Supplementary Information for

8-Nitroguanosines as chemical probes of the protein *S*-guanylation

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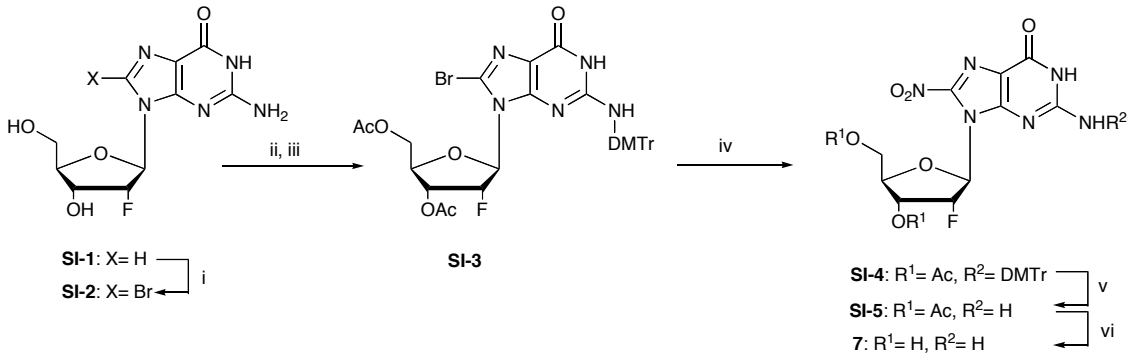
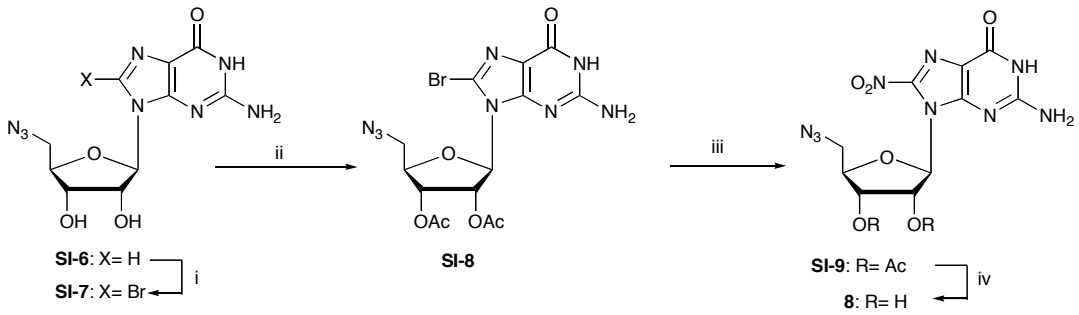
1. Figure S1: Synthetic scheme for 2'-deoxy-2'-fluoro-8-nitroguanosine **7**.



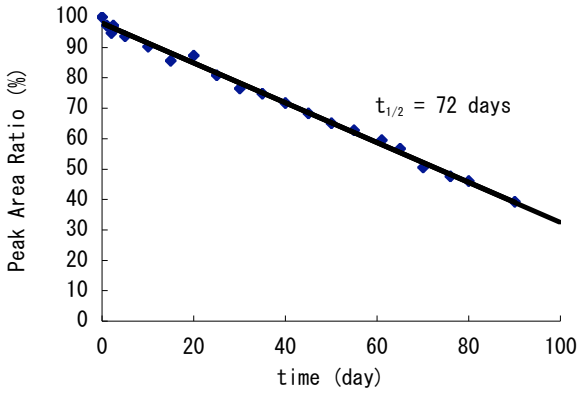
Reagents and conditions: (i) NBS, H_2O, 0°C, 2.5 h (47 %), (ii) Ac_2O, pyridine, room temp. 2 h (76 %), (iii) DMTr-Cl, pyridine; room temp., 16 h (79 %); (iv) KNO_2 (10 eq), 18-crown-6 (11 eq), DMF, 100 °C, 6 h (66 %); (v) p-TsOH (0.7 eq), $CHCl_3$, room temp. 20 min (86 %.); (vi) $MeNH_2$ (150 eq), MeOH, room temp. 2 h (60 %).

2. Figure S2: Synthetic scheme for 5'-azido-5'-deoxy-8-nitroguanosine **8**.



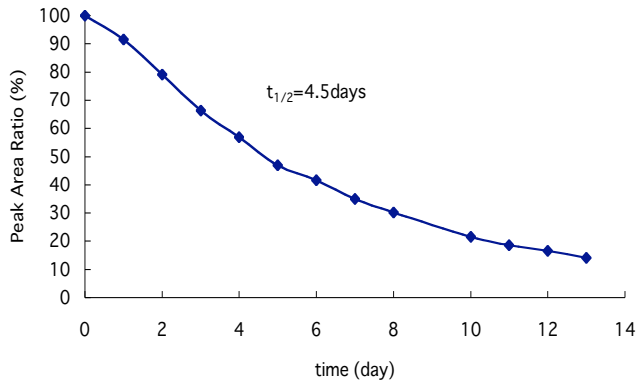
Reagents and conditions: (i) NBS, H_2O-CH_3CN (2 : 1), 0°C, 4 h (94 %), (ii) Ac_2O, pyridine, DMAP, 0°C, 1.5 h (97 %), (iii) KNO_2 (10 eq), DMF, 100 °C, 4 h (20 %); (iv) $MeNH_2$, MeOH, room temp. 30 min (quant.).

3. Figure S3: Concentration (peak area ratio to internal standard) vs time curve for 2'-deoxy-2'-fluoro-8-nitroguanosine **7** in Sodium phosphate buffer (50 mM, pH 7.4) at 37°C.



The initial concentration of **7** was 1 mM. 1 mM of uracil was employed as an internal standard.

4. Figure S4: Concentration (peak area ratio to internal standard) vs time curve for 2'-deoxy-2'-fluoro-8-nitroguanosine **8** in Sodium phosphate buffer (50 mM, pH 7.4) at 37°C.

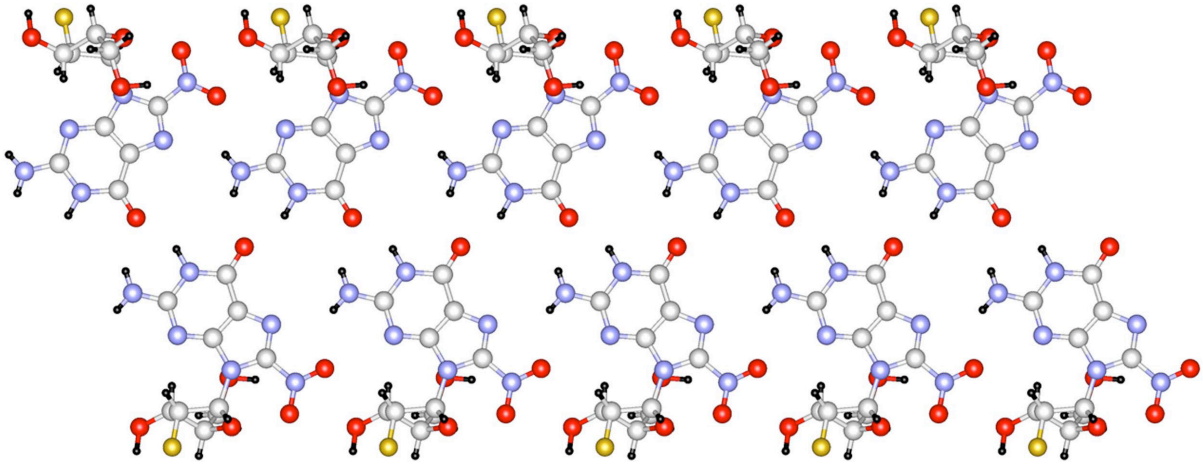


The initial concentration of **8** was 1 mM. 1 mM of uracil was employed as an internal standard.

5. Figure S5: Hydrogen bonding network in crystal structure of **7**.

Atom labels: hydrogen= black, carbon= gray, nitrogen= blue, oxygen= red, fluorine= yellow.

One of the oxygen atoms of a nitro group is involved in the hydrogen bonding with the neighboring $N\underline{H}_2$.



6. Synthetic procedures for compounds.

General procedures

Unless otherwise noted, the reactions were performed under inert atmosphere (Ar).

2'-Deoxy-2'-fluoroguanosine was purchased from Metkinen Chemistry (Finland).

Purity of **2** was checked with HPLC using conditions below:

Column: Develosil ODS HG-5 (4.6 mm i.d. x 150 mm), Eluent: MeOH/H_2O (5/95, constant flow for 3 min) - (gradient: 5/95 to 85/15 over10 min) - MeOH/H_2O (85/15), Flow rate: 1.0 mL/min

Synthesis of compound 4

8-Bromo-2',3',6'-*O*-acetyl-guanosine (2.00 g, 4.10 mmol) in pyridine (40 mL) was added 4,4'-dimethoxytrityl chloride (2.22 g, 6.56 mmol) at room temperature, and the mixture was stirred for 10 h. The reaction was diluted with iced water (30 mL) and extracted with dichloromethane (50 mL). The organic layer was dried (Na_2SO_4), and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with hexanes/EtOAc (1/1) and then with dichloromethane/methanol (20:1), to give 3.22 g (99%) of **4** as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-6.83 (complex, 13H), 5.71 (complex, 2H), 5.54 (m, 1 H), 4.22-4.00 (complex, 3 H), 3.76 (s, 6H), 2.09 (s, 3H), 2.02 (s, 6H); HRMS (FAB, matrix NBA):

2.09 (s, 3H), 2.02 (s, 6H); HRMS (FAB, matrix NBA): calcd for $C_{37}H_{37}O_{10}N_5Br$ $[M+H]^+$ 790.1724, found 790.1728.

Synthesis of compound 5 (Table 1, entry 2)

A mixture of **4** (100 mg, 0.126 mmol), KNO_2 (107 mg, 1.26 mmol), and 18-crown-6 (333 mg, 1.26 mmol) in DMF (10 mL) was stirred for 6 h at 100 °C. The mixture was diluted with chloroform (100 mL), and washed with H_2O (100 mL x 3) and brine (100 mL). The organic phase was dried (Na_2SO_4), and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with chloroform/acetonitrile (1/0 to 3/1), to give 23.3 mg (23%) of recovered **4**, and 57.2 mg (60%) of **5** as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) of **5**: δ 11.16 (s, 1H), 7.90 (s, 1H), 7.23-6.80 (complex, 13H), 5.59 (complex, 2H), 5.36 (m, 1H), 4.24-4.00 (complex, 3 H), 3.74 (s, 6H), 2.09 (s, 3H), 2.05 (s, 3H), 1.79 (s, 3H); HRMS (FAB, matrix NBA): calcd for $C_{37}H_{37}O_{12}N_6$ $[M+H]^+$ 757.2470, found 757.2477.

Synthesis of compound 6

A solution of *p*-toluenesulfonic acid monohydrate (100 mg) in chloroform (10 mL) was added dropwise over 25 min to a solution of **5** (190 mg, 0.250 mmol) in chloroform (60 mL) at room temperature. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with chloroform/methanol (1/0 to 20/1), to give 105 mg (quant.) of **6** as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.94 (br.s, 1H), 8.30 (br.s, 1H), 6.93 (br.s, 1H), 6.76 (m, 1H), 6.59 (m, 1H), 5.72 (m, 1H), 4.52-4.42 (complex, 3 H), 2.20 (s, 6H), 2.03 (s, 3H).

Synthesis of 8-nitroguanosine (2)

Methylamine (40% methanol solution, 5 mL) was added to a solution of **6** (102 mg, 0.224 mmol) in methanol (10 mL), and was stirred for 12 h at room temperature.

The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on Cosmosil^{TM} 75C_{18}-OPN (Nakalai tesque, Japan), eluting with water/methanol (9/1), to give 61.2 mg (83 %) of **2** as a yellow solid: ^{1}H NMR (300 MHz, D_2O): δ 6.76 (d, J = 6.0 Hz, 1H), 5.08 (m, 1H), 4.55 (m, 1H), 4.28 (m, 1H), 4.00-3.86 (complex, 2H); HPLC purity 99.5 %.

Synthesis of 8-bromo-2'-deoxy-2'-fluoroguanosine (SI-2)

A solution of 2'-deoxy-2'-fluoroguanosine (10.0 mg, 0.035 mmol) in water (1.1 mL) was added *N*-bromosuccinimide (9.4 mg, 0.053 mmol) at 0°C. The mixture was stirred for 2 h 20 min at the same temperature. The resulting precipitate was collected by filtration, washed with methanol to obtain **SI-2** (6.0 mg, 47 %) as colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.83 (s, 1H), 6.57 (s, 1H), 5.87 (m, 1H), 5.67 (m, 1H), 5.54 (d, J = 7.2 Hz, 1H), 4.86 (t, J = 5.7 Hz, 1H), 4.56 (m, 1H), 3.51-3.86 (complex, 3H); HRMS (FAB, matrix NBA): calcd for $C_{10}H_{12}O_4N_5BrF$ $[M+H]^+$ 364.0057, found 364.0063.

Synthesis of compound SI-3

A mixture of **SI-2** (5.0 mg, 0.014 mmol) and pyridine (0.5 mL) was added dropwise acetic anhydride (6.5 μL, 0.0069 mmol) at 0°C. After stirring for 2 h, the mixture was diluted with iced water, and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with chloroform/methanol (95/5), to give 4.1 mg (67%) of diacetate as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.57 (br.s, 1H), 6.68 (br.s, 1H), 6.16-5.86 (complex, 3H), 4.55 (dd, J = 2.4, 12.6 Hz, 1H), 4.45 (m, 1H), 4.28 (dd, J = 5.1, 12.6 Hz, 1H), 2.23 (s, 3H), 2.02 (s, 3H); HRMS (FAB, matrix NBA): calcd for $C_{14}H_{16}O_6N_5BrF$ $[M+H]^+$ 448.0268, found 448.0271.

A solution of the diacetate (36.5 mg, 0.081 mmol) and 4,4'-dimethoxytrityl chloride (137.7 mg, 0.407 mmol) in pyridine (4.0 mL) was stirred at room temperature for 16 h. The reaction mixture was diluted with iced water, and extracted with chloroform. The organic layer was washed with saturated aqueous $NaHCO_3$, brine. The resultant organic layer was dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by column chromatography on silica gel, eluting with chloroform/methanol (39/1), to afford 48.2 mg (79%) of **SI-3** as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26-6.78 (complex, 13H), 5.76 (dd, J = 3.6, 17.7 Hz, 1H), 5.40-5.22 (complex, 2H), 4.14-3.86 (complex, 3H), 3.75 (s, 3H), 3.74 (s, 3H), 2.14 (s, 3H), 2.01 (s, 3H); HRMS (FAB, matrix NBA): calcd for $C_{35}H_{34}O_8N_5BrF$ $[M+H]^+$ 750.1575, found 750.1577.

Synthesis of compound SI-4

A mixture of **SI-3** (16.8 mg, 0.022 mmol), KNO_2 (19.1 mg, 0.224 mmol), 18-crown-6 (65 mg, 0.246 mmol) in DMF (1.6 mL) was stirred for 6 h at 100 °C. The mixture was diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$, brine. The

EtOAc, and washed with saturated aqueous $NaHCO_3$, brine. The organic phase was dried (Na_2SO_4), and the volatiles were removed under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with chloroform/acetonitrile (5/0 to 3/1), to give 10.6 mg (66%) of **SI-4** as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-6.79 (complex, 13H), 6.21-5.34 (complex, 3H), 4.30-3.95 (complex, 3H), 3.75 (s, 6H), 2.16 (s, 3H), 2.02 (s, 3H); HRMS (FAB, matrix NBA): calcd for $C_{35}H_{34}O_{10}N_6F$ $[M+H]^+$ 717.2321, found 717.2325.

Synthesis of compound SI-5

A solution of *p*-toluenesulfonic acid monohydrate (2.0 mg) in chloroform (0.2 mL) was added dropwise to a solution of **SI-4** (10.6 mg, 0.015 mmol) in chloroform (1.0 mL) at room temperature. The mixture was stirred for 20 min at the same temperature, and then purified by column chromatography on silica gel, eluting with chloroform/methanol (1/0 to 9/1), to give 5.3 mg (86%) of **SI-5** as a yellow solid: ^{1}H NMR (300 MHz, CD_3OD): δ 6.75 (dd, J = 1.8, 24.0 Hz, 1H), 5.95 (ddd, J = 5.7, 8.7, 18.0 Hz, 1H), 5.75 (m, 1H), 4.48 (dd, J = 3.0, 12.0 Hz, 1H), 4.39 (ddd, J = 3.0, 5.4, 8.7 Hz, 1H), 4.27 (dd, J = 5.4, 12.0 Hz, 1H); HRMS (FAB, matrix NBA): calcd for $C_{14}H_{16}O_8N_6F$ $[M+H]^+$ 415.1014, found 415.1014.

Synthesis of 2'-deoxy-2'-fluoro-8-nitroguanosine (7)

Methylamine (40% methanol solution, 0.2 mL) was added to a solution of **SI-5** (5.3 mg, 0.013 mmol) in methanol (0.5 mL), and was stirred for 2 h at room temperature.

The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on Cosmosil™ 75C_{18}-OPN (Nakalai tesque, Japan), eluting with water/methanol (95/5), to give 61.2 mg (83 %) of **1** as a yellow solid: ^{1}H NMR (300 MHz, CD_3OD): δ 6.74 (dd, J = 2.4, 22.5 Hz, 1H), 5.73 (ddd, J = 2.4, 5.4, 54.0 Hz, 1H), 4.00 (m, 1H), 3.90 (dd, J = 2.7, 12.3 Hz, 1H), 3.73 (dd, J = 4.8, 12.3 Hz, 1H); HRMS (FAB, matrix NBA+NaI): calcd for $C_{10}H_{11}O_6N_6FNa$ $[M+Na]^+$ 353.0622, found 353.0624; HPLC purity 97.0 %.

Synthesis of 5'-azide-5'-deoxy-8-bromoguanosine (SI-7)

5'-azide-5'-deoxyguanosine **SI-6** (400 mg, 1.3 mmol) was dissolved in water/acetonitrile (2/1 (30 mL) and cooled in ice bath. To this solution was added NBS (174 mg, 2.0 mmol) and stirred for 4 h. The most of solvent was removed under reduced pressure, and resulting precipitate was collected to afford a brown solid. Purification of the solid by column chromatography (acetonitrile/ water = 8/1) yielded product **SI-7** (376

chromatography (acetonitrile/ water = 8/1) yielded product **SI-7** (376 mg, 94%) as an orange solid. $[\alpha]_D^{15.5}$ +5.41° (c=1.25, DMSO); ^{1}H NMR (300MHz, DMSO-d_6) δ 10.83 (s, 1H); 6.61 (s, 2H); 5.73 (d, J = 6.3 Hz, 1H); 5.57 (s, 1H); 5.29 (s, 1H); 5.17 (m, 1H); 4.14 (m, 1H); 3.99 (dt, J = 3.6, 8.1 Hz, 1H); 3.81 (dd, J = 8.4, 12.9 Hz, 1H); 3.44 (dd, J = 4.2, 12.9 Hz, 1H); ^{13}C NMR (125MHz, $CDCl_3$) δ 156.2, 154.2, 152.8, 122.1, 118.2, 90.7, 84.5, 71.8, 70.8, 52.1; HRMS (FAB, matrix NBA): calcd for $C_{10}H_{12}BrO_4N_8$ $[M+H]^+$ 387.0165, found 387.0172.

Synthesis of 2',3'-*O*-acetyl-5'-azido-5'-deoxy-8-bromoguanosine (SI-8)

A solution of **SI-7** (200 mg, 0.52 mmol) in pyridine (2 mL) was added acetic anhydride (0.14 mL, 1.5 mmol) and DMAP (6.3 mg, 0.05 mmol) at 0°C. After stirring for 1.5 h, the reaction was quenched with iced water. The mixture was extracted with chloroform, and the organic layer was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a yellow solid. Purification by column chromatography (chloroform/ methanol = 30/1) yielded **SI-8** as a light yellow solid (194 mg, 97%). $[\alpha]_D^{16.5}$ +8.06° (c=0.165, acetone); ^{1}H NMR (500MHz, $CDCl_3$) δ 11.96 (s, 1H); 6.66 (s, 2H); 6.40 (m, 1H); 6.28 (m, 1H); 5.97 (d, J = 5.7 Hz); 4.28 (m, 1H); 3.73 (m, 1H); 2.15 (s, 6H); ^{13}C NMR (125MHz, $CDCl_3$) δ 170.0, 169.9, 157.9, 153.8, 152.7, 121.8, 118.0, 88.5, 81.4, 72.0, 71.4, 51.7, 20.9, 20.8; HRMS (FAB, matrix NBA): calcd for $C_{14}H_{16}BrO_6N_8$ $[M+H]^+$ 471.0376, found 471.0379.

Synthesis of 2',3'-*O*-acetyl-5'-azido-5'-deoxy-8-nitroguanosine (SI-9)

A solution of **SI-8** (50 mg, 0.11 mmol) in DMF (2.5 mL) was added potassium nitrite (94 mg, 1.1 mmol). The mixture was heated at 100°C and stirred for 4 h. The reaction was diluted with water, and extracted with chloroform. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure to give a yellow solid. Purification by column chromatography (ethyl acetate/ acetonitrile/ hexanes =15/5/1) yielded **SI-9** as a light yellow solid (70 mg, 20%). $[\alpha]_D^{16}$ +12.4° (c=0.125, MeOH); ^{1}H NMR (500MHz, $CDCl_3$) δ 11.93 (s, 1H); 8.28 (s, 1H); 7.08 (m, 1H); 6.78 (d, J = 5.1 Hz, 1H); 6.67 (s, 1H); 5.67 (m, 1H); 4.30 (m, 1H); 3.76 (m, 2H); 2.22 (s, 3H); 2.20(s, 3H); ^{13}C NMR (125MHz, $CDCl_3$) δ 171.1, 170.0, 158.8, 155.8, 152.9, 120.6, 115.5, 89.1, 82.6, 72.3, 70.5, 51.6, 21.1, 20.7; HRMS (FAB, matrix NBA): calcd for $C_{14}H_{16}O_8N_9$ $[M+H]^+$ 438.1122, found 438.1130.

Synthesis of 5'-azido-5'-deoxy-8-nitroguanosine (8)

To a solution of **SI-9** (5.0 mg, 0.011 mmol) in methanol (0.5 mL) was added 40% methylamine methanol solution (0.25 mL). After stirring at room temperature for 30 minutes, the reaction mixture was concentrated under reduced pressure to give **8** as a red solid (5.1 mg,

quant.). This compound was used for next reaction without purification. $[\alpha]_D^{16}$ +10.8° (c=0.100, DMSO); ^{1}H NMR (500MHz, CD_3OD) δ 6.53 (d, J = 4.5 Hz, 1H); 5.22 (dd, J = 4.5, 6.0 Hz, 1H); 4.54 (t, J = 6.0 Hz, 1H); 4.08 (m, 1H); 3.66 (dd, J = 6.9, 13.2 Hz, 1H); 3.54 (dd, J = 3.6, 13.2 Hz, 1H); ^{13}C NMR (125MHz, $CDCl_3$) δ 161.8, 159.3, 153.8, 143.7, 116.6, 91.2, 84.2, 71.6, 71.5, 52.1; HRMS (FAB, matrix NBA): calcd for $C_{10}H_{12}O_6N_9$ $[M+H]^+$ 354.0911, found 354.0914; HPLC purity 95.8 %.

Synthesis of dye 11

To a solution of fluoresceinamine (150 mg, 0.43 mmol) in pyridine (2.0 mL) were added 10-undecynoic acid (79 mg, 0.43 mmol, 1.0 eq) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, 83 mg, 0.43 mmol). After stirring at room temperature for 13 h, the reaction mixture was poured into water, and acidified (pH < 2) by conc. HCl. After stirring for 1 h, precipitate was collected by filtration, and washed with water. The precipitate was recrystalized from hexanes/EtOAc to obtain **11** (79 mg, 36 %) as orange crystals. ^{1}H NMR (300MHz, DMSO-d_6) δ 10.33 (s, 1H); 10.10 (s, 2H); 8.32 (d, J = 1.8 Hz, 1H); 7.82 (dd, J = 1.8, 8.4 Hz, 1H); 7.19 (d, J = 8.7 Hz, 1H); 6.67 (d, J = 2.1 Hz, 2H); 6.56 (complex, 4H); 2.73 (t, J = 2.7 Hz, 1H); 2.37 (t, J = 7.2 Hz, 2H); 2.14 (dt, J = 2.7, 6.9 Hz, 2H); 1.63 (m, 2H); 1.44-1.30 (complex, 10H); MS (FAB, matrix NBA): m/z 512 $[M+H]^+$.

7. Procedures for preparation of the cell lysate, and reaction of the lysate with **8**.

Preparation of C6 cell lysate

C6 rat glioma cells cultured in a 100 mm dish were suspended in 500 μL PBS (10 mM Na phosphate buffer pH 7.4 and 150 mM NaCl) containing protease inhibitors and sonicated (5 sec×5) on ice. The suspension was centrifuged for 15 min at 20,000g. The supernatant was collected and dialyzed with 10 kDa cut off membrane against PBS (500 mL × 3) to remove low molecular weight thiol-containing compounds. Dithiothreitol was added to a final concentration became 0.1 mM. The solution was concentrated by ultrafiltration with Ultrafree-MC (5000 cut, Millipore, Bradford, MA). The protein concentration was measured by using a BCA protein assay kit (Pierce, Rockford, IL).

Reaction of compound 8 with C6 cell lysate, and fluorescent labeling of the product by the Huisgen reaction

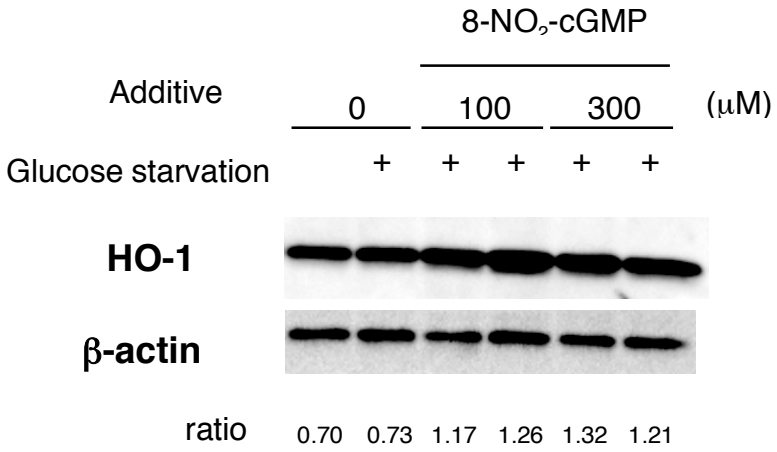
A 0.1 M solution of 5'-azido-5'-deoxy-8-nitroguanosine **8** (200 μL) in aqueous 0.6 M Na_2HPO_4 solution was added C6 cell lysate (20 μL, protein concentration 2.08 mg/mL).

After stirring at room temperature for 5 h, the reaction mixture was supplemented with 0.1% SDS aqueous solution and concentrated with a Microcon™ (Millipore, 10000 cut). The cell lysate treated with **8** was mixed with 0.5 M sodium phosphate buffer pH 8.0 (40 μg in 100 μl). Dye **11** (200 μg in acetonitrile 5 μl) was added and the solution was mixed gently. An acetonitrile solution of copper (I) bromide (10 mg/mL, 6.6 μL) was premixed with tris[(1-benzyl-1*H*-1,2,3-triazole-4-yl)methyl]amine (120 mg/mL in acetonitrile, 2.5 μL). The resulting Cu-complex solution (9 μL) was added to the reacted lysate mixture, and stirred overnight at room temperature. The reaction mixture was then centrifuged to remove residual Cu salt and dye. The supernatant was transferred to Microcon™ system (10000 cut, Millipore), washed with 0.1% SDS aqueous solution and concentrated. The labeled proteins on SDS-PAGE gel were quantitated by an FLA-2000 fluorescent image analyzer (Fuji Film, Tokyo, Japan).

8. Experimental procedures for detection of heme oxygenase-1 (HO-1) induction by compounds **1**, **2**, **7** and **8** in cells

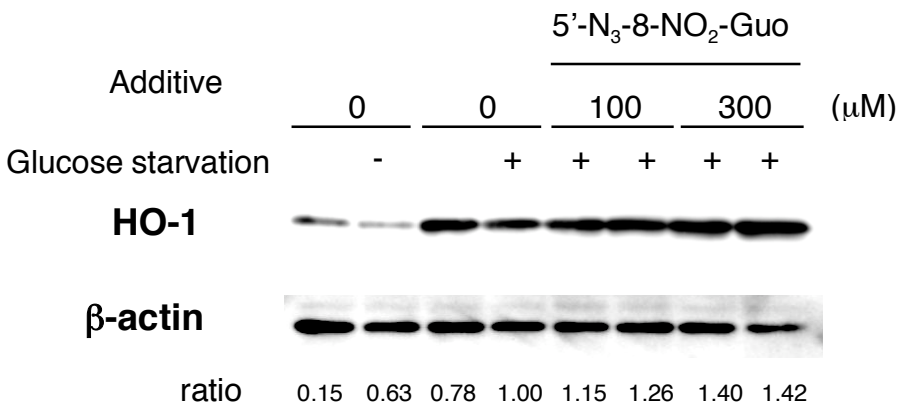
Human hepatoma cells (HepG2) were plated at densities of 1 x 10^5 cells/well in 12-well plates, and were cultured overnight at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin-streptomycin, 1% nonessential amino acid, and 10% fetal bovine serum, under a humidified 5% CO_2 atmosphere. Cells were then treated with nitroguanosine derivatives for 8 h in DMEM without glucose for the glucose starvation. The cells were lysed with 10 mM Tris-HCl (pH 7.4), containing 1% NP-40, 0.1 % deoxycholate, 0.1 % SDS, 150 mM NaCl, 1% glycerol, 0.1 mM DTPA, 1 mM amidinophenylmethanesulfonyl fluoride hydrochloride, 0.5 μg/mL leupeptin, and 0.1 mM E64. The cell lysates were subjected to SDS-PAGE and expression level of HO-1 was accessed by immunoblotting with anti-heme oxygenase 1 antibody (rabbit polyclonal antibody: SPA-896; Stressgen Bioreagents, MI). Immunoreactive bands were detected by using a chemiluminescence reagent (ECL Plus; GE Healthcare, Amersham Biosciences, Uppsala, Sweden) with a luminescent image analyzer (LAS1000UV mini; Fuji Film).

9. Figure S6: Heme oxygenase-1 induction by 8-NO_2-cGMP **1**.



HepG2 cells were treated with indicated concentration of **1** under glucose-starved condition for 8 h, and then were analysed by Western blotting. As a control, expression of β-actin is shown.
The ratio represents relative intensity of HO-1 and β-actin.

10. Figure S7: Heme oxygenase-1 induction by 5'-azido-5'-deoxy-8-nitroguanosine **8**.



HepG2 cells were treated with indicated concentration of **8** under glucose-starved condition for 8 h, and then were analysed by Western blotting. As a control, expression of β-actin is shown. The ratio represents relative intensity of HO-1 and β-actin.