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# Carborane Derivative Development for Boron Neutron Capture Therapy

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## Summary

Boron Neutron Capture Therapy [BNCT] is a binary method of cancer therapy based on the capture of neutrons by a boron-10 atom [$^{10}B$]. Cytotoxic $^{7}Li$ nuclei and $\alpha$-particles are emitted, with a range in tissue of 9 and 5 $\mu$m, respectively, about one cell diameter. The major obstacle to clinically viable BNCT is the selective localization of 5-30 ppm $^{10}B$ in tumor cells required for effective therapy.

A promising approach to BNCT is based on hydrophilic boron-rich oligomeric phosphate diesters, or "trailers" that have been shown to concentrate selectively in tumor tissue. Examples of these compounds were prepared previously at high cost using an automated DNA synthesizer. Direct synthesis methods are needed for the production of gram-scale quantities for further biological evaluation. The work accomplished as a result of the collaboration between Fluorochem, Inc. and UCLA demonstrates that short oligomers containing at least five carborane units with four phosphodiester linkages can be prepared in substantial quantities. This work was accomplished by the application of standard phosphoramidite coupling chemistry.

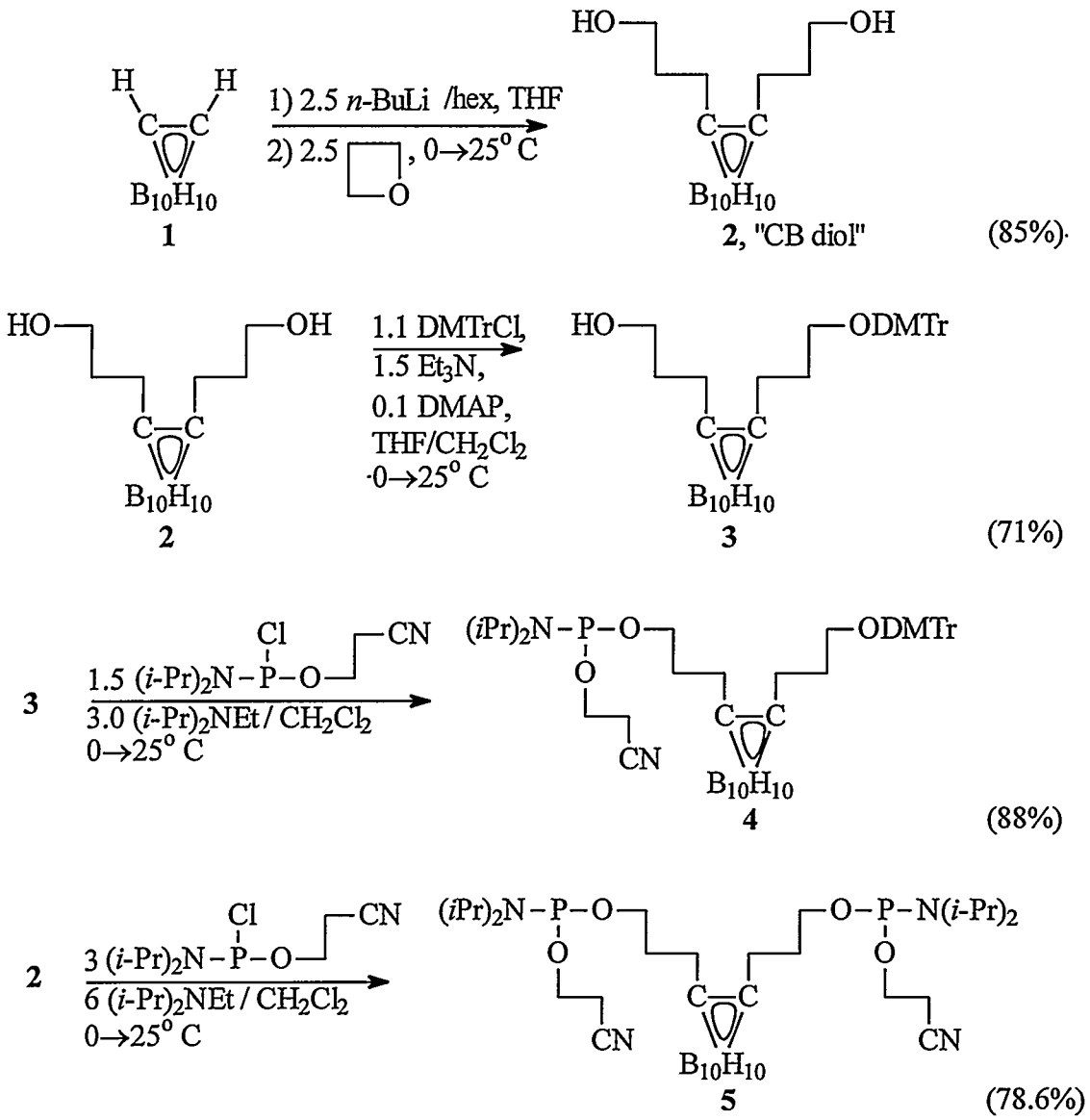
## Introduction and Background

Boron neutron capture therapy [BNCT] is a binary approach to cancer therapy based on the capture of neutrons by a boron-10 atom [$^{10}B$]. Cytotoxic $^{7}Li$ nuclei and α-particles are emitted, with a range in tissue of 9 and 5 μm, respectively, about one cell diameter.[1] The major obstacle to clinically viable BNCT is the selective localization of 5-30 ppm $^{10}B$ in tumor cells required for effective therapy.[2]

Recent evaluations have shown that homogeneous hydrophilic boron-rich oligomeric phosphate diesters are selectively concentrated in tumor tissue.[3] *In vivo* biodistribution studies show that intravenous injection of *nido*-carborone oligophosphate diesters into EMT-6 tumor-bearing mice results in the accumulation of significant amounts of boron in the tumor tissue. The *closo*-carborane oligomers have one negative charge in each repeating unit for the phosphate portion, while the more hydrophilic *nido*-carborane oligomers have an additional negative charge per carborane moiety in each repeating unit. Recently, the single-cell micro-injection of TC7 cells with homogeneous fluorescein-labeled *nido*-boronated oligomeric phosphate diesters resulted in the rapid localization of therapeutic quantities of boron in the cell nuclei.[4] Cell growth was essentially unaffected and the nucleus localization occurred without apparent toxicity.[4]

Boron-rich oligomeric phosphate diesters have been assembled thus far using an automated DNA synthesizer. The preparation of select derivatives on a micromolar scale has been accomplished with no changes to standard reagents or procedures in automated DNA synthesizer techniques.[5] This technique provides potential access to a wide variety of novel boron-rich oligophosphate diesters. However, high costs and low output render this method impractical for the production of the quantities necessary for additional biological evaluations. Consequently, all *in vivo* biological studies of these compounds have necessarily been carried out using small animals (mice) and very low injected doses of compound.[3] The objective of this research is to design and demonstrate synthetic procedures to manufacture boron-rich oligophosphate compounds in quantities sufficient for evaluation in animals using higher doses, and ultimately to provide the amounts of materials needed for clinical trials.

Recent developments in phosphoramidite coupling chemistry have provided new efficient synthetic procedures for DNA synthesis. Oligonucleotides have been synthesized in 1 mmol quantities via phosphoramidite nucleoside and highly loaded aminopolyethyleneglycol derivatized polystyrene.[6] New classes of nucleoside phosphitylating reagents have increased the effectiveness of nucleoside phosphite coupling reactions. With the introduction of N,N-diisopropylaminophosphoramidite synthons, several protecting groups have been developed.[7] Of the reported reagents, 2-cyanoethyl derivative can be synthesized effectively and removed easily for deprotection.[8]




Scheme 1. Preparation of starting materials

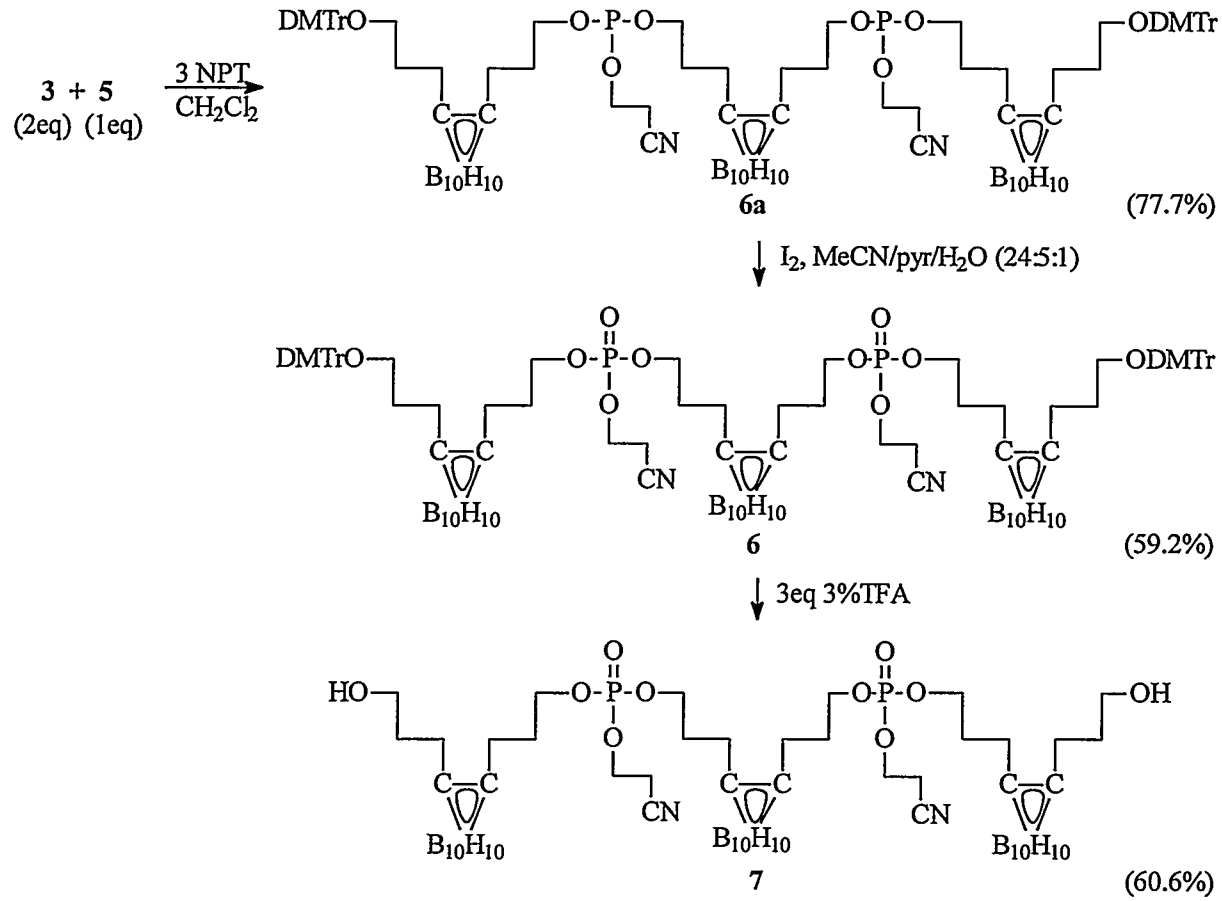
## Results and Discussion

The building blocks needed for the synthesis of boron-rich oligophosphate diesters were prepared as shown in Scheme 1. The key intermediate is the bis(propanol) *ortho*-carborane compound, CB diol **2**. In the first step, double deprotonation of *ortho*-carborane, **1**, with 2.5 equivalents butyl lithium followed by anionic ring-opening of 2.5 equivalents oxetane in THF provided the bis(propanol)-derivatized *ortho*-carborane cage, CB diol **2**, in up to 85% yield. One hydroxyl group of CB diol **2** can be protected selectively with a 4,4'-dimethoxytrityl group (DMTr) via reaction with 1.1 equivalents 4,4'-dimethoxytrityl chloride in triethyl amine with DMAP catalyst, yielding DMTr-mono-protected CB diol, **3**.[3] The DMTr group is used as a protecting group, because it can be removed efficiently under mildly acidic conditions. The ratios of 4,4'-dimethoxytrityl chloride, triethylamine and DMAP in

THF/methylene chloride solvent mixture were crucial for maximizing yields of **4**. The desired product was separated from unreacted starting material **2** and minor amounts of bis-DMTr-protected diol by flash column chromatography. Yields of 71% were obtained routinely in multi-gram preparations.

The (allyloxy)bis(dimethylamino)phosphine phosphitylating reagent suggested in the initial SBIR Phase I proposal is not presently commercially available. However, β-cyanoethyl diisopropylchlorophosphoramidite can be prepared based on published procedures or purchased commercially.[8,9] The chemistry of this class of nucleoside phosphitylating reagents is well known. Unlike the (allyloxy)bis(dimethylaminophosphine reagent which requires *N*-methylimidazolium triflate to mediate the reaction, the β-cyanoethyl reagent reacts smoothly in the presence of diisopropylethylamine. The DMTr-mono-protected CB diol **3** was treated with 1.5 equivalents of the phosphitylating reagent, 2-cyanoethyl diisopropylchloro-phosphoramidite, in the presence of 3 equivalents of diisopropylethylamine to produce the corresponding (phosphitylated)carborane derivative, **4**, in 88.2% yield. The unprotected CB diol, **2**, was treated with 3 equivalents of the same phosphitylating reagent, 2-cyanoethyl diisopropylchlorophosphoramidite, in the presence of 6 equivalents of diisopropylethylamine to produce the corresponding phosphitylated carborane derivative, **5**, in 78.6% yield.

With compounds **3** and **5** in hand, synthesis of oligomers was initially conducted in a controlled step-wise fashion. A trimer of the phosphate diester **6** was obtained in 2 steps. First, a phosphoramidite coupling reaction mediated by 3 equivalents of a tetrazole activator, NPT [5-(*p*-nitrophenyl)-1*H*-tetrazole], gave the carboranyl trimer diphosphite **6a**. The reaction was monitored by TLC (thin layer chromatography), and the compound was purified by column chromatography to give a 77.7% yield. This condensation reaction is promoted by 2-4 equivalents of an activator such a tetrazole or NPT in order to obtain acceptable reaction rates. However, using excess tetrazole compounds poses serious problems in large scale synthesis due to the fact that these compounds are expensive, toxic, potentially explosive and poorly soluble in organic solvents. A recent improvement for this coupling by use of catalytic amounts of activator in conjunction with 13X molecular sieves could be developed and optimized.[10]

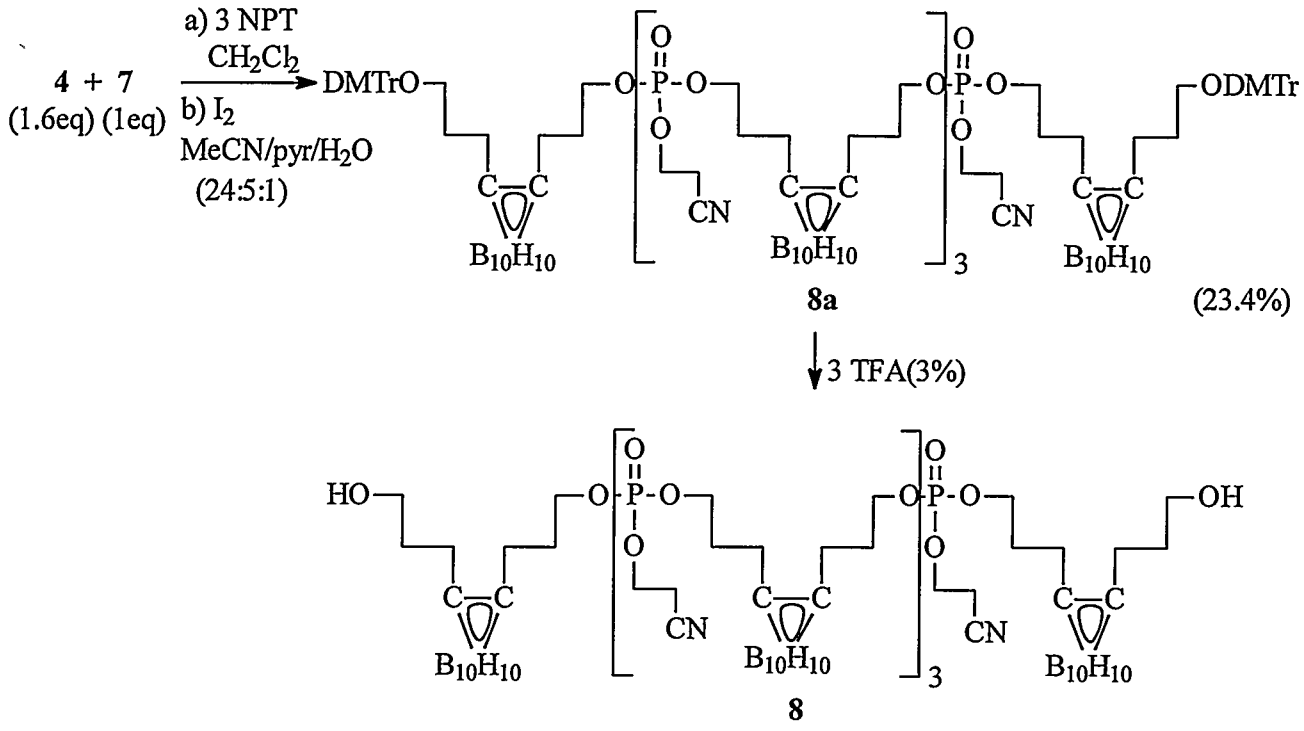



Scheme 2. Step-wise coupling to form trimers.

The second step consists of oxidation of the phosphorous atom from phosphite $P^{(III)}$ to phosphate $P^{(V)}$. The use of bis(trimethylsilyl)peroxide and trimethylsilyl triflate was proposed, but it was found that the oxidation occurs easily with a solution of aqueous iodine in pyridine. This method allows visual observation of the reaction endpoint by loss of iodine coloration. Thus, trimer carboranyl diphosphite **6a** was oxidized by aqueous iodine to the phosphotriester **6**. The compound was isolated in 59.2% yield after purification by column chromatography.

The DMTr protecting group was removed by reaction with 3 equivalents of 3% trifluoroacetic acid. Flash column chromatography gave a 60.6% yield of trimer carboranyl diol **7**.

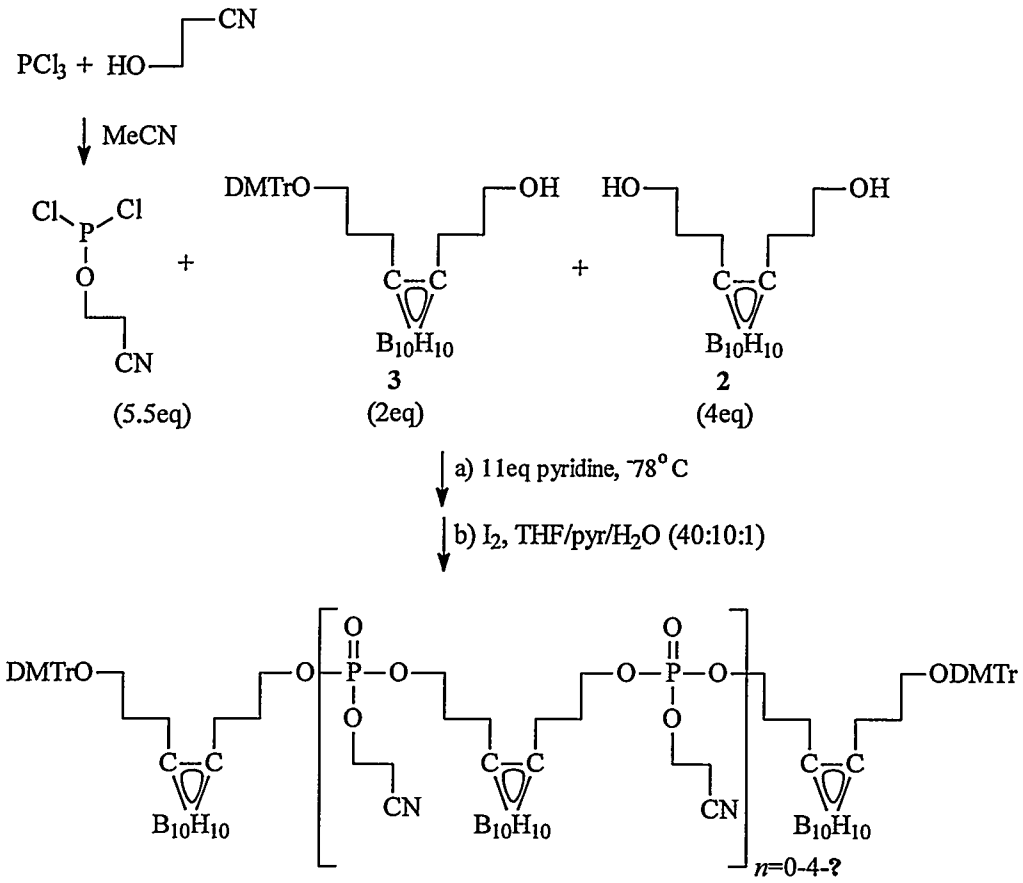
The terminal hydroxyl groups on diol **7** were condensed with carboranyl phosphoramidite **4** as in the preparation of **6a**. Thus, compound **7** and **4** were linked utilizing a phophoramidite coupling reaction in the presence of NPT, followed by oxidation with aqueous iodine to give bis(DMTr) protected pentamer **8a**, in 2 steps in 23.4% overall yield. Removal of the DMTr protecting group with 3% TFA in $CH_2Cl_2$ gives the pentameric carboranyl diol **8**.




Scheme 3. Step-wise coupling to boron-rich oligophosphate diesters.

Ongoing research by Hawthorne, et al is aimed at generating longer trailers by a similar route. For example, coupling of a free diol (e.g. pentamer 8) with 2 equivalents of phosphoramidite monomer 4 in the presence of NPT followed by oxidation with aqueous iodine and DMTr removal with 3% TFA, forms the septamer carboranyl diol.

The above approach develops precisely tailored oligomers. The resulting discrete species are needed to determine the relationship between biological properties and chain length. If it is ultimately determined that chain length is not critical for biological effectiveness, an alternate approach leading to statistical mixtures of chain lengths could significantly reduce the number of reaction steps required. To demonstrate the feasibility of this approach, an adaptation of the condensation of nucleosides by Letsinger was used.[11] A phosphite coupling reagent, 2-cyanoethyl dichlorophosphite, was synthesized from the reaction of excess phosphorous trichloride and 3-hydroxypropionitrile. An excess of this coupling reagent, 5.5 equivalents, was treated with 4 equivalents of CB diol 2 and 2 equivalents of DMTr-mono-protected carborane 3, in the presence of 11 equivalents pyridine. The phosphite groups were then oxidized with aqueous iodine to form DMTr-protected oligomer phosphate diesters. A TLC of the reaction mixture showed 5-6 distinct spots.




Scheme 4. Direct condensation reaction of dichlorophosphite coupling reagent with CB diol 2 and capping unit mono-DMTr-carborane 3.

## References


1 (a) Hawthorne, M. F. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 950-984. (b) Soloway, A. H.; Tjarks, W.; Barnum, B. A.; Rong, F.-G.; Barth, R. F.; Codogni, I. M.; Wilson, J. G. *Chem. Rev.* **1998**,*98*, 1515-1562.

2. (a) Javid M.; Brownell, G. L.; Sweet, W. H. *J. Clin. Invest.* **1952**, *31*, 604. (b) Tolpin, E. I.; Wellum, G. R.; Dohan, F. C., Jr.; Kornblith, P. L.; Zamenhof, R. G. *Oncology* **1975**, *32*, 223. (c) Kobayashi, T.; Kanda, K. *Radiat. Res.* **1982**, *91*, 77-94. (d) Gabel, D.; Foster F.; Fairchild, R. G. *Radiat. Res.*, **1987**, *111*, 14-15.

3. Kane, R. R.; Guan, L; Shelly, K.; Hawthorne, M. F. In *Advances in Neutron Capture Therapy*; Larsson, B., Crawford, J., Weinreich, R., Eds.; Elsevier: Amsterdam, 1997; Vol. II, Chemistry and Biology, p 362-367.

4. Nakanishi, A.; Guan, L; Kane, R. R.; Kasamatsu, H.; Hawthorne, M. F. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 238.

5 Kane, R. R.; Drechsel, K.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1993**, *115*, 8853.

6 Wright. P; Lloyd, D.; Rapp, W.; Andrus, A. *Tetrahedron Lett.* **1993**, *34*, 3373-3376.

7 (a) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862. (b) Sonveaux. E. *Protocols for Oligonucleotide Conjugates: Synthesis and Analytical Techniques.* Agrawal, S.; Ed.; Humana Press: Ottowa, **1993**, *26.*

8 (a) Sinha, M. D.; Biernat, J.; McManus, J.; Köster. H. *Nucleic Acids Res.*, **1984**, *12*,4539-4557. (b) Sinha, N. D.; Birna, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846. (c) Lyttle, M. H.; Wright, P. B.; Sinha, N. D.; Bain, J. D. Chamberlin, A. R. *J. Org. Chem.* **1991**, 4608-4615.

9. Tanimura, H.; Maeda, M.; Fukazawa, T.; Sekine, M.; Hata, T. *Nucleic Acids Res.*, **1989**, *17*, 8135-8147

10 Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1997**, *119*, 11758, and references therein.

11 Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. *J. Am. Chem. Soc.* **1975**, *97*, 3278-3279.