecific antibody delivery system.[19] These results demonstrate the vast potential of the novel boron-rich trailer molecules described in this communication and will be reported elsewhere.

Acknowledgment. Financial support by the National Institutes of Health (Grants CA31753-09 and CA53870-01) is gratefully acknowledged. RRK is supported in part by a postdoctoral traineeship (USHHS National Institutional Service Award CA-09056). KD thanks the Gottlieb Daimler-und Karl Benz-Stiftung for a predoctoral fellowship.

Supplementary Material Available: Full experimental details and compound characterization data for compound **2** and ^{11}B spectra of oligomer **13**, demonstrating its conversion from all-*closo* to all-*nido*-carboranes (6 pages). Ordering formation is given on any current masthead page.

(13) The carboranes in these oligomers are typically >95% *nido*, as shown by ^{11}B NMR.

(14) 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.

(15) 20% acrylamide (3% bis), 7 M urea.

(16) Spectra were measured on a Finnigan TSQ-700 with electrospray source in the Division of Immunology at the City of Hope, Duarte, CA (thanks to Toby O'Neil and Dr. Kristine M. Zwiderick for their help). For a recent discussions of electrospray mass spectroscopy, see: Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal.Chem.* **1990**, *62*(9), 882–899; Ganem, B. and Henion, J. D. *Chemtracts* **1993**, *6*(1), 1–22.

(17) Chen, C.-J.; Shively, J. E.; Kane, R. R.; Hawthorne, M. F., unpublished results.

(18) Primus, F. J.; Kane, R. R.; Hawthorne, M. F., unpublished results.

(19) Primus, F. J.; Rickard, K. J.; Kane, R. R.; Ng, L. L.; Pak, R. H.; Hawthorne, M. F., unpublished results.