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A NEW METHOD FOR SYNTHESIS OF 2-ALKOXYADENOSINE ANALOGS

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Abstract – O^6-Methylguanosine and 2-amino-6-chloropurine riboside derivative were treated with isoamylnitrite 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,[1] and it has been reported to possess antiviral activity[2] and antiplatelet properties[3] and furthermore has been used in pain therapy[4] and in treating B-cell proliferative diseases.[5] Additionally, many of the 2-alkoxyadenosine derivatives, including spongosine, are potent and selective agonists at the coronary artery A_2 adenosine receptor.[6-8] 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.[9,10] By applying this method, Ojha and co-workers have synthesized spongosine.[3] 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.[11] 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

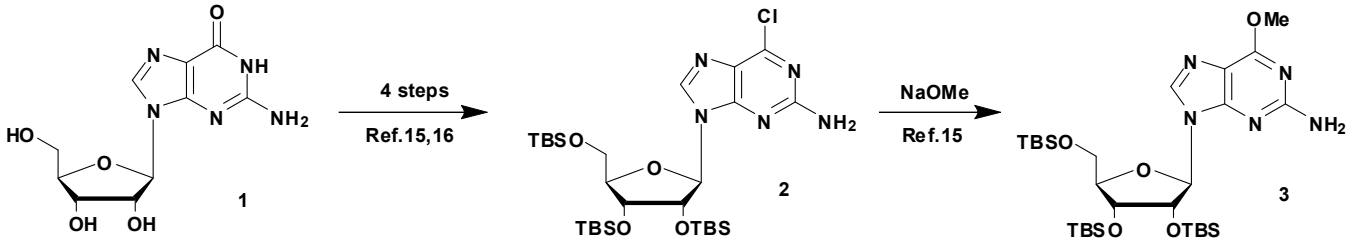
skeleton with various alkyl iodides was carried out in the presence of cesium carbonate to give 2-alkoxy-6-chloropurine riboside, followed by amination at the 6-position by using a solution of saturated ammonia in ethanol that afforded 2-alkoxyadenosine derivatives.[12] Ueeda *et al*. obtained 2-alkoxyadenosine derivatives by the reaction of 2-chloroadesnosine derivatives with lithium alkoxide, which was produced by alkyl alcohol treated with butyl lithium.[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.[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^6-methylguanosine derivative treated with sodium nitrite or isoamyl nitrite in the presence of carboxylic acid.[14,15] In addition, Huisgen *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.[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, *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**).[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 ^{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

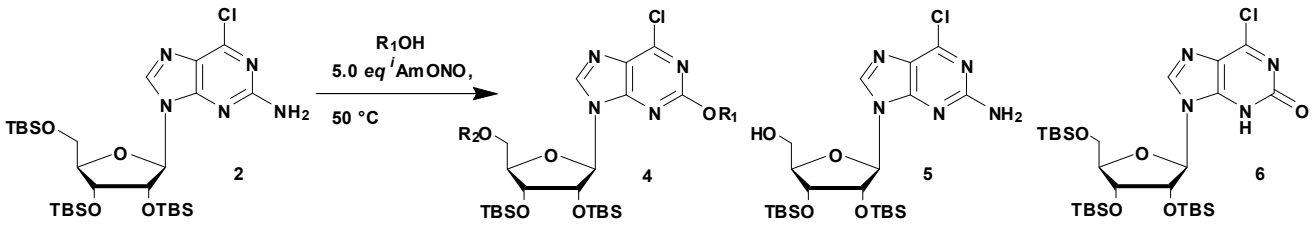
product of starting material (**2**). As to the selective elimination of 5'-TBS group, de Fallois *et al*. have reported that treating 3',5'-disilyl-arabinouridine under basic conditions such as KOH/EtOH or K_2CO_3/MeOH results in selective 5'-desilylation.[18] Whereas also in our reactive conditions, 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**)

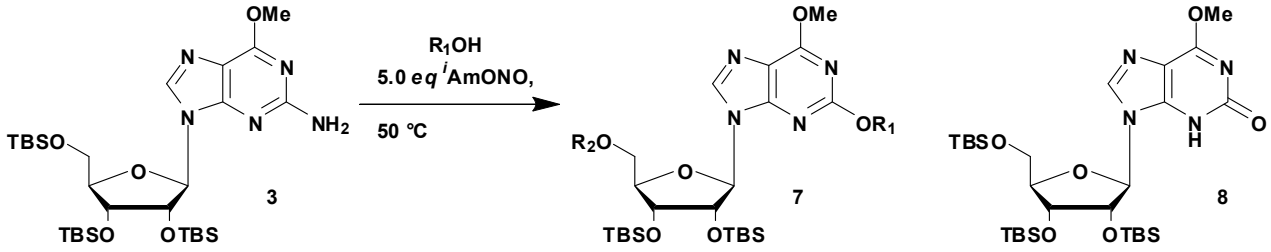
In a similar way in ethanol, compound **2** was treated with isoamyl nitrite to give the 2-ethoxylated compound **4c** in 55% yield and its 5′-desilylated derivative **4d** in 43% yield. Interestingly, the 5′-desilylated compound is hardly obtained using *n*-butanol, whereas the trisilylated derivative **4e** is obtained in 73% yield. In isopropanol, compound **4f** formed with 74% yield, and in addition, 1,3-dihydro-2*H*-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 increaseing 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 intoroduction of *t*-butoxy group into the nucleoside analogs at the 2-position in the purine skeleton.

Table 1. 2-Alkoxylation of **2** with alcohols



entry	solvent	product(s) and yield(s)	reaction time	by-product and yield
1	R_1 = Me	4a (R_2 = TBS): 12 % and 4b (R_2 = H): 77%	1 day	5: 3%
2	R_1 = Et	4c (R_2 = TBS): 55% and 4d (R_2 = H): 43%	1 day	
3	R_1 = *n*-Bu	4e (R_2 = H): 73%	1 day	
4	R_1 = *i*-Pr	4f (R_2 = TBS): 74%	1 day	6: 14%
5	R_1 = *t*-Bu	4g (R_2 = TBS): 37%	2.5 h	6: 37%

Table 2. 2-Alkoxylation of **3** with alcohols



entry	solvent	product(s) and yield(s)	reaction time	by-product and yield
1	R_1 = Me	7a (R_2 = TBS): 29% and 7b (R_2 = H): 70%	2 days	
2	R_1 = Et	7c (R_2 = TBS): 84%	2 days	8: 15%
3	R_1 = *n*-Bu	7d (R_2 = TBS): 67%	3 days	8: 21%
4	R_1 = *i*-Pr	7e (R_2 = TBS): 63%	5 days	8: 18% and 3 (SM rec.):15%
5	R_1 = *t*-Bu	7f (R_2 = TBS): 43%	4 days	8: 36%

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);[14,15] in the presence of isoamyl 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).[19] 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

1). Another possible mechanism for substitution involves unimolecular thermal decomposition of the diazonium ion, followed by capture of the resulting purinyl cation **Ic** by the nucleophile (Path 2). The alkoxylated reaction 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 **Ic** (X = OMe) may be more stabilized by the resonance effect due to electron-releasing nature than **Ic** (X = Cl). Hence, reaction barriers for resonance-stabilized purinyl carbocation **Ic** (X = OMe) was affected by the thermodynamic changes, however, additional contributions to the barriers arised from increased Marcus intrinsic barriers for reaction resulting mainly from the breaking of partial π-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, $H_2{}^{18}O$.[21] Hence, it appears that **Ia** was converted to the nitrogen eliminated-intermediate **Ic**, followed by the production of cyanoimine intermediate **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 **Ia**, **Ib**, and (or) **Id**. When the intermediates react with water molecules, compounds **6** and **8** were obtained.

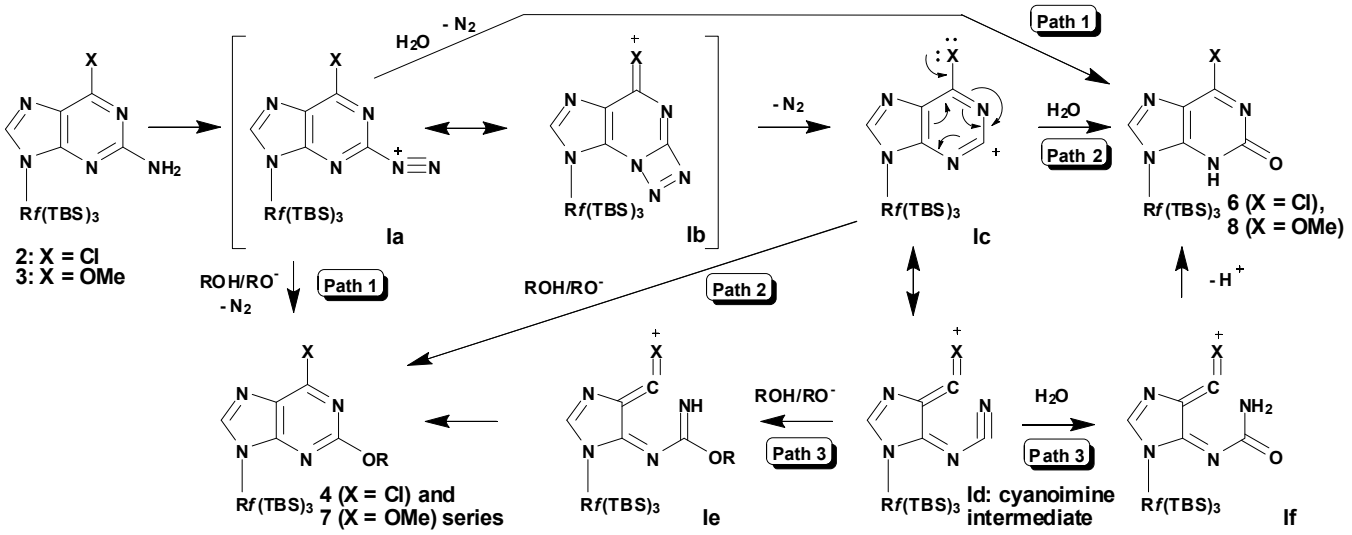
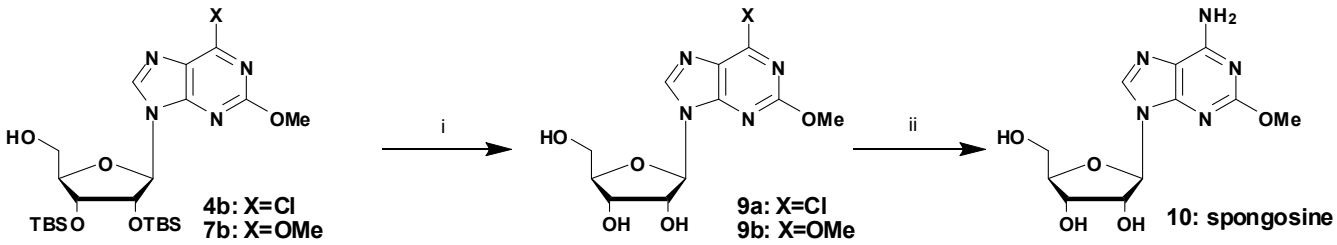


Chart 1. Possible reaction mechanism for the formation of **4** and **7**series along with by-products **6** and **8**

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]



Reagents and conditions: i, TBAF in THF, rt, 40 min; ii, liq. NH_3, MeOH, 120 °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^{TM} 400 Plus FT NMR System (BRUKER). Chemical shifts and coupling constants (*J*) were given in δ 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*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (4c: R_1 = TBS) and 6-chloro-2-methoxy-9-(2,3-bis-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (4d: R_1 = 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: R_1 = TBS:** ^{1}H NMR (400MHz, $CDCl_3$): δ 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 $C_{29}H_{56}ClN_4O_5Si_3$ $[M+H]^+$: 659.32415. Found 659.32380. **4b: R_1 = H:** ^{1}H NMR (400MHz, $CDCl_3$): δ 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 $C_{23}H_{41}ClN_4NaO_5Si_2$ $[M+Na]^+$: 567.21962. Found 567.21509. **5:** ^{1}H NMR (400MHz, $CDCl_3$): δ 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-2'), 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 $C_{22}H_{41}ClN_5O_4Si_2$ $[M+H]^+$: 530.23801. Found 530.23830.

6-Chloro-2-ethoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (4c: R_1 = TBS) and 6-chloro-2-ethoxy-9-(2,3-bis-*O*-*tert*-butyldimetylsilyl-*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, $CDCl_3$): δ 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 $C_{30}H_{57}ClN_4NaO_5Si_3$ $[M+Na]^+$: 695.32175. Found 695.32141. **4d: R_1 = H:** ^{1}H NMR (400MHz, $CDCl_3$): δ 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),

0.17 (3 H, s, TBS), 0.16 (3 H, s, TBS), -0.07 (3 H, s, TBS), -0.52 (3 H, s, TBS); HRMS (ESI) Calcd for $C_{24}H_{43}ClN_4NaO_5Si_2$ $[M+Na]^+$: 581.23527. Found 581.23015.

6-Chloro-2-butoxy-9-(2,3-bis-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (4e)

Compound **2** (161.1 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 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%). ^{1}H NMR (400MHz, $CDCl_3$): 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'), 3.95 (1 H, m, H-5'a), 3.72 (1 H, m, H-5'b), 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), -0.55 (3 H, s, TBS); HRMS (ESI) Calcd for $C_{26}H_{47}ClN_4NaO_5Si_2$ $[M+Na]^+$: 609.26657. Found 609.26495.

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

Compound **2** (161.1 mg, 0.25 mmol) was dissolved in dry ^{i}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 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:** ^{1}H NMR (400MHz, $CDCl_3$): 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-^{i}Pr), 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-^{i}Pr), 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 $C_{31}H_{59}ClN_4NaO_5Si_3$ $[M+Na]^+$: 709.33740. Found 709.33540. **6:** ^{1}H NMR (400MHz, $CDCl_3$): 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 $C_{28}H_{53}ClN_4NaO_5Si_3$ $[M+Na]^+$: 667.29045. Found 667.28795.

2-*tert*-Butoxy-6-chloro-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (4g) and

6-chloro-1,9-dihydro-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-2*H*-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:** ^{1}H NMR (400MHz, $CDCl_3$): 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), 0.57 (9 H, s, TBS), 0.01 (3 H, s, TBS), 0.00 (3 H, s, TBS), -0.04 (6 H, s, TBS), -0.23 (3 H, s, TBS), -0.47 (3 H, s, TBS); HRMS (ESI) Calcd for $C_{32}H_{61}ClN_4NaO_5Si_3$ $[M+Na]^+$: 723.35305. Found. 723.35348

2,6-Dimethoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (7a) and 2,6-dimethoxy-9-(2,3-bis-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-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:** ^{1}H NMR (400MHz, $CDCl_3$): 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 $C_{30}H_{58}N_4NaO_6Si_3$ $[M+Na]^+$: 677.35564. Found 677.35163. **7b:** ^{1}H NMR (400MHz, $CDCl_3$): δ 7.76 (1 H, s, H-8), 5.64 (1 H, d, *J* = 7.6, H-1'), 4.85 (1 H, dd, *J* = 7.6 and 4.8, 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 $C_{24}H_{44}N_4NaO_6Si_2$ $[M+Na]^+$: 563.26916. Found 563.26782.

2-Ethoxy-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (7c) and 1,9-dihydro-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-2*H*-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, $CDCl_3$): δ 8.06 (1 H, s, H-8), 5.87 (1 H, d, *J* = 4.8, H-1'), 4.39 (1 H, m, H-2'), 4.25-4.35 (2 H, m, 2-OEt), 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 $C_{31}H_{50}N_4NaO_6Si_3$ $[M+Na]^+$: 691.37129. Found 691.36924. **8:** ^{1}H NMR (400MHz, $CDCl_3$): δ 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'), 4.03 (1 H, m, H-4'), 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 $C_{29}H_{56}N_4NaO_6Si_3$ $[M+Na]^+$: 663.33999. Found 663.33790.

2-*n*-Butoxy-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-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, $CDCl_3$): δ 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 $C_{33}H_{64}N_4NaO_6Si_3$ $[M+Na]^+$: 719.40259. Found 719.40006.

2-Isopropoxy-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-purine (7e)

Compound **2** (160.0 mg, 0.25 mmol) was dissolved in dry ^{i}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,

$CDCl_3$): 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-^{i}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, m, H-4'), 3.84 (1 H, dd, *J* = 11.6 and 4.0, H-5'a), 3.67 (1 H, dd, *J* = 11.6 and 2.4, H-5'b), 1.28 (6 H, d, *J* = 6.4, 2-O-^{i}Pr), 0.82 (9 H, s, TBS), 0.80 (9 H, s, TBS), 0.67 (9 H, s, TBS), 0.01 (3 H, s, TBS), 0.00 (3 H, s, TBS), -0.03 (3 H, s, TBS), -0.04 (3 H, s, TBS), -0.19 (3 H, s, TBS), -0.32 (3 H, s, TBS); HRMS (ESI) Calcd for $C_{32}H_{62}N_4NaO_6Si_3$ $[M+Na]^+$: 705.38694. Found 705.38177.

2-*tert*-Butoxy-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimetylsilyl-*β*-D-ribofuranosyl)-9*H*-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 (74.3mg, 0.11 mmol, 43%). Evaporation of second fraction gave **8** as oil (58.3 mg, 0.09 mmol, 36%). **7e:** ^{1}H NMR (400MHz, $CDCl_3$): 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-*t*Bu^i), 0.82 (9 H, s, TBS), 0.82 (9 H, s, TBS), 0.63 (9 H, s, TBS), 0.02 (3 H, s, TBS), 0.02 (3 H, s, TBS), 0.00 (3 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 $C_{33}H_{64}N_4NaO_6Si_3$ $[M+Na]^+$: 719.40256. Found 719.40203.

6-Chloro-2-methoxy-9-(*β*-D-ribofuranosyl)-9*H*-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 CH_2Cl_2) to give crystals **9a** (127.4 mg, 0.38 mmol, 100%). ^{1}H NMR (400MHz, $CDCl_3$): 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 $C_{11}H_{13}ClN_4NaO_5$ $[M+Na]^+$: 339.04667. Found 339.04409.

2,6-Dimethoxy-9-(*β*-D-ribofuranosyl)-9*H*-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 CH_2Cl_2) to give crystals **9b** (47.1 mg, 0.12 mmol, 100%). ^{1}H NMR (400MHz, MeOH-d_4): 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 $C_{12}H_{16}N_4NaO_6$ $[M+Na]^+$: 335.09621. Found 335.09400.

2-Methoxyadenosine (**Spongosine: 9**)

Method A) Compound **9a** (120.0 mg, 0.38 mmol) was dissolved in NH_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 $CHCl_3$) to give crystals (47.3 mg, 0.16 mmol, 42%).

Method B) Compound **9b** (624.5 mg, 2.00 mmol) was dissolved in NH_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 $CHCl_3$) to give crystals (416.0 mg, 1.40 mmol, 70%).

^{1}H NMR (400MHz, DMSO-d_6): δ 7.90 (1 H, s, H-8), 7.09 (2 H, brs, NH_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 × 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 $C_{11}H_{15}N_5NaO_5$ $[M+Na]^+$: 320.09654. Found 320.09424.

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