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Synthesis and Hybridization Study of a Boranophosphate-Linked Oligothymidine Deoxynucleotide

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Abstract: A T_{15} oligodeoxynucleotide (ODN) fully substituted with a diastereomeric mixture of boranophosphate linkages was synthesized through silylation of its hydrogen phosphonate precursor on solid support followed by reaction with borane. The hybridization properties of this ODN with complementary RNA and DNA were determined by T_m analysis. This analog does not hybridize to any significant extent under the conditions tested.
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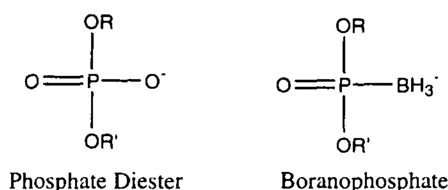
The boranophosphate internucleotidic linkage is an intriguing hydrolytically stable analog of the phosphodiester connection (Figure 1). Isoelectronic to phosphate diester and isosteric to methyl phosphonate, the boranophosphate linkage was first synthesized in dinucleotides.[1] Recently, a 14mer ODN containing a single incorporation of one enantiomer of boranophosphate was synthesized enzymatically.[2] This ODN bound to a complementary DNA molecule with slightly poorer binding affinity relative to an unmodified control.

Figure 1

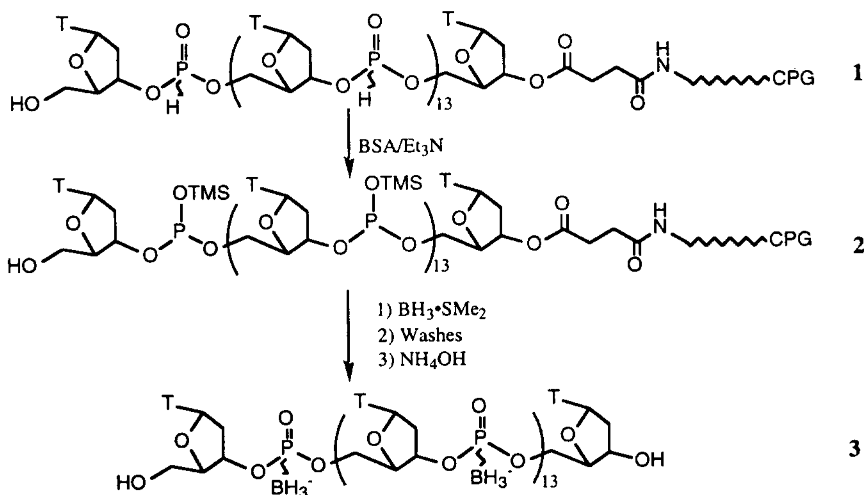


Phosphate Diester   Boranophosphate

Chemically synthesized ODNs bearing this linkage at all positions are of interest in the field of sequence specific inhibition of gene expression by an antisense mechanism.[3] Published synthetic methodology is inadequate for the synthesis of boranophosphate ODNs which are long enough to determine hybridization properties to complementary sequences.[4] Herein, we report the chemical synthesis of a T_{15} ODN fully substituted with boranophosphate in place of the normal phosphate diester linkage and the ability of this diastereomeric mixture to hybridize to RNA and DNA targets has been determined.[5]

The hydrogen phosphonate intermediate has been used as a convenient precursor for a number of ODN analogs including phosphorothioates,[6a] phosphoramidates,[6b] and hydroxymethyl phosphonates.[6c] The boranophosphate moiety was cleanly synthesized from the hydrogen phosphonate intermediate by *in situ* formation of silyloxy phosphite followed by reaction with $BH_3{\cdot}SMe$ (Scheme 1). The boranophosphate formation step was accomplished while the ODN was attached to the controlled pore glass solid support (CPG).

A T_{15} sequence bearing H-phosphonate linkages was synthesized using standard H-phosphonate chemistry.[7] The CPG-bound T_{15} ODN was treated with 1 M bistrimethylsilylacetamide (BSA)/0.5 M triethylamine (TEA) in dry THF for 30 min.[8] followed by 0.2 M BH_3•SMe in dry THF for 5 min.[9] After successive washings wi*h pyridine, MeOH, CH_2Cl_2, and ether, the solid support was treated with concentrated NH_4OH to release the ODN .



Scheme 1

Polyacrylamide gel electrophoresis (PAGE) analysis of the crude ODN showed one major band with a similar mobility to a T_{15} phosphate diester control. After PAGE purification, the ODN was analyzed by ^{31}P NMR, UV, and electrospray mass spectroscopy. Boranophosphate has a unique chemical shift in ^{31}P NMR (~93 ppm) compared to phosphate diester (~0 ppm) and phosphite (~145 ppm).[10] The ^{31}P NMR spectrum of **3** in D_2O is shown below (Figure 2) confirmed the sole presence of boranophosphate.

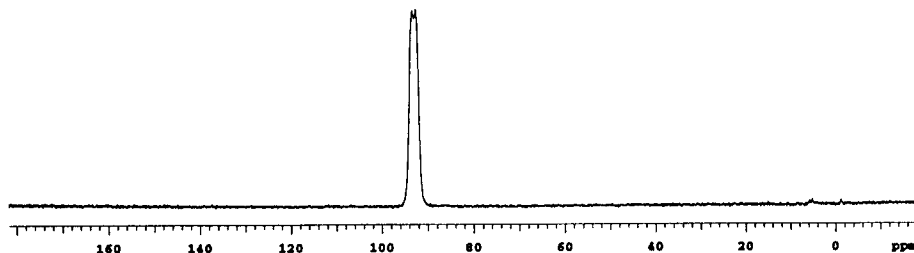


Figure 2

Electrospray mass spectroscopy analysis of **3** showed a MW of 4469.9 (theoretical value of 4469.0) demonstrating the lack of borane reaction on the thymine rings. The UV absorbance spectrum of the T_{15}

boranophosphate and the T_{15} phosphate diester ODN showed λ_{max}'s at 266 nm further supporting the conclusion that the thymine heterocycles are unaltered.

The hybridization properties of the boranophosphate ODN **3** with both complementary A_{15} RNA and dA_{15} DNA were determined by thermal denaturation studies (T_m). When hybridized with A_{15} RNA, the T_{15} boranophosphate ODN **3** displayed nondetectable binding at low ionic strength (0.1 M KCl, 0.1 M pH 7 phosphate buffer, Figure 3, curve b); however, the binding curve at a higher ionic strength (1 M KCl, 0.1 M pH 7 phosphate buffer) suggests that hybridization began to occur below 25°C (curve a). In contrast, the T_{15} phosphate diester control displayed T_m's of 31.5°C and 45.5°C, respectively (curves c and d). When hybridized with dA_{15} DNA, the T_{15} boranophosphate exhibited similar binding patterns (data not shown). In 0.1 M KCl with 0.1 M pH 7 phosphate buffer, no detectable binding was observed; while at the higher ionic strength condition (1 M KCl) partial hybridization began to be observed below 25°C. The control T_{15} diester ODN displayed T_m's of 37.0°C and 51°C, respectively, for the DNA target.

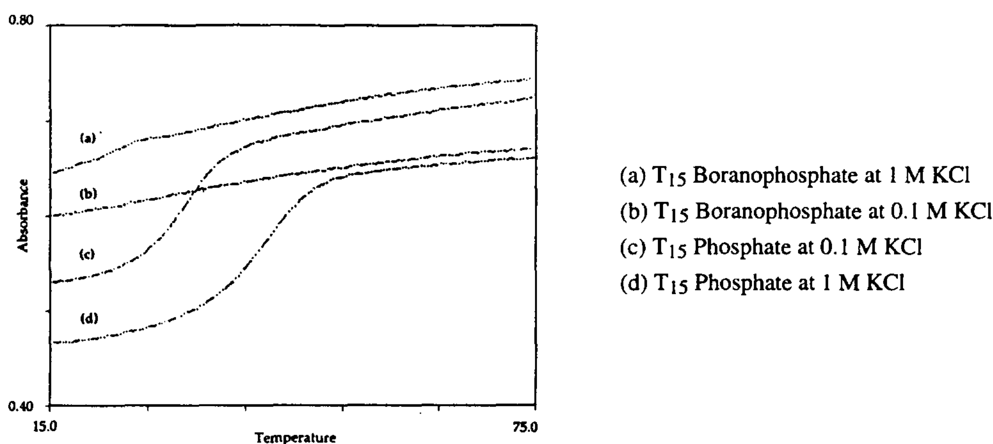


Figure 3. Binding Curves of T_{15} Phosphate Diester and T_{15} Boranophosphate with A_{15} RNA.

The oligothymidine ODN's binding property with DNA and RNA is a limited sequence evaluation of boranophosphate ODNs. However, prior work with the isosteric methylphosphonate ODN analog suggests that the oligothymidine sequence is valid for analog evaluation. An octathymidine ODN has been synthesized as a diastereomeric mixture of methylphosphonate linkages.[11] This diastereomeric mixture bound with a comparable T_m to complementary DNA relative to the native phosphodiester.[11] Our study demonstrates that the binding affinity of the diastereomeric mixture of the boranophosphate-linked oligothymidine ODN with complementary RNA and DNA is much poorer than the native phosphodiester ODN control. Consequently, diastereomeric mixtures of boranophosphate are unlikely to be useful replacements for phosphate diesters in antisense research.[3] The uses of the diastereomerically pure boranophosphates which are attainable from enzymatic methods[2] and potentially from stereocontrolled chemical synthesis, remain an open question.

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