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Solid-phase Synthesis of Oligodeoxyribonucleoside Boranophosphates by the Boranophosphotriester Method

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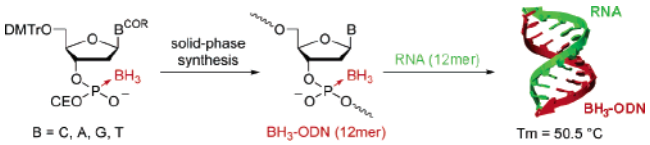

Oligodeoxyribonucleoside boranophosphates (BH_3−ODNs), containing four kinds of nucleobases, were synthesized by the solid-phase boranophosphotriester method. The 2′-deoxyribonucleoside 3′-boranophosphate monomers having 2-cyanoethyl (CE) groups as the phosphorus protecting groups were synthesized in good yields. A new condensing reagent, 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate, was found to be highly effective for the condensation reaction on the solid support. We also found that 1,8-bis(*N*,*N*-dimethylamino)naphthalene could accelerate the condensation reaction without causing β-elimination of the CE groups from the boranophosphate triesters. The internucleotidic CE groups were selectively removed by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under anhydrous conditions. The acetylation of the terminal 5′-hydroxy group was found to be effective to suppress the decomposition of the BH_3−ODNs during the DBU treatment on the solid support. Under optimized conditions for the solid-phase synthesis and the deprotection reactions, BH_3−ODNs (4mers and 12mers) containing four kinds of nucleobases were synthesized in good yields. The hybridization properties of the BH_3−ODN 12mers with the complementary native DNAs and RNAs were determined by the thermal denaturing studies. In contrast to the low thermal melting (Tm) value of the duplex composed of $T(_{PB}T)_{11}$ and native dA_{12} (12.8 °C), the duplex consisting of $d(C_{PB}A_{PB}G_{PB}T)_3$ and $d(ACTG)_3$ showed a higher Tm value (44.7 °C) under high-salt conditions. Furthermore, $d(C_{PB}A_{PB}G_{PB}T)_3$ formed a more stable duplex with the complementary RNA, $r(ACUG)_3$ with a Tm value of 50.5 °C. Thus, we first demonstrated that the binding affinity of BH_3−ODN to a complementary DNA or RNA is dramatically increased, owing to the inclusion of the four kinds of nucleobases.

Introduction

Oligodeoxyribonucleoside boranophosphate (BH_3−ODN), which is a new class of boron-modified nucleic acids, is regarded as a potentially useful antisense molecule.[1] This DNA analogue was first reported in 1990 by Shaw et al.,[2] and since then, BH_3−ODN has been attempted to be synthesized by many researchers so far.[3−5] The methods previously reported for the synthesis of BH_3−ODN are either enzymatic or chemical. In an enzymatic approach, only BH_3−ODN with a *S*p configuration can be synthesized because of the substrate specificity of the enzyme (T7 polymerase).[6,7] Furthermore, the enzymatic method is obviously not suitable for large-scale synthesis. On the other hand, the chemical synthesis of BH_3−ODN is accomplished by way of phosphite intermediates, which are obtained by the phosphoramidite[2] or *H*-phosphonate[3−5] method, followed by boronation. However, undesirable side reactions occur on the

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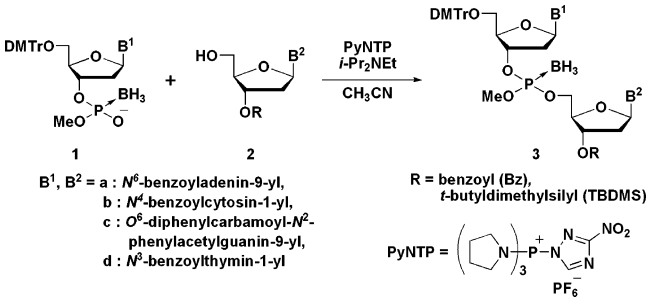
SCHEME 1



base moieties in the boronation step.[8,9] Therefore, these methods are applicable only to thymine derivatives, which are less reactive to borane reagents. On the other hand, we have recently reported a novel strategy for the chemical synthesis of BH_3−ODN in solution.[10,11] In this method, we used pre-boronated phosphorylating reagents to introduce BH_3 groups into a DNA backbone to completely avoid undesirable side reactions caused by borane reagents. In this paper, we wish to report the application of the boranophosphotriester method to the solid-phase synthesis of BH_3−ODNs. The physicochemical properties of the BH_3−ODNs, including four kinds of nucleobases thus synthesized, are also described.

Results and Discussion

Synthesis of a Novel Boranophosphorylating Reagent and Monomer Units. In the previous paper, we reported a boranophosphotriester method for the synthesis of dinucleoside boranophosphates in solution (Scheme 1).[11] In the method, a methyl group was employed as a phosphorus protecting group in the monomer unit **1**. The monomer **1** was allowed to condense with the nucleoside **2** in the presence of 3-nitro-1,2,4-triazol-1-yl-tris(pyrrolidin-1-yl)phosphonium hexafluorophosphate (PyNTP) as a condensing reagent;[12] the condensation proceeded quickly in the presence of a strong base such as diisopropylethylamine (*i*-Pr_2NEt).

Upon further investigating in detail the reaction conditions for the condensation previously reported, we found that the condensation reaction promoted by PyNTP proceeded even in the presence of a weak base such as 2,6-lutidine. This result indicates that a 2-cyanoethyl (CE) group, which is cleaved under strong basic conditions, can be used for a phosphorus protecting group instead of a methyl group. A methyl protecting group should be removed prior to cleavage of other base-labile protecting groups,[11] and 2 h are required for complete removal. On the contrary, a CE protecting group is quickly and selectively cleaved under anhydrous basic conditions without affecting other functional groups. Therefore, we synthesized a new CE-protected boranophosphorylating reagent **7** from PCl_3 according to Scheme 2.

The new boranophosphorylating reagent **7** was allowed to react with the properly protected nucleosides **8** in the presence of PyNTP and 2,6-lutidine at room temperature for 1−12 h. The resulting fully protected intermediates **9** were then treated with Et_3N in CH_2Cl_2 at room temperature for 1 h to remove one of the CE groups of **9**. After purification by silica gel column chromatography, the corresponding boranophosphate monomers **10** were obtained in good isolated yields (Table 1).

SCHEME 2

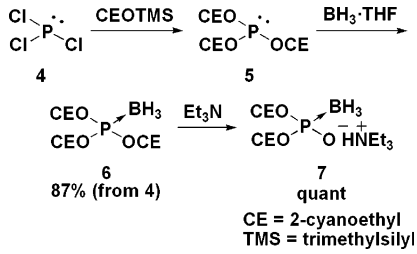
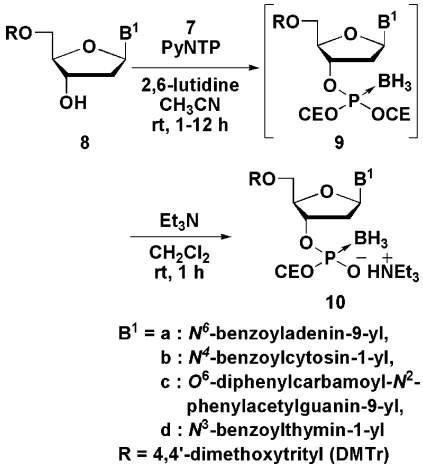


TABLE 1. Synthesis of Fully Base-Protected Monomers



entry	B^1	yield of **10** (%)
1	a	51
2	b	56
3	c	81
4	d	73

Solid-Phase Synthesis of Dinucleoside Boranophosphates. In the next stage, the solid-phase synthesis of dinucleoside boranophosphates was examined. In the present method, a DMTr cation assay could not be used to estimate the yields of the dimers, because the detritylation reaction had to be carried out in the presence of the $DMTr^+$ scavenger to avoid the decomposition of the product.[4,10,13] Therefore, the yields of the dimers were estimated by HPLC analyses after removal of all of the protecting groups.

The monomer **10** was condensed with N^3-benzoylthymidine anchored to a controlled pore glass (CPG) in the presence of PyNTP and 2,6-lutidine in CH_3CN for 20 min (Scheme 3). After removal of the 5′-*O*-DMTr group by treatment with 3% dichloroacetic acid (DCA) in Et_3SiH−CH_2Cl_2 (1:1, v/v) for 15 s, the dimer **12** on the CPG was then treated with concd NH_3 at 55 °C for 12 h. The HPLC analysis of the reaction mixture indicated that the formation of the deprotected dimer **13** as a major product, but some decomposed products were also observed. A similar result was obtained when NH_3/MeOH was

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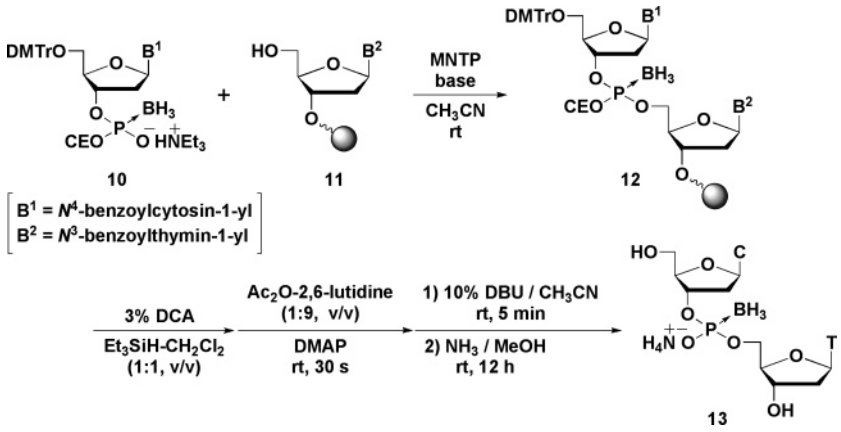
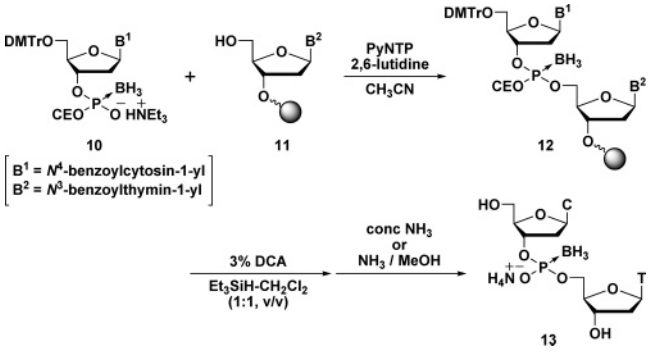
TABLE 2. Solid-Phase Synthesis of $dC_{PB}T$



entry	base	time of condensation	yield of **13** (%)
1	2,6-lutidine (10% in CH_3CN)	5 min	88
2	2,4,6-collidine (10% in CH_3CN)	5 min	90
3	2,4,6-collidine (10% in CH_3CN)	5 min × 2[a]	96
4	DMAN (0.15 M in CH_3CN)	1 min	92
5	DMAN (0.15 M in CH_3CN)	5 min	93
6	DMAN (0.15 M in CH_3CN)	10 min	95
7	DMAN (0.15 M in CH_3CN)	20 min	96

[a] A double coupling protocol.

SCHEME 3



used in the place of concd NH_3. The formation of the byproducts would arise from the decomposition of the dimer caused by the nucleophilic attack of the hydroxide ion or methoxide ion to the electrophilic phosphorus atom of the boranophosphotriester linkage. To suppress these side reactions, the CE group was removed by treatment with 10% 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in CH_3CN under anhydrous conditions prior to the removal of the other base-labile protecting groups. The resulting boranophosphodiester, which was more stable than the corresponding triester, was treated with concd NH_3. As a result, the decomposition of the dimer was almost suppressed, but a small amount of the degradation products was still observed by an HPLC analysis. During the DBU treatment, the free 5′-hydroxy group would be deprotonated to generate the alkoxide, which should readily attack the neighboring phosphorus atom before the elimination of the CE group. Therefore, the free 5′-hydroxy group was acetylated prior to the removal of the CE group. Upon applying this deprotection procedure, the degradation of the dimer was successfully suppressed.

Condensation Reaction on the Solid-Support. Because the deprotection conditions of the dimer on the solid support were established, the reaction conditions for the internucleotidic bond formation were reinvestigated. When the monomer **10** was condensed with **11** in the presence of PyNTP and 2,6-lutidine in CH_3CN for 20 min, the yield of the dimer **13** was estimated to be 78% on the basis of the HPLC analysis of the deprotected products. This yield is apparently insufficient for the synthesis of the longer BH_3−ODNs. Therefore, we decided to apply a more efficient condensing reagent to the present method.[12]

The reactivity of phosphonium-type condensing reagents is known to be affected by the core structure of the compounds.[14] Many types of phosphonium-type condensing reagents have been developed to date. Among these reagents, 2-(benzotriazol-1-yloxy)-1,1-dimethyl-2-pyrrolidin-1-yl-1,3,2-diazaphospholidiniumhexafluorophosphate (BOMP)[14] is the most reactive as a condensing reagent. However, BOMP was unfortunately found to not be effective in the present case because the hydroxybenzotriazole, which is generated during the activation process of the monomer, would not catalyze the boranophosphorylation reaction. Therefore, we tried to use a new condensing reagent involving the same core structure as that of BOMP and 3-nitro-1,2,4-triazol-1-yl group as a leaving group because 3-nitro-1,2,4-triazole, which would be generated from the reagent, is known to be a highly effective nucleophilic catalyst for the boranophosphotriester method.[10] Therefore, the new condensing reagent 1,3-dimethyl-2-(3-nitro-1,2,4-triazol-1-yl)-2-pyrrolidin-1-yl-1,3,2-diazaphospholidinium hexafluorophosphate (MNTP)[12] was applied to the present method.

The condensation reaction using MNTP was found to proceed faster than that using PyNTP on the solid support. For example, the dimer $dC_{PB}T$ **13** was obtained in 88% yield when the condensation was performed for 5 min in the presence of 2,6-lutidine (Table 2, entry 1). Then we focused our attention on the basicity of the coexisting base, because the use of a stronger base is advantageous for the activation of the 5′-hydroxy group

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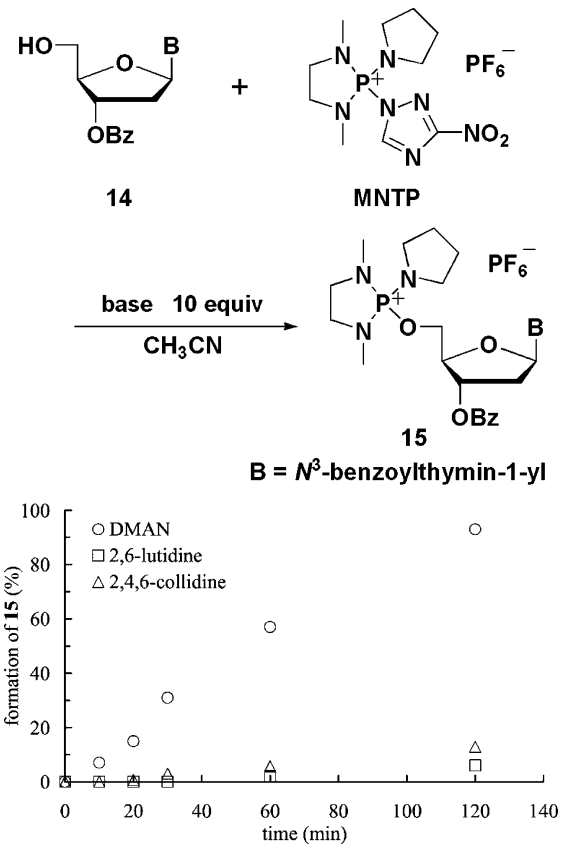

FIGURE 1. Reactions between MNTP and 5′-OH under DMAN, 2,6-lutidine, and 2,4,6-collidine.

of the nucleoside on the solid support, while it is required to avoid the β-elimination of the CE group. 2,4,6-Collidine (pK_a 7.59[15]), which is more basic than 2,6-lutidine (pK_a 6.75[15]), was found to be slightly effective for the acceleration of the condensation reaction (entry 2). A double coupling protocol (5 min × 2) gave a better result (entry 3). On the other hand, 1,8-bis(*N*,*N*-dimethylamino)naphthalene (DMAN, pK_a 12.1[16]) is known as a "proton sponge", which is capable of capturing a dissociable proton. As a result, we expected that DMAN could activate the 5′-hydroxy group of the nucleoside **11** but could not abstract the α-proton of the CE group in **10** and applied DMAN as a coexisting base. Actually, the condensation proceeded very quickly in the presence of DMAN, and the β-elimination of the CE group during the condensation was not observed; the dimer **13** was obtained in 92% yield by a 1-min condensation (entry 4). However, prolonged reactions were not effective to improve the yield of the dimer (entries 5−7). These results indicate that the 5′-hydroxy group, activated by DMAN, would be reacted with MNTP to some extent. To elucidate this undesired reaction, a model reaction of a nucleoside bearing the free 5′-hydroxy group with MNTP in the presence of DMAN was carried out and monitored by ^{31}P NMR spectroscopy (Figure 1). The signal of MNTP (24.9 ppm in CD_3CN) gradually decreased, and a new signal corresponding to the 5′-*O*-modified product **15** (33.2 ppm) was observed. After 2 h, about 90% of the starting nucleoside **14** was converted to **15**. In contrast, the 5′-*O*-modification by MNTP proceeded very slowly and was

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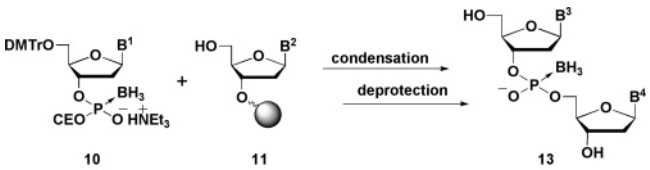
TABLE 3. Solid-Phase Synthesis of Dinucleoside Boranophosphates



B^1 = a : N^6-benzoyladenin-9-yl,
b : N^4-benzoylcytosin-1-yl,
c : O^6-diphenylcarbamoyl-N^2-phenylacetylguanin-9-yl,
d : N^3-benzoylthymin-1-yl
B^2 = d : N^3-benzoylthymin-1-yl

B^3 = e : adenin-9-yl,
f : cytosin-1-yl,
g : guanin-9-yl,
h : thymin-1-yl
B^4 = h : thymin-1-yl

entry	B^1	B^3	base	time of condensation	yield (%)
1	a	e	DMAN[a]	5 min	96
2	a	e	2,4,6-collidine[b]	5 min × 2	95
3	b	f	DMAN[a]	5 min	93
4	b	f	2,4,6-collidine[b]	5 min × 2	96
5	c	g	DMAN[a]	5 min	95
6	c	g	2,4,6-collidine[b]	5 min × 2	94
7	d	h	DMAN[a]	5 min	90
8	d	h	2,4,6-collidine[b]	5 min × 2	96

[a] 0.15 M in CH_3CN. [b] 10% in CH_3CN.

almost negligible within a short reaction time when 2,6-lutidine or 2,4,6-collidine was used. These findings are consistent with the results of the solid-phase synthesis of the dimers.

Next, dinucleoside boranophosphates including four kinds of nucleobases were synthesized on the solid-support (Table 3). In all the cases, the desired dimers were obtained in good yields by using the single coupling protocol with DMAN (5 min, method A) and the double coupling protocol with 2,4,6-collidine (5 min × 2, method B).

Solid-Phase Synthesis of d($C_{PB}A_{PB}G_{PB}T$). To compare the two coupling protocols described above, the solid-phase synthesis of a 4mer, d($C_{PB}A_{PB}G_{PB}T$), was carried out. The chain elongation cycle for the manual solid-phase synthesis is shown in Table 4.

After deprotection of all the protecting groups (NH_3/MeOH, rt, 24 h), the crude products were analyzed by reverse-phase HPLC (Figure 2). The HPLC profiles of the reaction mixtures obtained by using DMAN (Figure 2A) and 2,4,6-collidine (Figure 2B) were quite similar to each other, and for both cases, the yield of the tetramer was estimated to be about 90%.

In the next stage, the purification of d($C_{PB}A_{PB}G_{PB}T$) was attempted. Because the tetramers were obtained as mixtures of diastereomers, it was difficult to purify by reverse-phase HPLC. Therefore, the crude mixture, obtained by the coupling protocol using DMAN, was purified by anion exchange HPLC. After desalting by a C18 Sep-Pak cartridge, d($C_{PB}A_{PB}G_{PB}T$) was obtained in 30% yield (Figure 3). The isolated tetramer was successfully identified by MALDI TOF-MS (calcd for $[M - H]^-$, 1166.3525; found, 1166.3718).

Synthesis of BH_3−ODN 12mers. The solid-phase synthesis of d($C_{PB}A_{PB}G_{PB}T$)$_3$ and T($_{PB}$T)$_{11}$ was then attempted according to the chain elongation cycle described in Table 4 (method A). In these cases, cleavage of the oligomer from the solid support and removal of the base protecting groups were carried out by treatment with concd NH_3 (55 °C, 12 h) instead of using NH_3/MeOH (rt, 24 h), because more drastic conditions are generally required to complete the deprotection of longer oligomers. After deprotection, d($C_{PB}A_{PB}G_{PB}T$)$_3$ and T($_{PB}$T)$_{11}$ were purified by anion-exchange HPLC in a manner similar to the case of a 4mer,

TABLE 4. Chain Elongation Cycle of the Manual Solid-Phase Synthesis

step	manipulation	reagents and solvents	time
1	detritylation	3% DCA in CH_2Cl_2−Et_3SiH (1:1, v/v)	15 s
2	wash	(i) CH_2Cl_2, (ii) CH_3CN	
3	drying		10 min
4	condensation	**10** (0.1 M), MNTP (0.2 M) in base/CH_3CN	[a]
5	wash	(i) CH_3CN, (ii) CH_2Cl_2	
6		repeat steps 1−5 to synthesize the objective sequence	
7	detritylation	3% DCA in CH_2Cl_2−Et_3SiH (1:1, v/v)	15 s
8	capping	Ac_2O−2,6-lutidine (1:9, v/v) + DMAP (10 mg/mL)	30 s
9	decyanoethylation	10% DBU in CH_3CN (v/v)	5 min
10	deprotection	NH_3/MeOH (rt, 0.1 mM)	24 h

[a] 5 min using DMAN (method A) and 5 min × 2 using 2,4,6-collidine (method B).

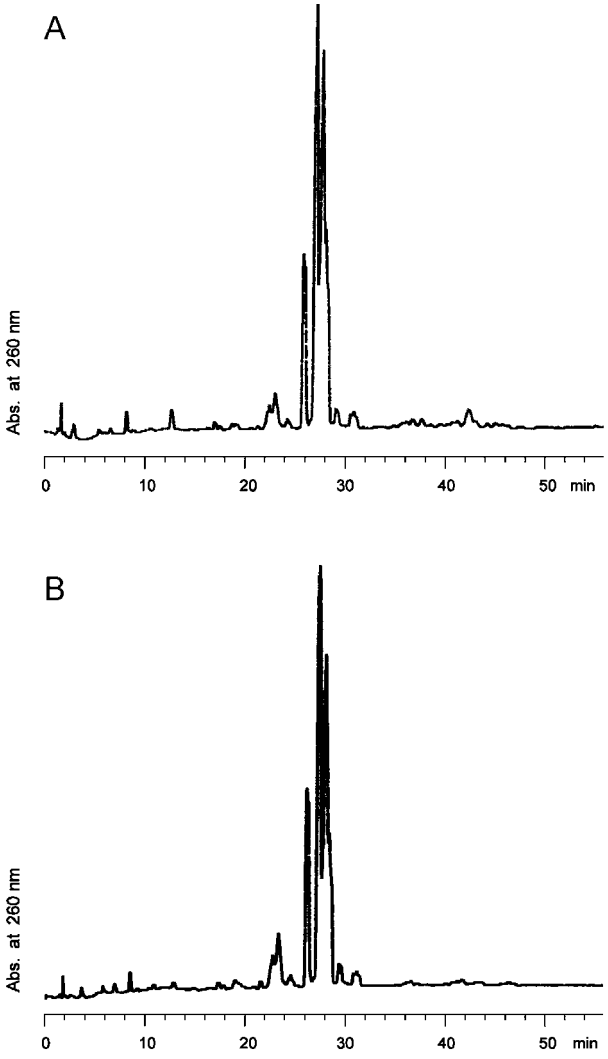


FIGURE 2. Reverse-phase HPLC profiles of crude mixtures of $d(C_{PB}A_{PB}G_{PB}T)$, synthesized by using coupling protocols with DMAN (5 min; A) and 2,4,6-collidine (5 min × 2; B).

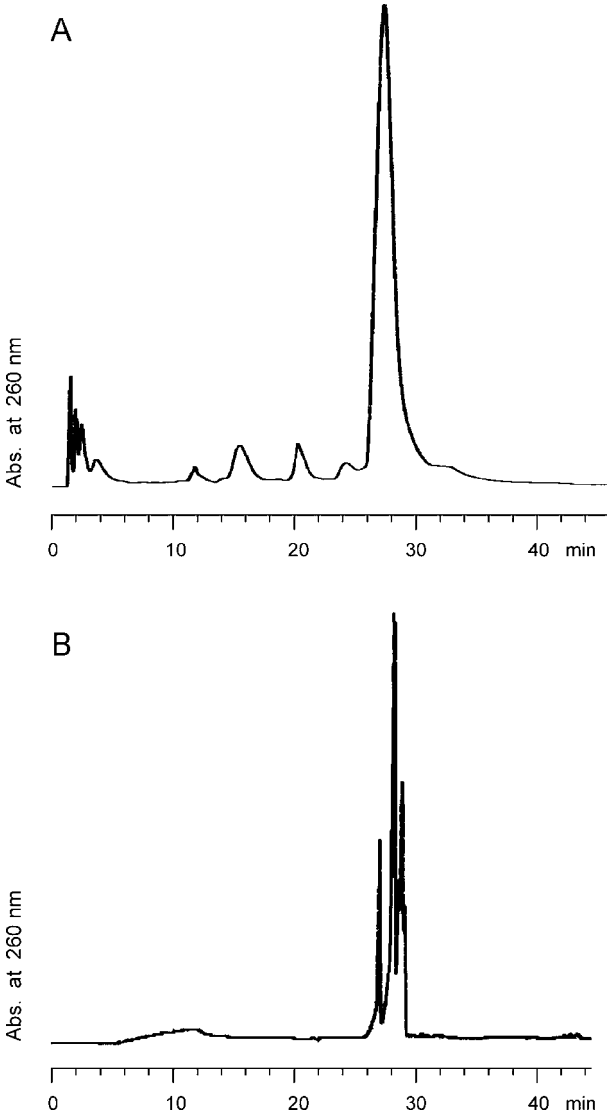


FIGURE 3. Anion-exchange HPLC profile of crude $d(C_{PB}A_{PB}G_{PB}T)$ obtained by using DMAN as a base in the condensation (A) and reverse-phase HPLC profile after purification by anion-exchange HPLC (B).

$d(C_{PB}A_{PB}G_{PB}T)$; the 12mers were not eluted from the HPLC column. Therefore, the 12mers were purified by 15% PAGE/7 M urea (PAGE = polyacrylamide gel electrophoresis). After the elution of the oligomers from the gel, followed by desalting by a C18 Sep-Pak cartridge, $d(C_{PB}A_{PB}G_{PB}T)_3$ and $T(_{PB}T)_{11}$ were isolated in 16 and 8% yields, respectively. The 12mers were identified by MALDI TOF-MS (calcd for $d(C_{PB}A_{PB}G_{PB}T)_3$ $[M - H]^-$, 3621.06; found, 3621.62 and calcd for $T(_{PB}T)_{11}$ $[M - H]^-$, 3564.00; found, 3562.98).

Thermal Denaturation Studies. The hybridization properties of the BH_3−ODN 12mers with complementary DNAs and RNAs were determined by thermal denaturation measurements at high and low ionic strengths (1 and 0.1 M NaCl, phosphate buffer, pH 7.0, respectively; Table 5). At a low ionic strength, no detectable binding of $T(_{PB}T)_{11}$ with dA_{12} was detected, while under high ionic strength conditions, a thermal melting (Tm)

TABLE 5. Comparison of the Tm Values of Duplexes Consisting of BH_3−ODNs and Natural DNAs and RNAs

entry	BH_3−ODNs	counterparts	Tm (°C)	ΔTm[a] (°C)
	100 mM NaCl, NaH_2PO_4−Na_2HPO_4 buffer (pH 7.0)			
1	$T(_{PB}T)_{11}$	dA_{12}	[b]	[b]
2	$d(C_{PB}A_{PB}G_{PB}T)_3$	$d(ACTG)_3$	39.7	−14.6
3	$d(C_{PB}A_{PB}G_{PB}T)_3$	$r(ACUG)_3$	45.0	−7.4
	1 M NaCl, NaH_2PO_4−Na_2HPO_4 buffer (pH 7.0)			
4	$T(_{PB}T)_{11}$	dA_{12}	12.8	−31.3
5	$d(C_{PB}A_{PB}G_{PB}T)_3$	$d(ACTG)_3$	44.7	−13.3
6	$d(C_{PB}A_{PB}G_{PB}T)_3$	$r(ACUG)_3$	50.5	−4.3

[a] The difference of the native control's Tm. [b] The clear Tm values were not observed.

of the corresponding duplex was observed at 12.8 °C. This result is consistent with those previously reported by Caruthers et al. and Shaw and Sergueev.[4,5] On the other hand, $d(C_{PB}A_{PB}G_{PB}T)_3$ showed an amazingly high binding affinity with $d(ACTG)_3$, compared with the duplex of $T(_{PB}T)_{11}$ and dA_{12}, even at a low ionic strength (Tm = 39.7 °C). At a high ionic strength, the duplex showed a Tm of 44.7 °C. Although the binding affinity of the BH_3−ODN, $d(C_{PB}A_{PB}G_{PB}T)_3$, was less than that of the corresponding unmodified phosphodiester ODN (54.3 and 58.0 °C at low and high ionic strengths, respectively), the duplex stability apparently increased by introducing cytosine and guanine bases in the BH_3−ODN. Furthermore, upon hybridizing with a complementary RNA, $r(ACUG)_3$, $d(C_{PB}A_{PB}G_{PB}T)_3$ formed a more stable complex under the same conditions; 45.0 and 50.5 °C at low and high ionic strengths, respectively), while the complex of a control of unmodified natural phosphodiester exhibited slightly lower Tm (52.4 and 54.8 °C at low and high ionic strengths, respectively). Thus, we first proved that the binding affinity of BH_3−ODN with complementary DNA and RNA highly increased by incorporating four kinds of nucleobases, especially cytosine and guanine bases BH_3−ODNs having longer mixed sequences or GC-rich sequences are expected to form sufficiently stable complexes with complementary DNAs and RNAs.

Conclusion

We have developed a new method for the synthesis of BH_3−ODNs on a solid support. The present boranophosphotriester method enables us to synthesize BH_3−ODNs with all possible nucleobases, A, C, G, and T. Although BH_3−ODNs sometimes gave a pessimistic forecast for antisense molecules because of their low affinity for the target nucleic acids, we demonstrated that the duplex stability of BH_3−ODNs with complementary DNAs and RNAs is useful enough to apply them as antisense molecules.

Experimental Section[17]

Tris(2-cyanoethyl) Phosphite 5.[18] To a solution of tris(2-cyanoethyl) phosphite (24.7 g, 102.7 mmol) in THF (103 mL) under an argon atmosphere was added dropwise a 0.93 M solution of BH_3·THF in THF (121.5 mL, 113 mmol) at 0 °C, and the solution was stirred for 1 h at room temperature. After the mixture was concentrated to dryness under reduced pressure, the residue was chromatographed on silica gel (150 g) using AcOEt as an eluent to give **6** (22.9 g, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.31 (6H, m), 2.79 (6H, t, J = 5.7 Hz), 1−0 (3H, bq); ^{31}P NMR ($CDCl_3$)-δ 119.8−116.9 (m).

Tris(2-cyanoethyl) Boranophosphate 6. To a solution of tris-(2-cyanoethyl) phosphite (24.7 g, 102.7 mmol) in THF (103 mL) under an argon atmosphere was added dropwise a 0.93 M solution of BH_3·THF in THF (121.5 mL, 113 mmol) at 0 °C, and the solution was stirred for 1 h at room temperature. After the mixture was concentrated to dryness under reduced pressure, the residue was chromatographed on silica gel (150 g) using AcOEt as an eluent to give **6** (22.9 g, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 4.31 (6H, m), 2.79 (6H, t, J = 5.7 Hz), 1−0 (3H, bq); ^{31}P NMR ($CDCl_3$)-δ 119.8−116.9 (m).

Triethylammonium Bis(2-cyanoethyl) Boranophosphate 7. To a solution of **6** (4.93 g, 19.3 mmol) in dry CH_2Cl_2 (19 mL) was added Et_3N (26.9 mL, 193 mmol) at room temperature. The mixture was warmed at 60 °C and stirred for 1 h. The mixture was then cooled to room temperature and concentrated to dryness under reduced pressure. Excess Et_3N was removed by repeated coevaporation with toluene and concentrated to dryness under reduced pressure to give **7** (5.86 g, quant) as a colorless oil. IR (KBr) 3406, 2986, 2900, 2616, 2365, 2253, 1636, 1476, 1396, 1333, 1264, 1225, 1146, 1043, 1006, 912, 837, 768, 651, 555, 445; ^{1}H NMR ($CDCl_3$)-δ 12.58 (1H, br s), 4.14−4.04 (4H, m), 3.07 (6H, q, J = 7.2 Hz), 2.74−2.66 (4H, m), 1.34 (9H, t, J = 7.2 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 117.8, 58.1, 58.1, 45.4, 19.9, 19.9, 8.4; ^{31}P NMR ($CDCl_3$) δ 96.64 (q, J_{PB} = 127.37 Hz).

Triethylammonium 2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl Bis(2-cyanoethyl) Boranophosphate (10a). 5′-*O*-Dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosine (**8a**; 1.31 g, 2.00 mmol) and **7** (0.730 g, 2.40 mmol) were dried by repeated coevaporation with dry toluene, followed with dry pyridine, and finally dissolved in dry CH_3CN (20.0 mL). To the solution were successively added 2,6-lutidine (2.30 mL, 20.0 mmol) and PyNTP (2.40 g, 4.80 mmol). After being stirred at room temperature for 6 h, the mixture was diluted with $CHCl_3$ (50 mL). The reaction mixture was washed with saturated $NaHCO_3$ (3 × 50 mL), and the aqueous layer was back-extracted with $CHCl_3$ (2 × 50 mL). The organic layer and the washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. After repeated coevaporation with toluene, the residue was chromatographed on silica gel using EtOAc as an eluent to give bis-(2-cyanoethyl)-5′-*O*-dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl boranophosphate (**9a**), containing a small amount of phosphite. Then to a solution of crude **9a** in CH_2Cl_2 (2.00 mL) was successively added Et_3N (2.80 mL, 20.0 mmol). After being stirred for 1 h, the mixture was concentrated to dryness under reduced pressure. The residue was chromatographed on silica gel by using a gradient of MeOH (0−4%) in CH_2Cl_2 with 0.5% Et_3N as an eluent. The fractions containing triethylammonium 2-cyanoethyl 5′-*O*-dimethoxytrityl-*N*^{6}-benzoyl-2′-deoxyadenosin-3′-yl boranophosphate (**10a**) were combined and concentrated to dryness. Excess Et_3N was removed by repeated coevaporation with toluene and concentrated to dryness under reduced pressure to give **10a** (908 mg, 51%) as a colorless foam. IR (KBr) 3412, 2952, 2838, 2365, 1701, 1610, 1581, 1510, 1457, 1400, 1300, 1251, 1177, 1150, 1072, 1036, 944, 911, 833, 795, 758, 710, 646, 584, 472; ^{1}H NMR ($CDCl_3$) δ 9.09 (1H, br s), 8.70 (1H, s, 8-H), 8.16 (1H, s), 8.01, 7.98 (2H, 2m), 7.61−7.13 (12H, m), 6.77 (4H, d, J = 9.0 Hz), 6.55 (1H, m), 5.14 (1H, m), 4.39 (1H, m), 4.08−3.91 (2H, m), 3.76 (6H, s), 3.41 (2H, m), 3.03 (6H, q, J = 6.0 Hz), 2.96−2.46 (4H, m), 1.31 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 164.4, 158.2, 152.3, 151.3, 151.3, 149.2, 144.2, 141.3, 141.2, 135.4, 135.3, 133.5, 132.6, 130.1, 129.9, 128.7, 128.0, 127.7, 127.7, 126.7, 123.1, 117.7, 113.0, 86.5, 85.9, 85.9, 85.8, 84.6, 84.5, 74.1, 74.1, 73.7, 73.7, 63.7, 63.6, 58.3, 58.3, 58.2, 58.1, 55.2, 45.5, 39.9, 20.2, 20.1, 20.0, 8.6; ^{31}P NMR ($CDCl_3$) δ 98.42−93.27 (m); HRMS FAB$^+$ *m/z* calcd for $C_{41}H_{43}BN_6O_8P$ $[M + H]^+$, 798.2973; found, 789.2975.

(17) A general statement describing materials and methods is provided in the Supporting Information.

(18) Goldstein, J. A.; McKenna, C.; Westheimer, F. H. *J. Am. Chem. Soc.* **1976**, *98*, 7327−7332.

Triethylammonium 2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{4}-benzoyl-2′-deoxycytidin-3′-yl Bis(2-cyanoethyl) Boranophosphate (10b). This compound was obtained from **8b** as a colorless foam (56% yield) in a manner similar to the synthesis of **10a**, except for the reaction time of boranophosphorylation (1 h). IR (KBr) 3426, 2953, 2838, 2679, 2367, 1698, 1652, 1564, 1507, 1486, 1394, 1305, 1253, 1178, 1149, 1116, 1072, 1037, 913, 832, 790, 706, 585; ^{1}H NMR ($CDCl_3$) δ 12.10 (1H, br s), 8.74 (1H, br s), 8.24−8.16 (1H, m), 7.88, 7.86 (2H, 2m), 7.62−7.08 (13H, m), 6.89−6.80 (4H, m), 6.32−6.24 (1H, m), 5.04 (1H, m), 4.41−4.30 (1H, m), 4.00−3.88 (2H, m), 3.79 (6H, s), 3.52−3.44 (2H, m), 3.04 (6H, q, J = 6.0 Hz), 2.90−2.78 (1H, m), 2.72−2.47 (2H, m), 2.39−2.24 (1H, m), 1.31 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 162.0, 158.6, 144.7, 144.0, 144.0, 135.4, 135.3, 135.1, 133.1, 130.1, 130.1, 128.9, 128.2, 128.0, 127.5, 127.1, 117.8, 113.2, 113.0, 96.3, 87.3, 87.1, 86.9, 86.9, 86.0, 85.8, 73.0, 72.6, 62.8, 58.2, 58.1, 55.2, 45.5, 41.2, 20.0, 20.0, 8.5; ^{31}P NMR ($CDCl_3$) δ 99.72−92.48 (m); HRMS FAB$^+$ *m*/*z* calcd for $C_{40}H_{43}BN_4O_9P$ [M + H]$^+$, 765.2861; found, 765.2868.

Triethylammonium 2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*O*^{6}-diphenylcarbamoyl-*N*^{2}-phenylacetyl-2′-deoxyguanosin-3′-yl Boranophosphate (10c). This compound was obtained from **8c** as a colorless foam (81% yield) in a manner similar to the synthesis of **10a**, except for the reaction time of boranophosphorylation (12 h). IR (KBr) 3411, 3060, 2952, 2838, 2668, 2366, 2254, 1741, 1618, 1509, 1450, 1387, 1334, 1303, 1250, 1223, 1183, 1061, 945, 912, 833, 790, 760, 700, 645, 584, 471; ^{1}H NMR ($CDCl_3$) δ 11.83 (1H, br s), 8.26−8.19 (1H, m), 8.09 (1H, s), 7.47−7.08 (24H, m), 6.74 (4H, d, J = 6.0 Hz), 6.45−6.37 (1H, m), 5.17−5.07 (1H, m), 4.39−4.29 (1H, m), 4.07−3.86 (4H, m), 3.71 (6H, s), 3.47−3.20 (2H, m), 2.98 (6H, q, J = 6.0 Hz), 2.87−2.44 (4H, m), 1.26 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 169.8, 158.4, 157.9, 155.8, 154.4, 151.6, 150.4, 149.7, 144.4, 143.9, 142.5, 141.7, 136.3, 135.9, 135.4, 134.2, 130.0, 129.5, 129.1, 128.7, 127.8, 126.8, 126.1, 125.2, 123.7, 121.4, 117.9, 113.5, 113.1, 86.4, 85.8, 84.4, 73.7, 63.7, 58.2, 57.5, 55.7, 55.1, 52.7, 45.6, 43.9, 39.9, 37.8, 21.4, 20.0, 8.5, 7.8; ^{31}P NMR ($CDCl_3$) δ 98.48−92.65 (m); HRMS FAB$^+$ *m*/*z* calcd for $C_{55}H_{54}BN_7O_{10}P$ [M + H]$^+$, 1014.3763; found, 1014.3763.

Triethylammonium 2-Cyanoethyl 5′-*O*-Dimethoxytrityl-*N*^{3}-benzoylthymidin-3′-yl Boranophosphate (10d). This compound was obtained from **8d** as a colorless foam (73% yield) in a manner similar to the synthesis of **10a**, except for the reaction time of boranophosphorylation (12 h). IR (KBr) 3434, 3065, 2952, 2838, 2610, 2365, 1748, 1702, 1660, 1606, 1509, 1445, 1394, 1280, 1252, 1178, 1148, 1072, 1035, 912, 832, 794, 764, 729, 690, 642, 586, 443; ^{1}H NMR ($CDCl_3$) δ 7.95−7.19 (16H, m), 6.84 (4H, d, J = 6.0 Hz), 6.43, 6.40 (1H, dd, $J_{1',2'}/J_{1',2''}$ = 7.5 and 7.5 Hz), 5.19−5.08 (1H, m), 4.32−4.24 (1H, m), 4.03−3.86 (2H, m), 3.79 (6H, s), 3.56−3.38 (2H, m), 2.96 (6H, q, J = 6.0 Hz), 2.70−2.38 (4H, m), 1.39, 1.35 (3H, 2s), 1.25 (9H, t, J = 6.0 Hz), 1−0 (3H, bq); ^{13}C NMR ($CDCl_3$) δ 169.01, 162.72, 158.52, 149.71, 144.08, 135.53, 135.46, 135.30, 135.23, 135.03, 134.85, 131.45, 130.39, 130.02, 128.97, 128.07, 127.92, 127.04, 117.66, 113.20, 111.21, 111.16, 87.06, 87.02, 85.82, 85.76, 85.57, 85.52, 84.90, 84.78, 74.40, 74.25, 74.21, 63.70, 63.61, 58.17, 55.29, 45.60, 40.27, 40.21, 20.20, 20.12, 20.05, 11.72, 11.63, 8.83; ^{31}P NMR ($CDCl_3$) δ 99.38−94.02 (m); HRMS FAB$^+$ *m*/*z* calcd for $C_{41}H_{43}BN_3O_{10}P$ [M]$^+$, 779.2779; found, 779.2780.

Typical Procedure for Manual Solid-Phase Synthesis. Each cycle of the chain elongation consisted of detritylation (3% DCA in CH_2Cl_2−Et_3SiH (1:1, v/v); 3 × 5 s), washing (CH_2Cl_2, followed by CH_3CN), drying (10 min), coupling (0.1 M monomer **10**, 0.2 M MNTP, and 0.15 M DMAN in CH_3CN; 5 min, method A or 10% 2,4,6-collidine in CH_3CN; 2 × 5 min, method B), and washing (CH_3CN, followed by CH_2Cl_2). After the chain elongation, the DMTr group was removed by treatment of 3% DCA in CH_2Cl_2−Et_3SiH (1:1, v/v) for 3 × 5 s and washed with CH_2Cl_2. Then the 5′-OH group was acetylated by the reaction with Ac_2O−2,6-lutidine (1:9, v/v) in the presence of DMAP (10 mg/mL) for 30 s and washed (CH_3CN), and the CE groups were cleaved by 10% DBU/CH_3CN (5 min) and washed with CH_3CN. The oligomer on the solid support was then treated with concd NH_3/MeOH for 24 h at room temperature (for the 4mer) or with concd NH_3 for 12 h at 55 °C (for the 12mer) to remove the protecting groups of the nucleobases and also to release the oligomers from solid support. The CPG resin was removed by filtration and washed with H_2O. The filtrate was concentrated to dryness. The residue was dissolved in H_2O (1 mL), washed with Et_2O (5 × 1 mL), and the combined washings were back-extracted with H_2O (1 mL). The combined aqueous layers were concentrated to dryness. The resulting crude product was analyzed by reverse-phase HPLC with a linear gradient of 0−20% CH_3CN in 0.1 M ammonium acetate buffer (pH 7.0) at 50 °C for 60 min at a rate of 0.5 mL/min.

Synthesis of d($C_{PB}A_{PB}G_{PB}T$). According to the typical procedure described above (method A), the crude d($C_{PB}A_{PB}G_{PB}T$) was purified by anion-exchange HPLC. The eluents were (A) 20% CH_3CN in 10 mM NaH_2PO_4−Na_2HPO_4 (pH 6.9) and (B) 20% CH_3CN in 10 mM NaH_2PO_4−Na_2HPO_4 (pH 6.9) containing 1 M NaCl. A linear gradient of 0−50% (B) in (A) was developed in a period of 50 min at 50 °C at a rate of 0.5 mL/min. The fractions containing d($C_{PB}A_{PB}G_{PB}T$) (R_t = 28 min) were combined, concentrated, and then desalted by a C18 Sep-Pak cartridge. The yield of the product was determined by a UV absorbance measurement at 260 nm with the molar extinction coefficient of an approximate value for native d(CAGT): 39 200. The yield of purified d($C_{PB}A_{PB}G_{PB}T$) was 30%: MALDI TOF-MS calcd for $C_{39}H_{59}B_3N_{15}O_{19}P_3$ [M − H]$^-$, 1166.3525; found, 1166.3718.

Synthesis of $T(_{PB}T)_{11}$. According to the typical procedure described above (method A), the crude $T(_{PB}T)_{11}$ was purified by preparative polyacrylamide gel (15% PAGE/7M Urea, 40 × 20 cm, 1 mm thickness, fixed at 40 W, 4 h). The band containing $T(_{PB}T)_{11}$ was cut into small pieces and extracted from the gel, and then the combined extracts were desalted by a C18 Sep-Pak cartridge. The yield of the product was determined by a UV absorbance measurement at 260 nm with the molar extinction coefficient of an approximate value for native T_{12}: 88 880 000. The yield of purified $T(_{PB}T)_{11}$ was 8%: MALDI TOF-MS calcd for $C_{120}H_{189}B_{11}N_{24}O_{71}P_{11}$ [M − H]$^-$, 3564.00; found, 3562.98.

Synthesis of d($C_{PB}A_{PB}G_{PB}T$)$_3$. According to the typical procedure described above (method A), the crude d($C_{PB}A_{PB}G_{PB}T$)$_3$ was purified by preparative polyacrylamide gel (15% PAGE/7M Urea, 40 × 20 cm, 1 mm thickness, fixed at 40 W, 4 h). The band containing d($C_{PB}A_{PB}G_{PB}T$)$_3$ was cut into small pieces and extracted from the gel, and then the combined extracts were desalted by a C18 Sep-Pak cartridge. The yield of the product was determined by a UV absorbance measurement at 260 nm, with the molar extinction coefficient of an approximate value for native d(CAGT)$_3$: 98 550 000. The yield of purified d($C_{PB}A_{PB}G_{PB}T$)$_3$ was 16%: MALDI TOF-MS calcd for $C_{117}H_{181}B_{11}N_{45}O_{59}P_{11}$ [M − H]$^-$, 3621.06; found, 3621.62.

UV Melting Experiments. BH_3−ODN was mixed with either a DNA or a RNA target strand in a 1:1 molar ratio in a buffer of 100 mM NaH_2PO_4−Na_2HPO_4 (pH 7.0) containing 100 mM or 1 M NaCl. The total concentration of oligonucleotide was 8 μM in a final volume of 125 μL. The sample was degassed under reduced pressure for 15 min, then heated for 10 min at 90 °C, and cooled slowly to 0 °C prior to running the experiment. The heating rate was 0.5 °C/min, and the data were corrected for 0.25 °C interval. The melting temperatures of the duplexes were measured from the first derivative plots obtained from the thermal melting curves.

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Note Added after ASAP Publication. Et_3SiH was incorrectly listed as Et_3SH in Scheme 3 and Table 2 in the version published ASAP April 29, 2006; the corrected version was published ASAP May 9, 2006.

Supporting Information Available: ^1H and ^{31}P NMR spectra of **6**. ^1H, ^{13}C, and ^{31}P NMR spectra of **7**, **10a**, **10b**, **10c**, and **10d**. IR spectra of **7**, **10a**, **10b**, **10c**, and **10d**. UV melting curves of T_{12}/dA_{12}, $T(_{PB}T)_{11}/dA_{12}$, $d(CAGT)_3/d(ACTG)_3$, $d(C_{PB}A_{PB}G_{PB}T)_3/d(ACTG)_3$, $d(CAGT)_3/r(ACUG)_3$, and $d(C_{PB}A_{PB}G_{PB}T)_3/r(ACUG)_3$. General information for the experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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