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Communications

Carboranyl Oligonucleotides. 1. Synthesis of Thymidine(3',5')thymidine (*o*-Carboran-1-ylmethyl)phosphonate

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Summary: Using methyl (*o*-carboran-1-ylmethyl)phosphonate **6** as a novel and versatile borophosphonylating agent, 5'-*O*-(monomethoxytrityl)thymidine 3'-*O*-[methyl (*o*-carboran-1-ylmethyl)phosphonate] (**8**) and thymidine-(3',5')thymidine (*o*-carboran-1-ylmethyl)phosphonate (**12**) were synthesized. The internucleotide (*o*-carboran-1-ylmethyl)phosphonate linkage was resistant to cleavage by phosphorodiesterases. The dinucleotide **12** represents a new class of modified lipophilic oligonucleotide–bearing carboranyl residue, designed as a carrier for boron neutron capture therapy and for potential use in antisense oligonucleotide technology.

Boron neutron capture therapy (BNCT) is a binary system which combines two separately nonlethal constituents—a radiosensitizer containing stable boron-10 (^{10}B) isotope with nonionizing neutron radiation. When boron-10 is irradiated with low-energy neutrons, a nuclear reaction occurs yielding helium nuclei (α-particle), lithium-7 nuclei, and about 100 million times more energy than was put in. The generated radiation destroys malignant cells containing the boron compound resulting in a therapeutic effect.[1-4] Selectivity can be achieved with compounds which accumulate specifically in malignant cells and/or by aiming the neutron beam at the tumor mass which contains the boron carrier.

The major obstacles in BNCT are (1) reaching a high enough intracellular boron concentration and (2) selectivity toward the tumor cells. We hypothesize that *o*-carboran-1-yl-modified oligonucleotides may resolve these important problems. The oligonucleotides can bear several *o*-carboran-1-yl residues, each containing 10 boron atoms, which effectively increases the boron content per carrier molecule. Furthermore, these novel compounds can potentially target cancer cells by interacting with overexpressed or unique genes found in these cells (Figure 1). The *o*-carboran-1-yl residue is lipophilic, which should facilitate boron-modified oligonucleotide transport through cellular membranes.[4] An additional potential advantage of boron-modified oligonucleotides is the finding that oligonucleotides accumulate in the nucleus.[5] This is of special importance since microdosimetric calculations suggest that there is a five times greater chance for cell killing when boron-10 is primarily confined to the cell nucleus than when it is uniformly distributed throughout the cell.[6]

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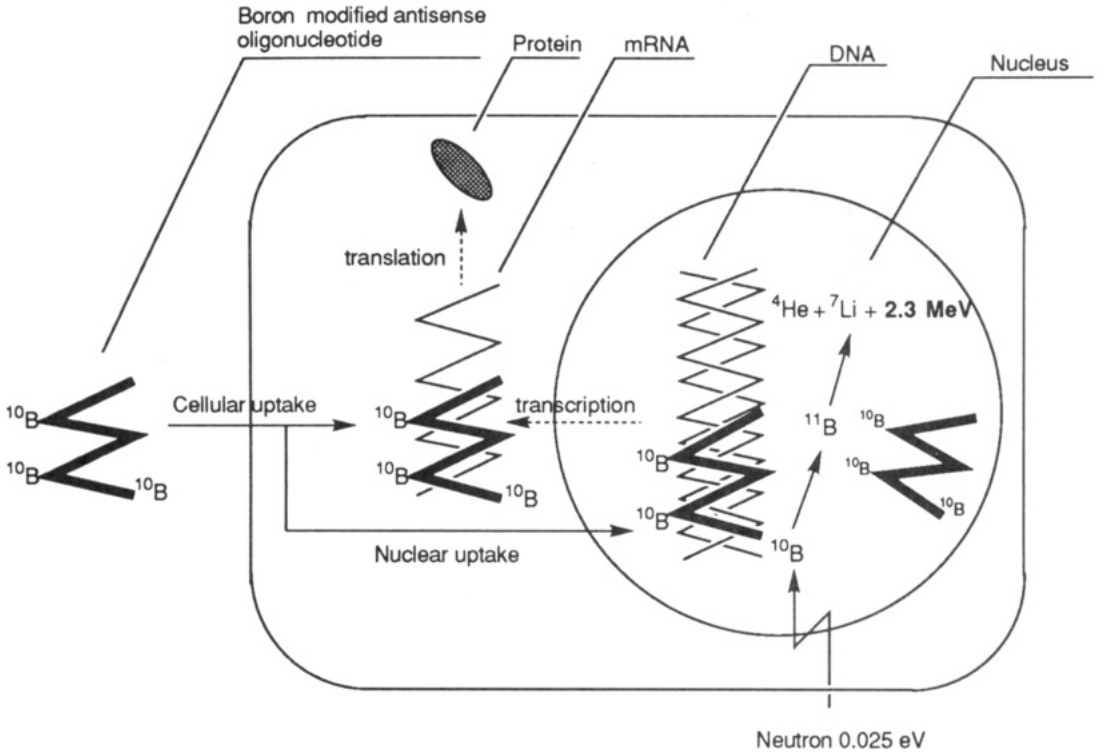

Figure 1. Hypothetical mechanism of action of boronated oligonucleotides for BNCT. A sequence-specific boron oligomer may interact with RNA in the cytoplasm or DNA or RNA in the nucleus. In addition, the oligomer may accumulate in the nucleus.

Although attempts to develop tumor-selective boron compounds date back to the 1960s[7] and despite extensive studies, the problem of selective delivery of boron carriers to tumor cells remains. Many classes of compounds have been synthesized for BNCT.[2,3,8] The first boron-containing nucleoside, 5-(dihydroxyboryl)-2′-deoxyuridine, was synthesized by Schinazi and Prusoff only in 1978.[9,10] Other boron-containing nucleosides such as carboranyluridines were subsequently prepared.[11,12] Sood et al. reported the synthesis of oligonucleotide boranophosphates and boranophosphate methyl ester analogues.[13,14] These derivatives allow insertion of only one boron atom per modification into the oligonucleotide chain. Similarly, Kane et al. recently reported on the synthesis of oligophosphates[15] and Powell et al. on oligonucleotides[16] bearing *o*-carboranyl residues as potential boron "trailers" for BNCT. In the latter case, the carboranyl cage was attached to the internucleotide linkage through an acid unstable phosphoramidate bond.

In this report, we describe the synthesis of thymidine-(3′,5′)thymidine (*o*-carboran-1-ylmethyl)phosphonate (**12**), the first oligonucleotide analogue bearing a 3′,5′-*O,O*-[(*o*-carboran-1-ylmethyl)phosphonate] internucleotide linkage instead of natural a 3′,5′-*O,O*-phosphodiester residue. Boron-containing oligonucleotides provide a new approach to boron "trailers" specificity toward tumors. The *o*-carboran-1-yl-modified oligonucleotides should serve as a foundation for the design of tumor-selective boron-rich carriers for BNCT utilizing the advantages of antisense oligonucleotide technology (AOT)[17] (Figure 1).

The synthesis of compound **12** and its monomer 5′-*O*-(monomethoxytrityl)thymidine 3′-*O*-[methyl (*o*-carboran-1-ylmethyl)phosphonate] (**8**) are shown in Figure 2. The key intermediate in the synthesis of **8** is borophosphonylating agent methyl (*o*-carboran-1-ylmethyl)phosphonate (**6**). Compound **6** was obtained in a three-step procedure. In the first step, propargyl bromide was reacted with trimethyl phosphite, using the Michaelis–Arbuzov type reaction, yielding dimethyl propargylphosphonate (**3**) in 45% yield.[18] As a major byproduct, dimethyl methylphosphonate was isolated [^{1}H NMR ($CDCl_3$) δ = 1.05 (d, 3H, J_{PH} = 18.5 Hz, CH_3), 3.4 (d, 6H, J_{PH} = 9.2 Hz, CH_3O)]. In the second step, product **3** was reacted with decaborane in acetonitrile according to the general reaction of decaborane and acetylene derivatives described by Heying et al.[19] The desired dimethyl (*o*-carboran-1-ylmethyl)phosphonate (**5**) was obtained in 40% yield.[20] The direct approach to **5** synthesis *via* the Michaelis–Arbuzov reaction between trimethyl phosphite and *o*-carboran-1-ylbromomethane was unsuccessful and led mainly

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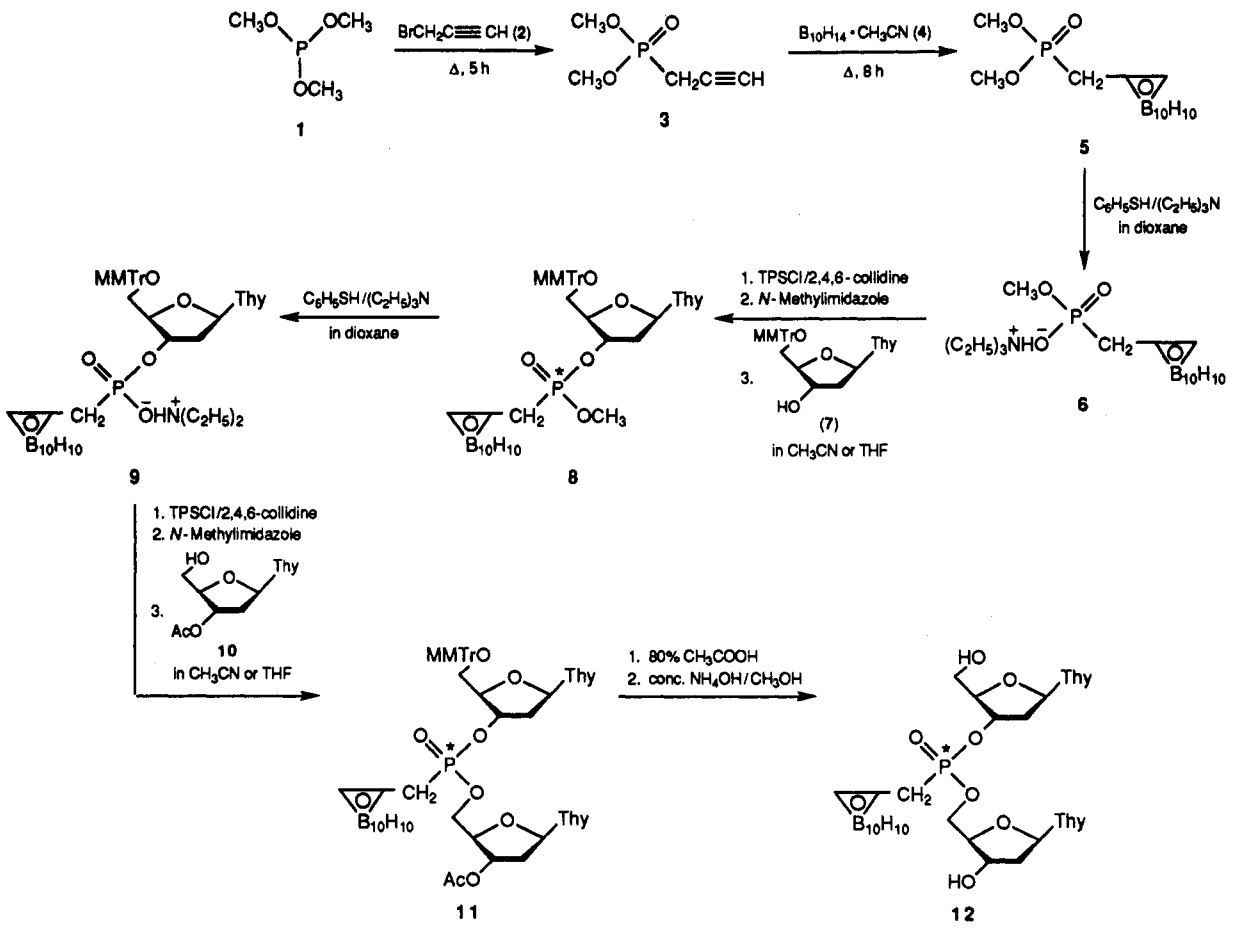

Figure 2. Synthesis of thymidine(3′,5′)thymidine (*o*-carboran-1-ylmethyl)phosphonates.

to dimethyl methylphosphonate. Compound **6** was obtained from **5** under treatment with a mixture of thiophenol/triethylamine/dioxane in 79% yield. This method is widely used in oligonucleotide chemistry to deblock internucleotide linkage protected with the methyl group.[21] In contrast, an approach for selective **5** demethylation by reaction with *tert*-butylamine[22] was only partially successful, since several undefined byproducts were detected [^{31}P NMR ($CDCl_3$) δ = −8.8, 23.4, 31.1]. This may be due to partial *closo* [*closo*-1,2-$C_2B_{10}H_{12}$ cage] to *nido* [(*nido*-7,8-$C_2B_9H_{11}$)$^-$ cage] carboranyl residue transformation.[15,23]

Fully protected monomer **8** was synthesized in the reaction of 5′-*O*-(monomethoxytrityl)thymidine (**7**) and **6**, in the presence of triisopropylbenzenesulfonyl chloride as the activating agent and 2,4,6-collidine and 1-methylimidazole. Product **8** was obtained in 45% yield after standard workup and chromatographic purification. Selective demethylation of **8** with thiophenol/triethylamine/dioxane was performed as described for **5**. After ≈5 min, the triethylammonium salt of 5′-*O*-monomethoxytrityl-thymidine 3′-*O*-(*o*-carboran-1-ylmethyl)phosphonate (**9**) was isolated by precipitation with hexanes in 80% yield. Crude **9** was sufficiently pure by TLC analysis to be used for 5′-*O*-(monomethoxytrityl)thymidine(3′,5′)-3′-*O*-acetylthymidine (*o*-carboran-1-ylmethyl)phosphonate (**11**) synthesis. The latter was synthesized by the reaction of **9** activated with triisopropylbenzenesulfonyl chloride with 3′-*O*-acetylthymidine (**10**), in the presence of 2,4,6-collidine in dry acetonitrile and 1-methylimidazole as the nucleophilic catalyst. The yield of **11** after chromatographic purification was 30%.[24] Deprotection of **11** was performed according to the literature procedures using concd NH_3-OH/CH_3OH to remove the 3′-*O*-acetyl group[25] and 80% CH_3COOH to remove the 5′-*O*-(monomethoxytrityl) group.[26] Crude thymidine(3′,5′)thymidine (*o*-carboran-1-ylmethyl)phosphonate (**12**) was purified by silica gel column chromatography and characterized by MS(FAB) analysis and by HPLC. The HPLC analysis of **12** revealed two pairs of peaks characterized by retention times of (R_f) 20.5 and 21.5 and 33.9 and 35.5 min, which probably correspond to the *nido* and *closo* form, respectively, of P-epimeric **12**. The observation of partial *closo* to *nido*

(20) Procedure for Synthesis of **5**. Decarborane (**4**) (0.01 mol, 1.2 g) was dissolved in dry CH_3CN (20 mL) and the resultant solution heated under reflux. After 15 min, **3** (0.02 mol, 2.8 g) was added to the boiling solution. Heating was continued for 8 h, and then the reaction mixture was left overnight at room temperature and filtered. The solvent was evaporated under reduced pressure, and the oily residue was redissolved in CH_2Cl_2 (25 mL). The resultant solution was washed with water (3 × 20 mL), the organic fraction was dried over $MgSO_4$, and the CH_2Cl_2 was evaporated. The oily residue was redissolved in CH_2Cl_2 (20 mL) and then precipitated with hexanes (250 mL). The precipitate was filtered, and hexanes were evaporated under reduced pressure yielding an oily residue which crystallized spontaneously. The crystals were washed with hexanes and dried under reduced pressure. For analysis, the resultant product was recrystallized from hexanes to yield 1.1 g (40%) as fine white flakes: mp 68–70 °C; ^{31}P NMR ($CDCl_3$) δ 20.7; ^{1}H NMR ($CDCl_3$) δ 0.8–3.4 (b signal, 10 H, $CCHB_{10}H_{10}$), 2.8 (d, 2H, J_{PH} = 20.3 Hz, PCH_2), 3.7 (d, 6H, J_{PH} = 10.2 Hz, CH_3OP), 4.4 (b s, 1H, CH); ^{13}C NMR ($CDCl_3$) δ 33.2 (d, J_{PC} = 144.2 Hz, PCH_2), 53.0 (d, J_{PC} = 6.8 Hz, CH_3OP), 59.84 and 67.8 (s and s, $CCHB_{10}H_{10}$). Anal. Calcd for $C_5H_{19}PO_3B_{10}$: C, 22.55; H, 7.19; B, 40.60. Found: C, 22.74; H, 7.21; B, 40.00.

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transformation of dinucleotide 11 during the deprotection leading to 12 is supported by the MS(FAB) analysis. The mass spectra of dinucleotide 12 characterized by t_R 33.9 and 35.5 and 20.5 and 21.5 min show ions [M + K] and [M - B], respectively. However, the *closo* to *nido* transformation does not seem to affect the stability of duplexes formed by oligonucleotides containing 5-*o*-carboran-1-yl-2′-*O*-deoxyuridine.[27] Work is under way on the synthesis of pure *closo* and *nido* form of 12 by modulating the deprotection conditions.

The dinucleotides 11 will serve, after selective deprotection and phosphitylation of its 3′-end with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite, as building blocks for the synthesis of longer oligonucleotides bearing one or more alternating (*o*-carboran-1-ylmethyl)phosphonate linkages by automatic synthesis on solid support. Results of enzymatic assays indicated that 11 was, as anticipated,[29,30] resistant to cleavage by bovine spleen phosphodiesterase (EC 3.16.1) and snake venom phosphodiesterase (EC 3.1.4.1).

Replacing one of the anionic oxygen atoms by the *o*-carboran-1-ylmethyl moiety generates a new center of chirality at the phosphorus of monomer 8 and oligonucleotides 11 and 12. Due to this modification and the nonstereoselectivity of the coupling reaction used, the (*o*-carboran-1-ylmethyl)phosphonate oligonucleotide is obtained as a mixture of diastereoisomers. The influence of absolute configuration at phosphorus of P-chiral antisense oligonucleotides on their physicochemical and biochemical properties has been previously studied.[28] Future work will take advantage of recent progress in stereocontrolled synthesis of P-chiral oligonucleotides analogues[30] to obtain longer, P-stereodefined oligomers for biological evaluation.

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Supplementary Material Available: Experimental procedures, characterization data, and ^1H, ^{13}C, and ^{31}P NMR spectra for compounds 1-12 (7 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.

(24) Procedure for Synthesis of 11. Compound 9 (16 mg, 0.02 mmol) and triisopropylbenzenesulfonyl chloride (8 mg, 0.025 mmol) were dissolved in dry CH_3CN (0.2 mL), and 2,4,6-collidine (5 μL, 0.035 mmol) was added with stirring. After 15 min at room temperature, a solution of 3′-*O*-acetylthymidine (10) (10 mg, 0.035 mmol) in dry CH_3CN (0.05 mL) followed by 1-methylimidazole (2 μL, 0.025 mmol) were added to the resultant mixture. The mixture was left overnight at room temperature and then CH_2Cl_2 (1 mL) was added. The resultant solution was washed with water (4 × 0.5 mL) and the organic fraction dried over $MgSO_4$ and evaporated to dryness. Crude product was purified by means of silica gel column chromatography using a stepwise 0–3% gradient of CH_3OH in CH_2Cl_2 as eluent. Fractions containing 11 were collected and the organic solvents evaporated to dryness. The residue was dissolved in CH_2Cl_2, and precipitated from hexanes, and dried under vacuum yielding the desired product, 6 mg (30%): TLC R_f 0.56 (9:1 CH_2Cl_2–CH_3OH); UV (95% C_2H_5OH) λ_{max} 265.0 nm, λ_{min} 245.0 nm, λ_{sh} 229.0 nm; MS/LSI (FB$^+$) 1016 [M + 2Li]; ^{31}P NMR ($CDCl_3$) δ 21.16 and 22.95; ^1H NMR ($CDCl_3$) δ 1.23 [d, 3H, J_{HH6} = 3 Hz, $CH_3(5)$], 1.44 [s, 3H, $CH_3(5)$], 1.50–1.72 and 2.24–2.48 (b m and b m, 2H and 2H, H2′), 0.6–3.2 (b signal, 10H, $CCHB_{10}H_{10}$), 1.88 (d, J_{PH} = 8.5 Hz, 2H, PCH_2), 2.07 (s, 3H, CH_3CO), 3.35–3.58 (m, 2H, H5′), 3.78 (s, 3H, CH_3OPh), 3.85–4.4 (mm, 4H, H5′ and H4′), 5.0 (b s, 1H, CH), 5.10–5.25 (b m, 1H, H3′), 6.0–6.4 (b mm, 3H, H3′, H1′), 6.70–6.85 and 7.10–7.50 (14H, arom).

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