Supporting Information

Caged Molecular Glues as Photoactivatable Tags for Nuclear Translocation of Guests in Living Cells

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1. General

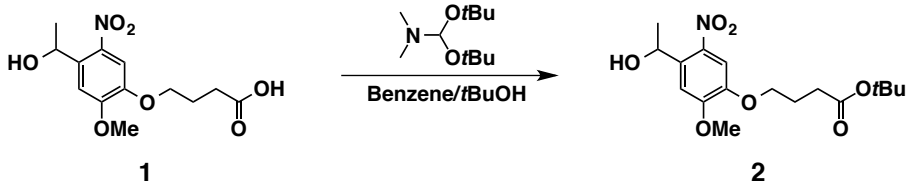
^{1}H and ^{13}C NMR spectra were recorded on a JEOL type GSX-270 or GSX-500 spectrometer, where chemical shifts for ^{1}H NMR spectroscopy were determined with respect to non-deuterated solvent residues; $CHCl_3$ (δ 7.26), $CHD_2(CD_3)SO$ (δ 2.50), CHD_2OD (δ 3.31), and HDO (δ 4.79), and those for ^{13}C NMR spectroscopy were determined with respect to $CDCl_3$ (δ 77.2), $(CH_3)_2SO$ (δ 39.5), and CD_3OD (δ 49.5). Matrix-assisted laser desorption/ionization time-of-flight mass (MALDI-TOF-MS) spectrometry was performed using α-cyano-4-hydroxy cinnamic acid (CCA) or sinapic acid (SA) as a matrix on an Applied Biosystems BioSpectrometry Workstation™ model Voyager-DE™ STR spectrometer or a Bruker Daltonics Autoflex™ Speed MALDI-TOF/TOF spectrometer. Electrospray ionization mass (ESI-MS) spectrometry was performed on a Thermo Scientific model Exactive orbitrap mass spectrometer. Recycling preparative gel permeation chromatography (GPC) was performed on a Japan Analytical Industry model LC-918 high performance liquid chromatography using a column set consisting of JAIGEL 1H, 2H, and 2.5H. Normal-phase column chromatography was performed using Kanto Chemical silica gel 60 N (particle size 63–210 μm) or Merck alumina 90 standardized. Photoirradiation was performed on Asahi Spectra models LAX-102 100-W or MAX-301 300-W xenon light sources and a 365 nm bandpass filter. Fluorescence spectra were recorded on a JASCO type FP-8500 spectrofluorometer. Confocal laser scanning microscopy was performed on a Carl-Zeiss model LSM 510 or a Leica model TCS SP8 confocal laser-scanning microscopes. Image analysis of agarose gels was performed on a FUJIFILM model LAS-3000 luminescent image analyzer. Electronic absorption and luminescence spectra were recorded on a Molecular Devices SpectraMax® Paradigm® multi-mode microplate detection platform. Flow cytometry analysis was conducted on a BD model LSR II cell analyzer. Zeta potential measurements were performed using a Malvern model Zetasizer Nano ZS particle size analyzer equipped with a 633 nm He-Ne laser light source.

Unless otherwise noted, reagents and solvents were used as received from commercial sources without further purification. Linearized plasmid DNA (l-pUC19; 2686 bp) was purchased from Clontech. TAMRA-labeled DNA (DNA-TAMRA) and its complementary strand were purchased from Operon Biotechnologies. Human hepatocellular carcinoma Hep3B cells (HB-8064) were purchased from ATCC. Eagle's minimal essential medium (EMEM) and 0.25% trypsin-EDTA were purchased from Life Technologies. Dulbecco's phosphate buffer saline (D-PBS) was purchased from Wako Pure Chemical Industries. Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific. Cell Counting Kit-8 and

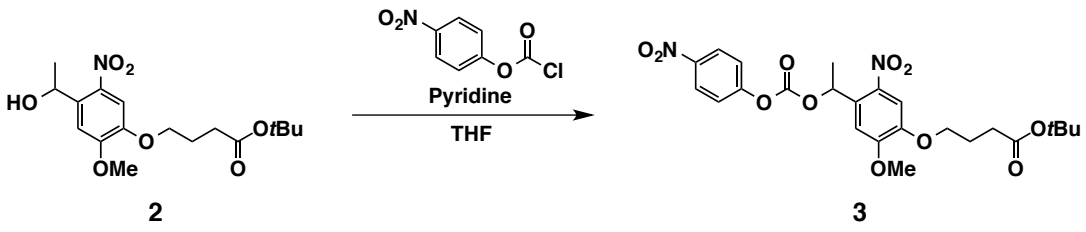
Hoechst 33342 were purchased from Dojindo. Lysotracker® Red was purchased from Lonza. DBCO-NHS ester and azide-PEG4-NHS ester were purchased from Click Chemistry Tools. Q-dot 655 ITK was purchased from Invitrogen.

2. Synthesis

2-1. Synthesis of ^{Caged}Glue-NBD

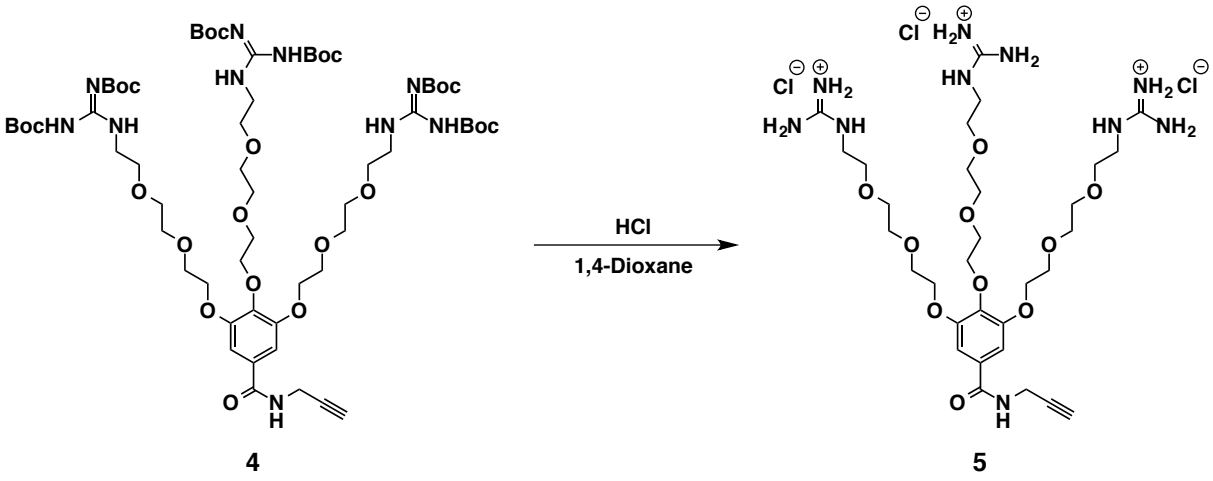


Compound 2. To a benzene/*t*BuOH (2.75 mL, v/v = 10/1) solution of **1** (1.00 g, 3.34 mmol) was added 1,1-di-*tert*-butoxytrimethylamine (6.1 mL, 25.5 mmol), and the mixture was stirred for 12 h at 80 °C. The reaction mixture was diluted with water (20 mL) and extracted with AcOEt (30 mL × 3). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel with AcOEt/hexane (1/2) as an eluent to allow isolation of **2** as yellow solid (700 mg, 59%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.45 (s, 9H; $C(CH_3)_3$), 1.57 (s, 3H; $CHCH_3$), 2.11–2.17 (m, 2H; $CH_2CH_2CH_2$), 2.28–2.29 (m, 1H; OH), 2.45 (t, J = 7.3 Hz, 2H; $COCH_2$), 3.98 (s, 3H; $ArOCH_3$), 4.08–4.11 (m, 2H; $ArOCH_2$), 5.54–5.59 (m, 1H; $ArCH(CH_3)OH$), 7.29 (s, 1H; ArH), 7.57 (s, 1H; ArH). ^{13}C NMR ($CDCl_3$; ppm): δ 24.5, 24.6, 28.2, 32.0, 56.5, 65.8, 68.6, 80.8, 108.8, 109.2, 137.3, 139.6, 147.1, 154.1, 172.5. MALDI-TOF-MS: *m*/*z* found: 338.08 ([M – *t*Bu + H + K^+] calcd: 338.06).

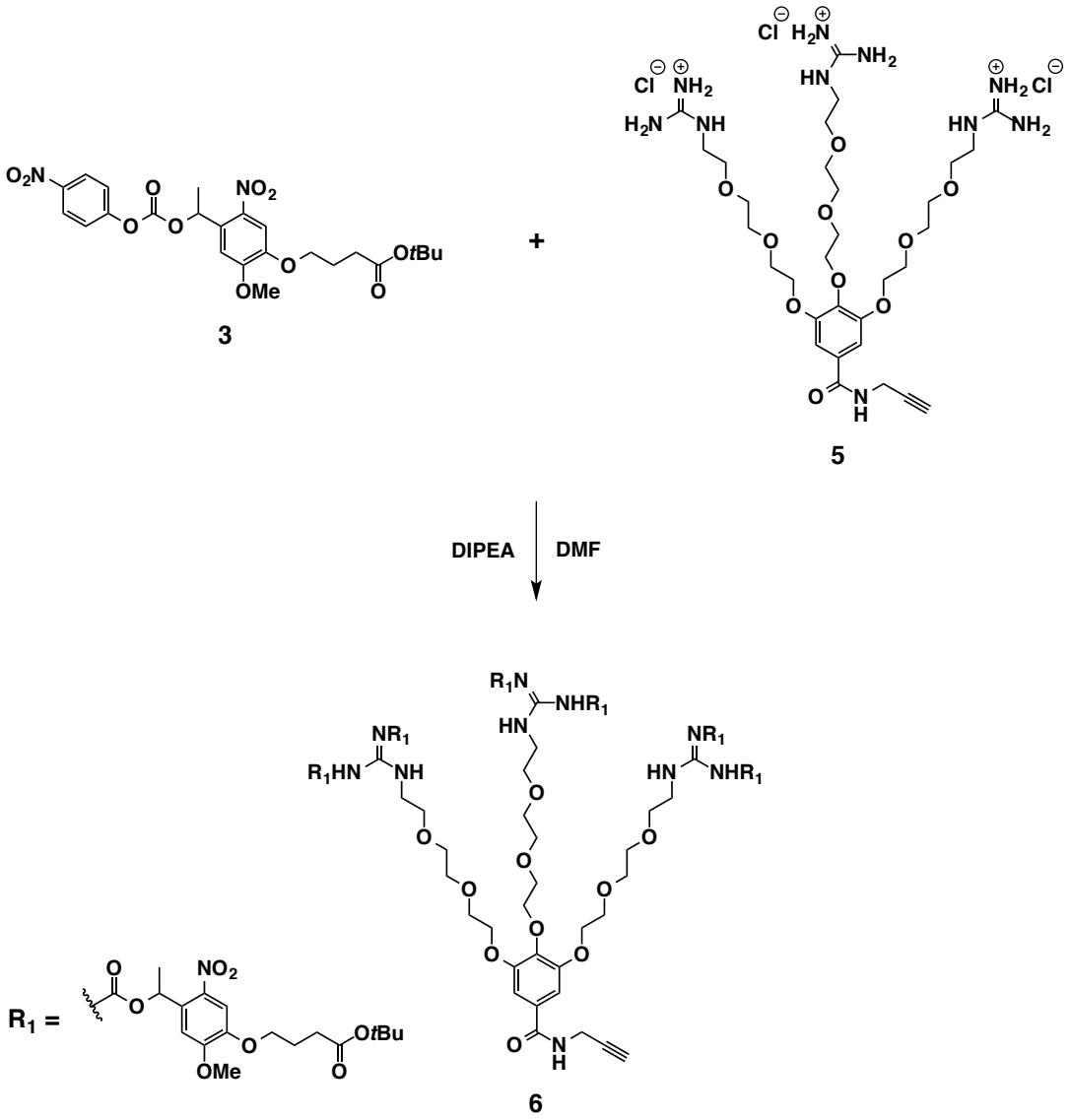


Compound 3. To a THF (2.5 mL) solution of a mixture of **2** (500 mg, 1.41 mmol) and pyridine (650 μL, 8.05 mmol) was dropwisely added a THF (2.5 mL) solution of 4-nitrophenyl chloroformate (529 mg, 2.62 mmol) at 0 °C under argon, and the mixture was stirred for 15 h at room temperature. Then, the reaction mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in AcOEt (50 mL) and washed with

saturated aqueous NH_4Cl (50 mL) followed by water (50 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel with AcOEt/hexane (1/4) as an eluent to allow isolation of **3** as yellow solid (665 mg, 91%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.46 (s, 9H; $C(CH_3)_3$), 1.77 (d, J = 6.3 Hz, 3H; $CHCH_3$), 2.10–2.20 (m, 2H; $CH_2CH_2CH_2$), 2.45 (t, J = 7.3 Hz, 2H; $COCH_2$), 4.00 (s, 3H; $ArOCH_3$), 4.12 (t, J = 6.3 Hz, 2H; $ArOCH_2$), 6.50–6.57 (m, 1H; $ArCH(CH_3)$), 7.11 (s, 1H; ArH), 7.35 (d, J = 9.0 Hz, 2H; ArH), 7.61 (s, 1H; ArH), 8.26 (d, J = 9.0 Hz, 2H; ArH). ^{13}C NMR ($CDCl_3$; ppm): δ 22.0, 24.3, 28.1, 31.7, 56.5, 68.4, 73.7, 80.5, 107.9, 108.9, 121.6, 125.3, 131.3, 139.9, 145.4, 147.8, 151.3, 154.1, 155.2, 172.1. MALDI-TOF-MS: m/z found: 543.37 ([M + Na^+] calcd: 543.16).

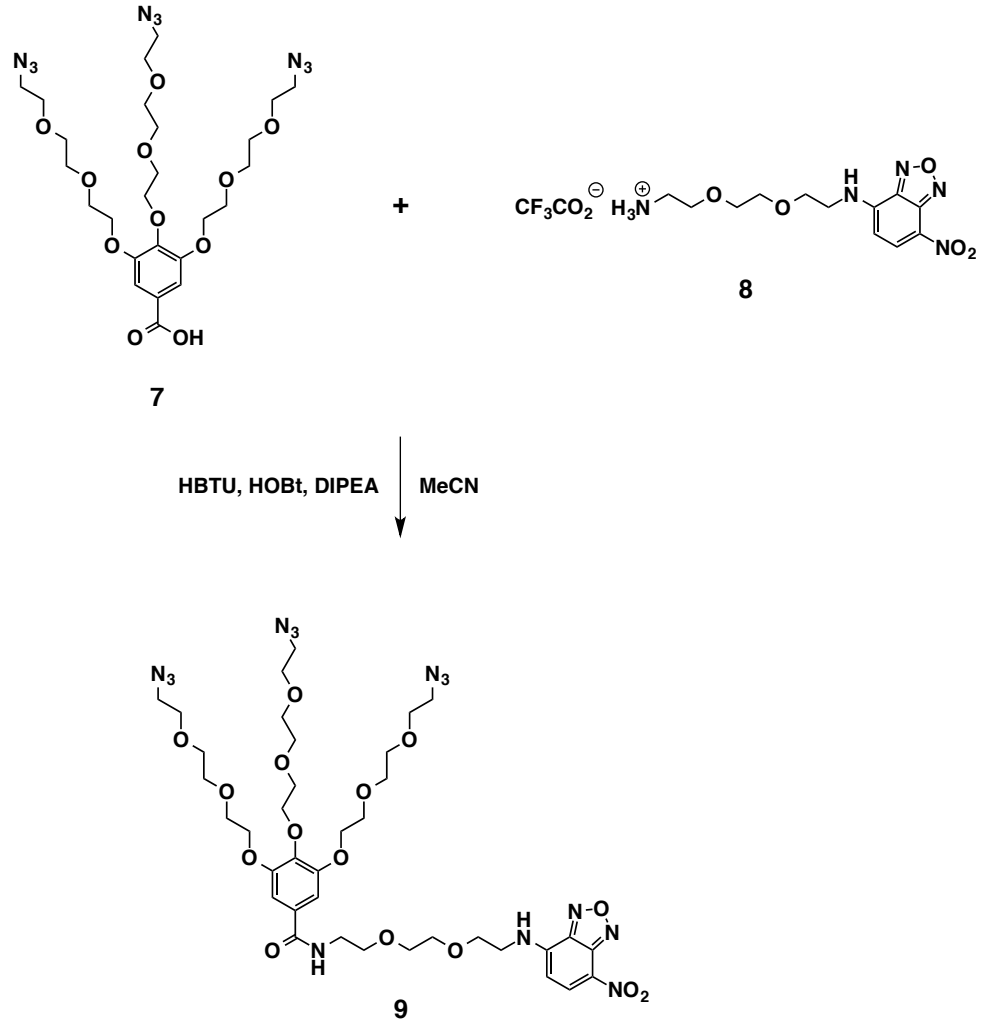


Compound 5. A 1,4-dioxane (1 mL) solution of HCl (4 M) was added to **4**[S1] (220 mg, 0.166 mmol), and the mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in MeOH (10 mL) and reprecipitated with Et_2O to allow isolation of **5** as colorless oil (140 mg, quant.). ^{1}H NMR (D_2O; ppm): δ 2.64 (t, J = 2.5 Hz, 1H; CH_2CCH), 3.34 (t, J = 5.0 Hz, 6H; CH_2NHCN), 3.61–3.95 (m, 24H; OCH_2), 4.14 (d, J = 3.0 Hz, 2H; $ArCONHCH_2$), 4.29–4.30 (m, 6H; $ArOCH_2$), 7.17 (s, 2H; ArH). ^{13}C NMR (D_2O; ppm): δ 38.7, 41.9, 69.1, 69.5, 69.9, 70.2, 70.4, 70.9, 72.4, 72.6, 72.7, 80.4, 107.3, 129.7, 140.6, 152.6, 157.9, 169.8. MALDI-TOF-MS: m/z found: 727.64 ([M – 3HCl + H^+] calcd: 727.41).



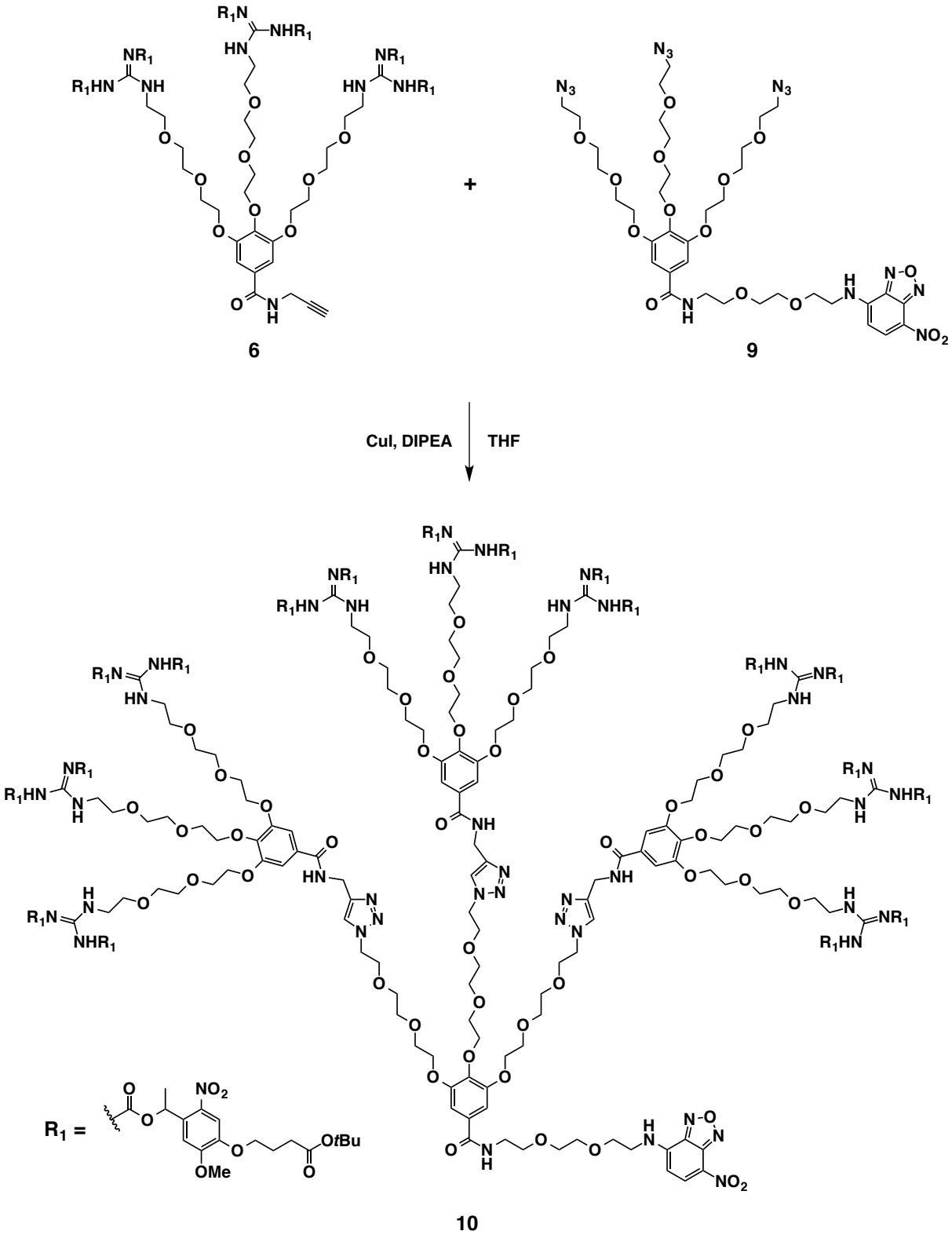
Compound 6. To a DMF (1 mL) solution of **5** (20 mg, 24 μmol) was successively added **3** (150 mg, 0.288 mmol) and diisopropylethylamine (DIPEA, 50 μL, 0.29 mmol), and the mixture was stirred overnight at 60 °C. Then, **3** (150 mg, 0.288 mmol) and DIPEA (50 μL, 0.29 mmol) were successively added to the reaction mixture, and the resultant mixture was stirred for 2 days at 60 °C. The reaction mixture was diluted with AcOEt (20 mL) and washed with water (20 mL × 3) followed by brine (20 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel with AcOEt/hexane (1/2 to 1/0) as an eluent to allow isolation of **6** as brown oil (20 mg, 28%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.43 (s, 54H; $C(CH_3)_3$), 1.57–1.68 (m, 18H; $CHCH_3$), 2.08–2.13 (m, 12H; $CH_2CH_2CH_2$), 2.40–2.44 (m, 12H; $COCH_2$), 3.51–4.34 (m, 69H; OCH_2, $ArOCH_2$,

$ArOCH_3$, CH_2NHCN, $ArCONHCH_2$, CH_2CCH), 6.35–6.46 (m, 6H; $ArCH(CH_3)$), 6.93–7.12 (m, 8H; ArH), 7.53–7.56 (m, 6H; ArH), 8.48–8.53 (m; guanidine-H), 9.07–9.14 (m; guanidine-H), 9.39 (br; guanidine-H), 11.69 (s; guanidine-H). ^{13}C NMR ($CDCl_3$; ppm): δ 21.2, 21.9, 22.1, 22.3, 22.4, 24.5, 24.5, 28.2, 31.8, 31.9, 41.1, 44.4, 56.6, 56.6, 56.7, 68.5, 68.8, 68.9, 69.3, 69.7, 69.8, 69.9, 70.6, 70.7, 71.0, 71.2, 71.3, 72.1, 72.2, 72.4, 72.4, 80.7, 80.7, 107.0, 108.3, 108.4, 108.6, 108.9, 128.9, 135.0, 139.5, 139.7, 139.8, 147.1, 147.2, 147.7, 154.2, 154.3, 154.4, 155.0, 155.1, 156.2, 160.6, 162.8, 163.0, 167.8, 172.3, 172.3. MALDI-TOF-MS: *m*/*z* found: 3035.83 ([M + Na^+] calcd: 3036.25), 2653.11 ([M – R_1 + H + Na^+] calcd: 2655.10), 2249.25 ([M – $2R_1$ + 2H + H^+] calcd: 2251.98), 1868.39 ([M – $3R_1$ + 3H + H^+] calcd: 1870.84).



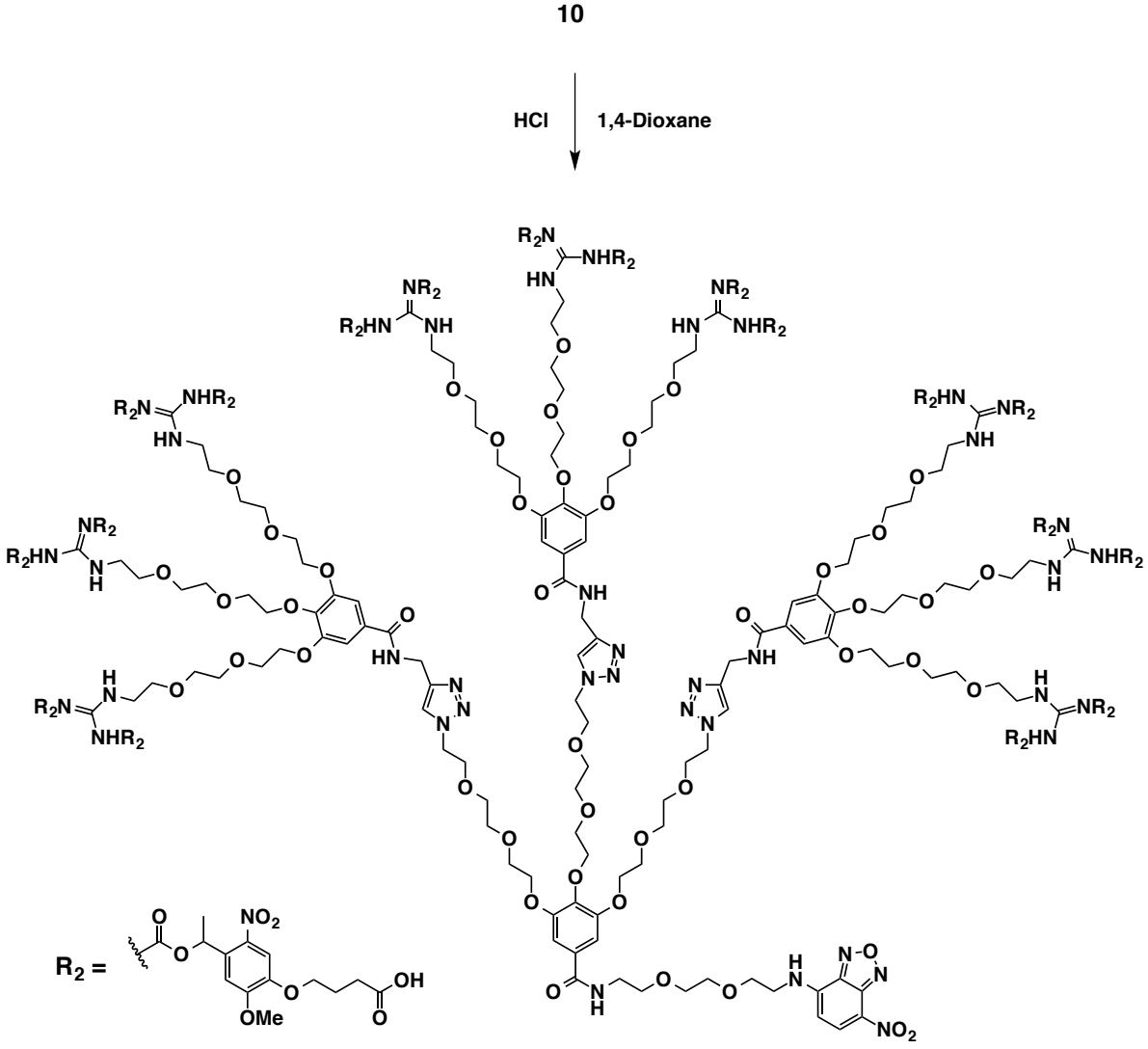
Compound 9. To a dry MeCN (10 mL) solution of a mixture of **7**[S2] (500 mg, 0.779 mmol)

and **8**[S3] (243 mg, 0.571 mmol) was successively added diisopropylethylamine (DIPEA, 386 μL, 2.24 mmol), 1-hydroxybenzotriazole monohydrate (HOBt, 105 mg, 0.686 mmol), and *o*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, 300 mg, 0.791 mmol), and the mixture was stirred for 12 h at room temperature. Then, the reaction mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in $CHCl_3$ (30 mL) and washed successively with aqueous $NaHSO_4$ (2 M, 30 mL), brine, saturated aqueous $NaHCO_3$ (30 mL), and brine (30 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on alumina with AcOEt as an eluent. The obtained fraction was subjected to recycling preparative GPC with $CHCl_3$ as an eluent to allow isolation of **9** as pale red oil (410 mg, 77%). ^{1}H NMR ($CDCl_3$; ppm): δ 3.39 (t, J = 5.0 Hz, 6H; CH_2N_3), 3.65–3.80 (m, 36H; OCH_2, $NHCH_2$), 4.17 (t, J = 5.0 Hz, 6H; $ArOCH_2$), 6.15 (d, J = 8.0 Hz, 1H; NBD), 7.00 (s, 2H; ArH), 8.48 (d, J = 9.0 Hz, 1H; NBD). ^{13}C NMR ($CDCl_3$; ppm): δ 39.8, 43.8, 50.7, 68.2, 69.2, 69.8, 69.9, 70.1, 70.3, 70.5, 70.6, 70.7, 70.8, 70.8, 72.5, 99.0, 107.1, 123.8, 129.7, 136.6, 141.5, 144.1, 144.1, 144.4, 152.5, 167.2. MALDI-TOF-MS: *m*/*z* found: 973.21 ([M + K^+] calcd: 973.35), 957.24 ([M + Na^+] calcd: 957.38).



Compound 10. To a THF (2 mL) solution of a mixture of **6** (51 mg, 17 μmol), **9** (4.8 mg, 5.1 μmol), and diisopropylethylamine (DIPEA, 10 μL, 58 μmol) was added copper(I) iodide (3.2 mg, 17 μmol), and the mixture was stirred for 15 h at room temperature. Then, the reaction

mixture was diluted with $CHCl_3$ (10 mL) and washed with saturated aqueous NH_4Cl (20 mL × 3) followed by brine (20 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was subjected to recycling preparative GPC twice with $CHCl_3$ as an eluent to allow isolation of **10** as orange oil (35 mg, 68%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.44 (s, 162H; $C(CH_3)_3$), 1.60–1.68 (br, 54H; $CHCH_3$), 2.09–2.13 (m, 36H; $CH_2CH_2CH_2$), 2.40–2.44 (m, 36H; $COCH_2$), 3.59–3.80 (br, 126H; OCH_2, CH_2NH), 3.90–3.95 (m, 54H; $ArOCH_3$), 4.08–4.12 (br, 54H; $ArOCH_2$), 4.47 (s, 6H; triazole-CH_2NH), 4.62 (s, 6H; OCH_2CH_2NH), 6.10 (s, 1H; NBD), 6.35–6.44 (br, 18H; $ArCH(CH_3)$), 6.96–7.13 (br, 26H; ArH), 7.54 (s, 18H; ArH), 7.72 (s, 1H; NBD), 8.48–8.51 (br, 3H; triazole-H). ^{13}C NMR ($CDCl_3$; ppm): δ 21.7, 21.9, 22.1, 24.4, 28.1, 29.6, 31.7, 41.0, 44.2, 50.2, 56.5, 68.3, 68.7, 69.1, 69.5, 70.1, 70.5, 72.0, 72.3, 80.5, 108.2, 108.7, 123.8, 131.6, 134.9, 139.6, 147.0, 147.6, 152.0, 152.4, 154.2, 154.9, 156.1, 160.7, 162.9, 172.2.



[Caged]Glue-NBD. A 1,4-dioxane (12 mL) solution of HCl (4 M) was added to **10** (41 mg, 4.4 μmol), and the mixture was stirred overnight at room temperature. Then, hexane (40 mL) was added to the reaction mixture, and the resultant suspension was filtered. The insoluble fraction, obtained by filtration, was dissolved in $CHCl_3$ and reprecipitated three times with Et_2O to allow isolation of **[Caged]Glue-NBD** as orange oil (34 mg, 85%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.42–1.78 (m, 54H; CHCH_3), 2.15 (br, 36H; $CH_2CH_2CH_2$), 2.53 (br, 36H; CH_2CO_2H), 3.30–4.35 (m, 240H; OCH_2, $ArOCH_2$, $ArOCH_3$, CH_2NH), 4.48 (br, 6H; triazole-CH_2NH), 4.61 (br, 6H; OCH_2CH_2-triazole), 6.06–6.56 (m, 19H; ArCH(CH_3), NBD), 6.86–7.23 (m, 26H; ArH), 7.43–7.59 (m, 18H; ArH), 7.91 (br, 3H; triazole-H), 8.33 (br, 1H; NBD), 8.49 (br; guanidine-H), 8.76 (br; guanidine-H), 9.94 (br; guanidine-H), 11.71 (br; guanidine-H). ^{13}C NMR ($CDCl_3$; ppm): δ 22.0, 22.3, 24.3, 30.4, 41.9, 51.8, 56.8, 56.9, 68.3, 68.7, 68.9, 69.8,

70.3, 70.6, 71.1, 71.7, 72.4, 73.6, 106.8, 108.2, 108.3, 108.6, 108.6, 108.9, 130.9, 131.6, 138.9, 139.5, 139.7, 147.2, 147.4, 147.5, 147.7, 152.2, 152.6, 154.2, 154.4, 154.5, 154.8, 167.0, 173.5.

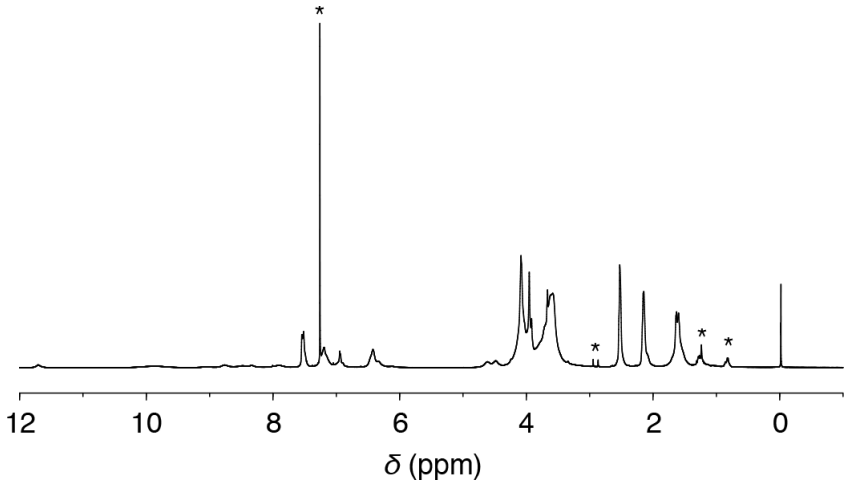


Figure S1. ^{1}H NMR spectrum of ^{Caged}Glue-NBD in $CDCl_3$ at 21 °C. The signals marked with asterisks at δ 0.82 and 1.23, 2.86 and 2.95, and 7.26 ppm are due to hexane, DMF, and $CHCl_3$, respectively.

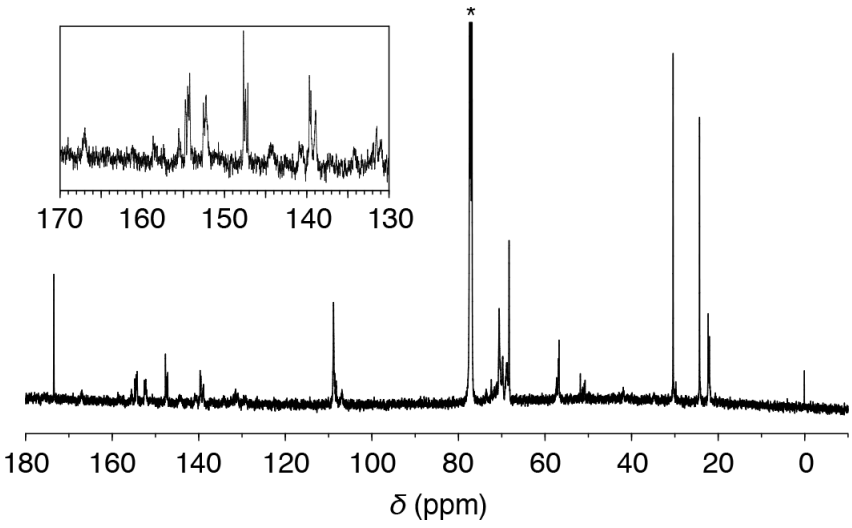
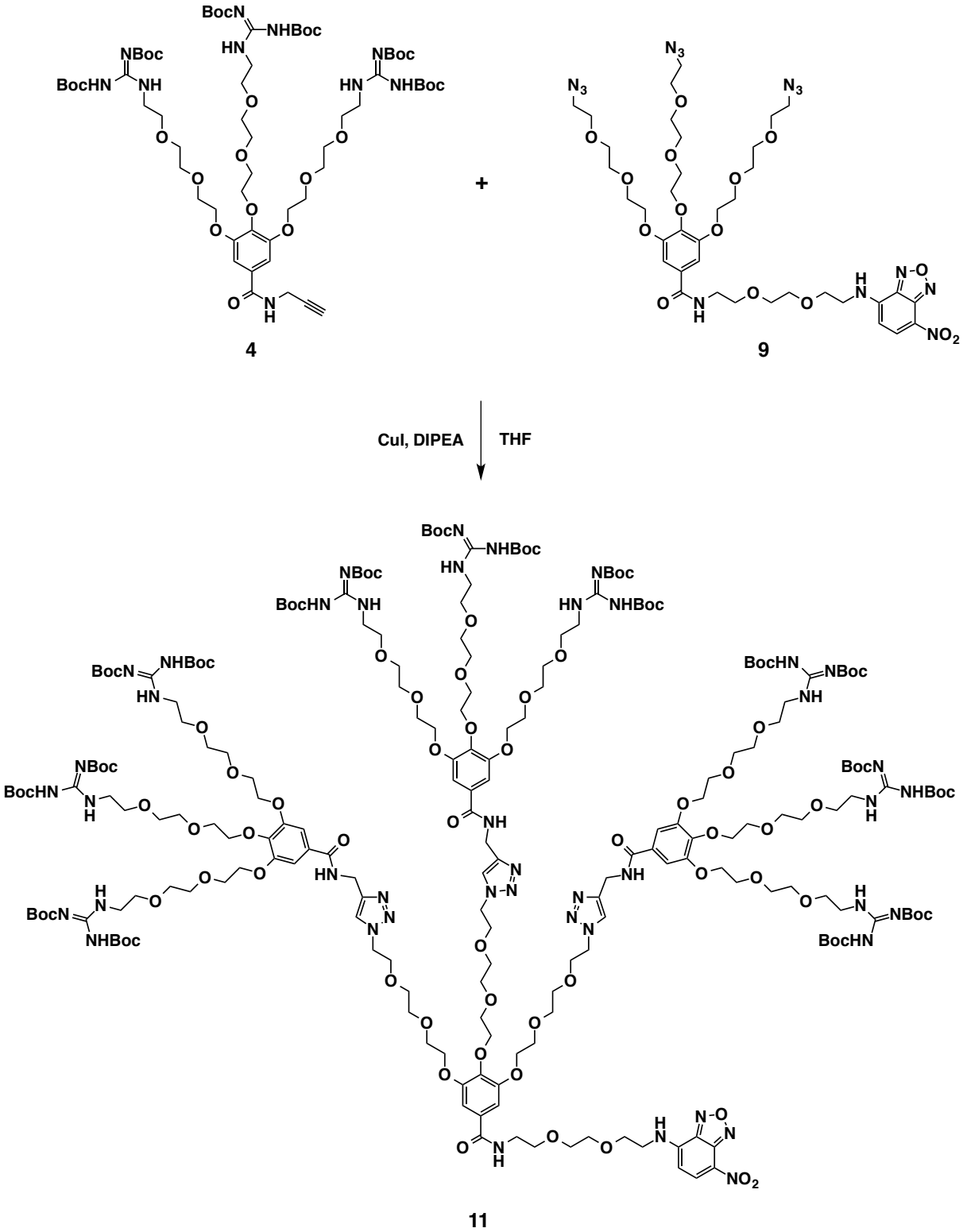


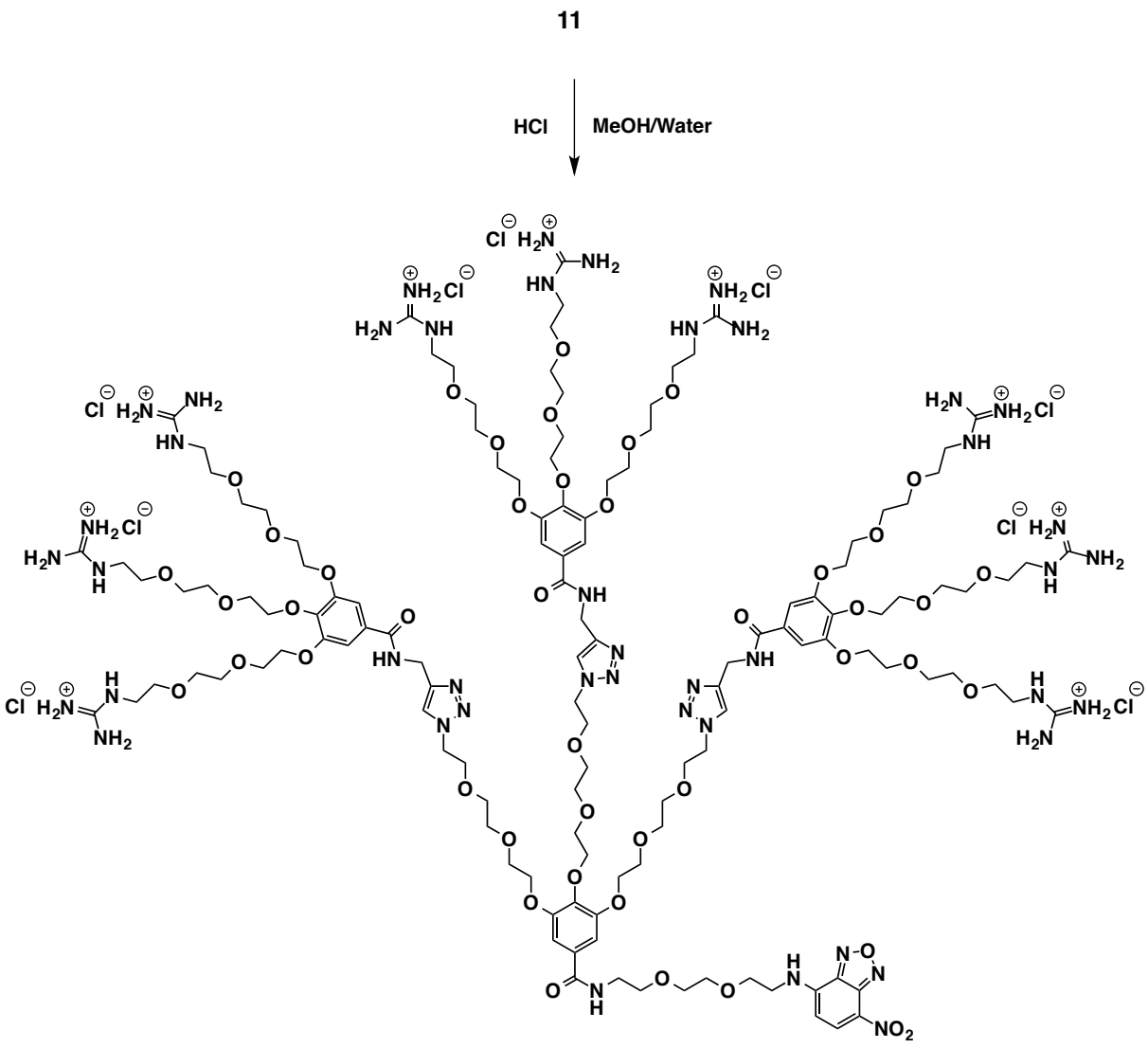
Figure S2. ^{13}C NMR spectrum of ^{Caged}Glue-NBD in $CDCl_3$ at 20 °C. The signal marked with an asterisk at δ 77.2 ppm is due to $CDCl_3$. The inset shows a magnified spectrum at δ 130–170 ppm.

2-2. Synthesis of Glue-NBD



Compound 11. To a THF (0.5 mL) solution of a mixture of **4** (24 mg, 18 μmol), **9** (5.0 mg, 5.4 μmol), and diisopropylethylamine (DIPEA, 4.0 μL, 23 μmol) was added copper(I) iodide

(CuI, 5.0 mg, 26 μmol), and the mixture was stirred for 7.5 h at room temperature. Then, THF (0.3 mL), DIPEA (4.0 μL, 23 μmol), and CuI (2.8 mg, 15 μmol) were successively added to the reaction mixture, and the resultant suspension was stirred for 3 days at room temperature. Then, $CHCl_3$ (1 mL) was added to the resultant mixture and filtered off with celite from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in $CHCl_3$ (2 mL) and washed with saturated aqueous NH_4Cl (2 mL × 2) followed by brine (2 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was subjected to recycling preparative GPC with $CHCl_3$ as an eluent to allow isolation of **11** as yellow-orange solid (15 mg, 56%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.46–1.48 (m, 162H; $C(CH_3)_3$), 3.58–3.83 (m, 126H; OCH_2), 4.06–4.15 (m, 24H; $ArOCH_2$), 4.44–4.48 (m, 6H; triazole-C*H*$_2$NH), 4.59–4.62 (m, 6H; OCH_2C*H*$_2$-triazole), 6.11 (br, 1H; NBD), 7.09–7.15 (m, 8H; ArH), 7.74–7.79 (m, 3H; triazole-H), 8.40 (d, J = 9.5 Hz, 1H; NBD), 8.59 (br, 9H; CH_2N*H*CN), 11.46 (br, 9H; $NHCO_2$). ^{13}C NMR ($CDCl_3$; ppm): δ 28.2, 28.4, 35.6, 40.7, 50.1, 68.9, 69.1, 69.5, 69.7, 69.9, 70.6, 70.7, 70.8, 70.9, 72.5, 79.4, 83.1, 107.0, 123.6, 129.2, 136.7, 141.7, 144.6, 145.1, 152.4, 152.6, 153.1, 156.4, 163.6, 167.0.



Glue-NBD

Glue-NBD. To a MeOH (2 mL) solution of **11** (14 mg, 2.9 μmol) was added hydrochloric acid (12 M, 2 mL), and the mixture was stirred for 15 h at room temperature. The reaction mixture was evaporated to dryness under reduced pressure, affording **Glue-NBD** as orange oil (12 mg, quant.). ^{1}H NMR (D_2O; ppm): δ 3.33 (br, 18H; CH_2NHCN), 3.43–3.92 (m, 114H; OCH_2), 4.10–4.14 (m, 18H; $ArOCH_2$), 4.49–4.57 (m, 12H; triazole-CH_2NH, OCH_2CH_2-triazole), 6.00 (br, 1H; NBD), 6.76–6.78 (m, 2H; ArH), 6.96–7.00 (m, 6H; ArH), 8.01–8.03 (m, 4H; triazole-H, NBD). ^{13}C NMR (D_2O; ppm): δ 34.2, 40.7, 48.6, 67.6, 68.3, 68.5, 68.9, 69.2, 69.6, 71.5, 105.4, 124.0, 127.7, 128.0, 138.9, 150.8, 151.1, 156.6, 167.7. MALDI-TOF-MS: *m*/*z* found: 3116.82 ([M – 9HCl + H^+] calcd: 3114.60).

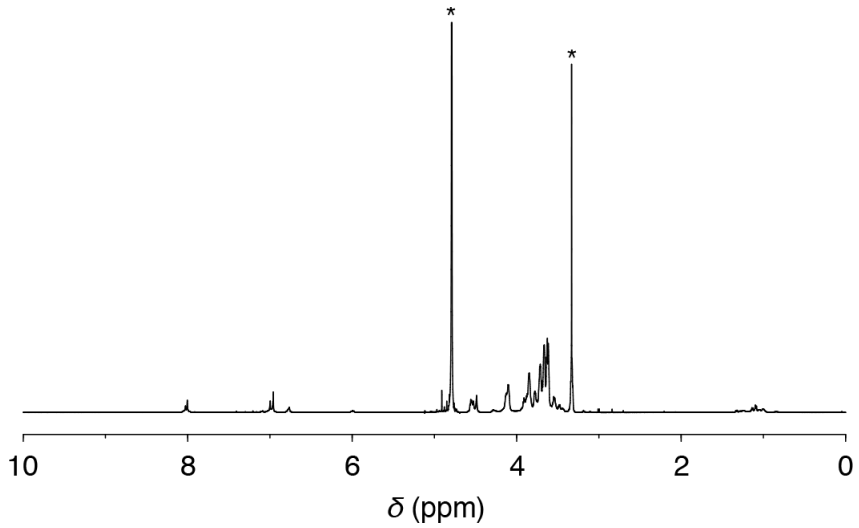


Figure S3. ^{1}H NMR spectrum of Glue-NBD in D_2O at 22 °C. The signals marked with asterisks at δ 3.34 and 4.79 ppm are due to CHD_2OD and water, respectively.

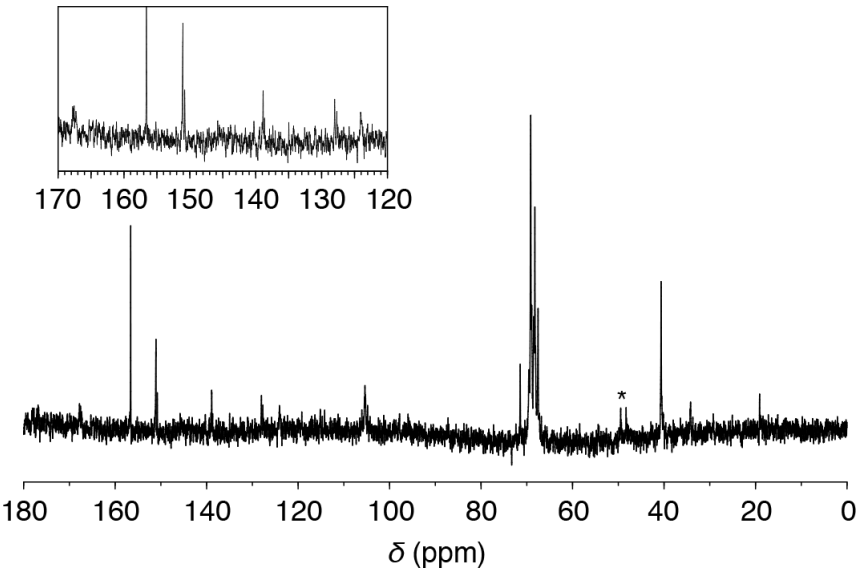
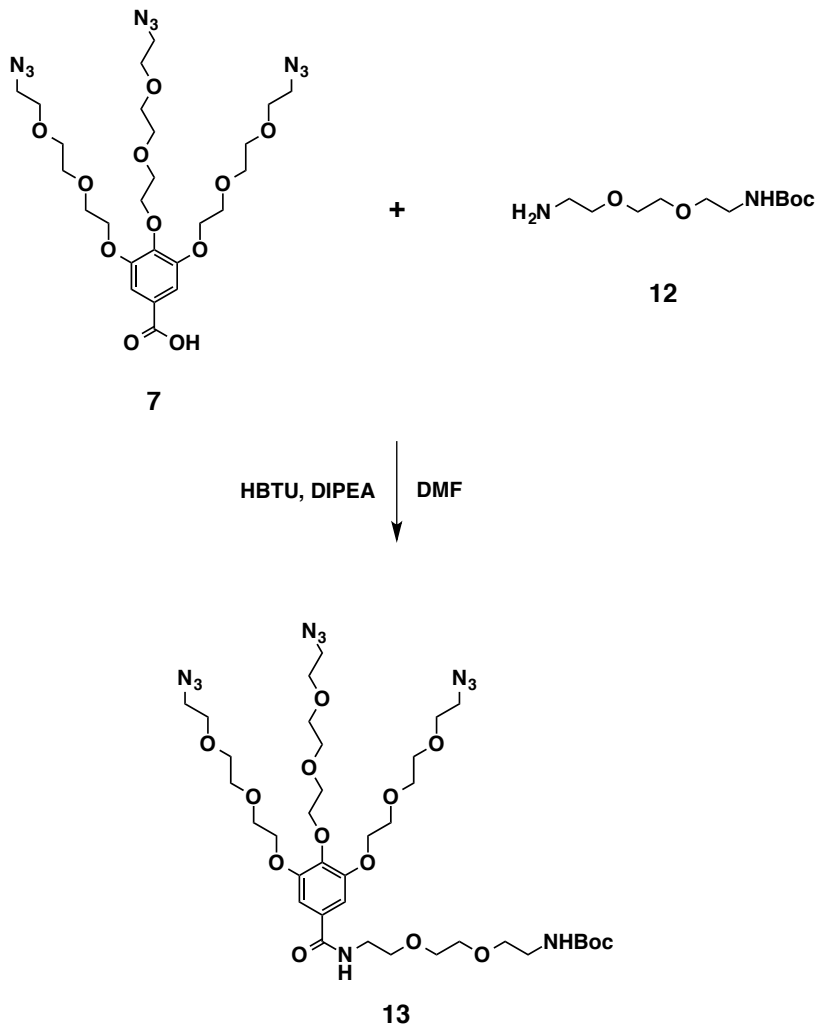


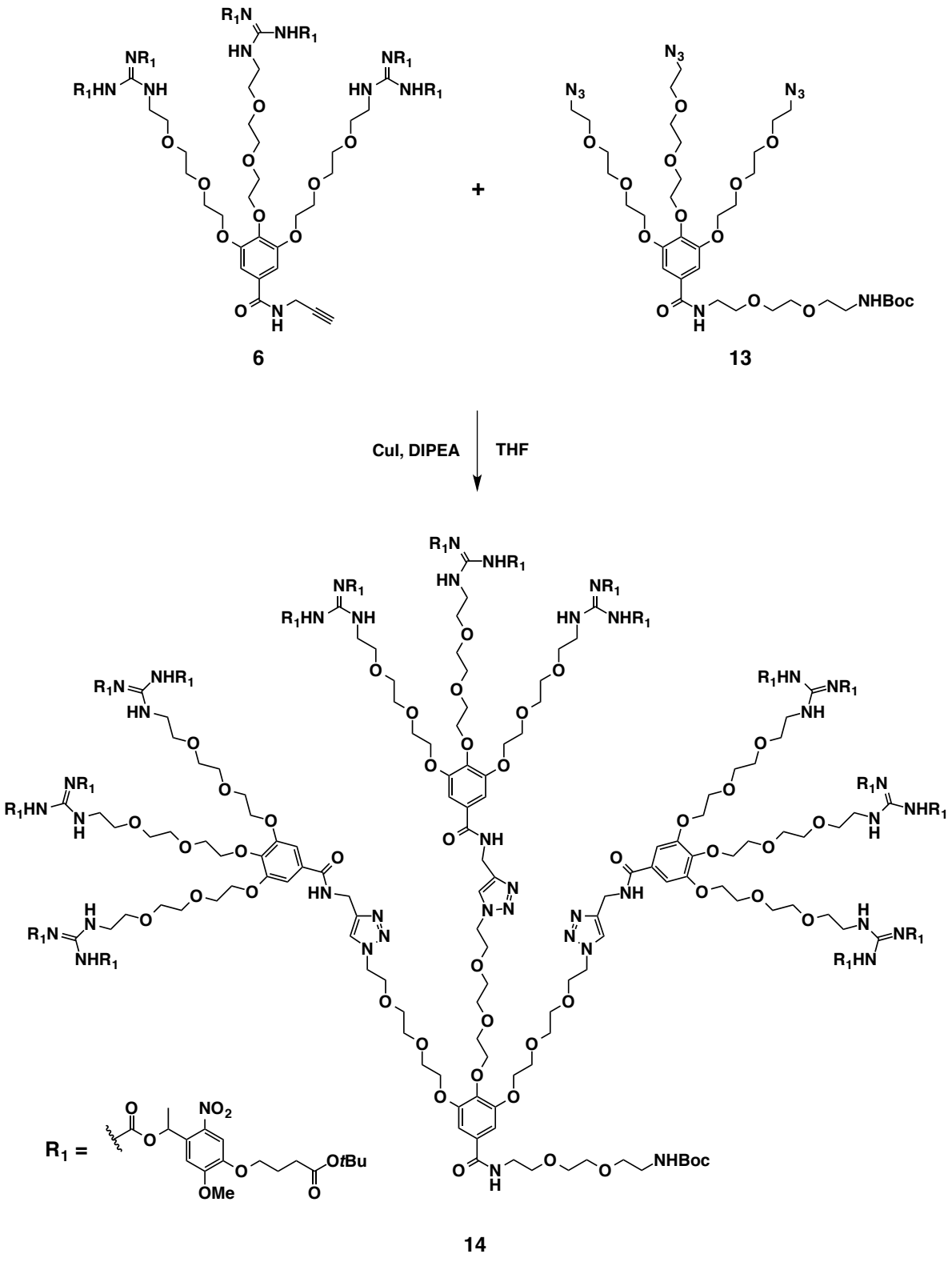
Figure S4. ^{13}C NMR spectrum of Glue-NBD in D_2O at 23 °C. The signal marked with an asterisk at δ 49.5 ppm is due to CD_3OD. The inset shows a magnified spectrum at δ 120–170 ppm.

2-3. Synthesis of ^{Caged}Glue-DBCO



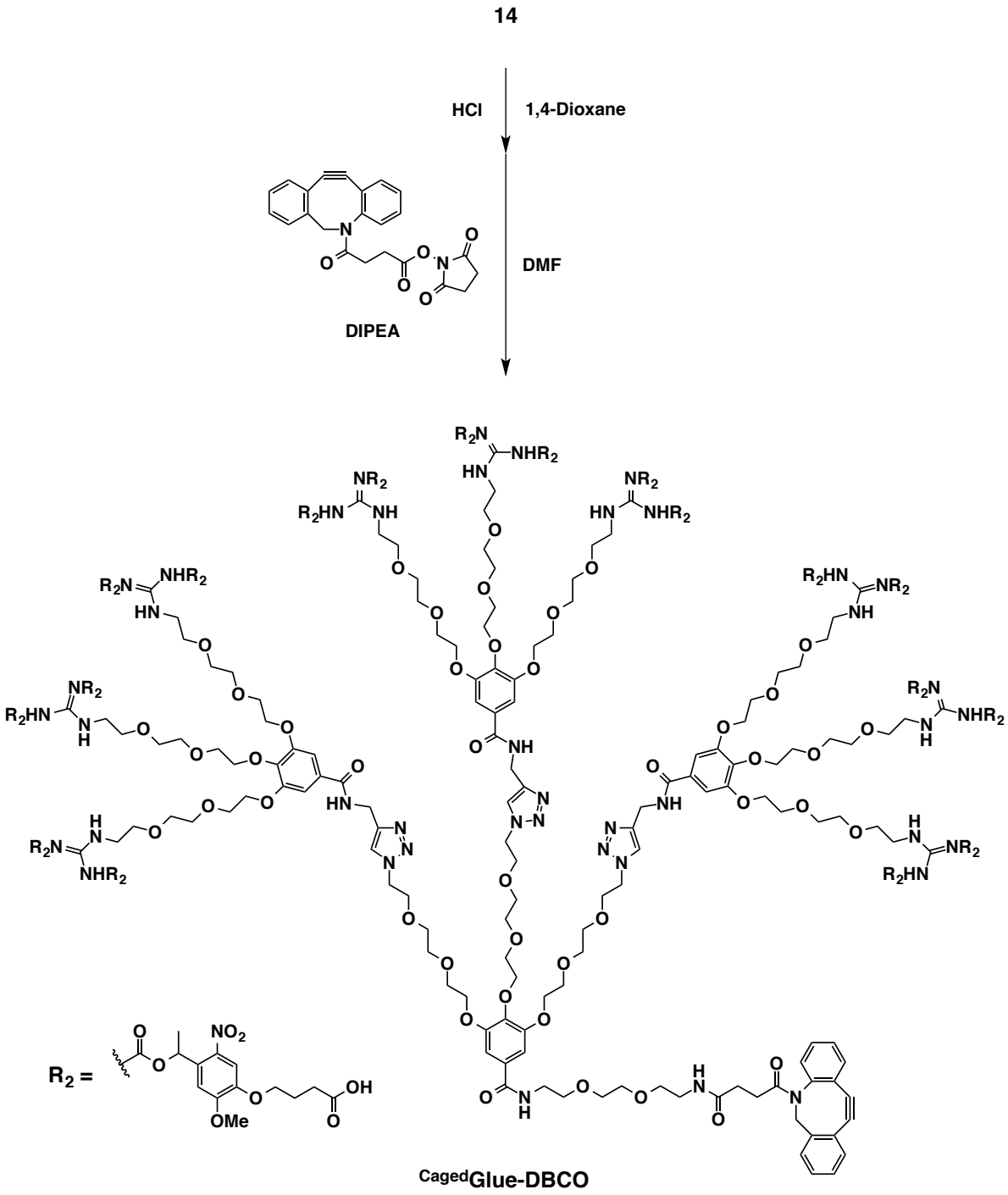
Compound 13. To a DMF (1.2 mL) solution of **7** (300 mg, 0.468 mmol) was added *o*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate (HBTU, 187 mg, 0.491 mmol), and the mixture was stirred for 5 min at room temperature. Then, a DMF (0.8 mL) solution of **12** (175 mg, 0.701 mmol) and diisopropylethylamine (DIPEA, 0.24 mL, 1.40 mmol) were successively added to the reaction mixture, and the resultant mixture was stirred for 70 h at room temperature. The reaction mixture was diluted with AcOEt (30 mL) and washed successively with aqueous $NaHSO_4$ (1 M, 30 mL), saturated aqueous $NaHCO_3$ (30 mL), water (30 mL), and brine (30 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on alumina followed by silica gel using AcOEt/MeOH (1/0 to 95/5) as an eluent to allow isolation of **13** as colorless oil (389 mg,

95%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.42 (s, 9H; $C(CH_3)_3$), 3.27–3.32 (m, 2H; CH_2NHCO_2), 3.38 (t, J = 4.8 Hz, 6H; CH_2N_3), 3.53–3.87 (m, 34H; OCH_2, $ArCONHCH_2$), 4.18–4.21 (m, 6H; $ArOCH_2$), 5.06 (br, 1H; $NHCO_2$), 6.68 (br, 1H; ArCONH), 7.06 (s, 2H; ArH). ^{13}C NMR ($CDCl_3$; ppm): δ 28.5, 40.0, 40.4, 50.8, 69.2, 69.9, 70.0, 70.2, 70.3, 70.4, 70.7, 70.8, 70.8, 70.9, 70.9, 72.5, 79.5, 107.2, 130.0, 141.5, 152.6, 156.1, 167.1. MALDI-TOF-MS: m/z found: 910.46 ([M + K^+] calcd: 910.40), 894.47 ([M + Na^+] calcd: 894.43).



Compound 14. To a THF (0.5 mL) solution of a mixture of **6** (36 mg, 12 μmol), **13** (3.1 mg, 3.6 μmol), and diisopropylethylamine (DIPEA, 6.0 μL, 35 μmol) was added copper(I) iodide (CuI, 5.0 mg, 26 μmol), and the mixture was stirred for 24 h at room temperature. Then, THF

(0.1 mL), DIPEA (4.8 μL, 28 μmol), and CuI (4.0 mg, 21 μmol) were successively added to the reaction mixture, and the resultant suspension was stirred for 22 h at room temperature. Then, $CHCl_3$ (1 mL) was added to the reaction mixture, and the resultant suspension was filtered off with celite from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in $CHCl_3$ (2 mL) and washed with saturated aqueous NH_4Cl (2 mL × 2) followed by brine (2 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was subjected to recycling preparative GPC with $CHCl_3$ as an eluent to allow isolation of **14** as white solid (15 mg, 43%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.43–1.44 (m, 171H; $C(CH_3)_3$), 1.57–1.74 (m, 54H; $CHCH_3$), 2.09–2.14 (m, 36H; $CH_2CH_2CH_2$), 2.40–2.44 (m, 36H; $COCH_2$), 3.22–3.26 (m, 2H; $OCH_2CH_2NHCO_2$), 3.45–4.25 (m, 238H; OCH_2, $ArOCH_2$, $ArOCH_3$, CH_2NHCN), 4.46–4.48 (m, 6H; triazole-CH_2NH), 4.63 (br, 6H; OCH_2CH_2-triazole), 5.15 (br, 1H; $OCH_2CH_2NHCO_2$), 6.35–6.45 (m, 18H; $ArCH(CH_3)$), 6.93–7.16 (m, 26H; ArH), 7.54–7.56 (m, 18H; ArH), 7.69–7.74 (m, 3H; triazole-H), 8.49–8.52 (m; guanidine-H), 9.11 (br; guanidine-H), 9.38 (br; guanidine-H), 11.69 (s; guanidine-H). ^{13}C NMR ($CDCl_3$; ppm): δ 21.9, 22.1, 22.4, 22.4, 22.8, 24.5, 24.5, 28.2, 28.5, 31.9, 34.6, 34.8, 41.1, 44.4, 50.2, 50.3, 56.6, 56.7, 56.8, 68.5, 68.7, 68.9, 69.3, 69.6, 69.7, 70.3, 70.6, 70.7, 70.9, 72.1, 72.2, 72.4, 72.5, 72.5, 72.7, 80.7, 106.8, 108.2, 108.3, 108.4, 108.9, 123.6, 131.8, 135.1, 139.5, 139.6, 139.7, 139.8, 147.1, 147.2, 147.7, 154.2, 154.3, 154.4, 155.1, 156.3, 160.6, 162.8, 162.9, 163.0, 166.9, 172.3.



[Caged]Glue-DBCO. A 1,4-dioxane (4 mL) solution of HCl (4 M) was added to **14** (15 mg, 1.5 μmol), and the mixture was stirred overnight at room temperature. Then, hexane (30 mL) was added to the reaction mixture, and the resultant suspension was filtered. The insoluble fraction, obtained by filtration, was dissolved in DMF (1 mL). Then, diisopropylethylamine (DIPEA, 2.0 μL, 12 μmol) and DBCO-NHS ester (2.4 mg, 6.0 μmol) were successively added to the resultant solution, and the mixture was stirred for 3 h at room temperature. DIPEA (2.0

μL, 12 μmol) and DBCO-NHS ester (1.0 mg, 2.5 μmol) were successively added to the reaction mixture, and the resultant mixture was stirred for 24 h at room temperature. Then, Et_2O (20 mL) was added to the reaction mixture, and the resultant suspension was filtered. The insoluble fraction, obtained by filtration, was dissolved in $CHCl_3$/MeOH (2 mL, v/v = 2/1) and reprecipitated with Et_2O. The precipitates formed were dissolved in $CHCl_3$ (2 mL), and the resultant solution was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, affording ^{Caged}**Glue-DBCO** as colorless oil (11 mg, 81%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.59–1.68 (m, 54H; CHC*H*$_3$), 1.89–1.92 (m, 1H; DBCO-COC*H*$_2$), 2.15–2.18 (m, 37 H; CH$_2$C*H*$_2$CH$_2$, DBCO-COC*H*$_2$), 2.37–2.42 (m, 1H; DBCO-COCH$_2$C*H*$_2$), 2.51–2.55 (m, 36H; C*H*$_2$CO$_2$H), 2.75–2.81 (m, 1H; DBCO-COCH$_2$C*H*$_2$), 3.25–3.26 (m, 2H; OCH$_2$C*H*$_2$NHCO), 3.34–4.25 (m, 238H; OCH$_2$, ArOCH$_2$, ArOCH$_3$, C*H*$_2$NHCN), 4.45 (br, 6H; triazole-C*H*$_2$NH), 4.60 (br, 6H; OCH$_2$C*H*$_2$-triazole), 5.06 (d, J = 13.5 Hz, 1H; DBCO-NCH$_2$), 6.34–6.44 (m, 18H; ArC*H*(CH$_3$)), 6.89–7.24 (m, 29H; ArH, DBCO-ArH), 7.34–7.38 (m, 3H; DBCO-ArH), 7.48–7.56 (m, 20H; ArH, DBCO-ArH), 7.73–7.77 (m, 3H; triazole-H), 8.51 (br; guanidine-H), 9.10 (br; guanidine-H), 9.37 (br; guanidine-H), 11.69 (br; guanidine-H). ^{13}C NMR ($CDCl_3$; ppm): δ 21.9, 22.1, 22.3, 22.4, 24.3, 24.4, 30.5, 41.1, 44.4, 51.9, 55.7, 56.6, 56.6, 56.7, 68.3, 68.9, 69.2, 69.7, 70.7, 72.1, 72.2, 72.5, 106.7, 108.4, 108.6, 108.9, 114.7, 125.5, 127.2, 127.8, 128.4, 128.8, 129.2, 131.9, 135.0, 135.2, 139.5, 139.6, 139.8, 147.0, 147.1, 147.6, 152.4, 154.4, 155.0, 156.2, 160.6, 162.8, 163.0, 173.5.

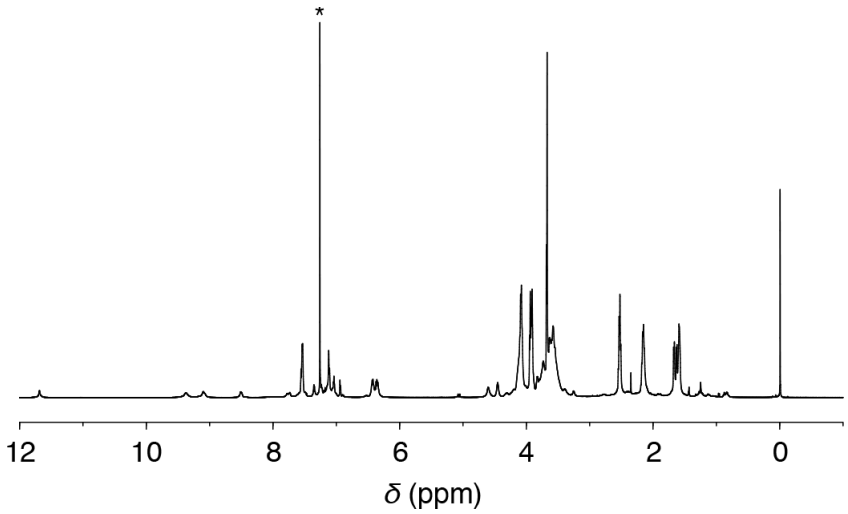


Figure S5. ^{1}H NMR spectrum of ^{Caged}Glue-DBCO in $CDCl_3$ at 21 °C. The signal marked with an asterisk at δ 7.26 ppm is due to $CHCl_3$.

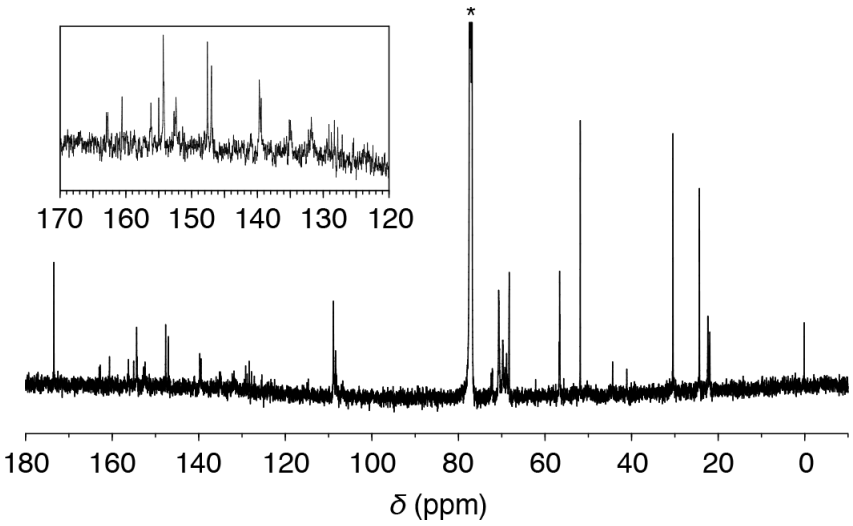
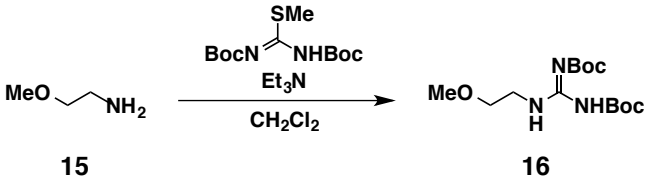


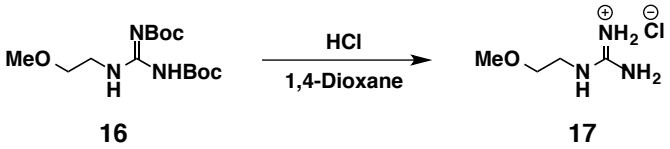
Figure S6. ^{13}C NMR spectrum of ^{Caged}Glue-DBCO in $CDCl_3$ at 21 °C. The signal marked with an asterisk at δ 77.2 ppm is due to $CDCl_3$. The inset shows a magnified spectrum at δ 120–170 ppm.

2-4. Synthesis of MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$



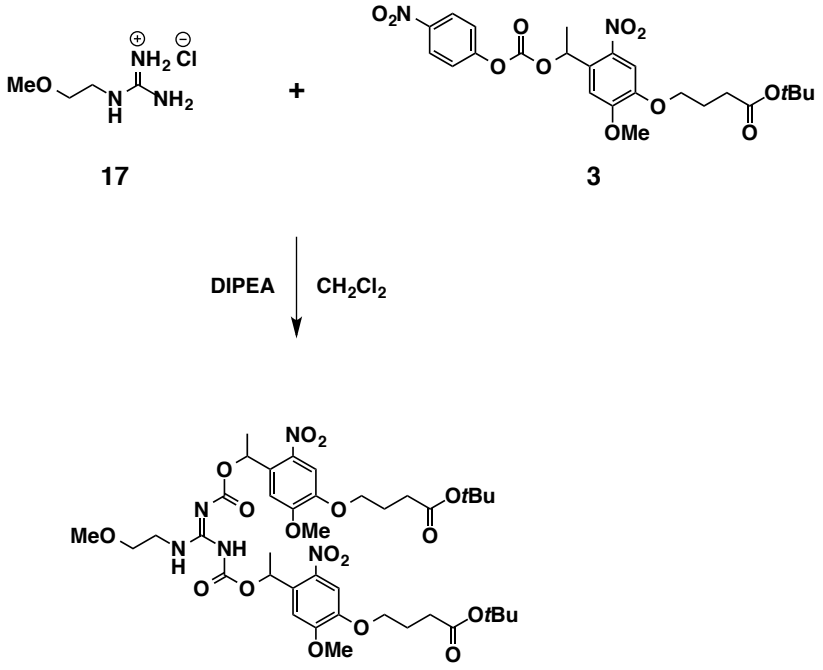
15 **16**

Compound 16. To a CH_2Cl_2 (10 mL) solution of **15** (647 mg, 8.61 mmol) was added 1,3-bis(*t*-butoxycarbonyl)-2-methylisothiourea (1.00 g, 3.44 mmol), and the mixture was stirred overnight at room temperature. Then, a CH_2Cl_2 (6 mL) solution of **15** (258 mg, 3.44 mmol) and triethylamine (Et_3N, 477 μL, 3.44 mmol) were successively added to the reaction mixture, and the resultant mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed successively with aqueous $NaHSO_4$ (1 M, 40 mL), saturated aqueous $NaHCO_3$ (40 mL), and brine (40 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, affording **16** as white solid (1.10 g, quant.). ^{1}H NMR ($CDCl_3$; ppm): δ 1.49 (s, 18H; $C(CH_3)_3$), 3.38 (s, 3H; OCH_3), 3.51 (t, J = 5.0 Hz, 2H; OCH_2), 3.62 (m, 2H; CH_2NH), 8.58 (br, 1H; CH_2NHCN), 11.48 (s, 1H; $NHCO_2$). ^{13}C NMR ($CDCl_3$; ppm): δ 28.2, 28.4, 40.8, 59.0, 70.7, 79.4, 83.1, 153.2, 156.4, 163.7. ESI-MS: m/z found: 356.14 ([M + K^+] calcd: 356.16), 340.17 ([M + Na^+] calcd: 340.18).



16 **17**

Compound 17. A 1,4-dioxane (15 mL) solution of HCl (4 M) was added to **16** (1.00 g, 3.15 mmol), and the mixture was stirred for 21 h at room temperature. Then, hexane (50 mL) was added to the reaction mixture, and the resultant suspension was filtered. The insoluble fraction, obtained by filtration, was dissolved in MeOH (10 mL), and the resultant solution was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, affording **17** as colorless oil (525 mg, quant.). ^{1}H NMR (DMSO-d_6; ppm): δ 3.27 (s, 3H; OCH_3), 3.30 (t, J = 5.5 Hz, 2H; OCH_2), 3.40 (t, J = 5.0 Hz, 2H; CH_2NH), 7.30 (br, 4H; CNH_2), 7.75 (br, 1H; CH_2NHCN). ^{13}C NMR (DMSO-d_6; ppm): δ 40.6, 58.1, 70.0, 157.3.



MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$

MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$. To a CH_2Cl_2 (1 mL) solution of a mixture of **17** (5.2 mg, 33 μmol) and **3** (51 mg, 98 μmol) was added diisopropylethylamine (DIPEA, 22 μL, 0.13 mmol), and the mixture was stirred for 2 days at room temperature. Then, DIPEA (90 μL, 0.52 mmol) was added to the reaction mixture, and the resultant mixture was stirred for 24 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (3 mL) and washed with water (4 mL × 2) followed by brine (4 mL). An organic extract separated was dried over Na_2SO_4 and filtered off from an insoluble fraction. The filtrate was evaporated to dryness under reduced pressure, and the residue was chromatographed on silica gel twice with CH_2Cl_2 and AcOEt/hexane (1/2) as eluents to allow isolation of **MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$** as colorless oil (20 mg, 69%). ^{1}H NMR ($CDCl_3$; ppm): δ 1.44 (s, 18H; $C(CH_3)_3$), 1.60–1.70 (m, 6H; $CHCH_3$), 2.09–2.16 (m, 4H; $CH_2CH_2CH_2$), 2.41–2.45 (m, 4H; CH_2CO_2), 3.29–3.33 (m, 3H; CH_2OCH_3), 3.42–3.58 (m, 3H; OCH_2, CH_2NH), 3.92–3.96 (m, 6H; $ArOCH_3$), 4.07–4.12 (m, 4H; $ArOCH_2$), 4.20–4.29 (m, 1H; CH_2NH), 6.37–6.50 (m, 2H; $ArCH(CH_3)$), 6.94–7.14 (m, 2H; ArH), 7.55–7.58 (m, 2H; ArH), 8.47 (br; guanidine-H), 9.15 (br; guanidine-H), 9.44 (br; guanidine-H), 11.71 (br; guanidine-H). ^{13}C NMR ($CDCl_3$; ppm): δ 22.1, 22.3, 22.5, 24.5, 24.5, 28.2, 31.9, 31.9, 41.2, 44.3, 56.5, 56.6, 56.6, 56.8, 59.0, 68.5, 68.5, 69.7, 69.8, 69.9, 70.1, 70.8, 70.9, 72.2, 80.7, 80.7, 108.0, 108.2, 108.3, 108.4, 108.6, 108.7, 108.8, 108.9, 109.0, 135.0, 135.3, 135.4, 139.4, 139.7, 139.8, 147.1, 147.2, 147.7, 147.8, 152.8, 154.3, 154.4, 155.1, 156.3, 160.6, 162.7, 162.8, 163.0, 172.3, 172.3. MALDI-TOF-MS: *m*/*z* found: 918.64 ([M + K^+] calcd: 918.34), 902.65 ([M + Na^+] calcd: 902.36).

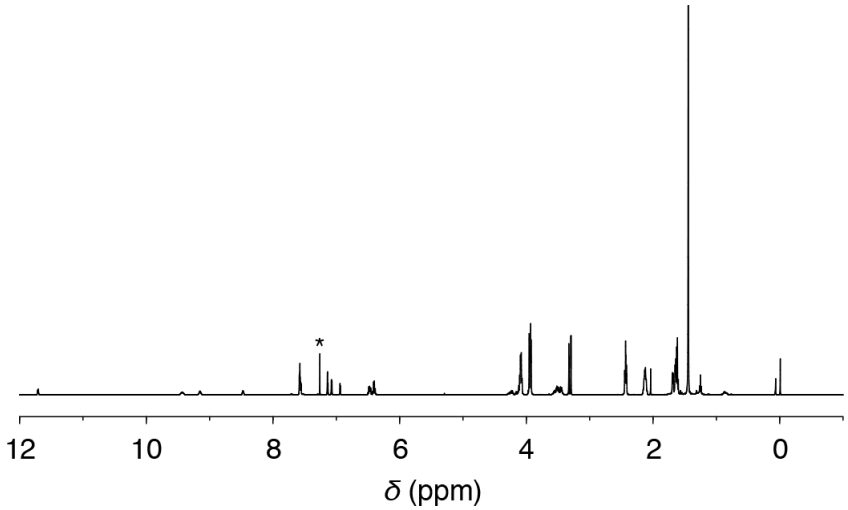


Figure S7. ^{1}H NMR spectrum of MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$ in $CDCl_3$ at 23 °C. The signal marked with an asterisk at δ 7.26 ppm is due to $CHCl_3$.

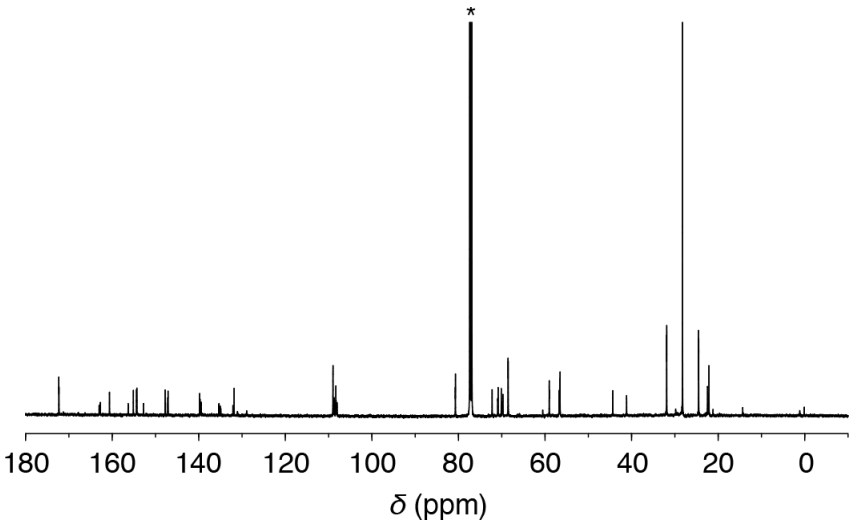


Figure S8. ^{13}C NMR spectrum of MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$ in $CDCl_3$ at 21 °C. The signal marked with an asterisk at δ 77.2 ppm is due to $CDCl_3$.

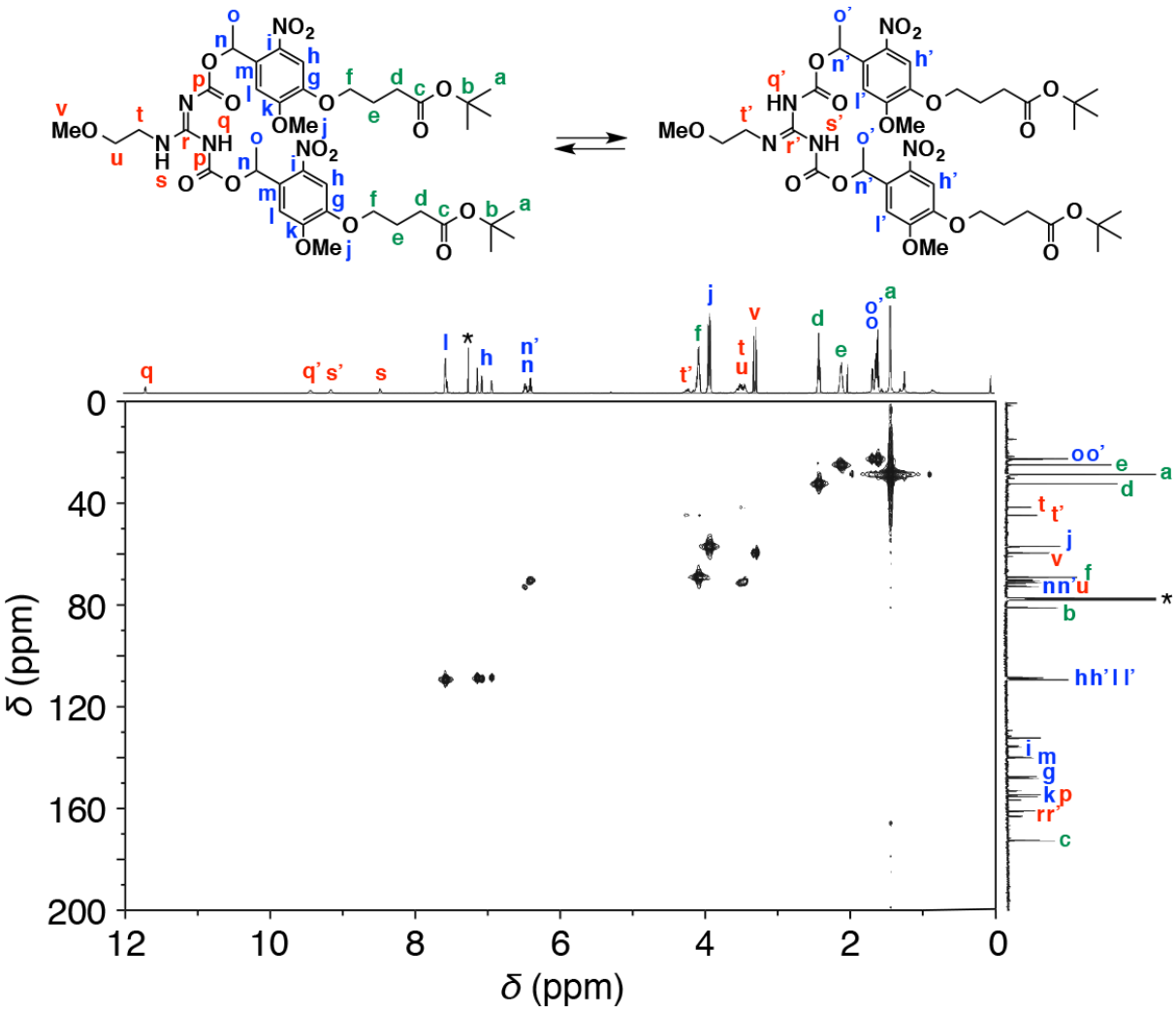


Figure S9. ^{1}H–^{13}C HMQC spectrum of MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$ in $CDCl_3$ at 21 °C. The signals marked with asterisks at δ 7.26 ppm in the ^{1}H NMR spectrum and δ 77.2 ppm in the ^{13}C NMR spectrum are due to $CDCl_3$.

=> The ^{1}H–^{13}C HMQC spectrum of MeO-ethyl-Gu(*t*Bu-^{BA}NVOC)$_2$ indicates the existence of its tautomer (Figure S9).[S4]

3. MALDI-TOF Mass Spectrometry

An aqueous solution of ^{Caged}Glue-NBD (50 μM) was exposed to UV light (λ = 365 nm) for 60 min and subjected to MALDI-TOF mass spectrometry using SA as a matrix. A reference sample using Glue-NBD (50 μM) was likewise prepared.

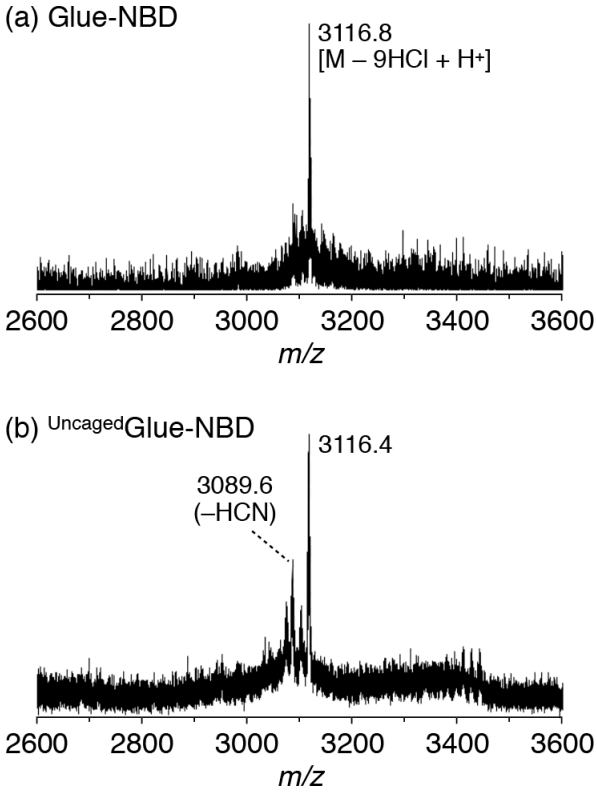


Figure S10. MALDI-TOF mass spectra using SA as a matrix of (a) Glue-NBD and (b) ^{Caged}Glue-NBD after 60-min UV exposure at 365 nm.

4. Zeta Potential Measurements

A Tris-HCl buffer (20 mM, pH 7.2) solution of ^{Caged}Glue-NBD (50 μM) was exposed to UV light (λ = 365 nm) for 60 min and subjected to zeta potential measurements. An analogous sample without UV exposure was prepared under conditions otherwise identical to the above procedure. A reference sample using Glue-NBD (50 μM) was likewise prepared.

5. Agarose Gel Electrophoresis

A Tris-HCl buffer (20 mM, pH 7.2) solution of a mixture of ^{Caged}Glue-NBD (1 μM) and l-pUC19 (0.06 nM) was exposed to UV light (λ = 365 nm) for 15, 30, 45, and 60 s and subjected to agarose gel electrophoresis. Likewise, reference samples without UV exposure in the absence and presence of ^{Caged}Glue-NBD (1 μM) were obtained under conditions otherwise identical to the above procedures. The gel was stained with ethidium bromide and fluorescently visualized using a FUJIFILM model LAS-3000 luminescent image analyzer (λ_{ext} = 310 nm).

6. Fluorescence Spectral Titration

6-1. Sequence of DNA-TAMRA

Sequence of TAMRA-labeled DNA (17-mer)

5'-AGCTCGGAAATCCACCG[Tamra-Q]-3'

Sequence of the Complementary DNA (17-mer)

5'-CGGTGGATTTCCGAGCT-3'

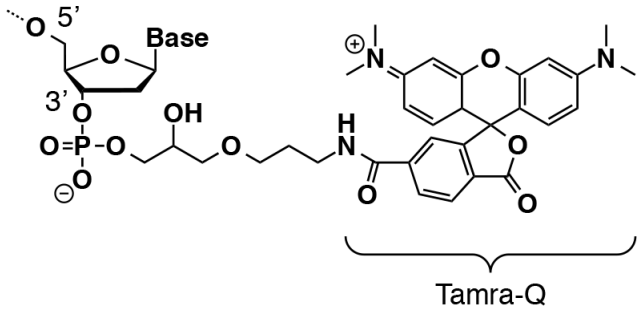


Figure S11. Terminal structure of the TAMRA (Tamra-Q)-labeled DNA.

6-2. Determination of the Association Constants

The association constants (K_{assoc}) were determined based on the Hill equation, by plotting the fractions of bound DNA against the concentrations of the titrant. The fractions of bound DNA (θ) were calculated from $(I - I_0)/(I_{sat} - I_0)$, where I_0, I, and I_{sat} represent the fluorescence intensities at 580 nm before titration, observed with titrants, and at the saturation point, respectively. The data were then fit to the Hill equation: $\theta = [T]^n/((K_d)^n + [T]^n)$, where [T] is the titrant concentration, K_d (= $1/K_{assoc}$) is the dissociation constant, and n is the Hill coefficient.[S5,S6]

7. Confocal Laser Scanning Microscopy

Human hepatocellular carcinoma Hep3B cells (5.0×10^3 cells/well; 8-chambered glass substrate, culture area = 0.8 cm^2/well) were incubated at 37 °C under 5% CO_2 for 24 h in Eagle's minimal essential medium (EMEM, 200 μL) containing 10% fetal bovine serum (FBS). The cell samples were rinsed with Dulbecco's phosphate buffer saline (D-PBS, 100 μL × 2) prior to use.

7-1. Cellular Uptake of ^{Caged}Glue-NBD, ^{Uncaged}Glue-NBD, and Glue-NBD

Hep3B cells were supplied with EMEM (200 μL) containing ^{Caged}Glue-NBD (10 μM) and incubated at 37 °C under 5% CO_2 for 3 h. The cells were rinsed with D-PBS (100 μL × 2), supplied with EMEM (10% FBS, 200 μL), and exposed to UV light (λ = 365 nm) for 2 min. Then, Hoechst 33342 (5 μg/mL) was supplied to the cells. After incubation of the resultant mixture at 37 °C under 5% CO_2 for 10 min, Lysotracker® Red (100 nM) was added to the cells, and the cells were incubated at 37 °C under 5% CO_2 for 20 min. Then, the cell sample was rinsed with D-PBS (100 μL × 2), supplied with EMEM (10% FBS, 200 μL), and subjected to confocal laser scanning microscopy (λ_{ext} = 488 and 543 nm; 710 nm, MaiTai laser, two-photon excitation). An analogous cell sample supplied with EMEM (200 μL) containing ^{Caged}Glue-NBD (10 μM) or Glue-NBD (10 μM) without UV exposure was likewise prepared under conditions otherwise identical to the above procedures.

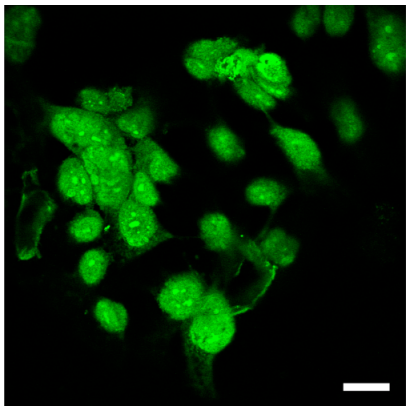


Figure S12. A confocal laser scanning micrograph upon excitation at 488 nm (λ_{obs} = 500–530 nm) of Hep3B cells after 3-h incubation at 37 °C in EMEM containing Glue-NBD (10 μM) followed by rinsing with D-PBS. Scale bar = 20 μm.

7-2. Preparation of ^{Caged}Glue-Modified Quantum Dots (^{Caged}Glue-QDs)

To a DMF (400 μL) solution of quantum dots (QDs) coated with amine-functionalized PEG (Q-dot 655 ITK, 500 nM) was added a DMF (100 μL) solution of azide-PEG4-NHS ester (125 μM), and the mixture was stirred at 25 °C for 1 h. The resulting solution was dialyzed for 24 h against DMF (500 mL) using a regenerated cellulose membrane (MWCO 3,000). An aqueous ^{Caged}Glue-DBCO (50 μM, 200 μL) solution was added to the post-dialysis solution (800 μL), and the mixture was stirred at room temperature for 3 h. The resulting solution was dialyzed for 24 h against DMF (500 mL) using a regenerated cellulose membrane (MWCO 25,000).

7-3. Cellular Uptake of ^{Caged}Glue-QD

Hep3B cells were incubated in EMEM (200 μL) containing ^{Caged}Glue-QD (10 nM) at 37 °C under 5% CO_2 for 3 h, rinsed with D-PBS (100 μL × 2), and then supplied with EMEM (10% FBS, 200 μL). Then, the cell sample was exposed to UV light at 365 nm for 2 min, followed by the treatment with Hoechst 33342 (5 μg/mL). After incubation at 37 °C under 5% CO_2 for 10 min, the resulting cell sample was subjected to confocal laser scanning microscopy (λ_{ext} = 405 and 488 nm). A reference cell sample without UV exposure was likewise prepared under conditions otherwise identical to the above procedures.

8. Flow Cytometry

Hep3B cells (5.0×10^3 cells/well; 96-well culture plate, culture area = 0.33 cm^2/well) were incubated in EMEM (10% FBS, 100 μL) at 37 °C under 5% CO_2 for 24 h. The cell sample was rinsed with D-PBS (100 μL × 2) and supplied with EMEM (100 μL) containing ^{Caged}Glue-NBD (10 μM). After incubation at 37 °C under 5% CO_2 for 30 min and 180 min, the cell sample was rinsed with D-PBS (100 μL) and then detached from the culture plate using 0.25% trypsin-EDTA (100 μL). The obtained suspension was centrifuged at 1,500 rpm for 5 min, and the supernatant was removed. The residue was rinsed with D-PBS (100 μL × 2), suspended in D-PBS (100 μL), and then subjected to flow cytometry (λ_{ext} = 488 nm). An analogous cell sample supplied with EMEM (100 μL) containing Glue-NBD (10 μM) was likewise prepared under conditions otherwise identical to the above procedures.

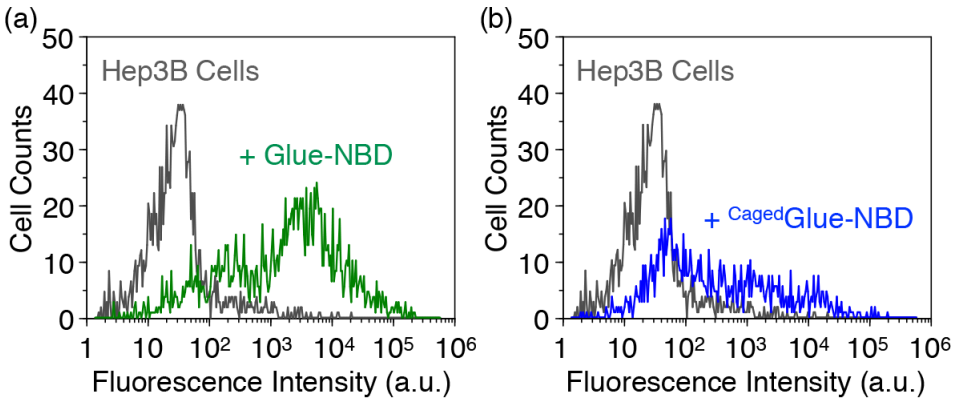


Figure S13. Flow cytometry histograms (λ_{ext} = 488 nm) of Hep3B cells before (black) and after incubation in EMEM containing (a) Glue-NBD (10 μM, green) and (b) ^{Caged}Glue-NBD (10 μM, blue) at 37 °C for 3 h.

9. Cell Viability Assay

Hep3B cells (5.0×10^3 cells/well; 96-well culture plate, culture area = 0.33 cm^2/well) were incubated in EMEM (10% FBS, 200 μL) at 37 °C under 5% CO_2 for 24 h. The cell sample was rinsed with D-PBS (100 μL × 2) and supplied with EMEM (100 μL) containing ^{Caged}Glue-NBD or Glue-NBD (0.1–10 μM). After incubation at 37 °C under 5% CO_2 for 3 h, the Cell Counting Kit-8 reagent (10 μL) was supplied to the cells followed by incubation of the resultant mixture at 37 °C under 5% CO_2 for 2 h. An analogous cell sample, exposed to UV light (λ = 365 nm) for 2 min after incubation with ^{Caged}Glue-NBD, was likewise prepared under conditions otherwise identical to the above procedures. The cell samples thus prepared were subjected to absorption spectroscopy (λ = 450 nm).

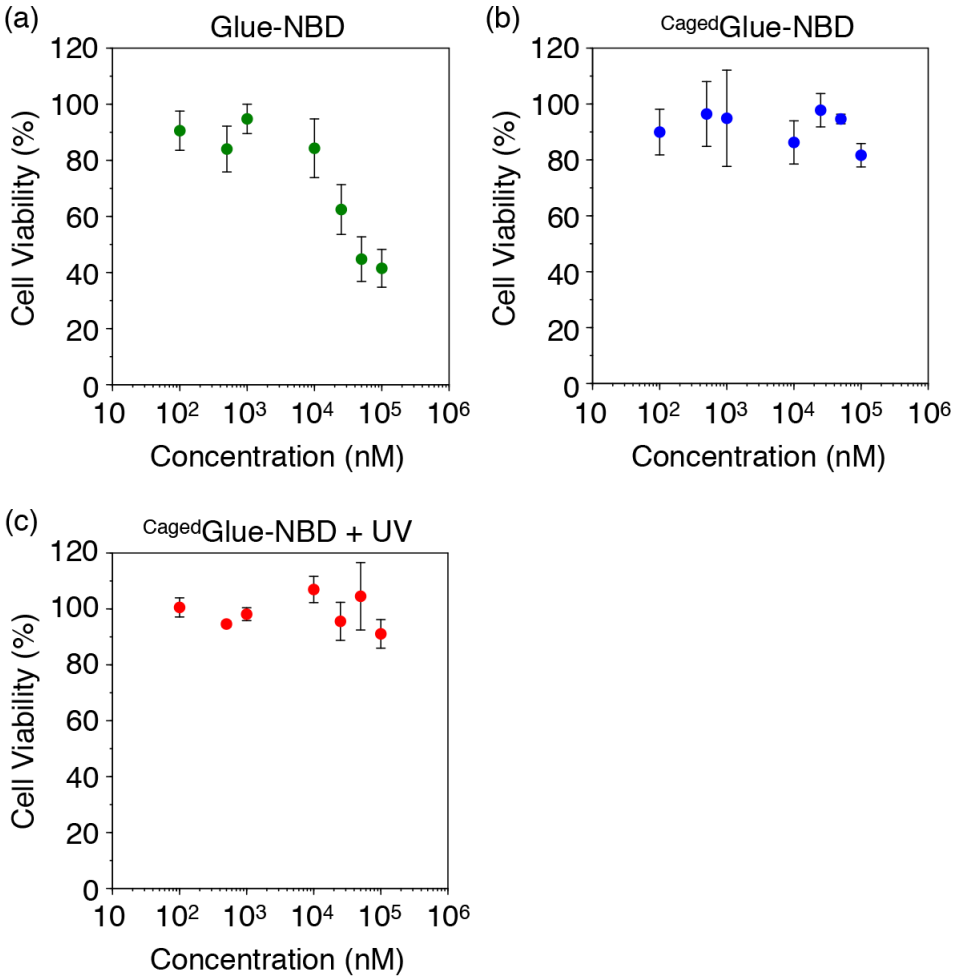


Figure S14. Viabilities of Hep3B cells after incubation in EMEM containing (a) Glue-NBD (0.1–10 μM), (b) ^{Caged}Glue-NBD (0.1–10 μM), and (c) ^{Caged}Glue-NBD (0.1–10 μM) followed by 2-min UV exposure at 365 nm.

10. References

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