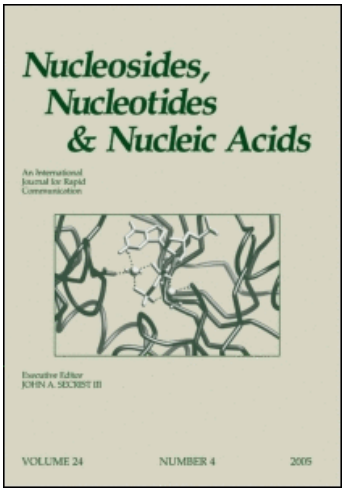

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A NEW METHOD FOR THE SYNTHESIS OF N^2-ALKYLGUANOSINES USING MITSUNOBU REACTION AS A KEY STEP

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ABSTRACT

Peracetylated guanosine was reacted with $POCl_3$ to give an 2-acetamido-6-chloro-9*H*-purine derivative, which was condensed with primary or secondary alcohols to give N^2-alkylated analogues. The products were treated with mercaptoethanol in the presence of sodium methoxide to afford N^2-alkylguanosines.

INTRODUCTION

Recently, N^2-alkylguanosine has attracted the attention of many chemists since the discovery of this type of compound in cells. For instance, the 5′-termini of nuclear RNAs involved a unique permethylated ribonucleoside of 2,2,7-tri-*N*-methylguanosine (**A**)[1]. An N^2-ethyl-2′-deoxyguanosine 3′-monophosphate (**B**) moiety has been reported to be involved in the granulocyte and lymphocyte DNA of alcohol abusers at a high level as the ultimate DNA adduct of acetaldehyde[2]. Robertson reported the formation of the N^2-substituent (**C**) by the reaction of deoxyguanosine with dehydroretronecine, a carcinogenic metabolite of the pyrrolizidine alkaloid monocrotaline[3].

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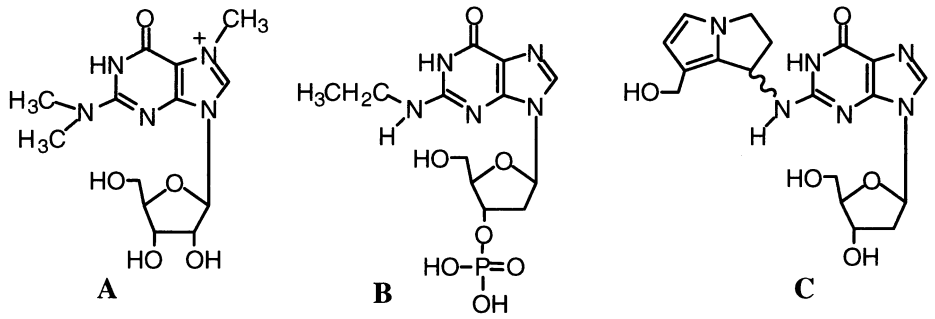
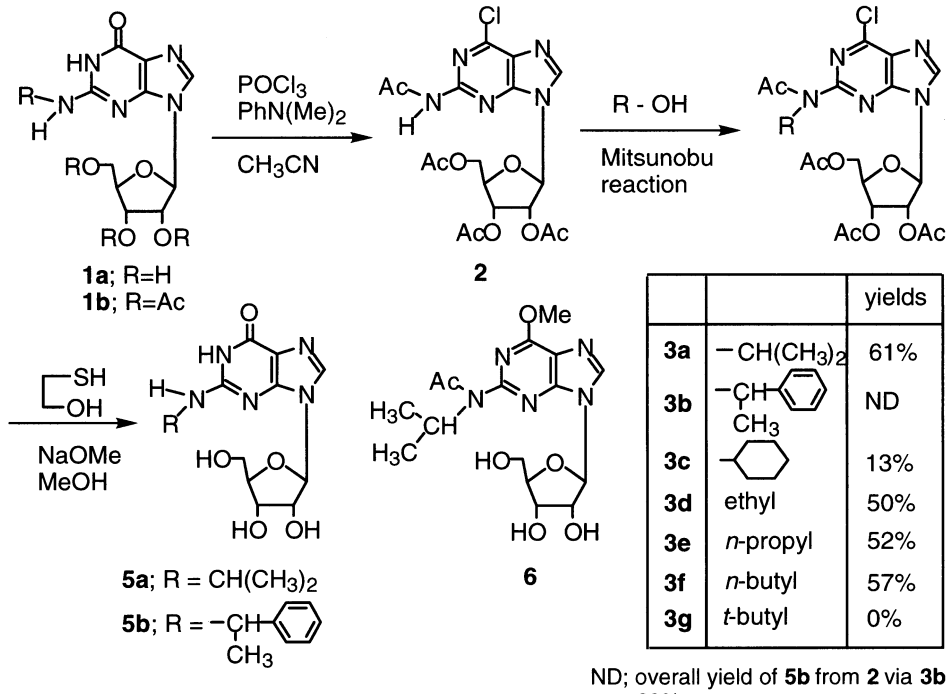


Also, interaction of N^2-substituted guanosine triphosphates with cellular and oncogenic ras-p21 proteins has been reported[4].

The synthesis of N^2-alkylguanosine has been achieved by alkylation of the O^6-silylated guanosine with 1,3-benzodithiolylium tetrafluoroborate (BDTF)[5]. In the case of N^2-ethyl-guanosine, one-step reaction of guanosine with acetaldehyde and $NaBH_3CN$ has been reported by Sako *et al*[6]. Also, reduction of the amide group of acyl nucleosides was performed with borane to give the corresponding *N*-alkyl nucleosides[7]. However, those methods are limited to a primary alkyl group. For the synthesis of the guanosine analogue bearing a secondary alkyl group, a nucleophilic displacement of inosine-2-sulfonic acid or 2-halogeno-inosines has been used[8]. Recently, the Mitsunobu reaction has been employed as a method for *N*-alkylation of a heterocyclic compound[9]. We already reported a new method for the synthesis of N^3-alkyl-5-fluorouracils[10]. In this paper, a novel method to introduce a secondary as well as a primary alkyl group onto the N^2 position of guanosine using the Mitsunobu reaction as a key step is described.

RESULTS

Peracetylation of guanosine (**1a**) gave N^2,$O^{2'}$,$O^{3'}$,$O^{5'}$-tetraacetyl-guanosine (**1b**), which was treated with $POCl_3$ (6.0 eq) in the presence of *N*,*N*-dimethylaniline (1.0 eq) in CH_3CN[11] at reflux temperature for 30 min to afford 2-acetamido-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**2**) in 69% yield. Then, compound **2** (10 mmol) was treated with 2-propanol (30 mmol), tributylphosphine (30 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide)[12] (30 mmol) in dry THF (200 mL) at 50 °C for 3 h and separated by a column of silica gel. From the first fraction 6-chloro-2-(N^2-isopropyl)acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**3a**) was obtained in 61% yield. Change of the solvent to 1,4-dioxane decreased the yield to 41%. When diisopropyl azodi-carboxylate and triphenylphosphine were employed as a condensing agent, the imino ether **4** as well as the desired **3a** was observed on thin-layer chromatography (tlc).



		yields
3a	$-CH(CH_3)_2$	61%
3b	−CH− (CH3)	ND
3c	−	13%
3d	ethyl	50%
3e	*n*-propyl	52%
3f	*n*-butyl	57%
3g	*t*-butyl	0%

Scheme.

It is supposed that the bulky agents proceed the condensation of 2-propanol with the imino form of **2**, which is less susceptable to steric hindrance. A trial to convert **3a** to the guanosine derivative was achieved by the method of Lee *et al*[13]. Thus, compound **3a** (1 mmol) was refluxed with 2-mercaptoethanol (0.3 ml, 4.3 mmol) and 0.06 M NaOMe in MeOH (50 mL, 3 mmol) under nitrogen atmosphere overnight. After work-up of the solution, the mixture was separated by a column of silica gel to give **5a** and **6** in 65% and 19% yields, respectively. The major product (**5a**) showed a similar absorption to that of N^2-methylguanosine on UV spectrum, and correlation between $Me_2C\underline{H}$- and C2 was observed on the HMBC spectrum as shown in the Figure. Thus, compound **5a** was proved to be N^2-isopropylguanosine. However, the ^{1}H-NMR spectrum of the minor product (**6**) showed the methyl signals of O^6-$C\underline{H}_3$ and N^2-$COC\underline{H}_3$, and HR-MS suggested that the formula of **6** should be $C_{16}H_{23}N_5O_6$. Combined with HMBC correlation, compound **6** was identified as 2-(N^2-isopropyl)acetamido-6-methoxy-9-(β-D-ribofuranosyl)purine. When **3a** was treated with NaOMe alone, **6** was obtained as the sole product in quantitative yield. Next, we explored the reaction of **2** with (±)-1-phenylethanol. Thus, **2** (2 mmol) was condensed with (±)-1-phenylethanol (6 mmol) using tributylphosphine (6 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide)

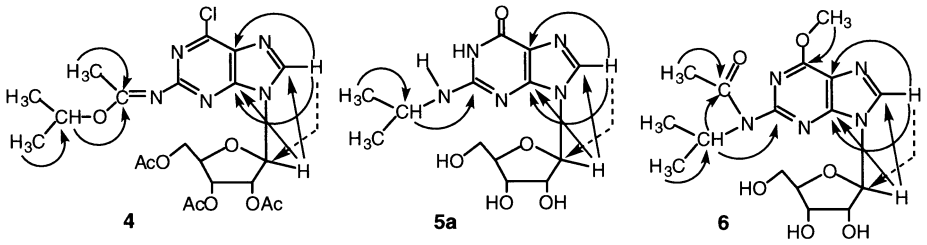


Figure. HMBC correlations of the base moieties of compounds **4**, **5a** and **6**.

(6 mmol) in dry THF (20 mL) at 50 °C for 1 h and subsequent treatment with mercaptoethanol and NaOMe afforded N^2-(1-phenylethyl)-guanosine (**5b**) as a mixture of diastereomers in 60% overall yield from **2**. However, reaction of **2** with cyclohexanol gave the condensate (**3c**) only in 13% yield and the reaction of **2** with *tert*-butanol was unsuccessful. These results suggest the difficulty to introduce a cycloalkyl and a tertiary alkyl group. On the other hand, treatment of **2** with primary alcohols using the similar conditions as described above gave **3d**–**f**. However, the yield of the products was in a range of 50–57%, which is less satisfactory compared with the methods already established in the earlier report[5–8].

As a conclusion, we have developed a method to obtain N^2-alkylguanosine in 4 steps. It became possible to introduce an alcohol on exocyclic amino of guanosine. It should be emphasized that the secondary alcohol is possible to condense with **2**. However, cyclic secondary alcohol such as cyclohexanol was proved to be less reactive under the reaction condition, indicating the difficulty to prepare the guanosine derivative bearing a cyclic secondary alkyl group.

EXPERIMENTAL

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrometer. Low resolution mass spectra were obtained on a Shimadzu-LKB 9000B mass spectrometer in the direct-inlet mode. High resolution mass spectra were obtained on a JMS AX-500 spectrometer in the direct-inlet mode. ^{1}H-NMR spectra were recorded on either Varian UNITY 200 (200 MHz) or Varian UNITY 600 (600 MHz) in $CDCl_3$ (or dimethyl sulfoxide (DMSO)-d_6) with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with silica gel 60 containing fluorescent indicator F_{254} were used for thin-layer chromatography and silica gel 60 (Merck 7734, 60–200 mesh) was employed for column chromatography.

6-Chloro-2-acetamido-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (2). To a solution of N^2,$O^{2'}$,$O^{3'}$,$O^{5'}$-tetraacetylguanosine (**1b**, 22.88 g, 63.8 mmol) in acetonitrile (500 ml) was added *N*, *N*-dimethylaniline (8 ml, 63.8 mmol) and phosporyl chloride (35 ml, 383 mmol) and the solution was refluxed for 30 min. Then the solution was concentrated to a small volume and the residue was partitioned between $CHCl_3$ (400 ml) and water (400 ml). The organic layer was washed with 5% $NaHCO_3$ (300 ml) and water (300 ml), dried over $MgSO_4$, and chromatographed over a column of silica gel (5.0 × 40 cm) using a 33–80% AcOEt in hexane to give a caramel (20.66 g, 69%). HR-MS *m/z*: 469.0965 (M^+, $C_{18}H_{20}ClN_5O_8$ requires 469.1001). UV λ_{max} (MeOH) nm: 288, 258. ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, br s, *N*-H), 8.13 (1H, s, H8), 6.09 (1H, d, *J* = 4.4 Hz, H1′), 5.91 (1H, dd, *J* = 4.4, 5.5 Hz, H2′), 5.76 (1H, dd, *J* = 5.1, 5.5 Hz, H3′), 4.40–4.55 (3H, m, H4′, H5′), 2.46 (3H, s, N-Ac), 2.16 (3H, s, Ac), 2.11 (3H, s, Ac), 2.10 (3H, s, Ac).

6-Chloro-2-(*N*-isopropyl)acetamido-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3a). Method 1. To a mixture of **2** (4.7 g, 10 mmol) and 2-propanol (4.5 ml, 30 mmol) in dry THF (200 ml) was added tributylphosphine (7.5 ml, 30 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide) (5.16 g, 30 mmol) and the solution was stirred at 50 °C for 3 h, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (3.0 × 60 cm) using a 25–66% AcOEt in benzene to give a caramel (3.10 g, 61%). HR-MS *m/z*: 511.1465 (M^+, $C_{21}H_{26}ClN_5O_8$ requires 511.1470). UV λ_{max} (MeOH) nm: 265. ^{1}H-NMR ($CDCl_3$) δ: 8.33 (1H, s, H8), 6.20 (1H, d, *J* = 4.9 Hz, H1′), 5.82 (1H, dd, *J* = 5.2, 5.5 Hz, H2′), 5.50 (1H, dd, *J* = 5.2, 5.5 Hz, H3′), 4.97 (1H, q, *J* = 6.9 Hz, CH), 4.49–4.51 (1H, m, H4′), 4.37–4.44 (2H, m, H5′), 2.16 (3H, s, *N*-Ac), 2.14 (3H, s, Ac), 2.08 (3H, s, Ac), 2.07 (3H, s, Ac), 1.30 (3H, d, *J* = 6.9 Hz, CH_3), 1.28 (3H, d, *J* = 6.9 Hz, CH_3).

Method 2. To a mixture of **2** (470 mg, 1 mmol) and 2-propanol (0.45 ml, 3 mmol) in dry THF (15 ml) was added triphenylphosphine (0.788 mg, 3 mmol) and diisopropyl azodicarboxylate (0.6 ml, 3 mmol) and the solution was stirred at 50 °C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.0 × 35 cm) using a 25–75% AcOEt in hexane. Evaporation of the first fraction gave to *N*-[6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purin-2-yl]-1-propoxyethylimine (**4**) as a caramel (94 mg, 18%). HR-MS *m/z*: 511.1468 (M^+, $C_{21}H_{26}ClN_5O_8$ requires 511.1470). UV λ_{max} (MeOH) nm: 288. ^{1}H-NMR ($CDCl_3$) δ: 8.15 (1H, s, H8), 6.25 (1H, d, *J* = 5.2 Hz, H1′), 5.87 (1H, t, *J* = 5.5 Hz, H2′), 5.57–5.59 (1H, m, H3′), 5.29–5.33 (1H, m, CH), 4.34–4.45 (3H, m, H4′, H5′), 2.15 (3H, s, Ac), 2.14 (3H, s, Ac), 2.08 (3H, s, Ac), 2.03 (3H, s, CH_3), 1.35 (3H, d, *J* = 6.3 Hz, CH_3), 1.34 (3H, d, *J* = 6.3 Hz, CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 170.2, 169.5 and 169.3 (C=O X 3), 165.8 (C=N), 161.5 (C6), 152.9 (C4),

151.8 (C2), 141.9 (C8), 128.2 (C5), 85.9 (C1′), 80.3 (C3′), 73.0 (C2′), 70.5 (C5′), 69.8 (CH), 62.9 (C4′), 21.68 and 21.72 (CH_3 X 2), 20.7, 20.5 and 20.4 ($\underline{C}H_3CO$ X 3), 18.2 ($\underline{C}H_3C{=}N$). From the second fraction a mixture of **3a** and triphenylphosphine oxide was obtained.

Conversion of 3a to 5a. To a solution of **3a** (512 mg, 1 mmol) in methanol (50 ml) was added 2-mercaptoethanol (0.3 ml, 4.3 mmol) and 28% sodium methoxide in methanol (0.58 ml, 3 mmol). The solution was stirred and heated at reflux temperature under a nitrogen atmosphere overnight. Then the solution was chromatographed over Amberlite IR-120 (NH_4^+ form) (10 ml) using a methanol, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.0×53 cm) using a 0–50% MeOH in AcOEt. First fraction was evaporated to give 2-isopropylamino-6-methoxy-9-(β-D-ribofuranosyl)purine (**6**) as a caramel (72 mg, 19%), HR-MS m/z: 381.1635 (M^+, $C_{16}H_{23}N_5O_6$ requires 381.1649), 249.1235 (M^+-ribofuranosyl, $C_{11}H_{15}N_5O_2$ requires 249.1226). UV λ_{max} (MeOH) nm: 259, λ_{max} (0.1 M NaOH) nm: 258. ^{1}H-NMR ($CDCl_3$) δ: 8.19 (1H, s, H8), 5.92 (1H, d, $J = 6.6$ Hz, H1′), 5.23 (1H, d, $J = 8.5$ Hz, 5′OH), 4.77–4.84 (3H, m, H2′, 2′OH, CH), 4.45–4.46 (1H, m, H3′), 4.31 (1H, br s, 3′OH), 4.29 (1H, q, $J = 1.9$ Hz, H4′), 4.17 (3H, s, OCH_3), 3.89–3.92 (1H, m, H5′a), 3.74–3.78 (1H, m, H5′b), 1.93 (3H, s, Ac), 1.25 (3H, d, $J = 6.6$ Hz, CH_3), 1.18 (3H, d, $J = 6.9$ Hz, CH_3). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 170.4 ($CH_3(\underline{C}O)$-N), 161.5 (C6), 153.3 (C2), 151.3 (C4), 143.4 (C8), 121.4 (C5), 91.1 (C1′), 87.1 (C3′), 74.3 (C2′), 71.5 (C5′), 62.7 (C4′), 54.9 (OCH_3), 48.5 (CH), 23.4 ($\underline{C}H_3(CO)$-N), 21.0 and 20.6 (CH_3 X 2). From the second fraction N^2-isopropylguanosine (**5a**) was obtained as white crystals (211 mg, 65%), mp 197.5–200 °C. *Anal* Calcd for $C_{13}H_{19}N_5O_5 \cdot 0.3\ H_2O$: C, 47.21; H, 5.97; N, 21.18. Found: C, 47.47; H, 5.95; N, 20.92. MS m/z: 193 (M^+-ribofuranosyl). UV λ_{max} (MeOH) nm: 256.5, λ_{max} (0.1 M NaOH) nm: 260.5. ^{1}H-NMR (DMSO-d_6)δ: 10.32 (1H, br s, N^1-H), 7.94 (1H, s, H8), 6.30 (1H, d, $J = 7.4$ Hz, N^2-H), 5.70 (1H, d, $J = 6.0$ Hz, H1′), 4.52 (1H, t, $J = 5.5$ Hz, H2′), 4.11 (1H, dd, $J = 3.6$, 4.9 Hz, H3′), 3.94–4.01 (1H, m, CH), 3.87 (1H, dd, $J = 4.4$, 8.0 Hz, H4′), 3.62 (1H, dd, $J = 4.4$, 11.8 Hz, H5′a), 3.51 (1H, dd, $J = 4.7$, 11.8 Hz, H5′b), 1.18 (6H, d, $J = 6.3$ Hz, CH_3 X2). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 156.5 (C6), 151.8 (C2), 150.1 (C4), 136.2 (C8), 116.7 (C5), 86.9 (C1′), 85.2 (C3′), 73.3 (C2′), 70.5 (C5′), 61.6 (C4′), 42.3 (CH), 22.4 and 22.3 (CH_3 X 2).

***N*^{2}-(1-Phenylethyl)guanosine (5b).** To a mixture of **2** (940 mg, 2 mmol) and (±)-1-phenyletanol (0.72 ml, 6 mmol) in dry THF (20 ml) was added tributylphosphine (1.5 ml, 6 mmol) and 1,1′-azobis(*N*, *N*-dimethylformamide) (1.03 g, 6 mmol) and the solution was stirred at 50 °C for 1 h. Then the solution was concentrated to a small volume, and chromatographed over a

column of silica gel G (2.2 × 50 cm) using a 25–75% AcOEt in benzene to give 6-chloro-2-[N^2-(1-phenylethyl)acetamido]-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**3b**) as a syrup. The product was dissolved in methanol (100 ml) and 2-mercaptoethanol (0.6 ml, 8.6 mmol) and 28% sodium methoxide in methanol (1.16 ml, 6 mmol) was added to the solution. The solution was refluxed under a nitrogen atmosphere overnight, then passed through a column of Amberlite IR-120 (NH_4^+ form) (20 ml). The eluant and washing was concentrated to a small volume and the residual solution was chromatographed over a column of silica gel G (2.6 × 24 cm) using a 0–50% EtOH in $CHCl_3$ to give N^2-(1-phenylethyl)guanosine as a mixture of diastereomers. Caramel (462 mg, 60%). MS *m/z*: 255 (M^+-ribofuranosyl). UV λ_{max} (MeOH) nm: 257, λ_{max} (0.1 M NaOH) nm: 262.5. ^{1}H-NMR (DMSO-d_6) δ: 10.36 (0.46 H, br s, A: N^1-H), 10.35 (0.54 H, br s, B: N^1-H), 7.92 (0.46 H, s, A: H8), 7.94 (0.54 H, s, B: H8), 7.24–7.41 (5H, m, Ph), 6.92–6.95 (1H, m, *N*-H), 5.66 (0.46 H, d, *J* = 5.8 Hz, A: H1′), 5.69 (0.54 H, d, *J* = 5.5 Hz, B: H1′), 5.01–5.16 (1H, m, CH), 4.40–4.48 (1H, m, H2′), 4.09–4.11 (1H, m, H3′), 3.84–3.88 (1H, m, H4′), 3.52–3.58 (1H, m, H5′a), 3.37–3.41 (1H, m, H5′b), 1.47 (1.38 H, d, *J* = 3.8, Hz, A: CH_3), 1.48 (1.62 H, d, *J* = 3.8, Hz, B: CH_3).

6-Chloro-2-(*N*-cyclohexyl)acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl) purine (3c). To a mixture of **2** (940 mg, 2 mmol) and cyclohexanol (0.63 ml, 6 mmol) in dry THF (20 ml) was added tributylphosphine (1.5 ml, 6 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide) (1.03 g, 6 mmol) and the solution was stirred at 50 °C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel (2.5 × 43 cm) using a 25–80% AcOEt in hexane. From the second fraction, **3c** was obtained as a caramel (147 mg, 13%), HR-MS *m/z*: 551.1807 (M^+, $C_{24}H_{30}ClN_5O_8$ requires 551.1783). UV λ_{max} (MeOH) nm: 269. ^{1}H-NMR ($CDCl_3$) δ: 8.33 (1H, s, H8), 6.19 (1H, d, *J* = 5.1 Hz, H1′), 5.83 (1H, dd, *J* = 5.1, 5.6 Hz, H2′), 5.51 (1H, dd, *J* = 5.1, 5.4 Hz, H3′), 4.32–4.55 (4H, m, H4′, H5′, one of cyclohexyl), 2.16 (3H, s, Ac), 2.14 (3H, s, Ac), 2.09 (3H, s, Ac), 2.08 (3H, s, Ac), 1.23–1.93 (10H, m, ten of cyclohexyl). Fourth fraction was evaporated to recover starting material in 52%. Also a small amount of the unknown products were obtained from the first and third fraction.

6-Chloro-2-(*N*-ethyl)acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3d). To a mixture of **2** (940 mg, 2 mmol) and ethanol (0.35 ml, 6 mmol) in dry THF (20 ml) was added tributylphosphine (1.5 ml, 6 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide) (1.03 g, 6 mmol) and the solution was stirred at 50 °C for 1 h, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.2 × 30 cm) using a 25–75% AcOEt in benzene to give a caramel (498 mg, 50%). HR-MS *m/z*: 497.1334 (M^+, $C_{20}H_{24}ClN_5O_8$ requires 497.1373). UV λ_{max} (MeOH)

nm: 288.5, 262. ^{1}H-NMR ($CDCl_3$) δ: 8.23 (1H, s, H8), 6.14 (1H, d, $J = 4.8$ Hz, H1′), 5.89 (1H, dd, $J = 4.8$, 5.5 Hz, H2′), 5.51 (1H, t, $J = 5.5$ Hz, H3′), 4.37–4.49 (3H, m, H4′, H5′), 4.16 (2H, q, $J = 7.0$ Hz, CH_2), 2.46 (3H, s, *N*-Ac), 2.16 (3H, s, Ac), 2.12 (3H, s, Ac), 2.10 (3H, s, Ac), 1.23 (3H, t, $J = 7.0$ Hz, CH_3).

6-Chloro-2-(*N*-propyl)acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3e). To a mixture of **2** (940 mg, 2 mmol) and *n*-propanol (0.45 ml, 6 mmol) in dry THF (20 ml) was added tributylphosphine (1.5 ml, 6 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide) (1.03 g, 6 mmol) and the solution was stirred at 50 °C for 1 h, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2.2×40 cm) using a 25–75% AcOEt in benzene to give a caramel (536 mg, 52%), HR-MS m/z: 511.1474 (M^+, $C_{21}H_{26}ClN_5O_8$ requires 511.1471). UV λ_{max} (MeOH) nm: 287.5, 263. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, s, H8), 6.15 (1H, d, $J = 4.8$ Hz, H1′), 5.86 (1H, dd, $J = 4.8$, 5.5 Hz, H2′), 5.50 (1H, t, $J = 5.5$ Hz, H3′), 4.38–4.51 (3H, m, H4′, H5′), 4.04–4.11 (2H, m, CH_2), 2.43 (3H, s, *N*-Ac), 2.16 (3H, s, Ac), 2.13 (3H, s, Ac), 2.09 (3H, s, Ac), 1.58–1.70 (2H, m, CH_2), 0.90 (3H, dd, $J = 7.3$, 7.7 Hz, CH_3).

6-Chloro-2-(*N*-butyl)acetamido-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (3f). To a mixture of **2** (470 mg, 1 mmol) and *n*-butanol (0.27 ml, 3 mmol) in dry THF (10 ml) was added tributylphosphine (0.75 ml, 3 mmol) and 1,1′-azobis(*N*,*N*-dimethylformamide) (0.52 g, 3 mmol) and the solution was stirred at 50 °C overnight, then concentrated to a small volume. The residual solution was chromatographed over a column of silica gel G (2×30 cm) using a 25–80% AcOEt in hexane to give a caramel (536 mg, 57%), HR-MS m/z: 525.1584 (M^+, $C_{22}H_{28}ClN_5O_8$ requires 525.1627). UV λ_{max} (MeOH) nm: 288, 263. ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, s, H8), 6.16 (1H, d, $J = 4.7$ Hz, H1′), 5.84 (1H, dd, $J = 4.9$, 5.5 Hz, H2′), 5.50 (1H, dd, $J = 5.2$, 5.5 Hz, H3′), 4.35–4.49 (3H, m, H4′, H5′), 4.11 (2H, t, $J = 7.4$ Hz, CH_2), 2.43 (3H, s, *N*-Ac), 2.16 (3H, s, Ac), 2.14 (3H, s, Ac), 2.09 (3H, s, Ac), 1.55–1.62 (2H, m, CH_2), 1.29–1.36 (2H, m, CH_2), 0.91 (3H, dd, $J = 7.4$, 7.1 Hz, CH_3).

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