A NEW METHOD FOR SYNTHESIS OF 2-ALKOXYADENOSINE ANALOGS

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Abstract – 6-Methylguanosine and 2-amino-6-chloropurine riboside derivative were treated with isoamyl nitrite in the presence of an appropriate alcohol to give the corresponding 2-alkoxy-6-methoxy (or chloro) purine riboside derivatives.

Adenosine derivatives comprising 2-alkoxyadenosine groups that have an alkoxy group at the 2-position of the purine moiety are known worldwide for their prominent bioactivity. For instance, 2-methoxyadenosine, commonly known as spongosine, was isolated from the Caribbean sponge, Cryptotethia crypta, in 1951, and it has been reported to possess antiviral activity and antiplatelet properties and furthermore has been used in pain therapy and in treating B-cell proliferative diseases. Additionally, many of the 2-alkoxyadenosine derivatives, including spongosine, are potent and selective agonists at the coronary artery A2 adenosine receptor. Therefore, because of the above-stated attributes, the de novo synthesis of 2-alkoxyadenosine analog(s) containing spongosine has been carried out by various research institutions.

Nair and co-workers developed a synthetic approach for generating 2-benzyloxyadenosine derivatives. In their approach, methylthiolation of the 2-position in 2-iodoadenosine derivatives occurs, and then oxidation with oxone gives a methylsulfonyl group, followed finally by benzyloxydation at the 2-position by using sodium benzyloxide. By applying this method, Ojha and co-workers have synthesized spongosine. A nucleophilic substitution approach was adopted by Savory and colleagues to synthesize spongosine, in which 6-chloro-2-nitropurine riboside was treated with sodium methoxide to give a 2,6-dimethoxy derivative, which then reacted with an ammonia solution. On the other hand, Adachi and co-workers reported that the 2-amino-6-chloropurine riboside derivative was converted to 6-chloro-2-hydroxypurine riboside, and the reaction of the hydroxyl group at the 2-position in the purine
skeleton with various alkyl iodides was carried out in the presence of cesium carbonate to give 2-alkoxy-6-chloropurine riboside, followed by amination at the 6-position by using a solution of saturated ammonia in ethanol that afforded 2-alkoxyadenosine derivatives.\textsuperscript{12} Ueeda \textit{et al.} obtained 2-alkoxyadenosine derivatives by the reaction of 2-chloroadenosine derivatives with lithium alkoxide, which was produced by alkyl alcohol treated with butyl lithium.\textsuperscript{8} Recently, Wannar and colleagues developed the effective synthetic approach for 2-methoxyadenosine, viz., spongosine, in which penta-acetylated adenosine was nitrated with tetrabutylammonium nitrite to give 2-nitroadenosine analog, followed by the methoxylation at the 2-position in the purine system with the use of potassium cyanide in methanol, providing the spongosine.\textsuperscript{13}

All of the abovementioned syntheses of 2-alkoxyadenosine introduce the primary or secondary alkoxy group into the 2-position of the purine nucleoside analogs, and there have been no reports, to date, concerning 2-tertiary alkoxy-substituted adenosine analogs. Moreover, past researches have shown that the synthesis of 2-alkoxyadenosine analogs requires multiple steps, or products with low yield are formed; hence, many synthetic issues remain to be improved. This background prompted us to explore the de novo synthesis of 2-alkoxyadenosine analogs, many of which result in physiologically active substances. Previously, the purin-2-ylcarboxylate was synthesized in our laboratory using the O\textsuperscript{6}-methylguanosine derivative treated with sodium nitrite or isoamyl nitrite in the presence of carboxylic acid.\textsuperscript{14,15} In addition, Huisgen \textit{et al.} reported that 2-amino-1-methylnaphthalene was treated with ethyl nitrite in the presence of ethanol or acetic acid solution to give the corresponding 2-ethoxy- (or 2-acetoxy-) 1-methylnaphthalene, respectively.\textsuperscript{16} Herein, by applying these methods to the alkoxylation at the 2-position in purine nucleoside analogs, we develop a one-pot de novo synthesis for the conversion of the 2-amino group in 2-amino-6-chloropurine riboside (2) or 2-amino-6-methoxypurine riboside (3) to the 2-alkoxy group, \textit{via} nonaqueous diazotization–dediazoniation reactions. Furthermore, the synthetic process was exploited in the synthesis of 2-methoxyadenosine (spongosine), which was prepared in high yields and short steps.

**Conversion of 2-amino group into 2-alkoxy group**

As previously reported, 2-amino-6-chloropurine riboside (2) was synthesized from guanosine (1) in 4 steps, followed by conversion into 2-amino-6-methoxypurine riboside (3).\textsuperscript{15,17} First of all, compound 2 was treated with isoamyl nitrite in the presence of MeOH to give the corresponding derivatives 4a and 4b in 12% and 77% yields, respectively, and 5 in 3% yield as the by-product (Table 1, entry 1). The structures of 4a, 4b, and 5 were identified by HRMS (ESI) and \textsuperscript{1}H NMR spectra, in which the characteristic absorptions due to TBS, methoxy, and amino groups were observed. As a result, compound 4b was 5′-desilylated nucleoside; in addition, the by-product 5 has been proven to be the 5′-desilylated
product of starting material (2). As to the selective elimination of 5'-TBS group, de Fallois et al. have reported that treating 3',5'-disilyl-arabinouridine under basic conditions such as KOH/EtOH or K₂CO₃/MeOH results in selective 5'-desilylation.¹⁸ Whereas also in our reactive conditions, the reaction of nucleoside analogs, which have an amino group at the 2-position, with isoamyl nitrite to give the diazonium salts and the corresponding isoamyl alkoxide ions. It appears that theses alkoxide ions allow the nucleophilic attack of the silicon of the 5'-TBS group which is the least sterically hindered TBS group.

![Scheme 1. Synthesis of 6-subsituted 2-aminopurine nucleosides (2 and 3)](image)

In a similar way in ethanol, compound 2 was treated with isoamyl nitrite to give the 2-ethoxylated compound 4c in 55% yield and its 5'-desilylated derivative 4d in 43% yield. Interestingly, the 5'-desilylated compound is hardly obtained using n-butanol, whereas the trisilylated derivative 4e is obtained in 73% yield. In isopropanol, compound 4f formed with 74% yield, and in addition, 1,3-dihydro-2H-purin-2-one analog (6) is the by-product with 14% yield (Table 1, entry 4). In t-butanol, production of by-product 6 increases to 37% yield (Table 1, entry 5). The decreased yield of the 2-alkoxy analog and the increased yield of the by-product 6 in the presence of bulky alcohols can be explained as follows: the formation of the intermediate diazonium salt results in water production. When secondary or tertiary alcohols are used, it is expected that some water molecules react at the 2-position of nucleoside analogs. The reaction with bulky alcohols has to overcome large steric hindrances, while water molecules are less sterically hindered compared with bulky alcohols such as isopropanol, n-butanol and t-butanol; therefore increasing yield of xanthosine derivatives. The 6-methoxy analog (3) showed the same trend as the 6-chloro nucleoside (2); in methanol, the 5'-desilylated substance 7b was obtained as the main product in 70% yield while also giving the trisilylated derivative 7a in 29% yield (Table 2, entry 1). In ethanol, 7c was acquired with 84% yield (Table 2, entry 2), and in the presence of other alcohols, except for methanol, compound 3 was alkoxylated to give the corresponding compound without undergoing 5'-desilylation. In t-butanol, corresponding 2-t-butoxy derivative (7f) was obtained in 43% yield (Table 2, entry 5). To the best of our knowledge, this is the first example in which 2-t-butoxylated nucleosides (4g, 7f) were first synthesized by the introduction of t-butoxy group into the nucleoside analogs at the 2-position in the purine skeleton.
Table 1. 2-Alkoxylation of 2 with alcohols

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>product(s) and yield(s)</th>
<th>reaction time</th>
<th>by-product and yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R₁ = Me</td>
<td>4a (R₂ = TBS): 12% and 4b (R₂ = H): 77%</td>
<td>1 day</td>
<td>5: 3%</td>
</tr>
<tr>
<td>2</td>
<td>R₁ = Et</td>
<td>4c (R₂ = TBS): 55% and 4d (R₂ = H): 43%</td>
<td>1 day</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R₁ = n-Bu</td>
<td>4e (R₂ = H): 73%</td>
<td>1 day</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>R₁ = i-Pr</td>
<td>4f (R₂ = TBS): 74%</td>
<td>1 day</td>
<td>6: 14%</td>
</tr>
<tr>
<td>5</td>
<td>R₁ = t-Bu</td>
<td>4g (R₂ = TBS): 37%</td>
<td>2.5 h</td>
<td>6: 37%</td>
</tr>
</tbody>
</table>

Table 2. 2-Alkoxylation of 3 with alcohols

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>product(s) and yield(s)</th>
<th>reaction time</th>
<th>by-product and yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R₁ = Me</td>
<td>7a (R₂ = TBS): 29% and 7b (R₂ = H): 70%</td>
<td>2 days</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>R₁ = Et</td>
<td>7c (R₂ = TBS): 84%</td>
<td>2 days</td>
<td>8: 15%</td>
</tr>
<tr>
<td>3</td>
<td>R₁ = n-Bu</td>
<td>7d (R₂ = TBS): 67%</td>
<td>3 days</td>
<td>8: 21%</td>
</tr>
<tr>
<td>4</td>
<td>R₁ = i-Pr</td>
<td>7e (R₂ = TBS): 63%</td>
<td>5 days</td>
<td>8: 18% and 3 (SM rec.):15%</td>
</tr>
<tr>
<td>5</td>
<td>R₁ = t-Bu</td>
<td>7f (R₂ = TBS): 43%</td>
<td>4 days</td>
<td>8: 36%</td>
</tr>
</tbody>
</table>

Discussion of the reaction mechanism for 2-alkoxylation

The reaction mechanism to obtain the 2-alkoxy (4a–g, 7a–f) and xanthosine analogs (6, 8) proceeds in the following three manners (Chart 1, Path 1-3): in the presence of isomyl nitrite, compound 2 or 3 underwent substitution as in the case for the Sandmeyer reaction, to give the corresponding diazonium salt (Ia) as the reaction intermediate (Chart 1). Intermediate Ia was converted to Ib by resonance-stabilized electron delocalization. The diazo group at the 2-position of the compound Ia undergoes nucleophilic attack by water, alcohol or alkoxide ions which were generated by alcohol (Path
Another possible mechanism for substitution involves unimolecular thermal decomposition of the diazonium ion, followed by capture of the resulting purinyl cation \( \text{Ic} \) by the nucleophile (Path 2). The alkoxylation of compound 3 series have a slower reaction rate (Table 2, 2–5 days) in comparison with compound 2 series (Table 1, 2.5 h ~ 1 day). The difference of the reaction rate among compounds (2 and 3 series) can be explained as follows: In the 6-methoxylated compound 3, the reactive intermediate \( \text{Ic} \) (X = OMe) may be more stabilized by the resonance effect due to electron-releasing nature than \( \text{Ic} \) (X = Cl). Hence, reaction barriers for resonance-stabilized purinyl carbocation \( \text{Ic} \) (X = OMe) was affected by the thermodynamic changes, however, additional contributions to the barriers arose from increased Marcus intrinsic barriers for reaction resulting mainly from the breaking of partial \( \pi \)-bonds to the carbocationic carbon atom at the transition state.\(^{20}\) On the other hand, Rayat et al. suggested that the reaction mechanism proceeded via a cyanoimine intermediate, because oxanosine as well as xanthosine were obtained when guanosine was treated with sodium nitrite solution, and their theory was based on a variety of experimental results with the use of the stable isotope compound, \( \text{H}_2\text{H}^{18}\text{O} \).\(^{21}\) Hence, it appears that \( \text{Ia} \) was converted to the nitrogen eliminated-intermediate \( \text{Ic} \), followed by the production of cyanoimine intermediate \( \text{Id} \) (Path 3). Taken together, alcohol or alkoxide ions were obtained in the 2-alkoxylated derivatives (4 and 7 series) by nucleophilic substitution reactions of intermediates \( \text{Ia} \), \( \text{Ib} \), and (or) \( \text{Id} \). When the intermediates react with water molecules, compounds 6 and 8 were obtained.

**Chart 1.** Possible reaction mechanism for the formation of 4 and 7 series along with by-products 6 and 8

**De novo synthesis of spongosine**

First, compound 4b or 7b was treated with tetrabutylammonium fluoride to give the desilylated product 9a or 9b, respectively. The next step involving the ammonolysis reaction in a sealed tube in the presence
of methanol produced the target material 2-methoxyadenosine, namely, spongosine (10) in 42% yield, while for 2,6-dimethoxylated compound 9b, the resulting spongosine (10) was obtained in 70% yield according to Savory’s method using methanolic ammonia solution.11

\[ \text{Reagents and conditions: i, TBAF in THF, rt, 40 min; ii, liq. NH}_3, \text{MeOH, 120 }^\circ\text{C, 1 d} \]

Scheme 2. Synthesis of 2-methoxyadenosine (spongosine: 10)

CONCLUSION

We developed a method for converting the 2-amino group bound to purine nucleoside analogs into the corresponding 2-alkoxylated compounds, in only one step. Our method involves a replacement reaction under mild conditions, compared with previous reports in which the 2-amino group was replaced by a 2-halogeno group, followed by nucleophilic substitution of the halogeno group with the alkoxide. It is also noteworthy that, to the best of our knowledge, this is the first reported synthesis of 2-tertiary alkoxylation to obtain the corresponding 2-tertiarybutoxy analogs. By using long-chain alcohols, the 2-alkoxylation reaction tends to be less reactive and by-product can be obtained in moderate yield. Further research is necessary on the effective synthesis of 2-alkoxyladesnosine analogs.

EXPERIMENTAL

Instrumentation

$^1$H NMR spectra were taken with a Ultrashield$^{\text{TM}}$ 400 Plus FT NMR System (BRUKER). Chemical shifts and coupling constants ($J$) were given in $\delta$ and Hz, respectively. High-resolution mass spectrometry was performed on a APEX IV mass spectrometer (BRUKER) with electrospray ionization mass spectroscopy (ESI-MS).

6-Chloro-2-methoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-$\beta$-D-ribofuranosyl)-9H-purine (4c: $R_1 = \text{TBS}$) and 6-chloro-2-methoxy-9-(2,3-bis-O-tert-butyldimethylsilyl-$\beta$-D-ribofuranosyl)-9H-purine (4d: $R_1 = \text{H}$)

Compound 2 (161.1 mg, 0.25 mmol) was dissolved in dry MeOH (4.0 mL), and isoamyl nitrite (146.4 mg,
1.25 mmol) was added to the solution at 0 °C, and then stirred for 1 day at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give oil (4a: 19.8 mg, 0.03 mmol, 12%, 4b: 101.9 mg, 0.19 mmol, 77% and 5: 3.9 mg, 3%). 4a: R1 = TBS: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.25 (1 H, s, H-8), 5.95 (1 H, d, $J = 5.2$, H-1’), 4.40 (1 H, m, H-2’), 4.19 (1 H, m, H-3’), 4.03 (1 H, m, H-4’), 3.95 (3 H, s, 2-OMe), 3.88 (1 H, dd, $J = 11.2$ and 3.2, H-5’a), 3.69 (1 H, dd, $J = 11.2$ and 2.4, H-5’b), 0.86 (9 H, s, TBS), 0.83 (9 H, s, TBS), 0.70 (9 H, s, TBS), 0.05 (3 H, s, TBS), 0.04 (3 H, s, TBS), 0.01 (3 H, s, TBS), 0.00 (3 H, s, TBS), -0.13 (3 H, s, TBS), -0.33 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{29}$H$_{56}$ClN$_4$O$_5$Si$_3$ [M+H]$^+$: 659.32415. Found 659.32380.

4b: R1 = H: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.03 (1 H, s, H-8), 5.80 (1 H, d, $J = 7.6$, H-1’), 5.41 (1 H, dd, $J = 11.2$ and 2.4, 5’-OH), 4.93 (1 H, dd, $J = 7.6$ and 4.8, H-2’), 4.33 (1 H, m, H-3’), 4.16 (1 H, m, H-4’), 4.09 (3 H, s, 2-OMe), 3.97 (1 H, m, H-5’a), 3.73 (1 H, m, H-5’b), 0.95 (9 H, s, TBS), 0.77 (9 H, s, TBS), 0.13 (3 H, s, TBS), 0.12 (3 H, s, TBS), -0.10 (3 H, s, TBS), -0.55 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{23}$H$_{41}$ClN$_4$NaO$_5$Si$_2$ [M+Na]$^+$: 567.21962. Found 567.21509.

5: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 7.81 (1 H, s, H-8), 6.02 (1 H, d, $J = 11.2$, 5’-OH), 5.72 (1 H, d, $J = 8.0$, H-1’), 5.17 (2 H, s, 2-NH$_2$), 4.92 (1 H, dd, $J = 8.0$ and 4.8, H-1’), 4.30 (1 H, m, H-3’), 4.17 (1 H, m, H-4’), 3.93 (1 H, m, H-5’a), 3.70 (1 H, m, H-5’b), 0.95 (9 H, s, TBS), 0.78 (9 H, s, TBS), 0.14 (3 H, s, TBS), 0.12 (3 H, s, TBS), 0.10 (3 H, s, TBS), 0.52 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{22}$H$_{41}$ClN$_5$O$_4$Si$_2$ [M+H]$^+$: 530.23801. Found 530.23830.

6-Chloro-2-ethoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9$^H$-purine (4c: R$_1$ = TBS) and 6-chloro-2-ethoxy-9-(2,3-bis-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9$^H$-purine (4d: R$_1$ = H)

Compound 2 (161.1 mg, 0.25 mmol) was dissolved in dry EtOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 1 day at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give tri-TBS analogue (4c) as oil (91.8 mg, 0.14 mmol, 55%). Evaporation of the second fraction gave bis-TBS analogue (4d) as oil (60.6 mg, 0.11 mmol, 43%). 4c: R$_1$ = TBS: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.34 (1 H, s, H-8), 6.05 (1 H, d, $J = 5.6$, H-1’), 4.53 (1 H, m, H-2’), 4.42-4.52 (2 H, m, 2-OEt), 4.30 (1 H, m, H-3’), 4.13 (1 H, m, H-4’), 3.99 (1 H, dd, $J = 11.6$ and 3.6, H-5’a), 3.81 (1 H, dd, $J = 11.6$ and 2.4, H-5’b), 1.46 (3 H, t, $J = 6.8$, 2-OEt), 0.97 (9 H, s, TBS), 0.94 (9 H, s, TBS), 0.79 (9 H, s, TBS), 0.16 (3 H, s, TBS), 0.15 (3 H, s, TBS), 0.12 (3 H, s, TBS), 0.11 (3 H, s, TBS), -0.03 (3 H, s, TBS), -0.24 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{30}$H$_{57}$ClN$_4$NaO$_5$Si$_3$ [M+Na]$^+$: 695.32175. Found 695.32141. 4d: R$_1$ = H: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.03 (1 H, s, H-8), 5.79 (1 H, d, $J = 7.6$, H-1’), 4.93 (1 H, dd, $J = 7.6$ and 4.8, H-2’), 4.50 (2 H, q, $J = 7.2$, 2-OEt), 4.33 (1 H, m, H-3’), 4.16 (1 H, m, H-4’), 3.97 (1 H, dd, $J = 12.8$ and 1.6, H-5’a), 3.72 (1 H, m, H-5’b), 1.45 (3 H, t, $J = 7.2$, 2-OEt), 0.95 (9 H, s, TBS), 0.77 (9 H, s, TBS),
6-Chloro-2-butoxy-9-(2,3-bis-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (4e)

Compound 2 (161.1 mg, 0.25 mmol) was dissolved in dry n-BuOH (4.0 mL) and isooamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 1 day at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (33% AcOEt in hexane) to give oil (107.0 mg, 0.18 mmol, 73%). 1H NMR (400MHz, CDCl3): δ 7.98 (1 H, s, H-8), 5.78 (1 H, d, J = 7.6, H-1’), 4.96 (1 H, dd, J = 7.6 and 4.4, H-2’), 4.43 (2 H, m, 2-OBu), 4.30 (1 H, m, H-3’), 4.16 (1 H, m, H-4’), 3.72 (1 H, m, H-5’a), 1.81 (2 H, m, 2-OBu), 1.51 (2 H, m, 2-OBu), 0.98 (3 H, t, J = 7.6, 2-OBu), 0.96 (9 H, s, TBS), 0.76 (9 H, s, TBS), 0.12 (3 H, s, TBS), 0.11 (3 H, s, TBS), -0.10 (3 H, s, TBS); HRMS (ESI) Calcd for C_{26}H_{47}ClN_{4}NaO_{5}Si_{2} [M+Na]^+: 609.26657. Found 609.26495.

6-Chloro-2-isopropoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (4f) and 6-chloro-1,9-dihydro-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2H-purin-2-one (6)

Compound 2 (161.1 mg, 0.25 mmol) was dissolved in dry iPrOH (4.0 mL) and isooamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 1 day at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 4f as oil (126.5 mg, 0.19 mmol, 74%). Evaporation of second fraction gave 6 as oil (22.5 mg, 0.04 mmol, 14%). 4f: 1H NMR (400MHz, CDCl3): δ 8.31 (1 H, s, H-8), 6.06 (1 H, d, J = 5.6, H-1’), 5.34 (1 H, septet, J = 6.0, 2-O-iPr), 4.52 (1 H, m, H-2’), 4.29 (1 H, m, H-3’), 4.12 (1 H, m, H-4’), 3.97 (1 H, dd, J = 11.2 and 3.6, H-5’a), 3.80 (1 H, dd, J = 11.2 and 2.4, H-5’b), 1.41 (6 H, d, J = 6.4, 2-O-iPr), 0.98 (9 H, s, TBS), 0.94 (9 H, s, TBS), 0.80 (9 H, s, TBS), 0.16 (3 H, s, TBS), 0.15 (3 H, s, TBS), 0.11 (6 H, s, TBS), -0.22 (3 H, s, TBS), -0.27 (3 H, s, TBS); HRMS (ESI) Calcd for C_{31}H_{59}ClN_{4}NaO_{5}Si_{3} [M+Na]^+: 709.33740. Found 709.33540. 6: 1H NMR (400MHz, CDCl3): δ 8.34 (1 H, s, H-8), 6.07 (1 H, d, J = 6.0, H-1’), 4.56 (1 H, dd, J = 6.0 and 4.4, H-2’), 4.27 (1 H, dd, J = 4.4 and 2.8, H-3’), 4.14 (1 H, m, H-4’), 4.01 (1 H, dd, J = 11.6 and 3.2, H-5’a), 3.80 (1 H, dd, J = 11.6 and 2.4, H-5’b), 0.97 (9 H, s, TBS), 0.93 (9 H, s, TBS), 0.80 (9 H, s, TBS), 0.17 (3 H, s, TBS), 0.16 (3 H, s, TBS), 0.10 (6 H, s, TBS), -0.04 (3 H, s, TBS), -0.25 (3 H, s, TBS); HRMS (ESI) Calcd for C_{28}H_{53}ClN_{4}NaO_{5}Si_{3} [M+Na]^+: 667.29045. Found 667.28795.

2-tert-Butoxy-6-chloro-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (4g) and
6-chloro-1,9-dihydro-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2H-purin-2-one (6)

Compound 2 (161.1 mg, 0.25 mmol) was dissolved in dry t-BuOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 1 day at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 4g as oil (64.9 mg, 0.09 mmol, 37%). Evaporation of second fraction gave 6 as oil (59.7 mg, 0.09 mmol, 37%).

4g: 1H NMR (400MHz, CDCl3): δ 8.09 (1 H, s, H-8), 5.97 (1 H, d, J = 6.8, H-1’), 4.36 (1 H, dd, J = 6.8 and 4.4, H-2’), 4.27 (1 H, dd, J = 4.4 and 1.6, H-3’), 3.94 (1 H, m, H-4’), 3.76 (1 H, dd, J = 11.2 and 3.2, H-5’a), 3.64 (1 H, dd, J = 11.2 and 2.4, H-5’b), 1.51 (9 H, s, t-Bu), 0.82 (9 H, s, TBS), 0.80 (9 H, s, TBS), 0.79 (9 H, s, TBS), -0.04 (3 H, s, TBS), -0.00 (3 H, s, TBS), -0.47 (3 H, s, TBS); HRMS (ESI) Calcd for C32H61ClN4NaO5Si3 [M+Na]+: 723.35305. Found. 723.35348

2,6-Dimethoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7a) and
2,6-dimethoxy-9-(2,3-bis-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7b)

Compound 3 (160.0 mg, 0.25 mmol) was dissolved in dry MeOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 2 days at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 7a as oil (47.3 mg, 0.07 mmol, 29%). Evaporation of second fraction gave 7b as oil (94.6 mg, 0.18 mmol, 70%).

7a: 1H NMR (400MHz, CDCl3): δ 8.10 (1 H, s, H-8), 5.88 (1 H, d, J = 4.8, H-1’), 4.36 (1 H, m, H-2’), 4.17 (1 H, m, H-3’), 4.00 (3 H, s, OMe), 3.97 (1 H, m, H-4’), 3.87 (3 H, s, OMe), 3.86 (1 H, dd, J = 11.2 and 4.0, H-5’a), 3.67 (1 H, dd, J = 11.2 and 2.4, H-5’b), 0.81 (9 H, s, TBS), 0.78 (9 H, s, TBS), 0.67 (9 H, s, TBS), 0.00 (3 H, s, TBS), -0.02 (3 H, s, TBS), -0.04 (3 H, s, TBS), -0.06 (3 H, s, TBS), -0.17 (3 H, s, TBS), -0.31 (3 H, s, TBS); HRMS (ESI) Calcd for C30H58N4NaO6Si3 [M+Na]+: 677.35564. Found 677.35163.

7b: 1H NMR (400MHz, CDCl3): δ 7.76 (1 H, s, H-8), 5.64 (1 H, d, J = 7.6, H-1’), 4.85 (1 H, m, H-2’), 4.20 (1 H, m, H-3’), 4.07 (3 H, s, OMe), 4.02 (1 H, m, H-4’), 3.92 (3 H, s, OMe), 3.84 (1 H, dd, J = 12.8 and 2.0, H-5’a), 3.59 (1 H, dd, J = 12.8 and 1.2, H-5’b), 0.83 (9 H, s, TBS), 0.64 (9 H, s, TBS), 0.00 (3 H, s, TBS), -0.01 (3 H, s, TBS), -0.23 (3 H, s, TBS), -0.671 (3 H, s, TBS); HRMS (ESI) Calcd for C24H44N4NaO6Si2 [M+Na]+: 563.26916. Found 563.26782.

2-Ethoxy-6-methoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7c) and
1,9-dihydro-6-methoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-2H-purin-2-one (8)

Compound 3 (160.0 mg, 0.25 mmol) was dissolved in dry EtOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 2 days at 50 °C. The mixture was
evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 7c as oil (140.8 mg, 0.21 mmol, 84%). Evaporation of second fraction gave 8 as oil (24.0 mg, 0.04 mmol, 15%). 7c: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.06 (1 H, s, H-8), 5.87 (1 H, d, $J = 4.8$, H-1'), 4.39 (1 H, m, H-2'), 4.18 (1 H, m, H-3'), 4.02 (3 H, s, 6-OMe), 3.97 (1 H, m, H-4'), 3.86 (1 H, dd, $J = 11.2$ and 4.0, H-5'a), 3.68 (1 H, dd, $J = 11.2$ and 2.4, H-5'b), 1.32 (9 H, s, TBS), 0.82 (9 H, s, TBS), 0.79 (9 H, s, TBS), 0.01 (3 H, s, TBS), 0.00 (3 H, s, TBS), -0.03 (3 H, s, TBS), -0.05 (3 H, s, TBS), -0.16 (3 H, s, TBS), -0.32 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{31}$H$_{50}$N$_4$NaO$_6$Si$_3$ [M+Na]$^+$: 691.37129. Found 691.36924.

8: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 7.44 (1 H, s, H-8), 5.69 (1 H, d, $J = 7.6$, H-1'), 4.46 (1 H, dd, $J = 8.0$ and 4.8, H-2'), 4.05 (1 H, m, H-3'), 4.02 (3 H, s, 6-OMe), 3.93 (1 H, dd, $J = 11.6$ and 2.4, H-5'a), 3.77 (1 H, dd, $J = 11.6$ and 1.6, H-5'b), 0.91 (9 H, s, TBS), 0.85 (9 H, s, TBS), 0.66 (9 H, s, TBS), 0.18 (3 H, s, TBS), 0.12 (3 H, s, TBS), 0.09 (3 H, s, TBS), 0.00 (3 H, s, TBS), -0.16 (3 H, s, TBS), -0.50 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{29}$H$_{56}$N$_4$NaO$_6$Si$_3$ [M+Na]$^+$: 663.33999. Found 663.33790.

2-n-Butoxy-6-methoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7d)
Compound 3 (160.0 mg, 0.25 mmol) was dissolved in dry $n$-BuOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 3 days at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 7d as oil (116.6 mg, 0.17 mmol, 67%). Evaporation of second fraction gave 8 as oil (33.7 mg, 0.05 mmol, 21%). 7d: $^1$H NMR (400MHz, CDCl$_3$): $\delta$ 8.07 (1 H, s, H-8), 5.87 (1 H, d, $J = 4.8$, H-1'), 4.35 (1 H, m, H-2'), 4.17-4.27 (2 H, m, 2-OBu), 4.16 (1 H, m, H-3'), 4.00 (3 H, s, 6-OMe), 3.96 (1 H, m, H-4'), 3.85 (1 H, dd, $J = 11.2$ and 4.0, H-5'a), 3.65 (1 H, dd, $J = 11.2$ and 2.4, H-5'b), 1.63-1.72 (2 H, m, 2-OBu), 1.30-1.42 (2 H, m, 2-OBu), 1.83 (1 H, t, $J = 7.6$, 2-OBu), 0.83 (9 H, s, TBS), 0.77 (9 H, s, TBS), 0.67 (9 H, s, TBS), 0.00 (3 H, s, TBS), -0.01 (3 H, s, TBS), -0.04 (3 H, s, TBS), -0.06 (3 H, s, TBS), -0.17 (3 H, s, TBS), -0.32 (3 H, s, TBS); HRMS (ESI) Calcd for C$_{33}$H$_{64}$N$_4$NaO$_6$Si$_3$ [M+Na]$^+$: 719.40259. Found 719.40006.

2-Isopropoxy-6-methoxy-9-(2,3,5-tri-O-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7e)
Compound 2 (160.0 mg, 0.25 mmol) was dissolved in dry $^1$PrOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 5 days at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 7e as oil (116.6 mg, 0.17 mmol, 67%). Evaporation of second fraction gave 8 as oil (28.8 mg, 0.05 mmol, 18%), and third fraction was SM rec. (2) (24.0 mg, 0.04 mmol, 15%). 7e: $^1$H NMR (400MHz,
CDCl₃): δ 8.04 (1 H, s, H-8), 5.88 (1 H, d, J = 5.2, H-1’), 5.21 (1 H, septet, J 6.4, 2-O-Pr), 4.37 (1 H, m, H-2’), 4.17 (1 H, m, H-3’), 4.01 (3 H, s, 6-OMe), 3.98 (1 H, d, J = 11.2 and 3.6, H-5’a), 3.66 (1 H, d, J = 11.2 and 2.8, H-5’b), 1.28 (6 H, d, J = 6.4, 2-O-pr), 0.82 (9 H, s, TBS), 0.80 (9 H, s, TBS), 0.63 (9 H, s, TBS), 0.00 (3 H, s, TBS), -0.20 (3 H, s, TBS), -0.42 (3 H, s, TBS); HRMS (ESI) Calcd for C₃₂H₆₂N₄NaO₆Si₃ [M+Na]+: 705.38694. Found 705.38177.

2-tert-Butoxy-6-methoxy-9-(2,3,5-tri-tert-butyldimethylsilyl-β-D-ribofuranosyl)-9H-purine (7f)
Compound 2 (160.0 mg, 0.25 mmol) was dissolved in dry t-BuOH (4.0 mL) and isoamyl nitrite (146.4 mg, 1.25 mmol) was added to the solution at 0 °C, and then stirred for 4 days at 50 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give 7f as oil (74.3 mg, 0.11 mmol, 43%). Evaporation of second fraction gave 8 as oil (58.3 mg, 0.09 mmol, 36%). 7e: ¹H NMR (400MHz, CDCl₃): δ 7.95 (1 H, s, H-8), 5.93 (1 H, d, J = 6.8, H-1’), 4.39 (1 H, dd, J = 6.4 and 4.4, H-2’), 4.14 (1 H, dd, J = 4.4 and 2.0, H-3’), 4.02 (1 H, s, 6-OMe), 3.97 (1 H, m, H-4’), 3.79 (1 H, dd, J = 11.2 and 3.6, H-5’a), 3.66 (1 H, dd, J = 11.2 and 2.8, H-5’b), 1.55 (9 H, s, 2-O-tBu’), 0.82 (9 H, s, TBS), 0.82 (9 H, s, TBS), 0.00 (3 H, s, TBS), -0.20 (3 H, s, TBS), -0.42 (3 H, s, TBS); HRMS (ESI) Calcd for C₃₃H₆₄N₄NaO₆Si₃ [M+Na]+: 719.40256. Found 719.40203.

6-Chloro-2-methoxy-9-(β-D-ribofuranosyl)-9H-purine (9a)
Compound 4b (208.6 mg, 0.38 mmol) was dissolved in THF (5.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (3.0 mL) was added to the solution, and then stirred for 20 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (17% MeOH in CH₂Cl₂) to give crystals 9a (127.4 mg, 0.38 mmol, 100%). ¹H NMR (400MHz, CDCl₃): δ 8.21 (1 H, s, H-8), 5.96 (1 H, d, J = 6.4, H-1’), 4.89 (1 H, dd, J = 6.4 and 5.2, H-2’), 4.52 (1 H, dd, J = 5.2 and 2.4, H-3’), 4.29 (1 H, m, H-4’), 4.03 (3 H, s, 2-OMe), 3.95 (1 H, dd, J = 12.8 and 2.0, H-5’a), 3.81 (1 H, dd, J = 12.8 and 1.6, H-5’b); HRMS (ESI) Calcd for C₁₁H₁₃ClN₄NaO₅ [M+Na]+: 339.04667. Found 339.04409.

2,6-Dimethoxy-9-(β-D-ribofuranosyl)-9H-purine (9b)
Compound 7b (166.8 mg, 0.31 mmol) was dissolved in THF (3.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (1.0 mL) was added to the solution, and then stirred for 40 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (14% MeOH in CH₂Cl₂) to give crystals 9b (47.1 mg, 0.12 mmol, 100%). ¹H NMR (400MHz, MeOH-d₄): δ 8.25 (1 H, s,

H-8), 5.94 (1 H, d, J = 5.6, H-1’), 4.75 (1 H, m, H-2’), 4.38 (1 H, m, H-3’), 4.13 (1 H, m, H-4’), 4.10 (3 H, s, OMe), 3.99 (3 H, s, OMe), 3.89 (1 H, dd, J = 12.8 and 2.8, H-5’a), 3.77 (1 H, dd, J = 12.8 and 3.2, H-5’b); HRMS (ESI) Calcd for C_{12}H_{16}N_{4}NaO_{6} [M+Na]^+: 335.09621. Found 335.09400.

2-Methoxyadenosine (Spongosine: 9)
Method A) Compound 9a (120.0 mg, 0.38 mmol) was dissolved in NH\textsubscript{3} (2.0 mL)/MeOH (5.0 mL), and then sealed and stirred for 1 d at 100 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% MeOH in CHCl\textsubscript{3}) to give crystals (47.3 mg, 0.16 mmol, 42%).
Method B) Compound 9b (624.5 mg, 2.00 mmol) was dissolved in NH\textsubscript{3} (5.0 mL)/MeOH (12.0 mL), and then sealed and stirred for 1 d at 120 °C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (17% MeOH in CHCl\textsubscript{3}) to give crystals (416.0 mg, 1.40 mmol, 70%).

\textsuperscript{1}H NMR (400MHz, DMSO-\textit{d}6): \(\delta\) 7.90 (1 H, s, H-8), 7.09 (2 H, brs, NH\textsubscript{2}), 5.54 (1 H, d, J = 6.4, H-1’), 5.16 (1 H, d, J = 6.4, OH), 4.89-4.98 (2 H, m, OH \times 2), 4.38 (1 H, m, H-2’), 3.90 (1 H, m, H-3’), 3.68 (1 H, m, H-4’), 3.58 (3 H, s, 2-OMe), 3.41 (1 H, m, H-5’a), 3.29 (1 H, m, H-5’b); HRMS (ESI) Calcd for C\textsubscript{11}H\textsubscript{15}N\textsubscript{5}NaO\textsubscript{5} [M+Na]^+: 320.09654. Found 320.09424.

REFERENCES


