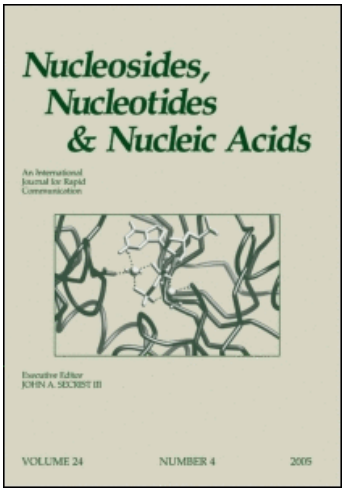


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A FACILE SYNTHESIS OF 5'-END SOLID-ANCHORED, 3'-END FREE OLIGODEOXYRIBONUCLEOTIDES VIA THE (5' → 3')-ELONGATED PHOSPHORAMIDITE STRATEGY

Akira Sakakura[a]; Yoshihiro Hayakawa[a]
[a] Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya, Japan

Online publication date: 28 February 2001

To cite this Article Sakakura, Akira and Hayakawa, Yoshihiro(2001) 'A FACILE SYNTHESIS OF 5'-END SOLID-ANCHORED, 3'-END FREE OLIGODEOXYRIBONUCLEOTIDES VIA THE (5' → 3')-ELONGATED PHOSPHORAMIDITE STRATEGY', Nucleosides, Nucleotides and Nucleic Acids, 20: 3, 213 — 227
To link to this Article: DOI: 10.1081/NCN-100002082
URL: http://dx.doi.org/10.1081/NCN-100002082

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(3), 213–227 (2001)

A FACILE SYNTHESIS OF 5′-END SOLID-ANCHORED, 3′-END FREE OLIGODEOXYRIBONUCLEOTIDES VIA THE (5′→3′)-ELONGATED PHOSPHORAMIDITE STRATEGY

Akira Sakakura and Yoshihiro Hayakawa*

Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

ABSTRACT

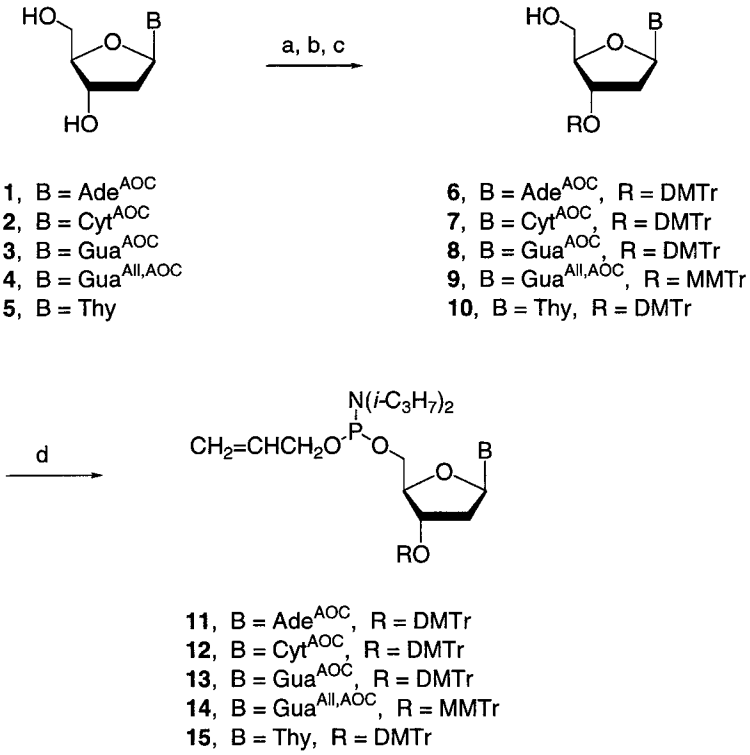
It is demonstrated that not only N^2- but also O^6-protection of the guanine base is necessary for obtaining the oligodeoxyribonucleotides in high yields and at a high purity in the solid-phase synthesis via the (5′ → 3′)-chain elongated phosphoramidite approach.

Development of an efficient synthesis of 5′-end solid-anchored, 3′-end free oligodeoxyribonucleotides is an important subject, because these substances may serve as key intermediates for the preparation of oligonucleotides with organic functions at the 3′-terminals, which are biologically attractive, for instance, as antisense molecules (1). One of the most convenient ways to synthesize such solid-anchored oligonucleotides is the (5′ → 3′)-directed chain elongation, starting from a nucleoside attached at the 5′-hydroxyl to solid supports, via the phosphoramidite method using suitably protected nucleoside 5′-phosphoramidites as building blocks (2). However, according to a report (3) and an examination we performed (4), the construction of the nucleotide chain by the (5′ → 3′)-elongation method is not conducted in a satisfactory yield. Thus, we made some investigations to elucidate the reasons causing a lowering of the yield when the conventional (5′ → 3′)-chain

*Corresponding author. Fax: 81-52-789-5646; E-mail: yoshi@info.human.nagoyau.ac.jp

elongation method is used. As the result, we found that a main reason is the use of the O^6-unprotected guanosine compound, which is harmed by the phosphoramidite resulting in a decrease in the yield and purity of the product. Consequently, we examined the synthesis of oligodeoxyribonucleotides via the (5′ → 3′)-elongation strategy using the N^2, O^6-protected guanosine phosphoramidite monomer and have realized an efficient method giving the product in a desirable yield with an excellent purity.

Deoxyribonucleoside 5′-phosphoramidites, **11–15**, requisite as monomer units for the synthesis of oligodeoxyribonucleotides, were prepared starting from the corresponding 3′,5′-*O*-free deoxyribonucleosides, **1–5** (5), with the allylic protector on the nucleoside base via the pathway shown in Scheme 1. Thus, the 5′-hydroxyl of the starting material was silylated with *tert*-butyldimethylsilyl chloride (TBDMS-Cl) by the assistance of imidazole (DMF, 25°C, 12 h), and the 3′-hydroxyl was then tritylated with *p*,*p*′-dimethoxytrityl chloride (DMTr-Cl) in the presence of 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) (pyridine, 25°C, 24 h) to give the 3′,5′-

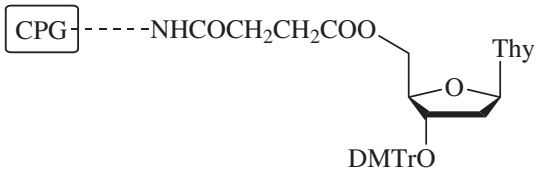


Scheme 1. Preparation of deoxyribonucleoside 5′-phosphoramidites. (a) t-$C_4H_9(CH_3)_2SiCl$, imidazole, DMF; (b) $C_6H_5(p\text{-}CH_3OC_6H_4)_2CCl$ or $p\text{-}CH_3OC_6H_4(C_6H_5)_2CCl$, DBU, pyridine; (c) $(n\text{-}C_4H_9)_4NF$, THF; (d) $CH_2{=}CHCH_2OP[N(i\text{-}C_3H_7)_2]_2$, diisopropylammonium 1*H*-tetrazolide, CH_3CN.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

O-protected nucleotide. In this tritylation, the use of DBU is important to obtain the desired product in high yield. The reaction in the absence of DBU or using triethylamine in place of DBU afforded the target compounds in lower yields. The 5′-*O*-TBDMS protecting group of the resulting product was removed by exposure to tetrabutylammonium fluoride (TBAF) (THF, 25°C, 1.5 h) and, finally, the phosphoramidite moiety was introduced to the resulting 5′-*O*-free product by the use of (allyloxy)[bis(diisopropylamino)]phosphine $\{(CH_2{=}CHCH_2O)P[N(i\text{-}C_3H_7)_2]_2\}$ and diisopropylammonium 1*H*-tetrazolide (6) to give the desired deoxyribonucleoside 5′-phosphoramidite. The overall yields of the phosphoramidites from the corresponding starting nucleosides were 56% for **11**, 81% for **12**, 28% for **13**, 47% for **14** (7), and 92% for **15**.

First, we carried out the synthesis of a 12mer, $^{5'}$TCACCATTATGC$^{3'}$ (**16**), using **11**, **12**, **13**, and **15** as building blocks, and benzimidazolium triflate (8) as the activator. The synthesis was carried out on an Applied Biosystems Model 392 DNA/RNA synthesizer, starting from thymidine (**17**) covalently attached at the 5′-hydroxyl to controlled pore glass (CPG) supports (500 Å pore size) via a long-chain



17

alkylamine linker. Table 1 indicates the reaction cycle for the chain elongation. The average coupling yield for one-base elongation determined by trityl assay was 99.6% (overall 95.7%) (9). After finishing the elongation, the product was treated with a mixture of the tris(dibenzylideneacetone)dipalladium(0)–chloroform complex $[Pd_2(dba)_3{\cdot}CHCl_3]$ (2.5 equiv/allyl), triphenylphosphine (25 equiv/allyl), and diethylammonium formate (150 equiv/allyl) at 50°C for 1 h to remove the allylic protecting groups (6,10) and then with concentrated ammonia at 25°C for 1 h to detach the product from the solid supports. HPLC of the crude product (Fig. 1) showed two conspicuous peaks at $t_r = 18.5$ and 20.0 min. Comparison of the authentic sample confirmed that the major product giving the peak at $t_r = 18.5$ min is the target oligonucleotide **16**, while the minor product providing the peak at $t_r = 20.0$ min could not be isolated in a pure form and therefore its structure could not be determined. However, digestion of the crude product with a mixture of snake venom phosphodiesterase and bacterial alkaline phosphatase provided important information for determining this product. HPLC of the digests obtained from the crude product showed peaks due to four kinds of parent nucleosides and one peak with a longer retention time than those of the four kinds of nucleosides. The region

Table 1. Reaction Cycle for the (5′→3′)-Directed Chain Elongation Method

Step	Operation	Reagent	Time, min
1	washing	CH_3CN	0.4
2	detritylation	3% Cl_3CCOOH/CH_2Cl_2	1.3
3	washing	CH_3CN	0.8
4	coupling	0.1 M phosphoramidite/CH_3CN + 0.2 M benzimidazolium triflate/CH_3CN	2.0
5	washing	CH_3CN	0.2
6	capping	Ac_2O-2,6-lutidine–THF (1:1:8) + 16% *N*-methylimidazole/THF	0.3
7	washing	CH_3CN	0.2
8	oxidation	1.0 M *t*-C_4H_9OOH/toluene	0.5
9	washing	CH_3CN	0.6

where the later peak was observed is the region where peaks due to derivatives suffering some modification at the nucleoside base appear. Further, the experimentally derived composition of the four bases was dA:dC:dG:T = 3.12:4.00:0.74:4.14, and, compared with the ratio calculated from the desired oligomer **16**, *i.e.*, dA:dC:dG:T = 3:4:1:4, the content of dG is rather low. On the basis of these findings, we conceived that **13** suffers some undesired reaction, perhaps phosphitylation, at its unprotected O^6-position of the guanine base to decrease the purity of the target compound (11).

Accordingly, we next examined the synthesis of the oligomer **16** in a similar manner to that described above, using the deoxyguanosine phosphoramidite **14** with an allyl protector at the O^6-position (12) in place of **13** without O^6-protection. According to the trityl assay, the coupling yield was 99.9% average and 99% overall. As the HPLC (Fig. 2) shows, the product has an excellent purity (ca. 85%) in a crude form. Little side product was detected in a long retention-time region. A similar result was obtained in the synthesis of a 15mer, 5′TGTCGACACCCAATT3′ (**18**) (see Fig. 2). Thus, as expected, the O^6-protection prevented the guanine moiety from undesired reactions.

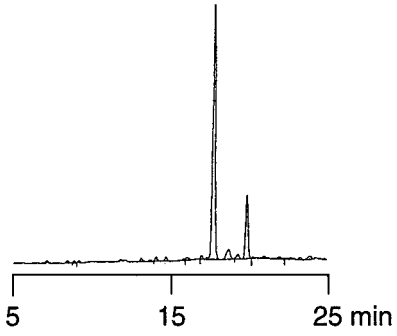


Figure 1. HPLC profile of the crude product of **16** obtained in the synthesis using **13** as the guanosine phosphoramidite.

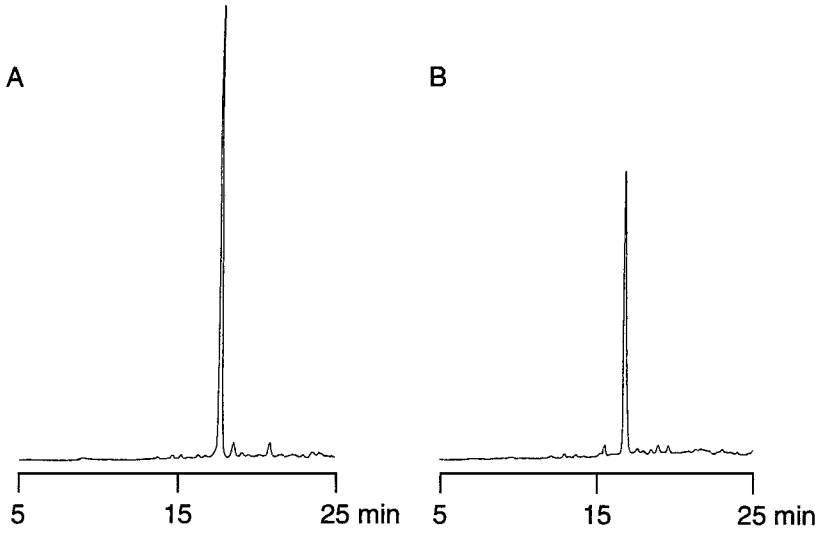


Figure 2. HPLC profiles of the crude products of **16** and **18** prepared using **14** as the guanosine monomer unit; (A) **16** and (B) **18**.

In conclusion, we revealed that a main reason why the existing synthesis of oligodeoxyribonucleotides through the (5′ → 3′)-chain elongation does not afford the target compounds in high yield and at a high quality is that the O^6-free guanosine base suffers some modification under conditions of phosphitylation with a nucleoside 5′-phosphoramidite in the presence of a promoter such as 1*H*-tetrazole or benzimidazolium triflate. Consequently, we used the N^2, O^6-protected guanosine phosphoramidite, in place of the O^6-unprotected derivative that has been conventionally used so far, to achieve an efficient synthesis of solid-anchored 3′-end free oligodeoxyribonucleotides through the (5′ → 3′)-chain elongation. This approach was effectively applied to the synthesis of some oligodeoxyribonucleotide derivatives with a covalent conjugation at the 3′-end positions, including oligode-oxyribonucleotide–amino acid and –peptide conjugates, and an oligonucleotide–intercalator conjugate (13).

EXPERIMENTAL SECTION

General Procedure and Materials

UV spectra were measured in methanol on a JASCO V-550 spectrometer. IR spectra were measured in KBr on a JASCO FT/IR-5300 spectrometer. NMR spectra were taken in $CDCl_3$ on a JEOL α-400 instrument. For ^{1}H NMR spectra, tetramethylsilane (TMS) was used as a standard. For ^{31}P NMR spectra, H_3PO_4 was used as an external standard. The chemical shifts are described as δ values in parts

per million (ppm). Elemental analyses were carried out in the Faculty of Agriculture, Nagoya University. High-performance liquid chromatography (HPLC) using an ODS-5 μm column with a 0.1 M ammonium acetate buffer containing 5–13% acetonitrile (v/v) (linear gradient in 30 min) was carried out on a JASCO PU-980 chromatograph with a JASCO UV-970-absorption detector. Fuji silysia silica gel BW-300S was used for column chromatography. Solid-phase synthesis was carried out on an Applied Biosystems Model 392 DNA/RNA synthesizer. Unless otherwise stated, reactions were carried out at ambient temperature. The organic extracts were dried over $MgSO_4$ before concentration. N^6-Allyloxycarbonyl-2′-deoxyadenosine (**1**), N^4-allyloxycarbonyl-2′-deoxycytidine (**2**), N^2-allyloxycarbonyl-2′-deoxyguanosine (**3**), and O^6-allyl-N^2-allyloxycarbonyl-2′-deoxyguanosine (**4**) were prepared by reported methods (5). Acetonitrile, pyridine, and dichloromethane were distilled from CaH_2. THF was distilled from benzophenone ketyl. Commercially supplied reagents were used without any purification, unless otherwise noted. A solution of a nucleoside 5′-phosphoramidite (0.1 M) and benzimidazolium triflate (0.2 M) in acetonitrile were prepared immediately before use. A 1:1:8 mixture of acetic anhydride, 2,6-lutidine and THF, and a 16% solution of *N*-methylimidazole in THF were also freshly prepared before use.

N^6-Allyloxycarbonyl-3′-*O*-*p*, *p*′-dimethoxytrityl-2′-deoxyadenosine 5′-(Allyl *N*,*N*-diisopropylphosphoramidite) (11)

To a DMF (22 mL) solution of **1** (7.24 g, 21.6 mmol) and imidazole (2.95 g, 43.3 mmol) was added TBDMS-Cl (2.92 g, 19.4 mmol) at 0°C. The resulting mixture was stirred at 25°C for 12 h. After addition of water (50 mL), the mixture was extracted with ethyl acetate (100 mL × 4). The combined organic extracts were washed with brine (50 mL), dried, and concentrated. The residue was subjected to silica gel (200 g) column chromatography with a 1:100:100 to 1:10:10 methanol–ethyl acetate–hexane mixture as the eluent, giving the 5′-*O*-silylated nucleoside as a viscous oil (7.53 g, 78%). To a solution of the silylation product (8.88 g, 19.8 mmol) and DBU (6.01 g, 5.90 mL, 39.5 mmol) in pyridine (45 mL) was added DMTr-Cl (13.4 g, 39.5 mmol) at 0°C and the mixture was stirred at 25°C for 24 h. The reaction mixture was concentrated under reduced pressure to give a residual oil. This material was diluted with ethyl acetate (500 mL) and washed with water (100 mL) followed by brine (100 mL). The organic solution was dried and concentrated. The resulting oily product was purified by column chromatography on silica gel (100 g) eluted with a 1:2 to 1:1 ethyl acetate–hexane mixture to afford the 3′-*O*-tritylated nucleoside as an amorphous solid (11.0 g, 74%). To a THF (40 mL) solution of the product (11.0 g, 14.6 mmol) was added a 1.0 M solution of TBAF in THF (30.0 mL, 30.0 mmol), and the mixture was stirred for 1.5 h. After addition of an aqueous solution saturated with NH_4Cl (50 mL), the aqueous layer was extracted with ethyl acetate (100 mL × 3). The combined organic extracts were washed with brine (50 mL), dried, and concentrated. Silica gel (100 g) column chromatography of the resulting crude product with a 1:1 mixture

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

of ethyl acetate and hexane followed by ethyl acetate to give N^6-allyloxycarbonyl-3′-*O*-*p*,*p*′-dimethoxytrityl-2′-deoxyadenosine (**6**) (9.33 g, 100%) as an amorphous solid: UV λ_{max} 268 (ε 21500), 237 nm (25400); IR 1755, 1610, 1585, 1510, 1465, 1250, 1220 cm^{-1}; ^1H NMR δ 1.75 (dd, J = 5.4, 13.7 Hz, 1 H), 2.70 (ddd, J = 5.4, 10.2, 13.7 Hz, 1 H), 3.31 (br dd, J = 11.2, 12.7 Hz, 1 H), 3.72 (br d, J = 12.7 Hz, 1 H), 3.78 (s, 3 H), 3.79 (s, 3 H), 4.06 (s, 1 H), 4.63 (d, J = 5.4 Hz, 1 H), 4.75 (d, J = 5.9 Hz, 2 H), 5.28 (dd, J = 1.5, 10.2 Hz, 1 H), 5.40 (dd, J = 1.5, 17.1 Hz, 1 H), 5.64 (br d, J = 11.2 Hz, 1 H), 5.98 (tdd, J = 5.9, 10.2, 17.1 Hz, 1 H), 6.32 (dd, J = 5.4, 10.2 Hz, 1 H), 6.84 (d, J = 8.8 Hz, 2 H), 6.85 (d, J = 8.8 Hz, 2 H), 7.23 (t, J = 7.3 Hz, 1 H), 7.31 (dd, J = 7.3, 7.3 Hz, 2 H), 7.36 (d, J = 8.8 Hz, 4 H), 7.47 (d, J = 7.3 Hz, 2 H), 7.99 (s, 1 H), 8.45 (s, 1 H), 8.65 (s, 1 H). Anal. Calcd for $C_{35}H_{35}N_5O_7$: C, 65.92; H, 5.53; N, 10.98. Found: C, 65.96; H, 5.56; N, 10.79. To a stirred solution of (**6**) (1.13 g, 1.78 mmol), diisopropylamine (90.3 mg, 125 μL, 0.892 mmol), 1*H*-tetrazole (62.2 mg, 0.887 mmol) in acetonitrile (10 mL) was added (allyloxy)bis(diisopropylamino)phosphine (940 mg, 3.26 mmol), and the mixture was stirred for 3 h. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with brine (70 mL × 2). The organic solution was dried and concentrated to give a viscous oil, which was dissolved in dichloromethane (8 mL). This solution was poured into pentane (300 mL) at −78°C with stirring to give a powder, which was collected by filtration. The powder was dissolved in dichloromethane, and then the solution was evaporated in vacuo to afford **11** as an amorphous solid (1.42 g, 97%): UV λ_{max} 268 (ε 21800), 238 (24900), 212 nm (42500); IR 1760, 1730, 1610, 1585, 1510, 1460 cm^{-1}; ^1H NMR δ 0.99 (d, J = 6.8 Hz, 3 H), 1.05 (d, J = 6.8 Hz, 3 H), 1.14 (d, J = 6.8 Hz, 6 H), 2.07–2.11 (m, 1 H), 2.23 (ddd, J = 5.4, 8.3, 13.7 Hz, 0.5 H), 2.30 (ddd, J = 5.4, 9.3, 13.7 Hz, 0.5 H), 3.29 (ddd, J = 2.9, 6.3, 11.2 Hz, 0.5 H), 3.39 (ddd, J = 2.4, 7.3, 11.7 Hz, 0.5 H), 3.43–3.52 (m, 2 H), 3.55 (ddd, J = 2.4, 4.9, 11.7 Hz, 0.5 H), 3.66 (ddd, J = 2.4, 5.9, 11.2 Hz, 0.5 H), 3.78 (s, 6 H), 3.98–4.17 (m, 3 H), 4.46 (d, J = 5.4 Hz, 0.5 H), 4.49 (d, J = 5.4 Hz, 0.5 H), 4.76 (d, J = 5.9 Hz, 2 H), 5.08 (br d, J = 10.3 Hz, 0.5 H), 5.12 (br d, J = 10.3 Hz, 0.5 H), 5.20 (dd, J = 2.0, 17.1 Hz, 0.5 H), 5.25 (dd, J = 2.0, 17.1 Hz, 0.5 H), 5.28 (d, J = 10.3 Hz, 1 H), 5.40 (d, J = 17.1 Hz, 1 H), 5.83 (tdd, J = 4.9, 10.3, 17.1 Hz, 0.5 H), 5.92 (tdd, J = 4.9, 10.3, 17.1 Hz, 0.5 H), 5.99 (tdd, J = 4.9, 10.3, 17.1 Hz, 1 H), 6.61 (dd, J = 5.9, 8.3 Hz, 0.5 H), 6.62 (dd, J = 5.9, 9.3 Hz, 0.5 H), 6.84 (d, J = 8.8 Hz, 4 H), 7.32–7.21 (m, 3 H), 7.36 (d, J = 8.8 Hz, 2 H), 7.37 (d, J = 8.8 Hz, 2 H), 7.46–7.49 (m, 2 H), 8.35 (s, 0.5 H), 8.37 (s, 0.5 H), 8.76 (s, 1 H); ^{31}P NMR δ 148.6. Anal. Calcd for $C_{44}H_{53}N_6O_8P$: C, 64.07; H, 6.48; N, 10.19. Found: C, 64.03; H, 6.50; N, 10.09.

N^4-Allyloxycarbonyl-3′-*O*-*p*, *p*′-dimethoxytrityl-2′-deoxycytidine 5′-(Allyl *N*,*N*-diisopropylphosphoramidite) (12)

To a solution of **2** (11.8 g, 37.9 mmol) and imidazole (5.18 g, 76.1 mmol) in DMF (38 mL) was added TBDMS-Cl (5.73 g, 38.0 mmol) at 0°C. The mixture was stirred at 25°C for 12 h. After addition of water (50 mL) at 0°C, the mixture was

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

extracted with ethyl acetate (100 mL × 4). The combined organic extracts were washed with brine (50 mL), dried, and concentrated. The residue was subjected to silica gel (200 g) column chromatography. Elution with a 1:1 to 5:1 ethyl acetate–hexane mixture gave the 5′-*O*-silylated nucleoside as a residual oil (14.9 g, 92%). To a solution of the product (10.7 g, 25.1 mmol) and DBU (7.13 g, 7.00 mL, 46.8 mmol) in pyridine (100 mL) was added DMTr-Cl (16.8 g, 49.6 mmol) at 0°C, and the mixture was stirred at 25°C for 24 h. The reaction mixture was concentrated under reduced pressure to give a viscous oil, to which was added water (500 mL). The mixture was extracted with ethyl acetate (500 mL × 2). The combined organic extracts were successively washed with a 0.1 M aqueous solution of $CuSO_4$ (200 mL × 2) to remove efficiently pyridine and DBU, an aqueous solution saturated with $NaHCO_3$ (200 mL × 3), and brine (200 mL × 2), and then concentrated. The resulting crude product was chromatographed on silica gel (320 g) column with a 1:3 to 1:1 mixture of ethyl acetate and hexane to afford the 3′-*O*-trityl nucleoside as an amorphous solid (18.2 g, 99%). To a solution of this product (7.57 g, 10.4 mmol) in THF (50 mL) was added a 1.0 M solution of TBAF in THF (18.0 mL, 18.0 mmol). The mixture was stirred at 25°C for 2 h and then diluted with ethyl acetate (200 mL). The organic mixture was successively washed with water (150 mL × 2), an aqueous solution saturated with NH_4Cl (150 mL × 2), an aqueous solution saturated with $NaHCO_3$ (150 mL × 2), and brine (150 mL × 2), and then dried and concentrated. Chromatography of the crude product on silica gel (200 g) using a 1:1 to 1:5 mixture of ethyl acetate and hexane gave N^4allyloxycarbonyl-3′-*O*-*p*,*p*′-dimethoxytrityl-2′-deoxycytidine (**7**) (6.00 g, 89%): UV λ_{max} 284 (ε 9300), 238 nm (35000); IR 1750, 1655, 1560, 1510, 1250, 1200 cm^{-1}; ^1H NMR δ 1.96 (m, 1 H), 2.18 (m, 1 H), 2.97 (br s, 1 H), 3.23 (dd, $J = 2.4$, 11.7 Hz, 1 H), 3.62 (d, $J = 11.7$ Hz, 1 H), 3.76 (s, 6 H), 3.90 (d, $J = 2.4$ Hz, 1 H), 4.35 (d, $J = 6.4$ Hz, 1 H), 4.64 (d, $J = 5.9$ Hz, 2 H), 5.27 (d, $J = 10.7$ Hz, 1 H), 5.33 (d, $J = 17.1$ Hz, 1 H), 5.91 (tdd, $J = 5.9$, 10.7, 17.1 Hz, 1 H), 6.22 (dd, $J = 6.4$, 7.8 Hz, 1 H), 6.81 (d, $J = 8.3$ Hz, 4 H), 7.20 (t, $J = 6.8$ Hz, 1 H), 7.18–7.22 (m, 1 H), 7.27 (dd, $J = 6.8$, 7.3 Hz, 2 H), 7.34 (d, $J = 8.3$ Hz, 4 H), 7.44 (d, $J = 7.3$ Hz, 2 H), 8.06 (d, $J = 7.3$ Hz, 1 H). Anal. Calcd for $C_{34}H_{35}N_3O_8$: C, 66.55; H, 5.75; N, 6.85. Found: C, 66.48; H, 5.98; N, 6.61. To a solution of **7** (5.60 g, 9.13 mmol), diisopropylamine (462 mg, 640 μL, 4.57 mmol), and 1*H*-tetrazole (318 mg, 4.54 mmol) in acetonitrile (50 mL) was added (allyloxy)bis(diisopropylamino)phosphine (3.37 g, 11.7 mmol), and the resulting mixture was stirred for 1 h. The reaction mixture was diluted with ethyl acetate (400 mL), washed with an aqueous solution saturated with $NaHCO_3$ (50 mL) followed by brine (50 mL), dried, and concentrated. The resulting crude material was subjected to silica gel (100 g) column chromatography and eluted with a 1:5:trace to 2:1:trace ethyl acetate–hexane–triethylamine mixture to afford **12** as an amorphous solid (7.35 g, 100%): UV λ_{max} 284 (ε 8800), 238 (31900), 212 nm (34000); IR 1695, 1610, 1510, 1465, 1250 cm^{-1}; ^1H NMR δ 0.99 (d, $J = 6.8$ Hz, 3 H), 1.08 (d, $J = 6.8$ Hz, 3 H), 1.13 (d, $J = 6.8$ Hz, 3 H), 1.15 (d, $J = 6.8$ Hz, 3 H), 1.72 (ddd, $J = 5.9$, 9.3, 13.7 Hz, 1 H), 1.86 (s, 3 H), 1.86–1. 92 (m, 1 H), 3.22–3.30 (m, 1 H), 3.45–3.56 (m, 2.5 H), 3.64 (m, 0.5 H), 3.79 (s, 6 H), 4.01–4.14 (m, 2 H),

4.33 (d, $J = 5.9$ Hz, 0.5 H), 4.35 (d, $J = 5.9$ Hz, 0.5 H), 5.07 (d, $J = 10.3$ Hz, 0.5 H), 5.10 (d, $J = 10.3$ Hz, 0.5 H), 5.17 (dd, $J = 2.0$, 17.1 Hz, 0.5 H), 5.22 (dd, $J = 2.0$, 17.1 Hz, 0.5 H), 5.80 (tdd, $J = 5.4$, 10.3, 17.1 Hz, 0.5 H), 5.86 (tdd, $J = 5.4$, 10.3, 17.1 Hz, 0.5 H), 6.41 (dd, $J = 5.4$, 9.3 Hz, 0.5 H), 6.46 (dd, $J = 5.4$, 9.3 Hz, 0.5 H), 6.82 (d, $J = 8.8$ Hz, 2 H), 6.83 (d, $J = 8.8$ Hz, 2 H), 7.21–7.35 (m, 5 H), 7.33 (d, $J = 8.8$ Hz, 2 H), 7.34 (d, $J = 8.8$ Hz, 2 H), 7.43–7.46 (m, 2 H), 7.52 (s, 0.5 H), 7.65 (s, 0.5 H), 8.17 (br s, 1 H); ^{31}P NMR δ 148.4, 148.6. Anal. Calcd for $C_{40}H_{50}N_3O_8P$: C, 65.65; H, 6.89; N, 5.74. Found: C, 65.61; H, 6.78; N, 5.69.

N^2-Allyloxycarbonyl-3′-*O*-*p*, *p*′-dimethoxytrityl-2′-deoxyguanosine 5′-(Allyl *N*,*N*-diisopropylphosphoramidite) (13)

To a solution of N^2-allyloxycarbonyl-3′, 5′-bis-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine (10.3 g, 17.8 mmol) in THF (120 mL) was added a 1.0 M solution of TBAF in THF (40.0 mL, 40.0 mmol). The resulting mixture was stirred at 25°C for 24 h. To the reaction mixture were added a 3:3:1 mixture of pyridine, methanol and water (50 mL) and Dowex 50W-X8 (30 mL), and stirring was continued for an additional 30 min. Dowex 50W-X8 was removed by filtration and the filtrate was concentrated. The residue was chormatographed on silica gel (300 g) column with a 1:20 mixture of methanol and dichloromethane as the eluent to afford **3** (6.10 g, 98%). To a solution of **3** (6.10 g, 17.4 mmol) and imidazole (3.69 g, 54.2 mmol) in DMF (20 mL) was added TBDMS-Cl (2.66 g, 17.6 mmol) at 0°C and the resulting mixture was stirred at 25°C for 24 h. The reaction mixture was diluted with a 1:1 mixture of ethyl acetate and hexane (600 mL), successively washed with water (300 mL), an aqueous solution saturated with NH_4Cl (200 mL), an aqueous solution saturated with $NaHCO_3$ (200 mL), and brine (200 mL), dried, and concentrated. The residue was purified by silica gel (300 g) column chromatography using a 1:30 mixture of methanol and dichloromethane to afford the 5′-*O*-silylated nucleoside as a viscous liquid (3.36 g, 42%). To a solution of the product (2.35 g, 5.04 mmol) and DBU (2.40 mL, 8.04 mmol) was added DMTr-Cl (5.30 g, 15.7 mmol). The mixture was stirred at 25°C for 24 h and concentrated. Water (600 mL) was added to the resulting residual oil and the aqueous mixture was extracted with dichloromethane (400 mL, 150 mL × 2). The combined organic extracts were successively washed with a 1.0 M solution of $CuSO_4$ (200 mL), an aqueous solution saturated with $NaHCO_3$ (200 mL), and brine (200 mL). After dryness, removal of the organic solvent gave a residual oil, which was subjected to silica gel (200 g) column chromatography eluted with a 1:2 to 1:5 ethyl acetate–hexane mixture to provide the 3′-*O*-tritylated nucleoside as a glassy material (3.30 g, 85%). To a solution of this product (3.90 g, 5.08 mmol) in THF (40 mL) was added a 1.0 M solution of TBAF in THF (6.00 mL, 6.00 mmol), and the resulting mixture was stirred at 25°C for 22 h. The reaction mixture was diluted with ethyl acetate (400 mL), successively washed with an aqueous NH_4Cl-saturated solution (200 mL), an $NaHCO_3$ solution (200 mL), and brine (200 mL), dried, and concentrated. The resulting residual

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

material was recrystallized from dichloromethane to give N^2-allyloxycarbonyl-3′-*O*-*p*, *p*′-dimethoxytrityl-2′-deoxyguanosine (**8**) (2.66 g, 80%): UV λ_{max} 237 nm (ε 24200); IR 1695, 1610, 1510, 1385, 1250, 1175 cm^{-1}; ^1H NMR δ 1.72 (dd, J = 5.4, 13.7 Hz, 1 H), 2.42 (ddd, J = 5.9, 9.8, 13.7 Hz, 1 H), 3.24 (dd, J = 10.7, 11.7 Hz, 1 H), 3.68 (d, J = 11.7 Hz, 1 H), 3.79 (s, 6 H), 4.06 (s, 1 H), 4.50 (d, J = 5.9 Hz, 1 H), 4.71 (d, J = 5.9 Hz, 2 H), 4.87 (d, J = 10.7 Hz, 1 H), 5.32 (d, J = 10.7 Hz, 1 H), 5.38 (d, J = 17.2 Hz, 1 H), 5.90 (tdd, J = 5.9, 10.7, 17.2 Hz, 1 H), 6.16 (dd, J = 5.4, 9.8 Hz, 1 H), 6.84 (dd, J = 1.5, 8.8 Hz, 4 H), 7.15–7.46 (m, 10 H), 7.65 (s, 1 H), 11.28 (s, 1 H). Anal. Calcd for $C_{35}H_{35}N_5O_8$: C, 64.31; H, 5.40; N, 10.71. Found: C, 64.45; H, 5.22; N, 10.60. To a suspension of **8** (1.23 g, 1.88 mmol), diisopropylamine (101 mg, 140 μL, 0.989 mmol), and 1*H*-tetrazole (66.3 mg, 0.947 mmol) in acetonitrile (15 mL) was added (allyloxy)bis(diisopropylamino)phosphine (940 mg, 3.26 mmol). After stirring at 25°C for 3 h, the reaction mixture was diluted with ethyl acetate (100 mL), washed with brine (50 mL × 2), dried, and concentrated. The residue was dissolved in dichloromethane (6 mL) and the resulting solution was poured into pentane (300 mL) at −78°C with stirring to give powder, which was collected by filtration. Chromatograhic purification of this crude powdery product on silica gel (150 g) column with a 1:1:trace to 5:1:trace ethyl acetate–hexane–diisopropylamine mixture provided **13** as an amorphous solid (1.59 g, 100%): UV λ_{max} 239 (ε 29200), 211 nm (40700); IR 1700, 1610, 1510, 1465, 1250 cm^{-1}; ^1H NMR δ 1.00 (d, J = 6.8 Hz, 3 H), 1.08 (d, J = 6.8 Hz, 3 H), 1.14 (d, J = 6.8 Hz, 6 H), 2.02–2.08 (m, 1 H), 2.32–2.40 (m, 1 H), 3.27 (ddd, J = 2.4, 6.3, 11.2 Hz, 0.5 H), 3.36 (ddd, J = 2.9, 7.8, 11.2 Hz, 0.5 H), 3.47–3.56 (m, 2.5 H), 3.61 (ddd, J = 2.0, 4.9, 11.2 Hz, 0.5 H), 3.74 (s, 6 H), 3.81 (m, 0.5 H), 3.84 (m, 0.5 H), 3.99–4.21 (m, 2 H), 4.43 (d, J = 9.8 Hz, 0.5 H), 4.45 (d, J = 9.8 Hz, 0.5 H), 4.67 (m, 2 H), 5.06 (d, J = 10.3 Hz, 0.5 H), 5.13 (d, J = 10.3 Hz, 0.5 H), 5.19 (dd, J = 2.0, 17.1 Hz, 0.5 H), 5.26 (dd, J = 2.0, 17.1 Hz, 0.5 H), 5.30 (d, J = 10.3 Hz, 1 H), 5.35 (d, J = 17.6 Hz, 1 H), 5.77–5.98 (m, 2 H), 6.56 (dd, J = 5.9, 8.3 Hz, 1 H), 6.73 (dd, J = 2.0, 8.8 Hz, 2 H), 6.75 (d, J = 8.8 Hz, 2 H), 7.17–7.22 (m, 3 H), 7.27–7.33 (m, 4 H), 7.38–7.44 (m, 2 H), 8.10 (s, 1 H); ^{31}P NMR δ 148.5.[14] Anal. Calcd for $C_{44}H_{53}N_6O_9P$: C, 62.83; H, 6.37; N, 10.00. Found: C, 62.76; H, 6.56; N, 9.93.

O^6-Allyl-N^2-allyloxycarbonyl-3′-*O*-*p*-dimethoxytrityl-2′-deoxyguanosine 5′-(Allyl *N*,*N*-diisopropylphosphoramidite) (14)

To a solution of **4** (3.30 g, 8.43 mmol) and imidazole (1.16 g, 17.0 mmol) in DMF (10 mL) was added TBDMS-Cl (1.27 g, 8.43 mmol) at 0°C and the mixture was stirred at 25°C for 12 h. After addition of water (50 mL), the mixture was extracted with ethyl acetate (50 mL × 3). The combined organic extracts were washed with brine (20 mL), dried, and concentrated. The resulting residual oil was subjected to silica gel (50 g) column chromatography with a 1:1 to 1:5 mixture of

ethyl acetate and hexane as eluent to give the 5′-*O*-silylated nucleoside as a viscous oil (3.75 g, 89%). To a solution of the product (3.75 g, 7.42 mmol) and DBU (1.58 g, 1.55 mL, 10.4 mmol) was added MMTr-Cl (3.21 g, 10.4 mmol) at 0°C. The resulting mixture was stirred at 25°C for 24 h and concentrated. The residue was dissolved in ethyl acetate (400 mL) and the solution was washed with an $NaHCO_3$-saturated aqueous solution (50 mL) followed by brine (50 mL), dried, and concentrated to give a viscous oil. This crude material was subjected to chromatography on silica gel (100 g) eluted with a 1:5 to 2:1 ethyl acetate–hexane mixture to give the tritylation product as an amorphous solid (4.10 g, 71%). To a solution of the product (4.10 g, 5.27 mmol) in THF (15 mL) was added a 1.0 M THF solution of TBAF (10.5 mL, 10.5 mmol), and the mixture was stirred at 25°C for 1.5 h. An aqueous NH_4Cl-saturated solution (50 mL) was added to the reaction mixture and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (50 mL × 3). The combined organic extracts were washed with brine (20 mL), dried, and concentrated to afford a residual liquid, which was subjected to column chromatography on silica gel (100 g) using a 1:20:20 to 1:5:5 methanol–ethyl acetate–hexane mixture to give O^6-allyl-N^2-allyloxycarbonyl-3′-*O*-*p*-dimethoxytrityl-2′-deoxyguanosine (**9**) (3.50 g, 100%): UV λ_{max} 269 (ε 18000), 236 nm (28000); IR 1755, 1720, 1610, 1510, 1245, 1195 cm^{-1}; ^1H NMR δ 1.76 (dd, J = 5.4, 13.2 Hz, 1 H), 2.69 (ddd, J = 5.4, 9.8, 13.2 Hz, 1 H), 3.29 (br dd, J = 9.8, 13.2 Hz, 1 H), 3.68 (br d, J = 13.2 Hz, 1 H), 3.77 (s, 3 H), 4.00 (s, 1 H), 4.65 (d, J = 5.9 Hz, 2 H), 4.62–4.66 (m, 1 H), 4.88 (br d, J = 9.8 Hz, 1 H), 5.06 (d, J = 5.9 Hz, 2 H), 5.22 (dd, J = 1.0, 10.7 Hz, 1 H), 5.26 (dd, J = 1.0, 10.2 Hz, 1 H), 5.32 (dd, J = 1.0, 17.1 Hz, 1 H), 5.44 (dd, J = 1.0, 17.1 Hz, 1 H), 5.92 (tdd, J = 5.9, 10.7, 17.1 Hz, 1 H), 6.11 (tdd, J = 5.9, 10.2, 17.1 Hz, 1 H), 6.24 (dd, J = 5.4, 9.8 Hz, 1 H), 6.84 (d, J = 8.8 Hz, 2 H), 7.23 (t, J = 7.3 Hz, 2 H), 7.30 (dd, J = 7.3, 7.3 Hz, 4 H), 7.36 (d, J = 8.8 Hz, 2 H), 7.41 (s, 1 H), 7.48 (d, J = 7.3 Hz, 4 H), 7.82 (s, 1 H). Anal. Calcd for $C_{37}H_{37}N_5O_7$: C, 66.96; H, 5.62; N, 10.55. Found: C, 66.96, H, 5.69, N, 10.51. To a solution of **9** (1.10 g, 1.66 mmol), diisopropylamine (86.6 mg, 120 μL, 0.86 mmol) and 1*H*-tetrazole (60.2 mg, 0.859 mmol), in acetonitrile (16 mL) was added (allyloxy)bis(diisopropylamino)phosphine (717 mg, 2.49 mmol). After stirring at 25°C for 2 h, the reaction mixture was diluted with ethyl acetate ((100 mL), washed with an aqueous solution saturated with $NaHCO_3$ (20 mL) followed by brine (20 mL), dried, and concentrated. The resulting residual oil was purified by chromatography on silica gel (30 g) with a 3:1:trace to 2:1:trace mixture of ethyl acetate, hexane, and triethylamine, giving **14** as an amorphous solid (1.05 g, 74%): UV λ_{max} 269 (ε 22000), 236 nm (35000); IR 1755, 1605, 1510, 1460, 1410, 1240, 1185 cm^{-1}; ^1H NMR δ 0.99 (d, J = 6.8 Hz, 3 H), 1.04 (d, J = 6.8 Hz, 3 H), 1.12 (d, J = 6.8 Hz, 6 H), 2.05–1.99 (m, 1 H), 2.19–2.31 (m, 1 H), 3.29 (ddd, J = 2.9, 6.3, 11.2 Hz, 0.5 H), 3.38 (ddd, J = 2.9, 7.3, 11.2 Hz, 0.5 H), 3.43–3.57 (m, 2.5 H), 3.63 (ddd, J = 2.4, 5.9, 11.2 Hz, 0.5 H), 3.78 (s, 3 H), 4.17–3.96 (m, 3 H), 4.44–4.51 (m, 1 H), 4.71 (d, J = 5.9 Hz, 2 H), 5.08 (d, J = 5.9 Hz, 2 H), 5.04–5.12 (m, 1 H), 5.19 (d, J = 17.1 Hz, 0.5 H), 5.22–5.27 (m, 0.5 H),

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

5.27 (d, $J = 10.7$ Hz, 2 H), 5.40 (d, $J = 17.1$ Hz, 1 H), 5.44 (d, J = 17.1 Hz, 1 H), 5.82 (tdd, $J = 4.9, 10.2, 17.1$ Hz, 0.5 H), 5.91 (tdd, $J = 4.9, 10.2, 17.1$ Hz, 0.5 H), 5.99 (tdd, $J = 5.9, 10.7, 17.1$ Hz, 1 H), 6.14 (tdd, $J = 5.9, 10.7, 17.1$ Hz, 1 H), 6.49 (dd, $J = 5.9, 8.8$ Hz, 1 H), 6.84 (d, $J = 8.8$ Hz, 2 H), 7.21–7.25 (m, 2 H), 7.28–7.32 (m, 4 H), 7.36 (d, $J = 8.8$ Hz, 1 H), 7.37 (d, $J = 8.8$ Hz, 1 H), 7.48–7.50 (m, 5 H), 8.12 (s, 0.5 H), 8.14 (s, 0.5 H); ^{31}P NMR δ 148.6.[14] Anal. Calcd for $C_{46}H_{55}N_6O_8P$: C, 64.93; H, 6.51; N, 9.88. Found: C, 65.00; H, 6.62; N, 9.78.

3′-*O*-*p*,*p*′-Dimethoxytritylthymidine 5′-(Allyl *N*,*N*-diisopropylphosphoramidite) (15)

A solution of 3′-*O*-*p*, *p*′-dimethoxytritylthymidine (**10**) (1.21 g, 2.22 mmol), diisopropylamine (86.6 mg, 120 μL, 0.86 mmol), 1*H*-tetrazole (72.9 mg, 1.04 mmol), and (allyloxy)bis(diisopropylamino)phosphine (894 mg, 3.10 mmol) in acetonitrile (10 mL) was stirred at 25°C for 3 h. The reaction mixture was diluted with ethyl acetate (200 mL), washed with brine (100 mL × 2), dried, and concentrated. The resulting residual material was dissolved in benzene (8 mL) and the solution was poured into pentane (300 mL) at −78°C with stirring. The occurring powder was collected by filtration and dissolved in dichloromethane (100 mL). Concentration of the solution was afforded **15** as an amorphous solid (1.50 g, 92%): UV λ_{max} 268 (ε 11300), 236 (21300), 210 nm (34000); IR 1695, 1610, 1510, 1465, 1250 cm^{-1}; ^{1}H NMR δ 0.99 (d, $J = 6.8$ Hz, 3 H), 1.08 (d, $J = 6.8$ Hz, 3 H), 1.13 (d, $J = 6.8$ Hz, 3 H), 1.15 (d, $J = 6.8$ Hz, 3 H), 1.72 (ddd, $J = 5.9, 9.3, 13.7$ Hz, 1 H), 1.86 (s, 3 H), 1.86–1.92 (m, 1 H), 3.22–3.30 (m, 1 H), 3.45–3.56 (m, 2.5 H), 3.64 (m, 0.5 H), 3.79 (s, 6 H), 4.01–4.14 (m, 2 H), 4.33 (d, $J = 5.9$ Hz, 0.5 H), 4.35 (d, $J = 5.9$ Hz, 0.5 H), 5.07 (d, $J = 10.3$ Hz, 0.5 H), 5.10 (d, $J = 10.3$ Hz, 0.5 H), 5.17 (dd, $J = 2.0, 17.1$ Hz, 0.5 H), 5.22 (dd, $J = 2.0, 17.1$ Hz, 0.5 H), 5.80 (tdd, J = 5.4, 10.3, 17.1 Hz, 0.5 H), 5.86 (tdd, $J = 5.4, 10.3, 17.1$ Hz, 0.5 H), 6.41 (dd, $J = 5.4, 9.3$ Hz, 0.5 H), 6.46 (dd, $J = 5.4, 9.3$ Hz, 0.5 H), 6.82 (d, $J = 8.8$ Hz, 2 H), 6.83 (d, $J = 8.8$ Hz, 2 H), 7.21–7.35 (m, 5 H), 7.33 (d, $J = 8.8$ Hz, 2 H), 7.34 (d, $J = 8.8$ Hz, 2 H), 7.43–7.46 (m, 2 H), 7.52 (s, 0.5 H), 7.65 (s, 0.5 H), 8.17 (br s, 1 H); ^{31}P NMR δ 148.6, 148.4. Anal. Calcd for $C_{40}H_{50}N_3O_8P$: C, 65.65; H, 6.89; N, 5.74. Found: C, 65.61; H, 6.87; N, 5.69.

Deprotection and Detachment of Oligodeoxyribonucleotides

Immediately before carrying out removal of the allylic protectors, a solution of the Pd catalyst in THF was prepared by heating a heterogeneous mixture of $Pd_2(dba)_3 \bullet CHCl_3$ (20.7 mg, 20.0 mmol), triphenylphosphine (52.5 mg, 200 μmol), diethylamine (87.7 mg, 124 μL, 1.20 mmol), and formic acid (55.3 mg, 45.3 μL, 1.20 mmol) in THF (2.0 mL) until being a clean orange solution. The protected

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

oligodeoxyribonucleotide anchored on the CPG supports were washed with acetonitrile (0.5 mL × 3) and dried in vacuo. To the solid supports was added a suitable volume of the Pd-catalyst solution (2.5 equiv of the Pd catalyst/allyl). The mixture was heated at 50°C for 1 h with occasional shaking. After the supernatant fluid was decanted, the resulting CPG supports were washed by the reported procedure (6). The resulting CPG supports were treated with conc. aqueous ammonia at 25°C for 1 h to give the oligodeoxyribonucleotide.

ACKNOWLEDGMENT

This work was supported in part by Grants-in-Aid for Scientific Research (No. 11101001) from the Ministry of Education, Science, Sports and Culture, by a Grant from "Research for the Future" Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301), and by contributions from the Asahi Glass Foundation and the Daiko Foundation.

REFERENCES

1. (a) Asseline, U.; Bonfils, E.; Kurfürst, R.; Chassignol, M.; Roig, V.; Thuong, N.T. *Tetrahedron* **1992**, *48*, 1233–1254, and references cited therein. (b) Marasco, Jr., C.J.; Angelino, N.J.; Paul, B.; Dolnick, B.J. *Tetrahedron Lett.* **1994**, *35*, 3029–3032. (c) Will, D.W.; Brown, T. *Tetrahedron Lett.* **1992**, *33*, 2729–2732. (d) Reed, M.W.; Adams, A.D.; Nelson, J.S.; Meyer, Jr., R.B. *Bioconjugate Chem.* **1991**, *2*, 217–225. (e) Gmeiner, W.H.; Luo, W.; Lown, J.W. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 487–490. (f) Perrouault, L.; Asseline, U.; Rivalle, C.; Thuong, N.T. Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Hélène, C. *Nature (London)* **1990**, *344*, 358–360. (g) Le Doan, T.; Perrouault, L.; Chassignol, M.; Thuong, N.T.; Hélène, C. *Nucleic Acids Res.* **1987**, *15*, 8643–8659. (h) Le Doan, T.; Perrouault, L.; Hélène, C. *Biochemistry* **1986**, *25*, 6736–6739. (i) Theisen, P.; McCollum, C.; Upadhya, K.; Jacobson, K.; Vu, H.; Andrus, A. *Tetrahedron Lett.* **1992**, *33*, 5033–5036. (j) Zuckermann, R.; Corey, D.; Schultz, P. *Nucleic Acids Res.* **1987**, *15*, 5305–5321. (k) McMinn, D.L.; Greensberg, M.M. *J. Am. Chem. Soc.* **1998**, *120*, 3289–3294, and references cited therein. (l) Chaloin, L.; Vidal, P.; Lory, P.; Méry, J.; Lautredou, N.; Divita, G.; Heitz, F. *Biochem. Biophys. Res. Commun.* **1998**, *243*, 601–608, and references cited therein. (m) Robles, J.; Maseda, M.; Beltrán, M.; Concernau, M.; Pedroso, E.; Grandas, A. *Bioconjugate Chem.* **1997**, *8*, 785–788, and references cited therein. (n) Soukchareun, S.; Tregear, G.W.; Haralambidis, J. Bioconjugate Chem. **1995**, *6*, 43–53, and references cited therein. (o) De la Torre, B.G.; Aviñó, A.; Tarrason, G.; Piulats, J.; Albericio, F.; Eritja, R. *Tetrahedron Lett.* **1994**, *35*, 2733–2736. (p) Judy, C.D.; Richardson, C.D.; Brousseau, R. *ibid.* **1991**, *32*, 879–882.
2. Previous oligodeoxyribonucleotide synthesis via the (5′ → 3′)-chain elongation phosphoramidite method: (a) van de Sande, J. H.; Ramsing, N.B.; Germann, M.W.; Elhorst, W.; Kalisch, B.W.; Kitzing, V.E.; Pon, R.T.; Clegg, R.C.; Jovin, T.M. *Science* **1988**, *241*, 551. (b) Horne, D.A.; Dervan, P.B. *J. Am. Chem. Soc.* **1990**, *112*, 2435–2437.

(c) Robles, J.; Pedroso, E.; Grandas, A. *Nucleic Acids Res.* **1995**, *23*, 4151–4161. (d) Wagner, T.; Pfleiderer, W. *Nucleosides Nucleotides* **1997**, *16*, 1657–1660.

3. Since the preparation of the nucleoside 5′-phosphoramidites is more tedious than that of the nucleoside 3′-phosphoramidites, the (5′ → 3′)-elongation strategy is less useful for the synthesis of simple oligonucleotides than the alternative (3′ → 5′)-elongation approach. By contrast, the (5′ → 3′)-elongation is obviously more efficient than the (3′ → 5′)-elongation for the synthesis of solid-anchored 3′-*O*-free oligonucleotides because of its directness.
4. Hayakawa, Y.; Harada, H.; Hirose, M.; Noyori, R.; Wakabayashi, S.; Miyazaki, K.; Kawase, Y.; Kato, I. *Nucleic Acids Symp. Ser.* **1991**, *25*, 63–64.
5. The compounds **1**, **2**, and **4** are known in literatures. **1** and **2**: Heidenhain, S.B.; Hayakawa, Y. *Synlett* **1998**, 853–854. **4**: Heidenhain, S.B.; Hayakawa, Y. *Nucleosides Nucleotides* **1999**, *18*, 1771–1787. The derivative **3** was obtained by the desilylation of 3′, 5′-di-*O*-*tert*-butyldimethylsilyl-N^2-allyloxycarbonyl-2′-deoxyguanosine, reported in the following ref 6, with $(n\text{-}C_4H_9)_4NF$ in THF.
6. Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691–1696.
7. Preparation of the N^2-allyloxycarbonyl-O^6-allyl-3′-*O*-dimethoxytrityl-2′-deoxyguanosine 5′-phosphoramidite was also attempted in a similar manner, but it could not be achieved because its synthetic precursor, N^2-allyloxycarbonyl-O^6-allyl-3′-*O*-dimethoxytrityl-2′-deoxyguanosine, is labile and rapidly decomposed, even under an argon atmosphere, to the 3′-*O*-free compound.
8. Hayakawa, Y.; Kataoka, M.; Noyori, R. *J. Org. Chem.* **1996**, *61*, 7996–7997.
9. A reason why this oligomer was prepared in a lower yield than the oligomer containing no guanosine moiety is that the guanosine phosphoramidite **13** allows the lower (97.5%) yield of the coupling than the other nucleoside phosphoramidite, **11**, **12**, or **14** (average 99.8%). Further, the yield of final coupling with the cytidine phosphoramidite **12** was 100.4%, which indicated that a certain side reaction takes place on the guanosine base.
10. Hayakawa, Y.; Kato, H.; Uchiyama, M.; Noyori, R. *J. Org. Chem.* **1986**, *51*, 2400–2402.
11. A similar side reaction at the O^6-position of the guanine base has been observed in the synthesis of oligodeoxyribonucleotides with the O^6-unprotected guanosine monomer via the (3′ → 5′)-chain elongation based on the phosphoramidite strategy. See, Pon, R.T.; Damha, M.J.; Ogilvie, K.K. *Nucleic Acids Res.* **1985**, *13*, 6447–6465. According to this report, the guanosine O^6-phosphityl product given by the use of the nucleoside 3′-phosphoramidite is decomposed by the treatment with a mixture of acetic anhydride and pyridine at 25°C for 0.5 min for capping the unreacted 5′-*O*-hydroxyl to give back the desired O^6-free derivative. In contrast, according to experiments carried out by us, the by-product afforded in the synthesis using the nucleoside 5′-phosphoramidite was not completed cleaved under the capping conditions even after 2 min.
12. The use of the O^6-protected guanosine building block to prevent the side reaction has been reported in the synthesis of oligodeoxyribonucleotides via the (3′ → 5′)-chain elongation. Pon, R.T.; Usman, N.; Damha, M.J.; Ogilvie, K.K. *Nucleic Acids Res.* **1986**, *14*, 6453–6470.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

13. (a) Sakakura, A; Hayakawa, Y.; Harada, H.; Hirose, M.; Noyori, R. *Tetrahedron Lett.* **1999**, *40*, 4359–4362. (b) Sakakura, A.; Hayakawa, Y. *Tetrhedron* in **2000**, *56*, 4427–4435.
14. Although the product was obtained as a mixture of two diastereomers, the ^{31}P NMR signals due to them are not separable to appear as a single peak.

Received July 17, 2000
Accepted October 27, 2000

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!