Heteroatom Chemistry
Volume 2, Number 1, 1991

Efficient Deanilidation of Phosphoranilidates by the Use of Nitrites and Acetic Anhydride†

Shigeyoshi Nishino, Yasuhiro Nagato, Yoshihiro Hasegawa, and Hisanao Yamamoto

Department of Chemistry, Faculty of Science, Tokyo Institute of Technology, O-okayama, Meguro-ku, Tokyo, Japan 152

Kazuo Kamaike and Yoshiharu Ishido*

Laboratory of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo, Japan 192-03

Received 28 June 1990.

ABSTRACT

Reagent systems of sodium– and tetrabutylammonium nitrite–acetic anhydride were proved to be extremely efficient for the deanilidation of nucleoside 3′-phosphoranilidates, whose reactions were rapid with the former and instant with the latter. It was further found that the reagent system is applicable to oligonucleotide synthesis provided that the exocyclic amino groups of 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxycytidine were protected by succinylation.

INTRODUCTION

The phosphoramidate approach has been widely accepted as a useful method for the synthesis of oligonucleotides bearing a 3′-terminal phosphoramidate function, whose amidate protecting group is properly stable and can be removed under neutral conditions by the use of an appropriate agent without damaging other protecting groups [2–11]. Isoamyl nitrite in a mixture of acetic acid and pyridine has been reported to be useful for the unmasking in the phosphoranilidate approach to the synthesis of oligonucleotides by Zielinski et al. [2] and Ohtsuka et al. [4]. In contrast to the phosphoranilidate, the corresponding anisidate is conspicuously susceptible to deamidation, although it still requires a 2–3 h time period for completion of the reaction under similar conditions [5, 6], and has been applied to the solid-phase synthesis of an oligonucleotide [7]. The addition of acetic anhydride to the solvent system was also shown by Hata et al. [8–10] to be efficient for the unmasking of a phosphoranilidate, which is completed in 3 h. In addition, deanilidation using carbon dioxide after sodiation with sodium hydride has been reported by Zielinski et al. [3], and is a useful method for the synthesis of an anti-sense oligonucleotide involving the phosphorothioate function by the use of carbon disulfide in place of carbon dioxide [11]. From the standpoint of the deanilidation reaction, it would be desirable to reduce the reaction time. We have recently established an efficient method for the unmasking of phosphoranilidates and our results are reported herein in full [12].

RESULTS AND DISCUSSION

We were interested in the decrease of molar ratio of acetic acid in the 4 : 5 mixture with pyridine, which brings about retardation of the reaction velocity in deanisidation [5]; i.e., protonation by the acidic species should be crucial in the two consecutive steps of *N*-nitrosation of phosphoranilidate and the P–N bond cleavage of *N*-nitrosophosphoranilidate involved therein. We were also interested in the significant effect of the addition of acetic

*To whom correspondence should be addressed.

†Partial Protection of Carbohydrate Derivatives. Part 27. For Part 26 see Ref. [1].

1042-7163/91/$3.50 + .25

anhydride to the mixture of acetic acid and pyridine on the P–N bond cleavage [8–10]. The acetic nitrous anhydride–sodium acetate system, which is easily prepared in situ from acetic anhydride and sodium nitrite, was chosen as a potential candidate reagent for improving the deanilidation reaction; the resulting two components were expected to behave as a strong nitrosating reagent and as a base in the reaction, respectively.

On treatment of a solution of 5′-*O*-dimethoxytritylthymidine 3′-(2-chlorophenyl) phosphoranilidate (**1**) with sodium nitrite (8 mol equiv) and acetic anhydride (200 mol equiv) in chloroform, it was demonstrated that the deanilidation was complete in about 10 min to give triethylammonium 5′-*O*-dimethoxytritylthymidine 3′-(2-chlorophenyl) phosphoranilidate (**2**) in 88% yield, as shown in Scheme 1. This interesting reaction was unsatisfactory to perform using a suspension of sodium nitrite in chloroform. Therefore, we next used tetrabutylammonium nitrite–acetic anhydride in pyridine, in view of the excellent solubility of the former in this organic solvent and because the nitrite ion paired with tetrabutylammonium ion should facilitate the formation of acetic nitrous anhydride because of its more naked or nucleophilic character toward the electrophilic acetic anhydride. Further improvement was then obtained by the use of ammonium nitrite (3 mol equiv) and acetic anhydride (4 mol equiv) in the reaction of **1** to give **2** (88% yield) (completed within one minute). The molar ratio of the reagents was optimized by the evaluation of the use of various proportions on the rate of the reaction. A similar reaction using the corresponding anisidate in place of the anilidate was found to be completed instantly (monitored by TLC).

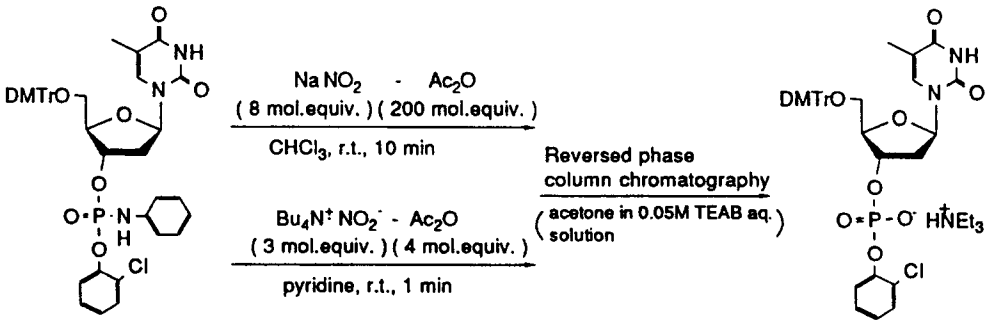
As we anticipated, because of the strong nitrosating activity of acetic nitrous anhydride, the reactions using N^6-benzoyl-5′-*O*-dimethoxytrityl-2′-deoxyadenosine, 5′-*O*-dimethoxytrityl-N^4-*o*-toluoyl-2′-deoxycytidine, and 5′-*O*-dimethoxytrityl-N^2-isobutyryl-2′-deoxyguanosine 3′-(2-chlorophenyl) phosphoranilidates were all confirmed to give considerable amounts of undesirable byproducts, probably because of nitrosation at the exocyclic acylamino group, in addition to the desired sites (monitored by TLC).

This system of tetrabutylammonium nitrite–acetic anhydride in pyridine, however, would be useful for oligonucleotide synthesis if we could prevent the undesirable nitrosation reaction by introducing a suitable protecting group onto the exocyclic amino groups. A potential methodology is to remove both active hydrogens on the amino groups by the use of a phthaloyl or a succinyl group; the latter might be better than the former judging from their relative stability toward a nucleophile. Incidentally, Hata et al. demonstrated the utility of phthaloyl and succinyl groups for minimizing the depurination of the 2′-deoxyadenylic acid unit in their oligodeoxynucleotide synthesis [13]. If this kind of approach were possible, it would provide us with a novel, efficient methodology for oligodeoxyribonucleotide and oligoribonucleotide syntheses in terms of the phosphoramidate approach.

It was significant to observe quantitative recovery (94% yield) of the starting material on treatment of 3′,5′-bis-*O*-dimethoxytrityl-N^6-succinyl-2′-deoxyadenosine [13] (**3**) with the reagent system after 2 h at room temperature. Therefore, we prepared 2′-deoxyribonucleosides 3′-phosphoranisidate derivatives as will be described below.

5′-O-Dimethoxytrityl-N⁶-succinyl-2′-deoxyadenosine 3′-(2-Chlorophenyl) Phosphoranisidate (**5**)

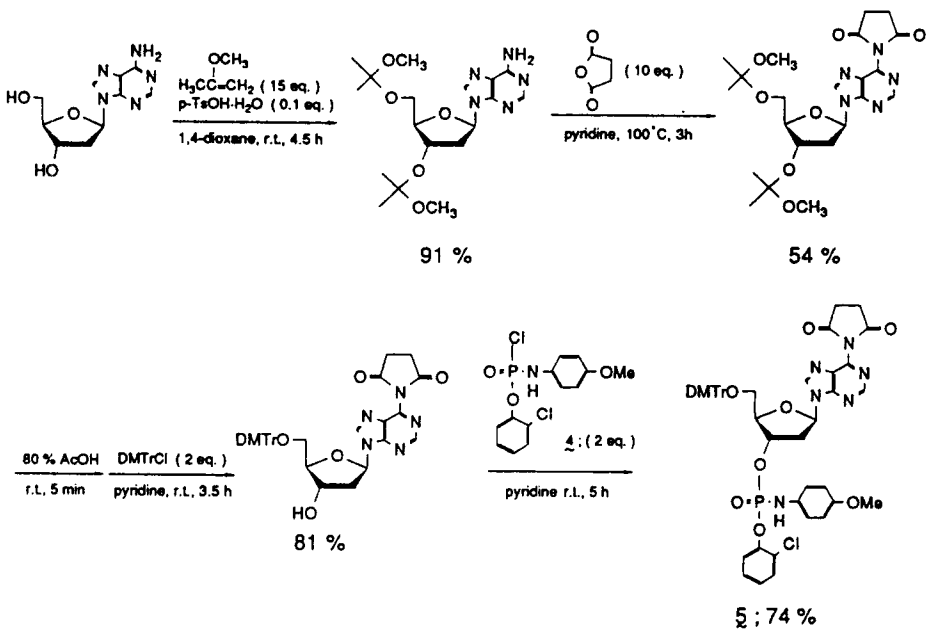
Compound **5** was prepared from 2′-deoxyadenosine through the sequence of reactions shown in Scheme 2; i.e., successive treatments with isopropenyl methyl ether in 1,4-dioxane in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate [14], with succinic anhydride in pyridine, with 80% aqueous acetic acid, with dimethoxytrityl chloride



1

2 ; 88 %

SCHEME 1



SCHEME 2

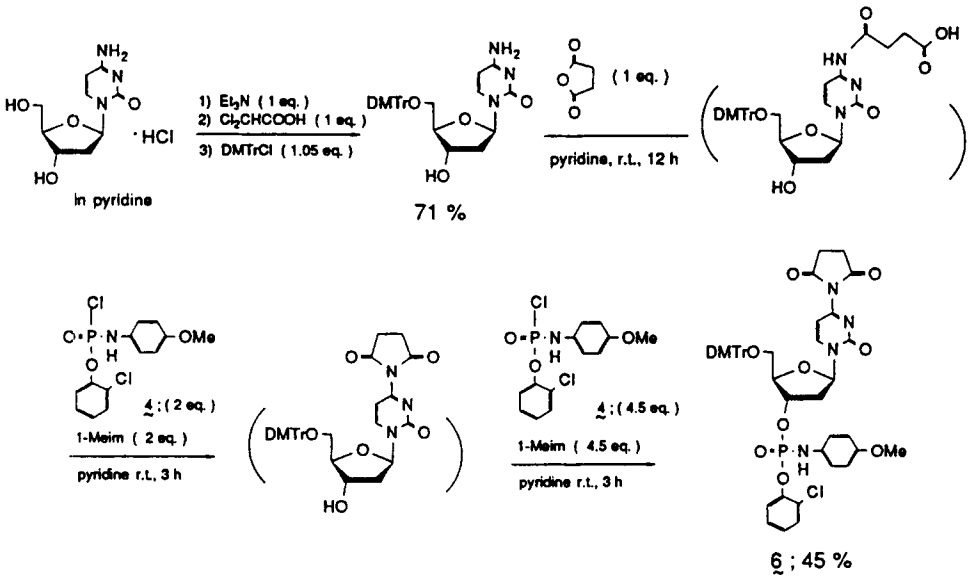
in pyridine [15], and finally with 2-chlorophenyl *N*-(4-methoxyphenyl) chlorophosphoramidate (**4**) in pyridine.

5'-O-Dimethoxytrityl-N⁴-succinyl-2'-deoxycytidine 3'-(2-Chlorophenyl) Phosphoranisidate (**6**)

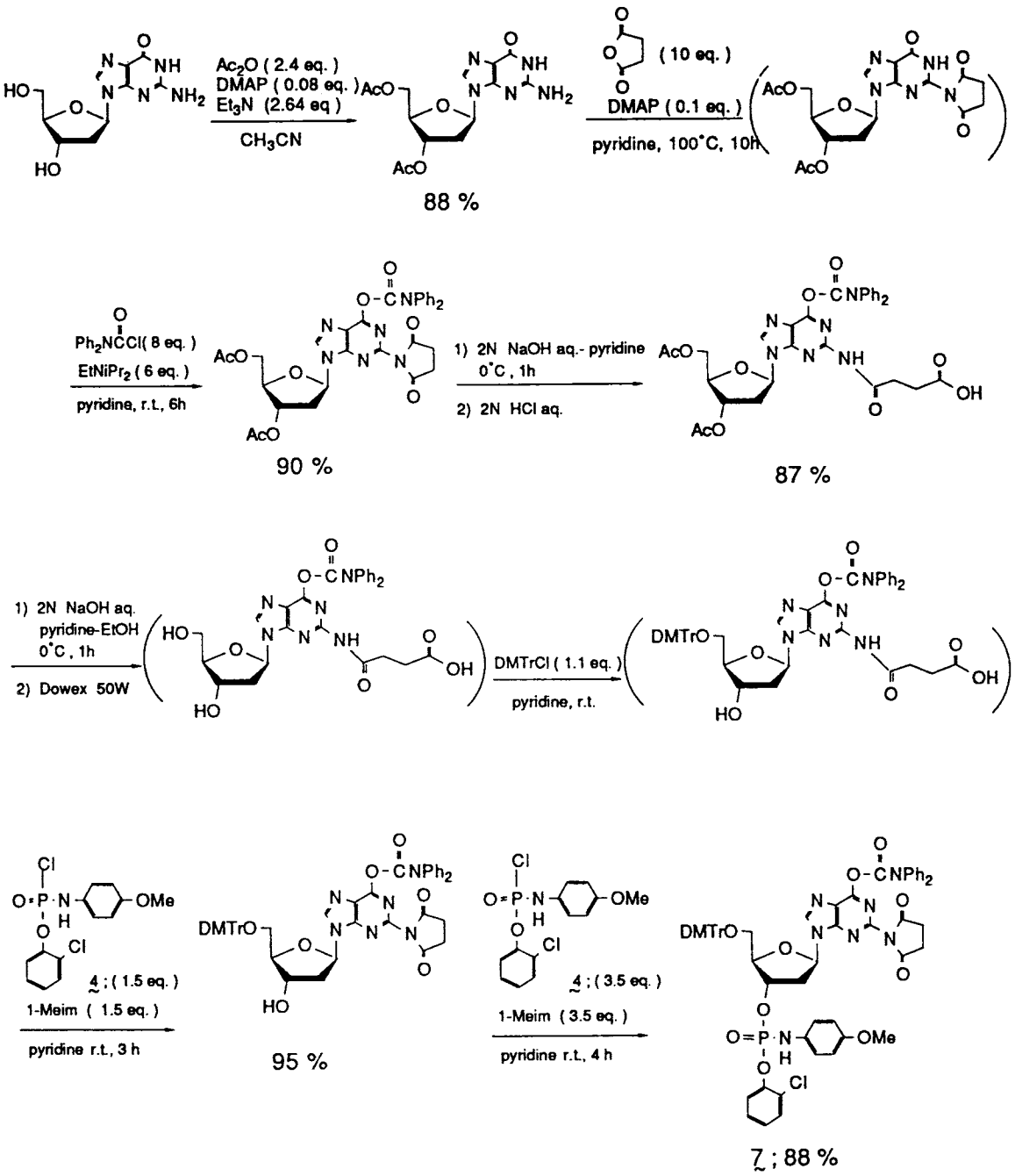
Compound **6** was prepared from 2'-deoxycytidine hydrochloride through the sequence of reactions shown in Scheme 3; i.e., successive treatments with dimethoxytrityl chloride in pyridine after the addition of triethylamine and then dichloroacetic acid, with succinic anhydride in pyridine, and with **4** in pyridine in the presence of an equimolar amount of 1-methylimidazole (1-Meim).

5'-O-Dimethoxytrityl-O⁶-diphenylcarbamoyl-N²-succinyl-2'-deoxyguanosine 3'-(2-Chlorophenyl) Phosphoranisidate (**7**)

Compound **7** was prepared by the sequence of reactions shown in Scheme 4, i.e., successive treatments with acetic anhydride in the presence of 4-



SCHEME 3

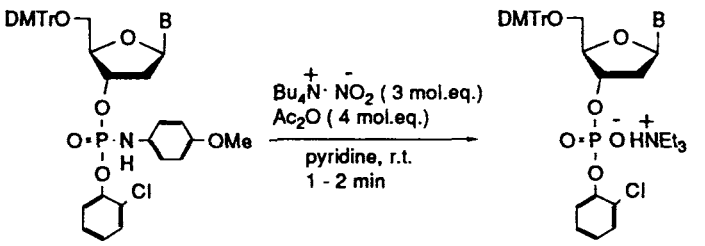


SCHEME 4

dimethylaminopyridine (DMAP) and triethylamine in acetonitrile [16]; with succinic anhydride in the presence of DMAP in pyridine; with diphenylcarbamoyl chloride in the presence of ethyl diisopropyl amine in pyridine [17]; with 2 M sodium hydroxide solution in aqueous pyridine followed by 2 M hydrochloric acid; with 2 M sodium hydroxide solution in aqueous pyridine–ethanol followed by Dowex 50 ion-exchange resin; with dimethoxytrityl chloride in pyridine; and finally with **4** in the presence of 1-Meim in pyridine in two successive steps.

5'-O-Dimethoxytritylthymidine 3'-(2-Chlorophenyl) Phosphoranisidate (**8**)

Compound **8** was prepared from thymidine by 5'-*O*-dimethoxytritylation [15], followed by phosphorylation [6] according to the methods reported.



5, 6, 7, 8

9 ; A^{Suc} = 98 %
10 ; C^{Suc} = 93 %
11 ; $^{Dpc}G^{Suc}$ = 85 %
12 ; T = 90 %

SCHEME 5

Deanisidation of **5**, **6**, **7**, *and* **8** *By the Use of Tetrabutylammonium Nitrite–Acetic Anhydride in Pyridine (See Scheme 5)*

All the derivatives were separatedly treated with tetrabutylammonium nitrite (3 mol equiv) and acetic anhydride (4 mol equiv) at room temperature in pyridine, and confirmed to afford the desired triethylammonium salts of the corresponding 2′-deoxyribonucleoside 3′-(2-chlorophenyl) phosphates, i.e., **9** (98% yield), **10** (93% yield), **11** (85% yield), and **12** (90% yield).

In conclusion, the present investigation proves the utility of the reagent system tetrabutylammonium nitrite–acetic anhydride in pyridine for the preparation of a 2′-deoxyribonucleoside bearing 3′-(2-chlorophenyl) phosphate as its triethylammonium salt, provided one uses the *N*-succinyl protecting group for the exocyclic amino groups of 2′-deoxyadenosine, 2′-deoxycytidine, and 2′-deoxyguanosine. This kind of approach should also be feasible in the cases of oligoribonucleotide syntheses. Furthermore, the application of this reagent system to the solid-phase approach is of particular interest in comparison with the use of isoamyl nitrite [7].

EXPERIMENTAL

TLC was conducted on Merck silica gel F_{254} by developing with 9:1 chloroform–methanol. Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chemicals, Co. Ltd.), by the use of chloroform–methanol, methylene chloride–methanol, or chloroform–methanol–triethylamine, and reversed-phase column chromatography was carried out on silanized silica gel (Kieselgel 60 silaniziert, 70–230 mesh, purchased from Merck) by the use of acetone–0.05 M triethylammonium hydrogencarbonate (bicarbonate; TEAB). High performance liquid chromatography (HPLC) was performed on LiChrosorb RP-18 (4 mm ID × 250 mm L) and NOVA PAK C-18 (4.6 mm ID × 150 mm L).

^{1}H-NMR spectra were recorded on a JEOL JNM FX-200 apparatus with tetramethylsilane (TMS) as the internal standard.

Elemental analyses were determined with a Perkin-Elmer 240-002 apparatus.

Synthesis of 5′-O-Dimethoxytritylthymidine 3′-(2-Chlorophenyl) Phosphoranilidate (**1**)

A solution of 5′-*O*-dimethoxytritylthymidine [15] (2.723 g, 5 mmol) in pyridine (5 mL × 3) was azeotropically evaporated, and the residue was dissolved in pyridine (25 mL), to which 1-methylimidazole (1-Meim; 1.2 mL, 15 mmol) and 2-chlorophenyl *N*-phenyl chlorophosphoramidate [3] (3.776 g, 12.5 mmol) were added, and the resulting mixture was stirred for 30 min at room temperature. The reaction mixture was diluted with methylene chloride (150 mL), and successively washed with a 5% aqueous solution of sodium bicarbonate (100 mL × 2) and water (100 mL). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness after filtering off the desiccant. The residue was purified by column chromatography on a column (4.5 cm ID × 15 cm L, methanol–methylene chloride system) of silica gel to give **1** (91% yield) as a mixture of diastereoisomers; R_f 0.47 and 0.52; ^{1}H-NMR ($CDCl_3$–TMS): δ 1.37 (1H, s, CH_3), 2.36–2.79 (2H, m, H-2′ and 2″), 3.24–3.62 (2H, m, H-5′ and 5″), 3.73 (6H, OCH_3 × 2), 4.29–4.39 (1H, m, H-4′), 5.13–5.33 (1H, m, H-3′), 6.46–6.53 (1H, m, H-1′), 6.76–7.46 (24H, m, Ph proton × 22, H-6 and P—N*H*), and 10.01 and 10.09 (1H, s × 2, N^3-*H*).

Anal. Calcd. for $C_{43}H_{41}N_3O_9PCl$: C, 63.74; H, 5.10; N, 5.19. Found: C, 63.54; H, 5.22; N, 5.11.

Deanilidation of **1** (*See Scheme 1*)

With Tetrabutylammonium Nitrite–Acetic Anhydride. To a solution of **1** (0.405 g, 0.5 mmol) in

pyridine (0.5 mL) was added a 0.8 M solution of tetrabutylammonium nitrite in pyridine (1.8 mL) and acetic anhydride (0.189 mL, 2 mmol) with stirring at room temperature. After 1 min of stirring, the resulting solution was treated with 0.5 M aqueous tetraethylammonium hydrogen carbonate (bicarbonate; TEAB) solution (14 mL), and the solution was extracted with methylene chloride (40 mL). The extract was washed with 0.5 M aqueous TEAB solution (20 mL × 2). The organic layer was evaporated to dryness, and the residue was chromatographed on a column (2.54 cm ID × 10 cm L; 20–40% acetone–0/05 M TEAB aqueous solution) of reversed-phase silica gel to give triethylammonium 5′-*O*-dimethoxytritylthymidine 3′-(2-chlorophenyl) phosphate (**2**; 0.368 g (88% yield)); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.25 (9H, t, J = 7.33 Hz, $N(CH_2CH_3)_3$), 2.32–2.47 (1H, m, H-2″), 2.58–2.72 (1H, m, H-2′), 2.99 (6H, q, $N(CH_2CH_3)_3$), 3.18–3.43 (2H, m, H-5′ and 5″), 3.77 (6H, s, OCH_3 × 2), 4.26–4.33 (1H, m, H-4′), 5.16–5.25 (1H, m, H-3′), 6.46 (1H, dd, $J_{1',2'}$ = 5 Hz, $J_{1',2''}$ = 6.5 Hz, H-1′), 6.77–7.67 (18H, m, Ph proton × 17 and H-6), and 9.43 (1H, br s, N^3-*H*).

A similar reaction by the use of sodium nitrite (0.276 g, 4 mmol) and acetic anhydride (9.4 mL, 100 mmol) toward a solution of **1** (0.405 g, 0.5 mmol), in chloroform (5 mL), however, took 10 min to complete, and the subsequent workup in the same way as above gave **2** (0.369 g, 88% yield).

*Examination of Stability of the Succinyl Protecting Group of 3′,5′-Bis-*O*-dimethoxytrityl-*N^6*-Succinyladenosine* (**3**)

To a solution of **3** [13] (0.248 g, 0.248 mmol) in pyridine (0.3 mL) 0.8 M tetrabutylammonium nitrite solution in pyridine (0.93 mL) and acetic anhydride (0.094 mL, 0.90 mmol) were added. The mixture was stirred for 2 h at room temperature. TLC test showed only one spot (R_f value = 0.68; 9:1 chloroform–methanol) at this reaction time, and the mixture was treated with 0.5 M TEAB aqueous solution (14 mL). The resultant mixture was extracted with methylene chloride (30 mL), and the extract was washed with the TEAB solution (15 mL × 2). The organic layer was dried over anhydrous magnesium sulfate and evaporated to dryness after filtering off the desiccant. The residue was chromatographed on a column (2.5 cm ID × 10 cm L) by the use of methanol–methylene chloride system to give **3** (0.2196 g, 94% yield).

^{1}H-NMR ($CDCl_3$–TMS): δ 1.83–1.98 (1H, m, H-2″), 2.23–2.41 (1H, m, H-2′), 2.98 (4H, s, CH_2CO × 2), 2.96–3.12 (1H, m, H-5′), 3.27 (1H, dd, $J_{4',5'}$ = 3.5 Hz, $J_{5',5''}$ = 10.5 Hz, H-5′), 3.76 (12H, s, OCH_3 × 4), 4.11–4.20 (1H, m, H-4′), 4.40–4.50 (1H, m, H-3′), 6.53 (1H, dd, $J_{1',2'}$ = 5.5 Hz, $J_{1',2''}$ = 9.0 Hz, H-1′), 6.62–7.72 (26 H, m, Ph proton × 26), 8.27 (1H, s, H-8), and 8.92 (1H, s, H-2).

*Syntheses of 5′-*O*-Dimethoxytrityl-2′-Deoxynucleosides 3′-(2-Chlorophenyl) Phosphoranisidates*

1) Synthesis of **5** *(See Scheme 2).* a) 3′,5′-Bis-*O*-(1-methoxy-1-methyl)ethyl-2′-deoxyadenosine: To a suspension of 2′-deoxyadenosine (1.2562 g, 5 mmol) in 1,4-dioxane (15 mL) *p*-toluenesulfonic acid monohydrate (0.0951 g, 0.5 mmol) and isopropenyl methyl ether (7.18 mL, 75 mmol) were added. The mixture was stirred for 4 h 30 min at room temperature, quenched with aqueous sodium hydrogencarbonate saturated solution (75 mL), and extracted with methylene chloride (150 mL). The organic layer was then evaporated to dryness, and chromatographed on a column of silica gel to give the title product (1.7896 g, 91% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.39 (12H, s, CH_3 × 4), 2.46–2.74 (2H, m, H-2′ and 2″), 3.24 (3H, s, OCH_3), 3.60 (1H, dd, $J_{5',5''}$ = 10.6 Hz, $J_{4',5'}$ = 3.8 Hz, H-5′), 3.71 (1H, dd, $J_{4',5''}$ = 3.7 Hz, H-5″), 4.16–4.27 (1H, m, H-4′), 4.58–4.71 (1H, m, H-3′), 6.40–6.53 (3H, m, H-1′ and NH_2), 8.22 (1H, s, H-8), and 8.35 (1H, s, H-2).

Anal. Calcd. for $C_{18}H_{29}N_5O_5$: C, 54.67; H, 7.39; N, 17.71. Found: C, 54.67; H, 7.39; N, 17.64.

b) 3′,5′-Bis-*O*-(1-methoxy-1-methyl)ethyl-N^6-succinyl-2′-deoxyadenosine: A solution of the product (0.3208 g, 0.81 mmol) obtained in (a) was twice evaporated azeotropically in pyridine (3 mL), and the residue was dissolved in pyridine (10 mL). The solution was treated with succinic anhydride (0.8116 g, 8.11 mmol) for 3 h at 100°C under stirring. The resulting solution was treated with aqueous sodium hydrogencarbonate saturated solution (75 mL), and was extracted with methylene chloride (150 mL). The organic layer was evaporated to dryness, and chromatographed on a column of silica gel to give the title product (0.2104 g, 54% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.36 (6H, s, CH_3 × 2), 1.39 (6H, s, CH_3 × 2), 2.50–2.82 (2H, m, H-2′ and 2″), 3.04 (4H, s, $COCH_2CH_2CO$), 3.17 (3H, s, OCH_3), 3.24 (3H, s, OCH_3), 3.60 (1H, dd, $J_{5',5''}$ = 10.7 Hz, $J_{4',5'}$ = 3.4 Hz, H-5′), 3.72 (1H, dd, $J_{4',5''}$ = 3.4 Hz, H-5″), 4.22–4.31 (1H, m, H-4′), 4.63–4.74 (1H, m, H-3′), 6.59 (1H, t, $J_{1',2'}$ H-1′), 8.60 (1H, s, H-8), 9.01 (1H, s, H-2).

c) 5′-*O*-Dimethoxytrityl-N^6-succinyl-2′-deoxyadenosine: A solution of the product (0.2104 g, 0.44 mmol) obtained in (b) in 80% aqueous acetic acid solution (6 mL) was stirred for 5 min at room temperature. The solution was evaporated, and the residue was evaporated from water repeatedly until no smell of acetic acid remained. The moisture in the resulting residue was removed by repeated evaporation from pyridine as described above. The residue was dissolved in pyridine (2.5 mL) and treated with dimethoxytrityl chloride (0.1641 g, 0.48 mmol and 0.160 g, 0.47 mmol after 1 h) under stirring. The mixture was treated with aqueous sodium hydrogencarbonate saturated solution (20 mL) after 3.5 h from the start of this reaction and extracted with methylene chloride (40 mL). After evaporation

to dryness, the extract was chromatographed on a column of silica gel to give a glass of the title product (0.2250 g, 81% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 2.47–2.59 (1H, m, H-2″), 2.74–2.78 (1H, m, H-2′), 3.00 (4H, s, $COCH_2CH_2CO$), 3.35–3.47 (2H, m, H-5′ and 5″), 3.76 (6H, s, OCH_3 × 2), 4.10–4.21 (1H, m, H-4′), 4.62–4.73 (1H, t, J = 6.5 Hz, H-1′), 6.76–6.82 and 7.14–7.40 (13H, m, Ph proton × 13), 8.33 (1H, s, H-8), 8.91 (1H, s, H-2).

Anal. Calcd. for $C_{35}H_{33}N_5O_7$: C, 66.13, H, 5.23; N, 11.02. Found: C, 66.27; H, 5.27; N, 11.21.

d) 5′-*O*-Dimethoxytrityl-N^6-succinyl-2′-deoxyadenosine 3′-(2-chlorophenyl) phosphoranisidate (**5**): The product (1.6899 g, 2.66 mmol) obtained in (c) was dried by repeated evaporation from pyridine azeotropically, and was dissolved in pyridine (10 mL). The solution was treated with 2-chlorophenyl *N*-(4-methoxyphenyl) chlorophosphoramidate (**4**) (1.7658 g, 5.32 mmol) at room temperature for 5 h with stirring. The resulting solution was then treated with water (5 mL), neutralized with aqueous sodium hydrogencarbonate saturated solution (100 mL), and extracted with methylene chloride (200 mL). After evaporation, the extract was chromatographed on a column of silica gel to give **5** (1.838 g, 74% yield) as a mixture of diastereoisomers; ^{1}H-NMR ($CDCl_3$–TMS): δ 2.69–2.94 (2H, m, H-2′ and 2″), 2.99 (4H, s, $COCH_2CH_2CO$), 3.23–3.48 (2H, m, H-5′ and 5″), 3.73 (9H, s, OCH_3 × 3), 4.38–4.55 (1H, m, H-4′), 5.43–5.60 (1H, m, H-3′), 6.37–6.51 (1H, m, H-1′), 6.68–6.83 and 6.97–7.52 (22H, m, Ph proton × 21 and P—N*H*), 8.20 and 8.25 (1H, s × 2, H-8), and 8.85–8.88 (1H, s × 2, H-2).

Anal. Calcd. for $C_{48}H_{44}N_6O_{10}PCl$: C, 61.90; H, 4.76; N, 9.02. Found: C, 61.74; H, 4.83; N, 9.15.

2) Synthesis of **6** *(See Scheme 3).* a) 5′-*O*-Dimethoxytrityl-2′-deoxycytidine: 2′-Deoxycytidine hydrochloride (5.2738 g, 20 mmol) was subjected to dehydration by repeated evaporation from pyridine and then dissolved in pyridine (100 mL). To this solution triethylamine (2.79 mL) and, after 30 min. stirring at room temperature, dichloroacetic acid (1.64 mL, 20 mmol) and dimethoxytrityl chloride (7.4543 g, 22 mmol) were added. After 1.5 h, the resulting mixture was subjected to the action of water (10 mL), neutralized with aqueous sodium hydrogencarbonate saturated solution (250 mL), and then extracted with methylene chloride (500 mL). The extract was evaporated and subjected to column chromatography to give a glass of the desired product (7.5177 g, 71% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 2.21 (1H, dt, $J_{1',2''}$ = 6.1 Hz, $J_{2',2''}$ = 13.4 Hz, H-2″), 2.44 (1H, ddd, $J_{1',2'}$ = 6.1 Hz, $J_{3',2'}$ = 5.1 Hz, H-2′), 3.36–3.43 (2H, m, H-5′ and 5″), 3.98–4.04 (1H, m, H-4′), 4.45–4.53 (1H, m, H-3′), 5.604 (1H, d, $J_{5,6}$ = 7.3 Hz, H-5), 6.22 (1H, t, H-1′), 6.82–6.86 and 7.19–7.44 (13H, m, DMTr-aromatic protons), and 7.95 (1H, d, H-6).

Anal. Calcd. for $C_{30}H_{31}O_6N_3$: C, 68.04; H, 5.90; N, 7.93. Found: C, 67.85; H, 5.85; N, 7.92.

b) **6**: The product (19.0304 g, 35.39 mmol) obtained in (a) was repeatedly subjected to azeotropic evaporation and dissolved in pyridine (108 mL), to which was added succinic anhydride (3.9555 g, 39.52 mmol), and the mixture was stirred for 17 h at room temperature. The solvent was evaporated and again re-evaporated from pyridine azeotropically. A solution of the residue in pyridine (100 mL) was treated with **4** (33.4126 g, 100.6 mmol) and 1-Meim (8.02 mL, 100.6 mmol) and, 3 days later, **4** (11.9342 g, 35.93 mmol) and 1-Meim (2.86 mL, 35.93 mmol). After stirring for 1.5 h, the solvent was evaporated and the residue was neutralized with aqueous sodium hydrogencarbonate saturated solution (300 mL). The mixture was extracted with methylene chloride (600 mL), and the organic layer was evaporated. The residue was subjected to column chromatographic purification on a column of silica gel to give **6** (6.8427 g, 21% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ *of less polar isomer*, 2.30–2.48 (1H, m, H-2″), 2.56–2.64 and 2.75–2.84 (4H, m, $COCH_2$—CH_2CO), 2.95–3.10 (1H, m, H-2′), 3.34–3.42 (2H, m, H-5′ and 5″), 3.75 (9H, s, OCH_3 × 3), 4.33–4.41 (1H, m, H-4′), 5.33–5.47 (1H, m, H-3′), 6.29 (1H, t, $J_{1',2'}$ = $J_{1',2''}$ = 6.1 Hz, H-1′), 6.72–7.50 (23H, m, Ph proton × 22, P—N*H*, and H-5), and 8.03 (1H, $J_{5,6}$ = 7.6 Hz, H-6); *of more polar isomer*, 2.32–2.49 (1H, m, H-2″), 2.51–2.64 and 2.69–2.78 (4H, m, $COCH_2CH_2CO$), 2.85–2.98 (1H, m, H-2′), 3.42–3.51 (2H, m, H-5′ and 5″), 3.72 (3H, s, OCH_3), 3.75 (6H, s, OCH_3 × 2), 4.37–4.46 (1H, m, H-4′), 5.32–5.48 (1H, m, H-3′), 6.29 (1H, t, $J_{1',2'}$ = $J_{1',2''}$ = 5.9 Hz, H-1′), 6.72–7.45 (23H, m, Ph proton × 21, P—N*H*, and H-5), and 8.11 (1H, d, $J_{5,6}$ = 7.6 Hz, H-6).

3) Synthesis of **7** *(See Scheme 4).* a) 3′,5′-Di-*O*-acetyl-N^2-succinyl-O^6-diphenylcarbamoyl-2′-deoxyguanosine: 3′,5′-Di-*O*-acetylguanosine [16] (3.5132 g, 10 mmol) was dissolved in pyridine (50 mL), after azeotropic evaporation and treated with succinic anhydride (8.0056 g, 80 mmol) and 4-dimethylaminopyridine (DMAP; 122.2 mg, 1 mmol) for 10 h at 100°C under stirring. The reaction mixture was allowed to cool at room temperature, and then treated with diphenylcarbamoyl chloride (18.5344 g, 80 mmol) and ethyl diisopropyl amine (10.45 mL, 60 mmol) for 4 h at room temperature under stirring. The resulting mixture was neutralized with aqueous sodium hydrogencarbonate saturated solution (100 mL) and extracted with methylene chloride (200 mL). The organic layer was evaporated and subjected to chromatography on a column of silica gel to give a glass of the title product (5.6523 g, 90% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 2.03 (3H, s, CH_3), 2.12 (3H, s, CH_3), 2.48–2.73 and 2.80–3.02 (6H, m, $COCH_2CH_2CO$, H-2′ and 2″), 4.29–4.63 (3H, m, H-4′, 5′, and 5″), 5.35–5.43 (1H, m, H-3′), 6.37–6.49 (1H, m, H-1′), 7.18–7.52 (10H, m, Ph proton × 10), and 8.33 (1H, s, H-8). The product (4.064 g, 6.46 mmol) thus obtained was dissolved in pyridine (38.71 mL) and treated with 2 M aqueous sodium hydrox-

ide solution (6.46 mL) for 1 h under a chilled condition with ice-bath with stirring. After neutralizing with 2 M hydrochloric acid, the mixture was concentrated in vacuo and then extracted with methylene chloride (200 mL). Precipitated sodium chloride was removed by decantation, the organic layer was washed with water (100 mL), the organic solvent was evaporated to dryness, and the residue was then subjected to chromatography on a column of silica gel by the use of chloroform–methanol system to give 3',5'-di-*O*-acetyl-O^6-diphenylcarbamoyl-N^2-(3-carboxypropionyl)-2'-deoxyguanosine (a glass, 3.678 g, 87% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.99 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 2.60–3.10 (6H, m, H-2', 2'', and $COCH_2CH_2CO$), 4.25 (3H, H-4', 5', and 5''), 5.40 (1H, m, H-3'), 6.40 (1H, t, $J_{1',2'} = J_{1',2''} = 6.27$ Hz, H-1'), 7.30–7.55 (10H, m, Ph protons), 8.15 (1H, s, H-8), and 9.24 (1H, s, N^2-*H*).

Anal. Calcd. for $C_{29}H_{30}N_6O_{10}$: C, 55.90; H, 4.85; N, 13.49. Found: C, 56.23; H, 4.64; N, 13.36.

b) 5'-*O*-Dimethoxytrityl-O^6-diphenylcarbamoyl-N^2-succinyl-2'-deoxyguanosine: The product (1.83 g, 2.81 mmol) obtained in (a) was dissolved in a mixture of pyridine (16.89 mL) and ethanol (16.89 mL), and the solution was treated with 2 M aqueous sodium hydroxide solution (2.81 mL) under cooling in an ice-bath for 1 h. To the resulting mixture Dowex 50W ion exchange resin was then added to neutralize the base, and the solution was evaporated, after filtering off the resin. The residue was evaporated azeotropically from pyridine three times and dissolved in pyridine (15 mL). Dimethoxytrityl chloride (0.948 g, 2.81 mmol) was added to the solution, after which the mixture was stirred for 2 h at room temperature and subjected to the action of 0.5 M aqueous TEAB solution. The resulting solution was extracted with methylene chloride (30 mL), and the organic layer was evaporated. The residue was then three times evaporated azeotropically from pyridine, and again dissolved in pyridine (15 mL). The solution was treated with **4** (1.399 g, 4.22 mmol) and 1-Meim (0.345 mL, 4.22 mmol) for 3 h at room temperature with stirring. The resulting solution was then treated with aqueous sodium hydrogencarbonate saturated solution (50 mL), and extracted with methylene chloride (100 mL). The organic layer was evaporated and the residue was chromatographed on a column of silica gel by the use of methylene chloride–methanol system to give the title product (2.250 g, 95% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 2.55–2.83 (2H, m, H-2' and 2''), 2.75 (4H, s, $COCH_2CH_2CO$), 3.25–3.45 (2H, m, H-5' and 5''), 3.75 (6H, s, $OCH_3 \times 2$), 4.15 (1H, m, H-4'), 4.57 (1H, m, H-3'), 6.50 (1H, t, $J_{1',2'} = J_{1',2''} = 4.56$ Hz, H-1'), 6.70–7.50 (23H, m, Ph protons), and 8.35 (1H, s, H-8).

Anal. Calcd. for $C_{48}H_{42}N_6O_9 \cdot H_2O$: C, 66.68; H, 5.08; N, 9.71. Found: C, 66.41; H, 5.46; N, 9.54.

c) **7**: The product (1.057 g, 1.25 mmol) obtained in (b) was dissolved in pyridine (5 mL), and the solution was treated with **4** (0.829 g, 2.50 mmol) and 1-Meim (0.205 mL, 2.50 mmol) for 4 h at room temperature with stirring. The mixture was subjected to the action of aqueous sodium hydrogencarbonate saturated solution (50 mL) and extracted with methylene chloride (80 mL). The organic layer was evaporated and chromatographed on a column of silica gel by the use of methylene chloride–methanol system to give the title product 1.255 g, 88% yield) as a glass of a diastereoisomer mixture; ^{1}H-NMR ($CDCl_3$–TMS): δ 2.55–3.00 (2H, m, H-2' and 2''), 2.87 and 2.88 (4H, s × 2, $COCH_2CH_2CO$), 3.30–3.65 (2H, m, H-5' and 5''), 3.70 (3H, s, OCH_3), 3.75 (6H, s, $OCH_3 \times 2$), 4.35–4.45 (1H, m, H-4''), 5.40–5.55 (1H, m, H-3'), 6.17–6.25 (1H, m, H-1'), 6.40–7.70 (32H, m, Ph proton × 31 and P—N*H*), and 8.22 and 8.25 (1H, s × 2, H-8)

Anal. Calcd. for $C_{61}H_{53}N_7O_{12}PCl \cdot H_2O$: C, 63.00; H, 4.74; N, 8.44. Found: C, 62.97; H, 4.86; N, 8.42.

4) Synthesis of **8**. 5'-*O*-Dimethoxythymidine [15] (2.2690 g, 4.03 mmol) was dissolved in pyridine (15 mL) after azeotropic evaporation from pyridine and treated with **4** (2.7672 g, 8.33 mmol) for 7 h at room temperature with stirring. The mixture was treated with water, neutralized with aqueous sodium hydrogencarbonate saturated solution (75 mL), and extracted with methylene chloride (150 mL). The extract was evaporated and the residue was chromatographed on a column of silica gel to give a glass of **8** (3.116 g, 92% yield) as a mixture of diastereoisomers; ^{1}H-NMR ($CDCl_3$–TMS): δ 1.35 (3H, s, CH_3), 2.34–2.77 (2H, m, H-2' and 2''), 3.21–3.58 (2H, m, H-5' and 5''), 3.73 (3H, s, NH—C_6H_4—CH_3), 3.76 (6H, s, $OCH_3 \times 2$ of DMtr groups), 4.22–4.28 (0.5H, m, H-4'), 4.30–4.37 (0.5H, m, H-4'), 5.32–5.48 (1H, m, H-3'), 6.38–6.51 (1H, m, H-1'), 6.68–7.45 (22H, m, Ph proton × 21 and P—N*H*), and 7.53 (1H, s, H-6).

Anal. Calcd. for $C_{44}H_{43}N_3O_{10}PCl$: C, 62.90; H, 5.16; N, 5.00. Found: C, 62.60; H, 5.01; N, 5.30.

Deanisidation Reactions of 5'-O-Dimethoxytrityl-2'-deoxyribonucleosides 3'-(2-Chlorophenyl) Phosphoroanisidates (See Scheme 5)

1) The Reaction of **5**: Compound **5** (0.529 g, 0.56 mmol) was dissolved in pyridine (2.0 mL), and 1 M solution of tetrabutylammonium nitrite in pyridine (1.14 mL) and acetic anhydride (0.214 mL, 2.24 mmol) were added to the solution. After the solution had been stirred for 2 min, it was treated with 0.5 M TEAB aqueous solution (50 mL), and extracted with methylene chloride (80 mL). The organic layer was evaporated and dissolved in 2:1 water–pyridine (60 mL). The solution was washed with diethyl ether (40 mL × 3). The aqueous layer

was evaporated, and the residue was dissolved in 0.5 M aqueous TEAB solution (40 mL). The organic layer was extracted with methylene chloride (80 mL) and the extract was evaporated. The residue was chromatographed on a column of reversed-phase silica gel by the use of 10–50% acetone in 0.05 M aqueous TEAB solution system to give a glass of triethylammonium 5′-*O*-dimethoxytrityl-N^6-succinyl-2′-deoxyadenosine 3′-(2′-chlorophenyl)phosphate (**9**) (0.441 g, 85% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.40 (9H, t, $N(CH_2CH_3)_3$), 2.60–3.42 (10H, m, $N(CH_2CH_3)_3$, H-2′, 2″, 5′, and 5″), 3.00 (4H, s, $COCH_2CH_2CO$), 3.76 (6H, s, $OCH_3 \times 2$), 4.42–4.51 (1H, m, H-4′), 5.14–5.33 (1H, m, H-3′), 6.55–6.66 (1H, m, H-1′), 6.73–6.91 and 7.07–7.39 (17H, m, Ph protons), 8.29 (1H, s, H-8), and 8.88 (1H, s, H-2).

2) The Reaction of **6**. Compound **6** (0.8546 g, 0.942 mmol) was dissolved in pyridine (1.9 mL), and 1 M solution of tetrabutylammonium nitrite in pyridine (2.83 mL) and acetic anhydride (0.356 mL, 3.77 mmol) were added to the solution, after which the solution was stirred for 2 min at room temperature. Subsequent workup as described above, followed by the reversed-phase column chromatography, gave a glass of triethylammonium 5′-*O*-dimethoxytrityl-N^4-succinyl-2′-deoxycytidine 3′-(2-chlorophenyl) phosphate (**10**) (0.7811 g, 93% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.25 (9H, t, $N(CH_2CH_3)_3$), 2.17–2.36 (1H, m, H-2″), 2.57–2.69 and 2.79–3.04 (11H, m, $N(CH_2CH_3)_3$, $COCH_2CH_2CO$, and H-2′), 3.20–3.43 (2H, m, H-5′ and 5″), 3.77 (6H, s, $OCH_3 \times 2$), 4.38–4.47 (1H, m, H-4′), 5.06–5.19 (1H, m, H-3′), 6.25–6.33 (1H, m, H-1′), 6.77–7.37 (18H, m, Ph proton × 17 and H-5), and 8.04 (1H, d, $J_{5,6}$ = 7.6 Hz, H-6).

3) The Reaction of **7**. Compound **7** (0.463 g, 0.405 mmol) was dissolved in pyridine (1.95 mL), and 1 M solution of tetrabutylammonium nitrite in pyridine (1.2 mL) and acetic anhydride (0.219 mL, 1.62 mmol) were added to the solution, after which the solution was stirred for 2 min at room temperature. Subsequent workup as above, followed by the reversed-phase column chromatography, afforded a glass of triethylammonium 5′-*O*-dimethoxytrityl-O^6-diphenylcarbamoyl-N^2-succinyl-2′-deoxyguanosine 3′-(2-chlorophenyl) phosphate (0.391 g, 85% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.22 (9H, t, $N(CH_2CH_3)_3$), 2.60–3.00 (2H, m, H-2″), 2.80–3.10 (10H, m, $N(CH_2CH_3)_3$ and $COCH_2CH_2CO$), 3.20–3.40 (2H, m, H-5′ and 5″), 3.73 (6H, s, $OCH_3 \times 2$), 4.40–4.45 (1H, m, H-4′), 5.14–5.25 (1H, m, H-3′), 6.45–6.55 (1H, t, $J_{1',2'} = J_{1',2''}$ = 5.7 Hz, H-1′), 6.70–7.75 (27H, m, Ph protons), and 8.25 (1H, s, H-8).

4) The Reaction of **8**. Compound **8** (1.1007 g, 1.31 mmol) was dissolved in pyridine (2.6 mL), and a 1 M solution of tetrabutylammonium nitrite in pyridine (3.93 mL) and acetic anhydride (0.494 mL, 5.24 mmol) were added to the solution, after which the mixture was stirred for 2 min at room temperature. Subsequent workup as described above, followed by the reversed-phase column chromatography, gave a glass of triethylammonium 5′-*O*-dimethoxytritylthymidine 3′-(2-chlorophenyl) phosphate (**12**) (0.9861 g, 90% yield); ^{1}H-NMR ($CDCl_3$–TMS): δ 1.25 (9H, t, $N(CH_2CH_3)_3$), 1.58 (3H, br s, CH_3), 2.29–2.48 (1H, m, H-2″), 2.57–2.73 (1H, m, H-2′), 2.98 (6H, q, $N(CH_2CH_3)_3$), 3.15–3.41 (2H, m, H-5′ and 5″), 3.78 (6H, s, $OCH_3 \times 2$), 4.28–4.36 (1H, m, H-4′), 5.12–5.25 (1H, m, H-3′), 6.33–6.55 (1H, m, H-1′), 6.76–6.95 and 7.59–7.68 (18H, m, Ph proton × 17 and H-6).

ACKNOWLEDGMENT

The authors thank Mrs. Noriko Hasegawa, Tokyo Institute of Technology, for elemental analyses. One of the authors (Y. I.) thanks the Science and Technology Agency, the Japanese Government, for Special Coordination Funds.

REFERENCES

[1] For Part 26, see K. Kamaike, Y. Hasegawa, I. Masuda, Y. Ishido, K. Watanabe, I. Hirao, K. Miura, *Tetrahedron, 46*, 1990, 163.
[2] W. S. Zielinski, Z. J. Lesnikowski, *Synthesis*, 1976, 185.
[3] Z. J. Lesnikowski, W. J. Stec, W. S Zielinski, *Synthesis*, 1980, 397.
[4] E. Ohtsuka, T. Tanaka, T. Wakabayashi, Y. Taniyama, M. Ikehara, *J. Chem. Soc., Chem. Commun.*, 1978, 824; E. Ohtsuka, T. Ono, M. Ikehara, *Chem. Pharm. Bull., 29*, 1981, 3274.
[5] E. Ohtsuka, S. Shibahara, M. Ikehara, *Chem. Pharm. Bull., 29*, 1981, 3440.
[6] E. Ohtsuka, Y. Taniyama, R. Marumoto, H. Sato, H. Hirosaki, M. Ikehara, *Nucleic Acids Res., 10*, 1982, 2597.
[7] E. Ohtsuka, Y. Taniyama, S. Iwai, T. Yoshida, M. Ikehara, *Chem. Pharm. Bull., 32*, 1984, 85; S. Iwai, E. Yamada, M. Asaka, Y. Hayase, H. Inoue, E. Ohtsuka, *Nucleic Acids Res., 15*, 1987, 3761.
[8] M. Sekine, T. Hata, *Tetrahedron Lett., 24*, 1983, 5741.
[9] M. Nishizawa, T. Kurihara, T. Hata, *Chem. Lett.*, 1984, 175.
[10] T. Hata, T. Kamimura, K. Urakami, K. Kohno, M. Sekine, I. Kumagai, K. Shinozaki, K. Miura, *Chem. Lett.*, 1987, 117.
[11] Z. J. Lesnikowski, W. Niewiarowski, W. S. Zielinski, W. J. Stec, *Tetrahedron, 40*, 1984, 15.
[12] S. Nishino, Y. Nagato, Y. Hasegawa, K. Kamaike, Y. Ishido, *Nucleic Acids Res., Symp. Ser., 20*, 1988, 73; S. Nishino, Y. Nagato, Y. Hasegawa, K. Kamaike, Y. Ishido, *Nucleic Acids Res., Symp. Ser., 21*, 1989, 29; K. Kamaike, Y. Hasegawa, I. Masuda, Y. Nagato, S. Nishino, Y. Ishido, The 1989 International Chemical Congress of Pacific Basin Societies, Abstract Part 1, BIOS-04-10 [Dec. 17–22 (1989)].

[13] A. Kume, M. Sekine, T. Hata, *Tetrahedron Lett., 23,* 1982, 4365; A. Kume, R. Iwase, M. Sekine, T. Hata, *Nucleic Acids Res., 12,* 1984, 8525.
[14] C. B. Reese, R. Saffhill, J. E. Sulston, *Tetrahedron, 26,* 1970, 1023.
[15] H. Schaller, G. Weimann, B. Lerch, H. G. Khorana, *J. Am. Chem. Soc., 85,* 1963, 3821; C. B. Reese, A. Ubasawa, *Tetrahedron Lett., 21,* 1980, 2265; C. B. Reese, A. Ubasawa, *Nucleic Acids Res. Symp. Ser., 7,* 1980, 5.
[16] A. Matsuda, M. Shinozaki, M. Suzuki, K. Watanabe, T. Miyasaka, *Synthesis,* 1986, 385.
[17] T. Kamimura, M. Tsuchiya, K. Urakami, K. Koura, M. Sekine, T. Hata, *J. Am. Chem. Soc., 106,* 1984, 4552.