Supporting Information

Sulfhydryl-Based Dendritic Chain Reaction

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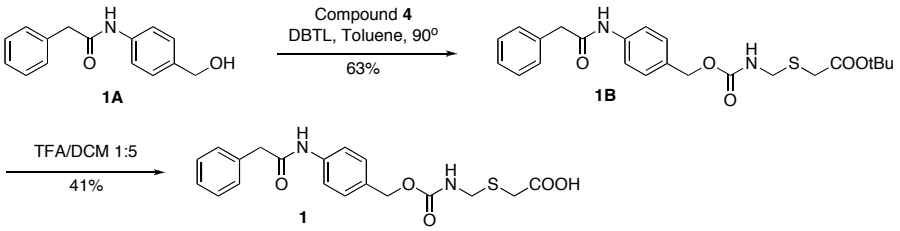
General methods. All reactions requiring anhydrous conditions were performed under an Argon atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either A.R. grade or purified by standard techniques. Thin layer chromatography (TLC): silica gel plates Merck 60 F_{254}: compounds were visualized by irradiation with UV light. Flash chromatography (FC): silica gel Merck 60 (particle size 0.040-0.063 mm), eluent given in parentheses. ^{1}H-NMR spectra were measured using Bruker Avance operated at 400MHz as mentioned. ^{13}C-NMR spectra were measured using Bruker Avance operated at 100 MHz as mentioned. The chemical shifts are expressed in δ relative to TMS (δ = 0 ppm) and coupling constants *J* in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma-Aldrich.

Abbreviations. AcOH- Acetic acid, ACN- Acetonitrile, DBTL- Dibutyltin dilaurate, DCM- Dichloromethane, DMF- N,N'-Dimethylformamide, Et_2O- Diethyl ether, EtOAc- Ethylacetate, Hex- n-Hexanes, MeOH- Methanol, NMM- N-Methylmorpholine, *p*-TsOH- *p*-Toluene sulfonic acid, THF- Tetrahydrofurane, TFA- Trifluoroacetic acid.



Compound **1B**

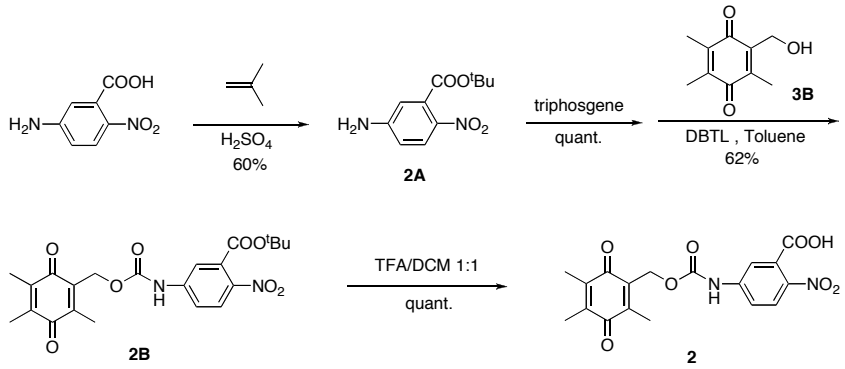
Compound **1A**[1] (190 mg, 0.79 mmol), compound **4** (200 mg, 0.86 mmol) and catalytic amount of DBTL were suspended in 4 mL toluene, and heated to 90°C for 20 min. The reaction mixture was monitored by TLC (EtOac/Hex 50:50). After completion, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/Hex 45:55) to give compound **1B** (220 mg, 63%) as a white powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.85 (1H, s), 7.41-7.19 (9H, m), 5.88 (1H, t, *J* = 6.4 Hz), 5.01 (2H, s), 4.41 (2H, d, *J* = 6.4 Hz), 3.66 (2H, s), 3.22 (2H, s), 1.44 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.7, 170.1, 156.6, 138.4, 135.2, 132.7, 129.9, 129.6, 129.5, 128.0, 120.5, 82.7, 67.2, 45.3, 45.1, 33.2, 28.5. MS (ESI+): *m/z* calc. for $C_{23}H_{28}N_2O_5S$: 444.5 ; found: 467.2 $[M+Na]^+$.

Compound **1**

Compound **1B** (50 mg, 0.11 mmol) was dissolved in 1.5 mL mixture of TFA/DCM 1:5, and stirred at RT for 20 min. After completion, the solvents were evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOac/AcOH 99:1) to give compound **1** (17 mg, 41%) as a white powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.32-7.11 (10H, m), 5.72 (1H, t, *J* = 6.4 Hz), 5.04 (2H, s), 4.39 (2H, d, *J* = 6.4 Hz), 3.68 (2H, s), 3.29 (2H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 175.5, 169.9, 157.0, 139.1, 134.7, 133.3, 133.0, 129.6, 129.4, 128.5, 118.5, 66.9, 45.5, 45.2, 34.0. MS (ESI+): *m/z* calc. for $C_{19}H_{20}N_2O_5S$: 388.4 ; found: 411. $[M+H]^+$.



Compound **2A**

Commercially available 5-amino-2-nitrobenzoic acid (1 g, 5.49 mmol) was dissolved in 17 mL of 1,4-dioxane in a sealed tube, and H_2SO_4 (2.2 mL) was added slowly. The reaction mixture was cooled to -40°C, and 17 mL of isobutylene were bubbled and the vessel was sealed. The mixture was warmed to room temperature and sttired overnight. After completion, the vessel was cooled to 0°C, opened slowly, and the mixture was diluted with 110 mL of EtOAc and was washed with 2M NaOH (110mL x 3) followed by brine (50 mL x 3).The organic layer was separated, dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 40:60) to give compound **2A** (785 mg, 60%) as a yellow powder.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.85 (1H, d, *J* = 9.6 Hz), 6.54 (2H, m), 4.56 (2H, brs), 1.53 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 166.8, 152.7, 134.3, 127.7, 114.5, 113.5, 83.9, 28.4. MS (ESI+): *m/z* calc. for $C_{11}H_{14}N_2O_4$: 238.2 ; found: $[M+Na]^+$.

Compound **2B**

Toluene was heated to reflux under argon atmosphere and triphosgene (78 mg, 0.26 mmol) in toluene was added. Then, a solution of compound **2A** (50 mg, 0.20 mmol) in toluene was slowly added dropwise with a syringe. The reaction mixture was stirred for 30 min at reflux and monitored by ^{1}H-NMR. After isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **3B**[3] (30 mg, 0.16 mmol) in toluene, followed by catalytic amount of DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux, stirred for 1 h under argon atmosphere and monitored by TLC (EtOAc/Hex 30:70). After completion, the solvent was removed under reduced pressure. The crude

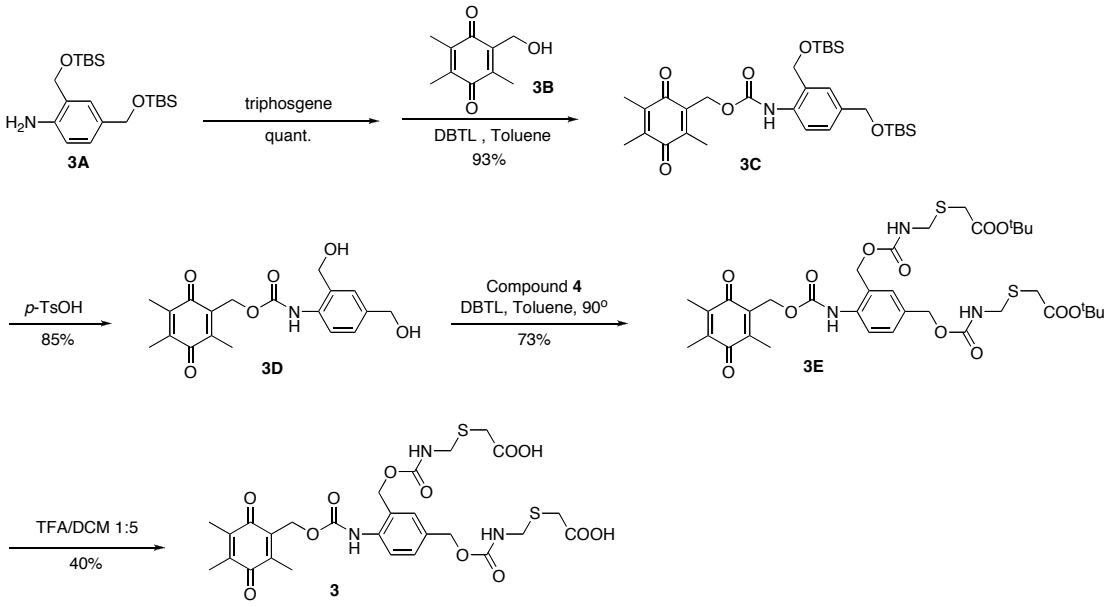
product was purified by column chromatography on silica gel (EtOAc/Hex 25:75) to give compound **2B** (44 mg, 62%) as yellow oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 7.94 (1H, d, *J* = 8.8 Hz), 7.64 (1H, dd, *J* = 8.8, 2.4 Hz), 7.55 (1H, d, *J* = 2.4 Hz), 7.18 (1H, s), 5.16 (2H, s), 2.19 (3H, s), 2.05 (6H, s), 1.56 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 187.9, 186.6, 165.4, 152.9, 145.9, 143.2, 142.6, 142.1, 141.5, 136.7, 132.4, 126.4, 119.6, 118.7, 84.7, 59.4, 28.5, 13.3, 13.1. MS (ESI+): *m/z* calc. for $C_{22}H_{24}N_2O_8$: 444.1 ; found: 467.1 $[M+Na]^+$.

Compound **2**

Compound **2B** (30 mg, 0.07 mmol) was dissolved in 1.5 mL mixture of TFA/DCM 1:1, and stirred at RT for 20 min. After completion, the solvents were evaporated under reduced pressure to give compound **2** (27 mg, 99%) as a yellow powder.

^{1}H NMR (400MHz, CD_3CN): δ = 8.58 (1H, s), 7.94 (1H, d, *J* = 9.2 Hz), 7.76 (1H, d, *J* = 2.4 Hz), 7.68 (1H, dd, *J* = 9.2, 2.4 Hz), 5.11 (2H, s), 2.13 (3H, s), 2.00 (6H, s). ^{13}C NMR (100MHz, CD_3CN): δ = 187.4, 186.3, 167.0, 165.0, 153.7, 145.6, 144.5, 142.4, 141.8, 141.2, 137.2, 130.8, 126.5, 120.2, 58.9, 12.4, 12.2. MS (ESI+): *m/z* calc. for $C_{18}H_{16}N_2O_8$: 388.1 ; found: 411.1 $[M+Na]^+$.



Compound **3C**

Toluene was heated to reflux under argon atmosphere and triphosgene (600 mg, 2.0 mmol) in toluene was added. Then, a solution of compound **3A**[2] (640 mg, 1.67 mmol)

in toluene was slowly added dropwise with a syringe. The reaction mixture was stirred for 30 min at reflux and monitored by ^{1}H-NMR. After isocyanate derivative was observed, the solvent was removed under reduced pressure. A solution of compound **3B**[3] (300 mg, 1.67 mmol) in toluene, followed by catalytic amount of DBTL, was added to the isocyanate residue. The reaction mixture was heated to reflux, stirred for 1 h under argon atmosphere and monitored by TLC (EtOAc/Hex 20:80). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 10:90) to give compound **3C** (914 mg, 93%) as an orange oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 8.30 (1H, s), 7.95 (1H, d, *J* = 8.4 Hz), 7.21 (1H, dd, *J* = 8.4, 1.2 Hz), 7.03 (1H, d, *J* = 1.2 Hz), 5.12 (2H, s), 4.69 (2H, s), 4.66 (2H, s), 2.16 (3H, s), 2.04 (6H, s), 0.92 (9H, s), 0.85 (9H, s), 0.07 (6H, s), 0.05 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 188.3, 186.4, 153.8, 145.2, 141.6, 141.5, 137.8, 137.4, 136.6, 128.7, 127.6, 127.3, 120.4, 66.1, 65.3, 58.3, 26.7, 26.4, 19.1, 18.7, 13.1, -4.5, -4.7. MS (ESI+): *m/z* calc. for $C_{31}H_{49}NO_6Si_2$: 587.3 ; found: 610.3 $[M+Na]^+$.

Compound **3D**

Compound **3C** (910 mg, 1.55 mmol) was dissolved in 10 mL MeOH, and catalytic amount of *p*-TsOH was added. The reaction mixture was stirred for 20 min in RT, and monitored by TLC (EtOAc/Hex 75:25). After completion, the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 70:30) to give compound **3D** (474 mg, 85%) as an orange powder.

^{1}H NMR (400MHz, $CDCl_3$ + CD_3OD): δ = 8.09 (1H, s), 7.74 (1H, s), 7.16 (1H, d, *J* = 8.0 Hz), 7.08 (1H, s), 5.04 (2H, s), 4.52 (2H, s), 4.48 (2H, s), 2.11 (3H, s), 1.98 (6H, s). ^{13}C NMR (100MHz, $CDCl_3$ + CD_3OD): δ = 188.2, 186.7, 154.5, 145.5, 141.8, 141.5, 139.4, 139.2, 137.4, 136.9, 128.2, 121.7, 64.8, 63.9, 58.6, 13.0. MS (ESI+): *m/z* calc. for $C_{19}H_{21}NO_6$: 359.1 ; found: 382.1 $[M+Na]^+$.

Compound **3E**

Compound **3D** (100 mg, 0.27 mmol), Compound **4** (193 mg, 0.83 mmol) and catalytic amount of DBTL were suspended in 4 mL toluene, and heated to 90°C for 20 min. The reaction mixture was monitored by TLC (EtOac/Hex 50:50). After completion, the solvent was evaporated under reduced pressure. The crude product was purified

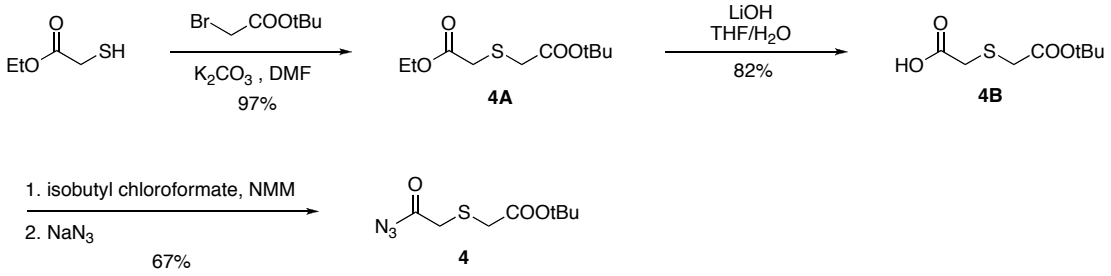
by column chromatography on silica gel (EtOac/Hex 40:60) to give compound **3E** (150 mg, 73%) as an orange oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 8.24 (1H, s), 7.78 (1H, d, *J* = 6.8 Hz), 7.25 (2H, m), 5.95 (1H, t, *J* = 6.4 Hz), 5.79 (1H, t, *J* = 6.4 Hz), 5.09 (2H, s), 5.00 (2H, s), 4.99 (2H, s), 4.40 (2H, d, *J* = 6.4), 4.35 (2H, d, *J* = 6.4 Hz), 3.21 (2H, s), 3.17 (2H, s), 2.14 (3H, s), 2.00 (6H, s), 1.43 (9H, s), 1.40 (9H,s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 188.2, 186.5, 170.9, 157.0, 156.6, 154.5, 145.5, 141.7, 141.4, 137.5, 132.7, 132.0, 130.5, 126.8, 123.3, 82.8, 69.9, 64.5, 58.7, 45.4, 35.3, 28.6, 13.1. MS (ESI+): *m/z* calc. for $C_{35}H_{47}N_3O_{12}S_2$: 765.3 ; found: 788.3 $[M+Na]^+$.

Compound **3**

Compound **3E** (30 mg, 0.04 mmol) was dissolved in 1.5 mL mixture of TFA/DCM 1:5, and stirred at RT for 20 min. After completion, the solvents were evaporated under reduced pressure. The crude product was purified by preparative RP-HPLC (10-90% ACN in water, 20 min) to give compound **3** (11 mg, 40%) as a yellowish powder.

^{1}H NMR (400MHz, CD_3CN): δ = 8.13 (1H, s), 7.65 (1H, s), 7.34 (2H, m), 6.32 (2H, m), 5.08 (2H, s), 5.05 (4H, s), 4.36 (2H, d, *J* = 6.4 Hz), 4.32 (2H, d, *J* = 6.4 Hz), 3.35 (2H, s), 3.33 (2H, s), 2.13 (3H, s), 2.09 (6H, s). ^{13}C NMR (100MHz, CD_3CN): δ = 188.0, 186.3, 174.9, 156.9, 156.5, 153.8, 146.1, 141.4, 141.3, 136.0, 135.1, 133.3, 130.5, 127.8, 122.2, 70.8, 64.6, 59.0, 45.2, 36.8, 13.0. MS (ESI-): *m/z* calc. for $C_{27}H_{31}N_3O_{12}S_2$: 653.1 ; found: 652.1 $[M-H]^-$.



Compound **4A**

Commercially available ethyl-2-mercaptoacetate (0.91 mL, 8.32 mmol) was dissolved in 7 mL DMF and cooled to 0°C under argon atmosphere. K_2CO_3 (1.4 g, 9.98 mmol) was then added, followed by dropwise addition of commercially available *t*-butyl

bromo acetate (1.23 mL, 8.32 mmol). The reaction mixture stirred at RT for 2 h. After completion, the reaction mixture diluted with Et_2O, and was washed with saturated aqueous solution of NH_4Cl, followed by brine. The organic layer dried over $MgSO_4$, and evaporated under reduced pressure to give compound **4A** (1.89 g, 97%) as colorless oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 4.12 (2H, quart, *J* = 7.2 Hz), 3.31 (2H, s), 3.23 (2H,s), 1.41 (9H, s), 1.22 (3H, t, *J* = 7.2 Hz). ^{13}C NMR (100MHz, $CDCl_3$): δ = 170.3, 169.4, 82.3, 61.9, 35.3, 33.9, 28.5, 14.6.

Compound **4B**

Compound **4A** (1.78 g, 7.6 mmol) was dissolved in 32 mL THF and cooled to 0°C. A solution of LiOH (202 mg, 8.4 mmol) in 8 mL water was then added slowly, and the reaction mixture stirred at RT for 2h and monitored by TLC (EtOAc/Hex 15:85). After completion, water was added and the basic mixture was washed with EtOAc. The aqueous mixture was then cooled to 0°C, acidified slowly with 1M HCl (pH = 2) and the product extracted with EtOAc. The organic layer dried over $MgSO_4$, and evaporated under reduced pressure to give compound **4B** (1.28 g, 82%) as colorless oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 3.42 (2H, s), 3.29 (2H, s), 1.44 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ =176.5, 169.9, 83.1, 35.6, 34.1, 28.7. MS (ESI-): *m/z* calc. for $C_8H_{14}O_4S$: 206.2 ; found: 205.1 $[M-H]^-$.

Compound **4**

Compound **4B** (400 mg, 1.94 mmol) was dissolved in 4 mL dry THF and cooled to (-15)°C. NMM (0.27 mL, 2.42 mmol) was added dropwise, followed by dropwise addition of isobutyl chloroformate, and the reaction mixture stirred at that temperature for 15 min. NaN_3 (253 mg, 3.88 mmol) in 2 mL water was added, and the reaction mixture was allowed to stir for 30 min at RT. After completion, the reaction mixture diluted with EtOAc and was washed with saturated aqueous solution of NH_4Cl. The organic layer dried over $MgSO_4$, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/Hex 10:90) to give compound **4** (300 mg, 67%) as colorless oil.

^{1}H NMR (400MHz, $CDCl_3$): δ = 3.32 (2H, s), 3.24 (2H, s), 1.43 (9H, s). ^{13}C NMR (100MHz, $CDCl_3$): δ = 177.2, 169.2, 82.7, 36.1, 35.3, 28.5. MS (ESI+): *m/z* calc. for $C_8H_{13}O_3S$: 231.2 ; found: 232.1 $[M+H]^+$.

General assay conditions

All stock solutions were prepared in ACN [10 mM]. In a typical assay, compound **3** [1000 μM] and compound **2** [500 μM], were incubated in TRIS buffer [pH = 7.2] with EDTA [10 mM]. Various amounts of ethyl-2-mercaptoacetate were added, and the release of 5-amino-2-nitrobenzoic acid was monitored using spectrophotometer at 405 nm.

Representative Example

To 80 μL of TRIS buffer [pH = 7.2] with EDTA [10 mM] were added 5 μL of Compound **2** [500 μM] and 10 μL of Compound **3** [1000 μM]. Finally, 5 μL of ethyl-2-mercaptoacetate, from a 1mM stock solution in ACN, was added [50 μM, 0.10 Eq.], and the release of 5-amino-2-nitrobenzoic acid was monitored using spectrophotometer at 405 nm.

Total assay volume : 100 μL.

Total water v/v% : 80%.

Total ACN v/v% : 20%.

References

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2. R. Erez and D. Shabat, *Org. & Biomol. Chem.*, **6**(15), 2669-2672 (2008).
3. L. Giraud and A. Giraud, *Synthesis*, (8), 1153-1160 (1998).