

## From boron analogues of amino acids to boronated DNA: potential new pharmaceuticals and neutron capture agents

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**Abstract** - We have been extensively involved in the synthesis of isoelectronic and isostructural boron analogues of the  $\alpha$ -amino acids. These have ranged from simple glycine analogues such as  $\text{H}_3\text{NBH}_2\text{COOH}$  and  $\text{Me}_2\text{NHBH}_2\text{COOH}$  to alanine analogues. A diverse variety of analogues, including precursors and derivatives (such as peptides) have expressed potent pharmacological activity, including anticancer, antiinflammatory, analgesic, and hypolipidemic activity in animal model studies and *in vitro* cell cultures.

More recently we have been involved in boronated nucleosides and (oligo)nucleotides. Synthetic oligonucleotide analogues or "antisense" agents can interact with a complementary nucleic acid sequence thereby blocking the biological effect of the target sequence. Toward this goal, we have prepared nucleosides which have been boronated on the pyrimidine and purine bases. We have also established that an entirely new class of nucleic acid derivatives is feasible in which one of the non-bridging oxygens in the internucleotide phosphodiester linkage can be replaced by an isoelectronic analogue, the borane group, ( $\text{BH}_3$ ). The boronated oligonucleotides can be viewed as hybrids of the normal oxygen oligonucleotides and the methylphosphonate oligonucleotides.

### INTRODUCTION

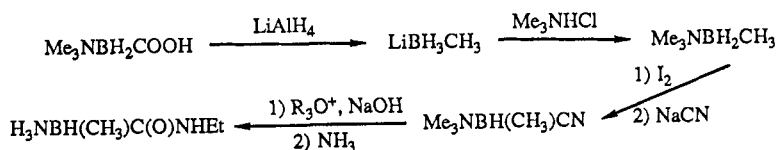
We have been extensively involved in the synthesis and pharmacological properties of boron analogues of biologically important molecules. Many of these are isoelectronic and isostructural with their naturally occurring carbon counterparts. Based upon four-coordinate boron, they generally possess sufficient hydrolytic and oxidative stability to be used in biological studies. These may be of use to probe fundamental biochemical events at the molecular level as well as in providing entirely new classes of compounds of potential medicinal value. They may also prove valuable in Boron Neutron Capture Therapy.

### AMINO ACIDS ANALOGUES

The amino acid analogues (ref. 1) have ranged from simple glycine and N-methyl substituted glycines such as  $\text{H}_3\text{NBH}_2\text{CO}_2\text{H}$  and  $\text{Me}_3\text{NBH}_2\text{CO}_2\text{H}$  to analogues of more complex amino acids such as alanine (amide),  $\text{H}_3\text{NB}(\text{Me})\text{HC}(\text{O})\text{NEtH}$  (ref. 2). The latter has been prepared from  $\text{Me}_3\text{NBH}_2\text{CO}_2\text{H}$  in a multistep process as outlined below.

Scheme 1

Synthesis of Boron Analog of Alanine



Other amides of common amino acids, likewise can be prepared (from the known  $\text{Me}_3\text{NBH}_2\text{R}'$ ) and these derivatives, as well as many other related precursors and derivatives of the simple glycines including peptides (ref. 3) are now commercially available (ref. 4).

A variety of potent pharmacological activity has been expressed (in model studies) by these amino acid analogues such as hypolipidemic, anticancer, and antiinflammatory activity (ref. 1).

In addition to amino acid analogues, we have synthesized analogues of other biomolecules such as choline and thiocholine derivatives, and analogues of potent therapeutic agents such as phosphonoacetate. Recently we have embarked on a new area of boronated nucleic acids which is described below.

### BORONATED NUCLEOSIDES AND NUCLEOTIDES

The synthesis of new classes of nucleic acid components has been stimulated by antiviral and antitumor activity associated with a wide variety of structurally divergent modified nucleic acids. In addition to therapeutic potential, modified nucleosides, mononucleotides and oligonucleotides may be useful as diagnostic agents and as probes for studying fundamental biological processes at the molecular level. For example, modified nucleoside mono-, di-, or triphosphates can act as substrates for various enzymes and interfere with their natural function. Modified oligonucleotides, "antisense" agents, may be used to specifically block gene expression and therefore may be useful as inhibitors of viral growth as well as other unwanted gene expression (ref.5) e.g. expression of oncogenes.

Of considerable added importance is the potential for boronated nucleic acids to localize in the nucleus and prove very effective in boron neutron capture therapy of cancer. Specifically designed boronated oligonucleotides may also function as antisense molecules and inhibit or completely block expression of oncogenes.

In order to be useful as antisense inhibitors, the nucleic acid analogs should possess the following properties (ref.6):

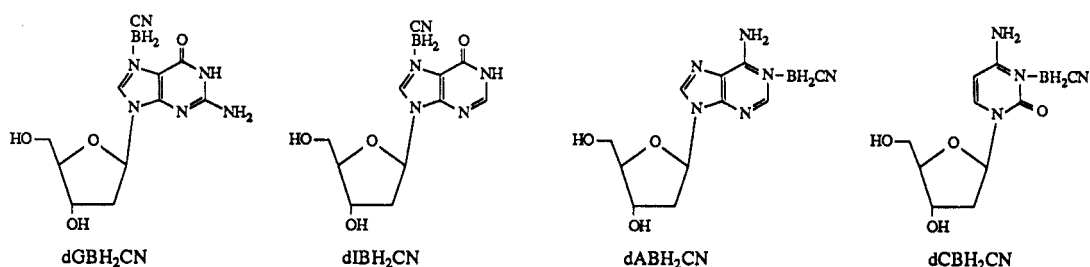
- Be sequence specific and able to form normal Watson-Crick base pairs.
- Be able to penetrate cell membranes.
- Be resistant to nucleases.

A number of modified oligonucleotides (ref.6) have been prepared to possess these properties although to various degrees. Some of the important modifications which have been incorporated into oligonucleotide backbone are phosphotriesters (-POR), methylphosphonates (-PCH<sub>3</sub>), phosphorothioates (-P=S), phosphorodithioates (S=P-S) and phosphoramidates (-PNR<sub>2</sub>). These analogs have been shown to inhibit various viruses e.g. HIV, Herpes Simplex viruses, etc., and expression of oncogenes. Many homo sequences of modified oligomers are also very effective in inhibiting viral growth indicating that in addition to the antisense mechanism, other mechanisms may also be operational. Boron containing oligonucleotides may also exhibit inhibitory effects by one or more of these mechanisms.

We have recently synthesized two new classes of nucleic acids which have been modified to contain boron.

#### Class I nucleosides with boronation of the base

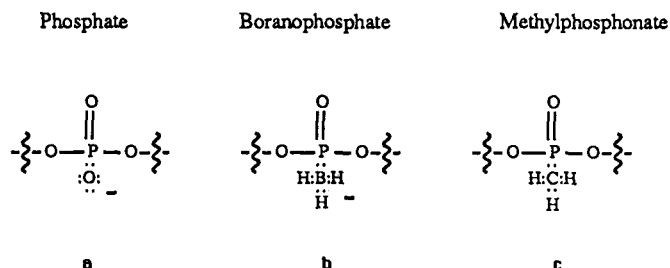
Class I is comprised of nucleosides which have been modified on the base to form cyanoborane adducts, [Figure 1](#). These modified nucleosides were prepared by the reaction of 3',5'-O-triisopropylsilyl-2'-deoxynucleosides with Ph<sub>3</sub>PBH<sub>2</sub>CN followed by deprotection. (ref.7) The reaction resulted in N<sup>7</sup>-cyanoborane adducts of dG and dI, a N<sup>1</sup>-adduct of dA and a N<sup>3</sup>-adduct of dC. The coordination site in dG and dI is away from the sites required for normal Watson-Crick base pairing. <sup>1</sup>H nmr studies indeed show that dG-N<sup>7</sup>-cyanoborane is able to form base pairs with dC as efficiently as a normal dG.



**Figure 1** Base Boronated Nucleosides

### Class II oligonucleotides with boronation on the backbone

We have now prepared examples of oligonucleotides with a boronated internucleotide backbone: the boranophosphates (Figure 2).



**Figure 2** Boronated Oligonucleotides: Comparison with normal nucleotides and methylphosphonates

The boranophosphate species is very closely related to the normal oxygen oligonucleotides (O-oligos, Fig. 2a) and the oligonucleotide methylphosphonates (Fig. 2c).

The borane (BH<sub>3</sub>) group is isoelectronic with oxygen and the boranophosphate internucleotide group is negatively charged like the normal O-oligos. The borane group is also isoelectronic, as well as isostructural, with the oligonucleotide methylphosphonates which are nuclease resistant. Thus, the boranophosphate oligo may represent an important and perhaps ideal "antisense" species in that the nucleotide bases are unaltered (and thus should maintain binding specificity) while the backbone remains negatively charged (and thus should be water soluble) like the O-oligo. Since BH<sub>3</sub> is much more hydrophobic than oxygen, it may impart a greater membrane permeability than the O-oligo, yet maintain nuclease resistance like the methylphosphonates. Although compounds containing boron-hydride bonds are susceptible to hydrolysis, the B-H bond in boranophosphates possesses unusual hydrolytic stability, as described below.

These oligonucleotides were prepared by the phosphite-triester method except the oxidation step with I<sub>2</sub>/H<sub>2</sub>O was replaced by reaction with Me<sub>2</sub>S·BH<sub>3</sub>, Scheme 2. The backbone protecting group and the 3'-acetate were removed by treatment with conc. NH<sub>4</sub>OH. The boranophosphate backbone is very stable in both acidic and basic medium. Thus, treatment of **1** with conc. NH<sub>4</sub>OH at 55°C does not result in any degradation of the dimer (except the removal of protecting groups).

Similarly, in acid the dimer is fairly stable. In 1N HCl:MeOH (1:1), <10% of the boranophosphate group was hydrolyzed to normal phosphate. In addition, it removed the 3'-acetate group. Since, these conditions were very drastic, we expect that under in vivo conditions or solid phase synthesis conditions, the boranophosphate group should be stable.

The boranophosphate group is also very resistant to cleavage by exonucleases. (Snake venom and calf spleen phosphodiesterases). Thus, under conditions where normal TpT dimer is > 95% cleaved, the boronated dimer **2** is >92% intact.

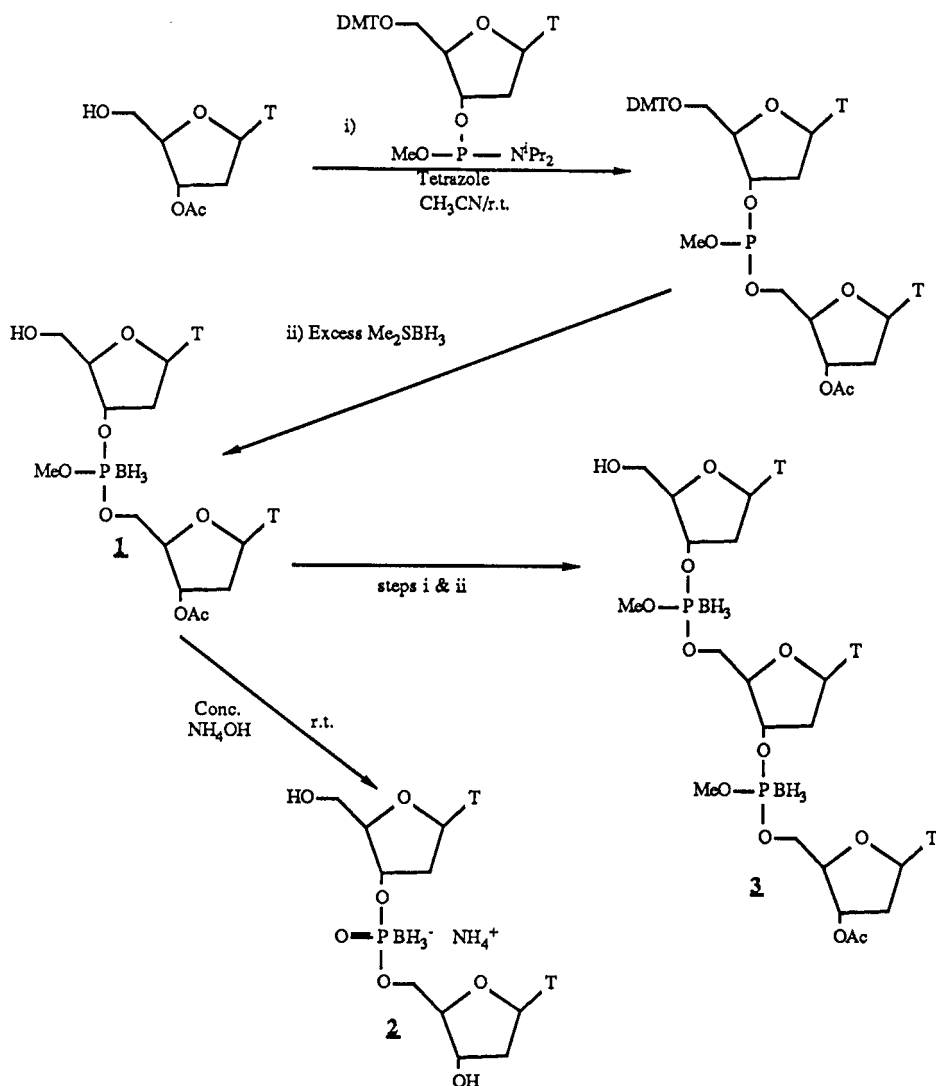
Further, we have also synthesized a trinucleotide, by repeating the steps 1 and 2 shown in Scheme 2. Synthesis of the trimer indicates that the boranophosphate backbone is compatible with reagents needed for chain elongation. The work described so far involves only thymidine. Synthesis of boronated oligonucleotides with other bases is in progress.

Potent biological activity has been found for these new species. The cytotoxicity of a number of boronated nucleosides and nucleotides in murine and human tumor cell lines has been determined. Significant activity has been observed. For example, in the human colorectal adenocarcinoma test, compound **2** in Scheme 2 gave a µg/mL ED<sub>50</sub> value of 0.875 compared to the standard commercial drug, 5-Fluorouracil of 3.09.

Antiinflammatory and hypolipidemic activity have also been expressed. For example, dC·BH<sub>2</sub>CN, shows a 52% inhibition of inflammation (in mice) at a dose of 8 mg/kg/day compared to the standard phenylbutazone at 47%. In cholesterol inhibition in mice, dG·BH<sub>2</sub>CN shows a lowering of serum cholesterol by 44% at a dose of 8 mg/kg/day after 16 days whereas the commercial drug clofibrate lowers serum cholesterol by 13% at a dose of 130 mg/kg/day.

These compounds are undergoing study for use in boron neutron capture therapy. In a preliminary *in vivo* experiment (mouse, Ehrlich Ascites Carcinoma) with the radiolabeled [ $^{14}\text{C}$ ] 2'-deoxycytidine- $^3\text{N}$ -cyanoborane, after 2 hrs, a tumor/blood ratio of 4.2 was observed and after 4 hrs, 8.5. Further testing of these species both *in vitro* and *in vivo* are underway.

**Scheme 2** Synthesis of Backbone Boronated Oligonucleotides



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