Methods

EGCG-5-TAMURA

NMR spectra were recorded on a JEOL Model ECP-400 (400 MHz for ^{1}H, 100 MHz for ^{13}C, 373 MHz for ^{19}F) in the indicated solvent. Chemical shifts were reported in parts per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane solutions in $CDCl_3$. ^{1}H NMR spectral data are reported as follows: $CDCl_3$ (7.26 ppm) or DMSO-d_6 (2.50 ppm), CD_3OD (3.30 ppm). ^{13}C NMR spectral data are reported as follows: $CDCl_3$ (77.0 ppm) or DMSO-d_6 (39.5 ppm). Multiplicities are reported by using the following abbreviations: s; singlet, d; doublet, t; triplet, q; quartet, m; multiplet, br; broad, *J*; coupling constants in Hertz. IR spectra was recorded on a Perkin-Elmer Spectrum One FT-IR spectrophotometer. Only the strongest and/or structurally important peaks are reported as the IR data given in cm^{-1}. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light, visualized by 10% ethanolic phosphomolybdic acid, 1% aqueous ceric sulfate solution, iodine, 0.75% aqueous KMn_2O_7 solution, 0.5% nynhydrin *n* –butanol solution or 5% ethanolic *p*-anisaldehyde solution. Flash column chromatography was performed on Silica Gel 60 N, purchased from Kanto Chemical Co. ESI-TOF Mass spectra were measured with Waters LCT Premier^{TM} XE. Analytical and preparative HPLC was carried out on a SSC-3461 pump with a SSC-5410 UV detector and a Waters 2475 fluorescence detector. THF, toluene and CH_2Cl_2 were dried by a Glass Contour. MeOH and EtOH were dried by distillation from magnesium turning with a catalytic amount of iodine.

2,4,6-Trihydroxybenzaldehyde

To a stirred solution of phloroglucinol (100 g, 658 mmol) in DMF (160 mL) and ethyl acetate (650 mL) was added dropwise $POCl_3$ (122 mL, 1.315 mol) at 0 ^{o}C for 2 h under Ar atmosphere. After being stirred at 40 ^{o}C for 20 h, the reaction mixture was filtered. The residue was washed with ice-cooled ethyl acetate, then suspended with hot H_2O at reflux temperature for 5 min, and cooled to room temperature. The solution was poured into ice-cooled H_2O and the aqueous layer was extracted with three portions of ethyl acetate. The combined organic layer was washed with H_2O and brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by recrystalization from H_2O to afford 2,4,6-trihydroxybenzaldehyde (60.7 g, 394 mmol, 70 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.68 (s, 2H), 10.67 (s, 1H), 9.91 (s, 1H), 5.75 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.9, 167.1, 164.5, 104.8, 94.3; FT-IR (solid) ν 3329, 3196, 2630, 1648, 1606, 1476, 1322, 1158, 1077, 830, 543 (cm^{-1}).

2-Benzyloxy-4,6-bis(methoxymethoxy)benzaldehyde

To a stirred solution of 2,4,6-trihydroxybenzaldehyde (10.0 g, 64.9 mmol) in MeCN (130 mL) was added dropwise DIEA (10.3 mL, 136 mmol) and dropwise MOMCl (23.7 mL, 136 mmol) at 0 ^{o}C under Ar atmosphere for over 40 min. After being stirred at same temperature for 80 min, the reaction mixture was poured into ice-cooled H_2O and the aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was used for the next reaction without further purification.

A solution of K_2CO_3 (18.0 g, 130 mmol) in dried and degassed DMF (65.0 mL) was stirred in a reaction vessel at room temperature under Ar atmosphere. To the reaction vessel was added a solution of the above residue at room temperature and stirred for 30 min, then BnBr (12.2 mL, 71.3 mmol) was added at the same temperature. After being stirred at 70 °C for 40 h, the reaction mixture was quenched with *N*,*N*-dimethylethane-1,2-diamine (773 μL, 7.13 mmol) at the same temperature, poured into ice-cooled H_2O and the aqueous layer were extracted with two portions of Et_2O. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography on silica gel (5% to 10% ethyl acetate in toluene) to afford 2-benzyloxy-4,6-bis(methoxymethoxy)benzaldehyde (10.0 g, 30.1 mmol, 47% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.46 (s, 1H), 7.47-7.30 (m, 5H), 6.45 (d, 1H, *J* = 1.9 Hz), 6.37 (d, 1H, *J* = 2.4 Hz), 5.24 (s, 2H), 5.16 (s, 2H), 5.13(s, 2H), 3.50 (s, 3H), 3.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.5, 163.4, 162.7, 161.0, 136.0, 128.5, 127.9, 127.0, 110.6, 96.0, 94.8, 94.7, 94.2, 70.5, 56.5, 56.3; FT-IR (neat) ν 3696, 2910, 1682, 1601, 1451, 1151, 1071, 1028, 927, 464, 479 (cm^{-1}).

2-Benzyloxy-4,6-dihydroxybenzaldehyde

To a stirred solution of 2-benzyloxy-4,6-bis(methoxymethoxy)benzaldehyde (10.0 g, 30.1 mmol) in MeOH (75.0 mL) was added dropwise 3 M aqueous HCl (20.0 mL) at room temperature under Ar atmosphere. After being stirred at 60 °C for 12 h, the reaction mixture was cooled to room temperature, poured into H_2O and the aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (20% to 25% ethyl acetate in hexane) to afford 2-benzyloxy-4,6-dihydroxybenzaldehyde (3.57 g, 14.6 mmol, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.42 (s, 1H), 10.16 (s, 1H) 7.40-7.35 (m, 5H), 5.97 (d, 1H, *J* = 1.9 Hz), 5.95 (d, 1H, *J* =2.4 Hz), 5.10 (s, 2H), 4.71 (d, 1H, *J* = 5.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.9, 163.4, 135.6, 128.8, 128.6, 128.4, 127.4, 127.0, 96.1, 91.8, 70.6; FT-IR (neat) ν 3357, 1635, 1454, 1385, 1259, 1198, 1175, 1103, 829, 794, 748, 703, 653, 574, 533, 487 (cm^{-1}).

2-Benzyloxy-4-(2-(2-chloroethoxy)ethoxy)-6-hydroxybenzaldehyde

To a stirred solution of 2-benzyloxy-4,6-dihydroxybenzaldehyde (1.00 g, 3.01 mmol) in dried and degassed DMF (12.3 mL) were added K_2CO_3 (668 mg, 4.91 mmol) and 2-(2-chloroethoxy)ethyl *p*-toluenesulfonate (1.14 g, 4.09 mmol) at room temperature under Ar atmosphere. After being stirred at 40 °C for 22 h, the reaction mixture was poured into ice-cooled 1 M aqueous HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (toluene) to afford 2-benzyloxy-4-(2-(2-chloroethoxy)ethoxy)-6-hydroxybenzaldehyde (**2-40**) (1.08 g, 3.06 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.48 (s, 1H), 10.16 (s, 1H), 7.40-7.33 (m, 5H), 6.05 (d, 1H, *J* = 1.9 Hz), 6.01 (d, 1H, *J* = 2.0 Hz), 5.08 (s, 1H), 4.17 (t, 2H, *J* = 4.4 Hz), 3.86 (t, 2H, *J* = 3.8 Hz), 3.81 (t, 2H, *J* = 5.8 Hz), 3.65 (t, 2H, *J* = 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.9, 167.0, 166.2, 162.6, 135.7, 128.7, 128.3, 127.3, 106.3, 93.7, 92.2, 71.5, 70.5, 69.3, 67.7, 42.6; FT-IR (neat) ν 2882, 1635, 1500, 1436, 1637, 1328, 1298, 1216, 1176, 1114, 1045, 922, 821, 741, 697, 665, 609, 542 (cm^{-1}).

6-Benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-hydroxybenzaldehyde

To a stirred solution of 2-benzyloxy-4-(2-(2-chloroethoxy)ethoxy)-6-hydroxybenzaldehyde (544 mg, 1.55 mmol) in dry CH_2Cl_2 (4.70 mL) was added dropwise Br_2 (80.0 μL, 1.55 mmol) at 0 °C under Ar atmosphere. After being stirred at the same temperature for 30 min, the reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$ and 10% aqueous $Na_2S_2O_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with 10% aqueous $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (20% ethyl acetate in hexane) to afford 6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-hydroxybenzaldehyde (542 mg, 1.26 mmol, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.95 (s, 1H), 10.12 (s, 1H), 7.41-7.32 (m, 5H), 6.12 (s, 1H), 5.13 (s, 1H), 4.22 (t, 2H, *J* = 4.8 Hz,), 3.92 (t, 2H, *J* = 4.8 Hz), 3.88 (t, 2H, *J* = 5.8 Hz), 3.65 (t, 2H, *J* = 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.8, 162.9, 162.2, 161.3, 135.2, 129.8, 128.8, 128.5, 127.8, 127.3, 106.6, 91.2, 89.4, 71.8, 70.9, 69.3, 69.2, 43.0; FT-IR (neat) ν 2882, 1638, 1563, 1501, 1550, 1416, 1385, 1296, 1218, 1120, 1089, 1030, 916, 790, 726, 699, 666, 609, 563 (cm^{-1}).

6-Benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)benzaldehyde

To a stirred solution of 6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-hydroxybenzaldehyde (1.71 g, 3.99 mmol) in dry and degassed DMF (12.0 mL) was added Cs_2CO_3 (2.60 g, 7.98 mmol) at 0 °C under Ar atmosphere. The reaction mixture was stirred at 0 °C for 30 min to generate phenolate, the reaction mixture was added dropwise PMBCl (650 μL, 4.79 mmol) at 0 °C for over 5 min. After being stirred at 40 °C for 10 h, the reaction mixture was poured into ice-cooled H_2O. The aqueous layer was extracted with two portions of Et_2O. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by recrystalization from ethyl acetate-hexane (suspended by ethyl acetate at 50 °C, then cooled to room temperature and added hexane) to afford 6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)benzaldehyde (1.62 g, 2.95 mmol, 74%).^{1}H NMR (400 MHz, $CDCl_3$) δ 10.34 (s, 1H), 7.53 (d, 2H, *J* = 8.7 Hz), 7.46-7.35 (m, 5H), 6.92 (d, 2H, *J* = 8.7 Hz), 6.45 (s, 1H), 5.20 (s, 1H), 4.97 (s, 2H), 4.21 (t, 2H, *J* = 4.3 Hz), 3.95 (t, 2H, *J* = 4.8 Hz), 3.88 (t, 2H, *J* = 5.8 Hz), 3.82 (s, 3H), 3.66 (t, 2H, *J* = 5.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.0, 161.7, 161.0, 159.8, 159.2, 135.6, 130.6, 128.7, 128.3, 128.2, 127.0, 114.9, 113.8, 100.6, 95.1, 76.3, 71.8, 71.1, 69.3, 69.2, 55.2, 43.9; FT-IR (solid) ν 1673, 1579, 1515, 1440, 1413, 1375, 1300, 1247, 1225, 1191, 1116, 1031, 963, 930, 904, 818, 740, 696, 663, 638, 609, 547, 481, 465 (cm^{-1}).

(*E*)-3-(6-Benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-1-(3,4,5-tris(benzyloxy)phenyl)prop-2-en-1-one

A solution of sodium-granule (116 mg, 5.04 mmol) in dry MeOH (640 μL) was stirred at 0 °C for 2 h under Ar atmosphere. To the reaction mixture was added dropwise a solution of 1-(3,4,5-tris(benzyloxy)phenyl)ethanone (479 mg, 1.09 mmol) in dry and degassed THF (3.00 mL) at 0 °C for over 20 min. Then the reaction mixture added dropwise a solution of

6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)benzaldehyde (500 mg, 0.909 mmol) in dry and degassed THF (1.50 mL) at 0 °C under Ar atmosphere. After being stirred at the same temperature for 10 min, the reaction mixture was poured into ice-cooled H_2O. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was washed with Et_2O to afford (*E*)-3-(6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-1-(3,4,5-tris (benzyloxy)phenyl)prop-2-en-1-one (743 mg, 0.766 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, 1H, *J* = 15.4 Hz), 7.95 (d, 1H, *J* = 15.4 Hz), 7.46-7.23 (m, 24H), 6.84 (d, 2H, *J* = 8.2 Hz), 6.50 (s, 1H), 5.19 (s, 2H), 5.11 (s, 2H), 4.93 (s, 4H), 4.86 (s, 2H), 4.19 (t, 2H, *J* = 4.8 Hz), 3.93 (d, 2H, *J* = 4.9 Hz), 3.89 (t, 2H, *J* = 5.8 Hz), 3.66 (t, 2H, *J* = 5.8 Hz), 3.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.6, 159.8, 159.1, 157.9, 157.5, 152.6, 142.2, 137.5, 136.7, 136.3, 135.8, 135.4, 133.9, 130.8, 128.8, 128.4, 128.1, 127.9, 127.6, 127.2, 123.7, 113.9, 113.6, 108.1, 100.6, 95.6, 75.3, 75.1, 71.9, 71.2, 71.1, 69.4, 69.4, 55.1, 43.0; FT-IR (solid) ν 3693, 3032, 2935, 1654, 1583, 1515, 1500, 1455、1425、1370、1323、1251, 1164, 1104, 1030, 1001, 852, 751, 697, 581, 489 (cm^{-1}).

3-(6-Benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-2-hydroxy-3-methoxy-1-(3,4,5-tris(benzyloxy)phenyl)propan-1-one

To a stirred aqueous solution of (*E*)-3-(6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-1-(3,4,5-tris(benzyloxy)phenyl)prop-2-en-1-one (740 mg, 0.770 mmol) in CH_2Cl_2 (15 mL) were added dropwise an aqueous solution of 3 M KOH (3.80 mL, 0.770 mmol), 30wt% aqueous H_2O_2 (8.70 mL, 77.0 mmol), and $Bu_4N \cdot HSO_4$ (553 mg, 1.54 mmol) at 0 °C under Ar atmosphere. After being stirred at room temperature for 8 d, the reaction mixture was poured into a mixture of ice-cooled 10% aqueous $Na_2S_2O_3$ and ethyl acetate. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was used for the next reaction without further purification.

To a stirred solution of the above residue in dry CH_2Cl_2 (3.90 mL) and MeOH (3.90 mL) was added $Sc(OTf)_3$ (75.8 mg, 0.154 mmol) at -78 °C under Ar atmosphere. After being stirred at the same temperature for 4 h, the reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (10% ethyl acetate in toluene) to afford 3-(6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-2-hydroxy-3-methoxy-1-(3,4,5-tris(benzyloxy) phenyl)propan-1-one (379 mg, 0.372 mmol, 48% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, 2H, *J* = 8.2 Hz), 7.44-7.21 (m, 20H), 7.00 (s, 2H), 6.89 (d, 2H, *J* = 6.4 Hz), 6.15 (s, 1H), 5.83 (t, 2H, *J* = 7.7 Hz), 5.02-4.77 (m, 10H), 4.01-3.89 (m, 2H), 3.78 (s, 3H), 3.76-3.71 (m, 4H), 3.54 (t, 2H, *J* = 6.3 Hz), 3.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0, 159.7, 157.4, 156.8, 156.5, 152.1, 142.5, 137.8, 137.5, 136.7, 136.3, 131.0, 130.8, 129.2, 129.0, 128.8, 128.4, 128.1, 127.8, 127.6, 127.4, 127.0, 125.3, 114.2, 113.9, 107.5, 95.7, 79.1, 75.1, 74.9, 73.8, 71.7, 70.8, 70.7, 69.3, 57.2, 55.2, 42.9, 21.4; FT-IR (neat) ν 3475, 3032, 2934, 1675, 1612, 1587, 1515, 1500, 1454, 1427, 1371, 1331, 1250, 1168, 1100, 1030, 978, 910, 827, 739, 697,

668, 484 (cm^{-1}).

1-(6-Benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-1-methoxy-3-oxo-3-(3,4,5-tris(benzyloxy)phenyl)propan-2-yl 3,4,5-tris(benzyloxy)benzoate

To a stirred solution of 3-(6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxybenzyl)oxy)phenyl)-2-hydroxy-3-methoxy-1-(3,4,5-tris(benzyloxy)phenyl)propan-1-one (378 mg, 0.371 mmol) in CH_2Cl_2 (2.60 mL) was added 3,4,5-tris(benzyloxy)benzoic acid (**2-20**) (196 mg, 0.445 mmol), EDCI (95.6 mg, 0. 557 mmol), and a catalytic amount of DMAP (9.00 mg, 0.0742 mmol) at 0 °C under Ar atmosphere. After being stirred at room temperature for 7 h, the reaction mixture was poured into ice-cooled 1M aqueous HCl. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (3% ethyl acetate in toluene) to afford 1-(6-benzyloxy)-3-bromo-4-(2-(2-chloroethoxy)ethoxy)
-2-((4-methoxybenzyl)oxy)phenyl)-1-methoxy-3-oxo-3-(3,4,5-tris(benzyloxy)phenyl)propan-2-yl 3,4,5-tris (benzyloxy)benzoate (419 mg, 0.291 mmol, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, 2H, *J* = 7.7 Hz,), 7.53-7.20 (m, 37H), 7.13 (s, 2H), 7.01 (d, 1H, *J* = 8.7 Hz), 6.80 (d, 1H, *J* =7.2 Hz), 6.16 (s, 1H), 5.43 (d, 1H, *J* = 8.7 Hz), 5.17-4.72 (m, 16H), 3.92 (m, 2H), 3.75-3.68 (m, 4H), 3.68 (s, 3H), 3.52 (t, 2H, *J* =5.3 Hz), 3.43 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.5, 165.9, 159.6, 157.1, 152.6, 152.2, 137.7, 137.4, 136.8, 136.7, 136.5, 130.7, 129.4, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.1, 113.9, 109.7, 107.6, 95.7, 75.4, 75.1, 75.1, 71.2, 70.8, 69.2, 57.7, 55.1, 42.9; FT-IR (neat) ν 3065, 3033, 2935, 2250, 1955, 1714, 1683, 1588, 1515, 1500, 1455, 1428, 1371, 1329, 1250, 1203, 1169, 1115, , 30, 982, 910, 825, 736, 697, 648, 588, 484 (cm^{-1}).

(2*R,3*S**)-5-benzyloxy-7-(2-(2-chloroethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate**

To a stirred solution of (2*R**,3*S**)-1-(6-benzyloxy-3-bromo-4-(2-(2-chloroethoxy)ethoxy)-2-((4-methoxy-benzyl)oxy)phenyl)-1-methoxy-3-oxo-3-(3,4,5-tris(benzyloxy)phenyl)propan-2-yl 3,4,5-tris(benzyloxy) benzoate (225 mg, 0.154 mmol) in dry CH_2Cl_2 (13.2 mL) was added dropwise TESH (1.45 mL) and TFA (775 μL) at 0 °C under Ar atmosphere. After being stirred at room temperature for 2 h, the reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (3% ethyl acetate in toluene) to afford (2*R**,3*S**)-5-benzyloxy-7-(2-(2-chloroethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (120 mg, 0.100 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.18 (m, 37H), 6.74 (s, 2H), 6.31 (d, 1H, *J* = 2.0 Hz), 6.29 (d, 1H, *J* = 1.9 Hz), 5.67 (br-s, 1H), 5.05-4.66 (m, 15H), 4.09 (t, 2H, *J* = 3.9 Hz), 3.82 (t, 2H, *J* = 4.8 Hz), 3.77 (t, 2H, *J* = 5.8 Hz), 3.61 (t, 2H, *J* = 4.8 Hz), 3.08 (ddd, 2H, *J* = 4.4, 15.2, 17.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.8, 158.7, 158.0, 155.5, 152.8, 152.3, 142.7, 138.4, 137.7, 137.4, 136.8, 136.7, 136.4, 133.2, 133.2, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.4, 127.1, 124.9, 109.2, 106.7, 101.0, 94.4, 93.9, 75.1, 75.0, 74.9,

71.5, 71.2, 71.0, 71.0, 70.0, 69.6, 68.2, 67.5, 42.6; FT-IR (neat) ν 3090, 3065, 3032, 2935, 2875, 2250, 1953, 1813, 1717, 1620, 1592, 1499, 1454, 1430, 1370, 1328, 1215, 1158, 1115, 1078, 1029, 910, 844, 816, 735, 697, 667, 620, 483 (cm^{-1}).

(2*R,3*S**)-7-(2-(2-Azidoethoxy)ethoxy)-5-(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (2-54)**

To a stirred solution of (2*R**,3*S**)-5-benzyloxy-7-(2-(2-chloroethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (120 mg, 0.100 mmol, 1.00 eq.) in dry DMF (1.00 mL) was added sodium azide (32.5 mg, 0.500 mmol, 5.00 eq.) and TBAI (55.4 mg, 0.150 mmol, 1.50 eq.) at room temperature under Ar atmosphere. After being stirred at 70 °C for 4 d, the reaction mixture was poured into H_2O. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (2% ethyl acetate in toluene) to afford (2*R**,3*S**)-7-(2-(2-azidoethoxy)ethoxy)-5-(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (89.4 mg, 0.0748 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.15 (m, 37H), 6.74 (s, 2H), 6.31 (d, 1H, *J* = 2.0 Hz), 6.29 (d, 1H, *J* = 1.9 Hz), 5.67 (br-s, 1H), 5.08-4.66 (m, 15H), 4.10 (t, 2H, *J* = 3.9 Hz), 3.83 (t, 2H, *J* = 4.8 Hz), 3.71 (t, 2H, *J* = 4.8 Hz), 3.38 (t, 2H, *J* = 4.8 Hz), 3.10 (ddd, 2H, *J* = 4.4, 15.2, 17.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 164.8, 158.8, 158.0, 155.6, 154.9, 152.9, 152.4, 142.8, 137.8, 137.5, 136.9, 136.8, 136.4, 128.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.2, 109.2, 106.8, 101.0, 94.4, 93.9, 75.1, 75.0, 71.2, 71.1, 70.2, 69.6, 68.3, 67.5, 50.7; FT-IR (neat) ν 3065, 3032, 2931, 2892, 2103, 1954, 1717, 1620, 1592, 1499, 1455, 1430, 1371, 1328, 1215, 1159, 1116, 1029, 911, 815, 736, 697, 480 (cm^{-1}).

(2*R,3*S**)-5-Benzyloxy-7-(2-(2-(2,2-dimethoxypropanamido)ethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate**

To a stirred solution of (2*R**,3*S**)-7-(2-(2-azidoethoxy)ethoxy)-5-(benzyloxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (10 mg, 8.4 μmol) in THF (100 μL) and H_2O (100 μL) was added Zn powder (1.6 mg, 25 μmol) and NH_4Cl (0.40 mg, 8.4 μmol) at 0 °C under Ar atmosphere. After being stirred at room temperature for 30 min, the reaction mixture was filtered through a pad of Celite. The filtrate mixture was used for the next reaction without removal of the solvent nor further purification.

To a solution of the above filtrate was added sodium 2,2-dimethoxypropanoate (1.4 mg, 9.2 μmol) and DMT-MM (2.5 mg, 9.2 μmol) at 0 °C under Ar atmosphere. After being stirred at room temperature for 10 min, the reaction mixture was poured into H_2O. The aqueous layer was extracted with two portions of ethyl acetate. The combined organic layer were washed with brine, dried over $MgSO_4$ and filtered. After removal of the solvent, the residue was purified by flush column chromatography (30% ethyl acetate in toluene) to afford (2*R**,3*S**)-5-benzyloxy-7-(2-(2-(2,2-dimethoxy-propanamido)ethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (**2-14**) (10.8 mg, 8.4 μmol, quant. in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.16 (m, 37H), 6.73 (s, 2H), 6.30 (br-s, 1H), 6.29 (br-s, 1H), 5.66 (br-s, 1H), 5.05-4.67 (m, 15H), 4.09 (dd, 2H, *J* = 6.9, 3.5 Hz), 3.79 (dd,

2H, *J* = 4.9, 4.3 Hz), 3.63 (t, 2H, *J* = 5.4 Hz), 3.51 (t, 2H, *J* = 5.3 Hz), 3.21 (s, 6H), 3.10 (ddd, 2H, *J* = 8.7, 6.5, 4.3 Hz), 1.46 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 170.0, 158.7, 155.5, 152.8, 152.3, 137.7, 136.8, 136.5, 133.1, 128.9, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.4, 127.1, 125.2, 124.9, 109.2, 106.7, 100.3, 75.0, 74.9, 71.2, 71.0, 69.9, 69.3, 68.2, 67.4, 55.9, 55.8, 50.2, 49.6, 38.8, 20.8; FT-IR (neat) ν 3034, 2938, 1716, 1689, 1620, 1592, 4500, 1454, 1430, 1370, 1328, 1234, 1193, 1159, 1116, 1030, 910, 733, 697, 604, 471 (cm^{-1}).

(2*R,3*S**)-5-Hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (2-13)**

H-Cube® was charged with $Pd(OH)_2$/C CatCart column and heated to 50 °C. The hydrogen pressure was set to 20 bar. (2*R**,3*S**)-5-Benzyloxy-7-(2-(2-(2,2-dimethoxypropanamido)ethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (30.0 mg, 23.0 μmol, 1.00 eq.) was dissolved in THF/MeOH (1/1, 30.0 mL) and the solution was pumped through the system with a flow rate of 1.0 mL/min. After passing through the instrument, the reaction mixture was collected under N_2 flow atmosphere and column was washed with MeOH (30.0 mL). The above residue were combined and concentrated *in vacuo*. The residue was dissolved in H_2O and lyophilized. The lyophilized solid was used for the next reaction without further purification.

To a stirred solution of the lyophilized solid in H_2O (4.70 mL) was added TFA (470 μL) at room temperature under Ar atmosphere. After being stirred at 50 °C for 2 h, the reaction mixture was cooled to room temperature and purified by reverse-phase column chromatography (VARIAN Bond Elut® C18) (0% then 30% MeCN in H_2O) to afford (2*R**,3*S**)-5-hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (10.8 mg, 17.6 μmol, 76% in 2 steps) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.79 (s, 2H), 6.40 (s, 2H), 6.00 (d, 1H, *J* = 2.4 Hz), 5.99 (d, 1H, *J* = 2.4 Hz), 5.37 (br-s, 1H), 4.98 (br-s, 1H), 3.97 (br-s, 2H), 3.68 (br-s, 2H), 3.52 (t, 2H, *J* = 5.8 Hz), 3.38-3.21 (m, 2H), 3.01-2.86 (br, 2H), 2.32 (s, 3H).

(*Z*)-*N*-(9-(2-carboxy-4-((15-((5-hydroxy-3-((3,4,5-trihydroxybenzoyl)oxy)-2-(3,4,5-trihydroxyphenyl) chroman-7-yl)oxy)-8-methyl-9-oxo-3,6,13-trioxa-7,10-diazapentadec-7-en-1-yl)carbamoyl)phenyl)-6-(dimethylamino)-3*H*-xanthen-3-ylidene)-*N*-methylmethanaminium

To a stirred solution of *N*-(9-(2-carboxy-4-((2,2-dimethyl-4-oxo-3,6,9-trioxa-5-azaundecan-11-yl) carbamoyl)phenyl)-6-(dimethylamino)-3*H*-xanthen-3-ylidene)-*N*-methylmethanaminium (1.9 mg, 3.00 μmol, 1.00 eq.) in dried CH_2Cl_2 (270 μL) was added dried TFA (30.0 μL) at 0 °C under Ar atmosphere. After being stirred at the same temperature for 30 min with being monitored by HPLC with fluorescence detection, the reaction mixture was evaporated *in vacuo*. The crude mixture was used for the next reaction without further purification.

To a solution of the above residue in degassed MeCN (400 μL) was added a solution of 5-hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-

trihydroxybenzoate (**2-13**) (2.10 mg, 3.41 μmol, 1.15 eq.) and a degassed pH = 3 buffer solution (pyridine: acetic acid = 1:20, 40 μL) at room temperature under Ar atmosphere. After being stirred at 60 °C for 4 h with monitored by HPLC with fluorescence detection, the reaction mixture was diluted with a degassed mixture of H_2O and TFA (999:1, 10 mL) and centrifuged to afford (*Z*)-*N*-(9-(2-carboxy-4-((15-((5-hydroxy-3-((3,4,5-trihydroxybenzoyl)oxy)-2-(3,4,5-trihydroxyphenyl)chroman-7-yl)oxy)-8-methyl-9-oxo-3,6,13-trioxa-7,10-diazapentadec-7-en-1-yl)carbamoyl)phenyl)-6-(dimethylamino)-*3H*-xanthen-3-ylidene)-*N*-methylmethanaminium (2.4 mg, 2.12 μmol, 71% in 2 steps) as an orange powder.

FT-IR (solid) ν 3272, 2141, 1944, 1672, 1601, 1537, 1453, 1376, 1311, 1205, 1036, 917, 851, 802, 725, 553 (cm^{-1}); HRMS (ESI-TOF) calcd. for $C_{58}H_{60}N_5O_{19}$ $[M+H]^+$ 1130.3883, found 1130.3844.

EGCG-7-FITC

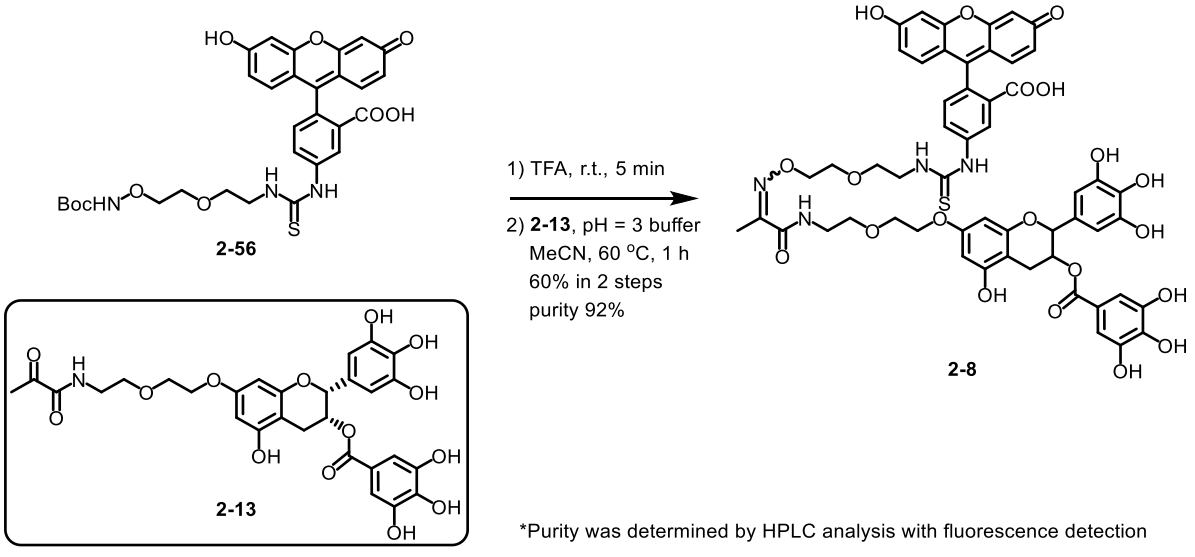
(2*R,3*S**)-5-Hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (2-13)**

H-Cube® was charged with $Pd(OH)_2$/C CatCart column and heated to 50 °C. The hydrogen pressure was set to 20 bar. (2*R**,3*S**)-5-Benzyloxy-7-(2-(2-(2,2-dimethoxypropanamido)ethoxy)ethoxy)-2-(3,4,5-tris(benzyloxy)phenyl)chroman-3-yl 3,4,5-tris(benzyloxy)benzoate (**2-14**) (30.0 mg, 23.0 μmol, 1.00 eq.) was dissolved in THF/MeOH (1/1, 30.0 mL) and the solution was pumped through the system with a flow rate of 1.0 mL/min. After passing through the instrument, the reaction mixture was collected under N_2 flow atmosphere and column was washed with MeOH (30.0 mL). The above residue were combined and concentrated *in vacuo*. The residue was dissolved in H_2O and lyophilized. The lyophilized solid was used for the next reaction without further purification.

To a stirred solution of the lyophilized solid in H_2O (4.70 mL) was added TFA (470 μL) at room temperature under Ar atmosphere. After being stirred at 50 °C for 2 h, the reaction mixture was cooled to room temperature and purified by reverse-phase column chromatography (VARIAN Bond Elut® C18) (0% then 30% MeCN in H_2O) to afford (2*R**,3*S**)-5-hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (**2-13**) (10.8 mg, 17.6 μmol, 76% in 2 steps) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 6.79 (s, 2H), 6.40 (s, 2H), 6.00 (d, 1H, *J* = 2.4 Hz), 5.99 (d, 1H, *J* = 2.4 Hz), 5.37 (br-s, 1H), 4.98 (br-s, 1H), 3.97 (br-s, 2H), 3.68 (br-s, 2H), 3.52 (t, 2H, *J* = 5.8 Hz), 3.38-3.21 (m, 2H), 3.01-2.86 (br, 2H), 2.32 (s, 3H).

5-(3-(15-(((2*R,3*S**)-5-Hydroxy-3-((3,4,5-trihydroxybenzoyl)oxy)-2-(3,4,5-trihydroxyphenyl)chroman-7-yl)oxy)-8-methyl-9-oxo-3,6,13-trioxa-7,10-diazapentadec-7-en-1-yl)thioureido)-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid**



*Purity was determined by HPLC analysis with fluorescence detection

To a stirred solution of 5-(3-(2,2-dimethyl-4-oxo-3,6,9-trioxa-5-azaundecan-11-yl)thioureido) -2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid(2.75 mg, 4.55 μmol) in dried TFA (45.0 μL) at room,

temperature under Ar atmosphere. After being stirred at the same temperature for 5 min with being monitored by HPLC with fluorescence detection, the reaction mixture was evaporated *in vacuo*. The crude mixture was used for the next reaction without further purification.

To a solution of the above residue in degassed MeCN (400 μL) was added a solution of 5-hydroxy-7-(2-(2-(2-oxopropanamido)ethoxy)ethoxy)-2-(3,4,5-trihydroxyphenyl)chroman-3-yl 3,4,5-trihydroxybenzoate (4.33 mg, 5.43 μmol) and a degassed pH = 3 buffer solution (pyridine: acetic acid = 1:20, 11 μL) at room temperature under Ar atmosphere. After being stirred at 60 °C for 1 h with monitored by HPLC with fluorescence detection, the reaction mixture was diluted with a degassed mixture of H_2O and TFA (999:1, 10 mL) and centrifuged to afford 5-(3-(15-(((2*R**,3*S**)-5-hydroxy-3-((3,4,5-trihydroxybenzoyl)oxy)-2-(3,4,5-trihydroxyphenyl)chroman-7-yl)oxy)-8-methyl-9-oxo-3,6,13-trioxa-7,10-diazapentadec-7-en-1-yl)thioureido)-2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoic acid (3.0 mg, 2.71 μmol, 60% in 2 steps, purity 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.72 (brs, 1H), 7.16 (d, 1H, *J* = 8.2 Hz), 6.79 (s, 2H), 6.67-6.52 (m, 7H), 6.40 (s, 2H), 6.00 (d, 1H, *J* = 2.4 Hz), 5.99 (d, 1H, *J* = 1.9 Hz), 5.37 (br-s, 1H), 4.98 (br-s, 1H), 4.29-4.26 (m, 2H), 3.97 (br-s, 2H), 3.73-3.58 (m, 8H), 3.53-3.48 (m, 2H), 1.90 (s, 3H); FT-IR (solid) ν 3272, 2141, 1944, 1672, 1601, 1537, 1453, 1376, 1311, 1205, 1036, 917, 851, 802, 725, 553 (cm^{-1}).

Reagents and compounds

EGCG (E4143), catalase (C100) and superoxide dismutase (SOD) (S5395) were purchased from Sigma-Aldrich (St. Louis, MO). Synthesized peptides, deduced from the extracellular domain corresponding to the 102–295 region of the human 67LR, were purchased from Thermo Electron GmbH (Ulm, Germany). siRNAs were purchased from Life Technologies™ (Carlsbad, CA). Silencer Select Negative Control No.1: Cat. # 4390843 and Silencer Select si-67LR: Cat. # 4392420 s194591 were used. Anti-67LR serum was obtained from a rabbit, which was immunized with a synthesized peptide corresponding to residues 161–170 of human 67LR.

Cell culture

Human multiple myeloma cells U266 were maintained in RPMI 1640 medium containing 10% FBS. HEK293 cells (Human Embryonic Kidney cell line; ATCC) and HepG2 cells (human hepatoma cell line; ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). All cell lines were maintained in a state of exponential growth at 37°C in humidified air with 5% CO_2. To avoid the effect of ROS (Reactive Oxygen Species) and EGCG instability, catalase and SOD were added. Each 67LR peptide (1 μM) and EGCG (1 μM) were mixed and incubated at room temperature for 15 min in DMEM supplemented with 200 U/ml catalase and 5 U/ml SOD. The mixture was then added to the HepG2 cells in DMEM supplemented with 2% FBS, 5 mg/ml BSA, 200 U/ml catalase and 5 U/ml SOD. Following an incubation of five days, the cells in each well were enumerated using a cell counter (Sysmex Corporation, Tokyo, Japan). In this study, cell death was not observed in HepG2 cells treated with or without EGCG. To assess Akt activities and cGMP levels, U266 cells were plated in 96-well plates and treated with 10 μM EGCGs. To measure Akt activation, cells were treated with EGCG for 1h, and to measure cGMP production, cells were treated with EGCG for 3h. Evaluation of Akt activity and cGMP induction was performed as previously described[10].

Thermodynamic analysis

Thermodynamic analysis was performed by using a TAM III Calorimeter (TA instruments) at 25°C. Prior to experiments, r67LR was dialyzed in 2% dimethyl sulfoxide (DMSO) and phosphate buffered saline, pH 7.4. After the solution was filtered by using a 0.45 μm syringe filter, titration was performed with EGCG and r67LR using 2.48 μL injections of 2 mM EGCG or EGCG analogue into a 40 μM r67LR solution. Injection of the ligands into the solution were subtracted from original titration data prior to data analysis. For all analyses, thermodynamic parameters (enthalpy: ΔH, entropy: ΔS and free energy: ΔG) were calculated from the injection of the ligands into the buffer and subtracted from raw titration data by using Nano Analyze version 2.4.1 (TA instruments). From the values of ΔH, the changes in ΔG and in ΔS were calculated using the following equation: $\Delta G = -RT \ln Ka = \Delta H - T\Delta S$, where R is the universal molar gas constant and T is the absolute temperature. Oligomer formation was evaluated as previously described [18, 19].

Cell-surface binding analysis of EGCG

Analysis of the interaction between EGCG and the 67LR-overexpressed HepG2 cells was performed using the surface plasmon resonance (SPR) biosensor SPR670 (Moritex Corp., Tokyo, Japan). The cells were immobilized on the sensor chip and the chip was equilibrated in PBS. EGCG was added at a flow rate of 30 ml/min. The cell surface binding was measured at 25°C for 2 min followed by dissociation. In this binding analysis, the SPR signal had a characteristic behavior as follows: The elevation of the SPR signal (the value of the changed resonance angle: resonance units) was immediately observed after the injection of the ligands (+EGCG). After the termination of the ligand exposure (–EGCG), the perfusion buffer was changed to the ligand-free running buffer. The SPR signal decreased due to the dissociation of ligands bound to the surface of the immobilized molecules, and the signal converged to a constant level.

Evaluation of the antioxidant activity

The antioxidant activity of EGCG and EGCG analogue were performed by evaluating Cu^{+} derived from the reduction of Cu^{2+}, which was added at known concentrations either to standard or to experimental samples. The oxygen radical absorbance capacity (ORAC) assay was performed. Briefly, 25 μL of 25 μM EGCG and EGCG analogue were resolved in 75 mM phosphate buffer (pH 7.0) and added to a 96-well plate. Fluorescein isothiocyanate (FITC) sodium (150 μL, 100 nM in phosphate buffer) was used as a target of free radical attack with 2,2′-azobisisobutyramidinium (25 μL, 200 mM in phosphate buffer), a free radical generator. Trolox was used as a standard anti-oxidant agent. The FITC fluorescence decay was monitored at 4-min intervals (Ex, 485 nm; Em, 535 nm). Fluorescent intensity was evaluated by using a plate reader (EnVision, PerkinElmer).

Fluorescence resonance energy transfer (FRET)

67LR (161–170) peptide and control competence-stimulating peptide (CSP of *Streptococcus pneumonia*) or HEK293 cells transfected with control siRNA or siRNA targeting 67LR were dissolved in 100 μL phosphate buffer saline in a the 96 well plate. A mixture (50 μL) of 7-FITC-labelled EGCG (8 μM) and

5-tetramethylrhodamine (TAMURA)-labelled EGCG (8 μM) was added. Fluorescent intensity was evaluated by using a plate reader (EnVision, PerkinElmer).

Western blot analysis

Western blotting was performed as previously described[11].

Statistics

Data were analysed with GraphPad Prism (version 4) using Student's *t*-tests when comparing two conditions, Dunnett's test when comparing with controls and Tukey's test for multiple comparisons. Values of $p < 0.05$ were considered significant. All of the in vitro data are representations of more than three independent experiments.