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Synthesis of Mixed Sequence Borane Phosphonate DNA

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For some time now,[1] oligodeoxyribonucleotides (ODNs) bearing internucleotide borane phosphonate linkages have been of considerable interest for applications in diagnostic and therapeutic areas because they mimic natural DNA in various biological processes.[1−5] The problem with this analogue is the lack of high yielding, chemical methods for its synthesis. In this paper, we report a new strategy that generates mixed sequence borane phosphonate DNA of high yield and purity.

To date, the most successful synthesis approach has been conversion of deoxyoligonucleotide H-phosphonates, via silylation followed by boronation, to oligomers having exclusively borane phosphonate internucleotide linkages.[2−5] Results with unprotected bases suggest that 10mers can be prepared with maximum yields of 20−30%.[5] Recently, an alternative method,[6] featuring mononucleotide borane phosphonates and a phosphotriester strategy, has been used to prepare borane phosphonate dinucleotides having all four bases but with coupling yields from 72 to 92%. Unless improved considerably, these results suggest that neither approach is viable for the preparation of oligomers rapidly in high yields on supports.

To overcome these challenges, we have developed a new strategy for synthesizing borane phosphonate DNA. Previous research has shown that the 5′-dimethoxytrityl group, which transiently protects each synthon during natural DNA chemical synthesis, is incompatible with the preparation of borane phosphonate DNA.[2] This observation forced us to explore alternative strategies. On the basis of earlier research,[7] we prepared 2′-deoxynucleosides having 5′-*O*-[benzhydroxybis(trimethylsilyloxy)]silyl protection and discovered that this silyl ether could be removed under conditions compatible with the synthesis of borane phosphonate DNA. As a result, this group has been substituted for dimethoxytrityl as a transient 5′-protecting group (Figure 1).

Another serious challenge has been protection of the 2′-deoxynucleoside bases. This is because borane reagents reduce the commonly used amide protecting groups to *N*-alkyl or aryl exocyclic amines or form stable, irreversible borane adducts with these bases. This problem has been solved by using a relatively new protecting group strategy.[8] Thus for adenine and cytosine, the exocyclic amines are protected with dimethoxytrityl and trimethoxytrityl, respectively. Protection of guanine with *N*2-(9-fluorenylmethoxycarbonyl) is based upon our observations that certain carbamates are resistant to reduction with borane. Similarly with thymine, the use of anisoyl on N3 eliminates possible reduction by borane reagents[2] and also protects from N-methylation of thymine via the methyl phosphate internucleotide linkage.

Using these appropriately protected 2′-deoxynucleoside phosphoramidites (Figure 1), a new, high yielding synthesis cycle has been developed.[9] Starting with a 2′-deoxynucleoside attached to polystyrene, the first step is condensation with **1a**−**d** in anhydrous acetonitrile and tetrazole to generate a family of dimers having a phosphite triester internucleotide linkage. These dimers are then reacted with either THF·BH_3 or a peroxyanion solution.[8] Removal of 5′-silyl protection with triethylammonium hydrogen fluoride generates a family of dinucleotides having any of the four bases and either a P−IV phosphonium borane adduct or a phosphate triester linkage. These dimers can then be extended using the same repetitive cycle to generate an ODN of the appropriate length.

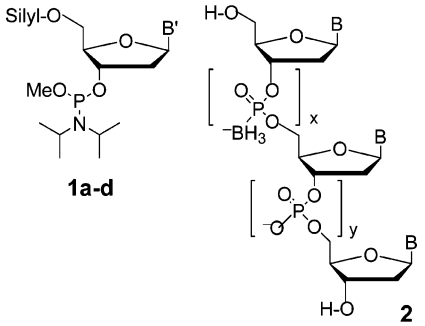

Figure 1. 5′-*O*-Silyl-2′-deoxynucleoside-3′-phosphoramidites and borane phosphonate ODNs. Silyl = benzhydroxybis(trimethoxysilyloxy)silyl. B′ = protected base. B = cytosine, thymine, adenine, and guanine. X,Y = combinations of phosphate and borane phosphonate linkages.

Table 1. Mass Spectrum Analysis of Oligodeoxynucleotides

		molecular weight	
no.	ODN[a]	calculated	observed
3	d[($T_pT_pT_b$)$_4T_pT$]	4185.7	4183.4[b]
4	d[(T_bT_p)$_6T_bT$]	4179.8	4177.5[b]
5	d[($G_pT_pG_bT_pG_pT_b$)$_2G_pT$]	4360.8	4360.7[b]
6	d[($G_bT_pG_bT_p$)$_3G_bT$]	4354.9	4355.2[b]
7	d(T_b)$_9$T	2960.8	2954.5[c]
8	d[(A_bT_b)$_4A_bT$]	3005.9	3000.7[c]
9	d[(C_bT_b)$_4C_bT$]	2885.8	2881.5[c]
10	d($T_bC_bT_bT_bA_bC_bT_bG_bA_bT$)	2973.9	2967.5[c]

[a] p = phosphate; b = borane phosphonate. [b] Perseptive Biosystems Voyager Biospectrometry Workstation using a previously published procedure.[8] [c] HPLC-ESI-Q-TOF-MS Instrument System.

Protecting groups are removed sequentially. Initially and with the ODN attached to the support, 80% acetic acid is used to eliminate trityl groups from adenine and cytosine[8] (the P−IV borane adduct is compatible with acetic acid). Next the oligomer is treated with a dithiolate[10] to remove internucleotide methyl protection. Finally, ammonium hydroxide eliminates carbamate and anisoyl groups from guanine and thymine, respectively, and generates **2**. Purification is by reverse phase HPLC. A typical result for a 10mer (compound **10**, Table 1) having all four bases and borane phosphonate internucleotide linkages is shown in Figure 2 (total reaction mixture). The major peak (excluding the first, anisic acid peak) is the product (99% coupling yield, isolated yield 88%). As expected

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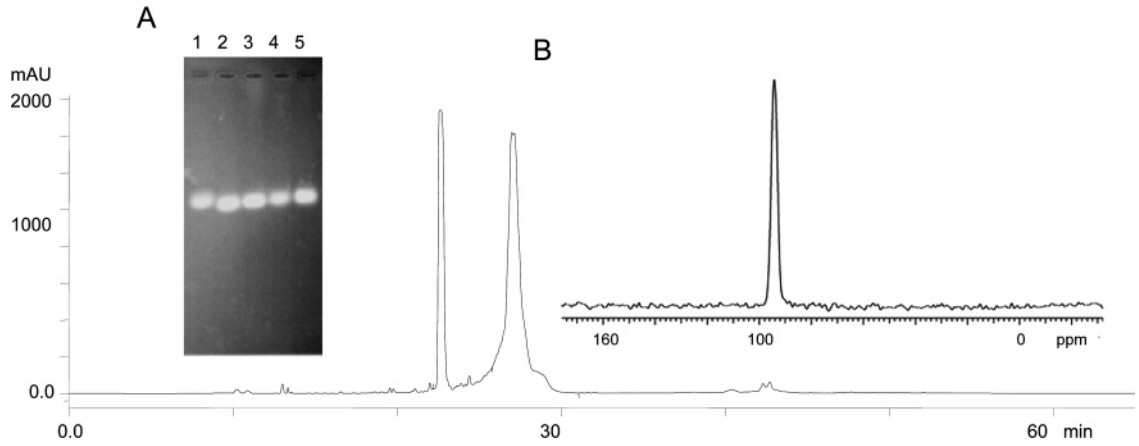

Figure 2. Reverse phase HPLC analysis of the total, crude reaction mixture from the synthesis of compound **10**. Inset A: Gel electrophoresis results from total, crude reaction mixtures. Lanes 1−5, **d(T_pT_p)$_4T_pT$**, **7**, **8**, **9**, and **10**, respectively. Inset B: Phosphorus NMR of compound **10**.

from many P-chiral centers and the resulting large number of stereoisomers, this peak is broad.

Numerous oligomers have been prepared using this synthesis strategy with isolated yields of 70−89%. Table 1 lists mass data for examples having combinations of 2′-deoxynucleoside bases and internucleotide phosphate and borane phosphonate linkages. The observed masses for all ODNs correspond to those as calculated. Similarly, phosphorus NMR analyses display a broad signal at 96 ppm (borane phosphonate) and a sharp peak at −2 ppm when phosphate is part of the backbone. By phosphorus NMR, when all internucleotide linkages are borane phosphonate (Table 1, compound **10**), phosphate cannot be detected (inset B, Figure 2). ^{11}B NMR spectra for all oligomers consist of a broad signal at −40 ppm (characteristic of the borane phosphonate linkage). Neither the mass spectral nor boron NMR data suggest the presence of boronated bases or sugars. When total, unpurified reaction mixtures are analyzed by gel electrophoresis, only one major band which corresponds to the product is observed (inset A, Figure 2). As expected from previous research,[1−6] these borane phosphonate ODNs are resistant to degradation by exonucleases and DNase I.

Now that a chemistry has been developed for synthesizing borane phosphonate DNA in high yield and having any combination of the four common bases as well as both phosphate and borane phosphonate internucleotide linkages, many biological and biochemical studies are possible. These include the use of borane phosphonate ODNs for various diagnostic and therapeutic applications as well as the further development of borane phosphonate oligomers having, among others, phosphorothioate, phosphorodithioate, and methyl phosphonate internucleotide linkages.

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Supporting Information Available: Procedures for the synthesis of **1a**−**d** and a table outlining the steps for one synthesis cycle. Also included are several HPLC profiles from the synthesis of various ODNs, a table summarizing both the total yields and coupling yields for various ODNs, and gel electrophoresis analysis of borane phosphonate ODNs following degradation with SVP and DNase I. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) For deoxyoligonucleotide synthesis, a modified Applied Biosystems Model 392 instrument is used. Each synthesis cycle consists of the following reactions with appropriate washes: 65 s coupling with phosphoramidite (0.05 M) and tetrazole (0.22 M) in acetonitrile, conversion of the phosphite triester to the borane adduct using 25 mM BH_3·THF in THF or to the phosphate triester with an aqueous peroxyanion buffer[8] and 70 s 5′-deprotection with 1.1 M HF/1.1 M triethylamine/0.02 M *N*-methyldiethanolamine in *N*,*N*-dimethylformamide (pH 9). Syntheses are completed on 3′-polystyrene (LV-PS-200 nmol) from Glen Research.
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