O-Allyl Protection of Guanine and Thymine Residues in Oligodeoxyribonucleotides

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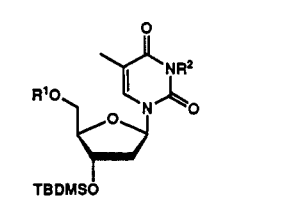
Received March 8, 1993

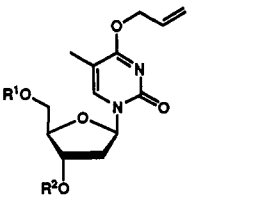
In oligonucleotide synthesis based on the phosphotriester approach, arenesulfonyl azole condensing agents often react with guanosine or thymidine at the O^6- or O^4-position, and the resulting sulfonates are displaced with azoles, generated in the reaction, to give the corresponding azolyl by-products.[2] Synthesis via the phosphoramidite method also brings about base modification at the highly nucleophilic oxygen atoms.[3] Accordingly, many protecting groups such as *o*-nitrophenyl,[4] 2-cyanoethyl,[5] 2-(*p*-nitrophenyl)ethyl,[5,6] and diphenylcarbamoyl[7] have been used to avoid these undesired side reactions. Strong bases, such as concentrated ammonia or diazabicyclo[5.4.0]undecene (DBU), are needed to remove these protectors, which in turn cause cleavage of internucleotide linkages. This paper describes the utility of an allyl protector for the oxygens which can be deblocked under very mild conditions by a palladium(0)-catalyzed reaction.

Protection. The allyl group was introduced to the O^4-position of the thymidine derivative **1** in two steps by base-aided condensation with 2-mesitylenesulfonyl chloride in dichloromethane followed by the reaction of the resulting sulfonate with allyl alcohol, giving **4** in 81% overall yield.[8] Attempted reaction of **1** with allyl alcohol in the presence of diethyl azodicarboxylate and triphenylphosphine in THF[9] gave the undesired $N(3)$-allyl derivative **2**, exclusively, in 98% yield. The two-step O-allylation of the N^2-unprotected and -allyloxycarbonylated deoxyguanosines **8** and **9** gave **10** and **11** in 87 and 80% overall yields, respectively.

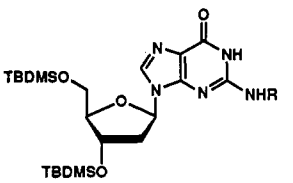
The structures of the *O*- and *N*-allyl products were determined by investigating the cross peaks between the allylic methylene protons and heteroaromatic carbons in the 500-MHz ^1H,^{13}C COLOC NMR spectra.[10] The spectrum of the *O*-allyl thymidine **5** (Figure 1) showed a single corresponding signal at δ 4.80/170 ppm, which arises from the coupling of the methylene protons and C-4 atom of thyminyl base. On the other hand, in the 2D-spectrum of the *N*-allyl isomer **3** (Figure 1), two signals were detected at δ 4.48/150 and 4.48/163 ppm due to the coupling of the methylene protons and the C-2 and C-4 atoms, respectively. Similarly, the *O*-allyl product **10** (Figure 2) showed only one ^1H–^{13}C coupling signal at δ 4.87/161 ppm.



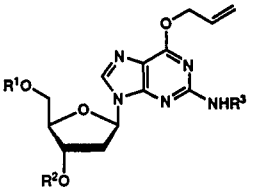
1, R^1 = DMTr; R^2 = H
2, R^1 = DMTr; R^2 = CH_2=$CHCH_2$
3, R^1 = H; R^2 = CH_2=$CHCH_2$



4, R^1 = DMTr; R^2 = TBDMS
5, R^1 = H; R^2 = TBDMS
6, R^1 = DMTr; R^2 = H
7, R^1 = H; R^2 = AOC



8, R = H
9, R = AOC



10, R^1 = R^2 = TBDMS; R^3 = H
11, R^1 = R^2 = TBDMS; R^3 = AOC
12, R^1 = DMTr; R^2 = TBDMS; R^3 = AOC
13, R^1 = H; R^2 = TBDMS; R^3 = AOC
14, R^1 = DMTr; R^2 = H; R^3 = AOC

Stability. The *O*-allyl protector is stable under the conditions for removal of the *O*-(dimethoxytrityl) (DMTr) or *O*-(*tert*-butyldimethylsilyl) (TBDMS) groups at the C-3′ and C-5′ positions. Thus, when **4** was exposed to dichloroacetic acid in dichloromethane, the detritylated product **5** was obtained in 96% isolated yield. In a similar manner, **12** was detritylated to give **13** in 83% yield. When **4** was treated with tetrabutylammonium fluoride (TBAF) in THF, the TBDMS group was selectively removed to produce **6** in 82% isolated yield. Desilylation of **12** afforded **14** in 97% yield.[11] The *O*-allyl protector is also left intact in the preparation of oligonucleotides via the phosphotriester approach. Condensation of the O^6-allyldeoxyguanosine-3′-phosphodiester **15** and the 5′-O-free O^4-allylthymidine derivative **7** using 1-(2,4,6-triisopropylbenzenesulfonyl)-3-nitrotriazole (TPS-NT) in pyridine afforded, after deblocking of the 5′-*O*-DMTr group by treatment with dichloroacetic acid, the dinucleoside phosphate **17** in 69% isolated yield. No triazolylation at the guanyl-O^6 or the thyminyl-O^4 position was observed in the coupling reaction. *O*-Allyl protection is also useful for internucleotide-bond formation by the phosphoramidite method. Thus, the 1*H*-tetrazole-assisted coupling of **16** and **7** was achieved without any side reactions. The oxidation with *tert*-butyl hydroperoxide (TBHP)[12] followed by detritylation gave **17** in 95% overall yield.

Deprotection. The allyl protector is extremely sensitive to a palladium(0) complex in the presence of a

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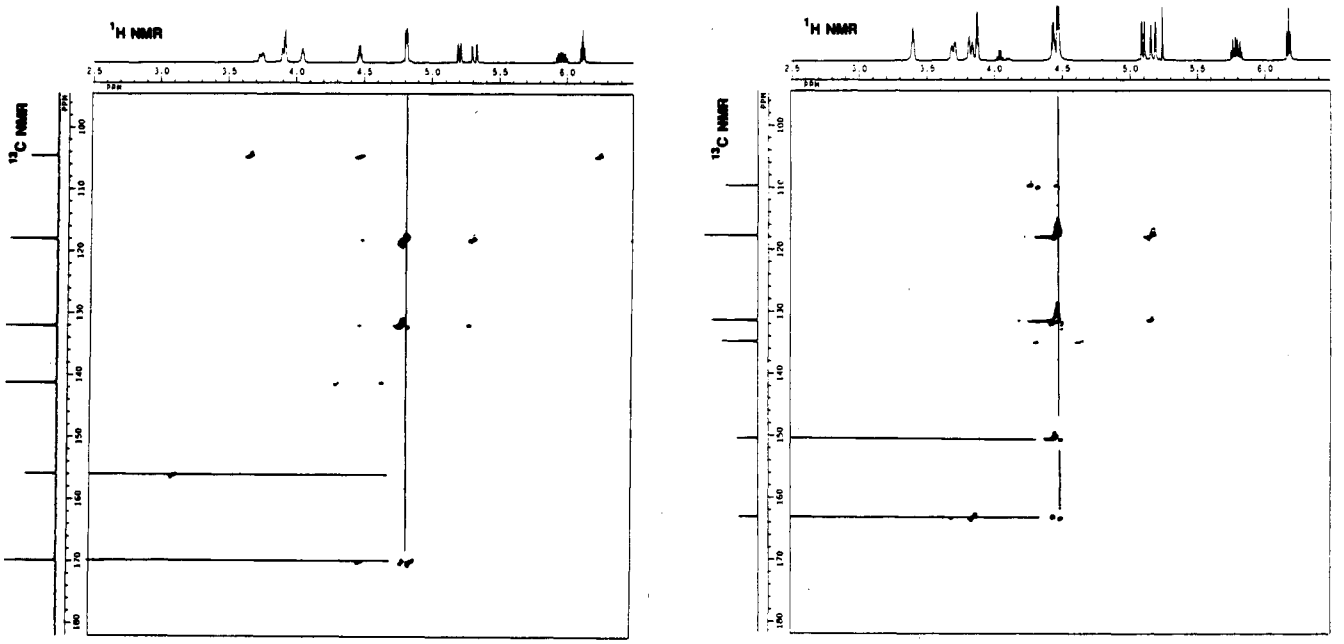


Figure 1. The 500-MHz ^1H, ^{13}C COLOC NMR spectra of the $N(3)$-allylthymidine **3** (left) and the O^4-allylthymidine **5** (right).

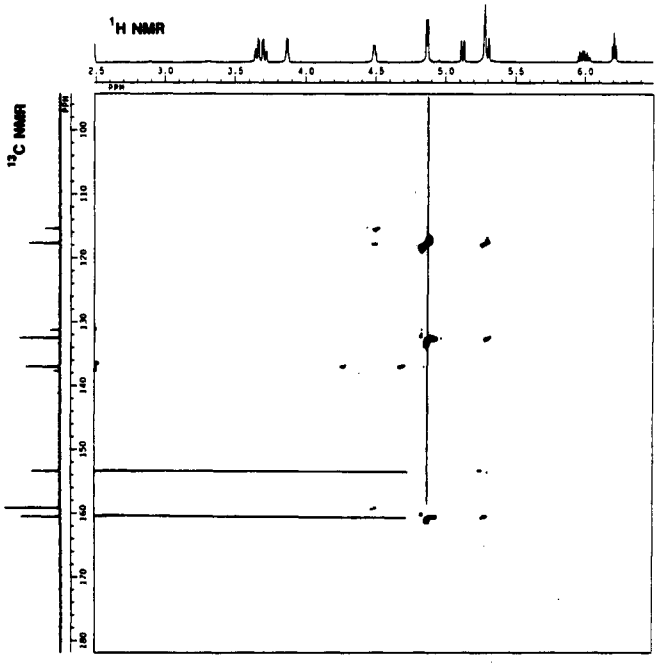
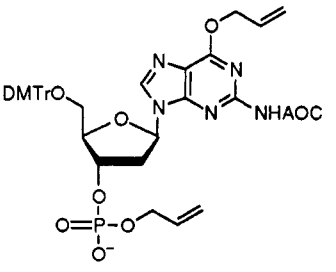


Figure 2. The 500-MHz ^1H, ^{13}C COLOC NMR spectrum of the O^6-allyldeoxyguanosine derivative **10**.

nucleophile.[13] Thus, when **4** was briefly treated with a mixture of 5 mol % of $Pd[P(C_6H_5)_3]_4$, 3 mol % of $P(C_6H_5)_3$, and excess diethylammonium hydrogencarbonate in dichloromethane at 25 °C, the allyl protector was removed selectively to give **1** in 98% yield. The deallylation of **10** was accomplished in a similar way to give **8** in 87% yield. Removal of the *O*-allyl and N^2-(allyloxycarbonyl) protectors in **11** was simultaneously carried out to produce **8** in quantitative yield. Further, the five allylic protectors in the multiprotected dimer **17** were deblocked simultaneously to afford the unprotected dGpT (**18**) in 90% isolated yield. The deprotection was accomplished without cleavage of the internucleotide linkage. The *N*-allyl group of **2**, unlike *O*-allylic groups, could not be removed by the palladium-catalyzed reaction.



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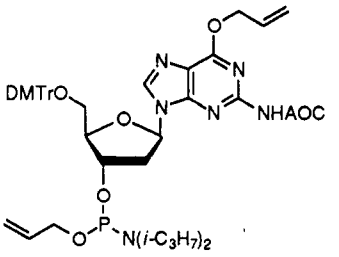

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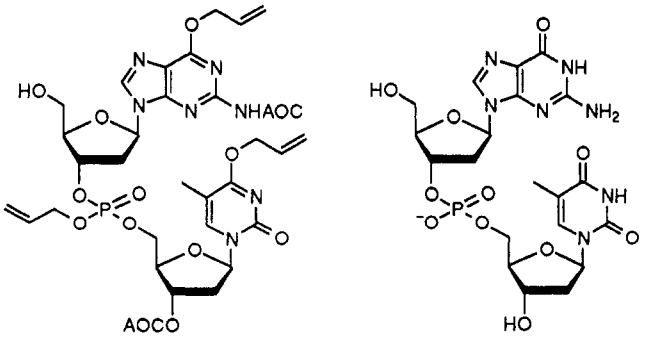

17 **18**

Experimental Section

General Methods. General experimental conditions and spectroscopic instrumentation used have been described.[13] Chromatography was performed on a column of E. Merck Kieselgel

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60 (70–230 mesh) using the stated solvent(s) as eluent. IR spectra were obtained in KBr unless otherwise noted. All UV and NMR spectra were measured in CH_3OH and $CDCl_3$, respectively.

Materials. The protected nucleosides 1,[14] 8,[13] and 9[13] were prepared by the known method.

Diethylammonium Hydrogencarbonate. Into a vigorously stirred mixture of diethylamine (106 g, 150 mL, 1.45 mmol), water (26.0 g, 1.44 mmol), and ether (300 mL) was introduced carbon dioxide gas through a glass tube at room temperature for 30 min. The occurring crystals were collected by filtration and dried in vacuo at room temperature. Diethylammonium hydrogencarbonate (121g, 62%) thus obtained was stored at −20 °C and employed without further purification in the Pd-catalyzed deprotection.

***N*(3)-Allyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p,p*′-dimethoxytrityl)thymidine (2).** A solution of 1 (5.03 g, 7.63 mmol), triphenylphosphine (3.05 g, 11.6 mmol), diethyl azodicarboxylate (1.99 g, 1.8 mL, 11.4 mol), and allyl alcohol (4.70 g, 5.5 mL, 80.9 mmol) in THF (50 mL) was stirred at room temperature overnight. The reaction mixture was diluted with a 1:1 mixture of ethyl acetate and hexane (150 mL) and washed with water (200 mL × 2) followed by brine (200 mL × 2). Concentration of the organic solution gave a residual oil, whose chromatography (1.5:1 ether/hexane containing a trace amount of triethylamine) afforded 2 (5.09 g, 98%): IR 1705, 1671 cm^{-1}; UV λ_{max} 270 (ε 10 000), 233 nm (22 000); ^1H NMR δ −0.07 and −0.01 (two s's, 6 H, $(CH_3)_2Si$), 0.80 (s, 9 H, t-C_4H_9Si), 1.51 (s, 3 H, CH_3C=), 2.10–2.37 (m, 2 H, H-2′ × 2), 3.23 (dd, 1 H, J = 10.8, 3.1 Hz, H-5′), 3.44 (dd, 1 H, J = 10.8, 3.1 Hz, H-5′), 3.76 (s, 6 H, CH_3O × 2), 3.94 (d, 1 H, J = 3.0 Hz, H-4′), 4.47 (dt, 1 H, J = 3.1, 6.2 Hz, H-3′), 4.53 (d, 2 H, J = 5.4 Hz, CH_2=$CHCH_2$), 5.16 (dd, 1 H, J = 10.0, 1.5 Hz, *cis*-CH_2=$CHCH_2$), 5.25 (dd, 1 H, J = 17.0, 1.5 Hz, *trans*-CH_2=$CHCH_2$), 5.87 (ddt, 1 H, J = 17.0, 10.0, 5.4 Hz, CH_2=$CHCH_2$), 6.35 (t, 1 H, J = 6.9 Hz, H-1′), 6.80 (d, 4 H, J = 8.9 Hz, ortho protons to CH_3O of DMTr), 7.23–7.39 (m, 9 H, aromatic protons of DMTr), 7.93 (s, 1 H, H-6). Anal. Calcd for $C_{40}H_{50}N_2O_7Si$: C, 68.73; H, 7.22; N, 4.01. Found: C, 68.90; H, 7.08; N, 3.90.

Preparation of *O*⁴-Allyl-3′-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-(*p,p*′-dimethoxytrityl)thymidine (4). A solution of 1 (1.86 g, 2.82 mmol), triethylamine (1.16 g, 5.83 mmol), DMAP (38.4 mg, 0.31 mmol), and 2-mesitylenesulfonyl chloride (1.28 g, 5.83 mmol) in dichloromethane (60 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with dichloromethane and washed with water followed by a sodium hydrogencarbonate solution. Concentration of the organic layer gave a foamy residue, which, after drying in vacuo, was dissolved in dichloromethane (30 mL). To the solution were added allyl alcohol (2.56 g, 3.00 mL, 44.1 mmol) and trimethylamine (2.62 g, 4.00 mL, 44.4 mmol)[15] at 0 °C, and the resulting mixture was stirred at the same temperature for 10 min. DBU (0.46 g, 0.45 mL, 3.01 mmol) was added, and stirring was continued for an additional 10 h at 0 °C. The reaction mixture was poured into dichloromethane, washed with water and brine, and concentrated. The crude product was chromatographed (1:2 ethyl acetate/hexane) to give 4 (1.59 g, 81% yield): IR1674, 1609 cm^{-1}; UV λ_{max} 282 (ε 9500), 228 nm (sh, 25 000); ^1H NMR δ −0.10 and −0.03 (two s's, 6 H, $(CH_3)_2Si$), 0.78 (s, 9 H, t-C_4H_9Si), 1.51 (s, 3 H, CH_3C=), 2.18 (ddd, 1 H, J = 13.9, 6.2, 5.4 Hz, H-2′), 2.48 (ddd, 1 H, J = 13.9, 6.2, 5.4 Hz, H-2′), 3.23 (dd, 1 H, J = 10.4, 3.0 Hz, H-5′), 3.50 (dd, 1 H, J = 10.4, 2.5 Hz, H-5′), 3.76 (s, 6 H, CH_3O × 2), 3.94 (dt, 1 H, J = 4.6, 2.3 Hz, H-4′), 4.45 (dd, 1 H, J = 10.9, 5.0 Hz, H-3′), 4.87 (d, 2 H, J = 5.4 Hz, CH_2=$CHCH_2$), 5.22 (dd, 1 H, J = 10.4, 1.5 Hz, *cis*-CH_2=$CHCH_2$), 5.34 (dd, 1 H, J = 17.3, 1.5 Hz, *trans*-CH_2=$CHCH_2$), 6.01 (ddt, 1 H, J = 17.3, 10.4, 5.4 Hz, CH_2=$CHCH_2$), 6.29 (t, 1 H, J = 5.9 Hz, H-1′), 6.80 (d, 4 H, J = 8.9 Hz, ortho protons to CH_3O of DMTr), 7.23–7.39 (m, 9 H, aromatic protons of DMTr), 7.93 (s, 1 H, H-6). Anal. Calcd for $C_{40}H_{50}N_2O_7Si$: C, 68.73; H, 7.22; N, 4.01. Found: C, 68.81; H, 7.00; N, 3.94.

***O*⁶-Allyl-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (10).** To a stirred solution of the compound 8 (10.7 g, 21.6 mmol) in a mixture of HMPA (30 mL) and dichloromethane (150 mL) were added triethylamine (12.0 mL, 8.71 g, 86.1 mmol), DMAP (580 mg, 4.25 mmol), and 2-mesitylenesulfonyl chloride (11.0 g, 50.2 mmol). After being stirred at room temperature overnight, the resulting mixture was diluted with ether (500 mL). The organic solution was washed with a saturated solution of sodium hydrogencarbonate (200 mL × 2) and brine (200 mL × 2), dried, and concentrated to give a semisolid product. The crude material was treated with hexane containing a small amount of ether, and the occurring crystalline *O*⁶-(2-mesitylenesulfonyl)-2′-deoxyguanosine intermediate (14.5 g, 99%) was collected by filtration. A solution of the sulfonylated product (4.65 g, 6.85 mmol) was mixed at 0 °C with molecular seives 3A (1.52 g), trimethylamine[15] (9.0 mL, 5.90 g, 99.9 mmol), and allyl alcohol (7.0 mL, 5.98 g, 103 mmol), and the mixture was stirred at the same temperature for 30 min. To this solution was added DBU (1.6 mL, 1.63 g, 10.7 mmol), and stirring was continued for an additional 12 h. The reaction mixture was warmed to room temperature, and the occurring precipitates and molecular sieves were removed by filtration. The filtrate was diluted with dichloromethane (100 mL) and washed with water (150 mL), a saturated ammonium chloride solution (100 mL × 2), and brine (100 mL × 2). The organic solution was dried and evaporated to give an oil, which was chromatographed by silica gel short column using a 1:2 mixture of ethyl acetate and hexane as an eluent to afford 10 as colorless crystals (3.23 g, 88% yield): IR1641, 1359 cm^{-1}; UV λ_{max} 283 (ε 11 000), 247 nm (11 000); ^1H NMR δ 0.07 and 0.10 (two s's, 12 H, $(CH_3)_2Si$ × 2), 0.91 (s, 18 H, t-C_4H_9Si × 2), 2.30–2.42 (m, 1 H, H-2′), 2.51–2.64 (m, 1 H, H-2′), 3.74 (dd, 1 H, J = 10.6, 4.1 Hz, H-5′), 3.81 (dd, 1 H, J = 10.6, 4.1 Hz, H-5′), 3.97 (dd, 1 H, J = 6.7, 3.8 Hz, H-4′), 4.58 (dt, 1 H, J = 5.6, 3.8 Hz, H-3′), 4.83 (br s, 2 H, NH_2), 5.00 (dt, 2 H, J = 5.9, 1.5 Hz, CH_2=$CHCH_2$), 5.26 (ddd, 1 H, J = 10.4, 2.9, 1.5 Hz, *cis*-CH_2=$CHCH_2$), 5.42 (ddd, 1 H, J = 16.9, 2.9, 1.5 Hz, *trans*-CH_2=$CHCH_2$), 6.12 (ddt, 1 H, J = 16.9, 10.4, 5.9 Hz, CH_2=$CHCH_2$), 6.32 (t, 1 H, J = 6.7 Hz, H-1′), 7.90 (s, 1 H, H-8). Anal. Calcd for $C_{25}H_{45}N_5O_4Si_2$: C, 56.02; H, 8.48; N, 13.07. Found: C, 56.02; H, 8.56; N, 13.14.

***O*⁶-Allyl-*N*²-(allyloxycarbonyl)-3′,5′-*O*-bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (11).** A mixture of 9 (11.2 g, 19.4 mmol), triethylamine (11 mL, 7.99 g, 78.9 mmol), DMAP (278 mg, 2.27 mmol), and 2-mesitylenesulfonyl chloride (5.54 g, 25.3 mmol) in dichloromethane (100 mL) was stirred at room temperature for 3 h. The mixture was poured into dichloromethane (200 mL), washed with water (100 mL × 2) and a sodium hydrogencarbonate solution (100 mL × 2), and dried. Concentration of the organic solution gave the sulfonylated intermediate as a residual oil (15.2 g), which was dried in vacuo. To a solution of the dried product in dichloromethane (100 mL) were added at 0 °C with stirring trimethylamine (27 mL, 17.7 g, 300 mmol) and allyl alcohol (20 mL, 17.1 g, 290 mmol). The resulting mixture was stirred at the same temperature for 10 min. To this solution was added DBU (3 mL, 3.05 g, 20.1 mmol), and stirring was continued overnight. The reaction mixture was warmed to room temperature, and the resulting precipitates were removed by filtration. The filtrate was diluted with dichloromethane (200 mL) and washed with water (200 mL × 2) and brine (200 mL × 2). The organic layer was concentrated to give an oil. Chromatography of the crude material on silica gel (100 g) eluted with a 1:2 mixture of ethyl acetate and hexane afford 11 as a colorless syrup (9.64 g, 80% overall): IR ($CHCl_3$)1754, 1607 cm^{-1}; UV λ_{max} 269 nm (ε 16 000); ^1H NMR δ 0.07 and 0.09 (two s's, 12 H, $(CH_3)_2Si$ × 2), 0.90 and 0.91 (tow s's, 18 H, t-C_4H_9Si × 2), 2.28–2.45 (m, 1 H, H-2′), 2.56–2.72 (m, 1 H, H-2′), 3.76 (dd, 1 H, J = 11.4, 3.5 Hz, H-5′), 3.87 (dd, 1 H, J = 11.4, 4.6 Hz, H-5′), 3.97 (dd, 1 H, J = 7.2, 3.6 Hz, H-4′), 4.62 (dt, 1 H, J = 6.3, 3.6 Hz, H-3′), 4.70 (d, 2 H, J = 5.4 Hz, CH_2=$CHCH_2$ of AOC), 5.06 (d, 2 H, J = 5.4 Hz, CH_2=$CHCH_2$ of allyl), 5.22–5.50 (m, 4 H, CH_2=$CHCH_2$ × 2), 5.97 (ddt, 1 H, J = 17.3, 10.4, 5.4 Hz, CH_2=$CHCH_2$ of AOC), 6.13 (ddt, 1 H, J = 16.8, 10.4, 5.4 Hz, CH_2=$CHCH_2$ of allyl), 6.39 (t, 1 H, J = 6.4 Hz, H-1′), 7.42 (br s, 1 H, NHCO), 8.10 (s, 1 H, H-8). Anal. Calcd for $C_{29}H_{49}N_5O_6Si_2$: C, 56.17; H, 7.98; N, 11.30. Found: C, 55.94; H, 8.20; N, 11.29.

Detritylation of 4, Giving *O*⁴-Allyl-3′-*O*-(*tert*-butyldimethylsilyl)thymidine (5). A solution of 4 (893 mg, 1.28 mmol)

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and dichloroacetic acid (3.12 g, 2.0 mL, 24.2 mmol) in dichloromethane (30 mL) was stirred at room temperature for 10 min. The reaction was quenched by addition of a saturated solution of sodium hydrogencarbonate (100 mL). The mixture was extracted with dichloromethane (50 mL × 2), and the organic extract was washed with a saturated solution of sodium hydrogencarbonate (100 mL × 2). Evaporation of the organic solution gave a residual oil, which was chromatographed (1:1 ethyl acetate/hexane) to afford **5** (485 mg, 96%): IR 3418, 1661, 1537 cm^{-1}; UV λ_{max} 284 (ϵ 7300), 207 nm (sh, 20 000); ^1H NMR δ 0.06 (s, 6 H, $(CH_3)_2Si$), 0.87 (s, 9 H, t-C_4H_9Si), 1.95 (s, 3 H, CH_3C=), 2.27 (ddd, 1 H, J = 13.4, 6.4, 3.9 Hz, H-2′), 2.47 (dt, 1 H, J = 13.4, 6.4 Hz, H-2′), 3.23 (dd, 1 H, J = 6.2, 3.1 Hz, 5′-OH), 3.71–3.78 (m, 1 H, H-5′), 3.91–3.95 (m, 2 H, H-4′ and 5′), 4.50 (dt, 1 H, J = 6.2, 3.9 Hz, H-3′), 4.88 (d, 2 H, J = 5.9 Hz, CH_2=CHCH_2), 5.26 (dd, 1 H, J = 10.4, 1.5 Hz, *cis*-CH_2=$CHCH_2$), 5.37 (dd, 1 H, J = 17.3, 1.5 Hz, *trans*-CH_2=$CHCH_2$), 5.95–6.10 (m, 2 H, CH_2=CHCH_2 and H-1′), 7.62 (s, 1 H, H-6). Anal. Calcd for $C_{19}H_{32}N_2O_6Si$: C, 57.53; H, 8.15; N, 7.06. Found: C, 57.35; H, 8.29; N, 7.23.

Detritylation of 12, Affording O^6-Allyl-N^2-(allyloxycarbonyl)-3′-O-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (13). Detritylation of **12** (183 mg, 0.23 mmol) was carried out by a similar procedure described above to give **13** (94 mg, 83%): ^1H NMR δ 0.10 (s, 6 H, $(CH_3)_2Si$), 0.91 (s, 9 H, t-C_4H_9Si), 2.22 (ddd, 1 H, J = 13.4, 6.2, 5.9 Hz, H-2′), 3.00 (ddd, 1 H, J = 13.4, 8.9, 5.4 Hz, H-2′), 3.76 (d, 1 H, J = 12.4 Hz, H-5′), 3.92 (dd, 1 H, J = 12.4, 2.0 Hz, H-5′), 4.07 (d, 1 H, J = 2.0 Hz, H-4′), 4.69 (d, 2 H, J = 5.4 Hz, CH_2=CHCH_2 of AOC), 4.75–4.85 (m, 2 H, H-3′ and 5′-OH), 5.07 (d, 2 H, J = 5.9 Hz, CH_2=CHCH_2 of allyl), 5.24–5.48 (m, 4 H, CH_2=$CHCH_2$ × 2), 5.88–6.18 (m, 2 H, CH_2=CHCH_2 × 2), 6.23 (dd, 1H, J = 8.5, 6.2 Hz, H-1′), 7.44 (br s, 1 H, NHCO), 7.86 (s, 1 H, H-8). Anal. Calcd for $C_{23}H_{35}N_5O_6Si$: C, 54.62; H, 6.99; N, 13.85. Found: C, 54.49; H, 6.94; N, 14.08.

Desilylation of 4, Giving O^4-Allyl-5′-O-(*p,p*′-dimethoxytrityl)thymidine (6). To a stirred solution of **4** (1.26 g, 1.80 mmol) in THF (30 mL) was added a 1.0 M THF solution of tetrabutylammonium fluoride (2.0 mL, 2.0 mmol). After 30 min, the mixture was poured into ethyl acetate (100 mL) and was washed with water (100 mL), a saturated solution of ammonium chloride (100 mL), and brine (100 mL). The organic solution was evaporated to afford a viscous oil. Chromatography (5:1 ethyl acetate/hexane to 10:1 dichloromethane/methanol containing a trace amount of triethylamine) of the crude material afforded **6** (860 mg, 82%): IR 3424, 1665, 1535, 1510 cm^{-1}; UV λ_{max} 282 (ϵ 9000), 233 nm (sh, 24 000); ^1H NMR δ 2.05 (s, 3 H, CH_3C=), 2.24 (dt, 1 H, J = 13.4, 6.4 Hz, H-2′), 2.63 (ddd, 1 H, J = 13.9, 5.9, 3.5 Hz, H-2′), 2.86 (br s, 1 H, 3′-OH), 3.35 (dd, 1 H, J = 10.4, 3.0 Hz, H-5′), 3.46 (dd, 1 H, J = 10.4, 3.0 Hz, H-5′), 3.77 (s, 6 H, CH_3O × 2), 4.10 (dd, 1 H, J = 5.4, 3.0 Hz, H-4′), 4.53 (dt, 1 H, J = 5.4, 3.0 Hz, H-3′), 4.86 (d, 2 H, J = 5.4 Hz, CH_2=CHCH_2), 5.24 (dd, 1 H, J = 10.4, 1.5 Hz, *cis*-CH_2=$CHCH_2$), 5.35 (dd, 1 H, J = 16.8, 1.5 Hz, *trans*-CH_2=$CHCH_2$), 6.01 (ddt, 1 H, J = 16.8, 10.4, 5.4 Hz, CH_2=CHCH_2), 6.38 (t, 1 H, J = 6.4 Hz, H-1′), 6.81 (d, 4 H, J = 8.9 Hz, ortho protons to CH_3O of DMTr), 7.20–7.39 (m, 9 H, aromatic protons of DMTr), 7.86 (s, 1 H, H-6). Anal. Calcd for $C_{34}H_{36}N_2O_7$: C, 69.84; H, 6.22; N, 4.79. Found: C, 69.71; H, 6.23; N, 4.99.

Desilylation of 12, Affording O^6-Allyl-N^2-(allyloxycarbonyl)-5′-O-(*p,p*′-dimethoxytrityl)-2′-deoxyguanosine (14). In a similar way as described above, **12** (303 mg, 0.37 mmol) was converted to **14** (253 mg, 97%): IR 3436, 1753, 1609, 1510 cm^{-1}; UV λ_{max} 269 (ϵ 18 000), 236 nm (sh, 28 000); ^1H NMR δ 2.58 (ddd, 1 H, J = 13.1, 6.2, 3.9 Hz, H-2′), 2.77 (dt, 1 H, J = 13.4, 6.2 Hz, H-2′), 3.13 (br s, 1 H, 3′-OH), 3.30–3.45 (m, 2 H, H-5′ × 2), 3.75 (s, 6 H, CH_3O × 2), 4.19 (dd, 1 H, J = 3.9, 1.9 Hz, H-4′), 4.62 (d, 2 H, J = 5.4 Hz, CH_2=CHCH_2 of AOC), 4.77 (br s, 1 H, H-3′), 5.04 (d, 2 H, J = 5.9 Hz, CH_2=CHCH_2 of allyl), 5.22–5.47 (m, 4 H, CH_2=$CHCH_2$ × 2), 5.92 (ddt, 1 H, J = 17.0, 10.8, 5.4 Hz, CH_2=CHCH_2 of AOC), 6.12 (ddt, 1 H, J = 17.0, 10.8, 5.4 Hz, CH_2=CHCH_2 of allyl), 6.55 (t, 1 H, J = 6.4 Hz, H-1′), 6.76 (d, 4 H, J = 8.4 Hz, ortho protons to CH_3O of DMTr), 7.16–7.48 (m, 9 H, aromatic protons of DMTr), 7.95 (s, 1 H, H-8). Anal. Calcd for $C_{38}H_{39}N_5O_8$: C, 65.78; H, 5.68; N, 10.06. Found: C, 65.92; H, 5.26; N, 10.06.

O^4-Allyl-3′-O-(allyloxycarbonyl)thymidine (7). To a solution of 1*H*-tetrazole (72.5 mg, 1.03 mmol) and triethylamine (150 μL, 1.08 mmol) in THF (10 mL) was added allyl chloroformate (110 μL, 1.04 mmol) at 0 °C. After the mixture was stirred for 20 min, the resulting precipitates were removed by filtration and washed with THF. The combined filtrate and washing were concentrated and dried in vacuo at room temperature to give an oil. A solution of the oil in THF (5 mL) was mixed with a solution of **6** (205 mg, 0.35 mmol) in DMF (10 mL), and the mixture was stirred at 50 °C for 3 h. The reaction mixture was cooled to room temperature and poured into a 1:1 mixture of ethyl acetate and hexane (100 mL). The organic layer was washed with an aqueous solution saturated with ammonium chloride (100 mL × 2) and brine (100 mL × 2) and concentrated to afford O^4-allyl-3′-O-(allyloxycarbonyl)-5′-O-(*p,p*′-dimethoxytrityl)thymidine as a colorless syrup. The syrup was dissolved in dichloromethane (5 mL), and to this was added dichloroacetic acid (0.6 mL, 7.27 mmol). The resulting mixture was stirred at room temperature for 20 min. The mixture was poured into a sodium hydrogencarbonate-saturated solution (100 mL) and extracted with dichloromethane (50 mL × 2). The combined organic extracts were washed with brine (50 mL × 2) and concentrated. The residue was purified by chromatography [1:1 to 3:1 ethyl acetate/hexane (gradient)] to give **7** (125 mg, 98%): IR3283, 1748, 1661 cm^{-1}; UV λ_{max} 283 (ϵ 7000), 204 nm (21 000); ^1H NMR δ 1.98 (s, 3 H, CH_3C=), 2.51 (ddd, 1 H, J = 10.8, 6.2, 2.3 Hz, H-2′), 2.68–2.79 (m, 1 H, H-2′), 3.32 (dd, 1 H, J = 6.9, 2.3 Hz, OH), 3.83–4.03 (m, 2 H, H-5′ × 2), 4.23 (d, 1 H, J = 2.3 Hz, H-4′), 4.65 (d, 2 H, J = 3.9 Hz, CH_2=CHCH_2 of AOC), 4.90 (d, 2 H, J = 3.9 Hz, CH_2=CHCH_2 of O^4-allyl), 5.25–5.46 (m, 5 H, CH_2=$CHCH_2$ × 2 and H-3′), 5.86–6.14 (m, 3 H, CH_2=CHCH_2 × 2 and H-1′), 7.56 (s, 1 H, H-6). Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.72; H, 6.06; N, 7.65. Found: C, 55.68; H, 5.82; N, 7.66.

Allyl O^6-Allyl-N^2-(allyloxycarbonyl)-5′-O-(*p,p*′-dimethoxytrityl)-2′-deoxyguanosyl-3′-Diisopropylphosphoramidite (16). A mixture of **14** (2.14 g, 3.08 mmol), diisopropylamine (0.18 g, 0.25 mL, 1.78 mmol), and 1*H*-tetrazole (121 mg, 1.72 mmol) in acetonitrile (10 mL) was stirred for 5 min at room temperature. To the resulting suspension was added allyloxy[bis(diisopropylamino)]phosphine[12b] (1.70 g, 5.88 mmol), and the mixture was stirred for an additional 4 h. After dilution with dichloromethane (100 mL), the organic solution was washed with water (100 mL × 2) followed by a saturated solution of sodium hydrogencarbonate (100 mL × 2). The aqueous layer was extracted with dichloromethane (50 mL × 2). Evaporation of the combined organic extracts gave a residual material, which was dissolved in a small amount of dichloromethane. The resulting solution was slowly added to vigorously stirred pentane (150 mL) at −78 °C. The occurring solids were collected by filtration, dissolved in a small amount of dichloromethane, and then concentrated to give **16** (2.53 g, 93%) as a colorless foam: IR 1759, 1719, 1609, 1510, 1250 cm^{-1}; UV λ_{max} 269 (ϵ 22 000), 236 nm (35 000); ^1H NMR δ 1.17 (d, 12 H, J = 6.4 Hz, $(CH_3)_2CH$ × 2), 2.49–2.70 (m, 1 H, H-2′), 2.79–2.92 (m, 1 H, H-2′), 3.28–3.41 (m, 2 H, $(CH_3)_2CH$ × 2), 3.48–3.68 (m, 2 H, H-5′ × 2), 3.76 (s, 6 H, CH_3O × 2), 3.98–4.35 (m, 3 H, CH_2=CHCH_2OP and H-4′), 4.63–4.78 (m, 3 H, CH_2=CHCH_2 of AOC and H-3′), 5.02–5.53 (m, 8 H, CH_2=CHCH_2 of allyl and CH_2=$CHCH_2$ × 3), 5.78–6.23 (m, 3 H, CH_2=CHCH_2 × 3), 6.40 (t, 1 H, J = 6.9 Hz, H-1′), 6.70–6.81 (m, 4 H, ortho protons to CH_3O of DMTr), 7.12–7.43 (m, 9 H, aromatic protons of DMTr), 7.95 and 7.97 (two s's, 1 H, H-8 of two diastereomers); ^{31}P NMR δ 148.43, 148.53. Anal. Calcd for $C_{47}H_{57}N_6O_9P$: C, 64.07; H, 6.53; N, 9.54. Found: C, 64.07; H, 6.62; N, 9.57.

Triethylammonium Salt of Allyl O^6-Allyl-N^2-(allyloxycarbonyl)-5′-O-(*p,p*′-dimethoxytrityl)-2′-deoxyguanosyl-3′-Phosphate (15). A mixture of **16** (1.24 g, 1.43 mmol), 2-cyanoethanol (0.52 g, 0.50 mL, 7.32 mmol), and 1*H*-tetrazole (416 mg, 5.93 mmol) in acetonitrile (15 mL) was stirred at room temperature. After 90 min, a 1.0 M solution of TBHP in dichloromethane (1.5 mL, 1.50 mmol) was added, and stirring was continued for an additional 1 h. The mixture was diluted with dichloromethane (100 mL) and washed with a saturated solution of sodium hydrogencarbonate (50 mL). The aqueous layer was extracted with dichloromethane (30 mL × 2). The combined organic extracts were concentrated to afford a brown

syrup. The syrup was dissolved in a mixture of triethylamine (10 mL) and dichloromethane (30 mL), and the solution was refluxed overnight. The reaction mixture was concentrated to afford a viscous oil. The crude product was purified by chromatography (ethyl acetate to 10% methanol/dichloromethane containing a trace amount of triethylamine) to afford the triethylammonium salt of **15** (1.94 g, 90%). ^{1}H NMR δ 1.30–1.50, 2.75–3.15, 3.27–3.77 (complex, H-2′ × 2, H-5′ × 2, CH_3O × 2, $(CH_3CH_2)_3N^+H$, and $(CH_3CH_2)_3N$ contaminated), 4.29–4.46 (m, 3 H, CH_2=CHCH_2OP and H-4′), 4.67 (d, 2 H, J = 5.6 Hz, CH_2=CHCH_2 of AOC), 4.99–5.62 (m, 9 H, H-3′, CH_2=CHCH_2 of allyl, and CH_2=CHCH_2 × 3), 5.82–6.23 (m, 3 H, CH_2=CHCH_2 × 3), 6.45 (t, 1 H, J = 6.9 Hz, H-1′), 6.69–6.80 (m, 4 H, ortho protons to CH_3O of DMTr), 7.12–7.43 (m, 9 H, aromatic protons of DMTr), 7.95 (s, 1 H, H-8), 12.25 (br s, 1 H, $(CH_3CH_2)_3N^+H$); ^{31}P NMR δ −0.37.

Allyl *O*^{6}-Allyl-*N*^{2}-(allyloxycarbonyl)-5′-*O*-(*p,p*′-dimethoxytrityl)-2′-deoxyguanylyl-(3′–5′)-*O*^{4}-allyl-3′-*O*-(allyloxycarbonyl)thymidine (17). By the Phosphotriester Method. A solution of **15** (228 mg, 0.25 mmol), **7** (94.2 mg, 0.25 mmol), and TPS-NT (97.8 mg, 0.26 mmol) in pyridine (2 mL) was stirred at room temperature. After 6 and 8 h, additional TPS-NT (92.3 mg, 0.24 mmol and 41.7 mg, 0.11 mmol, respectively) was added to the reaction mixture, and stirring was continued for total 14 h. The mixture was diluted with dichloromethane (100 mL), washed with water (50 mL × 2) followed by a saturated solution of sodium hydrogencarbonate (50 mL × 2), and concentrated. The residue was subjected to chromatography (ethyl acetate to 10% methanol/dichloromethane) to afford a colorless foam (203 mg). The crude product was dissolved in dichloromethane (5 mL). To this solution was added dichloroacetic acid (0.78 g, 0.50 mL, 6.1 mmol), and the resulting mixture was stirred at room temperature for 5 min. The reaction mixture was poured into a saturated solution of sodium hydrogencarbonate (50 mL) and was extracted with dichloromethane (50 mL × 2). Removal of solvents of the combined organic extracts gave a viscous oil, which was purified by preparative TLC (10:1 dichloromethane/methanol) to afford **17** (150 mg, 69%): IR3418, 1750, 1671, 1609, 1535 cm^{-1}; UV λ_{max} 269 nm (ϵ 19 000); ^{1}H NMR δ 2.00 (s, 3 H, CH_3C=), 2.11–2.35 (m, 1 H, H-2′), 2.51–2.82 (m, 2 H, H-2′ × 2), 3.12–3.28 (m, 1 H, H-2′), 3.78–4.01 (m, 2 H, H-5′ × 2), 4.30–5.53 (m, 26 H, H-3′ × 2, H-4′ × 2, H-5′ × 2, CH_2=CHCH_2 × 5), 5.84–6.21 (m, 5 H, CH_2=CHCH_2 × 5), 6.23–6.44 (m, 2 H, H-1′ × 2), 7.48 and 7.51 (two br s's, 1 H, NHCO of two diastereomers), 7.68 and 7.70 (two s's, 1 H, H-5 of two diastereomers), 7.94 and 7.96 (two s's, 1 H, H-8 of two diastereomers); ^{31}P NMR δ −1.48 and −0.96.

By the Phosphoramidite Method. To a stirred solution of **7** (100 mg, 0.27 mmol) and **16** (377 mg, 0.43 mmol) in acetonitrile (5 mL) was added 1*H*-tetrazole (149 mg, 2.13 mmol), and the resulting mixture was stirred at room temperature. After 10 min, to the mixture was added a 1.75 M solution of TBHP in dichloromethane (0.5 mL, 0.88 mmol), and stirring was continued overnight. The reaction was quenched by addition to a saturated solution of sodium hydrogencarbonate (100 mL), and the mixture was extracted with dichloromethane (100 mL ×2). The combined organic layers were washed with brine (100 mL) and concentrated. The resulting residue was dissolved in dichloromethane (10 mL), and to the solution was added dichloroacetic acid (1.09 g, 0.7 mL, 8.49 mmol). The mixture was stirred at room temperature for 10 min and poured into a saturated solution of sodium hydrogencarbonate (100 mL). The mixture was extracted with dichloromethane (50 mL × 2). The organic extract was washed with brine (50 mL × 2) and concentrated. The crude product was purified by preparative TLC (50:10:3 ethyl acetate/hexane/methanol) to afford **17** (227 mg, 95%).

Typical Procedure of Deallylation and Deallyloxycarbonylation. 3′-*O*-(*tert*-Butyldimethylsilyl)-5′-*O*-(*p,p*′-dimethoxytrityl)thymidine (1) from 4. To a heterogeneous mixture of **4** (155 mg, 0.22 mmol) and diethylammonium hydrogencarbonate (162 mg, 1.20 mmol) in dichloromethane (5 mL) was added dropwise by a syringe over 1 min a solution of $Pd[P(C_6H_5)_3]_4$ (55.6 mg, 48.1 μmol) and triphenylphosphine (7.7 mg, 29.4 μmol) in dichloromethane (5 mL), and the resulting mixture was stirred at room temperature. After 5 min, the reaction mixture was concentrated and the crude product was purified by short column chromatography to give **1** (143 mg, 98%).

Removal of Allylic Protector(s) of 10 and 11, Giving 3′,5′-*O*-Bis(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (8). In a similar way as described above, the allyl group of **10** (265 mg, 0.49 mmol) was removed to give **8** (202 mg, 87%). The compound **11** (189 mg, 0.32 mmol) was converted to **8** in quantitative yield (by TLC).

Deprotection of 17 Giving 2′-Deoxyguanylyl-(3′-5′)-thymidine (18). The Pd-catalyzed deprotection of **17** (192 mg, 0.22 mmol) was achieved in a manner similar to that described above. The water-soluble deprotected product **18** was obtained by extraction from the reaction mixture with water and concentration of the aqueous solution. The yield was 147 mg (3,539 OD_{260}; 90% yield). The product was identical in all respects with the commercially supplied authentic sample of **18**.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas No. 04226105 from the Ministry of Education, Science and Culture, Japan.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of **14**, **15**, and **17** and ^{31}P and P–H COSY NMR spectra of **15** and **17** (10 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.