Supporting Information

Effect of spermine-derived AGEs on oxidative stress and polyamine metabolism

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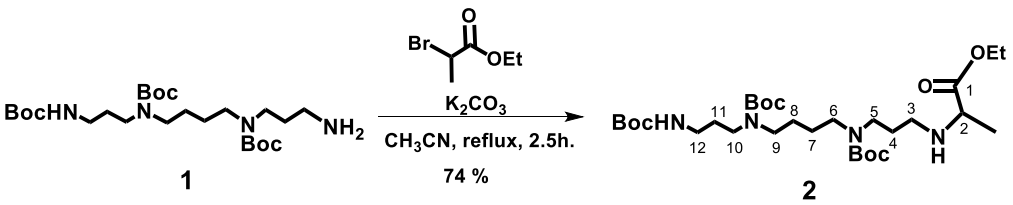
Materials

All commercially available reagents were used without further purification. ^{1}H and ^{13}C NMR spectra were measured on either a JEOL AL400 (400 MHz) or JNM-A500 (500 MHz) instrument with the chemical shifts represented as δ-values relative to the internal standards TMS or DSS. High- and low-resolution mass spectroscopy was acquired on a BRUKER microOTOF II mass spectrometer. HeLa cells (RCB0007), were provided by the RIKEN BRC through the National Bio-Resource Project of MEXT (Japan). Cell imaging fluorescence detection was observed using a Keyence BZ-X710 All-in-one Fluorescence Microscope®, and the data was analyzed using BZ-X Analyzer (Keyence, Japan) software.

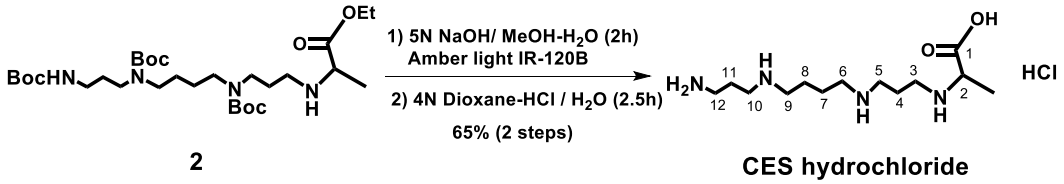
Synthetic Protocols

Synthesis of Tri-*N*-Boc-CES-benzylester **2**



Tri-*N*-Boc-spermine **1** (306mg, 0.61 mmol) and K_2CO_3 (0.59 mmol) were dissolved in CH_3CN (6.0 mL) before addition of ethyl 2-bromopropionate (11.0 mmol). The reaction mixture was then stirred for 2.5 h at 82 °C under reflux conditions. To workup, the mixture was cooled to rt and concentrated under vacuum. The residue was then redissolved in $CHCl_3$ and the organic layer was washed with H_2O, dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound, tri-*N*-Boc-CES-benzylester **2**, was purified by flash column chromatography to afford 273 mg (74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.18 (q, *J*=6.9, 7.1, 7.2, 2H, Et-C*H*$_2$), 3.33 (q, *J*=7.4, 10.5, 10.9, 1H, C*H*-2), 3.31-3.10 (m, 10H), 2.64-2.57 (m, 1H), 2.50-2.44 (m, 1H), 1.83 (broad s, 1H, BocN*H*), 1.71-1.67 (m, 4H), 1.49-1.44 (m, 3H), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$), 1.43 (s, 18H, Boc-C(C*H*$_3$)$_3$), 1.30 (d, 3H, *J*=7.4, 2-C*H*$_3$), 1.30 (t, 3H, *J*=7.2, 7.4, Et-C*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.6 (C-1), 156.0 (Boc-carbonyl), 155.5 (2C, Boc-carbonyl), 80.1 (Boc-*C*$(CH_3)_3$), 79.3 (Boc-*t*-butyl-*C*H), 79.2 (Boc-*t*-butyl-*C*H), 60.9 (C-2), 60.2 (Et-*C*H_2), 56.7 (C-6 or C-9), 56.6 (C-6 or C-9), 46.8 (C-5), 46.3 (C-10), 45,1 (C-3), 35.8 (C-12), 29.4 (C-4, C-7 or C-8), 28.4 (3C, Boc-C(*C*H_3)$_3$), 28.3 (C-4, C-7 or C-8), 27.2 (C-4, C-7 or C-8), 25.8 (C-11), 19.0 (2-*C*H_3), 14.2 (Et-*C*H_3); HRESI-MS m/z calcd for $C_{30}H_{59}N_4O_8$ $[M+H]^+$ 603.4327, found 603.4355.

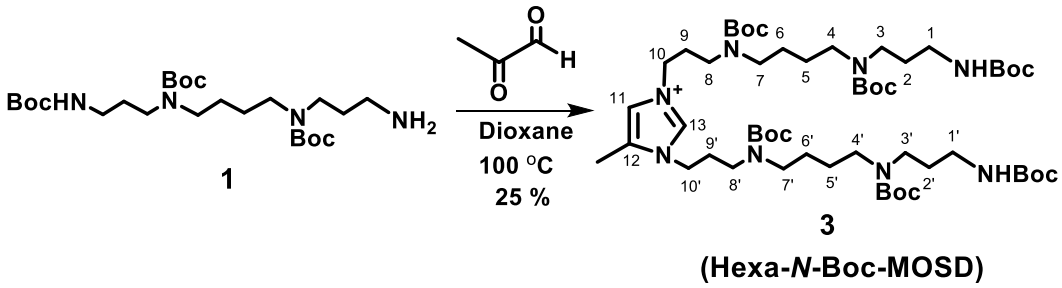
Synthesis of CES hydrochloride



2 **CES hydrochloride**

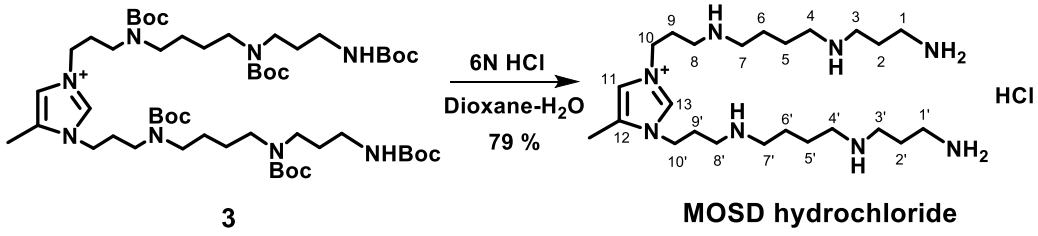
Tri-*N*-Boc-CES-benzylester **2** (93 mg, 0.15 mmol) was dissolved in MeOH (0.95 mL) before addition of 5M NaOH (0.93 mL). The reaction mixture was then stirred for 1 h at room temperature. To workup, the mixture was neutralized with Amberlite IR-120B, filtrated, then concentrated to afford tri-*N*-Boc-CES (66 mg). Following confirmation by ESI-MS, tri-*N*-Boc-CES was stirred in 4N dioxane hydrochloride (0.6 mL). After 5 min, precipitation was formed, which could then be dissolved with addition of H_2O (0.4 mL). The reaction mixture was stirred continuously for 1.5 h. To workup, the mixture was concentrated under vacuum to produce a crude syrup, which upon addition of diethyl ester, formed a precipitate. Filtration, drying, and subsequent collection of the white solid, CES hydrochloride, afforded 43 mg (65%). ^{1}H NMR (400 MHz, D_2O (10% CD_3OD)) δ 3.92 (q, *J*=7.2, 14.5, 1H, Et-C*H*$_2$), 3.13-2.91 (m, 12H), 2.02-1.88 (m, 4H), 1.64-1.58 (m, 4H), 1.41 (d, *J*=7.2, 3H, Et-C*H*$_3$); ^{13}C NMR (100 MHz, D_2O (10% CD_3OD) δ172.9 (carbonyl), 56.8 (C-2), 49.2 (C-6 or C-9), 48.8 (C-6 or C-9), 47.9 (C-10), 45.5 (C-5), 45.4 (C-3), 43.8 (C-12), 37.5 (C-11), 24.7 (C-4), 23.7 (2C, C-7, C-8), 15.1 (2-*C*H$_3$); HRESI-MS m/z calcd for $C_{13}H_{31}N_4O_2$ $[M+H]^+$ 275.2442, found 275.2453.

Synthesis of hexa-*N*-Boc-MOSD **3**



Tri-*N*-Boc-spermine **1** (200 mg, 0.40 mmol) was dissolved in anhydrous 1,4-dioxane (4.0 mL) before addition of methyl glyoxal (234 mL, 1.2 mmol). The reaction mixture was then stirred for 1.5 h at 100 °C under reflux conditions. To workup, the mixture was cooled to rt and concentrated under vacuum. The desired compound, hexa-*N*-Boc-MOSD **3** was purified by flash column chromatography to afford 104 mg (25%).^{1}H NMR (500 MHz, $CDCl_3$) δ4.22-4.14 (m, 3H), 3.49-3.48 (m, 2H), 3.31-3.10 (m, 20H), 2.31 (broad s, 3H, 12-CH_3), 2.17-2.11 (m, 3H), 1.65 (broad s, 5H), 1.45 (s, 18H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 18H, Boc-C(C*H*$_3$)$_3$), 1.44-1.43 (m, 30H); ^{13}C NMR (125 MHz, $CDCl_3$) δ156.2 (6C, Boc-carbonyl), 131.4 (C-13), 128.1 (C-12), 126.1 (C-11), 80.1 (4C, Boc-*C*-(CH_3)$_3$), 79.9 (2C, Boc-*C*(CH_3)$_3$), 50.9 (4C, C-4, C-4', C-7, C-7'), 47.3 (C-8 or C-8'), 47.0 (C-8 or C-8'), 45.0 (2C), 43.8 (4C, C-1, C-1', C-10, C-10'), 28.5 (2C, C-9, C-9'), 28.3 (18C, Boc-C(*C*H$_3$)$_3$), 26.1 (4C, C-5, C-5', C-6, C-6'), 26.0 (2C, C-3, C-3'), 9.27 (12-*C*H$_3$); HRESI-MS m/z calcd for $C_{54}H_{101}N_8O_{12}$ $[M]^+$ 1053.7533, found 1053.7539.

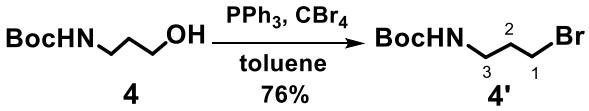
Synthesis of MOSD hydrochloride



3 **MOSD hydrochloride**

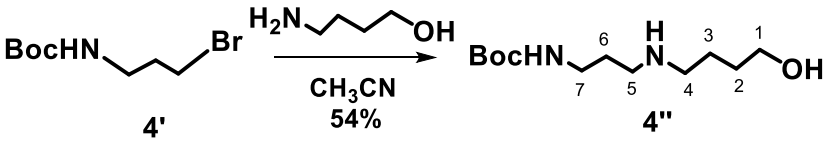
Hexa-*N*-Boc-MOSD **3** (104 mg, 0.10 mmol) was dissolved in 6N hydrochloride (1,4-dioxane-H_2O=1:1, 0.5 mL) and the mixture was stirred for 3 h at room temperature. The desired compound MOSD hydrochloride was purified by removing the solvent under vacuum and lyophilization to afford 53 mg (79%). ^{1}H NMR (500 MHz, D_2O (internal standard; DSS) δ8.81 (broad s, 1H), 7.31 (broad s, 1H), 4.28-4.22 (m, 4H), 3.16-3.10 (m, 20H), 2.31 (s, 3H), 2.29-2.25 (m, 3H), 2.09-2.06 (m, 4H), 1.77 (m, 9H); ^{13}C NMR (125 MHz, D_2O (internal standard; DSS)) δ137.7 (C-13), 135.0 (C=12), 122.0 (C-11), 49.8 (4C, C-4, C-4', C-7, C-7'), 49.0 (2C, C-8, C-8'), 47.3 (2C, C-3, C-3'), 47.1 (C-10), 46.4 (C-10'), 39.3 (2C, C-1, C-1'), 28.9 (C-2 or C-2'), 28.6 (C-2 or C-2'), 26.5 (2C, C-9, C-9'), 25.5 (4C, C-5, C-5', C-6, C-6'), 11.0 (12-$\underline{C}H_3$); HRESI-MS m/z calcd for $C_{24}H_{53}N_8$ $[M]^+$ 453.4388, found 453.4400.

Synthesis of compound 3-*N*-Boc-amino-1-propane bromide **4'**



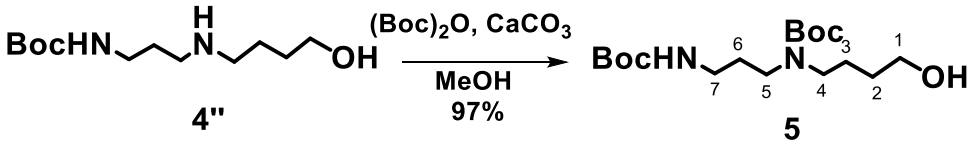
3-*N*-Boc-amino-1-propanol **4** (2.0 g, 14.4 mmol) and tetrabromomethane (4.5 g, 17.2 mmol) were dissolved in anhydrous toluene (50 mL) before addition of triphenylphosphane (5.7 g, 17.2 mmol). The reaction mixture was then stirred for 1.5 h at 100 °C under reflux conditions. To workup, the mixture was cooled to rt and ethyl acetate was added. The organic layer was washed with H_2O, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The desired compound **4'** was purified by flash column chromatography to afford 2.06 g (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ4.74 (broad s, 1H, Boc-N*H*), 3.44 (t, 2H, *J*=6.3, 6.8, C*H*$_2$-1), 3.27 (dd, 2H, *J*=6.3, 12.6, C*H*$_2$-3), 2.09-2.01 (m, 2H), 1.44 (s, 9H, Boc-*t*-butyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ155.9 (Boc-crbonyl), 79.3 (Boc-*C*$(CH_3)_3$), 38.9 (C-3), 32.6 (C-2), 30.8 (C-1), 28.3 (3C, Boc-C(*C*$H_3)_3$); HRESI-MS m/z calcd for $C_8H_{16}BrNNaO_2$ $[M+Na]^+$ 260.0257, found 260.0267.

Synthesis of *N*-Boc-spermidine-ol **4''**



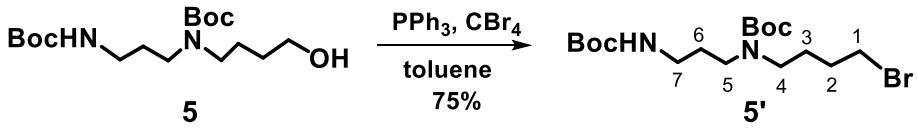
Compound **4'** (2.0 g, 8.4 mmol) was dissolved in anhydrous MeCN (30 mL) before addition of 4-amino-1-butanol (0.85 mL, 9.2 mmol). The reaction mixture was stirred overnight at 60 °C. To workup, the mixture was cooled to rt and concentrated under vacuum. The desired compound **4''** was purified by flash column chromatography to afford 3.2 g (54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ5.08 (broad s, 1H, Boc-N*H*), 3.69 (broad s, 1H, OH), 3.57 (t, 4H, *J*=5.3, 4.8, C*H*$_2$-1), 3.18 (dd, 2H, *J*=6.0, 12.3, C*H*$_2$-7), 2.65 (dd, 4H, *J*=5.3, 12.0, 4-C*H*$_2$, C*H*$_2$-5), 1.72-1.63 (m, 6H), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ156.1 (Boc-carbonyl), 79.0 (Boc-*C*$(CH_3)_3$), 62.4 (C-1), 49.5 (C-4), 46.5 (C-5), 38.3 (C-7), 32.2 (C-3), 29.8 (C-6), 28.3 (3C, Boc-C(*C*H$_3$)$_3$), 21.2 (C-2); HRESI-MS m/z calcd for $C_{12}H_{26}N_2NaO_3$ $[M+Na]^+$ 269.1836, found 269.1849.

Synthesis of *N*-di-Boc-spermidine-ol **5**



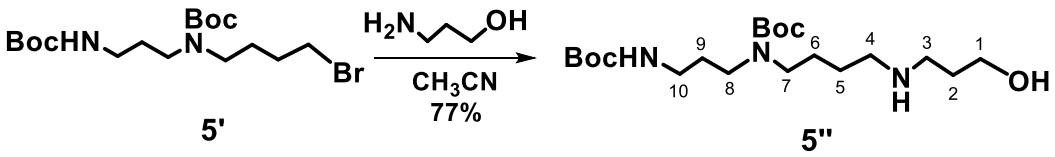
Compound **4''** (1.1 g, 4.5 mmol) was dissolved in anhydrous MeOH (46.0 mL) before addition of di-*tert*-butyl dicarbonate (1.5 g, 6.8 mmol) and $CaCO_3$ (230.0 mg, 2.3 mmol). The reaaction mixture was stirred for 1.5 h at room temperature. To workup, the mixture was concentrated under vacuum. The residue was then redissolved in H_2O and the product was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound **5** was purified by flash column chromatography to afford 1.5 g (97%).^{1}H NMR (400 MHz, $CDCl_3$) δ5.36 (broad s, 1H, Boc-N*H*), 3.65 (t, 2H, *J*=5.3, 6.3, C*H*$_2$-1), 3.25-3.09 (m, 6H), 1.67-1.49 (m, 6H), 1.46 (s, 9H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ156.0 (2C, Boc-carbonyl), 79.2 (2C, Boc-*C*(CH$_3$)$_3$), 62.2 (C-1), 46.6 (C-4), 44.0 (C-5), 37.5 (C-7), 29.5 (C-3), 28.7 (C-6), 28.2 (6C, Boc-C(*C*H$_3$)$_3$), 24.8 (C-2); HRESI-MS m/z calcd for $C_{17}H_{34}N_2NaO_5$ $[M+Na]^+$ 369.2360, found 369.2376.

Synthesis of *N*-di-Boc-spermidine-bromide **5'**



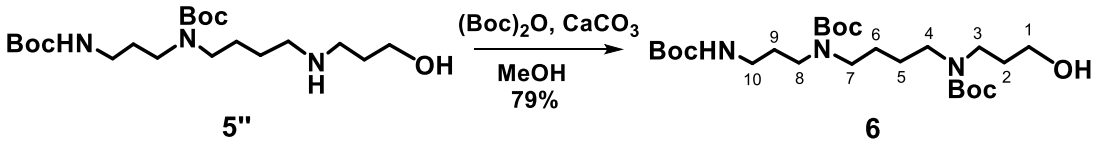
Compound **5** (1.5 g, 4.3 mmol) and tetrabromomethane (2.1 g, 6.5 mmol) were dissolved in anhydrous toluene (45 mL) before addition of triphenylphosphane (1.7 g, 6.5 mmol). The reaction mixture was stirred for 1.5 h at 100 °C under reflux conditions. To workup, the mixture was cooled to rt and ethyl acetate was added. The organic layer was then washed with H_2O, dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound **5'** was purified by flash column chromatography to afford 1.3 g (75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ5.28 (broad s, 1H, Boc-N*H*), 3.43 (t, 2H, *J*=6.8, 6.3, C*H*$_2$-1), 3.26-3.11 (m, 6H), 1.88-1.80 (m, 2H), 1.71-1.60 (m, 2H), 1.47 (s, 9H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ156.0 (2C, Boc-carbonyl), 79.7 (2C, Boc-*C*$(CH_3)_3$), 45.9 (C-4), 43.8 (C-5), 37.3 (C-7), 33.2 (C-1), 29.9 (C-2), 28.8 (C-6), 28.4 (6C, Boc-C(*C*H$_3$)$_3$), 26.9 (C-3); HRESI-MS m/z calcd for $C_{17}H_{33}BrN_2NaO_4$ $[M+Na]^+$ 431.1516, found 431.1537.

Synthesis of *N*-di-Boc-spermine-ol **5''**



Compound **5'** (1.3 g, 3.2 mmol) was dissolved in anhydrous MeCN (32 mL) before addition of 3-amino-1-butanol (0.27 mL, 2.8 mmol). The reaction mixture was stirred overnight at 60 °C. To workup, the mixture was cooled to rt and concentrated under vacuum. The desired compound **5''** was purified by flash column chromatography to afford 1.0 g (77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ5.44 (broad s, 1H, BocN*H*), 3.81 (t, 2H, *J*=4.8, 5.8, C*H*$_2$-1), 3.24-3.02 (m, 9H), 2.88 (t, 2H, *J*=5.8, C*H*$_2$-8), 2.64 (t, 2H, *J*=6.3, 7.2, C*H*$_2$-7), 1.73-1.66 (m, 4H), 1.53-1.44 (m, 3H), 1.46 (s, 9H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ156.1 (Boc-carbonyl), 156.0 (Boc-carbonyl), 79.4 (Boc-*C*$(CH_3)_3$), 78.8 (Boc-*C*$(CH_3)_3$), 64.2 (C-1), 49.8 (C-7), 49.3 (C-4), 46.8 (C-8), 43.7 (C-3), 37.4 (C-10), 30.4 (C-6), 27.2 (C-9), 26.8 (C-5), 28.4 (6C, Boc-C(*C*$H_3)_3$), 26.2 (C-2); HRESI-MS m/z calcd for $C_{20}H_{42}N_3O_5$ $[M+H]^+$ 404.3119, found 404.3138.

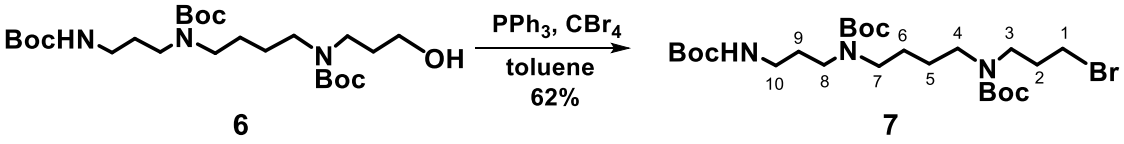
Synthesis of *N*-tri-Boc-spermine-ol **6**



5'' **6**

Compound **5''** (1.0 g, 2.5 mmol) was dissolved in anhydrous MeOH (25.0 mL) before addition of di-*tert*-butyl dicarbonate (0.8 g, 3.8 mmol) and $CaCO_3$ (124.0 mg, 1.3 mmol). The reaction mixture was stirred for 1 h at room temperature. To workup, the mixture was concentrated under vacuum. The residue was then redissolved in H_2O and the product was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound **6** was purified by flash column chromatography to afford 987.9 mg (79%).^{1}H NMR (400 MHz, $CDCl_3$) δ5.31 (broad s, 1H, Boc*N*H), 3.87 (broad s, 1H, 1-O*H*), 3.55 (broad s, 2H, C*H*$_2$-1), 3.73-3.10 (m, 10H), 2.01 (d, 1H, *J*=7.7, C*H*$_2$-9), 1.66 ((broad s, 4H, C*H*$_2$-5, C*H*$_2$-6), 1.53-1.44 (m, 4H), 1.46 (s, 18H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ157.0 (Boc-carbonyl), 156.0 (Boc-carbonyl), 155.4 (Boc-carbonyl), 78.0 (Boc-*C*(CH$_3$)$_3$), 79.5 (Boc-*C*(CH$_3$)$_3$), 79.8 (Boc-*C*(CH$_3$)$_3$), 58.2 (C-1), 46.8 (C-4 or C-7), 46.2 (C-4 or C-7), 43.7 (C-8), 42.6 (C-3), 37.4 (C-10), 30.6 (C-2), 28.4 (9C, Boc-C(*C*H$_3$)$_3$), 27.4 (C-5 or C-6), 27.1 (C-5 or C-6), 26.0 (C-9); HRESI-MS m/z calcd for $C_{25}H_{49}N_3NaO_7$ $[M+Na]^+$ 526.3463, found 526.3470.

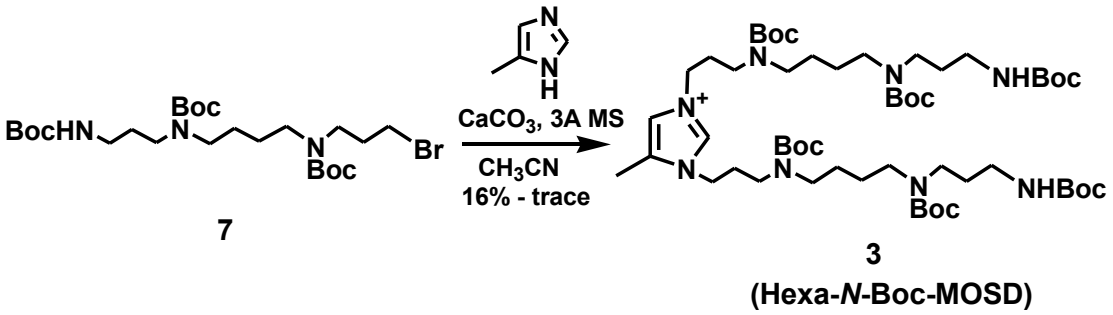
Synthesis of *N*-tri-Boc-spermine-bromide **7**



6 **7**

Compound **6** (988.0 g, 2.0 mmol) and tetrabromomethane (975.0 mg, 3.0 mmol) were dissolved in anhydrous toluene (20 mL) before addition of triphenylphosphane (770.0 mg, 3.0 mmol). The reaction mixture was stirred for 2 h at 100 °C under reflux conditions. To workup, the mixture was cooled to rt and ethyl acetate was added. The organic layer was then washed with H_2O, dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound **7** was purified by flash column chromatography to afford 681.0 mg (62%).^{1}H NMR (400 MHz, $CDCl_3$) δ5.34 (broad s, 1H, Boc*N*H), 3.40 (t, 2H, *J*=6.3, 6.8, C*H*$_2$-1), 3.32-3.10 (m 8H), 3.30 (t, 2H, *J*=6.8, 7.3, C*H*$_2$-10), 2.06 (broad s, 1H, C*H*$_2$-2), 1.66 ((broad s, 2H, C*H*$_2$-9), 1.49-1.44 (m, 2H), 1.45 (s, 18H, Boc-C(C*H*$_3$)$_3$), 1.44 (s, 9H, Boc-C(C*H*$_3$)$_3$), 1.29-1.24 (m,1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ155.9 (2C, Boc-carbonyl), 155.4 (Boc-carbonyl), 79.4 (2C, Boc-*C*$(CH_3)_3$), 78.8 (Boc-*C*$(CH_3)_3$), 47.5 (C-6), 46.7 (2C, C-7, C-4), 46.2 (C-3), 45.7 (C-8), 44.1 (C-10), 31.7 (C-1), 30.6 (C-2), 27.6 (9C, Boc-C(*C*H$_3$)$_3$), 25.7 (C-5), 25.4 (C-9); HRESI-MS m/z calcd for $C_{25}H_{48}BrN_3NaO_6$ $[M+Na]^+$ 588.2619, found 588.2645.

Synthesis of hexa-*N*-Boc-MOSD **3**



Compound **7** (47.7 mg, 0.084 mmol) with 3A molecular sieves (50 mg) was dissolved in anhydrous 1,4-dioxane (0.4 mL) under N_2. 4(5)-methyl imidazole (6.7 mg, 0.042 mmol) in anhydrous 1, 4-dioxane (0.3 mL) was then added. The reaction mixture was stirred for 16 h at 110 °C under reflux conditions. To workup, the mixture was cooled to rt before the molecular sieves were removed by filtration and the mixture concentrated under vacuum. The residue was then redissolved in $CHCl_3$ and the organic layer was washed with H_2O, dried over Na_2SO_4, filtered, and concentrated under vacuum. The desired compound, hexa-*N*-Boc-MOSD **3**, was purified by flash column chromatography to afford 7.1 mg (16%).

Comparison of MOSD-synthetic protocols

As highlighted in Scheme 3B of the manuscript, MOSD was synthesized through 2 different methods: one that mimics the natural glycation reaction (starting from compound **1**), and another that follows classical synthetic protocols (starting from compound **4**). By comparing the HPLC traces and mass spectrometry data (Figure S1), as well as the NMR spectrum (Figure S2) of the common intermediate, hexa-*N*-Boc-MOSD **3**, it can be concluded that both synthetic protocols may be used to produce the desired compound, MOSD.

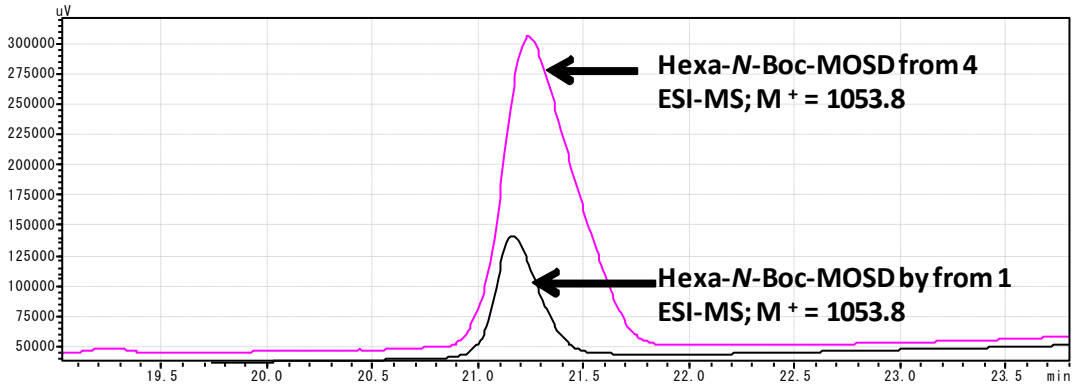


Figure S1. Comparison of HPLC traces run with hexa-*N*-Boc-MOSD **3** produced by methods either starting from compound **4** (pink), or from compound **1** (black). Also shown are the ESI-MS values of each peak. Chromatograms were obtained using reverse-phase HPLC employing a Shimadzu system (Tokyo, Japan), which consisted of two LC-20AP pumps and a APD-20AV photodiode array detector. An analytical column 4.6 x 250 nm, Cosmosil 5C_{18}-Ar-300 from Nacalai Tesque (Tokyo, Japan) was used. Samples were eluted using a combination of mobile phases A (100% H_2O with 0.1% TFA) and B (100% acetonitrile with 0.1% TFA) at a flow rate of 1.0 mL/ min. The detector was set to 220 nm.

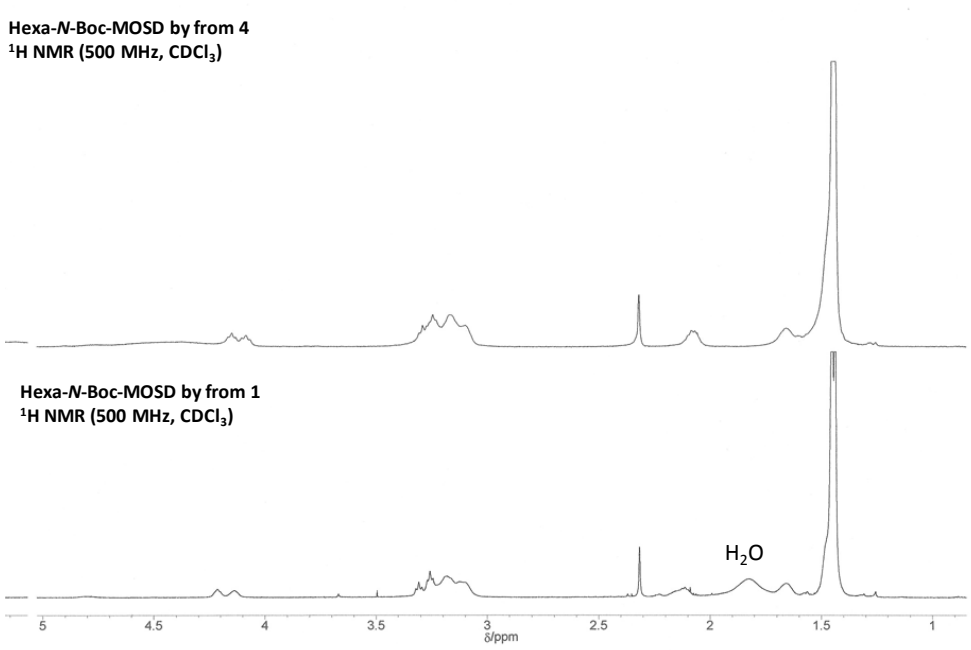


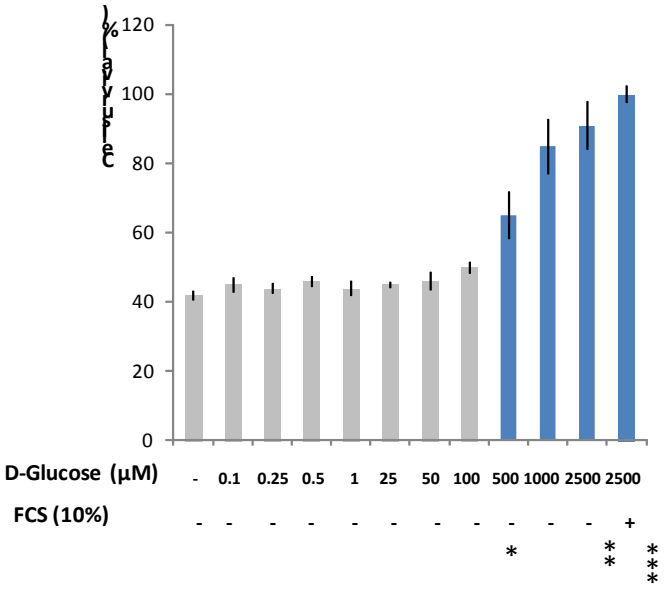
Figure S2. Comparison of NMR spectrum of hexa-*N*-Boc-MOSD **3** produced by methods either starting from compound **1** (bottom), or from com pound **4** (top).

Biological Studies

MTS assay

HeLa cells were cultured in DMEM (High glucose) (Wako, Tokyo Japan) supplemented with 10% (v/v) FBS and Penicillin-Streptomycin (Life Technologies, NY, USA) at 37 °C in a 5% CO_2-incubator. The MTS assay was run according to established procedure. Cells were first seeded onto 96-well plates (40,000 cells/ well) in 80 µL DMEM and incubated for 6 h. As indicated in Figure S3, the medium was replaced with 80 µL of various DMEM media (with and without FBS and antibody supplementation), followed by 20 µL of varying glucose concentrations. After incubation for 17 h at 37 °C under 5% CO_2, 20 µL of MTS reagent (CellTiter 96R Aqueous One Solution Cell Proliferation Assay, Promega) was added to each well and incubated for a further 3.5 h. The absorbance of each well at 450 nm was measured using a Bio-Rad Model 680 Microplate Reader.

Figure S3 Cell survival under various glucose concentration

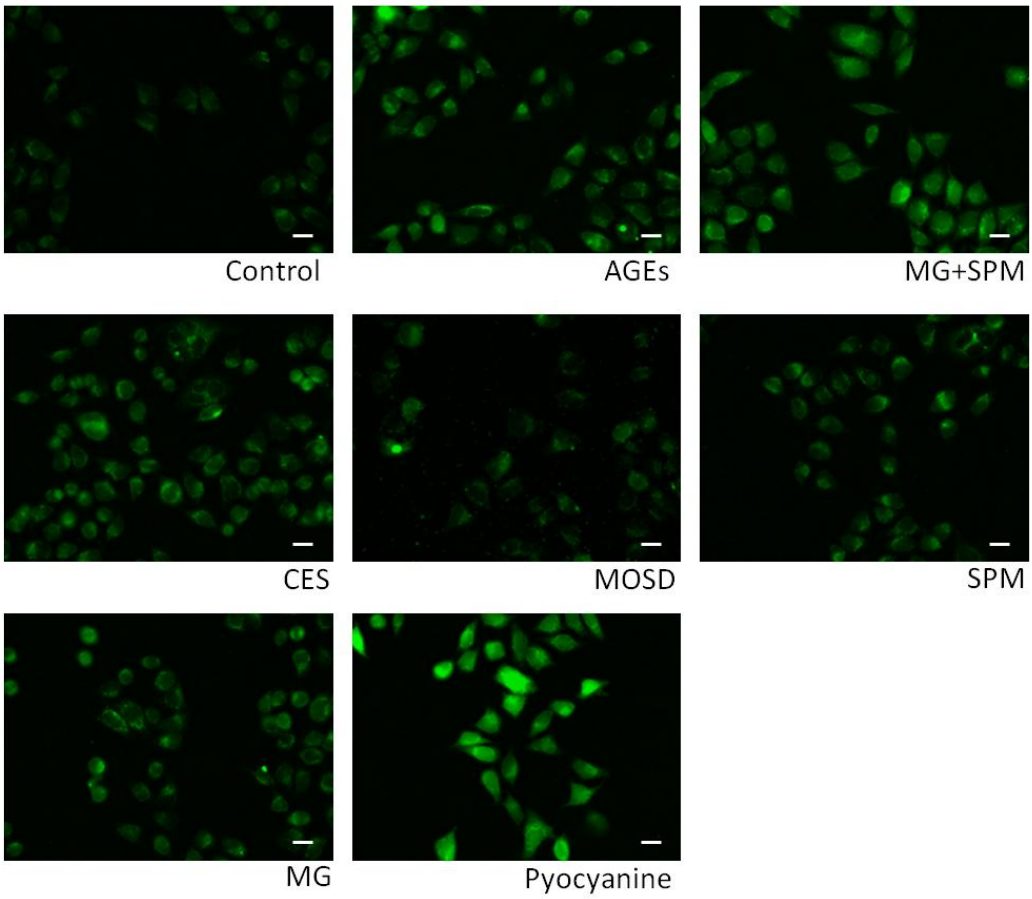


* Commercially low glucose medium concentration
** Commercially high glucose medium concentration
*** Normal condition of HeLa cell culture

ROS Detection

Commercial ROS-ID ™ Total ROS detection kit (Enzo, USA) was used. HeLa cells were seeded onto 8-well slide plates (30,000 cells/ well) in DMEM media and incubated overnight at 37 °C. Prior to the assay, media was replaced with 280 µL of DMEM containing 1 mM glucose without FBS. Solutions of 3mM Pyocyanine in DMF/PBS, 15 mM sample compounds in PBS, and 0.5 mM ROS detection reagent in DMF/PBS were prepared. For the positive control, 20 µL of Pyocyanine was added (final conc. 200 µM), immediately followed by 0.6 µL of ROS detection reagent (final conc. 1 µM). Cells were then incubated for 30 min at room temperature. For the samples under study, 20 µL of each compound was added (final conc. 1 mM) and the cells were incubated for 10 min at 37 °C. In the next step, 0.6 µL of ROS detection reagent was added (final conc. 1 µM), followed by incubation for a further 50 min at room temperature. For fluorescent microscopy, cell media was removed and cells were washed with PBS before analysis. The microscope filter lens used excitation and emission wavelengths of 470 nm/525 nm. Fluorescence intensity was calculated using Image J software (NIH, USA). Cell imaging data (Figure S4) was obtained by indiscriminately selecting approximately 10 cells and fluorescence intensity ratios (Figure 1a) were obtained as an average of 10 cells.

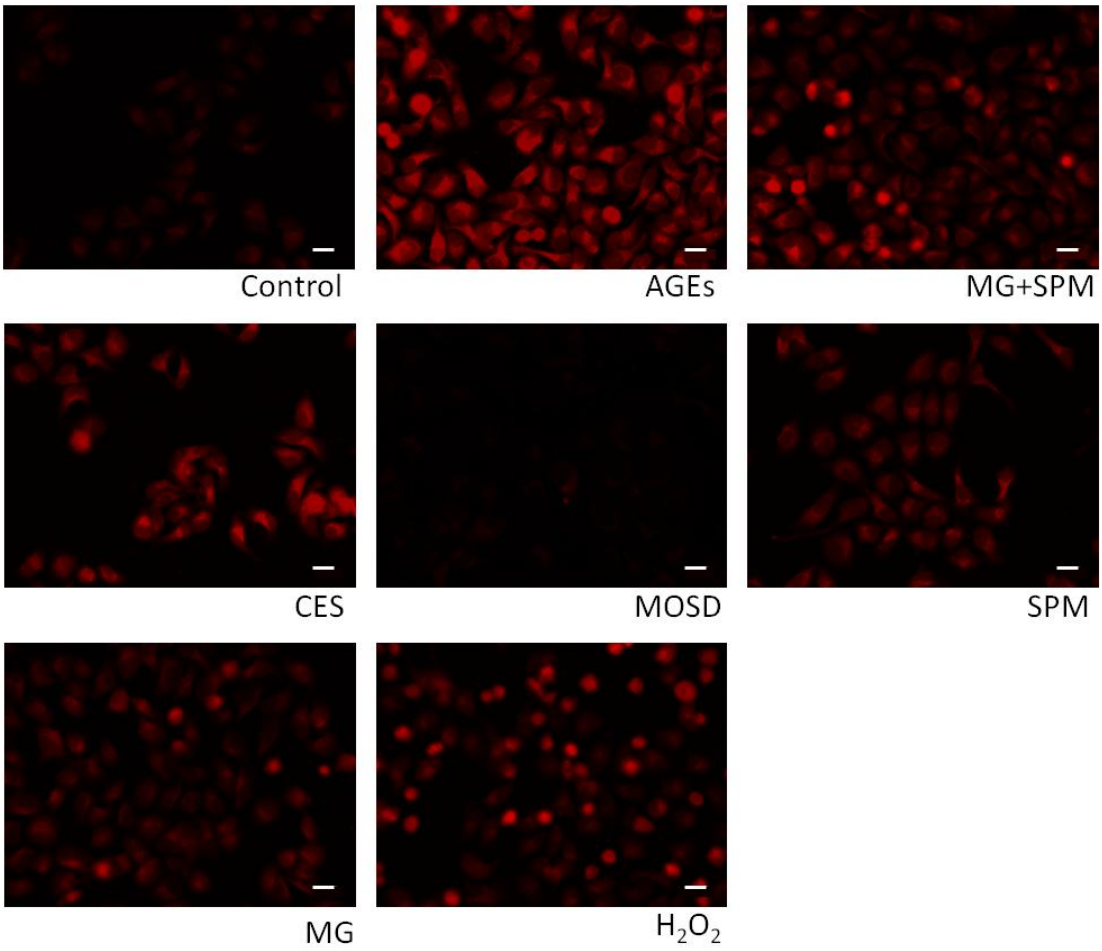
Figure S4 Fluorescence microscope pictures of ROS detection



Acrolein Detection

Acrolein detection reagent developed by our group was used. HeLa cells were seeded onto 8-well slide plates (30,000 cells/ well) in DMEM media and incubated for 6 h at 37 °C. Prior to the assay, media was replaced with 280 µL of DMEM containing 1 mM glucose without FBS. Solutions of 15 mM sample compounds in PBS and 160 mM acrolein detection reagent in PBS were prepared. For the sample compounds under study, 20 µL was added (final conc. 1 mM) and the cells were incubated for 17 h at 37 °C. In the next step, acrolein detection reagent was added (final conc. 10 µM), followed by incubation for a further 30 min at 37 °C. Finally, the medium was removed, and the cells were washed with PBS and fixated with 4% PFA. Cells were then incubated for 30 min at room temperature. For fluorescent microscopy, PFA was removed and cells were washed with PBS before analysis. The microscope filter lens used excitation and emission wavelengths of 545 nm/605 nm. Fluorescence intensity was calculated using Image J software (NIH, USA). Cell imaging data (Figure S5) was obtained by indiscriminately selecting approximately 10 cells and fluorescence intensity ratios (Figure 1b) were obtained as an average of 10 cells.

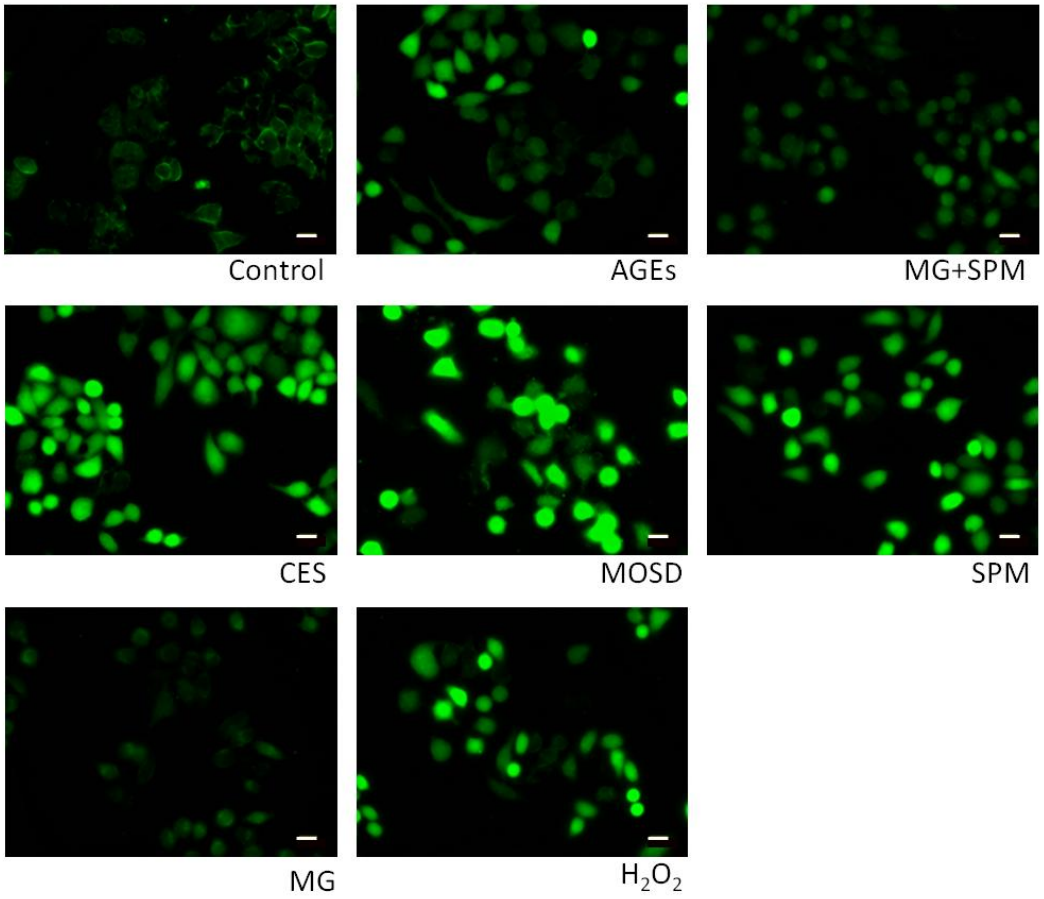
Figure S5 Fluorescence microscope pictures of acrolein detection



H_2O_2 Detection

Commercial OxiVision Green™ hydrogen peroxide sensor (ATT Bioquest, USA) was used. HeLa cells were seeded onto 8-well slide plates (30,000 cells/ well) in DMEM media and incubated overnight at 37 °C. Prior to the assay, media was replaced with 280 µL of DMEM containing 1 mM glucose without FBS. Solutions of 75 µM H_2O_2 in PBS, 15 mM sample compounds in PBS, and 160 mM H_2O_2 detection reagent in PBS were prepared. For the positive control, 20 µL of H_2O_2 was added (final conc. 50 µM) and the cells were then incubated for 5 min at 37 °C. For the sample compounds, 20 µL of each sample was added (final conc. 1 mM) and the cells were then incubated for 1.5 h at 37 °C. In all cases, 20 µL of H_2O_2 detection reagent was added (final conc. 10 µM), followed by incubation for a further 30 min at room temperature. For fluorescent microscopy, cell media was removed and cells were washed with PBS before analysis. The microscope filter lens used excitation and emission wavelengths of 470 nm/525 nm. Fluorescence intensity was calculated using Image J software (NIH, USA). Cell imaging data (Figure S6) was obtained by indiscriminately selecting approximately 10 cells and fluorescence intensity ratios (Figure 1c) were obtained as an average of 10 cells.

Figure S6 Fluorescence microscope pictures of H_2O_2 detection



Time-course study of acrolein levels influenced by MOSD addition

Typically, normal cells produce baseline levels of acrolein as a by-product of the polyamine metabolic pathway. With MOSD addition, acrolein levels were observed to fall significantly during time-course studies (from 2 to 24 hours). The assay to measure acrolein levels was performed as previously described. Fluorescence intensity values were measured cell at a time.

Figure S7 Time course of produced acrolein by MOSD