Supporting Information for

Cell Surface-Anchored Fluorescent Probe Capable of Real-Time Imaging of Single Mast Cell Degranulation Based on Histamine-Induced Coordination Displacement

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Figure S1 Fluorescence signal change of the coumarin probe 1a ~ 1d upon addition of CoCl₂ (blue bar), NiCl₂ (green bar), and CuCl₂ (yellow bar). The fluorescence intensity ($F_{\text{metal}}$) of the metal ion complex was measured at the saturation point in each experiment. Measurement conditions: [probe] = 5.0 µM, [metal ion] = 5 ~ 50 µM in HBSS, $\lambda_{\text{ex}}$ = 410 nm.

Figure S2 Plot the fluorescence signal change ($\lambda_{\text{ex}}$ = 500 nm) of 1d upon addition of (a) CoCl₂ and (b) NiCl₂. Measurement conditions: (a) [1d] = 5 µM, (b) [1d] = 1 µM in HBSS, $\lambda_{\text{ex}}$ = 410 nm. The data were analyzed by non-linear curve-fitting analysis assuming 1 : 1 binding to obtain the apparent stability constants ($K_a, \text{M}^{-1}$).
Figure S3 (a) Fluorescence spectral change of 2 upon addition of CoCl$_2$. (b) Plot of fluorescence intensity at 685 nm. (c) Fluorescent spectral change of 2 upon addition of NiCl$_2$. (d) Plot of fluorescence intensity at 680 nm. Measurement conditions: [2] = 0.5 µM in HBSS, $\lambda_{\text{ex}}$ = 625 nm.
Figure S4 Time trace plot of the fluorescence intensity ($\lambda_{em} = 680$ nm) of 2-Co(II) (a) and 2-Ni(II) (b) upon addition of histamine (1000 µM) Measurement conditions: [2-Co(II)] or [2-Ni(II)] = 2 µM (4 µM of CoCl$_2$ or NiCl$_2$) in HBSS. $\lambda_{ex} = 625$ nm. The fluorescence intensity at 680 nm was measured at every 5 sec.

Figure S5 (a) Fluorescence spectral change of 2-Ni(II) (solid lines) upon addition of histamine (0 to 2 mM). Measurement conditions: [2] = 5.0 µM with 2.0 equiv of Ni(II). [histamine] = 2 mM in HBSS, $\lambda_{ex} = 640$ nm. Dashed line indicates the fluorescence spectrum of 2 (5.0 µM) in HBSS. (b) Plot of fluorescence intensity at 690 nm.
Figure S6 Fluorescence HPLC analysis of histamine released from RBL-2H3 cells (a) with or (b) without stimulation by A23187 (5 µM) using o-phthalaldehyde method. The fluorescent histamine derivative was detected at 22 min.

Figure S7 Fluorescent HPLC analysis of histamine released from RBL-2H3 cells (a) without or (b) with the pre-treatment of epigallocatechin gallate (EGCG), an inhibitor of histidine decarboxylase (HDC), using o-phthalaldehyde method. The fluorescent histamine derivative was detected at 22 min.
Figure S8 Time trace of the fluorescence signal change of a rat peritoneal mast cell with stimulation by (a) 5 μM A23187 or (b) 100 μM substance P.
**Fluorescence titration**

Fluorescence titration with histamine was conducted with a solution of metal ion complex of the fluorescent probe in 3 mL of Hank’s balanced salt solution (HBSS, containing 1.3 mM Ca$^{2+}$ and 0.9 mM Mg$^{2+}$) at 25 °C. Typically, an aqueous stock solution of histamine dihydrochloride (0.6-300 mM, Sigma-Aldrich 53300) was added to a solution of 2-Co(II) (2 µM of 2 complexed with 4 µM of CoCl$_2$) in a quartz-cell with a micropipette, and the fluorescence emission spectra were recorded. Relative fluorescence quantum yields ($\Phi$) of the Cy5 derivative 2 was determined by using an EtOH solution of Nile Blue ($\Phi = 0.27$) as a standard.$^1$

**Cell culture**

RBL-2H3 cells were cultured in high-glucose Dulbecco’s Modified Eagle Medium (DMEM, 4.5 g of glucose/L) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 µg/mL) under a humidified atmosphere of 5% CO$_2$ in air. For all experiments, cells were harvested from subconfluent (<80%) cultures using a trypsin-EDTA solution and then resuspended in fresh medium. A subculture was performed every 2−3 days.

**Fluorescence imaging of degranulation of RBL-2H3 cell**

RBL-2H3 cells cultured in complete DMEM were treated with 3 (0.8 µM) and CoCl$_2$ (10 µM) in HBSS for 10 min at rt. The cells were washed twice with HBSS and subjected to imaging analysis. The fluorescence images were collected with a fluorescence microscope (LSM 700, ZEISS) with a 63x oil-immersion objective lens at Cy5 channel (excitation: 639 nm, emission: 640-700 nm). The data were analyzed by ZEN software (ZEISS) and Image J (NIH).

In the case of the fluorescence imaging under the conditions of histidine decarboxylase inhibition, the cells were pre-incubated in DMEM supplemented with epigallocatechin gallate (EGCG, 30 µM) for 48 hrs in CO$_2$ incubator, during which time the medium was changed at 24 and 48 hrs.

**Fluorescence detection of released histamine by o-phthalaldehyde method$^2$**

A suspension of RBL-2H3 cell (5.0×10$^5$ cells/mL) in HBSS (2 mL) was stimulated with A23187 (5 µM) and incubated for 20 min at 37 °C. After cooling to 4 °C for 15 min, the cell suspension was centrifuged (500 G, 10 min) at 4 °C. The supernatant (ca. 2 mL) was mixed with NaCl (0.75 g), 1 M NaOH aq (0.5 mL), and n-BuOH / CHCl$_3$ (3 : 2, v/v, 5 mL). After gentle agitation (5 min at rt) and the following centrifugation (300 G, 5 min at 4 °C), the organic layer (4 mL) was separated and mixed with n-heptane (2 mL) and 0.1 M HCl aq. (1.5 mL). After gentle agitation (5 min at rt) and the following centrifugation (270 G, 5 min at 4 °C), the aqueous phase (1 mL) was separated and mixed with 1 M NaOH aq (0.15 mL) and 0.2% o-phthalaldehyde in MeOH solution (0.1% w/v). The mixture was left to stand for 5 min at rt.
After termination of the reaction with 1 M H$_2$SO$_4$ aq. (0.14 mL), the solution (200 µL) was subjected to fluorescence HPLC analysis.

HPLC conditions:
YMC Triart C18, 4.6 mm x 250 mm, Flow rate: 1.0 mL/min, Detection (Hitachi L-2485): FL ex/em 360nm / 450 nm.
A: MeCN (0.1% TFA), B: H$_2$O (0.1% TFA)
A / B = 5 / 95 (0 min) → 5 / 95 (10 min) → 100 / 0 (30 min) → 100 / 0 (40 min) → 5 / 95 (50 min).

Preparation of rat peritoneal mast cells.

Rat peritoneal mast cells were obtained from Wistar rat (7 weeks, male). Briefly, 1x Tyrode-HEPES-Gelatin buffer (20 mL) was injected into rat peritoneal cavity, then massaged gently for 1 min. The buffer was recovered and centrifuged to obtain total rat peritoneal cells. The cells were purified by density-gradient centrifugation with Histodendz$^\text{TM}$ (50G, 15 min, 4 °C) to give rat peritoneal mast cells in Histodendz layer. The collected cells were immobilized on collagen coated grass base dish in RPMI 1640 (10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 µg/mL)) for fluorescence imaging.

All animal experiments were conducted according to relevant national and international guidelines contained in the 'Act on Welfare and Management of Animals' (Ministry of Environment of Japan) and 'Regulation of Laboratory Animals' (Kyushu University).

Fluorescence imaging of mast cell degranulation induced by secretagogues.

Rat peritoneal mast cells were treated with a solution of 3 (4 µM) and CoCl$_2$ (50 µM) in HBSS for 10 min at rt. After washing with HBSS (x1), the cells were treated with lysophosphatidylserine (2 µM), except for the experiment which used A23187 as a stimulant. In the case of the imaging of the antigen-induced degranulation, the cells were pre-sensitized with anti-dinitrophenyl (DNP) IgE (SPE-7, Sigma D8406) in RPMI for 4 h at 37 °C. The imaging was conducted upon stimulation of the cells with A23187 (5 µM), DNP-HSA (100 ng/mL), HSA (100 ng/mL), or substance P (100 µM). The fluorescence images were collected with a fluorescence microscope (LSM 700, ZEISS) equipped with a 63x oil-immersion objective lens at Cy5 channel (excitation: 639 nm, emission: 640-700 nm). The data were analyzed by ZEN software (ZEISS) and Image J (NIH).
General Materials and Methods for Organic Synthesis.

Unless otherwise noted, chemical reagents were purchased from commercial suppliers (Sigma-Aldrich, Tokyo Chemical Industry (TCI), Wako Pure Chemical Industries) and used without further purification. $^1$H NMR spectra were recorded using a Varian UNITY-400 (400 MHz) spectrometer (Varian, USA), and chemical shifts (δ, ppm) were referenced to residual solvent peak. ESI mass spectrometry was recorded using a Bruker microTOF II (Bruker Daltonics, USA) spectrometer. MALDI-TOF mass spectrometry was recorded using a Bruker autoflex III (Bruker Daltonics, USA) spectrometer. HPLC purification was conducted with a HITACHI L-7100 (Hitachi, Japan).

Scheme S1

Synthesis of 4

A mixture of 4-diethylamino salicylaldehyde (4.832 g, 25 mmol), Meldrum’s acid (3.603 g, 25 mmol), and piperidinium acetate (73 mg, 0.50 mmol) in dry EtOH (15 mL) was heated at reflux with stirring for 3.5 h. After cooling, the precipitate was filtrated and washed with EtOH
to give 4 (4.73 g, 72%) as an orange powder.

\[ ^1\text{H-NMR (400 MHz, DMSO-}d_6\text{): } \delta 1.12-1.15 (6\text{H, t, } J = 6.0 \text{ Hz}), 3.46-3.51 (4\text{H, m}), 6.57 (1\text{H, s}), 6.78-6.80 (1\text{H, d, } J = 9.2 \text{ Hz}), 7.62-7.65 (1\text{H, d, } J = 9.2 \text{ Hz}), 8.58 (1\text{H, s}), 12.47 (1\text{H, br}). \]

ESI-MS m/z 284.1 [M+Na]^+.

Synthesis of 5

\[ N,N'-\text{Dimethylethylenediamine (110 } \mu\text{L, 1.02 mmol) was added to a mixture of 4-bromo-2-nitroanisole (2.13 g, 9.2 mmol), NaI (2.76 g, 18.4 mmol), and CuI (95 mg, 0.50 mmol) in dry 1,4-dioxane (6 mL), and the mixture was stirred at 120 °C for 18 hr. After cooling to rt, the mixture was diluted with sat. NaHCO}_3 \text{aq. (100 mL) and extracted with EtOAc (30 mL×5). The combined organic layers were dried over Na}_2\text{SO}_4 \text{ and concentrated in vacuo to give 5 (2.51 g, 98%) as a red solid.} \]

\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{): } \delta 3.93 (3\text{H, s}), 6.83-6.85 (1\text{H, d, } J = 8.8 \text{ Hz}), 7.77-7.80 (1\text{H, dd, } J = 2.4, 8.8 \text{ Hz}), 8.09-8.10 (1\text{H, d, } J = 2.0 \text{ Hz}). \]

ESI-MS m/z 301.9 [M+Na]^+.

Synthesis of 6

To a solution of 5 (1.41 g, 5.1 mmol) in dry CH_2Cl_2 (15 mL) was added dropwise 1 M BBr_3 in CH_2Cl_2 (15 mL) at 0 °C, and the mixture was stirred at rt for 6 h. The reaction was quenched by slow addition of water (20 mL) at 0 °C. After stirring for 20 min at 0 °C, the resultant mixture was diluted with sat. NaHCO}_3 \text{aq. (100 mL) and extracted with CHCl}_3 (30 mL) and AcOEt (30 mL×5). The combined organic layers were dried over MgSO}_4 \text{ and concentrated in vacuo. The residue was dissolved in AcOEt / hexane (1 : 1) and filtered through silica plug. The filtrate was concentrated in vacuo to give 6 (1.25 g, 93%) as a red solid.} \]

\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{): } \delta 6.92-6.94 (1\text{H, d, } J = 8.8 \text{ Hz}), 7.79-7.82 (1\text{H, dd, } J = 2.0, 8.8 \text{ Hz}), 8.40 (1\text{H, d, } J = 1.6 \text{ Hz}), 10.48 (1\text{H, s}). \]

ESI-MS m/z 263.9 [M-H].

Synthesis of 7

To a solution of 6 (1.24 g, 4.7 mmol) in dry DMF (10 mL) was added portionwise NaH (400 mg, 10 mmol, 60% oil dispersion) at rt. Benzyl chloride (805 } \mu\text{L, 7.0 mmol) and KI (166 mg, 1.0 mmol) was added, and the mixture was stirred at 60 °C overnight. After quenching by water (20 mL) at 0 °C, the resultant mixture was diluted with sat. NaHCO}_3 \text{aq. (80 mL) and extracted with AcOEt (30 mL×5). The combined organic layers were dried over Na}_2\text{SO}_4 \text{ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO}_2, \text{ hexane / AcOEt = 10 : 1 → 7.5 : 1) to give 7 (1.40 g, 84%) as a yellow solid.} \]

\[ ^1\text{H-NMR (400 MHz, CDCl}_3\text{): } \delta 5.20 (1\text{H, s}), 6.85-6.87 (1\text{H, d, } J = 8.8 \text{ Hz}), 7.32-7.42 (5\text{H, m}), 7.72-7.75 (1\text{H, dd, } J = 2.0, 8.8 \text{ Hz}), 8.10 (1\text{H, d, } J = 2.0 \text{ Hz}). \]

ESI-MS m/z 378.0 [M+Na]^+. 

S10
Synthesis of 8

A solution of 7 (533 mg, 1.5 mmol), 4 (418 mg, 1.6 mmol), Ag₂CO₃ (1.24 g, 4.5 mmol), and PdCl₂ (26.6 mg, 0.15 mmol) in dry DMSO (5 mL) was stirred at 120 °C overnight. After cooling to rt, the mixture was diluted with sat. NaHCO₃ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were filtered through Celite, and the filtrate was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, hexane / AcOEt = 5 : 1 → 4 : 1 → 3 : 1) to give 8 (93 mg, 14%) as a yellow solid.

1H-NMR (400 MHz, CDCl₃): δ 1.20-1.23 (6H, t, J = 7.2 Hz), 3.40-3.45 (4H, m), 5.26 (2H, s), 6.51 (1H, s), 6.59-6.61 (1H, d, J = 8.8 Hz), 7.12-7.14 (1H, d, J = 8.8 Hz), 7.30-7.46 (6H, m), 7.70 (1H, s), 7.94-7.97 (1H, d, J = 9.2 Hz), 8.16-8.17 (1H, d, J = 2.0 Hz). ESI-MS m/z 467.2 [M+Na]+.

Synthesis of 9

A mixture of 8 (165 mg, 0.37 mmol) and SnCl₂·2H₂O (271 mg, 1.2 mmol) in AcOEt (4 mL) was stirred at 90 °C for 2 h. SnCl₂·2H₂O (271 mg, 1.2 mmol) was added, and the mixture was further stirred at 90 °C for 1 h. After cooling to rt, the mixture was diluted with sat. NaHCO₃ aq. (50 mL) and extracted with AcOEt (20 mL×3). The combined organic layers was dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, hexane / AcOEt = 2 : 1) to give 9 (48 mg, 31%) as a yellow amorphous powder.

1H-NMR (400 MHz, CDCl₃): δ 1.18-1.22 (6H, t, J = 6.8 Hz), 3.38-3.43 (4H, m), 5.10 (2H, s), 6.51 (1H, s), 6.56-6.58 (1H, d, J = 8.4 Hz), 6.85-6.87 (1H, d, J = 8.4 Hz), 7.00-7.02 (1H, d, J = 8.0 Hz), 7.12 (1H, d, J = 2.0 Hz), 7.28-7.44 (6H, m), 7.60 (1H, s). ESI-MS m/z 437.2 [M+Na]+.

Synthesis of 10

A solution of 9 (52 mg, 0.13 mmol), DIPEA (122 µL, 0.70 mmol), KI (50 mg, 0.30 mmol), and tert-butyl bromoacetate (110 µL, 0.75 mmol) in dry DMF (3 mL) was stirred at 100 °C overnight. After cooling to rt, the mixture was diluted with sat. NaHCO₃ aq. (50 mL) and extracted with AcOEt (20 mL×3). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, hexane / AcOEt = 4 : 1 → 3 : 1) to give 10 (62 mg, 77%) as a solid.

1H-NMR (400 MHz, CDCl₃): δ 1.18-1.22 (6H, t, J = 7.2 Hz), 1.40 (18H, s), 3.37-3.59 (4H, m), 4.10 (4H, s), 5.14 (2H, s), 6.50 (1H, s), 6.54-6.57 (1H, d, J = 11.2 Hz), 6.84-6.86 (1H, d, J = 8.0 Hz), 7.17 (1H, s), 7.23 (1H, s), 7.24 (1H, s), 7.28-7.29 (1H, d, J = 6.4 Hz), 7.33-7.37 (2H, t, J = 8.0 Hz), 7.42-7.44 (2H, d, J = 7.2 Hz), 7.57 (1H, s). ESI-MS m/z 665.3 [M+Na]+.
Synthesis of 1a

To a solution of 10 (19 mg, 0.030 mmol) in dry CH$_2$Cl$_2$ (2 mL) were added dropwise TFA (2 mL) at 0 °C, and the mixture was stirred at rt for 4 h. The solvent was removed in vacuo to give a crude carboxylate as a yellow oil. A suspension of the crude carboxylate and 10% Pd/C (5 mg) in MeOH (4 mL) was stirred at rt for 2.5 h under H$_2$ atmosphere. After filtration through Celite and washing with MeOH, the filtrate was concentrated in vacuo, and the residue was purified by HPLC to give 1a (0.37 mg, 2.3% as monoTFA salt) as a yellow solid.

HPLC conditions
Column: YMC-ODS-A 10 mm x 25 mm, Flow rate : 9.9 mL/min, Detection : UV(220 nm),
A: MeCN (0.1% TFA), B: H$_2$O (0.1% TFA)
A / B = 10 / 90 (0 min) → 10 / 90 (10 min) → 70 / 30 (40 min)→100 / 0 (50 min)→10 / 90 (60 min).
Retention time of 1a: 37.0 min

$^1$H-NMR (400 MHz, DMSO-$d_6$): $\delta$ 1.12-1.23 (6H, t, $J = 7.2$ Hz), 3.34-3.49 (4H, m), 3.96 (4H, s), 6.54 (1H, d, $J = 2.4$ Hz), 6.72-6.75 (2H, m), 6.88 (1H, s), 6.92-6.94 (1H, d, $J = 7.6$ Hz), 7.41-7.43 (1H, d, $J = 8.8$ Hz), 7.79 (1H, s).

HRMS (ESI) calcd for C$_{23}$H$_{25}$N$_2$O$_7$ [M+H]$^+$ 441.1662, found 441.1652.

Scheme S2

Synthesis of 11

A mixture of 4 (183 mg, 0.70 mmol), 5 (203 mg, 0.73 mmol), PdCl$_2$ (12.4 mg, 0.070 mmol), and Ag$_2$CO$_3$ (579 mg, 2.1 mmol) in dry DMSO (2.5 mL) was stirred at 120 °C overnight. After cooling to rt, the mixture was diluted with sat. NaHCO$_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, hexane / AcOEt = 5 : 2 → 2 : 1) to give 11 (38 mg , 15%) as a red solid.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.20-1.23 (6H, t, $J = 7.2$ Hz), 3.40-3.45 (4H, m), 3.98 (3H, s), 6.51-6.52 (1H, d, $J = 2.4$ Hz), 6.59-6.61 (1H, dd, $J = 2.4, 8.8$ Hz), 7.09-7.12 (1H, d, $J = 8.8$ Hz),
7.30-7.32 (1H, d, \( J = 8.8 \) Hz), 7.70 (1H, s), 8.00-8.03 (1H, dd, \( J = 2.4, 8.8 \) Hz), 8.16 (1H, d, \( J = 2.0 \) Hz). ESI-MS \( m/z \) 391.1 [M+Na]\(^+\).

**Synthesis of 12**

A solution of 11 (72 mg, 0.20 mmol) and SnCl\(_2 \cdot 2\)H\(_2\)O (300 mg, 1.3 mmol) in AcOEt (3 mL) was stirred at reflux for 4 h. After cooling to rt, the mixture was diluted with sat. NaHCO\(_3\) aq. (50 mL) and extracted with AcOEt (30 mL×6). The combined organic layers were dried over Na\(_2\)SO\(_4\) and concentrated in vacuo to give 12 (65 mg, 98%) as an orange amorphous powder.

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.18-1.22 (6H, t, \( J = 6.8 \) Hz), 3.38-3.43 (4H, m), 3.86 (3H, s), 6.51 (1H, s), 6.55-6.57 (1H, m), 6.79-6.81 (1H, d, \( J = 8.4 \) Hz), 7.03-7.05 (1H, d, \( J = 8.0 \) Hz), 7.09-7.10 (1H, d, \( J = 2.4 \) Hz), 7.25-7.28 (1H, d, \( J = 8.8 \) Hz), 7.60 (1H, s). ESI-MS \( m/z \) 361.2 [M+Na]\(^+\).

**Synthesis of 13**

A mixture of 12 (65 mg, 0.19 mmol), KI (125 mg, 0.75 mmol), DIPEA (216 µL, 1.5 mmol), and benzyl bromoacetate (235 µL, 1.5 mmol) in dry DMF (3 mL) was stirred at 100 °C for 24 h. After cooling to rt, the mixture was diluted with sat. NaHCO\(_3\) aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na\(_2\)SO\(_4\) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO\(_2\), hexane / AcOEt = 3 : 1 → 2 : 1) to give 13 as an orange oil (54 mg, 44%).

\(^1\)H-NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.19-1.23 (6H, t, \( J = 6.8 \) Hz), 3.39-3.44 (4H, m), 3.70 (3H, s), 4.21 (4H, s), 5.14 (4H, s, CH\(_2\)C\(_6\)H\(_5\)), 6.51 (1H, s), 6.55-6.57 (1H, d, \( J = 8.8 \) Hz), 6.81-6.84 (1H, d, \( J = 8.8 \) Hz), 7.11 (1H, s), 7.20-7.33 (12H, m), 7.42 (1H, s). ESI-MS \( m/z \) 657.2 [M+Na]\(^+\).

**Synthesis of 1b**

A suspension of 13 (35 mg, 0.055 mmol) and 10% Pd/C (12 mg) in MeOH (3 mL) was stirred at rt for 4 h under H\(_2\) atmosphere. After filtration through Celite and washing with MeOH, the filtrate was concentrated in vacuo. The residue was purified by HPLC to give 1b (9 mg, 29% calcd. as monoTFA salt) as a yellow solid.

**HPLC conditions**

Column: YMC actus C18 10 mm x 250 mm, Flow rate: 9.9 mL/min, Detection: UV(220 nm)

A: MeCN (0.1% TFA), B: H\(_2\)O (0.1% TFA)

A / B = 10 / 90 (0 min) → 10 / 90 (10 min) → 70 / 30 (40 min) → 0 / 100 (50 min) → 10 / 90 (60 min).

Retention time of 1b: 39.1 min

\(^1\)H-NMR (400 MHz, CD\(_3\)OD): \( \delta \) 1.20-1.23 (6H, t, \( J = 7.2 \) Hz), 3.46-3.49 (4H, m), 3.83 (3H, s),
4.10 (4H, s), 6.55 (1H, s), 6.73-6.75 (1H, d, $J = 9.28$ Hz), 6.96-6.98 (1H, d, $J = 8.4$ Hz), 7.21 (1H, s), 7.26-7.28 (1H, dd, $J = 2.0, 8.4$ Hz), 7.41-7.44 (1H, d, $J = 8.8$ Hz), 7.81 (1H, s). HRMS (ESI) calcd for C$_{24}$H$_{26}$N$_2$O$_7$ [M+H]$^+ 455.1818$, found 455.1817.

**Scheme S3**

**Synthesis of 14**

A mixture of 4 (131 mg, 0.50 mmol), $p$-iodoanisole (152 mg, 0.65 mmol), PdCl$_2$ (8.9 mg, 0.0050 mmol), and Ag$_2$CO$_3$ (413 mg, 1.5 mmol) in dry DMSO (5 mL) was stirred at 120 °C overnight. After cooling to rt, the mixture was diluted with sat. NaHCO$_3$ aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, hexane / AcOEt = 3 : 1 → 2 : 1) to give 14 (40 mg, 25%) as a yellow oil.

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.20-1.22 (6H, t, $J = 6.8$ Hz), 3.38-3.44 (4H, m), 4.83 (1H, s), 6.51-6.52 (1H, d, $J = 2.8$ Hz), 6.56-6.59 (1H, dd, $J = 2.4, 8.8$ Hz), 6.91-6.95 (2H, m), 7.27-7.29 (1H, d, $J = 8.8$ Hz), 7.61-7.64 (3H, m). ESI-MS $m/z$ 346.1 [M+Na]$^+$.  

**Synthesis of 15**

To a solution of 14 (126 mg, 0.39 mmol) in dry CH$_2$Cl$_2$ (5 mL) was added dropwise 1 M BBr$_3$ in CH$_2$Cl$_2$ (2.5 mL) at 0 °C, and the solution was stirred at rt for 1 h. After quenching with slow addition of water (20 mL) at 0 °C, the mixture was diluted with sat. NaHCO$_3$ aq. (100 mL) and extracted with CHCl$_3$ (30 mL) and AcOEt (30 mL×3). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, hexane / AcOEt = 2 : 1 → 1 : 1) to give 15 as a dark green solid (109 mg, 90%).

$^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 1.19-1.24 (6H, t, $J = 6.8$ Hz), 3.38-3.44 (4H, m), 4.83 (1H, s), 6.52 (1H, s), 6.56-6.59 (1H, dd, $J = 2.4, 8.8$ Hz), 6.84-6.87 (2H, d, $J = 8.8$ Hz), 7.27-7.29 (1H, d, $J = 8.8$ Hz), 7.56-7.59 (2H, d, $J = 8.8$ Hz), 7.62 (1H, s). ESI-MS $m/z$ 332.1 [M+Na]$^+$.
Synthesis of 16

A solution of 15 (62 mg, 0.20 mmol), paraformaldehyde (42 mg, 1.4 mmol calcd. as HCHO), AcONa (115 mg, 1.4 mmol), and di-tert-butyl iminodiacetate (159 mg, 0.19 mmol) in H\textsubscript{2}O / i-PrOH (1:2, 4.5 mL) was stirred at 105 °C for 18 h. After cooling to rt, the mixture was diluted with sat. NaHCO\textsubscript{3} aq. (100 mL) and extracted with AcOEt (30 mL×3). The combined organic layers were dried over Na\textsubscript{2}SO\textsubscript{4} and concentrated in vacuo. The residue was purified by flash column chromatography (SiO\textsubscript{2}, hexane / AcOEt = 7 : 2) to give crude 16 (84 mg, 52%) as a 1 : 1 mixture with di-tert-butyl iminodiacetate. This material was used for the next reaction without further purification.

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \(\delta\) 1.17-1.21 (6H, t, \(J = 6.8\) Hz), 1.44 (18H, s), 3.37-3.44 (8H, m), 3.96 (2H, s), 6.51 (1H, s), 6.54-6.57 (1H, m), 6.89-6.91 (1H, d, \(J = 8.4\) Hz), 7.26 (1H, s), 7.36 (1H, s), 7.48-7.51 (1H, dd, \(J = 2.0, 8.4\) Hz), 7.57 (1H, s), 9.70 (1H, br). ESI/MS \(m/z\) 589.3 [M+Na]\textsuperscript{+}.

Synthesis of 1c

To a solution of crude 16 (48 mg, \textit{ca.} 0.059 mmol) in dry CH\textsubscript{2}Cl\textsubscript{2} (2 mL) was added dropwise TFA (2 mL) at 0 °C, and the solution was stirred at rt for 8 h. After removal of the solvent in vacuo, the residue was purified by HPLC to give 1c as a yellow solid (23 mg, 69% as monoTFA salt).

HPLC conditions
Column: YMC actus C18 10 mm x 250 mm, Flow rate : 9.9 mL/min, Detection : UV(220 nm)
A: MeCN (0.1% TFA), B: H\textsubscript{2}O (0.1% TFA)
A / B = 10 /90 (0 min) \rightarrow 10 / 90 (10 min) \rightarrow 60 / 40 (40 min) \rightarrow 0 / 100 (50 min) \rightarrow 10/90(60 min).
Retention time of 1c: 35.5 min

\textsuperscript{1}H-NMR (400 MHz, DMSO-d\textsubscript{6}): \(\delta\) 1.11-1.14 (6H, t, \(J = 6.8\) Hz), 3.42-3.44 (4H, m), 3.81 (4H, s), 4.20 (2H, s), 6.55 (1H, s), 6.71-6.73 (1H, d, \(J = 9.2\) Hz), 6.87-6.89 (1H, d, \(J = 8.0\) Hz), 7.46-7.48 (1H, d, \(J = 9.6\) Hz), 7.58-7.61 (1H, d, \(J = 8.8\) Hz), 7.64 (1H, s), 7.95 (1H, s). HRMS (ESI) calcd for C\textsubscript{24}H\textsubscript{27}N\textsubscript{2}O\textsubscript{7} [M+H]\textsuperscript{+} 455.1818, found 455.1819.
Scheme S4

**Synthesis of 17**

To a crude 16 (32 mg, ca. 0.039 mmol) in dry DMF (2 mL) was added dropwise NaH (4 mg, 0.10 mmol, 60% oil dispersion) at 0 °C. Iodomethane (6.5 µL, 0.10 mmol) was added, and the solution was stirred at 0 °C for 15 min. After dilution with brine (30 mL), the mixture was extracted with AcOEt (10 mL×3). The combined organic layers were dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, Hexane / AcOEt = 3 : 1 → 2 : 1) to give 17 (23 mg, quant.) as a yellow oil.

^1H-NMR (400 MHz, CDCl₃): δ 1.18-1.22 (6H, t, J = 7.2 Hz), 1.43 (18H, s), 3.38-3.43 (4H, m), 3.46 (4H, s), 3.81 (3H, s), 3.97 (2H, s), 6.51 (1H, s), 6.55-6.58 (1H, dd, J = 2.4, 8.8 Hz), 6.86-6.89 (1H, d, J = 8.4 Hz), 7.26-7.29 (1H, d, J = 8.8 Hz), 7.68-7.72 (3H, m). ESI-MS m/z 603.3 [M+Na]⁺.

**Synthesis of 1d**

A solution of 17 (23 mg, 0.040 mmol) in dry CH₂Cl₂ (2 mL) was added dropwise to TFA (2 mm) at 0 °C, and the solution was stirred at rt for 6 h. After removal of the solvent in vacuo, the residue was purified by HPLC to give 1d (18 mg, 77% as monoTFA salt) as a yellow solid.

HPLC conditions
Column: YMC actus C18 10 mm x 250 mm, Flow rate: 9.9 mL/min, Detection: UV(220 nm)
A: MeCN (0.1% TFA), B: H₂O (0.1% TFA)
A / B = 10 / 90 (0 min) → 10 / 90(10 min) → 70 / 30(40 min) → 0 / 100(50 min) →10 /90 (60 min).
Retention time of 1d: 36.2 min

^1H-NMR (400 MHz, CD₃OD): δ 1.20-1.24 (6H, t, J = 6.8 Hz), 3.48-3.52 (4H, m), 3.94 (3H, s), 4.09 (4H, s), 4.62 (2H, s), 6.56 (1H, s), 6.74-6.77 (1H, d, J = 8.8 Hz), 7.15-7.17 (1H, d, J = 8.8 Hz), 7.44-7.46 (1H, d, J = 8.8 Hz), 7.79 (1H, s), 7.81-7.84 (1H, d, J = 8.4 Hz), 7.92-7.93 (1H, d, J = 3.2 Hz). HRMS (ESI) calcd for C₂₅H₂₉N₂O₇ [M+H]⁺ 469.1975, found 469.1975.
Scheme S5

Synthesis of 18

A solution of 4-methoxyphenylhydrazine hydrochloride (5.24 g, 30 mmol) and 3-methyl-2-butanone (3.21 mL, 30 mmol) in dry EtOH (100 mL) was stirred at reflux overnight. After cooling to rt, the mixture was concentrated in vacuo. The residue was diluted with CH$_2$Cl$_2$ (100 mL), and the solution was washed with water (50 mL×2) and dried over MgSO$_4$. The solvent was removed in vacuo to give 18 (5.15 g, 91%) as a red solid.

$^1$H-NMR (400 MHz, CDCl$_3$): δ 1.27 (6H, s), 2.22 (3H, s), 3.81 (3H, s), 6.78-6.81 (2H, m), 7.39-7.42 (1H, d, $J = 8.4$ Hz). ESI-MS m/z 190.1 [M+H]$^+$.

Synthesis of 19

To a solution of 18 (1.376 g, 7.27 mmol) in dry CH$_2$Cl$_2$ (20 mL) was added dropwise 1 M BBr$_3$ in CH$_2$Cl$_2$ (20 mL) over 1 hr at 0 °C, and the mixture was stirred at rt for 1.5 h. The reaction was quenched by slow addition of water (30 mL) at 0 °C. After stirring for 10 min at 0 °C, the resultant mixture was diluted with sat. NaHCO$_3$ aq. (100 mL) and extracted with CH$_2$Cl$_2$ (50 mL×4). The combined organic layers were dried over MgSO$_4$ and concentrated in vacuo to give 19 (1.22 g, 95%) as a brown solid.

$^1$H-NMR (400 MHz, CDCl$_3$): δ 1.26 (6H, s), 2.23 (3H, s), 6.59 (1H, br), 6.72-6.74 (1H, d, $J = 2.4$, 8.4 Hz), 6.78-6.79 (1H, d, $J = 2.4$ Hz), 7.32-7.34 (1H, d, $J = 8.0$ Hz). ESI-MS m/z 176.1 [M+H]$^+$. 

S17
Synthesis of 20

A solution of 19 (1.213 g, 6.9 mmol) and iodoethane (0.90 mL, 8.7 mmol) in dry MeCN (20 mL) was stirred at reflux for 17 h. After cooling to rt, the solution was concentrated in vacuo to give a solid. The solid was triturated in CHCl₃/hexane and filtrated followed by washing with CHCl₃ to give 20 (1.575 g, 69%) as a grey solid.

¹H-NMR (400 MHz, DMSO-d₆): δ 1.38-1.42 (3H, t, J = 7.6 Hz), 1.46 (6H, s), 2.72 (3H, s), 4.37-4.42 (2H, m), 6.92-6.95 (1H, dd, J = 2.4, 8.8 Hz), 7.13 (1H, d, J = 2.4 Hz), 7.71-7.32 (1H, d, J = 8.8 Hz), 10.25 (1H, s). ESI-MS m/z 204.1 [M-I]+.

Synthesis of 21

A mixture of 20 (100 mg, 0.30 mmol), N-(3-(phenylamino)-2-propenylidene)aniline monohydrochloride (38 mg, 0.15 mmol), AcONa (37 mg, 0.45 mmol), Ac₂O (0.5 mL) and AcOH (0.25 mL) was stirred at 100 °C for 1 hr. After cooling to rt, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, chloroform / MeOH = 150 : 1 → 100 : 1 → 50 : 1 → 20 : 1 → 10 : 1) to give a red solid. The solid was suspended in CHCl₃/Et₂O and filtrated followed by washing with Et₂O to give 21 (80 mg, 83%) as a red solid.

¹H-NMR (400 MHz, DMSO-d₆): δ 1.38-1.42 (6H, t, J = 7.2 Hz), 1.74 (12H, s), 2.30 (6H, s), 4.13-4.15 (4H, m), 6.38-6.41 (2H, d, J = 13.6 Hz), 6.90-6.96 (1H, t, J = 12.8 Hz), 7.03-7.08 (6H, m), 8.07-8.13 (2H, t, J = 13.2 Hz). ESI-MS m/z 527.3 [M-I]+.

Synthesis of 22

A solution of 21 (71 mg, 0.11 mmol) and 1 M NaOH aq. (1.5 mL) in MeOH (10 mL) was at rt for 1.5 h. After neutralization with 1N HCl aq. (1.5 mL) at 0 °C, the mixture was concentrated in vacuo. The residue was suspended in MeOH/CHCl₃ and filtrated. The filtrate was concentrated in vacuo to give the crude 22 (82 mg) as a red solid. This material contained insoluble material but was used for the next step without further purification.

¹H-NMR (400 MHz, CD₃OD): δ 1.33-1.37 (6H, t, J = 7.2 Hz), 1.66 (12H, s), 4.02-4.08 (4H, m), 6.10-6.13 (2H, d, J = 13.6 Hz), 6.42-6.48 (1H, m), 6.74-6.76 (2H, d, J = 8.4 Hz), 7.03-7.05 (2H, d, J = 8.8 Hz), 7.97-8.04 (2H, t, J = 12.8 Hz). ESI-MS m/z 443.3 [M-I]+.

Synthesis of 23

A mixture of 22 (84 mg, 0.15 mmol), paraformaldehyde (30 mg, 1.0 mmol calcld. as HCHO), AcONa (82 mg, 1.0 mmol) and di-tert-butyl iminodiacetate (76 mg, 0.31 mmol) in H₂O/i-PrOH (1:2, 4.5 mL) was stirred at 105 °C overnight. After cooling to rt, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (SiO₂, chloroform / MeOH = 100 : 1 → 50 : 1 → 25 : 1 → 10 : 1) to give 23 as a metallic red solid (30 mg, 19%).

¹H-NMR (400 MHz, CD₂OD): δ 1.34-1.37 (6H, t, J = 7.2 Hz), 1.48 (36H, s), 1.68 (12H, s),
3.41 (8H, s), 3.96 (4H, s), 4.04-4.10 (4H, m), 6.15-6.19 (2H, d, J = 12.4 Hz), 6.52-6.67 (1H, t, J = 12.0 Hz), 6.93 (2H, s), 7.01 (2H, s), 8.06-8.12 (2H, t, J = 13.2 Hz). ESI-MS m/z 957.6 [M-I]⁺.

Synthesis of 2

To the solution of 23 (30 mg, 0.028 mmol) in dry DMF (1 mL) was added NaH (4.0 mg, 0.10 mmol, 60% oil dispersion) at 0 °C. Iodomethane (16 µL, 0.26 mmol) was added, and the solution was stirred at rt for 40 min. The reaction was quenched with water (2 mL) at 0 °C. After addition of 1 M HCl aq. (100 µL), the mixture was concentrated by evaporation to give a crude dimethoxy derivative of 23. To a solution of the crude dimethoxy compound in CH₂Cl₂ (2 mL) was added dropwise TFA (2 mL) at 0 °C. After stirring for 10 h at 0 °C, the solvent was removed in vacuo to give the crude 2. The crude was purified by HPLC system to give 2 (7.6 mg, 31% from 23 as monoTFA salt) as a blue solid.

HPLC conditions
Column: YMC-ODS-A 10 mm x 250 mm, Flow rate: 9.9 mL/min, Detection: UV(220 nm)
A: MeCN (0.1% TFA), B: H₂O (0.1% TFA)
A / B = 5 / 95 (0 min) → 5 / 95(10 min)→55 / 45(40 min)→100 / 0(50 min) → 5 / 95( 60 min)
Retention time of 2 : 37.1 min
¹H-NMR (400 MHz, CD₃OD): δ 1.37-1.41 (6H, t, J = 6.8 Hz), 1.75 (12H, s), 3.94 (6H, s), 3.97 (8H, s), 4.11-4.13 (4H, d, J = 7.6 Hz), 4.49 (4H, s), 6.22-6.26 (2H, d, J = 14.0 Hz), 6.55-6.61 (1H, t, J = 12.4 Hz), 7.32 (2H, s), 7.38 (2H, s), 8.15-8.21 (2H, t, J = 12.8 Hz).
HRMS (ESI) calcd for C₄₁H₅₄N₄O₁₀ (m/z =2) [M+H-CF₃COO]⁺ 381.1915, found 381.1926.
**Synthesis of 24**

To an ice-cooled solution of 3-bromopropyl chloride (756 µL, 7.5 mmol) in dry CH₂Cl₂ (6 mL) was added dropwise N-Boc-piperazine (1676 mg, 9.0 mmol) in dry CH₂Cl₂ (10 mL) over 10 min. The solution was stirred for 1 h at 0 °C. After addition of water (10 mL), the mixture was diluted with sat. NaHCO₃ aq. (100 mL) and extracted with CHCl₃ (30 mL×3).
The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, hexane / AcOEt = 1 : 1 → 1 : 2) to give 24 (1.97 g, 82%) as a white solid.

$^1$H-NMR (400 MHz, CDCl$_3$): δ 1.45 (9H, s), 2.89-2.92 (2H, t, $J = 6.8$ Hz), 3.44 (6H, brs), 3.60 (2H, brs), 3.62-3.66 (2H, t, $J = 6.8$ Hz). ESI-MS $m/z$ 343.1, 345.0 [M+Na]$^+$ (The two molecular ion peaks were detected due to the two bromide isotopes $^{79}$Br and $^{81}$Br).

Synthesis of 25
A solution of 24 (964 mg, 3.0 mmol) and 19 (482 mg, 2.75 mmol) in dry MeCN (15 mL) was stirred at reflux for 40 h. After cooling to rt, the solution was concentrated in vacuo. The residue was suspended in MeOH/AcOEt and filtrated followed by washing with AcOEt to give 25 (778 mg, 57%) as a pale brown solid.

$^1$H-NMR (400 MHz, CD$_3$OD): δ 1.45 (9H, s), 1.53 (6H, s), 2.82-2.84 (2H, d, $J = 7.2$ Hz), 3.15-3.18 (2H, t, $J = 6.8$ Hz), 3.38-3.52 (6H, m), 4.67-4.70 (2H, t, $J = 6.4$ Hz), 6.96-6.99 (1H, dd, $J = 8.8$ Hz), 7.07-7.08 (1H, d, $J = 2.4$ Hz), 7.66-7.68 (1H, d, $J = 8.8$ Hz). The methyl protons at C2 position were not detectable due to deuterium exchange with CD$_3$OD. ESI-MS $m/z$ 416.2 [M-Br]$^+$. 

Synthesis of 26
A solution of 25 (765 mg, 1.54 mmol), N-(3-(phenylamino)-2-propenylidene)aniline monohydrochloride (199 mg, 0.77 mmol), AcONa(180 mg, 2.2 mmol), Ac$_2$O (2.2 mL) and AcOH (1.1 mL) was stirred at 100 °C for 1 h. After cooling to rt, the solution was concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, chloroform / MeOH = 200 : 1 → 150 : 1 → 100 : 1 → 50 : 1 → 20 : 1 → 10 : 1) to give 26 (628 mg, 79%) as a metallic red solid.

$^1$H-NMR (400 MHz, CD$_3$OD): δ 1.43 (18H, s), 1.71 (12H, s), 2.30 (6H, s), 2.99 (4H, brs), 3.39-3.57 (16H, m), 4.45 (4H, brs), 6.55 (2H, brs), 7.06-7.07 (4H, m), 7.13 (1H, brs), 7.34 (2H, brs), 8.03 (2H, brs). ESI-MS $m/z$ 951.5 [M-Br]$^+$. 

Synthesis of 27
To a solution of 26 (625 mg, 0.61 mmol) in MeOH (50 mL) was added 1 M NaOH aq. (4.0 mL) at 0 °C, and the mixture was stirred at rt for 1.5 h. After neutralization with 1 M HCl aq. (4.0 mL) at 0 °C, the mixture was concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, chloroform / MeOH = 50 : 1 → 20 : 1 → 10 : 1 → 5 : 1) to give 27 (461 mg, 80%) as a metallic red amorphous powder.

$^1$H-NMR (400 MHz, CD$_3$OD): δ 1.44 (18H, s), 1.68 (12H, s), 2.89-2.92 (4H, t, $J = 7.2$ Hz), 3.34-3.56 (16H, m), 4.32-4.35 (4H, t, $J = 7.2$ Hz), 6.19-6.22 (2H, d, $J = 13.6$ Hz), 6.48-6.54 (1H,
t, $J = 12.4$ Hz), 6.80-6.82 (2H, d, $J = 8.0$ Hz), 6.90 (2H, d, $J = 2.0$ Hz), 7.11-7.13 (2H, d, $J = 8.0$ Hz), 8.07-8.14 (2H, t, $J = 12.8$ Hz). ESI-MS $m/z$ 867.5 [M - Br]⁺.

**Synthesis of 28**

A mixture of 27 (310 mg, 0.33 mmol), paraformaldehyde (90 mg, 3.0 mmol calcd. as HCHO), AcONa (123 mg, 1.5 mmol) and di-tert-butyl iminodiacetate (162 mg, 0.66 mmol) in H$_2$O/i-PrOH (1 : 2, 4.5 mL) was stirred at 105 °C overnight. After cooling to rt, the mixture was diluted with brine (50 mL) and extracted with AcOEt (30 mL × 5). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, AcOEt / MeOH = 50 : 1 → 20 : 1 → 10 : 1 → 5 : 1 → 4 : 1 → 3 : 1 → 3 : 2) to give 28 (88 mg, 18%) as a metallic red solid.

$^1$H-NMR (400 MHz, CD$_3$OD): δ 1.44 (18H, s), 1.48 (36H, s), 1.63 (12H, s), 2.89-2.93 (4H, t, $J = 6.4$ Hz), 3.41-3.56 (24H, m), 3.94 (4H, s), 4.32 (4H, brs), 6.19-6.23 (2H, d, $J = 13.6$ Hz), 6.49-6.55 (1H, t, $J = 12.4$ Hz), 6.93 (2H, s), 7.05 (2H, s), 8.07-8.14 (2H, t, $J = 12.0$ Hz). ESI-MS $m/z$ 1381.8 [M - Br]⁺.

**Synthesis of 29**

To an ice-cooled solution of 28 (34 mg, 0.023 mmol) in dry DMF (1 mL) was added portionwise NaH (1.2 mg, 0.030 mmol calcd. as 60%) and iodomethane (16 µL, 0.26 mmol), and the mixture was stirred at rt for 40 min. After dilution with brine (50 mL) at 0 °C, the resultant mixture was extracted with AcOEt (10 mL × 3). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (SiO$_2$, chloroform / MeOH = 20 : 1 → 10 : 1 → 5 : 1) to give crude 29 (26.5 mg, a mixture of 29 and the corresponding monomethoxy derivative (2 : 1), 52%) as a blue solid.

$^1$H-NMR (400 MHz, CD$_3$OD): δ 1.45 (36H, s), 1.48 (18H, s), 1.72 (12H, s), 2.95-2.98 (4H, t, $J = 7.2$ Hz), 3.41-3.63 (30H, m), 3.88 (4H, s), 4.36 (4H, brs), 6.24-6.27 (2H, d, $J = 13.2$ Hz), 6.54 (1H, brs), 7.16 (2H, s), 7.48 (2H, s), 8.11-8.17 (2H, t, $J = 12.8$ Hz). ESI-MS $m/z$ 1409.9 [M-Br]⁺.

**Synthesis of 30**

To an ice-cold solution of crude 29 (26.5 mg, ca. 0.012 mmol of 29) in dry CH$_2$Cl$_2$ (2 mL) was added TFA (2 mL), and the solution was stirred at rt for 5 h. After removal of the solvent in vacuo, the residue was purified by HPLC to give 30 (10.7 mg, 67% as monoTFA salt) as a blue solid.

HPLC conditions
Column: YMC-ODS-A 10 mm x 250 mm, Flow rate: 9.9 mL/min, Detection: UV(220 nm)
**Synthesis of 3**

A mixture of 30 (3.9 mg, 2.9 µmol), N,N-disopropylethylamine (150 µL), SUNBRIGHT OE-080CS (Oleyl-O-poly(ethylene glycol)-succinyl-N-hydroxy-succinimidyl esters, 91.6 mg, 10.5 µmol, NOF Corp., Lot. No. = M128543, average M.W. = 8735) in dry DMF (1.5 mL) was stirred at 40 °C overnight. After removal of the solvent, the residue was purified by HPLC to give 3 (28.5 mg, 55% as monoTFA salt) as a blue solid.

HPLC conditions

Column: YMC-ODS-A 10mm x 250 mm, Flow rate: 9.9 mL/min, Detection: UV(220 nm)

A : MeCN (0.1% TFA), B: H2O (0.1% TFA)

A / B = 5 / 95 (0 min) → 5 / 95 (10 min) → 40 / 60 (50 min) → 100 / 0 (60 min) → 5 / 95 (70 min).

Retention time of 3 : 38.8 min

$^1$H-NMR (400 MHz, CD$_3$OD): $\delta$ 1.75 (12H, s), 2.98-3.01 (4H, t, $J = 6.8$ Hz), 3.22-3.26 (6H, m), 3.79-3.95 (10H, m), 3.97 (6H, s), 4.06 (8H, s), 4.38 (4H, brs), 4.58 (4H, s), 6.28-6.31 (2H, d, $J = 13.2$ Hz), 6.58-6.64 (1H, t, $J = 12.4$ Hz), 7.33 (2H, s), 7.46 (2H, s), 8.19-8.25 (2H, t, $J = 12.8$ Hz). ESI-MS m/z 985.5 [M-CF$_3$COO]$^+$.

$^1$H-NMR (400 MHz, CD$_3$OD): $\delta$ 0.88-0.91 (6H, t, $J = 7.2$ Hz), 1.05-1.56 (56H, m), 1.76 (12H, s), 1.93-2.03 (8H, m), 2.60-2.67 (4H, m), 3.44-3.97 (broad multiplet), 4.20-4.40 (18H, m), 5.33-5.38 (4H, m), 6.30-6.60 (3H, m), 7.33-7.75 (4H, m), 8.20 (2H, m).

MALDI-TOF-MS m/z calcd for (C$_{815}$H$_{1585}$N$_8$O$_{378}$) [M-CF$_3$COO]$^+$ 17535.5, found 17745.3 (broad signal in mass range of 17000 ~ 19000 as shown below).
References