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ONE-POT SYNTHESIS OF 2-NITROOXYALKOXYLATED INOSINE ANALOGS USING CYCLIC ETHER AND ISOAMYL NITRITE

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Abstract – An O^6 -methylguanosine derivative (**1**) was treated with isoamyl nitrite in the presence of a cyclic ether such as trimethylene oxide, tetrahydrofuran, or tetrahydropyran to give the corresponding 2-nitrooxyalkoxy-6-methoxypurine riboside derivatives (**2a–c**).

To date, 2-substituted inosine analogs, many of which form important intermediates of physiologically active substances, have been synthesized from appropriate nucleoside derivatives with an amino group at the 2-position *via* the diazonium salts using isoamyl nitrite.¹⁻³ For example, purin-2-yl carboxylate was synthesized in our laboratory using an O^6 -methylguanosine derivative treated with sodium nitrite or isoamyl nitrite in the presence of a carboxylic acid.^{4,5} Recently, we developed a method for the conversion of the 2-amino group of O^6 -methylguanosine derivatives to a 2-alkoxy group *via* non-aqueous diazotization–dediazonation reactions.⁶ This process was exploited for our synthesis of 2-methoxyadenosine (spongosine),⁶ which is known for its prominent bioactivity.⁷⁻¹⁰ Herein, by applying the above methods,⁴⁻⁶ we report a one-pot *de novo* nitrooxyalkoxylation at the 2-position of the purine skeleton using cyclic ethers and isoamyl nitrite. This is the first example of the synthesis of 2-nitrooxyalkoxylated nucleosides (**2a–c**, **5a–c**) *via* nucleophilic attack by a cyclic ether at the 2-position of purine nucleoside analog followed by further attack at the α -position of the cyclic ether by the nitrate ion, which results in cleavage of the cyclic ether ring. New methods for the preparation of nitrooxylated compounds are important because nitrooxylated aspirin derivatives have been found to be useful as anti-inflammatory agents with low gastrointestinal and general toxicity due to the release of nitric monoxide (NO) from the nitrooxylated moiety.^{11,12} Hence, the compounds described in this report could also act as NO-donor reagents.

The isoamyl nitrite-promoted substitution of the 2-amino group of O^6 -methylguanosine derivative **1**^{4,5} was carried out in three different solvent, trimethylene oxide ($n = 1$), THF ($n = 2$) or THP ($n = 3$), and the

Table 1. Synthesis of 2-nitrooxyalkoxylated purin riboside derivatives **2a–c**

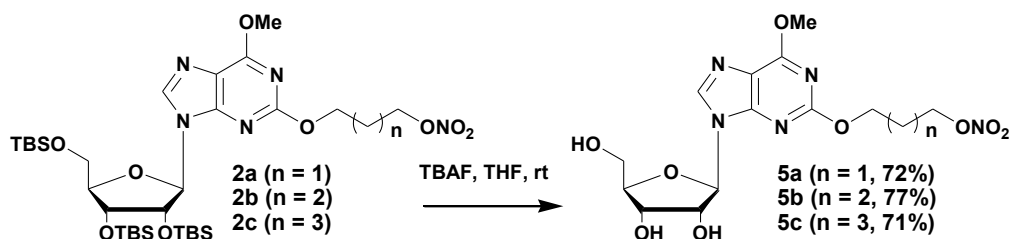
Entry	Cyclic ether	Time (day)	Yield (%)		
			2	3	4
1	trimethylene oxide (n = 1)	1	2a: 23, 2d: 12	20	43
2	THF (n = 2)	3	2b: 30	26	43
3	THP (n = 3)	4	2c: 24	19	39

7.5 eq. isoamyl nitrite was used and reacted at 50°C.

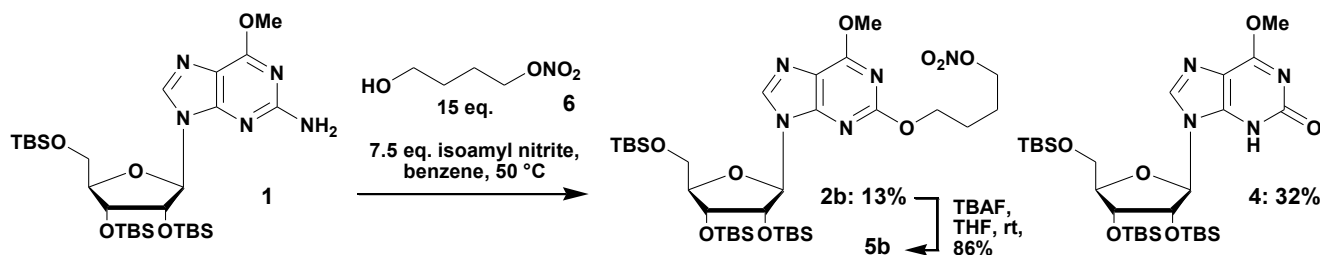
results are summarized in Table 1. First, compound **1** was treated with isoamyl nitrite in trimethylene oxide ($n = 1$) to give the O^6 -methylinosine analog (**2a**) nitrooxypropoxylated at the 2-position of the purine system, in a 23% yield. The reaction also yielded a non-substituted O^6 -methylinosine derivative (**3**), an O^6 -methylxanthosine analog (**4**), and an inosine analog (**2d**) a result of 3-[3-(nitrooxy)propoxy]-propoxylation of the 2-position, as by-products in 20, 43, and 12% yields, respectively (Table 1, entry 1). The structures of **2a**, **3**, **4**, and **2d** were identified by HR-MS (ESI), ^1H NMR spectroscopy, and ^1H - ^1H COSY spectroscopy. Similarly, compound **1** was treated in THF ($n = 2$) with isoamyl nitrite to give the 2-nitrooxybutoxylated compound **2b** in a 30% yield and by-products **3** and **4** in 26 and 43% yields, respectively (Table 1, entry 2). In THP ($n = 3$), the reaction resulted in the production of 2-nitrooxypentoxylated derivative **2c** and undesired products **3** and **4** in 24, 19, and 39% yields, respectively (Table 1, entry 3). The reaction times for these analogs decreased with the reduction of the cyclic ether ring size: the six- ($n = 3$), five- ($n = 2$), and four- ($n = 1$) membered ring systems required 4, 3, and 1 day(s), respectively, for completion of the reaction. These results may be due to the higher ring strain in the smaller ring systems. Especially trimethylene oxide ($n = 1$) has the most strained ring, therefore, is so reactive to produce double-reacted product 2-{3-[3-(nitrooxy)propoxy]propoxy}purine analog (**2d**) in 12% yield.

Deprotective reactions of the TBS groups at the 2', 3', and 5'-position of the ribose moieties of **2a–c** were carried out in high yields using conventional methods to provide compounds **5a–c** (Scheme 1). The molecular formulae of **5a–c** were determined by HRMS (ESI) and ^1H NMR, ^1H - ^1H COSY, and FT-IR spectroscopy. In particular, the nitrate ester structures (RONO₂) of **5a–c** were confirmed by their IR spectra (**5a**: 1602, 1275, 859 cm⁻¹, **5b**: 1602, 1279, 864 cm⁻¹, **5c**: 1597, 1277, 861 cm⁻¹).

While we synthesized compound **5b** through an alternative method (Scheme 2). The O^6 -methylguanosine derivative (**1**) was treated with isoamyl nitrite in the presence of 15.0 equivalents of 4-nitrooxybutan-1-ol



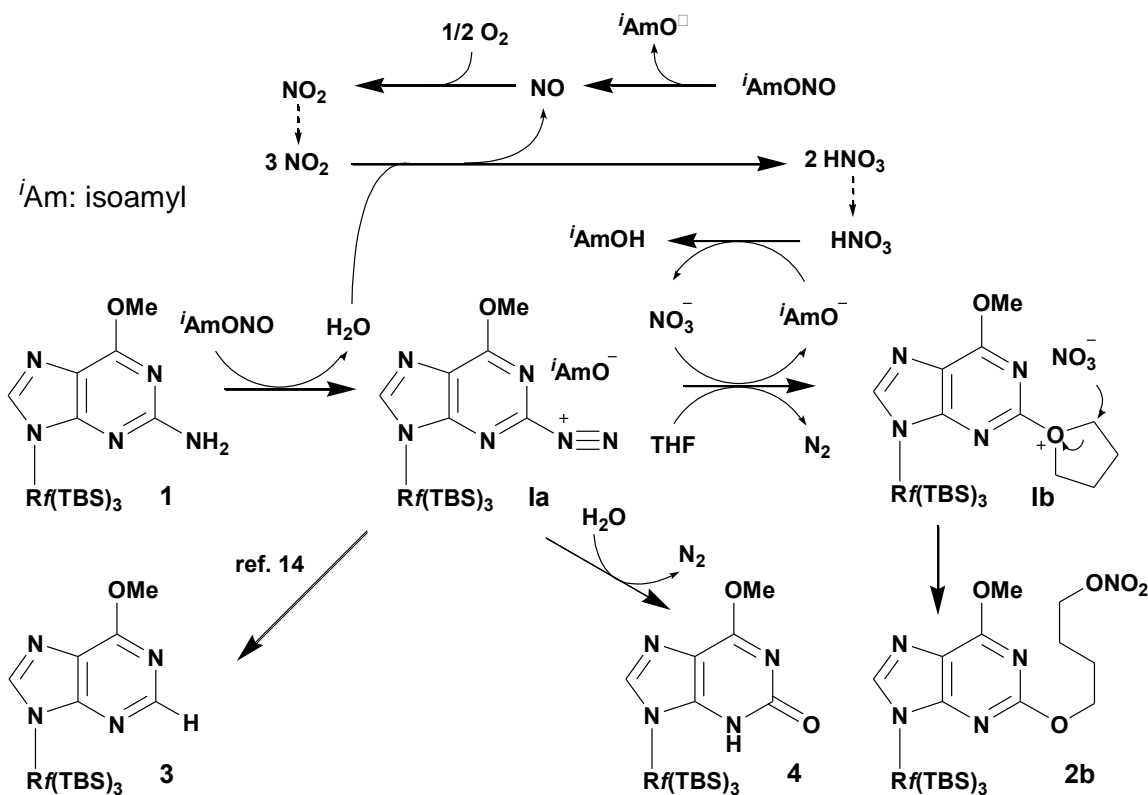
Scheme 1. Desilylation of 2-nitroxyalkoxylated purin riboside derivatives **2a–c**



Scheme 2. Synthesis of 2-nitroxybutoxylated purin riboside derivatives **2b** and **5b**

(**6**) in benzene to obtain the desired 2-nitroxybutoxylated product (**2b**) in a relatively low yield (13%) and the by-product *O*⁶-methylxanthosine analog **4** in a 32% yield. Moreover, compound **2b** was treated with tetrabutylammonium fluoride to obtain the desilylated product (**5b**) in an 86% yield. The ¹H NMR and HRMS data of **2b** and the ¹H NMR, ¹H-¹H COSY, ¹³C NMR, HRMS, and FT-IR data of **5b** were found to be identical with the corresponding synthesized products (**2b** in Table 1 and **5b** in Scheme 1). The plausible reaction mechanism to obtain the 2-nitroxybutoxyinosine (**2b**), inosine (**3**), and xanthosine analogs (**4**) is described in Scheme 3.^{4,6} In the presence of isoamyl nitrite, compound **1** underwent substitution, as in the Sandmeyer reaction, to give one water molecule and the corresponding diazonium salt (**Ia**) as the reaction intermediate.¹³ The diazo group at the 2-position of compound **Ia** was then nucleophilically attacked by the oxygen atom of THF, which simultaneously replaced the counter anion isoamyl oxide ion with the nitrate ion that is generated in the reaction system to providing the nitrogen-eliminated intermediate **Ib**. Then, nitrate esterification at the α -position of the THF moiety of **Ib** with the use of a nitrate ion occurred to afford the nitrooxybutoxylated product, **2b**. If diazonium salt **Ia** was attacked by a water molecule, the xanthosine derivative **4** was formed.⁶ When intermediate **Ia** underwent the thermal homolysis, **Ia** was converted to the transient species, purine-2-yl radical, followed by the production of the inosine analog **3** as previously reported by Nair *et al.*¹⁴ The production of nitrate ions in the reactive system can be explained based on the Ostwald process.^{15,16} As shown in Scheme 3, the NO radical, which is derived from isoamyl nitrite, reacts with oxygen to produce NO₂. The resulting NO₂ reacts with water, which is produced during diazonium salt formation *via* compound **1** and isoamyl nitrite, to give nitric acid (HNO₃) and NO. Then, the HNO₃ molecule is ionized by a reaction with an isoamyl oxide ion to provide a nitrate ion (NO₃⁻). We consider the reactions to provide compounds **2b**, **3**,

and **4** to be competitive; therefore, the yields of the desired products (**2a–c**) are relatively low. Further research on the effective synthesis of 2-nitrooxyalkoxyinosine analogs is necessary.



Scheme 3. Plausible reaction mechanism for the formation of compounds **2b**, **3**, and **4**

EXPERIMENTAL

Instrumentation

^1H NMR spectra were taken with a UltrashieldTM 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 spectrometry (ESI-MS).

6-Methoxy-2-[3-(nitrooxy)propoxy]-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-9H-purine (2a), 6-Methoxy-2-{3-[3-(nitrooxy)propoxy]propoxy}-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-9H-purine (2d), 6-Methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-9H-purine (3) and 1,9-dihydro-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-2H-purin-2-one (4)

Compound **1** (320.0 mg, 0.50 mmol) was dissolved in dry trimethylene oxide (8.0 mL) and isoamyl nitrite (489.7 μL , 3.75 mmol) was added to the solution at 0 $^\circ\text{C}$, and then stirred for 1 day at 50 $^\circ\text{C}$. The mixture

was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give **3** as oil (61.4 mg, 0.10 mmol, 20%). Evaporation of second fraction gave **2a** as oil (86.3 mg, 0.12 mmol, 23%). Then, evaporation of third fraction gave **2d** as oil (47.1 mg, 0.06 mmol, 12%) and finally fourth fraction gave **4** as oil (137.2 mg, 0.22 mmol, 43%). **3**: ^1H NMR (400 MHz, CDCl_3): δ 8.51 (1H, s, H-8), 8.42 (1H, s, H-2), 5.99 (1H, d, $J = 4.8$, H-1'), 4.52 (1H, m, H-2'), 4.22 (1H, m, H-3'), 4.10 (3H, s, 6-OCH₃), 4.06 (1H, m, H-4'), 3.94 (1H, dd, $J = 11.6$ and 4.0, H-5' a), 3.76 (1H, dd, $J = 11.2$ and 2.0, H-5' b), 0.88 (9H, s, OTBS), 0.83 (9H, s, OTBS), 0.71 (9H, s, OTBS), 0.08 (3H, s, OTBS), 0.07 (3H, s, OTBS), 0.04 (3H, s, OTBS), 0.03 (3H, s, OTBS), -0.11 (3H, s, OTBS), -0.33 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{29}\text{H}_{57}\text{N}_4\text{O}_5\text{Si}_3$ $[\text{M}+\text{Na}]^+$: 647.34507. Found 647.33660. **2a**: ^1H -NMR (400 MHz, CDCl_3): δ 8.20 (1H, s, H-8), 6.02 (1H, d, $J = 4.8$, H-1'), 4.68 (2H, m, 2-OCH₂CH₂CH₂ONO₂), 4.51 (1H, m, H-2'), 4.48 (2H, m, 2-OCH₂CH₂CH₂ONO₂), 4.31 (1H, m, H-3'), 4.15 (3H, s, 6-OCH₃), 4.11 (1H, m, H-4'), 3.99 (1H, dd, $J = 11.6$ and 3.6, H-5' a), 3.81 (1H, dd, $J = 11.6$ and 2.4, H-5' b), 2.28 (2H, m, 2-OCH₂CH₂CH₂ONO₂), 0.95 (9H, s, OTBS), 0.92 (9H, s, OTBS), 0.81 (9H, s, OTBS), 0.14 (3H, s, OTBS), 0.13 (3H, s, OTBS), 0.12 (3H, s, OTBS), 0.11 (3H, s, OTBS), -0.03 (3H, s, OTBS), -0.40 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{33}\text{H}_{64}\text{N}_5\text{O}_9\text{Si}_3$ $[\text{M}+\text{H}]^+$: 744.38498. Found 744.38499. **2d**: ^1H -NMR (400 MHz, CDCl_3): δ 8.18 (1H, s, H-8), 6.02 (1H, d, $J = 4.8$, H-1'), 4.57 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 4.48 (1H, m, H-2'), 4.42 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 4.31 (1H, m, H-3'), 4.15 (3H, s, 6-OCH₃), 4.11 (1H, m, H-4'), 3.99 (1H, dd, $J = 11.6$ and 4.0, H-5' a), 3.81 (1H, dd, $J = 11.2$ and 2.4, H-5' b), 3.62 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 3.54 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 2.11 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 1.97 (2H, m, 2-OCH₂CH₂CH₂OCH₂CH₂CH₂ONO₂), 0.96 (9H, s, OTBS), 0.93 (9H, s, OTBS), 0.83 (9H, s, OTBS), 0.18 (3H, s, OTBS), 0.15 (3H, s, OTBS), 0.13 (3H, s, OTBS), 0.12 (3H, s, OTBS), -0.03 (3H, s, OTBS), -0.19 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{35}\text{H}_{67}\text{N}_5\text{NaO}_{10}\text{Si}_3$ $[\text{M}+\text{Na}]^+$: 824.40879. Found 824.40789. **4**: ^1H -NMR (400 MHz, CDCl_3): δ 7.56 (1H, s, H-8), 5.70 (1H, d, $J = 7.6$, H-1'), 4.61 (1H, m, H-2'), 4.30 (1H, dd, $J = 7.6$ and 4.4, H-3'), 4.16 (1H, m, H-4'), 4.13 (3H, s, 6-OCH₃), 4.05 (1H, dd, $J = 12.0$ and 2.0, H-5' a), 3.88 (1H, dd, $J = 12.0$ and 1.6, H-5' b), 1.00 (9H, s, OTBS), 0.95 (9H, s, OTBS), 0.80 (9H, s, OTBS), 0.27 (3H, s, OTBS), 0.21 (3H, s, OTBS), 0.14 (3H, s, OTBS), 0.12 (3H, s, OTBS), -0.03 (3H, s, OTBS), -0.40 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{29}\text{H}_{57}\text{N}_4\text{O}_6\text{Si}_3$ $[\text{M}+\text{H}]^+$: 641.35846. Found 641.35804.

6-Methoxy-2-[4-(nitrooxy)butoxy]-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl- β -D-ribofuranosyl)-9H-purine (2b), 6-Methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl- β -D-ribofuranosyl)-9H-purine (3) and 1,9-dihydro-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl- β -D-ribofuranosyl)-2H-purin-2-one (4)

Compound **1** (1280.1 mg, 2.00 mmol) was dissolved in dry tetrahydrofuran (60.0 mL) and isoamyl nitrite

(1999.0 μL , 15.0 mmol) was added to the solution at 0 $^{\circ}\text{C}$, and then stirred for 3 days at 50 $^{\circ}\text{C}$. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give **3** as oil (329.7 mg, 0.52 mmol, 26%). Evaporation of second fraction gave **2b** as oil (458.7 mg, 0.60 mmol, 30%). Then, evaporation of third fraction gave **4** as oil (547.0 mg, 0.86 mmol, 43%). **2b**: $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.06 (1 H, s, H-8), 5.89 (1H, d, $J = 4.8$, H-1'), 4.43 (2H, m, 2-OCH₂CH₂CH₂CH₂ONO₂), 4.35 (1H, m, H-2'), 4.27 (2H, m, 2-OCH₂CH₂CH₂CH₂ONO₂), 4.18 (1H, m, H-3'), 4.01 (3H, s, 6-OCH₃), 3.98 (1H, m, H-4'), 3.86 (1H, dd, $J = 11.2$ and 3.2, H-5' a), 3.68 (1H, m, H-5' b), 1.82 (4H, m, 2-OCH₂CH₂CH₂CH₂ONO₂), 0.85 (9H, s, OTBS), 0.82 (9H, s, OTBS), 0.67 (9H, s, OTBS), 0.05 (3H, s, OTBS), 0.04 (3H, s, OTBS), 0.00 (3H, s, OTBS), -0.01 (3H, s, OTBS), -0.13 (3H, s, OTBS), -0.34 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{33}\text{H}_{63}\text{N}_5\text{NaO}_9\text{Si}_3$ $[\text{M}+\text{Na}]^+$: 780.38258. Found 780.37893.

6-Methoxy-2-[4-(nitrooxy)pentoxy]-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-9H-purine (2c), 6-Methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-9H-purine (3) and 1,9-dihydro-6-methoxy-9-(2,3,5-tri-*O*-*tert*-butyldimethylsilyl)- β -D-ribofuranosyl)-2H-purin-2-one (4)

Compound **1** (640.05 mg, 1.00 mmol) was dissolved in dry tetrahydrofuran (16.0 mL) and isoamyl nitrite (1000.0 μL , 7.5 mmol) was added to the solution at 0 $^{\circ}\text{C}$, and then stirred for 4 days at 50 $^{\circ}\text{C}$. The mixture was evaporated, and the residue was purified by silica gel column chromatography (25% AcOEt in hexane) to give **3** as oil (118.6 mg, 0.19 mmol, 19%). Evaporation of second fraction gave **2c** as oil (192.3 mg, 0.24 mmol, 24%). Then, evaporation of third fraction gave **4** as oil (248.9 mg, 0.39 mmol, 39%). **2c**: $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 8.18 (1H, s, H-8), 6.02 (1H, d, $J = 5.2$, H-1'), 4.49 (1H, m, H-2'), 4.48 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 4.38 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 4.31 (1H, m, H-3'), 4.15 (3 H, s, 6-OCH₃), 4.11 (1H, m, H-4'), 3.99 (1 H, dd, $J = 11.6$ and 3.6, H-5' a), 3.81 (1H, dd, $J = 11.6$ and 2.4, H-5' a), 1.91 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 1.81 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 1.65 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 0.95 (9H, s, OTBS), 0.93 (9H, s, OTBS), 0.81 (9H, s, OTBS), 0.14 (3H, s, OTBS), 0.13 (3H, s, OTBS), 0.12 (3H, s, OTBS), 0.11 (3H, s, OTBS), -0.03 (3H, s, OTBS), -0.20 (3H, s, OTBS); HRMS (ESI) Calcd for $\text{C}_{34}\text{H}_{65}\text{N}_5\text{NaO}_9\text{Si}_3$ $[\text{M}+\text{Na}]^+$: 794.39823. Found 794.39869.

6-Methoxy-2-[3-(nitrooxy)propoxy]-9-(β -D-ribofuranosyl)-9H-purine (5a)

Compound **2a** (86.3 mg, 0.12 mmol) was dissolved in THF (2.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (0.6 mL) was added to the solution, and then stirred for 15 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (10% MeOH in AcOEt) to give needle crystals **5a** (33.7 mg, 0.08 mmol, 72%). $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 8.21 (1H, s, H-8), 5.87 (1H, d, $J = 5.6$, H-1'), 4.61 (1H, m, H-2'), 4.60 (2H, m, 2-OCH₂CH₂CH₂ONO₂),

4.42 (2H, m, 2-OCH₂CH₂CH₂ONO₂), 4.25 (1H, dd, $J = 5.2$ and 3.6 , H-3'), 4.04 (3H, s, 6-OCH₃), 4.02 (1H, m, H-4'), 3.77 (1H, dd, $J = 12.4$ and 3.2 , H-5' a), 3.65 (1H, dd, $J = 12.4$ and 3.6 , H-5' b), 2.15 (2H, m, 2-OCH₂CH₂CH₂ONO₂); ¹³C-NMR (100MHz, CD₃OD): δ 163.3, 162.4, 154.2, 142.4, 118.4, 90.6, 87.4, 75.5, 72.1, 71.5, 65.3, 63.1, 55.0, 27.7; HRMS (ESI) Calcd for C₁₄H₁₉N₅NaO₉ [M+Na]⁺: 424.10750. Found 424.10650; FT-IR (Nujol) cm⁻¹: 1602, 1275, 859.

6-Methoxy-2-[3-(nitrooxy)butoxy]-9-(β -D-ribofuranosyl)-9H-purine (5b)

Compound **2b** (153.7 mg, 0.20 mmol) was dissolved in THF (2.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (1.0 mL) was added to the solution, and then stirred for 15 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (10% MeOH in AcOEt) to give needle crystals **5b** (64.7 mg, 0.15 mmol, 77%). ¹H-NMR (400 MHz, CD₃OD): δ 8.19 (1H, s, H-8), 5.85 (1H, d, $J = 5.6$, H-1'), 4.61 (1H, m, H-2'), 4.48 (2H, m, 2-OCH₂CH₂CH₂CH₂ONO₂), 4.34 (2H, m, 2-OCH₂CH₂CH₂CH₂ONO₂), 4.25 (1H, dd, $J = 5.2$ and 3.6 , H-3'), 4.03 (3H, s, 6-OCH₃), 4.02 (1H, m, H-4'), 3.78 (1H, dd, $J = 12.4$ and 3.2 , H-5' a), 3.65 (1H, dd, $J = 12.4$ and 3.2 , H-5' b), 1.78- 1.87 (4H, m, 2-OCH₂CH₂CH₂CH₂ONO₂); ¹³C-NMR (100 MHz, CD₃OD): δ 163.2, 162.6, 154.2, 142.3, 118.3, 90.6, 87.4, 75.4, 74.4, 72.2, 68.6, 63.1, 55.0, 26.4, 24.8; HRMS (ESI) Calcd for C₁₅H₂₁N₅NaO₉ [M+Na]⁺: 438.12315. Found 438.12253; FT-IR (Nujol) cm⁻¹: 1602, 1279, 864.

6-Methoxy-2-[3-(nitrooxy)pentoxy]-9-(β -D-ribofuranosyl)-9H-purine (5c)

Compound **2c** (192.3 mg, 0.24 mmol) was dissolved in THF (4.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (1.2 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 (10% MeOH in AcOEt) to give needle crystals **5c** (74.4 mg, 0.17 mmol, 71%). ¹H-NMR (400 MHz, CD₃OD): δ 8.18 (1H, s, H-8), 5.85 (1H, d, $J = 5.6$, H-1'), 4.62 (1H, m, H-2'), 4.41 (2H, $J = 6.8$, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 4.31 (2H, $J = 6.0$, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 4.26 (1H, m, H-3'), 4.02 (3H, s, 6-OCH₃), 4.01 (1H, m, H-4'), 3.77 (1H, dd, $J = 12.4$ and 2.8 , H-5' a), 3.65 (1H, dd, $J = 12.4$ and 2.2 , H-5' b), 1.66-1.80 (4H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂), 1.45-1.55 (2H, m, 2-OCH₂CH₂CH₂CH₂CH₂ONO₂); ¹³C-NMR (100 MHz, CD₃OD): δ 163.2, 162.7, 154.2, 142.3, 118.3, 90.6, 87.4, 75.4, 74.6, 72.2, 68.9, 63.1, 55.0, 29.6, 27.6, 23.5; HRMS (ESI) Calcd for C₁₆H₂₃N₅NaO₉ [M+Na]⁺: 452.13880. Found 452.13777; FT-IR (Nujol) cm⁻¹: 1597, 1277, 861.

6-Methoxy-2-[4-(nitrooxy)butoxy]-9-(2,3,5-tri-*O*-tert-butylidimethylsilyl- β -D-ribofuranosyl)-9H-purine (2b) and 1,9-dihydro-6-methoxy-9-(2,3,5-tri-*O*-tert-butylidimethylsilyl- β -D-ribofuranosyl)-2H-purin-2-one (4)

Compound **1** (236.5 mg, 0.37 mmol) and 4-nitrooxybutan-1-ol (**6**) (748.9 mg, 5.54 mmol) were dissolved in dry benzene (5.9 mL) and isoamyl nitrite (369.8 μ L, 2.78 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 **2b** as oil (34.9 mg, 0.05 mmol, 13%). Then, evaporation of second fraction gave **4** as oil (75.1 mg, 0.12 mmol, 32%).

6-Methoxy-2-[3-(nitrooxy)butoxy]-9-(β -D-ribofuranosyl)-9H-purine (5b**)**

Compound **2b** (30.4 mg, 0.04 mmol) was dissolved in THF (2.0 mL), and 1.0 M tetrabutylammonium fluoride-THF solution (0.2 mL) was added to the solution, and then stirred for 15 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (10% MeOH in AcOEt) to give crystals **5b** (14.4 mg, 0.03 mmol, 86%).

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