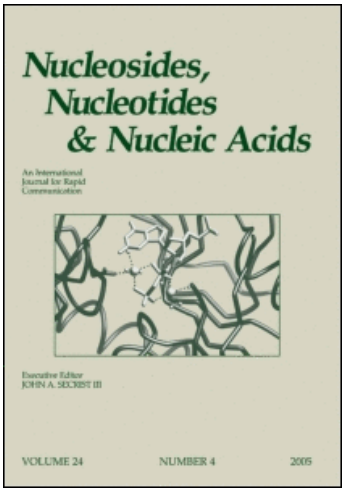

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Sophie B. Heidenhain[a]; Yoshihiro Hayakawa[a]
[a] Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Nagoya, Japan

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IMPROVED METHODS FOR THE PREPARATION OF 2'-DEOXYRIBONUCLEOSIDE AND RIBONUCLEOSIDE 3'-PHOSPHORAMIDITES WITH ALLYLIC PROTECTORS

Sophie B. Heidenhain and Yoshihiro Hayakawa*,[a]

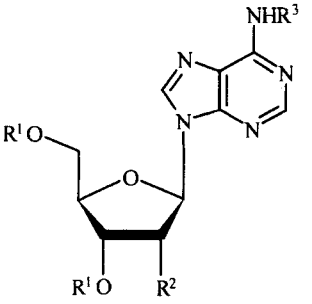
Laboratory of Bioorganic Chemistry, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-01, Japan

Abstract: Improved methods of preparing 2'-deoxyribonucleoside and ribonucleoside 3'-phosphoramidites with allylic protecting groups, which serve as useful building blocks, particularly for the synthesis of base-labile derivatives, are developed.

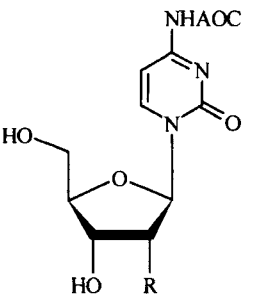
The development of useful protecting groups for nucleoside bases is an important subject in the chemical synthesis of oligonucleotides.[1] Several years ago, we developed the allyloxycarbonyl (AOC) protector, which can be cleanly removed by a Pd(0)-catalyzed reaction under almost neutral conditions[2,3,4] to give DNA and RNA oligomers of excellent quality. Further, this protection is useful for the preparation of base-labile derivatives, including oligoDNAs containing (5*R*)-5,6-dihydro-5-hydroxythymidine which is known as a mutagenic and/or cytotoxic modification of RNA,[5] oligoDNAs with modified backbones, such as phosphonodiesters,[6] phosphotriesters,[6] and phosphorothiodiesters,[7] 2'-deoxy-3-isoadenosine-incorporated nucleotides,[8] cytidine-5'-monophosphono-*N*-acetylneuraminic acid (CMP-Neu5Ac),[9] branched-type RNAs,[10] and N^4-acetylcytidine-incorporated oligoRNAs.[11] The AOC protection reported previously, however, has a drawback in that the introduction of the protector requires rather expensive reagents and time-consuming multi-operations. For instance, the protection of adenine and cytosine bases is achieved using allyl 1-benzotriazolyl carbonate (AOC-OBT)[3,4,10] or 1-(allyloxycarbonyl)tetrazole (AOC-Tet),[4,10] neither of

[a] *Tel: 81-52-789-4848; Fax: 81-52-789-4765; E-mail: yoshi@info.human.nagoya-u.ac.jp (Yoshihiro Hayakawa).*

which is commercially available and both of which must therefore be prepared from allyloxycarbonyl chloride (AOC-Cl) and the rather expensive 1-(hydroxy)benzotriazole or 1*H*-tetrazole. Further, the preparation of the *N*-AOC-adenosines demands the activation of the amino function of the nucleobase by the use of *tert*-butyllithium[3] or *tert*-butylmagnesium chloride,[10] which are both extremely moisture-sensitive and require special care, such as working under an argon atmosphere. The protection of the guanine base is much more tedious. Historically, only the N^2-amino group of the guanine base has been protected. Recently, however, many researchers have reported side reactions at the amide function during the formation of the internucleotide linkage via most of the available synthetic methods, including the phosphotriester method[12] and the phosphoramidite approach.[13] Therefore, we considered an additional protection of the amide function (the O^6-position) is of common interest. The amide protection also allows a higher solubility of guanosine derivatives in acetonitrile,[14] which is required for the performance of an efficient synthesis. We also recently developed AOC and allyl protectors for the N^2- and O^6-functions of the guanine base, respectively.[15] In this method, the introduction of the allylic protectors to the 2'-deoxyguanosine derivative was achieved from the 3',5'-*O*-bis-*tert*-butyldimethylsilyl (TBDMS)-protected derivative via (1) N^2-allyloxycarbonylation using AOC-Cl and *tert*-butylmagnesium chloride in a mixture of HMPA and THF, (2) the O^6-sulfonylation with mesitylenesulfonyl chloride in a mixture of HMPA and dichloromethane, and (3) the replacement of the sulfonyloxy function by allyl alcohol via the assistance of trimethylamine. However, there are drawbacks of this strategy. The allylation of the O^6-group (the step 2 and 3) requires several operation steps, including the isolation of the O^6-sulfonylated intermediate and several different reagents. Also undesirable is the use of toxic HMPA as a reaction



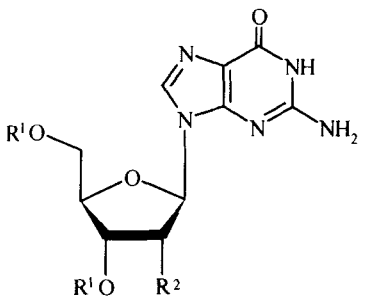
1: $R^1 = (CH_3)_3Si$; $R^2 = R^3 = H$
2: $R^1 = (CH_3)_3Si$; $R^2 = H$; $R^3 = AOC$
3: $R^1 = R^2 = H$; $R^3 = AOC$
4: $R^1 = H$; $R^2 = OH$; $R^3 = AOC$



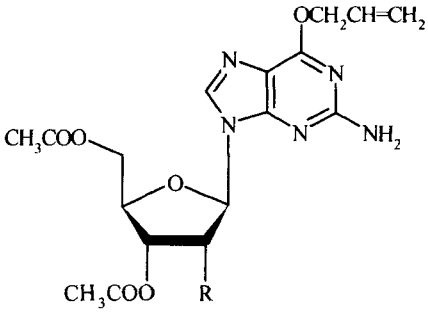
5: R = H
6: R = OH

solvent in the N^2-allyloxycarbonylation and O^6-sulfonylation. In addition, the TBDMS protection and its deblocking demand the expensive TBDMS chloride and tetrabutylammonium fluoride, respectively. There is thus a need for improvement of these methods to eliminate these drawbacks. Accordingly, this paper presents convenient methods for the preparation of 2'-deoxyribonucleoside and ribonucleoside phosphoramidites with allylic protecting groups.

N^6-Allyloxycarbonylation of 2'-deoxyadenosine was achieved in one pot by successive treatment with (1) an excess of hexamethyldisilazane (HMDS) and a catalytic amount of $(NH_4)_2SO_4$[16] in refluxing dioxane, giving the bis-silylated compound **1**; (2) AOC-Cl and *N*-methylimidazole, giving **2**; and (3) triethylamine in methanol to remove the transient silyl protector, affording **3** in 77% isolated yield. In a similar manner, N^6-AOC-adenosine **4**, N^4-AOC-2'-deoxycytidine **5**, and N^4-AOC-cytidine **6** were prepared from the parent nucleosides in 89–99% overall yields.[17] This method, however, could not be applied to the amino group of the guanine base,[18] so that alternative means were necessary for the preparation of N^2-AOC-protected guanosine derivatives.



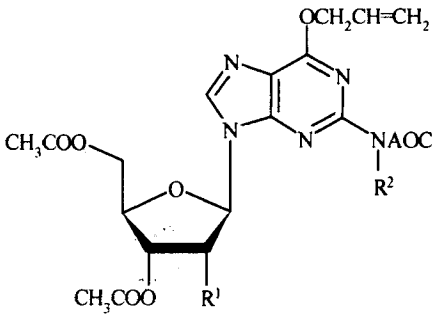
7: R^1 = TBDMS, R^2 = H
8: R^1 = TBDMS, R^2 = OTBDMS
9: R^1 = CH_3CO, R^2 = H
10: R^1 = CH_3CO, R^2 = CH_3COO



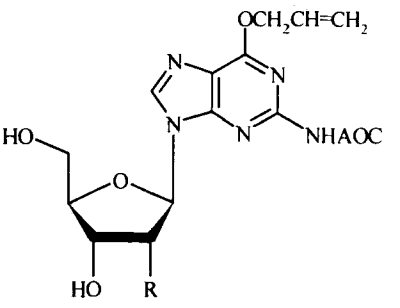
11: R = H
12: R = CH_3COO

The O^6-allylation via the Mitsunobu reaction[19] of **7** and **8**, whose sugar hydroxyls are protected by TBDMS groups, could not be accomplished. By contrast, we found that the *O*-acetylated derivatives, **9** and **10**, undergo the reaction with allyl alcohol to give the desired O^6-allylated products, **11** and **12**. Thus, the reaction of **9** and allyl alcohol by the assistance of $C_2H_5OCON{=}NCOOC_2H_5$ and triphenylphosphine in dioxane (25 °C, 60 min) afforded **11** in 80–91% yield. A similar reaction of **10** (60 °C, 3 h) gave **12** in 78% yield. The product **12** exhibited UV and ^1H NMR spectra superimposable to those of the authentic sample prepared via a different route, *i.e.*, the O^6-sulfonylation of **10** with mesitylenesulfonyl chloride in dichloromethane by the

assistance of triethylamine and 4-*N*,*N*-dimethylaminopyridine followed by the replacement of the sulfonyloxy function by allyl alcohol in the presence of *N*-methylmorpholine and DBU.[20] This result confirmed the O^6-allyl guanine structure of **12** and excluded another possible the N^1-allyl structure. Further, the O^6-allylated guanine structure of **11** was confirmed by the UV absorption at λmax 249 and 283 nm, which are identical with that observed in **12**.



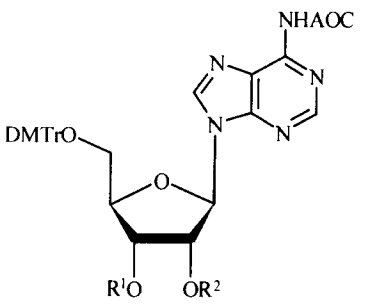
13: R^1 = H, R^2 = H
14: R^1 = H, R^2 = AOC
15: R^1 = CH_3COO; R^2 = H
16: R^1 = CH_3COO; R^2 = AOC



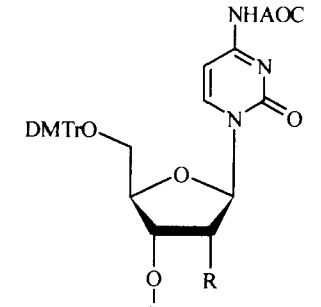
17: R = H
18: R = OH

Introduction of the AOC protecting group to the N^2-function of **11** was accomplished by treatment with 2.1 equiv. of *tert*-butylmagnesium chloride and 3.0 equiv. of AOC-Cl in THF (25 °C, 3 h) to give the N^2,N^2-bis-allyloxycarbonylated product **14** in 75% yield.[21] Similarly, the bis-AOC compound **16** was obtained from **12** in 66% yield.[21] Treatment of **14** with a 0.05 M solution of sodium hydroxide in ethanol removed the acetyl protector and one of the AOC groups selectively to give **17**.[22] In a similar manner, **16** was converted to **18**. The overall yields of **17** from **9** and **18** from **10** were 47% and 52%, respectively.

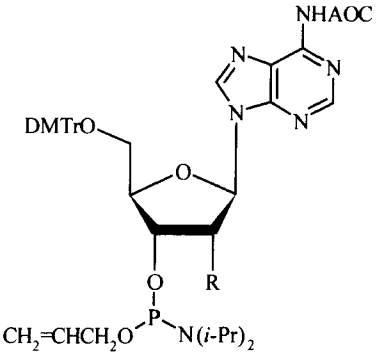
This new approach has some advantages in comparison with the previously reported procedure.[15] The allylation is achieved in a single step, a smaller amount of *tert*-butylmagnesium chloride is required for the N^2-allyloxycarbonylation,[23] and the use of toxic HMPA is avoided in both steps. Further, protection and removal of the acetyl protector are carried out by the use of less expensive reagents than those employed for the use of the TBDMS protectors. The allyloxycarbonylation using pyridine, triethylamine, 4-(dimethylamino)pyridine, 2-picoline, or 2,6-lutidine in place of the Grignard reagent as a promoter resulted in failure.



19: $R^1 = R^2 = H$
20: $R^1 = H$; R^2 = TBDMS
21: R^1 = TBDMS; $R^2 = H$



22: R = H
23: R = OTBDMS

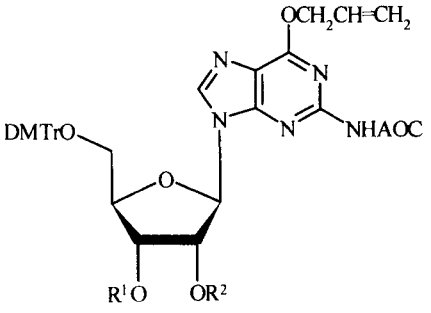


24: R = H
25: R = OTBDMS

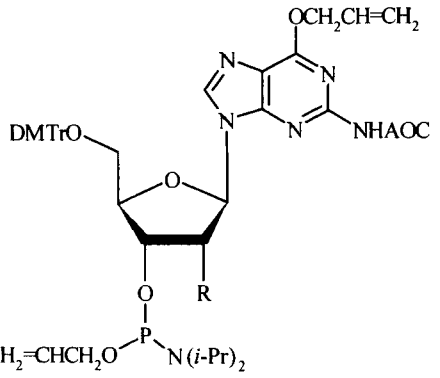
The AOC-protected adenosine and cytidine compounds, **3**, **4**, **5**, and **6**, can be converted to their phosphoramidites, **22**,[4] **23**,[11,24] **24**,[4] and **25**,[24] which are requisite as monomer units for oligonucleotide synthesis by the reported methods or their modifications. For example, **25** was obtained from **4** via (1) tritylation of the 5'-hydroxyl group by *p,p'*-dimethoxytrityl chloride in a 1:1 mixture of pyridine and DMF, giving **19** (77% yield); (2) silylation with *tert*-butyldimethylsilyl chloride and imidazole in DMF, producing **20** (33% yield) together with the 3'-*O*-silylated derivative **21** (45% yield); and (3) the condensation of **20** and $(CH_2{=}CHCH_2O)P[N(i\text{-}C_3H_7)_2]_2$ in the presence of diisopropylammonium tetrazolide (81% yield).

The guanine nucleosides **17** and **18** were derived to their 3'-phosphoramidites, **30** and **31**, as follows. The compound **17** was converted to the phosphoramidite **30** by the reported process.[15] Analogously, **18** was transformed to the 3'-phosphoramidite **31** via the steps of (1) the 5'-*O*-protection with *p,p'*-dimethoxytrityl chloride in a mixture of pyridine and DMF (55 °C, 1 h), giving **26** (74–78% yield); (2) the 2'-*O*-silylation using *tert*-butyldimethylsilyl chloride in the presence of imidazole (60 °C, 1 h) followed by the chromatographic separation from the 3'-*O*-silylated product **28**, affording **27** (29% yield);

and (3) the condensation with $(CH_2{=}CHCH_2O)P[N(i\text{-}C_3H_7)_2]_2$ promoted by diisopropylammonium tetrazolide (25 °C, 12 h), furnishing **31** (72% yield).



26: R^1 = H, R^2 = H
27: R^1 = H, R^2 = TBDMS
28: R^1 = TBDMS, R^2 = H
29: R^1 = TBDMS, R^2 = TBDMS



30: R = H
31: R = OTBDMS

We developed convenient methods for the preparation of nucleoside 3'-phosphoramidites with allylic protection that are superior to the previous methods in terms of operational simplicity and cost-performance. Thus, the advantage of the new approach in the preparation of adenosine and cytidine derivatives is that the introduction of the AOC protector can be performed by use of commercially available, inexpensive AOC-Cl and *N*-methylimidazole in place of AOC-OBT or AOC-Tet. On the other hand, the merit of the synthesis of guanosine analogs is that the one-step O^6-allylation is achieved with the acetyl group replacing the previously used TBDMS group for the protection of the sugar moiety. The N^2-allyloxycarbonylation requires a lesser amount of *tert*-butylmagnesium chloride for activation of the NH_2 function in comparison with the previous method, and toxic HMPA is not used in the synthesis.

EXPERIMENTAL SECTION

General Procedures and Materials. All melting points (mp) are uncorrected. UV spectra were measured in methanol on a JASCO V-550 spectrometer. ^{1}H NMR and ^{31}P NMR spectra were taken in DMSO-d_6 or $CDCl_3$ on a JEOL α-270 or α-400 instrument. For ^{1}H NMR spectra, the DMSO or $CDCl_3$ signal 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. Elementary analyses were carried out in the Faculty of Agriculture of Nagoya University. TLC was performed on a precoated silica gel 60 F_{254} plate supplied from Merck. 3',5'-*O*-Diacetyl-2'-deoxyguanosine (**9**),[25]

2',3',5'-*O*-triacetylguanosine (**10**),[16] (allyloxy)bis(diisopropylamino)phosphine,[4] and diisopropylammonium tetrazolide[26] were prepared by reported methods. Nucleoside derivatives commercially supplied and prepared by our hands were employed to reaction after coevaporation with a dry solvent. Commercially supplied reagents including hexamethyldisilazane (HMDS) (Chisso), *N*-methylimidazole (Nacalai), imidazole (Nacalai), allyloxycarbonyl chloride (AOC-Cl) (TCI), *tert*-butyldimethylsilyl chloride (ShinEtsu), and *p,p'*-dimethoxytrityl chloride (Lancaster) were used without any purification. Other substances were dried under high vacuum at room temperature unless otherwise stated. Acetonitrile was used after distillation, refluxing over phosphorus pentoxide, distillation, treatment with CaH_2, and distillation under argon atmosphere. Dioxane and pyridine were dried over sodium and CaH_2, respectively. The organic extracts were dried over magnesium sulfate before concentration. The reactions were carried out at ambient temperature unless otherwise noted.

N^6-[(Allyloxy)carbonyl]-2'-deoxyadenosine (3). A mixture of 2'-deoxyadenosine (15.1 g, 60.0 mmol), HMDS (160 mL), and ammonium sulfate (a catalytic amount) in dioxane (160 mL) was heated under reflux for 2.5 h. The reaction mixture was concentrated and the resulting residual material was coevaporated twice with dry toluene to give an oily product, which was dissolved in dichloromethane (400 mL). To this solution were added *N*-methylimidazole (12.0 mL, 12.4 g, 150 mmol) and AOC-Cl (12.0 mL, 13.6 g, 110 mmol) and the resulting mixture was stirred for 12 h. To this mixture, *N*-methylimidazole (2.40 mL, 2.50 g, 30.0 mmol) and AOC-Cl (3.10 mL, 3.50 g, 30.0 mmol) were added again. After 8 h, the reaction was quenched by addition of a phosphate buffer solution of pH 7. The whole mixture was extracted with dichloromethane (50 mL x 3) and the combined organic extracts were dried. Concentration of the organic solution gave a viscous oil, which was dissolved in methanol (300 mL) containing triethylamine (80 mL). The resulting solution was stirred for 12 h and then evaporated to afford an oil. This crude material was subjected to silica gel column chromatography with a 1:25 mixture of methanol and dichloromethane as eluent to give **3** (15.5 g, 77% yield) as a colorless foam; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); UV λ_{max} 267 nm; ^{1}H NMR (270 MHz, DMSO-d_6) 2.30–2.36 (m, 1H), 2.72–2.79 (m, 1H), 3.49–3.64 (m, 2H), 3.86–3.89 (m, 1H), 4.42–4.44 (m, 1H), 4.65 (d, 2H, J = 4.9 Hz), 4.98–5.00 (m, 1H), 5.23 (dd, 1H, J = 1.5 and 10.7 Hz), 5.33 (d, 1H, J = 4.9 Hz), 5.40 (dd, 1H, J = 1.5 and 17.1 Hz), 5.92–6.00 (m, 1H), 6.42–6.45 (m, 1H), 8.62 (s, 1H), 8.68 (s, 1H), 10.60 (s, 1H). Anal. Calcd for $C_{14}H_{17}N_5O_5 \cdot 1/3\ H_2O$: C, 49.27; H, 5.22; N, 20.52. Found: C, 49.29; H, 4.94; N, 20.52.

N^6-[(Allyloxy)carbonyl]adenosine (4). A mixture of adenosine (15.0 g, 56.0 mmol), HMDS (130 mL), and ammonium sulfate (a catalytic amount) in dry dioxane (140 mL) was heated under reflux for 3 h. The reaction mixture was evaporated to give an oil, which was coevaporated with dry toluene (50 mL x 2). The resulting residue was dissolved in dichloromethane (500 mL). To this solution were added *N*-methylimidazole (14.5 mL, 14.9 g, 180 mmol) followed by AOC-Cl (18.5 mL, 21.0 g, 170 mmol). The solution was stirred overnight and to resulting mixture were added *N*-methylimidazole (4.50 mL, 4.60 g, 56.0 mmol) and AOC-Cl (6.00 mL, 6.80 g, 56.0 mmol). After 12 h, the mixture was mixed with a phosphate buffer solution of pH 7 (300 mL) and extracted with dichloromethane (50 mL x 3). The combined organic layers were dried and evaporated to give a glassy oil, which was dissolved in methanol (500 mL). To the resulting solution was added triethylamine (130 mL) and the mixture was stirred for 12 h. The resulting colorless precipitates were collected by filtration, washed thoroughly with ether, and dried over P_2O_5 in vacuum. Concentration of the filtrate to one third of the volume gave an additional solid material, which was collected by filtration, washed with ether, and dried. The combined precipitates are **4** (17.5 g, 89% yield), mp 164–166 °C; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); UV λ_{max} 267 nm (ε 18,000); ^{1}H NMR (270 MHz, DMSO-d_6) 3.55–3.71 (m, 2H), 3.95–3.97 (m, 1H), 4.17 (m, 1H), 4.61–4.66 (m, 3H), 5.12 (t, 1H, J = 5.6 Hz), 5.21-5.22 (m, 1H), 5.23 (d, 1H, J = 10.6 Hz), 5.38 (dd, 1H, J = 1.4 and 15.8 Hz), 5.52 (d, 1H, J = 6.3 Hz), 5.90–6.04 (m, 2H), 8.63 (s, 1H), 8.68 (s, 1H), 10.66 (s, 1H). Anal. Calcd for $C_{14}H_{17}N_5O_6$: C, 47.86; H, 4.88; N, 19.93. Found: C, 47.86; H, 4.86; N, 20.14.

N^4-[(Allyloxy)carbonyl]-2'-deoxycytidine (5). 2'-Deoxycytidine hydrochloride (5.18 g, 19.6 mmol) was dissolved in a mixture of HMDS (50 mL) and dioxane (50 mL) containing ammonium sulfate (a catalytic amount) and the resulting solution was heated at 110 °C for 1 h. After cooling to room temperature, the mixture was passed through a Celite 545 pad and the filtrate was evaporated to give a viscous oil. To a solution of this oil in dichloromethane (200 mL) were successively added *N*-methylimidazole (2.2 mL, 2.6 g, 27.6 mmol) and AOC-Cl (2.8 mL, 3.18 g, 26.4 mmol). The resulting mixture was stirred for 1 h. The reaction was quenched by addition of a phosphate buffer of pH 7 (150 mL). The separated aqueous solution was extracted with dichloromethane (30 mL x 3) and the combined organic layers were concentrated. The obtained material was dissolved in a mixture of methanol (100 mL) and triethylamine (30 mL) and the solution was stirred for 12 h. Evaporation afforded a residual oil containing crystals. To this crude material was added a mixture of ether (50 mL) and dichloromethane (15 mL) and the mixture was vigorously stirred for 20 min. The resulting precipitate was collected by filtration and dried under high vacuum. These treatments gave **5** (5.80 g, 95% yield) as a

colorless powder. An analytical sample, mp 133-134 °C, was obtained by purification via the silica gel column chromatography with a 1:25 mixture of methanol and dichloromethane as eluent; R_f 0.4 (a 1:9 methanol–dichloromethane mixture); UV λ_{max} 241, 292 nm; ^{1}H NMR (270 MHz, DMSO-d_6) 1.96-2.04 (m, 1H), 2.25–2.29 (m, 1H), 3.52–3.58 (m, 2H), 3.83–3.85 (m, 1H), 4.20-4.21 (m, 1H), 4.62 (d, 2H, J = 5.4 Hz), 5.00 (t, 1H, J = 5.1 Hz), 5.20–5.24 (m, 2H), 5.36 (dd, 1H, J = 1.5 and 17.6 Hz), 5.86–5.99 (m, 1H), 6.06–6.11 (m, 1H), 7.00–7.03 (d, 1H, J = 7.4 Hz), 8.30–8.32 (d, 1H, J = 7.4 Hz), 10.78 (s, 1H). Anal. Calcd for $C_{13}H_{17}N_3O_6 \cdot 1/2H_2O$: C, 48.75; H, 5.66; N, 13.12. Found: C, 48.69; H, 5.67; N, 13.11.

N^4-[(Allyloxy)carbonyl]cytidine (6). A mixture of cytidine (15.0 g, 61.7 mmol), HMDS (150 mL), and ammonium sulfate (a catalytic amount) in dioxane (150 mL) was heated under reflux for 3 h. The reaction mixture was evaporated and the resulting material was coevaporated with dry toluene. The oily residue was dissolved in dichloromethane (500 mL). To this solution were added *N*-methylimidazole (6.70 mL, 6.90 g, 84.0 mmol) and AOC-Cl (8.90 mL, 10.1 g, 83.9 mmol) and the mixture was stirred for 1 h. Evaporation of the reaction mixture gave an oil, which was dissolved in a mixture of methanol (500 mL) and triethylamine (130 mL). The mixture was stirred for 12 h. Evaporation afforded a yellowish solid, which was recrystallized from a mixture of methanol (30 mL) and ethyl acetate (250 mL). Filtration of the crystals followed by washing with ethyl acetate and drying at 50 °C in vacuum gave **6** (20.0 g, 99% yield) as a colorless powder, mp 103–107 °C; R_f 0.3 (a 1:9 methanol–dichloromethane mixture); UV λ_{max} 242, 291 nm; ^{1}H NMR (400 MHz, DMSO-d_6) 3.57–3.97 (m, 5H), 4.62–4.64 (m, 2H), 5.03 (d, 1H, J = 5.4 Hz), 5.15 (t, 1H, J = 4.9 Hz), 5.22–5.38 (m, 2H), 4.47 (d, 1H, J = 4.4 Hz), 5.77 (d, 1H, J = 2.4 Hz), 5.91–5.98 (m, 1H), 7.00 (d, 1H, J = 7.3 Hz), 8.40 (d, 1H, J = 7.3 Hz), 10.80 (s, 1H). Anal. Calcd for $C_{13}H_{17}N_3O_7 \cdot 1/2\ H_2O$: C, 46.43; H, 5.39; N, 12.49. Found: C, 46.03; H, 5.16; N, 12.49.

O^6-Allyl-N^2-[(Allyloxy)carbonyl]-2'-deoxyguanosine (17). A mixture of **9** (3.55 g, 10.1 mmol), triphenylphosphine (4.24 g, 16.2 mmol), and allyl alcohol (4.8 mL, 4.10 g, 70.6 mmol) in dioxane (60 mL) was stirred for 10 min. To this mixture was added diethyl azodicarboxylate (3.50 mL, 3.71 g, 19.2 mmol) and stirring was continued for an additional 3 h. The reaction mixture was concentrated to give a yellowish oil. Addition of dichloromethane (15 mL) formed crystals, which were removed by filtration, and the filtrate was concentrated to give an oil. Silica gel column chromatography of this crude material was done with a 3:7 mixture of ethyl acetate and hexane and then with a 3:2 mixture of ethyl acetate and hexane as eluent. Concentration of fractions eluted with the latter solvent afforded **11** (3.08 g, 78% yield) as a colorless amorphous solid; R_f 0.32 (a 3:1 ethyl acetate–hexane mixture); UV λ_{max} 249, 283 nm; ^{1}H NMR (400 MHz,

DMSO-d_6) 2.02 (s, 3H), 2.08 (s, 3H), 2.44–2.49 (m, 1H), 2.97–3.05 (m, 1H), 4.16–4.31 (m, 3H), 4.94 (d, 2H, J = 5.4 Hz), 5.26 (d, 1H, J = 10.3 Hz), 5.31 (d, 1H, J = 6.4 Hz), 5.40 (d, 1H, J = 17.1 Hz), 6.06–6.13 (m, 1H), 6.22 (dd, 1H, J = 5.9 and 8.3 Hz), 6.48 (br s, 2H), 8.08 (s, 1H). This product was contaminated by a small amount of triphenylphosphine oxide but was employed to the next step without further purification. The product **11** was dissolved in 60 mL THF and AOC-Cl (2.50 mL, 2.84 g, 23.6 mmol) was added. The resulting solution was cooled to 5 °C and to this a 1.4 M solution of *tert*-butylmagnesium chloride in THF (12 mL, 16.8 mmol) was added dropwise. The solution was stirred for 3 h and the reaction was quenched by addition of methanol (3 mL). The mixture was diluted with ethyl acetate and washed successively with an aqueous solution saturated with ammonium chloride, an aqueous solution saturated with sodium hydrogencarbonate, and brine. The combined organic layers were concentrated and the resulting light-yellow oil was subjected to silica gel column chromatography, if necessary, with a 2:3 mixture of ethyl acetate and hexane to give oily **14** (3.30 g, 75% overall yield from **11**); R_f 0.21 (a 1:1 ethyl acetate–hexane mixture); UV λ_{max} 254 nm; ^{1}H NMR (400 MHz, DMSO-d_6) 1.97 (s, 3H), 2.08 (s, 3H), 2.56–2.61 (m, 1H), 3.05–3.12 (m, 1H), 4.13–4.29 (m, 3H), 4.63–4.72 (m, 4H), 5.04 (d, 2H, J = 5.4 Hz), 5.15–5.45 (m, 7H), 5.80–5.89 (m, 2H), 6.06–6.12 (m, 1H), 6.38–6.42 (m, 1H), 8.69 (s, 1H); FAB-MS m/z 560 (M^+ + 1). This product was dissolved in 95 % ethanol (40 mL) and the solution was mixed with a 0.1 M sodium hydroxide solution in 95 % ethanol (40 mL). The mixture was stirred for 2 h and then neutralized with an 80% aqueous solution of acetic acid. Concentration gave an amorphous material, which was chromatographed on a short silica gel column with a 5:50:50 to 10:50:50 mixture of methanol, ethyl acetate, and hexane to give **17** (1.85 g, 47% from **9**); R_f 0.27 (a 1:5:5 ethanol–ethyl acetate–dichloromethane mixture); UV λ_{max} 254 (shoulder, ε 12,900), 268 nm (14,000); ^{1}H NMR (400 MHz, DMSO-d_6) 2.23–2.29 (m, 1H), 2.69–2.75 (m, 1H), 3.47–3.61 (m, 2H), 3.82–3.85 (m, 1H), 4.41 (m, 1H), 4.61–4.62 (m, 2H), 4.85 (t, 1H, J = 5.6 Hz), 5.05 (d, 2H, J = 5.9 Hz), 5.21–5.48 (m, 5H), 5.92–6.02 (m, 1H), 6.10–6.20 (m, 1H), 6.20–6.32 (m, 1H), 8.41 (s, 1H), 10.32 (s, 1H). Anal. Calcd for $C_{17}H_{21}N_5O_6$: C, 52.17; H, 5.41; N, 17.89. Found: C, 52.18; H, 5.65; N, 17.80.

O^6-Allyl-N^2-[(allyloxy)carbonyl]guanosine (18). The compound **10** (4.90 g, 12.0 mmol) was mixed with triphenylphosphine (5.40 g, 20.6 mmol) and allyl alcohol (7.50 mL, 6.41 g, 110 mmol) in dry dioxane (90 mL) and the suspension was heated at 80 °C for 45 min. To the mixture was added diethyl azodicarboxylate (3.50 mL, 3.71 g, 21.3 mmol). The resulting homogeneous mixture was heated at 60 °C for additional 3 h. Evaporation of the reaction mixture gave a yellow oil, which was treated with dichloromethane (15 mL). The solution was left in a refrigerator and the resulting

crystals were filtered off and washed with dichloromethane. The filtrate was concentrated to produce an oily material, which was purified by silica gel column chromatography. Firstly undesired products were eluted with a 3:7 mixture of ethyl acetate and hexane. Then the target compound **12**, a colorless amorphous solid (4.30 g, 80% yield), was eluted with a 3:2 mixture of ethyl acetate and hexane. **12**: R_f 0.48 (a 3:1 ethyl acetate–hexane mixture); UV λ_{max} 249, 283 nm; ^{1}H NMR (400 MHz, DMSO-d_6) 2.03 (s, 6H), 2.12 (s, 3H), 4.25–4.42 (m, 3H), 4.95 (d, 2H, J = 5.9 Hz), 5.26 (d, 1H, J = 10.3 Hz), 5.40 (dd, 1H, J = 1.5 and 19.0 Hz), 5.54–5.56 (m, 1H), 5.86–5.89 (m, 1H), 6.07–6.12 (m, 2H), 6.55 (s, 2H); 8.11 (s, 1H). Thus obtained **12** was contaminated by a slight amount of triphenylphosphine oxide. To a solution of this product in THF (60 mL) was added AOC-Cl (3.00 mL, 3.41g, 28.3 mmol). After cooling to 5 °C, to the resulting homogeneous mixture was added dropwise a 1.4 M solution of *tert*-butylmagnesium chloride in THF (18.0 mL, 25.2 mmol). The solution was stirred for additional 60 min and the reaction was quenched by adding methanol (3 mL). The mixture was diluted with ethyl acetate and washed with an aqueous solution saturated with ammonium chloride, an aqueous solution saturated with sodium hydrogencarbonate, and brine. The organic layers were evaporated and the resulting colorless oil was purified by silica gel column chromatography, if necessary, with a 2:3 ethyl acetate–hexane mixture to afford oily **16** (3.90 g, 66% overall yield from **12**); R_f 0.53 (a 1:1 ethyl acetate–hexane mixture); UV λ_{max} 254 nm; ^{1}H NMR (400 MHz, DMSO-d_6) 1.96 (s, 3H), 2.02 (s, 3H), 2.10 (s, 3H), 4.40–4.18 (m, 3H), 4.68–4.67 (m, 4H), 5.04 (d, 2H, J = 5.4 Hz), 5.60–5.15 (m, 6H), 5.74–5.73 (m, 1H), 5.91–5.81 (m, 3H), 6.12–6.06 (m, 1H), 6.27 (d, 1H, J = 3.4 Hz), 8.69 (s, 1H); FAB-MS m/z 618 (M^+ +1). The product **16** was mixed with ethanol (35 mL) and a 0.1 M solution of sodium hydroxide in 95 % ethanol (35 mL) and stirred for 30 min. The solution was neutralized with 80% acetic acid and evaporated. The residual oil was purified by chromatography on a short silica gel column with a 1:5:5 methanol–ethyl acetate–hexane mixture to afford **18** (2.55 g, 52% overall yield from **10**) as an amorphous solid; R_f 0.32 (a 1:5:5 ethanol–ethyl acetate–dichloromethane mixture); UV λ_{max} 254 (shoulder), 269 nm; ^{1}H NMR (400 MHz, DMSO-d_6) 3.52–3.54 (m, 1H), 3.62–3.65 (m, 1H), 3.90–3.91 (m, 1H), 4.17 (m, 1H), 4.61–4.62 (m, 3H), 4.93 (br s, 1H), 5.04–5.05 (m, 2H), 5.16–5.48 (m, 6H), 5.88 (d, 1H, J = 5.9 Hz), 5.91–6.00 (m, 1H), 6.12–6.19 (m, 1H), 8.43 (s, 1H), 10.38 (s, 1H). FAB-MS ($C_{17}H_{21}N_5O_7$) m/z 408 (M^+ + 1). The elementary analysis gave no desired data.

***N*^{6}-[(Allyloxy)carbonyl]-5'-*O*-(*p,p'*-dimethoxytrityl)adenosine (19).** A solution of **4** (15.0 g, 42.7 mmol) in a 1:1 mixture of pyridine and DMF (150 mL) was chilled to 0 °C and to this was added in small portions *p,p'*-dimethoxytrityl chloride

(15.6 g, 46.0 mmol) over 20 min. The resulting homogeneous mixture was stirred at the same temperature for 20 min and then at room temperature for 12 h. The mixture was poured into an ice-water mixture (1.2 L). The occurring precipitates were collected by filtration, washed with water, and dried at 50 °C in a high vacuum. This crude yellow solid was purified by silica gel column chromatography eluted with a 4:6 mixture of ethyl acetate and hexane, removing by-products, and then with a 1:9 mixture of methanol and ethyl acetate to collect the desired product. The latter fractions were evaporated to give **19** (21.5 g, 77% yield) as a yellowish foam; R_f 0.76 (a 1:9 methanol–dichloromethane mixture); UV λ_{max} 236, 268 nm; ^{1}H NMR (270 MHz, DMSO-d_6) 3.22–3.24 (m, 2H), 3.71 (s, 6H), 4.06–4.11 (m, 1H), 4.30–4.34 (m, 1H), 4.64–4.66 (m, 2H), 4.73–4.78 (m, 1H), 5.25–5.27 (m, 2H), 5.42 (dd, 1H, J = 1.5 and 17.3 Hz), 5.60 (d, 1H, J = 5.3 Hz), 5.90–6.04 (m, 2H), 6.79–6.84 (m, 4H), 7.18–7.36 (m, 9H), 8.56 (s, 2H), 10.65 (s, 1H). Anal. Calcd for $C_{35}H_{35}N_5O_8 \cdot 1/2\ H_2O$: C, 63.44; H, 5.48; N, 10.57. Found: C, 63.31; H, 5.30; N, 10.30.

***N*^{6}-[(Allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl)adenosine (20) and *N*^{6}-[(Allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-3'-*O*-(*tert*-butyldimethylsilyl)adenosine (21).** To a solution of **19** (19.8 g, 30.3 mmol) in DMF (35 mL) were added imidazole (4.4 g, 64.6 mmol) followed by *tert*-butyldimethylsilyl chloride (4.85 g, 32.2 mmol), and the mixture was stirred overnight. The reaction mixture was diluted with ethyl acetate (400 mL), washed with water (150 mL x 3), dried, and evaporated. The resulting colorless foam was subjected to silica gel column chromatography with a 2:8 mixture of ethyl acetate and hexane eluting the bis-silylated product, with a 35:65 mixture of the same solvents affording the desired products **20** and **21**, and with ethyl acetate giving the starting material. Concentration of the fractions containing the desired products gave **20** (7.68 g, 10.0 mmol, 33% yield) and **21** (10.5 g, 13.6 mmol, 45% yield). The recovered starting material **19** was 1.98 g (10%). **20**: R_f 0.64 (a 3:2 mixture of ethyl acetate and hexane; UV λ_{max} 236 (ε 24,800), 268 nm (20,800); ^{1}H NMR (270 MHz, DMSO-d_6) –0.15 (s, 3H), –0.05 (s, 3H), 0.73 (s, 9H), 3.27–3.68 (m, 2H), 3.71 (s, 6H), 4.11–4.28 (m, 2H), 4.63–4.65 (m, 2H), 4.86 (t, 1H, J = 4.8 Hz), 5.20 (s, 1H), 5.21 (dd, 1H, J = 1.7 and 10.6 Hz), 5.36 (dd, 1H, J = 1.7 and 17.3 Hz), 5.89–6.04 (m, 2H), 7.19–7.47 (m, 4H), 6.80–6.84 (m, 9H), 8.54 (s, 1H), 8.62 (s, 1H), 10.65 (s, 1H). Anal. Calcd for $C_{41}H_{49}N_5O_8Si$: C, 64.13; H, 6.43; N, 9.12. Found: C, 64.12; H, 6.40; N, 8.72. **21**: R_f 0.4 (a 3:2 mixture of ethyl acetate and hexane); UV λ_{max} 236 (ε 23,700), 268 nm (20,500); ^{1}H NMR (270 MHz, DMSO-d_6) 0.04 (s, 3H), 0.08 (s, 3H), 0.84 (s, 9H), 3.33–3.39 (m, 1H), 3.11–3.16 (m, 1H), 3.71 (s, 6H), 4.00–4.06 (m, 1H), 4.48–4.51 (m, 1H), 4.64–4.66 (m, 2H), 4.86–

4.90 (m, 1H), 5.22 (d, 1H, J = 10.6 Hz), 5.37–5.43 (m, 2H), 5.89–6.05 (m, 2H), 6.82–6.85, (m, 4H), 7.19–7.39 (m, 9H), 8.54 (s, 1H), 8.57 (s, 1H), 10.67 (s, 1H). Anal. Calcd for $C_{41}H_{49}N_5O_8Si$: C, 64.13; H, 6.43; N, 9.12. Found: C, 64.13; H, 6.41; N, 9.19.

N^6-[(Allyloxy)carbonyl]-2'-*O*-(*tert*-butyldimethylsilyl)-5'-*O*-(*p,p'*-dimethoxytrityl)adenosine 3'-(Allyl *N,N'*-diisopropylphosphoramidite) (25). To a solution of **20** (1.03 g, 1.34 mmol) in acetonitrile (7.0 mL) were added (allyloxy)bis(diisopropylamino)phosphine (430 μL, 404 mg, 1.40 mmol) followed by diisopropylammonium tetrazolide (115 mg, 0.67 mmol). The reaction mixture was stirred overnight and diluted with dichloromethane. The resulting mixture was washed with a phosphate buffer solution of pH 7 and dried. Evaporation of the organic layers afforded a colorless foam, which was chromatographed on silica gel column. Elution with a 3:7 mixture of ethyl acetate and hexane gave **25** (1.04 g, 81% yield) as a mixture of two diastereomers; R_f 0.42 (a 3:7 ethyl acetate–hexane mixture); UV λ_{max} 236 (ε 22,900), 268 nm (19,700); ^{1}H NMR (400 MHz, $CDCl_3$) –0.21 (s, 3H), –0.02 (s, 3H), 0.75 and 0.77 (2 s's, 9H), 1.15–1.28 (m, 12 H), 3.44–3.62 (m, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 3.96–4.26 (m, 2H), 4.41–4.49 (m, 3H), 4.76–4.78 (m, 2H), 4.97–5.44 (m, 6H), 5.89–6.09 (m, 3H), 6.77–6.85 and 7.20–7.73 (2 m's, 13H), 8.16–8.19 (m, 2H), 8.64 and 8.66 (2 s's, 1H); ^{31}P NMR (400 MHz, $CDCl_3$) 148.8, 151.0. Anal. Calcd for $C_{50}H_{67}N_6O_9PSi$: C, 62.87; H, 7.07; N, 8.80. Found: C, 62.75; H, 7.25; N, 8.65.

O^6-Allyl-N^2-[(allyloxy)carbonyl]-5'-*O*-(*p,p'*-dimethoxytrityl)guanosine (26). To a solution of **18** (1.73 g, 4.25 mmol) in a 1:1 mixture of pyridine and DMF (20 mL) was added *p,p'*-dimethoxytrityl chloride (1.54 g, 4.55 mmol) and the mixture was heated at 55 °C for 1.5 h. The reaction mixture was poured into an aqueous solution saturated with sodium hydrogencarbonate (100 mL) and extracted with ethyl acetate (30 mL x 3). The combined organic layers were dried and evaporated to give an oil, which was purified by silica gel column chromatography with a 3:7 mixture of ethyl acetate and hexane to elute by-products and then with a 3:2 mixture of ethyl acetate and hexane to elute the target product. Concentration of the latter fractions gave **26** (2.31 g, 77% yield) as a colorless foam; R_f 0.61 (a 3:1 mixture of ethyl acetate and hexane); UV λ_{max} 237 (ε 26,800), 269 nm (17,400); ^{1}H NMR (400 MHz; DMSO-d_6) 3.16–3.56 (m, 2H), 3.69 (s, 3H), 3.70 (s, 3H), 4.02–4.03 (m, 1H), 4.33–4.37 (m, 1H), 4.59–4.60 (m, 2H), 4.69–4.72 (m, 1H), 5.04 (d, 2H, J = 4.9 Hz), 5.10 (d, 1H, J = 5.9 Hz), 5.22 (d, 1H, J = 10.7 Hz), 5.29 (d, 1H, J = 10.7 Hz), 5.38 (d, 1H, J = 17.6 Hz), 5.47 (d, 1H, J = 17.1 Hz), 5.57 (d, 1H, J = 5.9 Hz), 5.92–5.99 (m, 2H), 6.13–6.20 (m, 1H), 6.72–6.78 (m, 4H), 7.16 (m, 9H), 8.33 (s, 1H), 10.34 (s, 1H). Anal. Calcd for $C_{38}H_{39}N_5O_9$: C, 64.31; H, 5.54; N, 9.87. Found: C, 64.32; H, 5.46; N, 9.69.

O^6-Allyl-N^2-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl)guanosine (27), O^6-allyl-N^2-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-3'-*O*-(*tert*-butyldimethylsilyl)guanosine (28), and O^6-allyl-N^2-[(allyloxy)carbonyl]-5'-*O*-(*p*,*p*'-dimethoxytrityl)-2',3'-*O*-[bis(*tert*-butyldimethylsilyl)]guanosine (29). To a solution of **26** (1.38 g, 1.94 mmol) in DMF (2 mL) were added imidazole (0.29 g, 4.3 mmol) and *tert*-butyldimethylsilyl chloride (0.32 g, 2.1 mmol) and the mixture was stirred at 60 °C for 1 h. Then the reaction mixture was poured into ice water (50 mL) and extracted with ethyl acetate (20 mL x 3). The combined organic layers were dried and evaporated to give a colorless foam, which was subjected to silica gel column chromatography. Firstly the bis-silylated product **29** was eluted with a 2:8 mixture of ethyl acetate and hexane and subsequently **27** and **28** were eluted with a 1:3 mixture of ethyl acetate and hexane. The product **28** was treated with a 1:5:5 triethylamine-methanol-ethyl acetate mixture. By this treatment, partial isomerization of **28** to **27** took place, giving a mixture of **27** and **28**. The resulting mixture was subjected again to silica gel chromatography under the conditions described above. Overall, there were obtained **27** (457 mg, 29% yield), **28** (213 mg, 12% yield), and **29** (613 mg, 38% yield). **27**: R_f 0.34 (a 3:7 mixture of ethyl acetate and hexane); UV λ_{max} 237 (ε 24,300), 269 nm (15,900); ^{1}H NMR (400 MHz, DMSO-d_6) –0.16 (s, 3H), –0.05 (s, 3H), 0.74 (s, 9H), 3.21–3.24 (m, 1H), 3.35–3.56 (m, 1H), 3.70 (s, 3H), 3.71 (s, 3H), 3.74–4.05 (m, 1H), 4.24–4.28 (m, 1H), 4.57–4.58 (m, 2H), 4.88–4.89 (m, 1H), 4.96–4.98 (m, 1H), 5.04 (d, 2H, J = 5.9 Hz), 5.21 (dd, 1H, J = 1.71 and 10.5 Hz), 5.30 (d, 1H, J = 10.2 Hz), 5.37 (dd, 1H, J = 1.5 and 17.1 Hz), 5.45 (dd, 1H, J = 1.5 and 17.1 Hz), 5.92–5.99 (m, 2H), 6.12–6.19 (m, 1H), 6.75–6.80 (m, 4H), 7.15–7.34 (m, 9H), 8.33 (s, 1H), 10.25 (s, 1H). Anal. Calcd for $C_{44}H_{53}N_5O_9Si$: C, 64.14 ; H, 6.48; N, 8.50. Found: C, 64.13; H, 6.43; N, 8.35. **28**: R_f 0.22 (a 3:7 mixture of ethyl acetate and hexane); UV λ_{max} 239 (ε 27,800), 269 nm (17,200); ^{1}H NMR (400 MHz, DMSO-d_6) 0.00 (s, 3H), 0.05 (s, 3H), 0.81 (s, 9H), 3.23–3.35 (m, 2H), 3.69 (s, 3H), 3.70 (s, 3H), 3.88-3.92 (m, 1H), 4.44–4.46 (m, 1H), 4.58–4.60 (m, 2H), 4.80–4.84 (m, 1H), 5.03–5.05 (m, 2H), 5.21 (dd, 1H, J = 1.5 and 10.7 Hz), 5.28 (d, 1H, J = 10.3 Hz), 5.31–5.41 (m, 2H), 5.44 (dd, 1H, J = 1.5 and 17.1 Hz), 5.87–5.88 (m, 1H), 5.91–5.99 (m, 1H), 6.12–6.19 (m, 1H), 6.75–6.79 (m, 4H), 7.16–7.31 (m, 9H), 8.37 (s, 1H), 10.32 (s, 1H). Anal. Calcd for $C_{44}H_{53}N_5O_9Si$: C, 64.14 ; H, 6.48; N, 8.50. Found: C, 64.13; H, 6.30; N, 8.21. **29**: R_f 0.67 (a 3:7 mixture of ethyl acetate and hexane); UV λ_{max} 236 (ε 25,900), 269 nm (16,700); ^{1}H NMR (400 MHz, DMSO-d_6) –0.33 (s, 3H), –0.08 (s, 3H), –0.03 (s, 3H), 0.05 (s, 3H), 0.72 (s, 9H), 0.81 (s, 9H), 3.26–3.56 (m, 2H), 3.67 (2 s's, 6H), 3.86–3.90 (m, 1H), 4.31–4.33 (m, 1H), 4.55–4.61 (m, 2H), 5.03 (d, 2H, J = 5.9 Hz), 5.13–5.14

(m, 1H), 5.22 (dd, 1H, J = 1.5 and 10.2 Hz), 5.29 (dd, 1H, J = 1.5 and 10.2 Hz), 5.37 (d, 1H, J = 1.5 Hz), 5.46 (dd, 1H, J = 1.5 and 17.1 Hz), 5.86–5.87 (m, 1H), 5.90–6.10 (m, 1H), 6.11–6.20 (m, 1H), 6.76–6.84 (m, 4H), 7.15–7.33 (m, 9H), 8.39 (s, 1H), 10.26 (s, 1H). Anal. Calcd for $C_{50}H_{67}N_5O_9Si_2$: C, 64.01 ; H, 7.20; N, 7.46. Found: C, 64.28; H, 7.00; N, 7.16.

***O*^{6}-Allyl-*N*^{2}-[(allyloxy)carbonyl]-5'-*O*-(*p,p'*-dimethoxytrityl)-2'-*O*-(*tert*-butyldimethylsilyl)guanosine 3'-(Allyl *N,N'*-diisopropylphosphoramidite) (31).** To a solution of **27** (98 mg, 0.12 mmol) in acetonitrile (1.0 mL) were added (allyloxy)bis(diisopropylamino)phosphine (41 μL, 40 mg, 0.13 mmol) and diisopropylammonium tetrazolide (11 mg, 0.06 mmol). After stirring for 12 h, the reaction mixture was diluted with dichloromethane and extracted with a phosphate buffer solution of pH 7. The organic layer was dried and concentrated to give a colorless foam, which was subjected to silica gel column chromatography eluted with a 1:3 mixture of ethyl acetate and hexane, giving **31** (a mixture of two diastereomers, 87.7 mg, 72% yield) as colorless foam; R_f 0.59 (a 3:7 ethyl acetate–hexane mixture); UV λ_{max} 237 (ε 24,000), 268 nm (16,700); ^{1}H NMR (400 MHz, DMSO-d_6) –0.32 (s, 3H), –0.10 (2 s's, 3H), 0.67 (s, 9H), 1.00–1.20 (m, 12 H), 3.39–3.60 (m, 2H), 3.70 (s, 3H), 3.71 (s, 3H), 3.95–4.18 (m, 4H), 4.22–4.57 (m, 2H), 4.95–5.49 (m, 10H), 5.68–5.75 (m, 1H), 5.86–5.99 (m, 3H), 6.11–6.21 (m, 1H), 6.73–6.80 (m, 4H), 7.18–7.34 (m, 9H), 8.34 and 8.36 (2 s's, 1H), 10.16 and 10.19 (2 s's, 1H); ^{31}P NMR (400 MHz, DMSO-d_6) 149.0, 150.5. Anal. Calcd for $C_{53}H_{71}N_6O_{10}PSi$: C, 62.95; H, 7.08; N, 8.31. Found: C, 62.94; H, 6.88; N, 8.22.

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