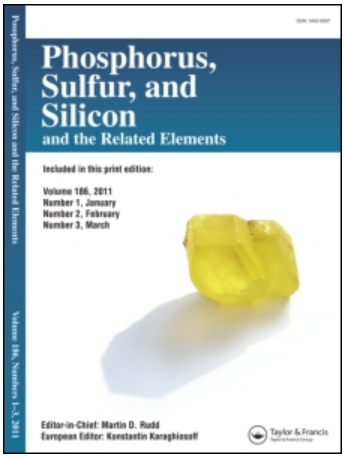

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis and Biochemical Activity of New Oligonucleotide Analogs

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Borane phosphonate deoxyoligonucleotides are synthesized from 5′-O-benzhydroxybis(trimethylsilyloxy)silyl-2′-deoxynucleoside-3′-phosphoramidites. The exocyclic amines of adenine and cytosine are protected with dimethoxytrityl and trimethoxytrityl, respectively, whereas guanine protection is with N2-(9-fluorenylmethoxycarbonyl) or N2-trimethoxytrityl. Thymine is protected with N3-anisoyl. Using these synthons and under standard conditions via activation with tetrazole, condensations in excess of 99% are observed. Oxidation with either THF•BH_3 or a peroxyanion solution followed by cleavage of the silyl ether with fluoride completes a cycle. Following synthesis of an appropriate oligomer, protecting groups are removed using sequentially acetic acid, a dithiolate and ammonium hydroxide. Oligodeoxynucleotide 10 mers and 12 mers having any combination of borane phosphonate and phosphate internucleotide linkages as well as all four 2′-deoxynucleotides are synthesized in isolated yields of 70–80% and characterized by phosphorus NMR and mass spectrometry.

Keywords Borane phosphonate DNA

INTRODUCTION

Oligodeoxyribonucleotides (ODNs) bearing internucleotide borane phosphonate linkages (Figure 1, compound **1**) have been of considerable interest for applications in diagnostic and therapeutic areas as

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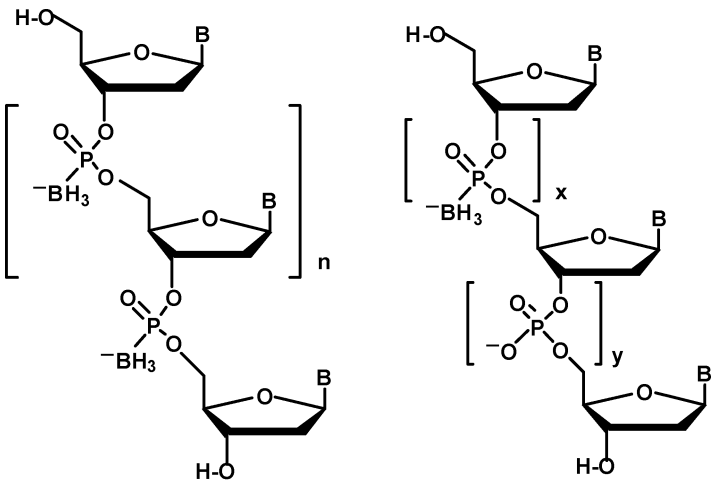


FIGURE 1 Borane phosphonate (a) and chimeric borane phosphonate/phosphate (b) ODNs. B = thymine, cytosine, adenine, and guanine. X, Y = combinations of phosphate and borane phosphonate linkages.

they mimic natural DNA in various biological processes.[1] The challenge over the years has been the development of a synthesis strategy that generates ODNs having all four bases and in yields sufficient to allow at least the preparation of 10mers. Until recently, the most successful strategy was the use of unprotected bases where 10 mers can be prepared in 20–30% yield.[2] An alternative approach that may prove useful involves the initial formation of mononucleotide borane phosphonates where these monomers are then joined via a phosphotriester condensation route to generate dimers with yields from 72–92%.[3] Both methods must be improved considerably in order to generate a route for preparing oligomers rapidly in high yields on supports.

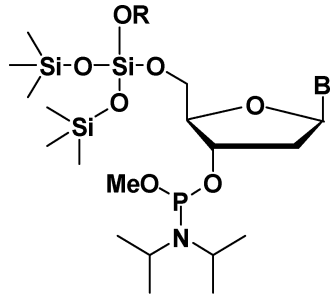
RESULTS

The synthetic challenge with this analog is the development of an orthogonal synthesis strategy. Specifically there are several key considerations. Of utmost importance, all base and phosphorus protecting groups must be stable toward boronation under conditions where these 2′-deoxynucleoside bases are not reduced or form irreversible complexes with borane. A second is the design of a transient 2′-deoxynucleoside hydroxyl protecting group, preferably 5′, which can be removed following

each condensation cycle under conditions that do not modify the growing, support bound borane phosphonate oligonucleotide. Additionally the ideal synthesis strategy would not only generate borane phosphonate ODNs but also chimeric oligomers having other internucleotide linkages such as phosphate (compound **2**).

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.[4] This observation forced us to explore alternative strategies. On the basis of earlier research,[5] we prepared 2′-deoxynucleosides having 5′-O-[benzhydroxy bis(trimethylsilyloxy) silyl protection (Figure 2, compounds **3a–e**) and discovered that the 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.

Another serious challenge is 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, which cannot be removed from the bases, or form stable, irreversible base adducts. This problem has been solved by using N-trityl protection on the exocyclic amines—a strategy that is common in the peptide field[6] but used only sparingly in polynucleotide synthesis.[7,8] Thus for adenine and cytosine, the exocyclic amines are protected with dimethoxytrityl and trimethoxytrityl, respectively. For guanine, two strategies have been studied. Initially we successfully used the



3a-e

FIGURE 2 5′-O-Silyl-2′-deoxynucleoside-3′-phosphoramidites. B = (3a) N3-anisoylthymine; (3b) N4-trimethoxytritylcytosine; (3c) N6-dimethoxytrityladenine; (3d) N2-(9-fluorenylmethoxycarbonyl) guanine; and (3e) N2-trimethoxytritylguanine. R = diphenylmethyl.

N2-(9-fluorenylmethoxycarbonyl)protecting group. However, overall yields are low (15% to the completely protected 2′-deoxynucleoside) and there is a risk, although minimal, of borane reduction. We, therefore, turned to the use of N2-trimethoxytrityl protection[7] where the overall yield is much higher (45–50% to the completely protected 2′-deoxynucleoside) and there is no risk of base reduction or complex formation with borane. We also introduced anisoyl on the N3 of thymine in order to prevent N3 methylation via the methylphosphate protecting group.

Using these appropriately protected 2′-deoxynucleoside phosphoramidites (Figure 2, compounds **3a–e**), a new high yielding synthesis cycle has been developed (Figure 3 and Table I). Starting with an appropriately protected 2′-deoxynucleoside attached to polystyrene (compound **4**), the first step is condensation with **3a–e** in anhydrous acetonitrile and tetrazole to generate a family of dimers (compound **5**) 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 (TEAHF) generates a family of dinucleotides and having any of the four

TABLE I Synthesis Cycle for Borane Phosphonate ODNs

Reaction	Wash/reagents/solvents	Time (sec)
Coupling	0.1 M Compound **3** in acetonitrile and 0.45 M tetrazole in acetonitrile (1:1)	5 to column, 60 wait
Wash	Acetonitrile	30
Boranation	25 mM BH3·THF in THF	30
	Peroxyanion Solution[a]	
or		
Oxidation		120
Wash	Tetrahydrofuran	30
	Dichloromethane,	30
	Acetonitrile	45
	Dimethylformamide	35
5′-Deprotection	1.1 M HF/1.1 M Triethylamine/0.02 M N-methyldiethanolamine in dimethylformamide (pH 9)[b]	25 to column, 45 wait
Wash	Dimethylformamide	40
	Acetonitrile	
		60
	0.4 M Tetrazole in Acetonitrile	3

[a]Peroxyanion Solution: Solution A = 3% (w/v) aqueous LiOH (10 mL), 1.5 M 2-amino-2-methyl-1-propanol in water (15 mL), and dioxane (17.5 mL). Solution B = *m*-chloroperbenzoic acid (1.78 g), dioxane (32.5 mL), and aqueous 30% hydrogen peroxide (12 mL). Equal volumes are mixed just prior to synthesis.

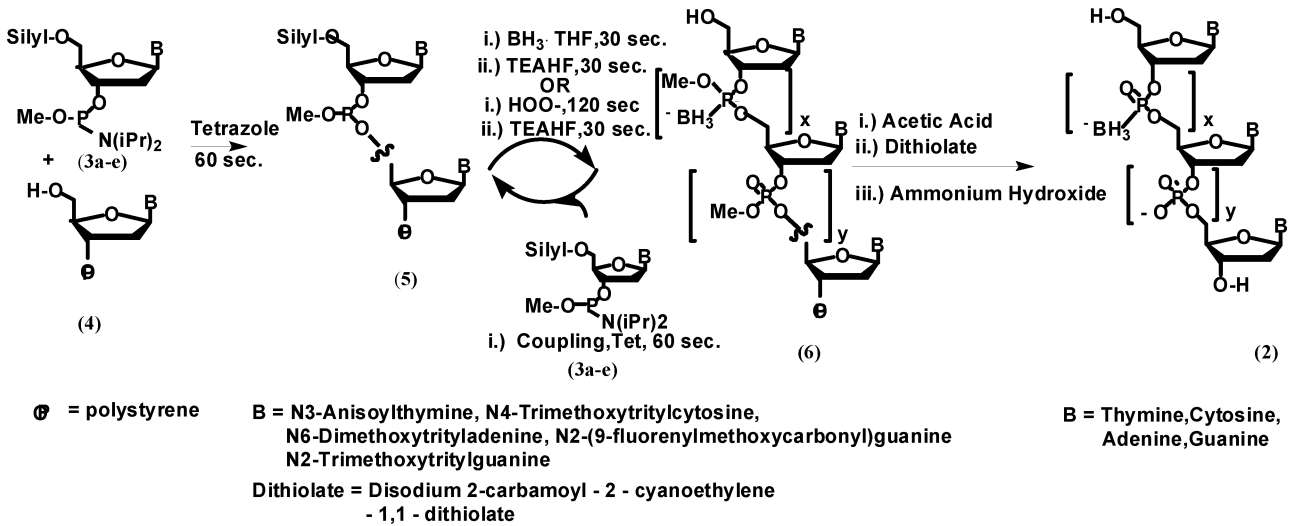
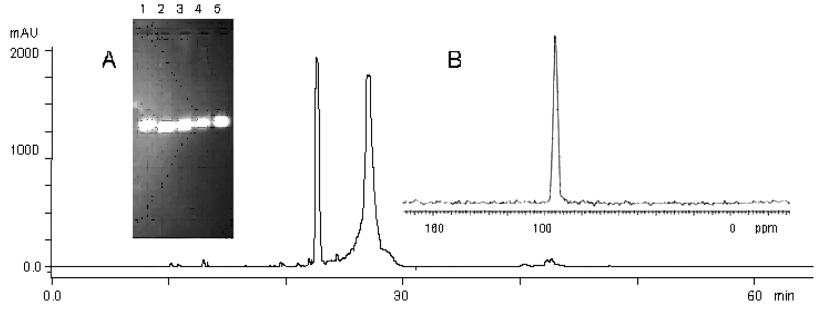


FIGURE 3 Synthesis cycle used to prepare borane phosphonate and chimeric borane phosphonate ODNs. B = appropriately protected bases (compounds 3a–e, 4, 5 and 6, see the legend to Figure 2) or thymine, cytosine, adenine and guanine (compound 2).

bases and either a P-IV phosphonium borane adduct or a phosphate triester linkage to oligonucleotides (compound **6**). These dimers can then be extended using the same repetitive cycle to generate a chimeric ODN having any defined sequence and length, as well as borane phosphonate and phosphate internucleotide linkages. For the preparation of a similar ODN having only borane phosphonate internucleotide linkages, the synthesis proceeds as outlined in Figure 3 except that oxidation is exclusively with $THF{\bullet}BH_3$.

The removal of protecting groups so as to generate compounds **1** or **2** proceeds via a three step procedure. Initially and with the ODN attached to the support (compound **6**), 80% acetic acid is used to eliminate trityl groups from adenine, cytosine, and guanine (the P-IV borane adduct is compatible with acetic acid). Next the oligomer is treated with a dithiolate[9] to remove internucleotide methyl protection. Finally ammonium hydroxide eliminates carbamate, if compound **3d** is used in the synthesis cycle, the anisoyl group from thymine and generates **1** or **2** by cleavage of the ODN from the support. Purification is by reverse phase HPLC. Typical results for total reaction mixtures are shown in Figure 4 (10 mer) and 5 (12 mer) for ODNs having all four bases and borane phosphonate internucleotide linkages. The major peaks in each case (excluding the first, anisic acid peak) are the products (99% coupling yields, isolated yields 75–88%). As expected from the many P-chiral



1 = d(T_PT_P)$_4T_PT$ **4 = d[(C_bT_b)$_4C_bT$]**

2 = d(T_bT)$_9T$ **5 = d($T_bC_bT_bT_bA_bC_bT_bG_bA_bT$)**

3 = d[(A_bT_b)$_4A_bT$] **b = Borane Phosphonate; p = Phosphate**

FIGURE 4 Reverse Phase HPLC analysis of the total crude reaction mixture from the synthesis of compound 14. (A) Gel electrophoresis results from total, crude reaction mixtures. Lanes 1–5, d(T_pT_p)$_4T_pT$, 11, 12, 13 and 14, respectively. (B) Phosphorus NMR of compound 14.

centers and the resulting large number of stereoisomers, these product peaks are broad.

These ODNs have been fully characterized by NMR, mass spectral analyses, and polyacrylamide gel electrophoresis. Phosphorus NMR analyses display a broad signal at 96 ppm (borane phosphonate) and a sharp peak at -2 ppm when some of the internucleotide linkages are phosphate (Figure 1, compound **2**). By phosphorus NMR, when all internucleotide linkages are borane phosphonate (Figure 1, compound **1**), phosphate cannot be detected. For example, the insets to Figures 4 and 5 display the phosphorus NMR spectra of the 10 mer and 12 mer, respectively, as prepared from the pooled product fractions of the reverse phase HPLC column eluates. In each case, phosphate peaks are not observed. ^{11}B NMR spectra for all oligomers synthesized consist of a broad signal at −40 ppm which is characteristic of the borane phosphonate linkage. Table II lists mass data for ODNs having various combinations of 2′-deoxynucleoside bases and internucleotide phosphate and borane phosphonate linkages. The observed masses for all ODNs correspond to those as calculated. These results show that ODNs as prepared by this method have the predicted structures and are free of any boronated bases or sugars. When further characterized by gel electrophoresis (Figure 4A), only one major band for each ODN, which corresponds to the product, is observed. This result is especially striking as total reaction mixtures are analyzed. Since only one major, intense band is observed for each reaction mixture, very few oligomers of lesser length are present. These results, as do the reverse phase HPLC

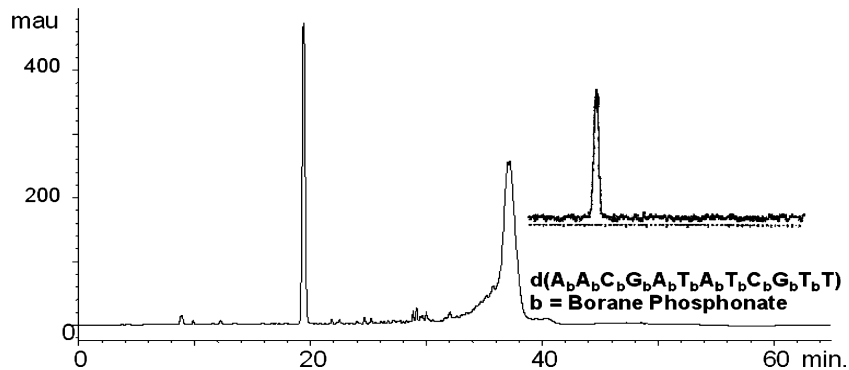


FIGURE 5 Reverse Phase HPLC analysis of the total crude reaction mixture from the synthesis of compound 15. The inset is the phosphorus NMR of compound 15.

TABLE II Mass Spectrum Analysis of Oligodeoxynucleotides

No	ODN[a]	Molecular weight	
		Calculated	Observed
7	$d[(T_pT_pT_b)_4T_pT]$	4185.7	4183.4_b
8	$d[(T_bT_p)_6T_bT]$	4179.8	4177.5_b
9	$d[(G_pT_pG_bT_pG_pT_b)_2G_pT]$	4360.8	4360.7_b
10	$d[(G_bT_pG_bT_p)_3G_bT]$	4354.9	4355.2_b
11	$d(T_b)_9T$	2960.8	2954.5_c
12	$d[(A_bT_b)_4A_bT$	3005.9	3000.7_c
13	$d[(C_bT_b)_4C_bT]$	2885.8	2881.5_c
14	$d(T_bC_bT_bT_bA_bC_bT_bG_bA_bT)$	2973.9	2967.5_c
15	$d(A_bA_bC_bG_bA_bT_bA_bT_bC_bG_bT_bT)$	3609.5	3617.0_c

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

analyses, further indicate that coupling yields are extremely high and lead primarily only to the product ODNs.

Preliminary results suggest that borane phosphonate ODNs may prove useful for both biochemical research and therapeutic applications. For example, borane phosphonate ODNs are resistant to degradation by exonucleases and DNase I. Results with DNase I are summarized in Figure 6. Under conditions where natural DNA is completely degraded, ODNs having borane phosphonate linkages (fully modified, every other or every third linkage) are essentially resistant to degradation. Similar results not shown were obtained with snake venom phosphodiesterase. When borane phosphonate ODNs are allowed to form duplexes with complementary RNA and then treated with *E. coli* RNase H, the RNA is degraded (Figure 7 summarizes several experiments). Under conditions where natural DNA stimulates complete RNA degradation by RNase H, similar experiments with fully modified borane phosphonate ODNs lead to 38% RNA hydrolysis. Oligomers having borane phosphonate linkages at every other or every third position are almost as satisfactory as natural DNA for stimulating RNase H degradation of RNA.

DISCUSSION

These results demonstrate that a new, high yielding method has been developed for the synthesis of borane phosphonate DNA. The synthesis strategy also demonstrates that chimeras containing both borane phosphonate and phosphate internucleotide linkages can be prepared by these procedures. Because the approach involves a simple oxidation

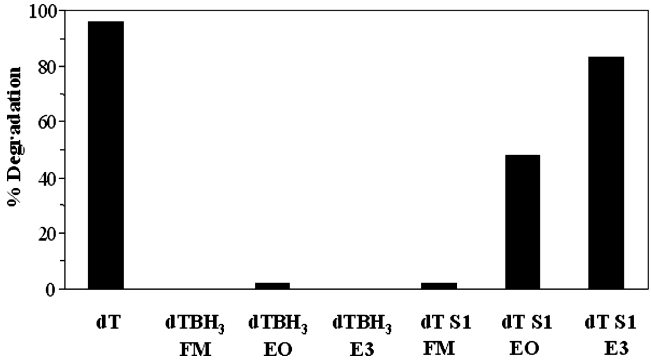


FIGURE 6 A summary of experiments addressing the lability of borane phosphonate DNA to DNase I. $dTBH_3$ FM, $dTBH_3$ EO, $dTBH_3$ E3: Borane phosphonate ODNs having fully modified (FM), every other (EO) and every third (E3) borane phosphonate internucleotide linkages. dTSI FM, dTSI EO, dTSI E3: phosphorothioate ODNs having fully modified (FM), every other (EO) and every third (E3) phosphorothioate internucleotide linkages. All other linkages are phosphate. All ODNs including the unmodified oligomer (dT) and complementary dA are twelve nucleotides in length.

step as the key variable for the synthesis of this chimera, other analogs containing phosphorothioate or selenophosphate should also be possible. Of considerable importance is the observation that borane phosphonate ODNs are active with RNase H. An especially exciting observation

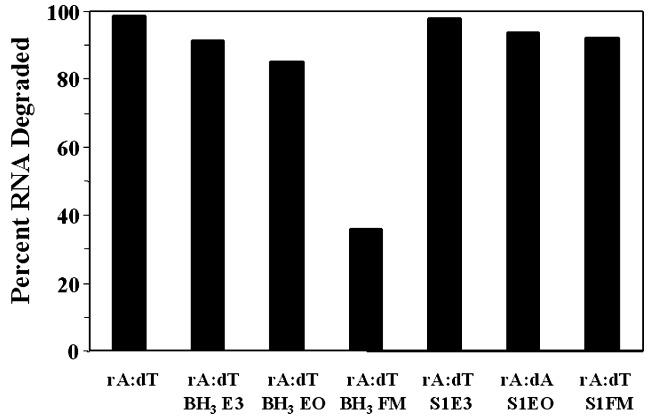


FIGURE 7 A summary of experiments addressing the ability of borane phosphonate ODNs to stimulate the degradation of complementary RNA by *E. coli* RNase H. All oligomers are 12 nucleotides in length. See the legend to Figure 6 for abbreviations.

is that ODNs having every other linkage or every third linkage as borane phosphonate are very active in stimulating RNase H activity. These ODNs are essentially resistant to degradation by nucleases as well. When taken together, these results strongly suggest that borane phosphonate chimeras having a minimum number of borane phosphonate linkages may prove extremely useful for cell biology research and for various therapeutic drug applications.

EXPERIMENTAL

Synthesis of N6-Dimethoxytrityl-2′-deoxyadenosine

2′-Deoxyadenosine (2.69 g, 10 mmol) was coevaporated three times with pyridine and dried in vacuo for 12 h. Anhydrous pyridine (50 mL) and chlorotrimethylsilane (50 mmol) were added. After the mixture had been stirred at room temperature for 2 h, dimethoxytrityl chloride (3.7 g,11 mmol) was added. The reaction was stirred overnight (~16 h) at room temperature. Water (60 mL) and aqueous ammonium hydroxide (2 mL, 28–30%) were added and the reaction mixture was stirred for 30 min. The crude product was extracted into dichloromethane. The organic layer was washed two times with a 5% aqueous solution of sodium bicarbonate and dried with anhydrous sodium sulfate. The organic layer containing product was filtered from salts and purified by column chromatography using chloroform/pyridine (99.9:0.1) and a gradient of methanol (0–6%).

Yield 99%. ^{1}H NMR (DMSO-d_6) δ 8.42 (s, 1H), 8.32 (s, 1H), 7.28–7.26 (m, 5H), 7.19 (d, 4H), 6.84 (d, 4H), 6.32 (t, 1H), 5.31 (d, 1H), 5.12 (t, 1H), 3.86–3.84 (m, 1H), 3.71 (s, 6H), 3.61–3.47 (m, 2H), 2.79–2.74 (m, 1H), 2.27–2.22 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 157.69, 153.68, 151.16, 148.07, 145.34, 140.37, 137.27, 129.77, 128.39, 127.71, 126.47, 121.05, 113.00, 88.04, 84.12, 70.92, 69.61, 61.83, 54.99; HRMS (FAB) calcd. for $C_{31}H_{31}N_5O_5$ (M^+) 553.2325. found 553.2309.

Synthesis of N4-Trimethoxytrityl-2′-deoxycytidine

2′-Deoxycytidine (2.64 g, 10 mmol) was coevaporated three times with pyridine and dried *in vacuo* for 12 h. Anhydrous pyridine (50 mL) and chlorotrimethylsilane (50 mmol) were added. After the mixture was stirred at room temperature for 2 h, trimethoxytrityl chloride (3.85 g, 10.5 mmol) was added. The reaction was stirred overnight (~16 h) at room temperature. Water (60 mL) and aqueous ammonium hydroxide (2 mL, 28–30%) were added, and the reaction mixture was stirred for 30 min. The crude product was extracted into dichloromethane,

the organic layer was washed two times with 5% aqueous solution of sodium bicarbonate, and dried with anhydrous sodium sulfate. The product was filtered and purified by column chromatography using chloroform/pyridine (99.9:0.1) with a gradient of methanol (0–6%).

Yield 95%. ^{1}H NMR (DMSO-d_6) δ 8.29 (s, 1H), 7.69 (d, 1H), 7.10 (d, 4H), 6.82 (d, 4H), 6.21 (d, 1H), 6.04 (t, 1H), 5.17 (d, 1H), 4.94 (t, 1H), 4.16–4.14 (m, 1H), 3.72 (s, 9H), 3.51–3.48 (m, 2H), 2.05–1.86 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 163.29, 157.37, 154.07, 139.53, 137.26, 129.78, 112.70, 96.38, 87.18, 84.67, 70.65, 68.92, 61.51, 54.98; HRMS (FAB) calcd. for $C_{31}H_{33}N_3O_7$ (M^+) 559.2319, found 559.2331.

Synthesis of N2-(9-Fluorenylmethoxycarbonyl)-2′-deoxyguanosine

2′-Deoxyguanosine (3.11 g, 11.0 mmol) was twice co-evaporated with 50 mL pyridine and then suspended in 80 mL pyridine. The reaction was started by the dropwise addition of chlorotrimethylsilane (6.5 mL, 75 mmol) with a syringe. The reaction proceeded for 1 h during which the deoxynucleoside was taken into solution. At this point 9-fluorenylmethyl chloroformate (3.5 g, 14.3 mmol) was added and the solution stirred for another 1.5 h. When complete, the reaction mixture was quenched with 20 mL water and stirring for 1 h. Following an aqueous work-up, the crystalline product was dissolved into dichloromethane, filtered and washed with chloroform. The product is a white solid.

Yield 65%. ^{1}H NMR (DMSO-d_6): δ 2.6 (m, 2H), 3.5 (m, 2H), 3.8 (d, $J = 2.5$ Hz), 4.3 (m, 1H), 4.4 (d, $J = 6.2$ Hz, 2H), 5.3 (m, 1H), 6.2 (t, 1H), 7.3 (t, 2H), 7.4 (t, 2H), 7.8 (d, $J = 7.0$ Hz, 2H), 7.9 (d, $J = 7.6$ Hz, 2H). MS: calcd. = 489, Found (ESI+) = 512 ($M+Na^+$).

Synthesis of 5′-O-[benzhydroxy-*bis* (trimethylsilyloxy]silyl-2′-deoxynucleoside

N-protected 2′-deoxynucleoside (10 mmol) was dried in vacuo for 6 h and then dissolved in anhydrous N,N-dimethylformamide (100 mL). Imidazole (20 mmol) was added to the mixture and the flask was placed on ice and stirred. Benzhydroxy-*bis*(trimethylsilyloxy)silyl chloride (10 mmol) was added slowly over 1 h via syringe. The flask was then removed from ice and allowed to stir at room temperature for ~4 h. The reaction was monitored by TLC and additional aliquots of the silyl chloride (1 mmol) were added until there was no presence of the starting material. Distilled water (60 mL) was added and the solvent was removed in vacuo to a final volume of 50 mL. The remaining solution was dissolved in

dichloromethane and rinsed with an aqueous solution of 5% sodium bicarbonate saturated with sodium chloride. The organic layer was dried over anhydrous sodium sulfate. The product was filtered and purified by column chromatography. Elution initially was with chloroform/benzene (9:1) followed by a gradient of methanol in chloroform (for N-trityl analogues, 0.1% pyridine was added to the eluting system). The product eluted in 5–10% methanol.

5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl]-N3anisoyl-2′-deoxythymidine

Yield 65.8%; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 2H), 7.60 (s, 1H), 7.37–7.22 (m, 10H), 6.94 (d, 2H), 6.31 (t, 1H), 5.94 (s, 1H), 4.32–4.29 (m, 1H), 3.90–3.87 (m, 1H), 3.86 (s, 3H), 3.82–3.76 (m, 2H), 2.26–2.20 (m, 1H), 1.98–1.92 (m, 1H), 1.88 (s, 3H), 0.10 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 168.06, 165.30, 163.08, 149.53, 143.97, 143.90, 135.64, 133.26, 128.53, 127.64, 127.60, 126.51, 126.41, 124.44, 114.66, 111.03, 86.78, 85.11, 77.44, 72.18, 63.26, 55.83, 36.71, 13.05, 1.73; HRMS (ESI) calcd. for $C_{37}H_{47}N_2O_{10}Si_3$ (M^+-H) 763.2544, found 763.2533.

5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl]-N4trimethoxytrityl-2′-deoxycytidine

Yield 43.5%; ^{1}H NMR (DMSO-d_6) δ 8.40 (bs, 1H), 7.48 (d, 1H), 7.39–7.22 (m, 10H), 7.15 (d, 6H), 6.83 (d, 6H), 6.27 (d, 1H), 6.06 (t, 1H), 5.96 (s, 1H), 5.26 (d, 1H), 4.11–4.05 (m, 1H), 3.80–3.78 (m, 1H), 3.71 (s, 9H), 3.68–3.61 (m, 2H), 2.05–1.98 (m, 1H), 1.74–1.68 (m, 1H), 0.05 (s, 18H); ^{13}C NMR (DMSO-d_6) δ 163.28, 157.39, 153.95, 144.06, 138.67, 137.25, 129.81, 129.56, 128.27, 127.16, 125.80, 112.69, 96.48, 86.15, 84.73, 76.07, 70.84, 68.97, 63.28, 54.95, 40.05, 1.43; HRMS (ESI) calcd. for $C_{50}H_{62}N_3O_{10}Si_3$ (M^++H) 948.3737, found 948.3725.

5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl]-N6dimethoxytrityl-2′-deoxyadenosine

Yield 67.9%; ^{1}H NMR (DMSO-d_6) δ 8.30 (s, 1H), 7.89 (s, 1H), 7.34–7.15 (m, 19H), 6.83 (d, 4H), 6.31 (t, 1H), 5.89 (s, 1H), 5.37 (d, 1H), 4.39–4.34 (m, 1H), 3.86–3.83 (m, 1H), 3.80–3.77 (m, 1H), 3.70 (s, 6H), 3.62–3.58 (m, 1H), 2.79–2.74 (m, 1H), 2.28–2.23 (m, 1H), −0.03 and −0.04 (2xs, 18H); ^{13}C NMR (DMSO-d_6) δ 157.67, 153.54, 151.14, 148.13, 145.29, 144.00, 140.02, 137.21, 129.66, 128.30, 128.09, 127.61, 126.99, 126.41, 125.75, 120.97, 112.94, 86.58, 83.72, 75.94, 70.61, 69.55, 63.21, 54.93, 38.39, 1.27; HRMS (ESI) calcd. for $C_{50}H_{60}N_5O_8Si_3$ (M^++H) 942.3744, found 942.3773.

5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl]-N2-(9-fluorenylmethoxycarbonyl)-2′-deoxyguanosine

Yield 24.3%; ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H), 11.33 (s, 1H), 7.95 (s, 1H), 7.92 (d, 2H), 7.82 (d, 2H), 7.44 (t, 2H), 7.37–7.27 (m, 10H), 7.22–7.19 (m, 2H), 6.22 (t, 1H), 5.92 (s, 1H), 5.37 (d, 1H), 4.52–4.46 (m, 2H), 4.36–4.31 (m, 2H), 3.86–3.83 (m, 1H), 3.77–3.64 (m, 2H), 2.48–2.25 (m, 2H), 0.00 and −0.01 (2xs, 18H); ^{13}C NMR (DMSO-d_6) δ 155.02, 154.44, 148.60, 147.30, 144.02, 143.22, 140.73, 136.92, 128.14, 127.79, 127.06, 125.75, 125.40, 120.13, 119.91, 86.64, 82.88, 75.98, 70.48, 67.39, 63.28, 46.08, 39.62, 1.30; HRMS (ESI) calcd. for $C_{44}H_{52}N_5O_9Si_3$ (M^++H) 878.3067, found 878.3051.

Synthesis of 5′-O-silyl-N protected-2′-deoxynucleoside-3′-O-phosphoramidites

Protected 2′-deoxynucleoside (2 mmol) was dried in vacuo for 6 h and then dissolved in anhydrous dichloromethane (20 mL). Methyl tetraisopropylphosphorodiamidite (2.1 mmol) was added and the mixture was stirred. Tetrazole (2 mmol) was added slowly over 1 h and the solution was allowed to stir for an additional 3 h. A small amount of triethylamine (approximately 0.4 mL) was added to neutralize the solution and the solvent was removed *in vacuo*. The crude product was isolated by chromatography with benzene followed by a gradient of ethyl acetate (0–40 or 100%) in benzene containing 0.1% triethylamine. Triethylamine was excluded during purification of compound **1d** in order to prevent the elimination of the Fmoc protecting group.

Compound **3a** *($B' = Thy^{An}$).* Yield 67.7%; ^{31}P NMR ($CDCl_3$) δ 150.51, 149.50; ^{13}C NMR ($CDCl_3$) δ168.10, 165.26, 163.07, 149.60, 144.03, 143.93, 135.62, 133.24, 128.52, 128.50, 127.65, 127.57, 126.56, 126.41, 126.39, 124.63, 114.65, 111.06, 86.90, 86.54, 86.48, 85.32, 77.28, 74.07, 63.39, 55.82, 50.65, 50.49, 43.28, 43.22, 43.16, 43.10, 40.21, 24.83, 24.76, 13.04, 1.76, 1.72; HRMS (ESI) calcd. for $C_{44}H_{65}N_3O_{11}Si_3P$ (M^++H) 926.3659, found 926.3632.

Compound **3b** *($B' = C^{TMT}$).* Yield 61.3%; ^{31}P NMR ($CDCl_3$) δ 150.3, 150.06; ^{13}C NMR ($CDCl_3$) δ 165.40, 158.70, 155.46, 144.00, 140.88, 136.83, 129.89, 128.36, 128.33, 127.40, 127.33, 126.43, 126.38, 126.36, 113.64, 94.79, 86.16, 86.11, 76.94, 73.73, 73.56, 73.47, 73.30, 69.69, 62.96, 62.89, 55.28, 50.67, 50.51, 43.15, 43.08, 43.02, 42.96, 40.85, 23.08, 23.06, 1.61; HRMS (ESI) calcd. for $C_{57}H_{78}N_4O_{11}Si_3P$ (M^++H) 1109.4707, found 1109.4667.

Compound **3c** *($B' = A^{DMT}$).* Yield 86.4%; ^{31}P NMR ($CDCl_3$) δ 149.77, δ 149.64; ^{13}C NMR ($CDCl_3$) δ 158.42, 154.30, 152.41, 148.80, 145.72,

144.26, 144.21, 138.53, 137.76, 130.32, 129.02, 128.41, 128.39, 128.04, 127.34, 126.95, 126.57, 126.51, 126.49, 121.48, 113.30, 86.89, 86.67, 84.63, 84.58, 76.98, 74.37, 74.20, 74.06, 73.89, 70.76, 63.20, 55.41, 50.77, 50.71, 50.60, 50.54, 43.17, 43.04, 39.63, 24.93, 24.86, 24.80, 24.77, 1.72, 1.69; HRMS (ESI) calcd. for $C_{57}H_{76}N_6O_9Si_3P$ (M^++H) 1103.4713, found 1103.4715.

Compound **3d** *(B′ = G^{Fmoc}).* Yield 37.1%; ^{31}P NMR ($CDCl_3$) δ 149.84, 149.69; ^{13}C NMR ($CDCl_3$) δ 155.86, 153.55, 153.49, 148.47, 148.42, 146.44, 146.38, 144.13, 144.06, 143.02, 142.99, 142.96, 141.53, 137.16, 128.44, 128.42, 128.27, 127.47, 127.39, 126.52, 126.39, 124.98, 121.25, 120.36, 87.07, 86.77, 84.10, 77.07, 74.51, 74.33, 74.06, 68.46, 63.31, 63.24, 50.70, 50.53, 46.81, 43.15, 43.02, 40.19, 24.89, 24.83, 24.76, 1.71, 1.67; HRMS (ESI) calcd. for $C_{51}H_{68}N_6O_{10}Si_3P$ (M^++H) 1039.4036, found 1039.4038.

Synthesis of N2-Trimethoxytrityl-2′-deoxyguanosine

2′-Deoxyguanosine (3.2 g; 11 mmol) was twice co-evaporated with 50 mL pyridine and dried *in vacuo* for 12 h. Anhydrous pyridine (60 mL) and chlorotrimethylsilane (7.1 mL; 56 mmol) were added. After the mixture was stirred at room temperature for 2 h, trimethoxytrityl chloride (4.3 g; 12 mmol) was added. The reaction was stirred overnight (~16 h) at room temperature. Pyridine hydrochloride was filtered. Water (60 mL) and aqueous ammonium hydroxide (2 mL) were added and the reaction mixture was stirred for 30 min. The crude product was extracted into dichloromethane, the organic layer was washed two times with 5% aqueous solution of sodium bicarbonate and dried with anhydrous sodium sulfate. The product was filtered and purified by column chromatography using chloroform/pyridine (99.9:0.1) with a gradient of methanol (0–25%). Yield 68.5%.

Synthesis of 5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl] -N2-trimethoxytrityl-2′-deoxyguanosine

N-Trimethoxytrityl-2′-deoxyguanosine (4.6 g; 7.7 mmol) was dried in vacuo for 12 h and then dissolved in anhydrous N,N-dimethylformamide (80 mL). Imidazole (1.05 g; 15.4 mmol) was added to the mixture and the flask was placed on ice and stirred. Benzhydroxy-bis(trimethylsilyloxy)silyl chloride (4 mL; 9.9 mmol) was added slowly over 2 h via syringe. The flask was then removed from ice and allowed to stir at room temperature for 6 h. The reaction was monitored by TLC. Distilled water (60 mL) was added and solvents were

removed in vacuo to a final volume of 50 mL. The remaining solution was dissolved in dichloromethane and rinsed with aqueous solution of 5% sodium bicarbonate saturated with sodium chloride and dried with anhydrous sodium sulfate. The product was filtered and purified by column chromatography using chloroform/pyridine (99.9:0.1) with a gradient of methanol (0-20%). Yield 47.4%.

Synthesis of 5′-O-silyl-N-trimethoxytrityl-2′-deoxyguanosine 3′-O-phosphoamidite (3e)

5′-O-[benzhydroxy-bis(trimethylsilyloxy)silyl]-2′-deoxyguanosine (3.6 g; 3.6 mmol) was dried in vacuo for 12 h and then dissolved in anhydrous dichloromethane (30 mL). Methyl tetraisopropylphosphorodiamidite (1.2 mL; 4 mmol) was added with stirring. 0.4 M solution of tetrazole in acetonitrile (3.2 mL; 3.6 mmol of tetrazole) was added slowly over 2 h and the reaction mixture was stirred for an additional 2 h. A small amount of triethylamine (approximately 0.4 mL) was added to neutralize the solution and the solvents were removed *in vacuo*. The crude product was isolated by column chromatography with benzene/triethylamine (99:1) followed by gradient of ethyl acetate (0–100%). Yield 75%.

REFERENCES

[1] H. Li, K. Porter, F. Huang, and B. R. Shaw, *Nucleic Acids Res.*, **23,** 4495 (1995).
[2] D. S. Sergueev. Z. A. Sergueeva, and B. R. Shaw, *Nucleosides, Nucleotides, Nucleic Acids*, **20,** 789 (2001).
[3] M. Shimizu, T. Wada, N. Oka, and K. J. Saigo, *J. Org. Chem.*, **69,** 5261 (2004).
[4] A. P. Higson, A. B. Sierzchala, H. A. Brummel, Z. Zhao, and M. H. Caruthers, *Tetrahedron Lett.*, 3899 (1998).
[5] S. A. Scaringe, F. E. Wincott, and M. H. Caruthers, *J. Am. Chem. Soc.*, **120,** 11, 820 (1998).
[6] E. Grons and J. Meinhofer, In *Protection of Functional Groups in Peptide Synthesis* (Academic Press, New York, 1987), Vol. 3.
[7] H. Schaller and H. G. Khorana, *J. Am. Chem. Soc.*, **85,** 3828 (1963).
[8] A. B. Sierzchala, D. J. Dellinger, J. R. Betley, T. Wyrzykiewicz, C. M. Yamada, and M. H. Caruthers, *J. Am. Chem. Soc.*, **125,** 13, 427 (2003).
[9] B. J. Dahl, K. Bjergarde, L. Henrikson, and O. Dahl, *Acta Chem. Scand.*, **44,** 639 (1990).