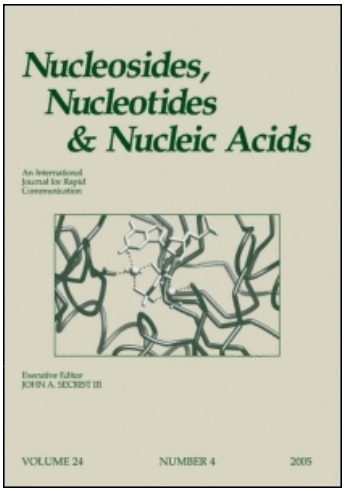

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

EFFICIENT METHODS FOR THE SYNTHESIS OF [2-^{15}N]GUANOSINE AND 2'-DEOXY[2-^{15}N]GUANOSINE DERIVATIVES

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Online publication date: 26 February 2001

To cite this Article Kamaike, Kazuo , Kinoshita, Kazutomo , Niwa, Katsuyuki , Hirose, Kumi , Suzuki, Kazuhiro and Ishido, Yoshiharu(2001) 'EFFICIENT METHODS FOR THE SYNTHESIS OF [2-^{15}N]GUANOSINE AND 2'-DEOXY[2-^{15}N]GUANOSINE DERIVATIVES', Nucleosides, Nucleotides and Nucleic Acids, 20: 1, 59 — 75
To link to this Article: DOI: 10.1081/NCN-100001437
URL: http://dx.doi.org/10.1081/NCN-100001437

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(1&2), 59–75 (2001)

EFFICIENT METHODS FOR THE SYNTHESIS OF [2-^{15}N]GUANOSINE AND 2′-DEOXY[2-^{15}N]GUANOSINE DERIVATIVES

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ABSTRACT

The nucleophilic addition–elimination reaction of 2′,3′,5′-tri-*O*-acetyl-2-fluoro-O^6-[2-(4-nitrophenyl)ethyl]inosine (**8**) with [^{15}N]benzylamine in the presence of triethylamine afforded the N^2-benzyl[2-^{15}N]guanosine derivative (**13**) in a high yield, which was further converted into the N^2-benzoyl[2-^{15}N] guanosine derivative by treatment with ruthenium trichloride and tetrabutylammonium periodate. A similar sequence of reactions of 2′,3′,5′-tri-*O*-acetyl-2-fluoro-O^6-[2-(methylthio)ethyl]inosine (**9**) and the 6-chloro-2-fluoro-9-(β-D-ribofuranosyl)-9*H*-purine derivative (**11**), which were respectively prepared from guanosine, with potassium [^{15}N]phthalimide afforded the N^2-phthaloyl [2-^{15}N]guanosine derivative (**15**; 62%) and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-[^{15}N]phthalimido-9*H*-purine (**17**; 64%), respectively. Compounds **15** and **17** were then efficiently converted into 2′,3′,5′-tri-*O*-acetyl [2-^{15}N]guanosine. The corresponding 2′-deoxy derivatives (**16** and **18**) were also synthesized through similar procedures.

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Dedicated to Professor Dr. Jan Michalski on the occasion of his 80th birthday.

INTRODUCTION

NMR studies of ^{15}N-labeled oligonucleotides have provided useful information regarding nucleic acid structures, nucleic acid–drug binding, and nucleic acid–protein interactions (1). Methodologically, the chemical introduction of a ^{15}N-labeled exocyclic amino group to nucleosides can be classified into three categories: 1) the synthesis of an appropriately ^{15}N-labeled heterocycle, followed by its glycosylation with an appropriately functionalized D-ribofuranosyl or 2-deoxy-D-ribofuranosyl derivative, to yield the desired ^{15}N-labeled nucleoside (2); 2) the derivatization of an intact nucleoside to the corresponding ^{15}N-labeled nucleoside through the reaction of its activated intermediates with [^{15}N]ammonia or with [^{15}N]benzylamine (3); and 3) the synthesis of an appropriately ^{15}N-labeled nucleoside intermediate, followed by its conversion into the ^{15}N-labeled nucleoside (4). The second approach might be much more promising than the others from the synthetic viewpoint, and to be specific about this category, the synthesis of 2′-deoxy [2-^{15}N]guanosine was performed through the nucleophilic addition–elimination reaction of 2′-deoxy-2-fluoro-3′,5′-di-*O*-methoxyacetyl-*O*^{6}-[2-(phenylthio) ethyl] inosine with [^{15}N]ammonia, which was generated in situ from [^{15}N]ammonium sulfate by the addition of sodium methoxide [see Kieper et al. (3)]. However, it would be more synthetically advantageous if it were possible to perform the second approach with the use of a small excess of a solid nucleophile, such as [^{15}N]phthalimide and/or potassium [^{15}N]phthalimide. We have previously reported an efficient method for the synthesis of [4-^{15}N]cytidine, 2′-deoxy[4-^{15}N]cytidine, [6-^{15}N]adenosine, and 2′-deoxy[6-^{15}N]adenosine derivatives, respectively, which is characterized by the nucleophilic substitution reaction of their azolyl derivatives with [^{15}N]phthalimide in the presence of triethylamine or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (5).

We now report herein efficient methods for the synthesis of [2-^{15}N]guanosine and 2′-deoxy[2-^{15}N]guanosine dervatives, from guanosine and 2′-deoxyguanosine derivatives, through the nucleophilic addition–elimination reaction of their 2-fluoro derivatives with [^{15}N]benzylamine and potassium [^{15}N]phthalimide, respectively (see Scheme 1).

RESULTS AND DISCUSSION

Synthesis of [2-^{15}N]Guanosine Derivatives Through the Nucleophilic Addition–Elimination Reaction of 2′,3′,5′-Tri-*O*-acetyl-2-fluoro-*O*^{6}-[2-(4-nitrophenyl)ethyl]inosine (8) with [^{15}N]Benzylamine

The introduction of the ^{15}N-label into the amino group of guanosine was carried out in four steps, using guanosine as the starting material, through the nucleophilic addition–elimination reaction of **8** with [^{15}N]benzylamine. After protecting the hydroxyl groups of the sugar moiety of guanosine by acetylation with acetic anhydride (6), the Mitsunobu reaction using 2-(4-nitrophenyl)ethanol led to 2′,3′,

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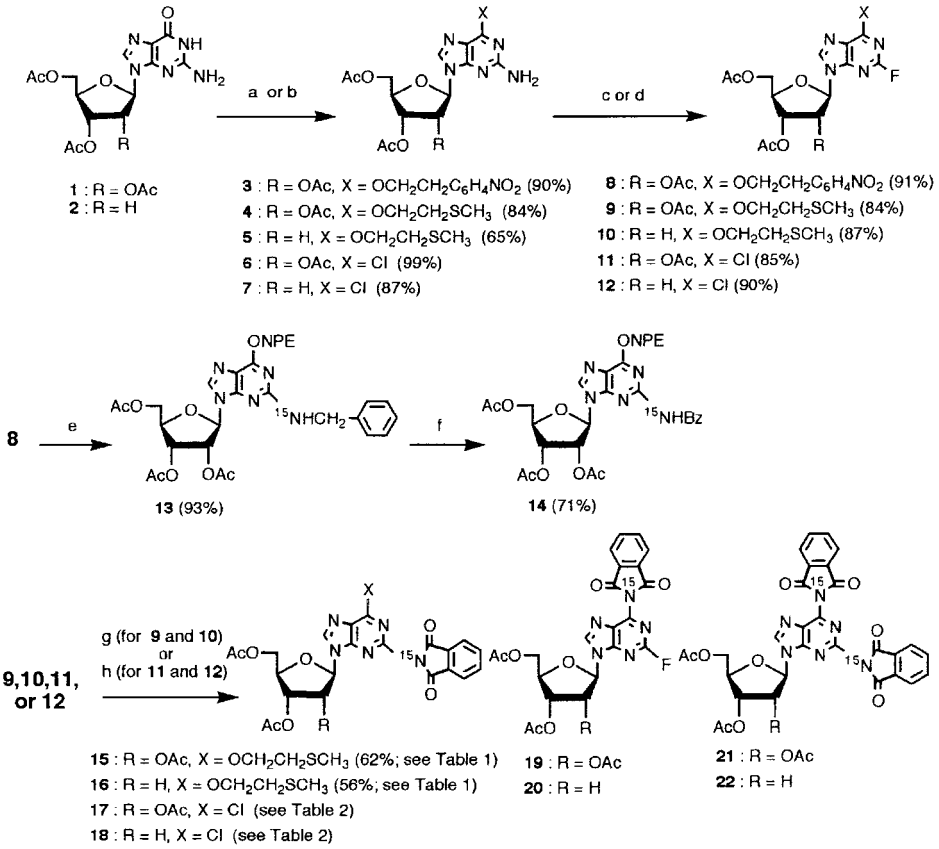

Scheme 1. Conditions: a) 2-(4-nitrophenyl)ethanol or 2-(methylthio)ethanol/diethyl azodicarboxylate/Ph_3P/1,4-dioxane, r.t., 1.5–3 h. b) $OPCl_3/Et_4NCl/CH_3CN$, 80°C, 5–40 min. c) 25% HF/pyridine/*tert*-butyl nitrite, in ice-bath, 1 h and then r.t., 4–9 h. d) 45% HF/pyridine/*tert*-butyl nitrite, in ice-bath, 30 min. e) [^{15}N]benzylamine (1.2 equiv)/Et_3N (1.2 equiv)/DMF, r.t., 2 h. f) $RuCl_3/Bu_4NIO_4$/2:2:3 CH_2Cl_2–CH_3CN–H_2O, r.t., 2 h. g) potassium [^{15}N]phthalimide (1.5 equiv)/DMF, 90°C, 1.5–2 days; Ac_2O/pyridine, r.t., 1 h (see Tab. 1). h) potassium [^{15}N]phthalimide (1.2 equiv)/DMF (see Tab. 2).

5′-tri-*O*-acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (**3**) (7). Successive diazotization and fluorination reactions at the 2-position of **3** were achieved by treatment with *tert*-butyl nitrite and a 25% HF/pyridine solution (8), giving the corresponding 2-fluoro compound (**8**) in 91% yield. The nucleophilic addition–elimination reaction of **8** with [^{15}N]benzylamine (1.2 equiv), which was prepared from [^{15}N]benzamide by the method of Horneman (9), in the presence of triethylamine (1.2 equiv) in DMF gave 2′,3′,5′-tri-*O*-acetyl-N^2-benzyl-O^6-[2-(4-nitrophenyl)ethyl] [2-^{15}N]guanosine (**13**) in 93% yield. *N*-Debenzylation of **13** was attempted under a variety of reductive conditions, but none proved to be successful. Incidentally, Jones et al. reported the deblocking of the *N*-benzyl group involving its oxidation to the corresponding benzoyl group prior to the *N*-debenzoylation reaction [see Gao and Jones (3)]. Based

on this procedure, therefore, the oxidation of the N^2-benzyl group of **13** was efficiently achieved by treatment with ruthenium trichloride (0.02 equiv) and tetrabutylammonium periodate (4 equiv) (10) in 2:2:3 methylene chloride/acetonitrile/water at room temperature for 2 h, giving the N^2-benzoyl[2-^{15}N]guanosine derivative (**14**) in 71% yield.

Synthesis of [2-^{15}N]Guanosine and 2′-Deoxy[2-^{15}N]Guanosine Derivatives Through Nucleophilic Addition-Elimination Reactions of 2-Fluoro-O^6-[2-(methylthio)ethyl]inosine (9) and -2′-Deoxyinosine Derivatives (10) with Potassium [^{15}N]phthalimide

Next, we performed the syntheses of the [2-^{15}N]Guanosine and 2′-deoxy[2-^{15}N]guanosine derivatives through the nucleophilic addition–elimination reactions of **9** and **10** with potassium [^{15}N]phthalimide as the ^{15}N-labeling reagent (11). Compounds **9** and **10** were prepared in 84% and 87% yields by the O^6-(2-methylthio) ethylation (7) of 2′,3′,5′-tri-*O*-acetylguanosine (**1**) and 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (**2**), followed by treatment with *tert*-butyl nitrite and 25% HF/pyridine (8) without serious side reactions, for example the depurination of **10** (12), respectively.

The nucleophilic addition–elimination reaction of **9** with potassium [^{15}N]phthalimide (1.5 equiv) was performed in DMF under the conditions summarized in Entries 1–6 of Table 1, giving 2′,3′,5′-tri-*O*-acetyl-O^6-[2-(methylthio)-ethyl]-N^2-phthaloyl[2-^{15}N]guanosine (**15**). By following the progress of the

Table 1. Nucleophilic Addition–Elimination Reactions of 2-Fluoro-6-[(2-methylthio)ethyl]-9*H*-purine Derivatives (**9** and **10**) with Potassium [^{15}N]phthalimide

Entry	2-Fluoro-6-[(2-methylthio) ethyl]-9*H*-purine Derivative	K [^{15}N]Phth[a] (equiv)	Temperature (°C)	Time (h)	Yield (%)[b]	Recovery (%)
					15	**9**
1	**9**	1.5[c]	135	1.5	30	17
2		1.5[c]	110	5	45	28
3		3.0[c]	110	6.5	46	12
4		1.5[c]	100	24	64	19
5		1.5	90	36	62	24
6		1.5	80	36	33	47
					16	**10**
7	**10**	1.5	90	48	56	–

[a]K [^{15}N]Phth = potassium [^{15}N]phthalimide.
[b]Yield of **15** or **16** after the nucleophilic addition–elimination reaction and treatment with acetic anhydride/pyridine.
[c]Reaction of **9** with potassium phthalimide.

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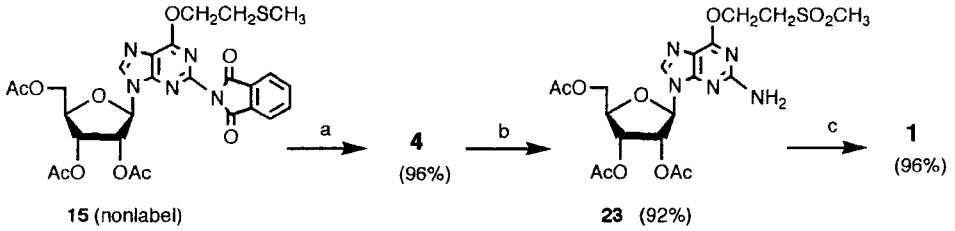

Scheme 2. Conditions: a) 0.5 *M* $NH_2NH_2 \cdot H_2O$/1:4 AcOH-pyridine, r.t., 30 min; Ac_2O/pyridine, r.t., 1 h. b) $RuCl_3/Bu_4NIO_4$/2:2:3 $CH_2Cl_2-CH_3CN-H_2O$, r.t., 30 min. c) 0.1 *M* DBU/CH_3CN, r.t. 1 h.

reaction by TLC, it was revealed to be accompanied by the partial conversion of **15** to the 2-(*o*-carboxybenzoyl) derivative and the formation of small amounts of several by-products. Therefore, acetic anhydride and pyridine were added to the reaction mixture to induce the ring closure of the *o*-carboxybenzoyl function back to **15**. After purification by column chromatography on silica gel, **15** was isolated. Among the results thus obtained, the most favorable results were obtained at 100°C (Entry 4; 64% yield for **15**) and 90°C (Entry 5; 62% yield for **15**) with the recovery of **9** (19 and 24%, respectively).

In a similar manner, the reaction of **10** with potassium [^{15}N]phthalimide (1.5 equiv) at 90°C for 48 h, followed by treatment with acetic anhydride and pyridine, gave the 2′-deoxy-O^6-[2-(methylthio)ethyl]-N^2-phthaloyl[2-^{15}N]guanosine derivative (**16**) in 56% yield (Tab. 1, Entry 7).

The 2-(methylthio)ethyl and phthaloyl groups of the guanine moiety were deprotected easily. In the model experiment, nonlabeled **15** was converted into **1** in 85% yield via the four-step reaction. This involved the unmasking of the pathaloyl group with hydrazine hydrate (13) followed by the treatment with acetic anhydride in pyridine, giving 2′,3′,5′-tri-*O*-acetyl-O^6-[2-(methylthio)ethyl]guanosine (**4**), oxidation of the sulfide function of **4** to the sulfone derivative (**23**) with ruthenium trichloride (0.02 equiv) and tetrabutylammonium periodate (4 equiv) in 2:2:3 methylene chloride/acetonitrile/water at room temperature for 30 min (10,14), and finally, treatment with 0.1 *M* DBU in acetonitrile to remove the 2-(methylsulfonyl)ethyl group (see Scheme 2).

Synthesis of [2-^{15}N]Guanosine and 2′-Deoxy[2-^{15}N]Guanosine Derivatives Through Nucleophilic Addition–Elimination Reactions of 6-Chloro-2-fluoro-9*H*-purine Derivatives (11 and 12) with Potassium [^{15}N]phthalimide

Furthermore, to develop a more facile synthetic method of [2-^{15}N]guanosine and 2′-deoxy[2-^{15}N]guanosine derivatives, nucleophilic addition–elimination reactions of **11** and **12** with potassium [^{15}N]phthalimide were carried out (15).

Compounds **11** and **12** were prepared in 85% and 90% yields from the 6-chloro derivatives (**6** and **7**) (16) by treatment with *tert*-butyl nitrite and 45% HF/pyridine (8) in an ice-bath for 30 min, respectively. In these cases, a higher concentration of hydrogen fluoride was required due to the lower reactivity of the amino groups of **6** and **7**, in contrast with **3**, **4**, and **5**. Nucleophilic addition–elimination reactions of **11** with potassium [^{15}N]phthalimide (1.2–1.5 equiv) were performed in DMF, and the results thus obtained are summarized in Entries 1–5 of Table 2. As easily noticed from these results, the introduction of an electron-withdrawing group to the 6-position of the guanine moiety enhanced the reactivity of the 2-fluoro function, and the reaction temperature could be lowered, in contrast to the reactions of **9** and **10**. In place of the efficiency of the reaction, the 2-fluoro-6-[^{15}N]phthalimido-9-(β-D-ribofuranosyl)-9*H*-purine derivative (**19**) and the 2,6-di[^{15}N]phthalimido-9-(β-D-ribofuranosyl)-9*H*-purine derivative (**21**) were also produced in addition to the 6-chloro-2-[^{15}N]phthalimido-9-(β-D-ribofuranosyl)-9*H*-purine derivative (**17**); all these products were purified by column chromatography on silica gel. The most favorable results were obtained at 40°C (Entries 3 and 5; 58% and 64% yields for **17**, respectively) and at room temperature (Entry 4; 61% yield for **17**), respectively. A slight improvement was attained with the addition of potassium [^{15}N]phthalimide in small portions, as in Entry 5, in comparison with Entry 3.

The reactions of the corresponding 2′-deoxy derivative (**12**) with potassium [^{15}N]phthalimide were similarly performed, and the results thus obtained are summarized in Entries 6–8 of Table 2. They were, however, induced less efficiently as compared to that of **11**, presumably due to the lack of the 2′-hydroxyl function, and

Table 2. Nucleophilic Addition–Elimination Reactions of 6-Chloro-2-fluoro-9*H*-purine Derivatives (**11** and **12**) with Potassium [^{15}N]phthalimide

Entry	6-Chloro-2-fluoro-9*H*-purine Derivative	K [^{15}N] Phth[a] (equiv)	Temperature (°C)	Time (h)	Yield (%)			Recovery (%)
					17	**19**	**21**	**11**
1	**11**	1.5[b]	90	0.5	32	18	29	–
2		1.2[b]	60	1.5	50	17	17	–
3		1.2[b]	40	8.5	58	16	8	–
4		1.2[b]	r.t.	30	61	5	9	–
5		0.5 + 0.4 + 0.3[c]	40	2 + 3.5 + 3.5	64	8	10	–
					18	**20**	**22**	**12**
6	**12**	1.2	60	1.5	37	27	7	11
7		0.6 + 0.3 + 0.3[c]	40	1.5 + 2 + 18	27	15	6	5
8		1.2	r.t.	48	26	21	4	24

[a]K [^{15}N]Phth = potassium [^{15}N]phthalimide.
[b]Reaction of **11** with potassium phthalimide.
[c]Potassium [^{15}N]phthalimide was added portionwise.

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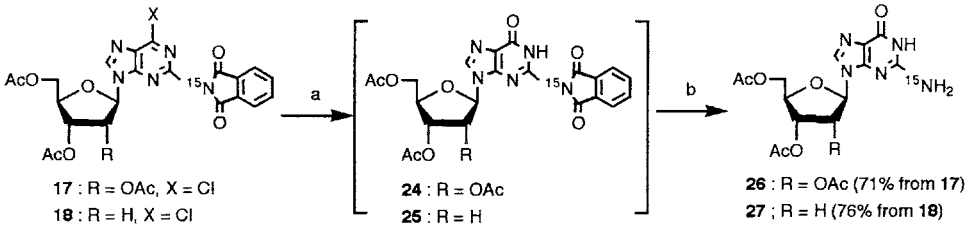

Scheme 3. Conditions: a) sodium 2-cyanoethoxide/THF, 45°C, 1 h; Ac_2O/pyridine/DMF, r.t., 2 h. b) 0.5 *M* $NH_2NH_2 \cdot H_2O$/1:4 AcOH-pyridine, r.t., 30 min; Ac_2O/pyridine, r.t., 1 h.

gave the 2-fluoro-6-[^{15}N]phthalimido-9-(β-D-2-deoxyribofuranosyl)-9*H*-purine derivative (**20**) and the 2,6-di[^{15}N]phthalimido-9(β-D-2-deoxyribofuranosyl)-9*H*-purine derivative (**22**) in addition to the objective 6-chloro-2-[^{15}N]phthalimido-9-(β-D-2-deoxyribofuranosyl)-9*H*-purine derivative (**18**); all these products were purified by column chromatography on silica gel. To accelerate the reaction and to minimize the side reactions, **12** was reacted with potassium [^{15}N]phthalimide at a higher temperature (60°C) and the progress of the reaction was followed by TLC. The reaction was quenched when the spot of the desired ^{15}N-labeled product **18** became the largest on TLC, and the resulting mixture was subjected to chromatographic separation to give **18** (37% yield), **20** (27% yield), **22** (7% yield), and **12** (11% recovery yield) (Entry 6). The reaction time could not be extended until **12** disappeared from the reaction mixture, due to the increasing formation of several other undesirable by-products, which made the separation of the objective products difficult, in spite of the addition of potassium [^{15}N]phthalimide in small portions, as experienced in the case of Entry 7.

^{15}N-Labeled **17** and **18** were converted to 2′,3′,5′-tri-*O*-acetyl[2-^{15}N]guanosine (**26**) and 3′,5′-di-*O*-acetyl-2′-deoxy[2-^{15}N]guanosine (**27**) in 71% and 76% yields via the four-step reaction, respectively. This involved treatment with sodium 2-cyanoethoxide in THF (17) and *O*-acetylation with acetic anhydride in pyridine, giving 2′,3′,5′-tri-*O*-acetyl-N^2-phthaloyl[2-^{15}N]guanosine or 3′,5′-di-*O*-acetyl-N^2-phthaloyl-2′-deoxy[2-^{15}N]guanosine, unmasking of the phthaloyl group with hydrazine hydrate, and finally, *O*-acetylation with acetic anhydride in pyridine (see Scheme 3).

In conclusion, the present investigation provides useful synthetic intermediates leading to an oligonucleotide functionalized by ^{15}N-labels in the exocyclic amino groups of guanosine and 2′-deoxyguanosine, in addition to those of adenosine, 2′-deoxyadenosine, cytidine, and 2′-deoxycytine, as previously reported (5).

EXPERIMENTAL

Column chromatography was performed on silica gel (Wakogel C-300, purchased from Wako Pure Chem) by the use of toluene/ethyl acetate, methanol/

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chloroform, and *n*-hexane/ethyl acetate system. TLC was conducted on Merck silica gel F_{254} by developing with 1:9 methanol/chloroform or 1:4 *n*-hexane/ethyl acetate. Melting points were determined by a Yanaco Micro-melting-point apparatus, and are uncorrected. ^{1}H NMR spectra were recorded on a Varian GEMINI-300 apparatus with $CDCl_3$ or DMSO-d_6 as an internal standard. ^{15}N NMR spectra were recorded on a Brucker DRX 500-2 apparatus with liquid $^{15}NH_3$ as an external standard. Mass spectra were recorded on a VG AutoSpecE apparatus. Elemental analyses were achieved with a Perkin-Elmer 240-002 apparatus.

2′,3′,5′-Tri-*O*-acetyl-O^6-[2-(4-nitrophenyl)ethyl]guanosine (3). 2′,3′,5′-Tri-*O*-acetylguanosine (**1**) (6) (2.046 g, 5 mmol) was, after azeotropic evaporation from pyridine (5 mL × 3), dissolved in dried 1,4-dioxane (40 mL), and triphenylphosphine (1.967 g, 7.5 mmol) and 2-(4-nitrophenyl)ethanol (1.672 g, 10 mmol) were added to the solution. After stirring for 10 min, diethyl azodicarboxylate (1.2 mL, 7.5 mmol) was added dropwise (7). The solution was stirred at room temperature for 2 h and the solvent was then removed in vacuo. The residue was dissolved in chloroform (100 mL) and was washed with water (50 mL × 3). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of 2:1 toluene/ethyl acetate system to give **3** (2.765 g, 99% yield), as a glass: ^{1}H NMR ($CDCl_3$) δ 2.08, 2.09, 2.14 (3s, 9H, COCH_3 × 3), 3.27 (t, 2H, $J = 6.7$ Hz, CH_2CH$_2$PhNO$_2$), 4.39–4.44 (m, 3H, H-4′, 5′, and 5″), 4.72 (t, 2H, $J = 6.7$ Hz, CH$_2$CH_2PhNO$_2$), 4.93 (br s, 2H, NH_2), 5.79 (t, 1H, $J_{2',3'} = J_{3',4'} = 4.7$ Hz, H-3′), 5.94–6.00 (m, 2H, H-1′ and 2′), 7.48 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group), 7.71 (s, 1H, H-8), and 8.17 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group). *Anal.* calcd. for $C_{24}H_{26}N_6O_{10}\cdot 0.45H_2O$: C, 50.87; H, 4.79; N, 14.83. Found: C, 51.13; H, 4.95; N, 14.51.

2′,3′,5′-Tri-*O*-acetyl-O^6-[2-(methylthio)ethyl]guanosine (4). Compound **4** was obtained as a glass in 84% yield (2.030 g) by treating **1** (2.046 g, 5 mmol) with triphenylphosphine (1.967 g, 7.5 mmol), 2-(methylthio)ethanol (0.87 mL, 10 mmol), and diethyl azodicarboxylate (1.2 mL, 7.5 mmol) in 1,4-dioxane (40 mL) and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.067, 2.07, 2.11 (3s, 9H, COCH_3 × 3), 2.20 (s, 3H, SCH_3), 2.91 (t, 2H, $J = 7.3$ Hz, CH$_2$CH_2SCH$_3$), 4.32–4.46 (m, 3H, H-4′, 5′, and 5″), 4.63 (t, 2H, $J = 7.3$ Hz, CH_2CH$_2$SCH$_3$), 4.98 (br s, 2H, NH_2), 5.76 (t, 1H, $J_{2',3'} = J_{3',4'} = 4.9$ Hz, H-3′), 5.94 (t, 1H, H-2′), 5.99 (d, 1H, $J_{1',2'} = 4.9$ Hz, H-1′), and 7.69 (s, 1H, H-8). *Anal.* calcd. for $C_{19}H_{25}N_5O_8S\cdot 0.3H_2O$: C, 46.68; H, 5.28; N, 14.32. Found: C, 46.74; H, 5.34; N, 14.12.

3′,5′-Di-*O*-acetyl-2′-deoxy-O^6-[2-methylthio)ethyl]guanosine (5). Compound **5** was obtained as a glass in 65% yield (2.763 g) by treating **2** (3.513 g, 10 mmol) with triphenylphosphine (3.934 g, 15 mmol), 2-(methylthio)ethanol (1.74 mL, 20 mmol), and diethyl azodicarboxylate (2.4 mL, 15 mmol) in 1,4-dioxane (100 mL) and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.02, 2.06 (2s, 6H, COCH_3 × 2), 2.15 (s, 3H, SCH_3), 2.46 (ddd, 1H, $J_{1',2'} = 6.2$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2',3'} = 2.5$ Hz, H-2′), 2.86 (t, 2H, $J = 7.3$ Hz, CH$_2$CH_2SCH$_3$),

2.89–2.96 (m, 1H, H-2″), 4.26–4.40 (m, 3H, H-4′, 5′, and 5″), 4.58 (t, 2H, J = 7.3 Hz, CH_2CH$_2$SCH$_3$), 5.00 (br s, 2H, NH_2), 5.34–5.37 (m, 1H, H-3′), 6.23 (dd, 1H, $J_{1',2'} = 6.2$ Hz, $J_{1',2''} = 7.9$ Hz, H-1′), and 7.70 (s, 1H, H-8). *Anal*. calcd. for $C_{17}H_{23}N_5O_6S{\cdot}0.1H_2O$: C, 47.79; H, 5.47; N, 16.39. Found: C, 47.72; H, 5.52; N, 16.15.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloro-9*H*-purine (6). Compound **6** was obtained as a glass in 99% yield (2.075 g) by treating **1** (2.005 g, 4.9 mmol) with tetraethylammonium chloride (1.657 g, 10 mmol), *N,N*-dimethylaniline (0.63 mL, 5 mmol), and phosphoryl chloride (2.7 mL, 29 mmol) in dried acetonitrile (10 mL) according to the method of Robins (16): ^{1}H NMR ($CDCl_3$) δ 2.08, 2.10, 2.14 (3s, 9H, COCH_3 × 3), 4.36–4.47 (m, 3H, H-4′, 5′, and 5″), 5.21 (br s, 2H, NH_2), 5.75 (t, 1H, $J_{2',3'} = J_{3',4'} = 5.0$ Hz, H-3′), 5.97 (t, 1H, H-2′), 6.01 (d, 1H, $J_{1',2'} = 5.0$ Hz, H-1′), and 7.87 (s, 1H, H-8).

9-(3,5-Di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-2-amino-6-chloro-9*H*-purine (7). Compound **7** was prepared from **2** by modifying the method of Robins (6) for the synthesis of **6**. Compound **2** (0.351 g, 1 mmol) was dissolved in dried acetonitrile (8 mL), and tetraethylammonium chloride (0.248 g, 1.5 mmol), *N,N*-dimethylaniline (0.76 mL, 6 mmol), and phosphoryl chloride (0.56 mL, 6 mmol) were added to the solution. After stirring for 5 min at 80°C, the solution was diluted with chloroform (40 mL) and poured slowly over 5% aqueous sodium hydrogencarbonate solution (50 mL). The organic layer was washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **7** (0.322 g, 87% yield) as a glass: ^{1}H NMR ($CDCl_3$) δ 2.07, 2.12 (2s, 6H, COCH_3 × 2), 2.55 (ddd, 1H, $J_{1',2''} = 6.2$ Hz, $J_{2',2'} = 14.2$ Hz, $J_{2',3'} = 2.5$ Hz, H-2′), 2.96 (ddd, 1H, $J_{1',2''} = 7.8$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2'',3'} = 6.3$ Hz, H-2″), 4.32–4.47 (m, 3H, H-4′, 5′, and 5″), 5.30 (br s, 2H, NH_2), 5.39–5.43 (m, 1H, H-3′), 6.28 (dd, 1H, H-1′), and 7.91 (s, 1H, H-8). *Anal.* calcd. for $C_{14}H_{16}N_5O_5Cl{\cdot}0.6H_2O$: C, 44.18; H, 4.56; N, 18.40. Found: C, 44.44; H, 4.52; N, 18.09.

2′,3′,5′-Tri-*O*-acetyl-2-fluoro-*O*^{6}- [2-(4-nitrophenyl)ethyl]inosine (8). A stirred solution of **3** (2.793 g, 5.0 mmol) in 25% HF/pyridine (12 mL) was prepared in an ice-bath and *tert*-butyl nitrite (1.8 mL, 15.0 mmol) was added. After stirring for 1 h, the solution was allowed to warm up to room temperature where it was kept for 12 h, diluted with chloroform (200 mL), and poured slowly over 5% aqueous sodium hydrogencarbonate solution (100 mL). The organic layer was washed with water (100 mL), dried over anhydrous magnesium sulfate, and evaporated to dryness. The residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **8** (2.554 g, 91% yeild) as a white solid: m.p. 162–163°C (from 1:1 *n*-hexane/ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.03, 2.10, 2.11 (3s, 9H, COCH_3 × 3), 3.28 (t, 2H, J = 6.7 Hz, CH_2CH$_2$PhNO$_2$), 4.34–4.42 (m, 3H, H-4′, 5′, and 5″), 4.80 (t, 2H, J = 6.7 Hz, CH$_2$CH_2PhNO$_2$), 5.55 (dd, 1H, $J_{3',4'} = 4.5$ Hz, H-3′), 5.80 (t, 1H, $J_{1',2'} = J_{2',3'} = 5.4$ Hz, H-2′), 6.10 (d, 1H,

H-1′), 7.46 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group), 8.04 (s, 1H, H-8), and 8.12 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group). *Anal.* calcd. for $C_{24}H_{24}N_5O_{10}F$: C, 51.34; H, 4.31; N, 12.47. Found: C, 51.22; H, 4.48; N, 12.47.

2′,3′,5′-Tri-*O*-acetyl-2-fluoro-O^6-[2-(methylthio)ethyl]inosine (9). Compound **9** was obtained as a syrup in 84% yield (1.019 g) by treating **4** (1.208 g, 2.5 mmol) with *tert*-butyl nitrite (0.89 mL, 7.5 mmol) in 25% HF/pyridine (6 mL) and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.05, 2.12, 2.123 (3s, 9H, COCH_3 × 3), 2.22 (s, 3H, SCH_3), 2.94 (t, 2H, $J = 7.1$ Hz, $CH_2CH_2SCH_3$), 4.35–4.43 (m, 3H, H-4′, 5′, and 5″), 4.73 (t, 2H, $J = 7.1$ Hz, $CH_2CH_2SCH_3$), 5.79 (dd, 1H, $J_{2',3'} = 5.5$ Hz, $J_{3',4'} = 4.4$ Hz, H-3′), 5.97 (t, 1H, H-2′), 6.12 (d, 1H, $J_{1',2'} = 5.5$ Hz, H-1′), and 8.04 (s, 1H, H-8). *Anal.* calcd. for $C_{19}H_{23}N_4O_8SF$: C, 46.91; H, 4.77; N, 11.52. Found: C, 46.77; H, 4.82; N, 11.41.

3′,5′-Di-*O*-acetyl-2′-deoxy-2-fluoro-O^6-[2-(methylthio)ethyl]inosine (10). Compound **10** was obtained as a syrup in 87% yield (0.745 g) by treating **5** (0.850 g, 2 mmol) with *tert*-butyl nitrite (0.7 mL, 6 mmol) in 25% HF/pyridine (4.8 mL) and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.05, 2.08 (2s, 6H, COCH_3 × 2), 2.17 (s, 3H, SCH_3), 2.58 (ddd, 1H, $J_{1',2'} = 5.9$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2',3'} = 2.5$ Hz, H-2′), 2.85 (ddd, 1H, $J_{1',2''} = 7.9$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2'',3'} = 6.3$ Hz, H-2″), 2.91 (t, 2H, $J = 7.1$ Hz, $CH_2CH_2SCH_3$), 4.27–4.36 (m, 3H, H-4′,5′, and 5″), 4.70 (t, 2H, $J = 7.1$ Hz, $CH_2CH_2SCH_3$), 5.34–5.37 (m, 1H, H-3′), 6.33 (dd, 1H, H-1′), and 8.04 (s, 1H, H-8). *Anal.* calcd. for $C_{17}H_{21}N_4O_6SF$: C, 47.69; H, 4.94; N, 13.09. Found: C, 47.97; H, 5.02; N, 12.83.

9-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-fluoro-9*H*-purine (11). A stirred solution of **6** (2.139 g, 5.0 mmol) in 45% HF/pyridine (10 mL) was prepared in an ice-bath and *tert*-butyl nitrite (1.8 mL, 15.0 mmol) was added. After stirring for 30 min, the solution was diluted with chloroform (200 mL), and poured slowly over 5% aqueous sodium hydrogencarbonate solution (100 mL). The organic layer was washed with 2 *M* hydrochloric acid (100 mL), followed by water (100 mL), 5% aqueous sodium hydrogencarbonate solution (100 mL), and water (100 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **11** (1.827 g, 85% yield) as a pale yellow solid [see Robins (8)]. ^{1}H NMR ($CDCl_3$) δ 2.08, 2.15, 2.16 (3s, 9H, COCH_3 × 3), 4.36–4.47 (m, 3H, H-4′, 5′, and 5″), 5.55 (dd, 1H, $J_{2',3'} = 5.6$ Hz, $J_{3',4'} = 4.3$ Hz, H-3′), 5.81 (t, 1H, H-2′), 6.16 (d, 1H, $J_{1',2'} = 5.6$ Hz, H-1′), and 8.27 (s, 1H, H-8). *Anal.* calcd. for $C_{16}H_{16}N_4O_7ClF$: C, 44.61; H, 3.74; N, 13.01. Found: C, 44.44; H, 3.76; N, 12.82.

9-(3,5-Di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)-6-chloro-2-fluoro-9*H*-purine (12). Compound **12** was obtained as a syrup in 90% yield (2.246 g) by treating **7** (2.477 g, 6.7 mmol) with *tert*-butyl nitrite (2.4 mL, 20.1 mmol) in 45% HF/pyridine (13.4 mL) and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.10, 2.14 (2s, 6H, COCH_3 × 2), 2.68 (ddd. 1H, $J_{1',2'} = 5.9$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2',3'} = 2.5$ Hz, H-2′), 2.88 (ddd, 1H, $J_{1',2''} = 7.8$ Hz, $J_{2',2''} = 14.2$ Hz,

$J_{2'',3'} = 6.3$ Hz, H-2″), 4.36–4.40 (m, 3H, H-4′, 5′, and 5″), 5.39–5.42 (m, 1H, H-3′), 6.41 (dd, 1H, H-1′), and 8.30 (s, 1H, H-8). *Anal.* calcd. for $C_{14}H_{14}N_4O_5ClF{\cdot}0.3H_2O$: C, 44.49; H, 3.89; N, 14.82. Found: C, 44.53; H, 3.78; N, 14.71.

2′,3′5′-Tri-*O*-acetyl-N^2-benzyl-O^6-[2-(4-nitrophenyl)ethyl] [2-^{15}N]guanosine (13). Compound **8** (0.561 g, 1 mmol) was dissolved in dried *N,N*-dimethylformamide (5 mL), and triethylamine (0.17 mL, 1.2 mmol) and [^{15}N]benzylamine (0.130 g, 1.2 mmol), which was prepared from [^{15}N]benzamide (99% ^{15}N-enriched, purchased from ISOTEC, Inc.) by the method of Horneman (9) in 85% yield, were added to the solution. After stirring for 2 h at room temperature, the solution was diluted with chloroform (50 mL) and washed with 1 *M* hydrochloric acid (25 mL), followed by water (25 mL), 5% aqueous sodium hydrogencarbonate solution (25 mL), and water (25 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **13** (0.604 g, 93% yield) as a glass: ^{1}H NMR ($CDCl_3$) δ 2.04, 2.08, 2.10 (3s, 9H, COCH_3 × 3), 3.22 (t, 2H, $J = 6.7$ Hz, CH_2CH$_2$PhNO$_2$), 4.32–4.42 (m, 3H, H-4′, 5′, and 5″), 4.62–4.70 (m, 4H, CH$_2$CH_2PhNO$_2$ and CH_2Ph), 5.41 (br dt, 1H, $J_{15N,H} = 90.9$ Hz, $J_{15NH,CH2Ph} = 6.2$ Hz, ^{15}N^2-*H*), 5.72 (t, 1H, $J_{2',3'} = J_{3',4'} = 5.5$ Hz, H-3′), 5.93 (d, 1H, $J_{1',2'} = 4.1$ Hz, H-1′), 6.01 (dd, 1H, H-2′), 7.27–7.68 (m, 7H, Ph-*H* of 2-(4-nitrophenyl)ethyl and benzyl groups), 7.65 (s, 1H, H-8), and 8.13 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group); ^{15}N NMR ($CDCl_3$) δ 89.94 (N^2); EI mass spectrum, *m/z* 649.4 (M^+).

2′,3′,5′-Tri-*O*-acetyl-N^2-benzoyl-O^6-[2-(4-nitrophenyl)ethyl][2-^{15}N] guanosine (14). Tetrabutylammonium periodate (10) (0.48 mmol, 0.208 g) and catalytic amount of ruthenium trichloride (1 mg) were added to a bilayer solution of **13** (0.12 mmol, 0.078 g) in 2:2:3 methylene chloride/acetonitrile/water (1.2 mL) at room temperature [see Gao and Jones, and Sarfati and Kansal (3); Gao and Sharpless (14)]. After stirring for 2 h, the mixture was diluted with chloroform (30 mL). The organic layer was washed with water (30 mL), followed by 5% aqueous sodium hydrogencarbonate solution (30 mL) and water (30 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **14** (0.056 g, 71% yield) as a glass: ^{1}H NMR ($CDCl_3$) δ 2.00, 2.07, 2.12 (3s, 9H, COCH_3 × 3), 3.29 (t, 2H, $J = 6.8$ Hz, CH_2CH$_2$PhNO$_2$), 4.41–4.50 (m, 3H, H-4′, 5′, and 5″), 4.80 (t, 2H, $J = 6.8$ Hz, CH$_2$CH_2PhNO$_2$), 5.95–5.97 (m, 2H, H-2′ and 3′), 6.06 (d, 1H, $J_{1',2'} = 3.8$ Hz, H-1′), 7.43–7.54 (m, 5H, Ph-*H* of 2-(4-nitrophenyl)ethyl and benzoyl groups), 7.93 (s, 1H, H-8), 7.95–7.98 (m, 2H, Ph-*H* of the benzoyl group), 8.11 (d, 2H, $J = 8.8$ Hz, Ph-*H* of the 2-(4-nitrophenyl)ethyl group), and 8.78 (d, 1H, $J_{15N,H} = 89.1$ Hz, ^{15}N^2-*H*); ^{15}N NMR ($CDCl_3$) δ 135.56 (N^2); EI mass spectrum, *m/z* 663.8 (M^+).

2′,3′,5′-Tri-*O*-acetyl-O^6-[2-(methylthio)ethyl]-N^2-phthaloylguanosine (15) (Tab. 1, Entry 4). Compound **9** (0.486 g, 1 mmol) was dissolved in dried *N,N*-dimethylformamide (10 mL), and potassium phthalimide (0.278 g, 1.5 mmol) was added to the solution. The mixture was then stirred for 24 h at 100°C. After

cooling down to room temperature, acetic anhydride (1 mL) and pyridine (1 mL) were added to the solution, which was then stirred at room temperature for 1 h. The mixture was quenched with water (2 mL), diluted with chloroform (40 mL), and washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **9** (0.092 g, 19% recovery yield) and **15** (0.392 g, 64% yield).

Compound **15** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.08, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 2.21 (s, 3H, SCH_3), 3.01 (t, 2H, J = 7.1 Hz, $CH_2CH_2SCH_3$), 4.41–4.45 (m, 3H, H-4′, 5′, and 5″), 4.78 (t, 2H, J = 7.1 Hz, $CH_2CH_2SCH_3$), 5.64 (dd, 1H, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 4.1 Hz, H-3′), 5.88 (t, 1H, H-2′), 6.24 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 7.80–7.83, 7.98–8.00 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.15 (s, 1H, H-8). *Anal.* Calcd. for $C_{27}H_{27}N_5O_{10}S$: C, 52.85; H, 4.43; N, 11.41. Found: C, 52.74; H, 4.70; N, 11.15.

Conversion of 2′,3′,5′-tri-*O*-acetyl-O^6-[2-(methylthio)ethyl]-N^2 phthaloylguanosine (15) into 2′,3′,5′-tri-*O*-acetylguanosine (1). Compound **15** (0.276 g, 0.45 mmol) was treated by 0.5 *M* hydrazine hydrate in 1:4 acetic acid/pyridine (1.35 mL) with stirring for 30 min at room temperature. To the resulting mixture, acetone (2 mL) was added. After stirring for 15 min, the reaction mixture was evaporated to dryness. The residue was, after azeotropic evaporation from pyridine (5 mL × 3), dissolved in dried pyridine (1.5 mL), and acetic anhydride (0.5 mL) was added to the solution, which was then stirred at room temperature for 1 h. The mixture was evaporated and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **4** (0.209 g, 96% yield). Tetrabutylammonium periodate (10) (1.0 mmol, 0.433 g) and catalytic amount of ruthenium trichloride (1 mg) were added to a bilayer solution of **4** (0.25 mmol, 0.121 g) in 2:2:3 methylene chloride/acetonitrile/water (2.5 mL) at room temperature [see Gao and Jones, and Sarfati and Kansal (3); Gao and Sharpless (14)]. After stirring for 30 min, the mixture was diluted with chloroform (30 mL). Organic layer was washed with water (20 mL), followed by 5% aqueous sodium hydrogencarbonate solution (20 mL) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of *n*-hexane/ethyl acetate system to give 2′,3′,5′-tri-*O*-acetyl-O^6-[2-(methylsulfonyl)ethyl]guanosine (**23**) (0.120 g, 92% yield) as a glass: ^{1}H NMR ($CDCl_3$) δ 2.08, 2.09, 2.14 (s, 9H, COCH_3 × 3), 3.11 (s, 3H, SO_2CH_3), 3.51 (t, 2H, J = 5.5 Hz, $CH_2CH_2SO_2CH_3$), 4.34–4.48 (m, 3H, H-4′, 5′, and 5″), 4.91 (t, 2H, J = 5.5 Hz, $CH_2CH_2SCH_3$), 5.03 (br s, 2H, NH_2), 5.77 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 4.8 Hz, H-3′), 5.96 (t, 1H, H-2′), 5.99 (d, 1H, $J_{1',2'}$ = 4.8 Hz, H-1′), and 7.71 (s, 1H, H-8). *Anal.* calcd. for $C_{19}H_{25}N_5O_{10}S{\cdot}H_2O$: C, 42.77; H, 5.10; N, 13.13 Found: C, 43.02; H, 5.12; N, 13.05.

Compound **23** (0.062 g, 0.12 mmol) was treated by 0.1 *M* DBU in acetonitrile (1.2 mL) with stirring for 1 h at room temperature. After acetic acid (7 μL,

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0.12 mmol) was added, the resulting mixture was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **1** (0.047 g, 96% yield).

2′,3′,5′-Tri-*O*-acetyl-*O*^{6}-[2-(methylthio)ethyl-*N*^{2}-phthaloyl[2-^{15}N] guanosine (15[^{15}N]) (Tab. 1, Entry 5). Compound **15[^{15}N]** was obtained as a glass in 62% yield (0.381 g) by treating **9** (0.486 g, 1 mmol) with potassium [^{15}N]phthalimide (0.279 g, 1.5 mmol, 99% ^{15}N-enriched, purchased from ISOTEC, Inc.) in dried *N,N*-dimethylformamide (10 mL) for 36 h at 90°C and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.08, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 2.21 (s, 3H, SCH_3), 3.01 (t, 2H, J = 7.1 Hz, $CH_2CH_2SCH_3$), 4.41–4.45 (m, 3H, H-4′, 5′, and 5″), 4.78 (t, 2H, J = 7.1 Hz, $CH_2CH_2SCH_3$), 5.64 (dd, 1H, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 4.1 (Hz, H-3′), 5.88 (t, 1H, H-2′), 6.24 (d, 1H, $J_{1',2'}$ = 5.5 Hz, H-1′), 7.80–7.83, 7.98–8.01 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.15 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 182.18 (N^2); low-resolution FAB mass spectrum, *m/z* 615.2 $(M + H)^+$.

3′,5′-Di-*O*-acetyl-2′-deoxy-*O*^{6}-[2-(methylthio)ethyl-*N*^{2}-phthaloyl[2-^{15}N]-guanosine (16[^{15}N]) (Tab. 1, Entry 7). Compound **16[^{15}N]** was obtained as a glass in 56% yield (0.160 g) by treating **10** (0.214 g, 0.5 mmol) with potassium [^{15}N]phthalimide (0.112 g, 0.6 mmol) in dried *N,N*-dimethylformamide (2.5 mL) for 48 h at 90°C and subsequent work-up as described earlier: ^{1}H NMR ($CDCl_3$) δ 2.05, 2.10 (2s, 6H, COCH_3 × 2), 2.20 (s, 3H, SCH_3), 2.66 (ddd, 1H, $J_{1',2'}$ = 6.2 Hz, $J_{2',2''}$ = 14.2 Hz, $J_{2',3'}$ = 2.5 Hz, H-2′), 2.87–2.96 (m, 1H, H-2″), 3.00 (t, 2H, J = 7.2 Hz, $CH_2CH_2SCH_3$), 4.32–4.42 (m, 3H, H-4′, 5′, and 5″), 4.77 (t, 2H, J = 7.2 Hz, $CH_2CH_2SCH_3$), 5.38–5.42 (m, 1H, H-3′), 6.47 (dd, 1H, $J_{1',2'}$ = 6.2 Hz, $J_{1',2''}$ = 7.9 Hz, H-1′), 7.80–7.83, 7.96–7.99 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.18 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 184.11 (N^2); EI mass spectrum, *m/z* 556.1 (M^+).

Reaction of 11 with potassium phthalimide (Tab. 2, Entry 4). Compound **9** (0.243 g, 0.5 mmol) was dissolved in dried *N,N*-dimethylformamide (2.5 mL), and potassium phthalimide (0.111 g, 0.6 mmol) was added to the solution. After stirring for 30 h at room temperature, the mixture was quenched with water (2 mL), diluted with chloroform (40 mL) and washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of *n*-hexane/ethyl acetate system to give 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-phthalimido-9*H*-purine (**17**) (0.170 g, 61% yield), 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-fluoro-6-phthalimido-9*H*-purine (**19**) (0.014 g, 5% yield), and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-diphthalimido-9*H*-purine (**21**) (0.030 g, 9% yield).

Compound **17** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 4.42–4.49 (m, 3H, H-4′, 5′, and 5″), 5.61 (dd, 2H, $J_{2',3'}$ = 5.6 Hz, $J_{3',4'}$ = 3.9 Hz, H-3′), 5.86 (t, 1H, H-2′), 6.27 (d, 1H, $J_{1',2'}$ = 5.6 Hz, H-1′), 7.82–7.85, 7.98–8.01 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.38 (s, 1H, H-8). *Anal.*

calcd. for $C_{24}H_{20}N_5O_9Cl{\cdot}0.5H_2O$: C, 50.85; H, 3.73; N, 12.35. Found: C, 50.92; H, 3.79; N, 12.27.

Compound **19** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.15, 2.16 (3s, 9H, COCH_3 × 3), 4.40–4.48 (m, 3H, H-4′, 5′, and 5″), 5.59 (dd, 2H, $J_{2',3'} = 5.8$ Hz, $J_{3',4'} = 3.8$ Hz, H-3′), 5.86 (t, 1H, H-2′), 6.25 (d, 1H, $J_{1',2'} = 5.8$ Hz, H-1′), 7.84–7.88, 8.00–8.04 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.30 (s, 1H, H-8). *Anal.* calcd. for $C_{24}H_{20}N_5O_9F{\cdot}1.3H_2O$: C, 51.03; H, 4.03; N, 12.40. Found: C, 51.25; H, 4.08; N, 12.12.

Compound **21** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 4.39–4.49 (m, 3H, H-4′, 5′, and 5″), 5.65 (dd, 2H, $J_{2',3'} = 5.8$ Hz, $J_{3',4'} = 3.7$ Hz, H-3′), 5.92 (t, 1H, H-2′), 6.34 (d, 1H, $J_{1',2'} = 5.8$ Hz, H-1′), 7.80–7.84, 7.97–8.02 (2m, 8H, Ph-*H* of the phthaloyl group), and 8.40 (s, 1H, H-8). *Anal.* calcd. for $C_{32}H_{24}N_6O_{11}{\cdot}1.9H_2O$: C, 54.69; H, 3.99; N, 11.96. Found: C, 54.43; H, 3.69; N, 11.85.

Reaction of 11 with potassium [^{15}N]phthalimide (Tab. 2, Entry 5). Compound **11** (0.349 g, 0.81 mmol) was dissolved in dried *N,N*-dimethylformamide (4 mL), and potassium [^{15}N]phthalimide (75 mg, 0.405 mmol) was added to the solution. After stirring for 2 h at 40°C, potassium [^{15}N]phthalimide (60 mg, 0.324 mmol) was added to the solution. Furthermore, after stirring for 3.5 h at 40°C, potassium [^{15}N]phthalimide (45 mg, 0.243 mmol) was added to the solution, which was then stirred for 3.5 h at 40°C. The mixture was quenched with water (2 mL), diluted with chloroform (40 mL), and washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of ***n***-hexane/ethyl acetate system to give 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-[^{15}N]phthalimido-9*H*-purine (**17 [^{15}N]**) (0.290 g, 64%), 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-fluoro-6-^{15}N]phthalimido-9*H*-purine (**19[^{15}N]**) (0.035 g, 8%), and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-di[^{15}N] phthalimido-9*H*-purine (**21 [^{15}N]**) (0.054 g, 10%).

Compound **17 [^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 4.42–4.49 (m, 3H, H-4′, 5′, and 5″), 5.61 (dd, 1H, $J_{2',3'} = 5.6$ Hz, $J_{3',4'} = 3.9$ Hz, H-3′), 5.86 (t, 1H, H-2′), 6.27 (d, 1H, $J_{1',2'} = 5.6$ Hz, H-1′), 7.82–7.85, 7.98–8.01 (2 m, 4H, Ph-*H* of the phthaloyl group), and 8.38 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 178.00 (N^2); low-resolution FAB mass spectrum, *m/z* 559.1 $(M + H)^+$.

Compound **19 [^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.15, 2.16 (3s, 9H, COCH_3 × 3), 4.40–4.48 (m, 3H, H-4′, 5′, and 5″), 5.59 (dd, 2H, $J_{2',3'} = 5.8$ Hz, $J_{3',4'} = 3.8$ Hz, H-3′), 5.86 (t, 1H, H-2′), 6.25 (d, 1H, $J_{1',2'} = 5.8$ Hz, H-1′), 7.84–7.88, 8.00–8.04 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.30 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 170.20 (N^6); low-resolution FAB mass spectrum, *m/z* 543.2 $(M + H)^+$.

Compound **21 [^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.09, 2.10, 2.13 (3s, 9H, COCH_3 × 3), 4.39–4.49 (m, 3H, H-4′, 5′, and 5″), 5.65 (dd, 2H, $J_{2',3'} = 5.8$

Hz, $J_{3',4'} = 3.7$ Hz, H-3′), 5.92 (t, 1H, H-2′), 6.34 (d, 1H, $J_{1',2'} = 5.8$ Hz, H-1′), 7.80–7.84, 7.97–8.02 (2m, 8H, Ph-*H* of the phthaloyl group), and 8.40 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 170.55 (N^6) and 178.44 (N^2); low-resolution FAB mass spectrum, *m/z* 671.2 $(M + H)^+$.

Reaction of 12 with potassium [^{15}N]phthalimide (Tab. 2, Entry 6). Compound **12** (0.223 g, 0.6 mmol) was dissolved in dried *N,N*-dimethylformamide (3 mL), and potassium phthalimide (0.134 g, 0.72 mmol) was added to the solution. After stirring for 1.5 h at 60°C, the mixture was quenched with water (2 mL), diluted with chloroform (40 mL), and washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness, and the residue was subjected to chromatographic separation on a column of silica gel by the use of *n*-hexane/ethyl acetate system to give **12** (0.024 g, 11% recovery yield), 9-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-6-chloro-2-[^{15}N]phthalimido-9*H*-purine (**18 [^{15}N]**) (0.111 g, 37%), 9-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-2-fluoro-6-[^{15}N]phthalimido-9*H*-purine (**20 [^{15}N]**) (0.079 g, 27%), and 9-(3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranosyl)-2,6-di[^{15}N]phthalimido-9*H*-purine (**22[^{15}N]**) (0.025 g, 7%).

Compound **18 [^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.04, 2.10 (2s, 6H, COCH_3 × 2), 2.70 (ddd, 1H, $J_{1',2'} = 6.0$ Hz, $J_{2',2''} = 14.1$ Hz, $J_{2',3'} = 2.4$ Hz, H-2′), 2.90–3.00 (m, 1H, H-2″), 4.33–4.40 (m, 3H, H-4′, 5′, and 5″), 5.38–5.40 (m, 1H, H-3′), 6.48 (dd, 1H, $J_{1',2'} = 6.0$ Hz, $J_{1',2''} = 7.8$ Hz, H-1′), 7.80–7.84, 7.94–7.99 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.40 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 178.11 (N^2); low-resolution FAB mass spectrum, *m/z* 500.9 $(M + H)^+$.

Compound **20[^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.10, 2.16 (2s, 6H, COCH_3 × 2), 2.71 (ddd, 1H, $J_{1',2'} = 5.9$ Hz, $J_{2',2''} = 14.2$ Hz, $J_{2',3'} = 2.4$ Hz, H-2′), 2.85–2.95 (m, 1H, H-2″), 4.36–4.40 (m, 3H, H-4′, 5′, and 5″), 5.41–5.44 (m, 1H, H-3′), 6.48 (dd, 1H, $J_{1',2'} = 5.9$ Hz, $J_{1',2''} = 8.0$ Hz, H-1′), 7.83–7.87, 8.01–8.05 (2m, 4H, Ph-*H* of the phthaloyl group), and 8.33 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 170.22 (N^6); low-resolution FAB mass spectrum, *m/z* 485.0 $(M + H)^+$.

Compound **22 [^{15}N]** was a glass: ^{1}H NMR ($CDCl_3$) δ 2.05, 2.12 (2s, 6H, COCH_3 × 2), 2.74 (ddd, 1H, $J_{1',2'} = 6.0$ Hz, $J_{2',2''} = 14.3$ Hz, $J_{2',3'} = 2.2$ Hz, H-2′), 2.94–3.05 (m, 1H, H-2″), 4.35–4.40 (m, 3H, H-4′,5′, and 5″), 5.42–5.45 (m, 1H, H-3′), 6.55 (dd, 1H, $J_{1',2'} = 6.0$ Hz, $J_{1',2''} = 7.9$ Hz, H-1′), 7.80–7.85, 7.96–8.03 (2 m, 8H, Ph-*H* of the phthaloyl group × 2), and 8.42 (s, 1H, H-8); ^{15}N NMR ($CDCl_3$) δ 170.56 (N^6) and 178.62 (N^2); low-resolution FAB mass spectrum, *m/z* 613.1 $(M + H)^+$.

2′,3′,5′-Tri-*O*-acetyl[2-^{15}N]guanosine (26 [^{15}N]). Compound **17 [^{15}N]** (85 mg, 0.15 mmol) was treated for 1 h at 45°C with sodium 2-cyanoethoxide, which was prepared from 2-cyanoethanol (0.17 mL, 2.5 mmol) and sodium hydride (54 mg, 2.25 mmol) in THF (22.5 mL). The reaction was quenched with acetic acid (0.16 mL, 2.7 mmol). The solvents were removed in vacuo. The residue was dissolved in dried *N,N*-dimethylformamide (3 mL)/pyridine (2 mL), and acetic anhydride (2 mL) was added to the solution. After stirring for 2 h at room temperature,

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the mixture was quenched with water (2 mL), diluted with chloroform (40 mL), and washed with 5% aqueous sodium hydrogencarbonate solution (20 mL × 2) and water (20 mL). After drying over anhydrous magnesium sulfate, the organic layer was evaporated to dryness. The residue was treated for 30 min at room temperature with 0.5 *M* hydrazine hydrate in 1:4 acetic acid/pyridine (1.5 mL). The reaction was quenched with acetone (1 mL). The solvents were removed in vacuo. The residue was dissolved in dried pyridine (3 mL), and acetic anhydride (1 mL) was added to the solution. After stirring for 1 h at room temperature, the mixture was quenched with methanol (0.5 mL). The solvents were removed in vacuo, and the residue was subjected to chromatographic separation on a column of silica gel by the use of methanol/chloroform system to give **26[^{15}N]** (44 mg, 71%) as a white solid: m.p. 227–229°C; UV (MeOH) λ_{max} 275 (sh), 255 nm, and λ_{min} 223 nm; ^{1}H NMR (DMSO-d_6) δ 2.03, 2.04, 2.11 (3s, 9H, COC*H*3 × 3), 4.21–4.40 (m, 3H, H-4′, 5′, and 5″), 5.65 (dd, 1H, $J_{2',3'} = 6.1$ Hz, $J_{3',4'} = 3.9$ Hz, H-3′), 5.78 (t, 1H, $J_{1',2'} = 6.1$ Hz, H-2′), 5.98 (t, 1H, H-1′), 6.25 (br d, 2H, $J_{15N,H} = 91.2$ Hz, ^{15}N^2-H_2), 7.92 (s, 1H, H-8), and 10.92 (br s, 1H, N^1-*H*); ^{15}N NMR (DMSO-d_6) δ 73.40 (N^2); low-resolution FAB mass spectrum, *m/z* 410.6 $(M + H)^+$.

3′,5′-Di-*O*-acetyl-2′-deoxy[2-^{15}N] guanosine (27 [^{15}N). Compound **27 [^{15}N]** was obtained as a white solid in 76% yield (40 mg) from **18[^{15}N]** (75 mg, 0.15 mmol) as described above: m.p. softening from 240°C, −300°C (dec.) (crystallized from methanol); UV (MeOH) λ_{max} 275 (sh), 255 nm, and λ_{min} 222 nm; ^{1}H NMR (DMSO-d_6) δ 2.03, 2.07 (2s, 6H, COCH_3 × 2), 2.41–2.50 (m, 1H, H-2′), 2.86–2.96 (m, 1H, H-2″), 4.15–4.28 (m, 3H, H-4′, 5′, and 5″), 5.28–5.30 (m, 1H, H-3′), 6.13 (dd, 1H, $J_{1',2'} = 5.9$ Hz, $J_{1',2''} = 8.6$ Hz, H-1′), 6.49 (br d, 2H, $J_{15N,H} = 89.7$ Hz, ^{15}N^2-H_2), 7.91 (s, 1H, H-8), and 10.67 (br s, 1H, N^1-*H*); ^{15}N NMR (DMSO-d_6) δ 72.59 (N^2); low-resolution FAB mass spectrum, *m/z* 352.7 $(M + H)^+$.

ACKNOWLEDGMENTS

The authors thank Dr. Yasuo Shida for MS measurements, Mrs. Chiseko Sakuma for ^{15}N NMR measurements, and Mr. Haruhiko Fukaya for elemental analyses, all from Analytical Center, Tokyo University of Pharmacy and Life Science.

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Received July 8, 2000
Accepted September 5, 2000

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