6-O-(Pentafluorophenyl)-2'-deoxyguanosine: A Versatile Synthon for Nucleoside and Oligonucleotide Synthesis

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The 6-O-(pentafluorophenyl)-2'-deoxyguanosine derivative **2a** can be used to generate in high yield 6-O-methyl-2'-deoxyguanosine, 2,6-diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine, and related derivatives. Further, after appropriate protection and derivatization, **2a** can be incorporated into oligonucleotides and there used for postsynthetic oligonucleotide modification. This approach is particularly useful for preparation of oligonucleotides containing 2,6-diaminopurine residues or their 6-alkylamino derivatives. In addition, reaction of **2a**, or oligonucleotides containing it, with 4-(dimethylamino)pyridine (DMAP) gives a fluorescent guanine–DMAP adduct.

We recently have reported high-yield synthesis of the 6-O-(pentafluorophenyl)-2'-deoxyguanosine derivative **2a** by reaction of deoxyguanosine (**1**) with trifluoroacetic anhydride in pyridine, followed by treatment with pentafluorophenol.[1] The 6-pyridyl intermediate presumably involved in this transformation has been produced by a variety of routes, but generally does not undergo substitution in high yield.[2-8] The strongly basic conditions usually required promote Zincke-type cleavage of the pyridyl group, as evidenced by the dark red colors which accompany such reactions.[9] We reasoned that acidic phenols such as pentafluorophenol or 4-nitrophenol might react with the 6-pyridyl intermediate in pyridine solution without requiring a stronger base, thereby avoiding Zincke cleavage. In fact, under these conditions there is no evidence of Zincke reaction, and the replacement proceeds in high yield. We now report the use of **2a** as a synthon for the high-yield preparation of 6-substituted 2-aminopurine nucleosides and its incorporation into oligonucleotides for postsynthetic transformation.

Synthesis of 6-Substituted 2'-Deoxyguanosine Derivatives. Conversion of **2a** to 2,6-diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**4**) or to 6-O-methyl-2'-deoxyguanosine (**6**) is effected by heating **2a** with, respectively, concentrated aqueous ammonia or sodium methoxide in methanol. The high yields for these transformations are in marked contrast to the modest yields in which either **4** or **6** are obtained from other methods.[1,6,10-12] This route has been used recently for conversion of [1-^{15}N]- and [2-^{15}N]-2'-deoxyguanosines[13] to the corresponding ^{15}N-labeled O^6-MeG derivatives for incorporation into DNA fragments for ^{15}N NMR studies.[14] The fluorescent 6-DMAP derivatives **5a** and **5b** also are obtained in high yield by heating **2a** with 4-(dimethylamino)pyridine (DMAP) in acetonitrile.

Postsynthetic Modification of Oligonucleotides. In addition to serving as a synthon for preparation of these nucleoside derivatives, **2a** also can be incorporated into oligonucleotides, where it can be used for postsynthetic modification. Treatment of **2a** with 4,4'-dimethoxytrityl chloride followed by phosphonylation or phosphitylation gives the H-phosphonate or cyanoethyl amidite derivative **3a** or **3b**.[15] The lability of the N2-trifluoroacetyl group limits the yield of **3a** but is useful after the oligonucleotide synthesis for the final deprotection. More stable N2-acyl groups, such as the isobutyryl group, for example, are very difficult to remove from 2,6-diaminopurine or O^6-methylguanine residues in an oligonucleotide. Furthermore, amino protection of **3a** or **3b** may not be necessary. There are recent reports that, for both phosphoramidite[17,18] and H-phosphonate[19,20] oligonucleotide synthesis, amino protection is not always required. Note that the overall yield for conversion of deoxyguanosine (**1**) to **3a** is comparable to that for standard protection of deoxyguanosine. Thus, this synthon is available for routine use in oligonucleotide synthesis.[21]

We have incorporated **3a** into two oligonucleotides, d[(DMT)GGTT(6-PFP)GTTGG] (**7**, Figure 1A) and d-[(DMT-6-PFP)GGGTTATTGG] (**8**), and explored some postsynthetic modification reactions of these molecules. In general, displacement of the pentafluorophenyl group in these oligomers proved to be significantly slower than was the case for the monomer (**2a**). For example, reaction of **7** with a 25% solution of DBU in methanol to give the corresponding O^6-MeG derivative was less than two-thirds complete even after 5 days. Similarly, overnight treatment of support-bound **7** with aqueous ammonia at room temperature gave little conversion to the corresponding 2,6-

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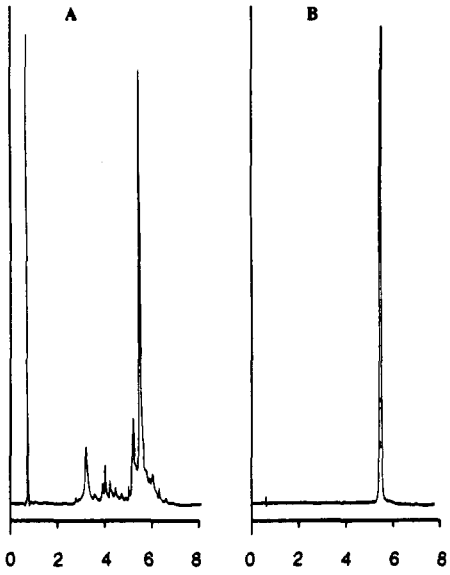

Figure 1. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) crude d[(DMT)GGTT(6-PFP)GTTGG] (**7**), and (B) pure **7**, using a gradient of 2–40% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min.

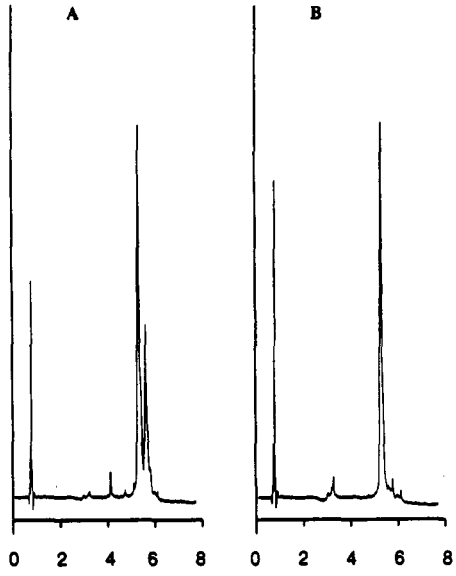

Figure 2. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) the mixture of **7** and d[(DMT)GGTT(2-NH_2)-ATTGG] (**DMT-9**) from purification of **7**, and (B) after heating this mixture with aqueous ammonia for 40 h, using a gradient of 2–40% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min.

diaminopurine oligomer (**DMT-9**). The crude mixture, shown in Figure 1A, was purified by HPLC to give pure **7** (Figure 1B) and a mixture of **7** and **DMT-9** (Figure 2A). Complete conversion of the latter mixture to the 2,6-diaminopurine molecule was effected by heating in aqueous ammonia at 60 °C for 48 h (Figure 2B). HPLC purification and detritylation using 0.1 M acetic acid gave pure **9** (Figure 3A), which was characterized by enzymatic degradation using either venom phosphodiesterase and bacterial alkaline phosphatase or nuclease P1 and calf alkaline phosphatase (Figure 3B).

Heating support-bound **7** or **8** at 60 °C for 39 h in a 10% solution of 1,8-diaminooctane in acetonitrile gave the diaminooctane derivative **10** or **11**, respectively. HPLC of the crude products are shown in Figure 4. The products were eluted from the support with aqueous ammonia (neither was soluble in the diaminooctane/acetonitrile solution), deprotected, and purified by HPLC (Figure 5).

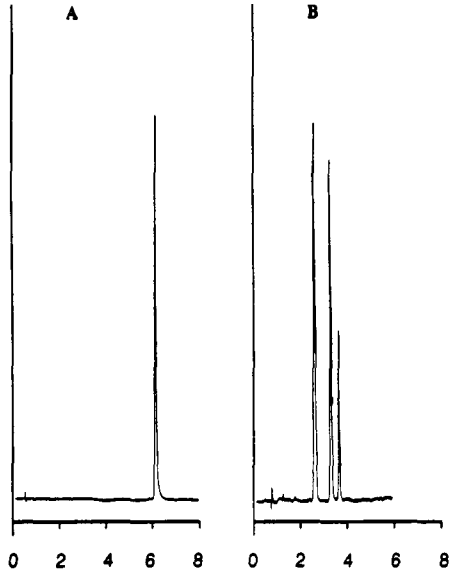

Figure 3. HPLC (monitored at 280 nm) on the C-3 Ultrapore column of (A) **9** after purification and detritylation using a gradient of 2–10% CH_3CN/0.1 M TEAA in 5 min at 2 mL/min, and (B) on the C-18 Nova-Pak column after enzymatic degradation with either venom phosphodiesterase and bacterial alkaline phosphatase (BAP) or nuclease P1 and calf alkaline phosphatase (CAP), using a gradient of 2–15% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min, the peaks in order of elution are dG, dT, and **4a**.

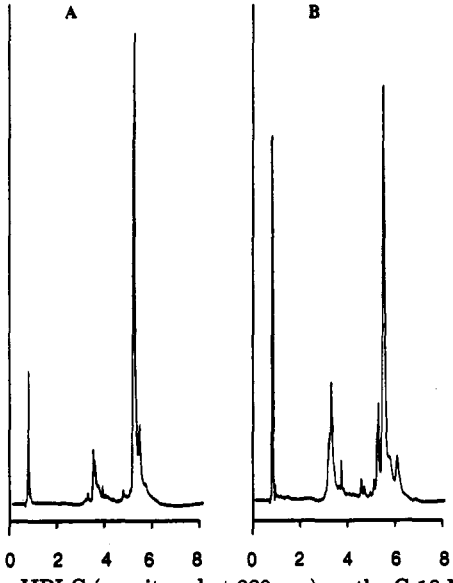

Figure 4. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) crude d{(DMT)GGTT[6-$NH(CH_2)_8NH_2$]GTTGG} (**DMT-10**), and (B) crude d{[DMT-6-$NH(CH_2)_8NH_2$]-GGGTTATTGG} (**DMT-11**) obtained from heating, respectively, support-bound **7** or **8** with 10% 1,8-diaminooctane in acetonitrile, using a gradient of 2–40% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min.

Each was characterized by enzymatic degradation (Figure 6), but **10** was completely degraded only by nuclease P1, a random endonuclease. Venom phosphodiesterase, a 5'-exonuclease, apparently was blocked by the bulky aminooctane moiety. In the case of **11**, where the aminooctane group was at the 5' terminus, both enzymes gave complete degradation.

Treatment of either pure **7** or support-bound **8** with a saturated solution of DMAP in acetonitrile at 60 °C for 2 d gave the 6-DMAP derivative **12** or **13**, respectively. HPLC of the crude products are shown in Figure 7. These molecules are sufficiently stable for deprotection using

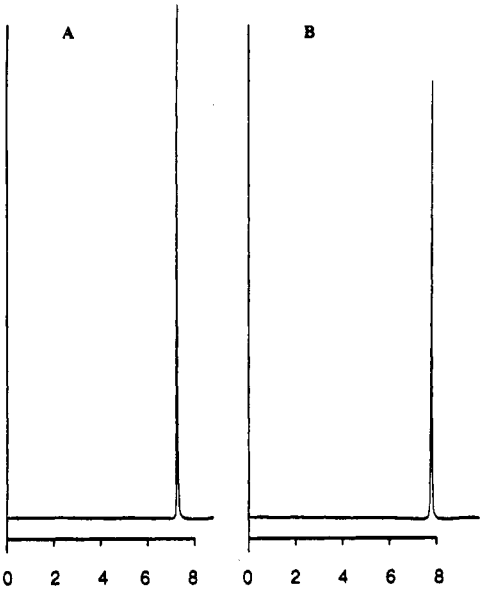

Figure 5. HPLC (monitored at 280 nm) on the C-3 Ultrapore column of (A) pure **10**, and (B) pure **11**, using a gradient of 2–10% CH_3CN/0.1 M TEAA in 5 min at 2 mL/min.

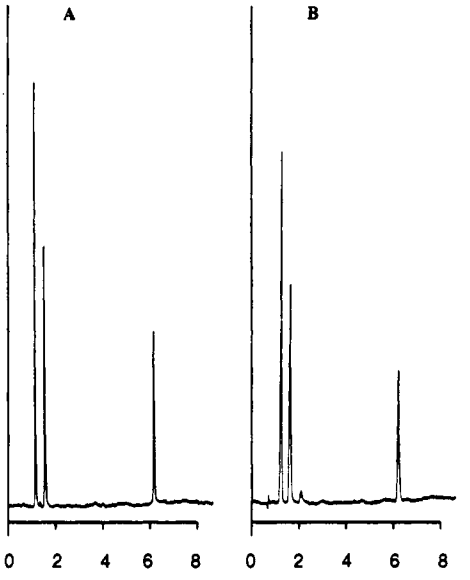

Figure 6. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) **10** after degradation with nuclease P1 and CAP, and (B) **11** after degradation with venom phosphodiesterase and BAP (nuclease P1/CAP gave the same results) using a gradient of 2–15% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min, the peaks in order of elution are dG, dT, dA (for **11**), and **4b**.

aqueous ammonia at room temperature and HPLC purification. The pure DMAP adducts are shown in Figure 8. Each was characterized by enzymatic degradation (Figure 9). The bulky DMAP group, like the aminooctane group, prevented complete degradation of **12** by venom phosphodiesterase, where this group is in the middle of the molecule, but had no effect on degradation of **13**, where it is at the 5′ end.

The extended conjugation in the guanine–DMAP adduct gives rise to absorption maxima in the areas of 310 and 350 nm, well above those of normal nucleic acid bases. The absorption spectra of **5b**, **12**, and **13** are shown in Figure 10. Furthermore, **5b**, like other 6-pyridylpurines,[23] is fluorescent. The fluorescence spectra of **5b**, **12**, and **13** are

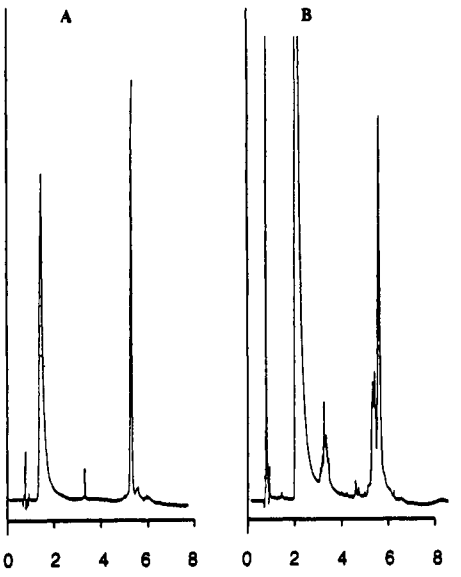

Figure 7. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) crude d{(DMT)GGTT[6-DMAP]GTTGG} (**DMT-12**), and (B) crude d{[DMT-6-DMAP]GGGTTATTGG} (**DMT-13**), obtained from heating, respectively, pure **7** or support-bound **8** with DMAP in acetonitrile, using a gradient of 2–40% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min.

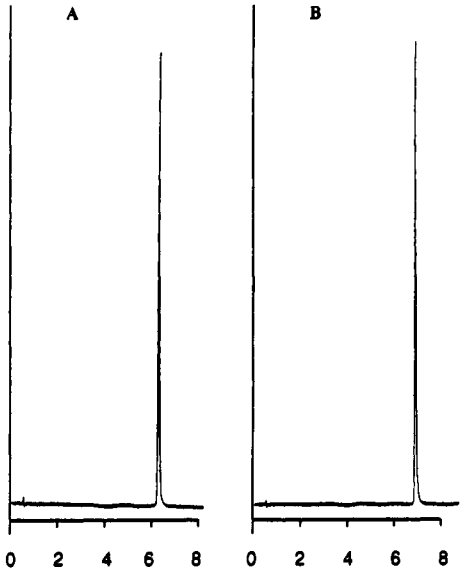

Figure 8. HPLC (monitored at 280 nm) on the C-3 Ultrapore column of (A) pure **12**, and (B) pure **13**, using a gradient of 2–10% CH_3CN/0.1 M TEAA in 5 min at 2 mL/min.

shown in Figure 11. In addition to the very different relative intensities displayed, there is also a shift in emission wavelength from 514 nm for **5b** to 500 nm for **12** and **13**. The corresponding Stoke's shifts are 9700 cm^{-1} for **5b** and 8170 and 8410 cm^{-1}, respectively, for **12** and **13**. This decrease in the Stoke's shift for the guanine–DMAP residues in these DNA fragments, relative to that of the monomer, is consistent with studies of bisbenzimide fluorescence, which showed that the environment in a DNA fragment is less polar than is that of the bulk solvent.[24] The intensity differences suggest that the quenching mechanisms in these molecules differ, perhaps due to the different neighboring bases, different solvent accessibility, and/or to the possible higher-order structures that each may be capable of forming.[25]

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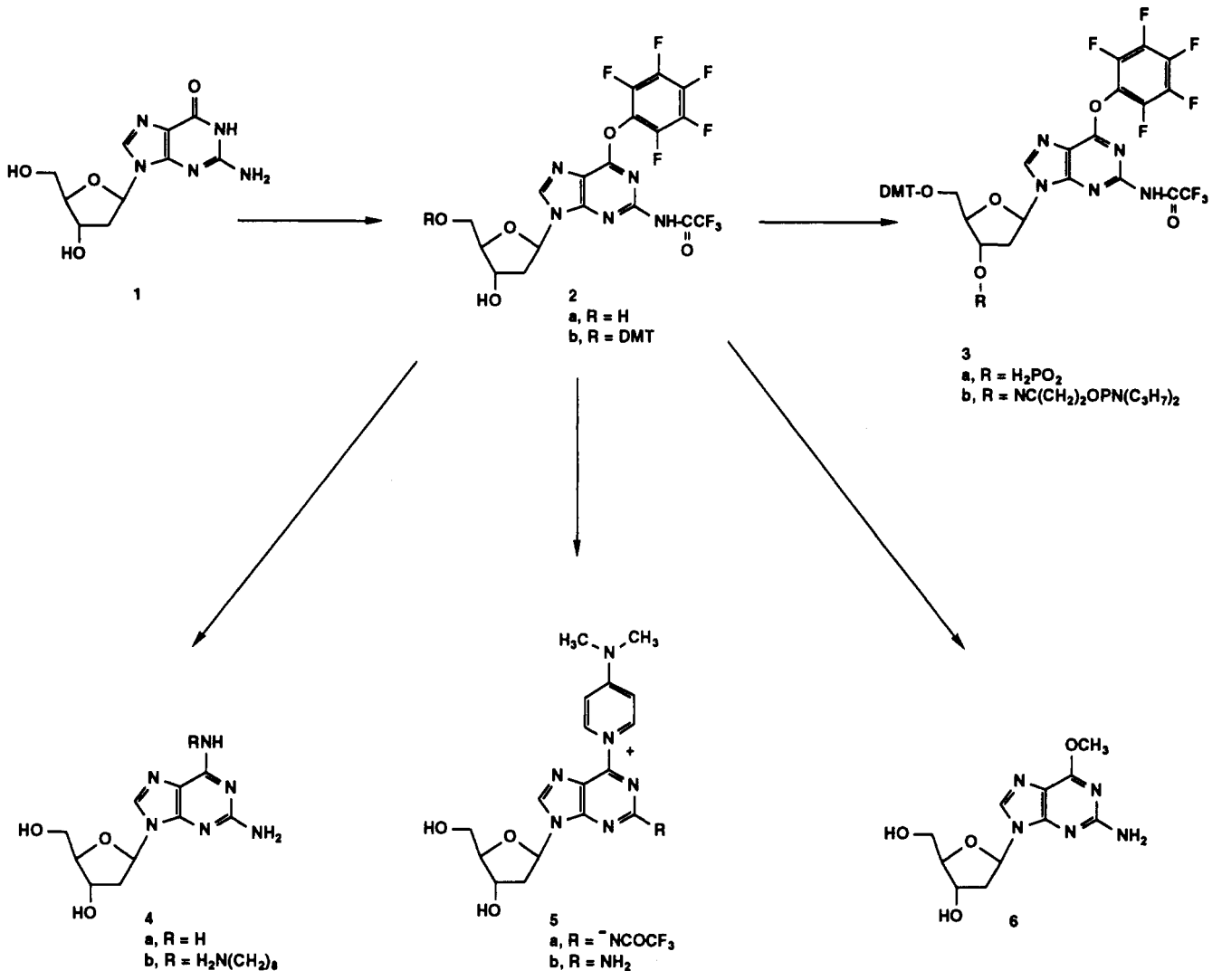
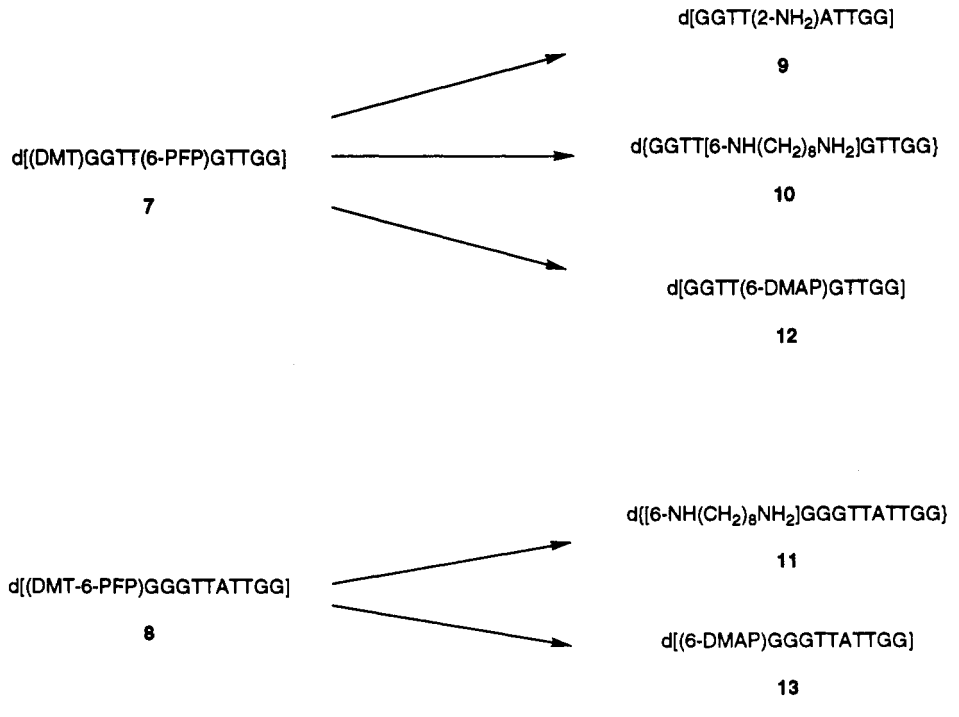
Scheme I



Scheme II



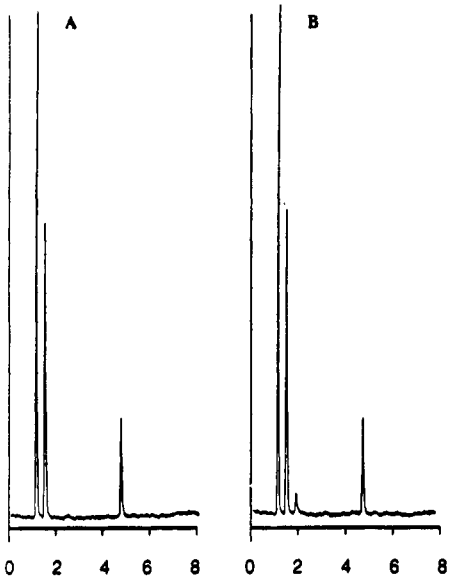


Figure 9. HPLC (monitored at 280 nm) on the C-18 Nova-Pak column of (A) **12**, and (B) **13** after degradation with nuclease P1 and CAP (degradation of **13** with venom phosphodiesterase and BAP gave the same results) using a gradient of 2–15% CH_3CN/0.1 M TEAA in 5 min at 4 mL/min, the peaks in order of elution are dG, dT, dA (for **13**) and **5b**.

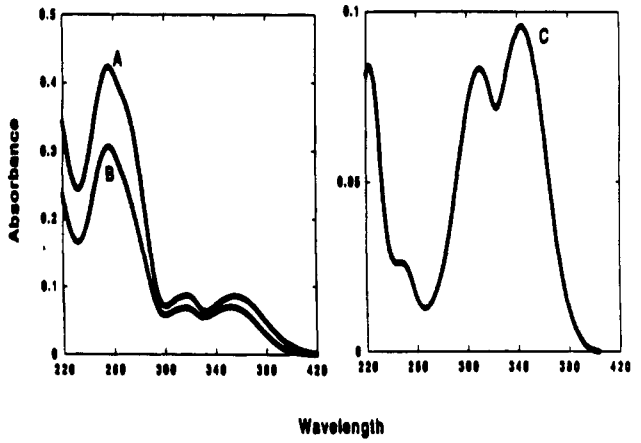


Figure 10. UV–vis spectra of (A) **12**, (B) **13**, and (C) **5b** in 10 mM phosphate, 16 mM sodium, 0.1 mM EDTA, pH 7, at room temperature. The spectra were recorded using a Perkin-Elmer λ 4C spectrometer.

The 6-*O*-(pentafluorophenyl)-2′-deoxyguanosine derivative **2a**, which can be obtained in high yield in a one-flask procedure from 2′-deoxyguanosine, has proven to be a useful synthon for preparation of the 2,6-diaminopurine and 2-amino-6-methoxypurine nucleosides **4** and **6**. Moreover, **2a** may be incorporated into synthetic oligonucleotides and used for postsynthetic modification to afford molecules containing either 2,6-diaminopurine residues, and their 6-aminoalkyl analogs, or fluorescent guanine–DMAP residues.

Experimental Section

General Methods and Reagents. General reagents were purchased from Aldrich Chemical Co.; 2′-deoxyguanosine was obtained from Life Science Resources. TLC was performed on Kieselgel 60F 254 DC-Plastikfolien plates in the solvent systems indicated. Analytical HPLC was carried out using Waters C-18 Nova-Pak cartridges (8 × 100 mm) under the conditions listed in Figure 1. Co-injections were performed to identify the components of the mixtures shown in the figures. Oligonucleotide trityl-on preparative separations employed a Waters C-18 reversed-phase column (19- × 150-mm steel column or a 25- × 100-mm Nova-Pak cartridge) with a gradient of 2–40% acetonitrile/0.1 M triethylammonium acetate (TEAA) in 45 min at a flow rate of 4 mL/min. Detritylation was effected using 0.1 M acetic acid for 20–40 min. The second purification used a Beckman Ultrapore C-3 reversed-phase column (10 × 250 mm) with a gradient of 2–20% acetonitrile/0.1 M triethylammonium acetate in 45 min at a flow rate of 2 mL/min.

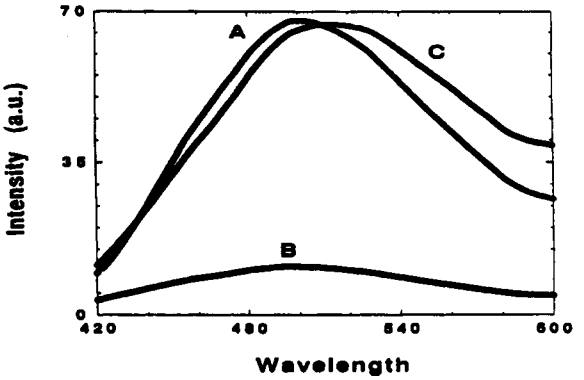


Figure 11. Fluorescence spectra of (A) **12**, (B) **13**, and (C) **5b** in 10 mM phosphate, 16 mM sodium, 0.1 mM EDTA, pH 7, at room temperature. The concentrations of these species were normalized to an absorbance at 350 nm of 0.0192. The spectra were recorded using a Perkin-Elmer MPF-66 spectrometer.

Enzymatic Degradation. A 1 OD_{260} sample of each oligonucleotide was treated with either 10 μL (0.01 unit) of snake venom phosphodiesterase, 10 μL (10 units) of bacterial alkaline phosphatase (BAP), and 75 μL of 0.1 M TEAA buffer (pH 10) or 10 μL (8 units) of nuclease P1, 4 μL (58 units) of calf alkaline phosphatase (CAP), and 75 μL of 0.1 M TEAA buffer (pH 6.5). The mixtures produced were analyzed on the C-18 Nova-Pak cartridge using a gradient of 2–15% acetonitrile/0.1 M TEAA in 5 min at a flow rate of 4 mL/min.

2-(Trifluoroacetamido)-6-[(pentafluorophenyl)oxy]-9-[5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-β-D-*erythro*-pentofuranosyl]purine (2b). To 2.2 g (4.2 mmol) of **2a** dried by evaporation of pyridine and dissolved in 50 mL of pyridine was added 1.9 g (6.3 mmol) of 4,4′-dimethoxytrityl chloride. The mixture was stirred at room temperature for 20 h, and 5 mL of methanol was then added. The mixture was concentrated to about 10 mL, dissolved in 80 mL of diethyl ether, and washed with three 50-mL portions of water. The organic layer was concentrated and the residue purified by chromatography on silica gel using a gradient of 0–5% methanol in methylene chloride containing 5% pyridine in 50 min at a flow rate of 40 mL/min. Concentration of appropriate fractions gave 3.3 g (4.0 mmol, 95%) of **2b**, which was used below without further purification. TLC of **2b** in 10% CH_3OH/CH_2Cl_2 gave an R_f of 0.58, while **2a** had an R_f of 0.25.

2-(Trifluoroacetamido)-6-[(pentafluorophenyl)oxy]-9-[5-*O*-(4,4′-dimethoxytrityl)-2-deoxy-3-*O*-(H-phosphonyl)-β-D-*erythro*-pentofuranosyl]purine (3a). To a stirred solution of 2.2 mL (25 mmol) of PCl_3 in 200 mL of methylene chloride at room temperature under a nitrogen atmosphere was added dropwise 24 mL (222 mmol) of *N*-methylmorpholine followed by 5.7 g (82 mmol) of triazole. After 30 min at room temperature the reaction mixture was cooled in an ice–salt bath and, after an additional 30 min in the cold, 4.1 g (5 mmol) of **2a**, dried three times by evaporation of acetonitrile and dissolved in 150 mL of methylene chloride, was added dropwise over 15 min. The reaction mixture was stirred at room temperature for 30 min and then partitioned with 200 mL of a solution of pyridine/water (10/90). The aqueous layer was further extracted with two 100-mL portions of methylene chloride. The combined organic layers were concentrated, and the residue was purified by chromatography on silica gel using a gradient of 0–18% methanol in methylene chloride containing 3% pyridine in 30 min at a flow rate of 48 mL/min. The product fractions were immediately partitioned with cold 0.1 M DBU-bicarbonate, and the aqueous layer extracted

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with three 100-mL portions of methylene chloride. The combined organic layers were concentrated to give 3.4 g (3.2 mmol, 65%) of **3a**, which was used below without further purification. TLC of **3a** in 20% CH_3OH/CH_2Cl_2 gave an R_f of 0.79, while in this solvent system **2b** had an R_f of 0.31. HPLC of **3a** gave an elution time of 5.62 min using a gradient of 10–80% CH_3CH/0.1 M TEAA in 5 min at 4 mL/min.

2,6-Diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-purine (4a). To 0.44 g (0.83 mmol) of **2a** was added 30 mL of concentrated aqueous ammonia. The mixture was heated (sealed tube) at 55 °C for 38 h, cooled, and concentrated to dryness. The residue was purified by reversed-phase HPLC using a gradient of 2–15% acetonitrile in water in 30 min at a flow rate of 16 mL/min. Concentration of appropriate fractions gave 0.18 g (79%) of **4a** which was identical to material prepared according to the literature.[1,11]

2-(Trifluoroacetimido)-6-[4-(dimethylamino)pyridyl]-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (5a) and 2-Amino-6-[4-(dimethylamino)pyridyl]-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine Trifluoroacetate (5b). To a solution of **2a** (1.6 g, 3 mmol) in 60 mL of acetonitrile was added 3.7 g (30 mmol) of DMAP. The mixture was heated at reflux for 18 h, during which time **5a** precipitated. The mixture was cooled to room temperature and filtered to give **5a** (1.18 g, 2.5 mmol, 84%). UV (H_2O), max 337 nm. Anal. Calcd for $C_{19}H_{20}N_7O_4F_3 \cdot {}^1/_2H_2O$: C, 47.90; H, 4.44; N, 20.58; F, 11.96. Found: C, 48.18; H, 4.36; N, 20.39; F, 11.99. Because of the limited solubility of **5a** in all solvents examined, the ^1H NMR spectrum was determined by allowing a suspension of **5a** in D_2O to stand overnight to hydrolyze to **5b**, which is soluble, δ (ppm) 9.05 (d, 2, J = 7.3 Hz, Ar), 8.28 (s, 1, Hg), 7.04 (d, 2, J = 7.7 Hz, Ar), 6.35 ("t", 1, J_{app} = 5.9 Hz, $H_{1'}$), 4.66 (m, 1, $H_{3'}$), 4.17 (m, 1, $H_{4'}$), 3.84 (m, 2, $H_{5'}$ and $H_{5''}$), 3.38 (s, 6, NMe_2), 2.78 and 2.56 (m and m, 1 and 1, $H_{2'}$ and $H_{2''}$). UV (H_2O, **5b**), max 312 and 442 nm; min 324 nm.

6-O-Methyl-2'-deoxyguanosine (6). To a solution of **2a** (0.53 g, 1 mmol) in 20 mL of methanol was added 0.54 g (10 mmol) of sodium methoxide. The mixture was heated at 55 °C for 36 h and concentrated to dryness. The residue was dissolved in water and neutralized by addition of acetic acid. The mixture was concentrated to a small volume and purified by reversed-phase HPLC using a gradient of 2–10% acetonitrile in water in 30 min at a flow rate of 16 mL/min. Concentration of appropriate fractions gave 0.24 g (85%) of **6**, which was identical to material prepared according to the literature.[1]

Oligonucleotide Synthesis. The syntheses of d[(DMT)-GGTT(6-PFP)GTTGG] (**7**) and d[(DMT-6-PFP)-GGGTTATTGG] (**8**) were carried out by the H-phosphonate method described in detail elsewhere.[26,27] After completion of the syntheses both **7** and **8** were oxidized, but the 5'-DMT moiety was not removed.

d[(DMT)GGTT(6-PFP)GTTGG] (7). A portion of support-bound **7** was treated with concentrated aqueous ammonia at room temperature for 18 h. After filtration and lyophilization crude **7** was purified by HPLC to give 532 OD_{260} of pure **7** and 381 OD_{260} of a mixture of **7** and a longer retention (HPLC) component identified as the corresponding 2,6-diaminopurine adduct (**DMT-9**).

d[GGTT(2-NH_2)ATTGG] (9). The 381 OD_{260} mixture obtained from ammonolysis and purification of **7** was treated with concd aqueous ammonia at 60 °C for 2 d. HPLC of the crude product now showed a single main component, which was purified by HPLC to give a 211 OD_{260} fraction of the pure 2,6-diamino adduct. This was detritylated by treatment with 13 mL of 0.1 M acetic acid (solution pH 3.3) for 20 min, after which the solution was neutralized by addition of a few drops of aqueous ammonia and lyophilized. Final HPLC purification (C-3 column) gave a pure fraction of 102 OD_{260} of **9** along with a 50 OD_{260} fraction that contained a minor impurity. Enzymatic degradation of a sample of **9** using either venom phosphodiesterase and BAP or nuclease P1 and CAP gave the expected ratio of dG, dT, and 2,6-diamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine.

d{GGTT[6-NH(CH_2)$_8$$NH_2$]GTTGG} (10). A portion of **7**, still attached to the support, was treated with 2 mL of a 10% solution of 1,8-diaminooctane in acetonitrile in a sealed vial at 60 °C for 46 h. The mixture was filtered and the support washed with acetonitrile. HPLC showed that the filtrate did not contain nucleotidic material. The support was further washed with aqueous ammonia to elute the product. After standing for 3 d in the ammonia solution the mixture was concentrated and lyophilized and the residue purified (C-18) to give a 332 OD_{260} fraction of the pure product. This was lyophilized and then detritylated by treatment with 10 mL of 0.1 M acetic acid (solution pH 3.3) for 25 min, after which the solution was neutralized by addition of a few drops of aqueous ammonia and lyophilized. Final HPLC purification (C-3) gave 191 OD_{260} of pure **10**. Enzymatic degradation of a sample of **10** using nuclease P1 and CAP gave the expected ratio of dG, dT, and **4b**.

d{[6-NH(CH_2)$_8$$NH_2$]GGGTTATTGG} (11). A portion of **8**, still attached to the support, was treated with 2 mL of a 10% solution of 1,8-diaminooctane in acetonitrile in a sealed vial at 60 °C for 46 h. The mixture was filtered and the support washed with acetonitrile. HPLC showed that the filtrate did not contain nucleotidic material. The support was further washed with concentrated aqueous ammonia to elute the product. After standing for 3 d in the ammonia solution the mixture was concentrated and lyophilized and the residue purified (C-18) to give a 201 OD_{260} fraction of the pure product. This was lyophilized and then detritylated by treatment with 10 mL of 0.1 M acetic acid (solution pH 3.3) for 22 min, after which the solution was neutralized by addition of a few drops of aqueous ammonia and lyophilized. Final HPLC purification (C-3) gave 137 OD_{260} of pure **11**. Enzymatic degradation of a sample of **11** using either venom phosphodiesterase and BAP or nuclease P1 and CAP gave the expected ratio of dG, dT, dA, and **4b**.

d[GGTT(6-DMAP)GTTGG] (12). A 190 OD_{260} portion of **7** was treated with 5 mL of a saturated solution of DMAP in a 1:4 mixture of water and acetonitrile at 60 °C for 44 h. HPLC showed complete conversion of **7** to a new component with a slightly shorter retention. The mixture was concentrated to dryness, and the dried residue was triturated with anhydrous acetonitrile (5×) and pentane (3×). The residue was then treated with aqueous ammonia at room temperature for 2 d, concentrated, and lyophilized. HPLC purification (C-18) gave a pure fraction of 144 OD_{260}. This was lyophilized and then detritylated by treatment with 18 mL of 0.1 M acetic acid (solution pH 3.2) for 15 min, after which the solution was neutralized by addition of a few drops of aqueous ammonia and lyophilized. Final HPLC purification (C-3) gave 95 OD_{260} of pure **12**. Enzymatic degradation of a sample of **12**, using nuclease P1 and CAP, gave the expected ratio of dG, dT, and **5**.

d[(6-DMAP)GGGTTATTGG] (13). A portion of **8**, still attached to the support, was treated with 4 mL of saturated DMAP in acetonitrile in a sealed vial at 60 °C for 48 h. The mixture was cooled and filtered, and the support was washed with acetonitrile. The support was then treated with aqueous ammonia at room temperature for 3 d and filtered, and the filtrate was concentrated and lyophilized. Purification by HPLC (C-18) gave a pure fraction of 131 OD_{260}. This was lyophilized and then detritylated by treatment with 14 mL of 0.1 M acetic acid (solution pH 3.3) for 13 min, after which the solution was neutralized by addition of a few drops of aqueous ammonia and lyophilized. Final HPLC purification (C-3) gave 76 OD_{260} of pure **13**. Enzymatic degradation of a sample of **13** using either venom phosphodiesterase and BAP or nuclease P1 and CAP gave the expected ratio of dG, dT, dA, and **5**.

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