Supplementary Materials for:

Preliminary *in vitro* biological evaluation of a strategy for passive intracellular glutathione-derived glyoxalase I inhibitor delivery: a novel approach

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1. Supplementary Materials and Methods for Synthesis

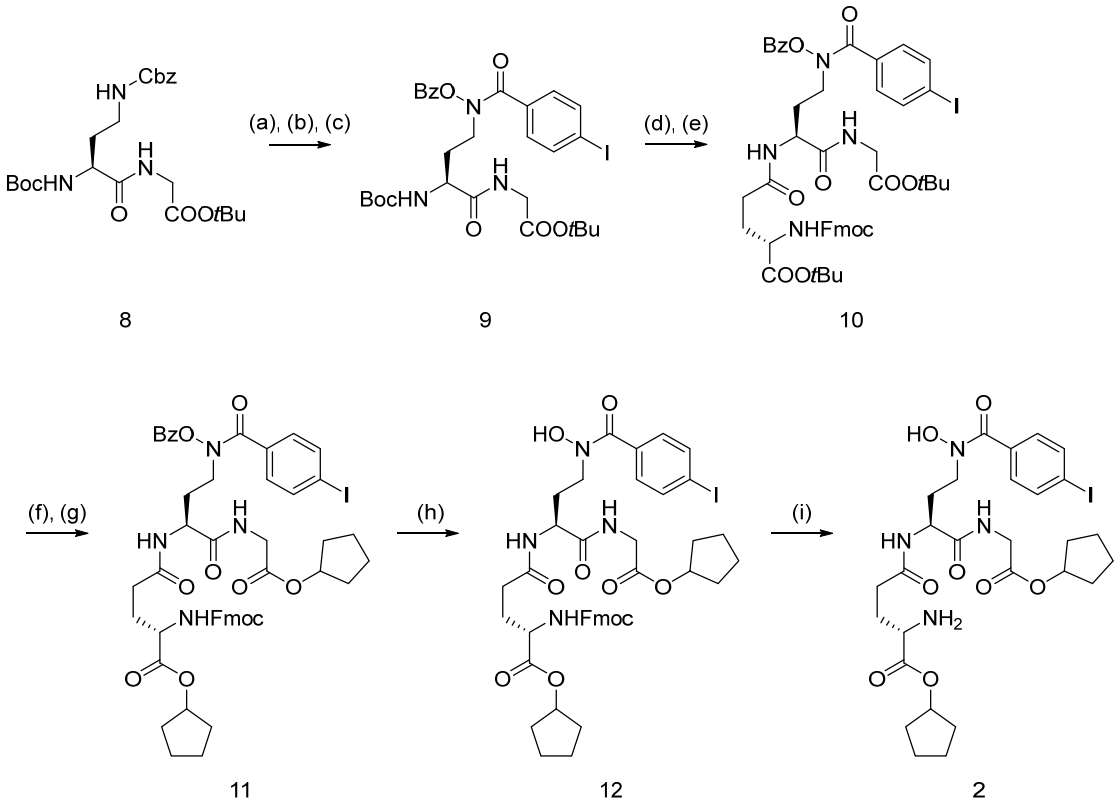
1-1. General

(*S*)-4-(((Benzyloxy)carbonyl)amino)-2-((*tert*-butoxycarbonyl)amino)butanoic acid (Boc-Dab(Z)-OH), Fmoc-Glu-O*t*Bu, and Boc-Glu(OBzl)-OH were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). [^{125}I]NaI was purchased from PerkinElmer (CT, USA). All other reagents were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), Nacalai Tesque Inc. (Kyoto, Japan), or FUJIFILM Wako Pure Chemical Industries, Co. (Tokyo, Japan) and used without further purification. ^{1}H-NMR spectra were recorded on an AV300M (Bruker BioSpin Co., Osaka, Japan) or AV4400N (Bruker BioSpin Co.) with tetramethylsilane as an internal standard. Mass spectra were acquired on a T100-LP (JEOL Ltd., Tokyo, Japan). The cyclopentyl group is represented as cyp.

1-2. Synthesis

NH_2-γ-Glu[-Dab(*N*-(*p*-bromobenzoyl)-*N*-hydroxy)-Gly-OH]-OH (1)

Compound **1** was synthesized according to the method of More *et al*.[1)]

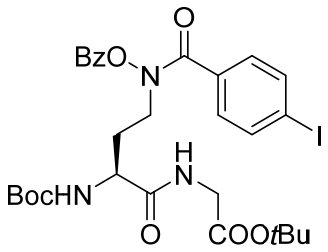


Scheme S1. Synthesis of **2**

(a) H_2, 5% Pd/C, methanol; (b) Benzoyl peroxide 25% water (BPO), carbonate buffer : dichloromethane (CH_2Cl_2) (1 : 1); (c) *p*-Iodobenzoyl chloride, *N*-methylmorpholine (NMM), carbonate buffer : CH_2Cl_2 (1 : 1); (d) 4 M HCl / 1,4-dioxane; (e) Fmoc-Glu-O*t*Bu, Water soluble carbodiimide hydrochloride (WSC·HCl), 1-hydroxybenzotriazole hydrate (HOBt·H_2O), NMM, CH_2Cl_2; (f) Trifluoroacetic acid (TFA), CH_2Cl_2; (g) Cyclopentanol, 4-dimethylaminopyridine (DMAP), WSC·HCl; (h) NH_4OH, methanol, (i) 20% Piperidine / DMF

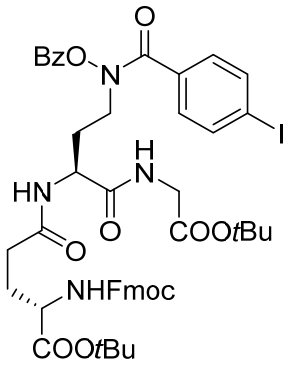
Boc-Dab(Cbz)-Gly-O*t*Bu (8)

Compound **8** was synthesized according to the method of More *et al*.[1)]



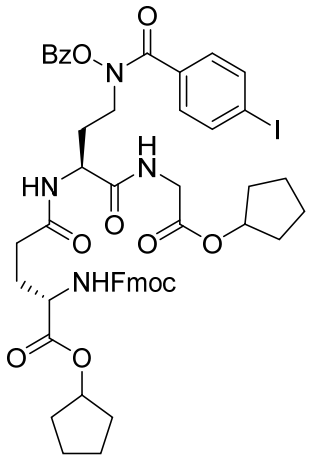
Boc-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-Gly-O*t*Bu (9)

Compound **8** (3.67 g, 7.88 mmol) was dissolved in methanol (39.5 mL), and 5% Pd/C (367 mg) was added under an Ar atmosphere. The mixture was stirred under a H_2 atmosphere at room temperature for 4 h. The reaction solution was then filtered, and the solvent was evaporated. After the residue was dissolved in dichloromethane (CH_2Cl_2; 79.0 mL), carbonate buffer (100 mM, pH 10.5; 79.0 mL) was added and stirred. To the stirred mixture, benzoyl peroxide 25% water (BPO; 3.02 g, 9.36 mmol) was gradually added and stirred at room temperature overnight. Subsequently, *p*-iodobenzoly chloride (2.49 g, 9.35 mmol) and *N*-methylmorpholine (NMM; 1.03 mL, 9.36 mmol) were added and stirred at room temperature overnight. The organic layer was separated and concentrated under vacuum. The residue was purified by silica gel column chromatography (33–50% ethyl acetate (EtOAc) / hexane) to obtain **9** as a white solid (1.61 g, 30.0%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.94–7.92 (2H, m), 7.69–7.60 (3H, m), 7.47–7.37 (4H, m), 7.08 (1H, t, J = 5.1 Hz), 5.41 (1H, d, J = 8.1 Hz), 4.39–4.24 (2H, m), 4.02–3.80 (3H, m), 2.19 (2H, q, J = 6.3 Hz), 1.45, 1.44 (18H, 2s); ESI-MS *m/z*: 682.12 (Calcd for $C_{29}H_{37}IN_3O_8$: 682.16).



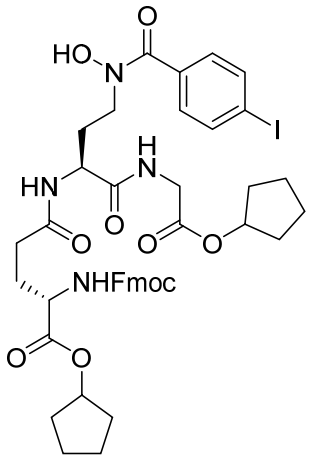
Fmoc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-Gly-O*t*Bu]-O*t*Bu (10)

Compound **9** (1.30 g, 1.51 mmol) was dissolved in 4 M HCl/1,4-dioxane (11.1 mL) at 4°C and stirred at room temperature for 35 min. After removing the solvent under vacuum, diethyl ether was added to precipitate a white solid, and the resulting precipitate was washed with diethyl ether. The residue was dissolved in CH_2Cl_2 (35.1 mL), and to the solution, Fmoc-Glu-O*t*Bu (974 mg, 2.29 mmol), water soluble carbodiimide hydrochloride (WSC·HCl; 549 mg, 2.86 mmol), 1-hydroxybenzotriazole hydrate (HOBt·H_2O; 438 mg, 2.86 mmol), and NMM (630 μL, 5.73 mmol) were added and stirred at room temperature overnight. The solvent was removed under vacuum, and the residue was dissolved in 100 mL of CH_2Cl_2 and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (33–67% EtOAc / hexane) to obtain **10** as a white solid (394 mg, 20.9%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.91–7.89 (2H, m), 7.80–7.75 (3H, m), 7.66–7.58 (5H, m), 7.47–7.28 (8H, m), 6.87 (1H, d, J = 7.2 Hz), 5.64 (1H, d, J = 7.5 Hz), 4.62 (1H, q, J = 7.2 Hz), 4.41–4.19 (4H, m), 4.02–3.79 (4H, m), 2.36–2.11 (5H, m), 1.99–1.89 (1H, m), 1.43, 1.42 (18H, 2s); ESI-MS *m/z*: 1011.27 (Calcd for $C_{48}H_{53}IN_4NaO_{11}$: 1011.26).



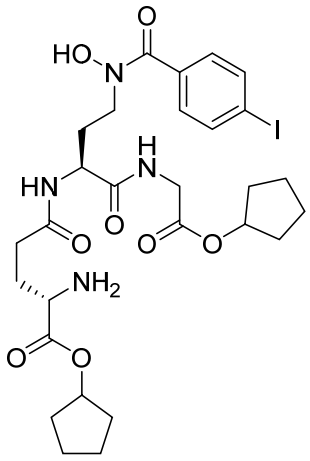
Fmoc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-Gly-Ocyp]-Ocyp (11)

To a solution of **10** (383 mg, 387 μmol) in CH_2Cl_2 (2.23 mL), trifluoroacetic acid (TFA; 20.1 mL) was added and stirred at room temperature for 3 h. After the solvent was removed under vacuum, the residue was dissolved in cyclopentanol (2.64 mL), and 4-dimethylaminopyridine (DMAP; 9.47 mg, 77.5 μmol) and WSC·HCl (222 mg, 1.16 mmol) were added at 4°C. The mixture then was stirred at room temperature overnight. The solvent was removed under vacuum, the residue was dissolved in 100 mL of CH_2Cl_2, and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (25–83% EtOAc / hexane) to obtain **11** as a white solid (41.5 mg, 10.6%). ^{1}H-NMR ($CDCl_3$) δ: 7.91–7.89 (2H, m), 7.80–7.75 (3H, m), 7.66–7.58 (5H, m), 7.46–7.30 (8H, m), 6.85 (1H, d, J = 7.2 Hz), 5.65 (1H, d, J = 7.2 Hz), 5.21–5.17 (2H, m), 4.62 (1H, q, J = 7.2 Hz), 4.40–4.17 (4H, m), 4.05–3.80 (4H, m), 2.36–2.11 (4H, m), 2.02–1.66 (18H, m); ESI-MS *m/z*: 1035.27 (Calcd for $C_{50}H_{53}IN_4NaO_{11}$: 1035.27).



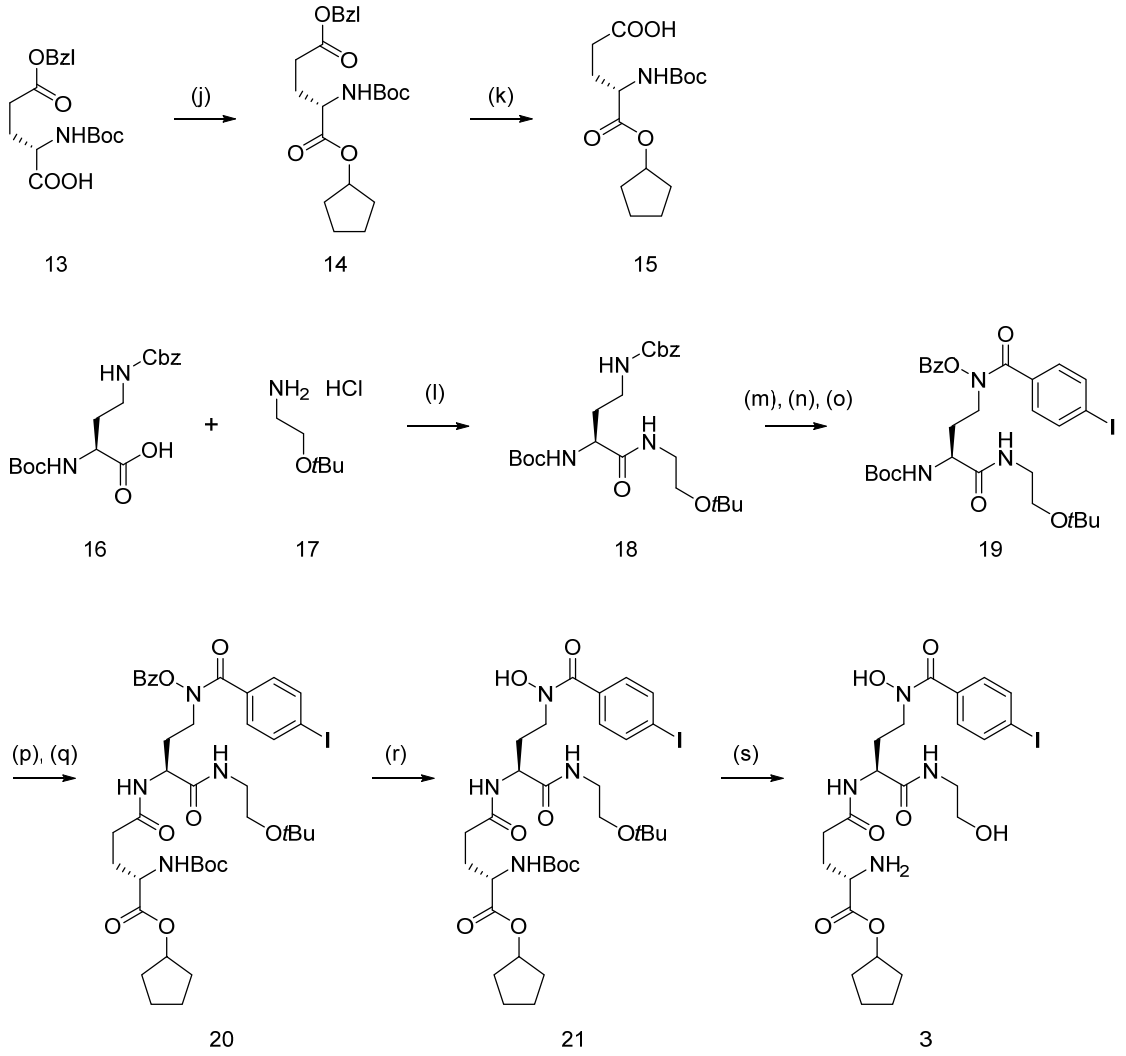
Fmoc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-Gly-Ocyp]-Ocyp (12)

To a solution of **11** (17.0 mg, 16.8 μmol) in methanol (337 μL), NH_4OH (28%; 187 μL) was added at 4°C under an Ar atmosphere, and stirred at room temperature for 1 h. The solvent was removed under vacuum, and the residue was dissolved in 100 mL of chloroform ($CHCl_3$) and washed three times with 100 mL of H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (1% methanol / $CHCl_3$) to obtain **12** as a white solid (13.0 mg, 85.2%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.91 (1H, brs), 7.83–7.70 (5H, m), 7.61–7.44 (5H, m), 7.43–7.28 (4H, m), 5.66 (1H, d, J = 8.1 Hz), 5.22–5.13 (2H, m), 4.61–4.56 (2H, m), 4.41–4.19 (4H, m), 4.03–3.73 (3H, m), 2.45–2.16 (4H, m), 2.05–1.66 (18H, m); ESI-MS *m/z*: 909.25 (Calcd for $C_{43}H_{50}IN_4O_{10}$: 909.26).



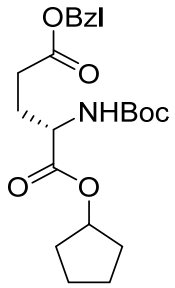
NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-Gly-Ocyp]-Ocyp (2)

Compound **12** (7.27 mg, 8.00 μmol) was dissolved in piperidine / DMF (20% (v/v), 100 μL) and stirred at room temperature for 30 min. The solution was then purified by reverse-phase HPLC (RP-HPLC). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 70% to 100% over 30 min at a flow rate of 1.5 mL/min. The eluate was monitored at 254 nm. Compound **2** was obtained as a yellow oil (1.5 mg, 27.3%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.71 (2H, d, J = 8.2 Hz), 7.42–7.39 (2H, m), 5.23–5.11 (2H, m), 4.65-4.53 (1H, m), 4.04–3.63 (5H, m), 2.36–1.63 (24H, m); HRMS (ESI) *m/z*: 687.18921 (Calcd for $C_{28}H_{39}IN_4O_8$ 687.18908).



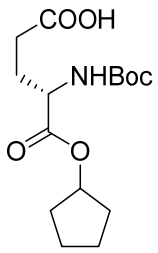
Scheme S2. Synthesis of **3**

(j) Cyclopentanol, WSC·HCl, DMAP, CH_2Cl_2; (k) H_2, 5% Pd/C, methanol; (l) WSC·HCl, HOBt·H_2O, NMM, CH_2Cl_2; (m) H_2, 5% Pd/C, methanol; (n) BPO, carbonate buffer : CH_2Cl_2 (1 : 1); (o) *p*-Iodobenzoyl chloride, NMM, carbonate buffer : CH_2Cl_2 (1 : 1); (p) 4 M HCl / 1,4-dioxane; (q) **15**, WSC·HCl, HOBt·H_2O, NMM, CH_2Cl_2; (r) NH_4OH, DMF; (s) *B*-Bromocatecholborane, CH_2Cl_2



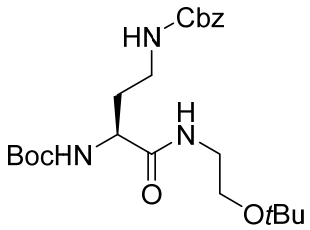
Boc-Glu(OBzl)-Ocyp (14)

Boc-Glu(OBzl)-OH (**13**; 675 mg, 2.00 mmol) was dissolved in CH_2Cl_2 (13.6 mL). To this solution, cyclopentanol (544 μL, 6.00 mmol), WSC·HCl (1.15 g, 6.00 mmol), and DMAP (48.9 mg, 0.400 mmol) were added at 4°C and stirred at room temperature overnight. After removing the solvent under vacuum, the residue was dissolved in 100 mL of CH_2Cl_2 and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (0–25% EtOAc / hexane) to obtain **14** as a white solid (642 mg, 79.2%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.38–7.30 (5H, m), 5.23–5.17 (1H, m), 5.12 (2H, s), 4.27 (1H, q, J = 7.2 Hz), 2.55–2.36 (2H, m), 2.24–2.12 (1H, m), 1.99–1.62 (9H, m), 1.46 (s, 9H, $C(CH_3)_3$); ESI-MS *m/z*: 428.17 (Calcd for $C_{22}H_{31}NO_6$: 428.21).



Boc-Glu-Ocyp (15)

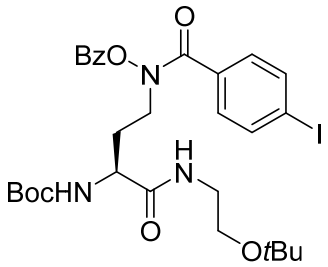
Compound **14** (625 mg, 1.54 mmol) was dissolved in methanol (15.4 mL) and 5% Pd/C (71.7 mg) was added under an Ar atmosphere. The mixture was stirred under a H_2 atmosphere at room temperature for 4 h. The reaction solution was then filtered, and the solvent was removed under vacuum to obtain **15** as a colorless oil. The product was used for the next reaction without further purification.



Boc-Dab(Cbz)-NH-CH_2-CH_2-O*t*Bu (18)

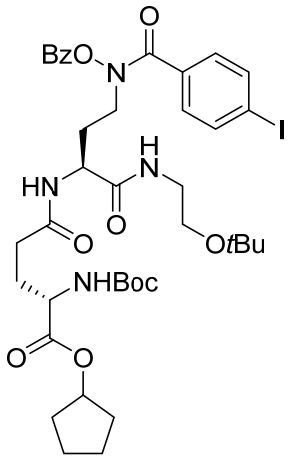
Boc-Dab(Cbz)-OH (**16**; 3.04 g, 8.63 mmol) was dissolved in CH_2Cl_2 (60 mL). To this solution, 2-(*tert*-butoxy)ethanamine·HCl (**17**; 1.97 g, 12.8 mmol), WSC·HCl (1.95 g, 10.2 mmol), HOBt·H_2O (1.56 g, 10.2 mmol), and NMM (2.82 mL, 25.7 mmol) were added at 4°C and stirred at room temperature overnight. After removing the solvent under vacuum, the residue was dissolved in 100 mL of $CHCl_3$ and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (25–67% EtOAc / hexane) to obtain **18** as a yellow oil (3.88 g, 99.7%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ:

7.37-7.29 (5H, m), 6.63 (1H, brs), 5.47 (1H, brs), 5.40 (1H, d, J = 7.5 Hz), 5.15–5.06 (2H, m), 4.19–4.10 (1H, m), 3.53–3.32 (5H, m), 3.10–3.06 (1H, m), 1.98–1.90 (1H, m), 1.83–1.75 (1H, m), 1.44 (9H, s), 1.18 (9H, s); ESI-MS *m/z*: 452.21 (Calcd for $C_{23}H_{38}N_3O_6$: 451.28).



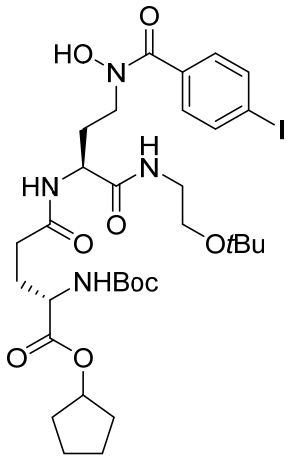
Boc-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-NH_2-CH_2-CH_2-O*t*Bu (19)

To a solution of **18** (1.06 g, 2.35 mmol) in methanol (23.6 mL), 5% Pd/C (140 mg) was added under an Ar atmosphere. The mixture was stirred under a H_2 atmosphere for 3 h. The reaction solution was then filtered and the solvent was removed under vacuum. After the residue was dissolved in CH_2Cl_2 (19.7 mL), carbonate buffer (100 mM, pH 10.5, 19.7 mL) was added and stirred. To the stirred mixture, BPO (761 mg, 2.36 mmol) was gradually added and stirred at room temperature overnight. Subsequently, *p*-iodobenzoly chloride (628 mg, 2.36 mmol) and NMM (311 μL, 2.83 mmol) were added and stirred at room temperature overnight. The organic layer was separated and concentrated under vacuum. The residue was purified by silica gel column chromatography (33% EtOAc / hexane) to obtain **19** as a white solid (434 mg, 27.8%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.94–7.89 (2H, m), 7.68–7.60 (3H, m), 7.46–7.36 (4H, m), 6.80–6.75 (1H, m), 5.40 (1H, d, J = 7.5 Hz), 4.32–4.14 (2H, m), 3.89–3.76 (1H, m), 3.44–3.32 (4H, m), 2.19 (1H, q, J = 6.3 Hz), 1.44 (9H, s), 1.17 (9H, s); ESI-MS *m/z*: 668.09 (Calcd for $C_{29}H_{39}IN_3O_7$: 668.18).



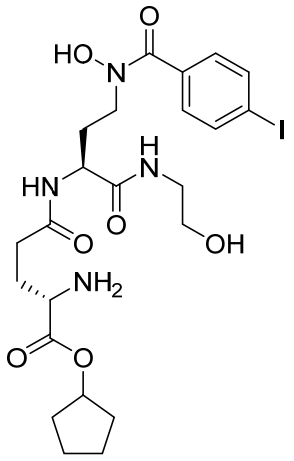
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-NH_2-CH_2-CH_2-O*t*Bu]-Ocyp (20)

Compound **16** (434 mg, 650 μmol) was dissolved in 4 M HCl / 1,4-dioxane (6.50 mL) at 4°C and the solution was stirred at room temperature for 30 min. After removing the solvent under vacuum, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. The residue was dissolved in CH_2Cl_2 (6.50 mL), and to the solution, **15** (308 mg, 977 μmol), WSC·HCl (150 mg, 782 μmol), HOBt·H_2O (120 mg, 784 μmol) and NMM (143 μL, 1.30 mmol) were added at 4°C and stirred at room temperature overnight. $CHCl_3$ (50 mL) was added to the solution, and washed sequentially with 50 mL of saturated aqueous $NaHCO_3$ and 50 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (33–67% EtOAc / hexane) to obtain **20** as a white solid (394 mg, 39.4%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.94–7.91 (2H, m), 7.68–7.61 (3H, m), 7.47–7.36 (4H, m), 7.08 (1H, brs), 6.91 (1H, d, J = 7.2 Hz), 5.29 (1H, d, J = 7.5 Hz), 5.21–5.17 (1H, m), 4.57 (1H, q, J = 7.2 Hz), 4.30–4.23 (2H, m), 3.84–3.72 (1H, m), 3.43–3.34 (4H, m), 2.36–2.32 (2H, m), 2.22–2.11 (2H, m), 1.93–1.67 (10H, m), 1.42 (9H, s), 1.17 (9H, s); ESI-MS *m/z*: 899.26 (Calcd for $C_{39}H_{53}ClIN_4O_{10}$: 899.25).



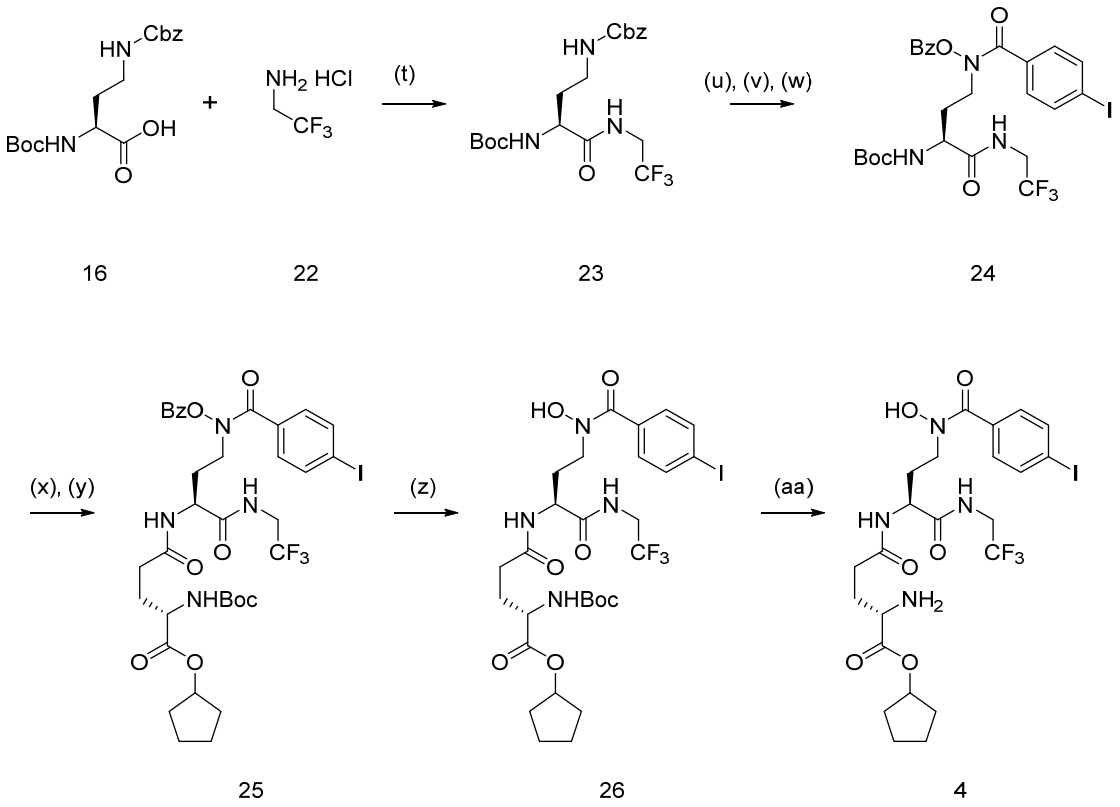
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH_2-CH_2-CH_2-O*t*Bu]-Ocyp (21)

To a solution of **20** (108 mg, 125 μmol) in DMF (1.50 mL), NH_4OH (28%, 833 μL) was added at 4°C under an Ar atmosphere and stirred at room temperature for 1 h. The solvent was diluted with 100 mL of $CHCl_3$ and washed three times with 100 mL of H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (1% methanol / $CHCl_3$) to obtain **21** as a white solid (75.9 mg, 79.9%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.79 (1H, brs), 7.83–7.72 (3H, m), 7.53–7.43 (3H, m), 5.24–5.16 (2H, m), 4.67–4.40 (2H, m), 4.30–4.20 (1H, m), 3.77–3.68 (1H, m), 3.44–3.33 (4H, m), 2.41–2.12 (4H, m), 1.95–1.66 (10H, m), 1.44 (9H, s), 1.16 (9H, s); ESI-MS *m/z*: 795.26 (Calcd for $C_{32}H_{49}ClIN_4O_9$: 795.22).



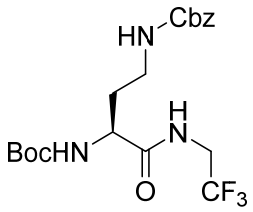
NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH_2-CH_2-CH_2-OH]-Ocyp (3)

B-Bromocatecholborane in CH_2Cl_2 (500 mM, 300 μL) was added to **21** (6.00 mg, 7.89 μmol) at 4°C.The mixture was vortexed at room temperature for 30 min. After H_2O (600 μL) was added, the solution was vortexed at room temperature for 20 min. The water layer was purified by RP-HPLC. The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 1.5 mL/min. The eluate was monitored at 254 nm. Compound **3** was obtained as a brown oil (1.18 mg, 24.8%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.82–7.78 (2H, m), 7.42 (2H, d, J = 8.1 Hz), 5.15–5.11 (1H, m), 4.65–4.53 (1H, m), 4.11–3.97 (2H, m), 3.67–3.43 (5H, m), 2.57–2.53 (2H, m), 2.40–1.66 (12H, m); HRMS (ESI) *m/z*: 605.14778 (Calcd for $C_{23}H_{34}IN_4O_7$: 605.14722).



Scheme S3. Synthesis of **4**

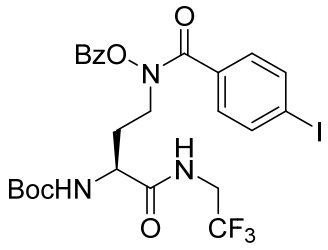
(t) WSC·HCl, HOBt, NMM, CH_2Cl_2; (u) H_2, 5% Pd/C, methanol; (v) BPO, carbonate buffer : CH_2Cl_2 (1 : 1); (w) *p*-Iodobenzoyl chloride, NMM, carbonate buffer : CH_2Cl_2 (1 : 1); (x) 4 M HCl / 1,4-dioxane; (y) **15**, WSC·HCl, HOBt, NMM, CH_2Cl_2; (z) NH_4OH, methanol; (aa) TFA, CH_2Cl_2



Boc-Dab(Cbz)-NH-CH_2-CF_3 (23)

Boc-Dab(Cbz)-OH (**16**; 2.60 g, 7.38 mmol) was dissolved in CH_2Cl_2 (52.0 mL). To this solution, 2,2,2-trifluoroethylamine·HCl (**22**; 1.50 g, 11.1 mmol), WSC·HCl (1.70 g, 8.87 mmol), HOBt (1.20 g, 8.88 mmol), and NMM (2.43 mL, 22.1 mmol) were added at 4°C and stirred at room temperature overnight. After removing the solvent under

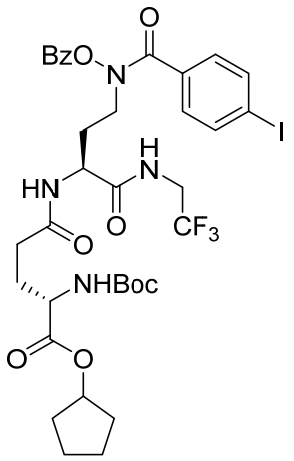
vacuum, the residue was dissolved in 100 mL of $CHCl_3$ and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$, and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (25–50% EtOAc / hexane) to obtain **23** as a white solid (2.24 g, 70.1%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.58 (1H, brs), 7.38–7.30 (5H, m), 5.43 (1H, d, *J* = 8.1 Hz), 5.32 (1H, brs), 5.17–5.05 (2H, m), 4.24–4.17 (1H, m), 4.02–3.77 (2H, m), 3.55–3.40 (1H, m), 3.14–3.07 (1H, m), 1.95–1.84 (2H, m), 1.44 (9H, s); ESI-MS *m/z*: 456.13 (Calcd for $C_{19}H_{26}F_3N_3NaO_5$: 456.17).



Boc-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-NH-CH_2-CF_3 (24)

To a solution of **23** (2.24 g, 5.17 mmol) in methanol (14.8 mL), 5% Pd/C (231 mg) was added under an Ar atmosphere. The mixture was stirred under a H_2 atmosphere for 3 h. The reaction solution was then filtered and the solvent was removed under vacuum. After the residue was dissolved in CH_2Cl_2 (50.0 mL), carbonate buffer (100 mM, pH 10.5; 50.0 mL) was added and stirred. To the stirred mixture, BPO (1.62 g, 5.02 mmol) was gradually added and stirred at room temperature overnight. Subsequently, *p*-iodobenzoly chloride (1.35 g, 5.07 mmol) and NMM (666 μL, 6.06 mmol) were added and stirred at room temperature overnight. The organic layer was separated, and concentrated under vacuum. The residue was purified by silica gel column chromatography (25% EtOAc /

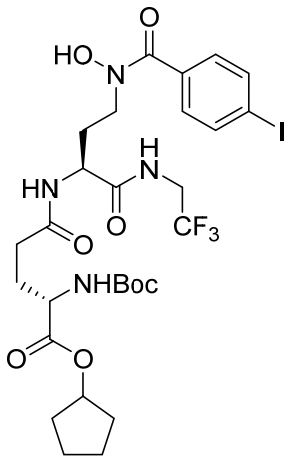
hexane) to obtain **24** as a pale yellow solid (2.17 g, 64.7%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.93–7.90 (2H, m), 7.69–7.61 (3H, m), 7.48–7.29 (5H, m), 5.42 (1H, d, *J* = 8.1 Hz), 4.39–4.18 (2H, m), 3.98–3.81 (3H, m), 2.17 (2H, q, *J* = 6.5 Hz), 1.45 (9H, s); ESI-MS *m/z*: 650.06 (Calcd for $C_{25}H_{28}F_3IN_3O_6$: 650.10).



Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-NH-CH_2-CF_3]-Ocyp (25)

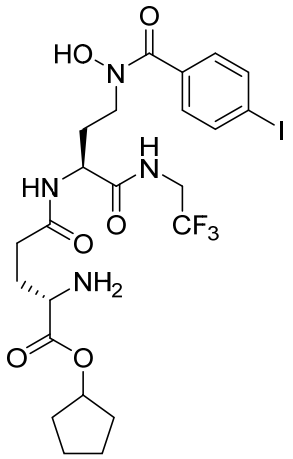
Compound **24** (1.00 g, 1.54 mmol) was dissolved in 4 M HCl / 1,4-dioxane (3.08 mL) at 4°C, and the solution was stirred at room temperature for 30 min. After removing the solvent under vacuum, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. The residue was dissolved in CH_2Cl_2 (15.4 mL), and to the solution, **15** (583 mg, 1.85 mmol), WSC·HCl (443 mg, 2.31 mmol), HOBt (312 mg, 2.31 mmol) and NMM (339 μL, 3.08 mmol) were added at 4°C and stirred at room temperature overnight. The solvent was removed under vacuum, and the residue was dissolved in 50 mL of EtOAc and washed sequentially with 50 mL of 10% aqueous citric acid, 50 mL of saturated aqueous $NaHCO_3$ and 50 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (25–50% EtOAc / hexane) to obtain **25** as

a dark brown solid (345 mg, 26.5%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.92–7.89 (2H, m), 7.69–7.61 (m, 3H, Ar), 7.48–7.35 (4H, m), 7.01 (1H, d, J = 6.5), 5.38–5.16 (3H, m), 4.55 (1H, q, J = 7.1 Hz), 4.26–4.19 (1H, m), 3.98–3.76 (4H, m), 2.44–2.14 (4H, m), 1.93–1.67 (10H, m) 1.44, (9H, s); ESI-MS *m/z*: 869.14 (Calcd for $C_{35}H_{42}F_3IN_4NaO_9$: 869.19).



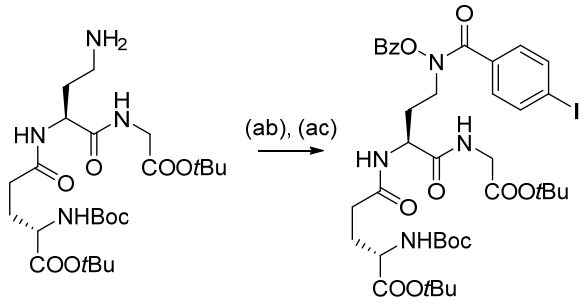
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CF_3]-Ocyp (26)

To a solution of **25** (345 mg, 0.408 mmol) in methanol (7.46 mL), NH_4OH (28%; 4.14 mL) was added at 4°C under an Ar atmosphere and stirred at room temperature for 1 h. The solvent was removed under vacuum, and the residue was dissolved in 50 mL of $CHCl_3$ and washed three times with 50 mL of H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (33–83% EtOAc / hexane) to obtain **26** as a white solid (69.4 mg, 22.9%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.86 (1H, brs), 8.14 (1H, brs), 7.76 (2H, d, J = 8.7 Hz), 7.55–7.41 (3H, m), 5.31–5.14 (2H, m), 4.56–4.40 (2H, m), 4.29–4.22 (1H, m), 4.02–3.51 (3H, m), 2.47–2.12 (4H, m), 1.94–1.68 (10H, m), 1.45 (9H, s); ESI-MS *m/z*: 741.17 (Calcd for $C_{28}H_{37}F_3IN_4O_8$: 741.16).

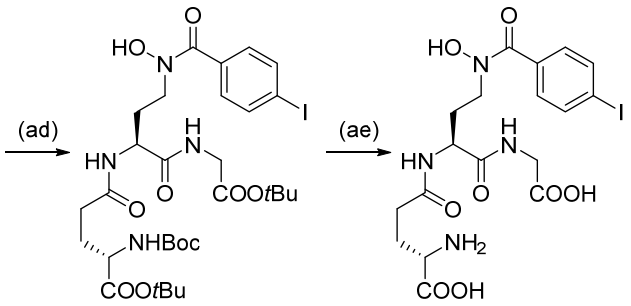


NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)- NH-CH_2-CF_3]-Ocyp (4)

To a solution of **26** (30.0 mg, 40.4 μmol) in CH_2Cl_2 (71.3 μL), TFA (336 μL) was added and stirred at room temperature for 1 h. After the solvent was removed under a stream of Ar gas, diethyl ether was added to precipitate a white solid, and the resulting precipitate was washed twice with diethyl ether. The precipitate was purified by RP-HPLC. The mobile phase of the HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 1.5 mL/min. The eluate was monitored at 254 nm. Compound **4** was obtained as a pale red solid (4.20 mg, 16.2%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.82–7.78 (2H, m), 7.42 (2H, d, J = 7.8 Hz), 5.15–5.11 (1H, m), 4.65–4.53 (1H, m), 4.05–3.44 (5H, m), 2.36–1.64 (14H, m); HRMS (ESI) *m/z*: 643.12431 (Calcd for $C_{23}H_{31}F_3IN_4O_6$: 643.12404).



27 28



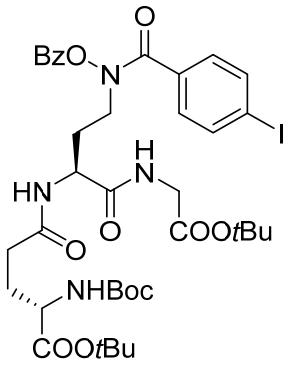
29 5

Scheme S4. Synthesis of **5**

(ab) BPO, carbonate buffer : CH_2Cl_2 (1 : 1); (ac) *p*-Iodobenzoyl chloride, NMM, carbonate buffer : CH_2Cl_2 (1 : 1); (ad) NH_4OH, methanol; (ae) TFA, CH_2Cl_2

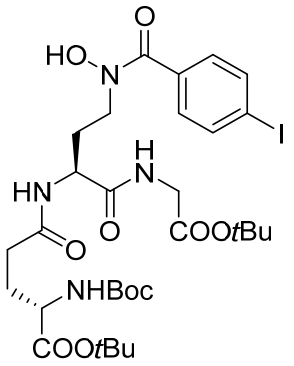
Boc-γ-Glu[-Dab-Gly-O*t*Bu]-O*t*Bu (27)

Compound **27** was synthesized according to the method of More *et al*.[1)]



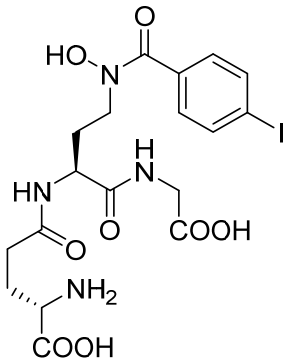
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-Gly-O*t*Bu]-O*t*Bu (28)

To a solution of **27** (170 mg, 329 μmol) in CH_2Cl_2 (4.00 mL), carbonate buffer (100 mM, pH 10.5; 4.00 mL) was added and stirred. To the stirred mixture, BPO (116 mg, 359 μmol) was gradually added and stirred at room temperature overnight. Subsequently, *p*-iodobenzoly chloride (175 mg, 657 μmol) and NMM (80.0 μL, 728 μmol) were added and stirred at room temperature overnight. The organic layer was separated, and concentrated under vacuum. The residue was purified by preparative thin-layer chromatography (PTLC) (60% EtOAc / hexane) to obtain **28** as a colorless oil (147 mg, 51.5%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.93–7.91 (2H, m), 7.69–7.60 (3H, m), 7.47–7.37 (5H, m), 6.92 (1H, d, J = 7.2 Hz), 5.26 (1H, d, J = 8.5 Hz), 4.62 (1H, q, J = 7.2 Hz), 4.42–4.33 (1H, m), 4.22–4.16 (1H, m), 4.04–3.81 (3H, m), 2.38–2.12 (5H, m), 1.93–1.83 (1H, m), 1.45, 1.42 (27H, 2s); ESI-MS *m/z*: 867.20 (Calcd for $C_{38}H_{52}IN_4O_{11}$: 867.27).



Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-Gly-O*t*Bu]-O*t*Bu (29)

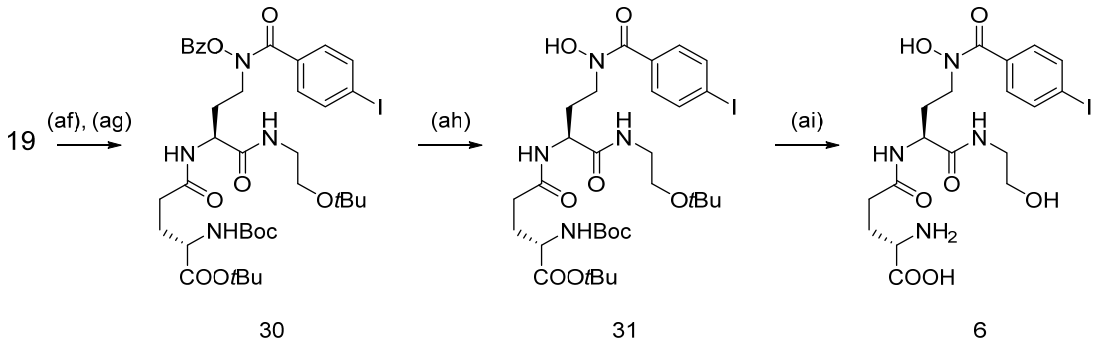
To a solution of **28** (70.0 mg, 80.8 μmol) in methanol (1.48 mL), NH_4OH (28%; 821 μL) was added at 4°C under an Ar atmosphere and stirred at room temperature for 1 h. The solvent was removed under vacuum, and the residue was purified by silica gel column chromatography (1% methanol / $CHCl_3$) to obtain **29** as a white solid (51.0 mg, 82.8%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.02 (1H, brs), 7.84–7.67 (3H, m), 7.53–7.42 (3H, m), 5.20 (1H, d, J = 8.4 Hz), 4.63–4.56 (2H, m), 4.24–4.16 (1H, m), 3.97–3.69 (2H, m), 3.54–3.46 (1H, m), 2.50–2.14 (3H, m), 1.98–1.87 (2H, m), 1.47, 1.45, 1.43 (27H, 3s); ESI-MS *m/z*: 761.25 (Calcd for $C_{31}H_{46}IN_4O_{10}$: 761.23).



NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-Gly-OH]-OH (5)

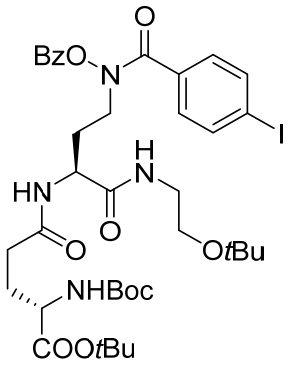
To a solution of **29** (20.0 mg, 26.2 μmol) in CH_2Cl_2 (600 μL), TFA (600 μL) was added at 4°C and stirred at room temperature for 3 h. After the solvent was removed under

a stream of Ar gas, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. The residue was purified by RP-HPLC. The mobile phase of RP-HPLC was consisted of 0.1% TFA/H_2O-methanol, and the percentage of methanol was maintained 30% for the first 5 min, then increased in a linear gradient to 60% for the next 30 min at a flow rate of 2.0 mL/min. The eluate was monitored at 254 nm. Compound **5** was obtained as a colorless oil (9.50 mg, 65.8%). ^{1}H-NMR (300MHz, CD_3OD) δ: 7.80 (2H, d, *J* = 8.2 Hz), 7.41 (2H, d, *J* = 8.2 Hz), 4.50–4.45 (1H, m), 4.05–3.91 (4H, m), 3.72–3.63 (1H, m), 2.58–2.54 (2H, m), 2.40–1.94 (4H, m); HRMS (ESI) *m/z*: 551.06335 (Calcd for $C_{18}H_{24}IN_4O_8$: 551.06388).



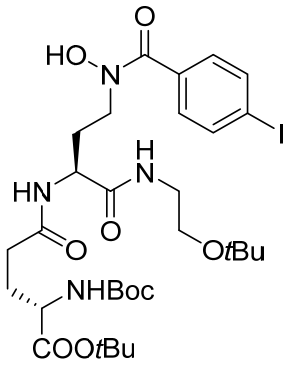
Scheme S5. Synthesis of **6**

(af) 4 M HCl / 1,4-dioxane; (ag) Boc-Glu-O*t*Bu, WSC·HCl, HOBt, NMM, CH_2Cl_2; (ah) NH_4OH, methanol; (ai) TFA, CH_2Cl_2



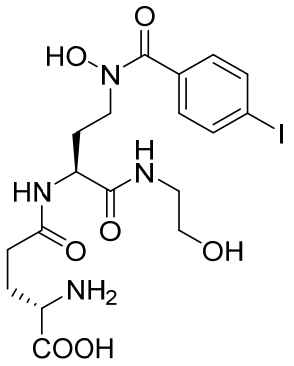
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-benzoyloxy)-NH-CH_2-CH_2-O*t*Bu]-O*t*Bu (30)

Compound **19** (3.13 g, 4.69 mmol) was dissolved in 4 M HCl / 1,4-dioxane (28.9 mL) at 4°C and stirred at room temperature for 30 min. After removing the solvent under vacuum, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. The residue was dissolved in CH_2Cl_2 (85.9 mL), and to the solution, Boc-Glu-O*t*Bu (1.70 g, 5.60 mmol), WSC·HCl (1.36 g, 7.09 mmol), HOBt (949 mg, 7.02 mmol) and NMM (1.42 mL, 12.9 mmol) were added and stirred at room temperature overnight. The solvent was removed under vacuum, and the residue was dissolved in 100 mL of EtOAc and washed sequentially with 100 mL of 10% aqueous citric acid, 100 mL of saturated aqueous $NaHCO_3$ and 100 mL of brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (33–67% EtOAc / hexane) to obtain **30** as a dark brown solid (2.04 g, 51.0%). ^{1}H-NMR ($CDCl_3$) δ: 7.93–7.90 (2H, m), 7.68–7.61 (3H, m), 7.47–7.36 (4H, m), 7.09 (1H, brs), 6.87 (1H, brs), 5.26–5.19 (1H, m), 4.60–4.54 (1H, m), 4.32–4.25 (2H, m), 3.85–3.73 (1H, m), 3.44–3.34 (4H, m), 2.40–2.12 (5H, m), 1.93–1.84 (1H, m), 1.46, 1.45 (18H, 2s), 1.17 (9H, s); ESI-MS *m/z*: 853.25 (Calcd for $C_{38}H_{54}IN_4O_{10}$: 853.29).



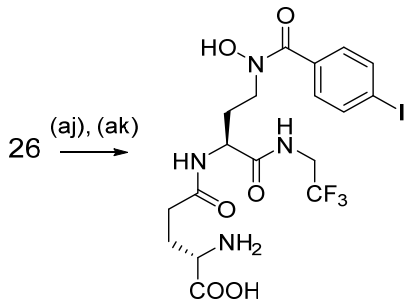
Boc-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CH_2-O*t*Bu]-O*t*Bu (31)

To a solution of **30** (1.92 g, 2.25 mmol) in methanol (28.9 mL), NH_4OH (28%; 16.1 mL) was added at 4°C under an Ar atmosphere and stirred at room temperature for 1.5 h. The solvent was removed under vacuum, and the residue was dissolved in 100 mL of $CHCl_3$ and washed three times with 100 mL of H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography (1% methanol / $CHCl_3$) to obtain **31** as a white solid (904 mg, 53.6%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.76 (1H, brs), 7.74 (2H, d, J = 8.5 Hz), 7.54–7.45 (3H, m), 5.17 (1H, d, J = 8.5 Hz), 4.68–4.43 (3H, m), 4.21–4.18 (1H, m), 3.53–3.33 (5H, m), 2.40–2.13 (5H, m), 1.93–1.84 (1H, m), 1.47, 1.44 (18H, 2s), 1.17 (9H, s); ESI-MS *m/z*: 749.19 (Calcd for $C_{31}H_{49}IN_4O_9$: 749.26).



NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CH_2-OH]-OH (6)

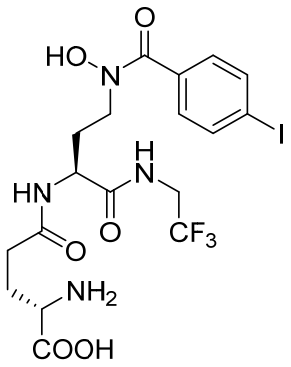
To a solution of **31** (1.75 mg, 2.34 μmol) in CH_2Cl_2 (4.0 μL), TFA (20.0 μL) was added at 4°C and stirred at room temperature for 3 h. After the solvent was removed under a stream of Ar gas, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. The precipitate was then purified by RP-HPLC. The mobile phase of the HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 60 min at a flow rate of 1.5 mL/min. The eluate was monitored at 254 nm. Compound **6** was obtained as a pale red oil (0.45 mg, 36%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.80 (2H, d, *J* = 8.5 Hz), 7.42 (2H, d, *J* = 8.1 Hz), 4.42–4.38 (1H, m), 4.10–3.97 (2H, m), 3.72–3.57 (5H, m), 2.57–2.50 (2H, m), 2.38–1.89 (6H, m); HRMS (ESI) *m/z*: 537.08428 (Calcd for $C_{18}H_{26}IN_4O_7$: 537.08462).



7

Scheme S6. Synthesis of **7**

(aj) TFA, CH_2Cl_2; (ak) 1 M NaOH

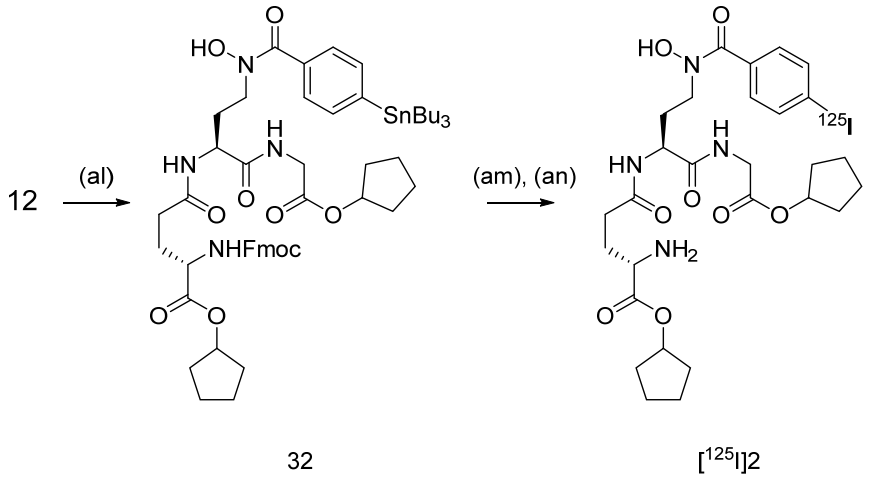


NH_2-γ-Glu[-Dab(*N*-(*p*-iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CF_3]-OH (7)

To a solution of **26** (8.87 mg, 12.0 μmol) in CH_2Cl_2 (21.0 μL), TFA (99.0 μL) was added and stirred at room temperature for 1 h. After the solvent was removed under vacuum, diethyl ether was added to precipitate a white solid and the resulting precipitate was washed with diethyl ether. To the precipitate, 1 M NaOH (130 μL) was added and the solution was stirred at room temperature for 1 min. The reaction mixture was neutralized with 1 M HCl and purified by RP-HPLC. The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored at 254 nm. Compound **7** was obtained as a brown oil (1.70 mg, 24.8%). ^{1}H-NMR (300 MHz, CD_3OD) δ: 7.82–7.78 (2H, m), 7.42 (2H, d, J = 7.8 Hz), 4.47–4.43 (1H, m), 4.11–

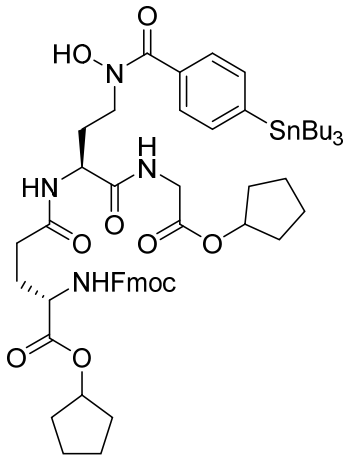
3.81 (3H, m), 3.68–3.52 (2H, m), 2.56–1.89 (4H, m); HRMS (ESI) *m/z*: 575.06123 (Calcd for $C_{18}H_{23}F_3IN_4O_6$: 575.06144).

1-3. Radiosynthesis



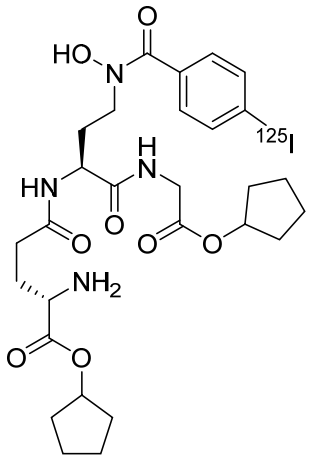
Scheme S7. Radiosynthesis of [^{125}I]**2**

(al) Bis(tributyltin), tris(dibenzylideneacetone)dipalladium(0), *N*-ethyldiisopropylamine, 1,4-dioxane; (am) [^{125}I]NaI, 1 M HCl, 3% H_2O_2, (an) 20% piperidine / DMF



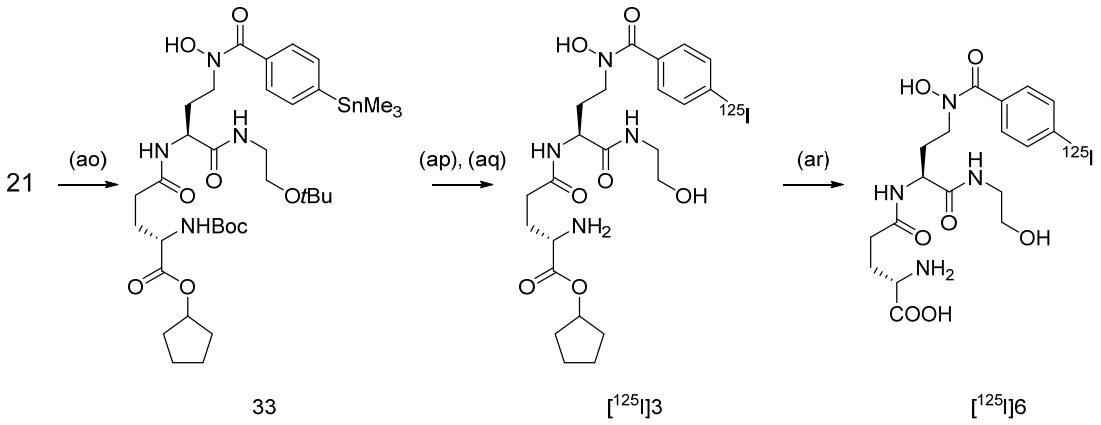
Fmoc-γ-Glu[-Dab(*N*-(*p*-(tributylstannyl)benzoyl)-*N*-hydroxy)-Gly-Ocyp]-Ocyp (32)

Bis(tributyltin) (21.1 μL, 42.2 μmol), tris(dibenzylideneacetone)dipalladium(0) (873 μg, 953 nmol) and *N*-ethyldiisopropylamine (16.4 μL, 96.4 μmol) were added to a solution of **12** (34.7 mg, 38.2 mmol) in 1,4-dioxane (1.91 mL) and stirred at room temperature for 18 h. The mixture was purified by PTLC (1% methanol / $CHCl_3$) to obtain **32** as a white solid (4.10 mg, 10.0%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.78 (1H, brs), 7.79–7.65 (5H, m), 7.61–7.58 (2H, m), 7.52–7.43 (3H, m), 7.37–7.27 (4H, m), 5.65 (1H, d, J = 8.1 Hz), 5.21–5.12 (2H, m), 4.65–4.53 (2H, m), 4.41–3.69 (7H, m), 3.53–3.47 (1H, m), 2.47–2.15 (4H, m), 2.05–1.66 (18H, m), 1.57–1.49 (6H, m), 1.37–1.24 (6H, m), 1.08–1.04 (6H, m), 0.90–0.85 (9H, m); ESI-MS *m/z*: 1095.40 (Calcd for $C_{55}H_{76}N_4NaO_{10}Sn$: 1095.45).



NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-Gly-Ocyp]-Ocyp ([^{125}I]2)

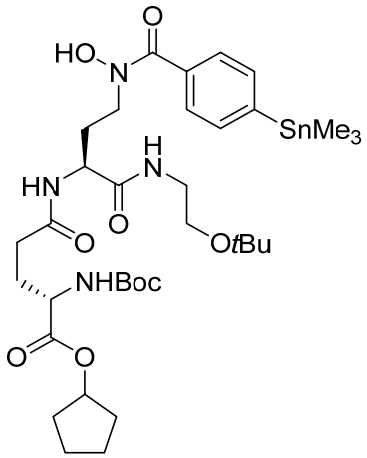
[^{125}]NaI (185 kBq), 1 M HCl (50 μL) and 3% H_2O_2 (50 μL) were added to a solution of **32** in ethanol (1 mg/mL, 50 μL), and stirred at room temperature for 30 min. After the reaction was terminated by the addition of saturated aqueous $NaHSO_3$ (100 μL), saturated aqueous $NaHCO_3$ (100 μL) was added, and the mixture was extracted three times by EtOAc (100 μL). The solvent was removed under vacuum. Piperidine / DMF (20% (v/v); 50 μL) was added to the residue, and the solution was vortexed at room temperature for 30 min. The mixture was purified by RP-HPLC to obtain [^{125}I]**2** (61.1 kBq, 33.0%). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 1.5 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.



33 [^{125}I]3 [^{125}I]6

Scheme S8. Radiosynthesis of [^{125}I]**3** and [^{125}I]**6**

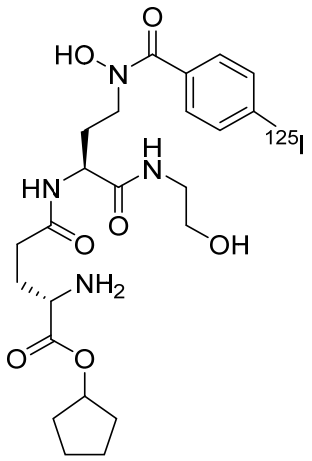
(ao) Hexamethylditin, bis(triphenylphosphine)palladium(II) dichloride ($Pd(TPP)_2Cl_2$), 1,4-dioxane; (ap) [^{125}I]NaI, 1 M HCl, 3% H_2O_2, (aq) *B*-Bromocatecholborane, CH_2Cl_2; (ar) 10 M NaOH



Boc-γ-Glu[-Dab(*N*-(*p*-(trimethylstannyl)benzoyl)-*N*-hydroxy)-NH-CH_2-CH_2-O*t*Bu]-Ocyp (33)

Hexamethylditin (46.5 µL, 224 µmol) and bis(triphenylphosphine)palladium(II) dichloride ($Pd(TPP)_2Cl_2$; 7.78 mg, 11.1 µmol) were added to a solution of **21** (69.4 mg, 91.3 µmol) in 1,4-dioxane (780 µL). The resulting mixture was stirred at 95°C until the solution was brown. The mixture was then filtered, and the filtrate was purified by PTLC (60% EtOAc / hexane) to obtain **33** as a brown solid (2.30 mg, 3.16%). ^{1}H-NMR (300

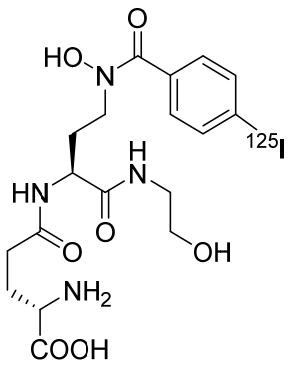
MHz, $CDCl_3$) δ: 8.76 (1H, brs), 7.77–7.65 (3H, m), 7.52–7.43 (3H, m), 5.24–5.16 (2H, m), 4.68–4.41 (2H, m), 4.28–4.18 (1H, m), 3.53–3.33 (5H, m) 2.40–2.13 (4H, m), 1.95–1.66 (10H, m), 1.44 (9H, s), 1.19 (9H, s), 0.31 (9H, s); ESI-MS *m/z*: 799.25 (Calcd for $C_{35}H_{59}N_4O_9Sn$: 799.33).



NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CH_2-OH]-Ocyp ([^{125}I]3)

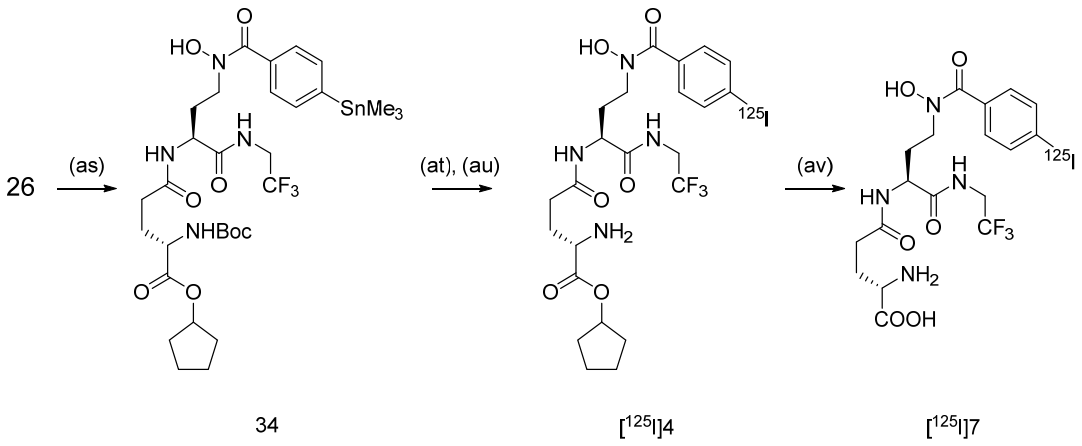
[^{125}]NaI (1.13 MBq), 1 M HCl (50 μL), and 3% H_2O_2 (50 μL) were added to a solution of **33** in ethanol (1 mg/mL, 50 μL) and vortexed at room temperature for 30 min. After the reaction was terminated by the addition of saturated aqueous $NaHSO_3$ (100 μL), saturated aqueous $NaHCO_3$ (100 μL) was added, and the mixture was extracted three times by EtOAc (100 μL). The solvent was removed under vacuum. *B*-bromocatechol-borane in CH_2Cl_2 (500 mM, 100 μL) was added to the residue at 4°C, and the solution was vortexed at room temperature for 30 min. Then, H_2O (100 μL) was added to the solution at 4°C and vortexed at room temperature for 20 min. The water layer was purified by RP-HPLC to obtain [^{125}I]**3** (124 kBq, 10.9%). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol was increasing

in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.



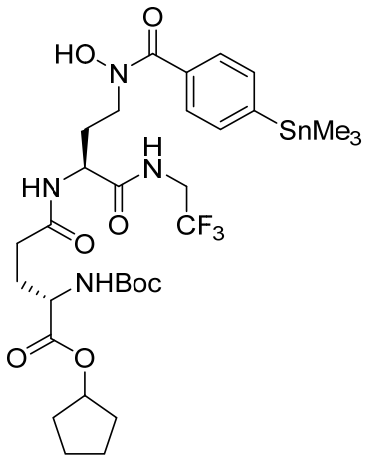
NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CH_2-OH]-OH ([^{125}I]6)

To a solution of [^{125}]**3** (25.1 kBq) in H_2O (291 μL), 10 M NaOH (9 μL) was added and vortexed at room temperature for 5 min. The solution was neutralized with 1 M HCl and purified by RP-HPLC to obtain [^{125}I]**6** (37.9 kBq, 75.5%). The mobile phase consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.



Scheme S9. Radiosynthesis of [^{125}I]**4** and [^{125}I]**7**

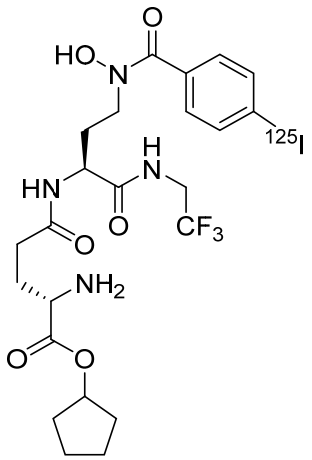
(as) Hexamethylditin, $Pd(TPP)_2Cl_2$, 1,4-dioxane; (at) [^{125}I]NaI, 1 M HCl, 3% H_2O_2; (au) *B*-Bromocatecholborane, CH_2Cl_2; (av) 10 M NaOH



Boc-γ-Glu[-Dab(*N*-(*p*-(trimethylstannyl)benzoyl)-*N*-hydroxy)-NH-CH_2-CF_3]-Ocyp (34)

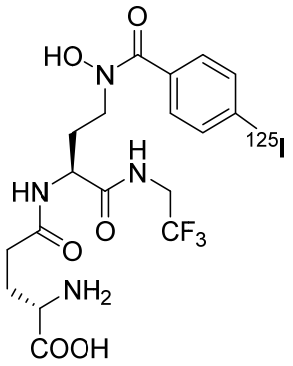
Hexamethylditin (45.2 μL, 218 μmol) and $Pd(TPP)_2Cl_2$ (7.59 mg, 10.8 μmol) were added to a solution of **26** (69.4 mg, 93.5 μmol) in 1,4-dioxane (757 μL). The resulting mixture was stirred at 95°C until the solution was brown. The mixture was then filtered, and the filtrate was purified by PTLC (60% EtOAc / hexane) to obtain **34** as a brown solid (21.3 mg, 29.2%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.88 (1H, brs), 8.16 (1H, brs), 7.69 (2H, d, J = 7.8 Hz), 7.52–7.42 (3H, m), 5.31–5.14 (2H, m), 4.56–4.41 (2H, m),

4.30–4.23 (1H, m), 4.06–3.52 (3H, m), 2.48–2.13 (4H, m), 1.94–1.68 (10H, m), 1.44 (9H, s), 0.31 (9H, s); ESI-MS *m/z*: 781.23 (Calcd for $C_{31}H_{48}F_3N_4O_8Sn$: 781.25).



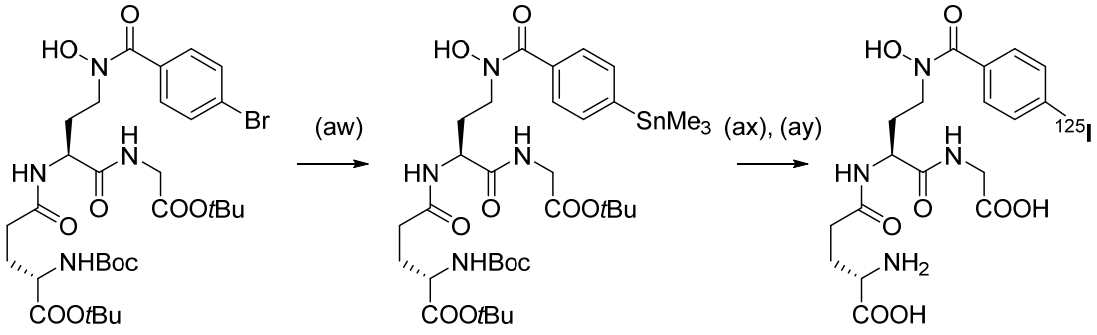
NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CF_3]-Ocyp ([^{125}I]4)

[^{125}]NaI (2.92 MBq), 1 M HCl (50 μL) and 3% H_2O_2 (50 μL) were added to a solution of **34** in ethanol (1 mg/mL, 50 μL), and vortexed at room temperature for 30 min. After the reaction was terminated by the addition of saturated aqueous $NaHSO_3$ (100 μL), saturated aqueous $NaHCO_3$ (100 μL) was added, and the mixture was extracted three times by EtOAc (100 μL). The solvent was removed under vacuum. *B*-Bromocatechol-borane in CH_2Cl_2 (500 mM, 100 μL) was added to the residue at 4°C, and the solution was vortexed at room temperature for 30 min. Then, H_2O (100 μL) was added to the solution at 4°C, and the mixture was vortexed at room temperature for 20 min. The water layer was then purified by RP-HPLC to obtain [^{125}I]**4** (653 kBq, 22.3%). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.



NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-NH-CH_2-CF_3]-OH ([^{125}I]7)

To a solution of [^{125}]**4** (250 kBq) in H_2O (291 μL), 10 M NaOH aq. (9 μL) was added and vortexed at room temperature for 5 min. The reaction mixture was neutralized with 1 M HCl and purified by RP-HPLC to obtain [^{125}I]**7** (197 kBq, 78.5%). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.



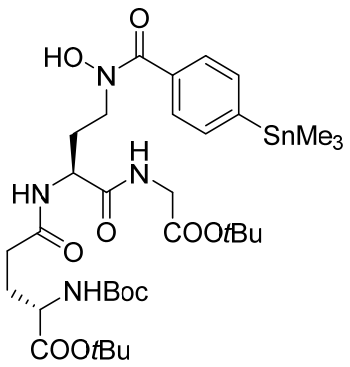
35 36 [^{125}I]5

Scheme S10. Radiosynthesis of [^{125}I]**5**

(aw) Hexamethylditin, $Pd(TPP)_2Cl_2$, 1,4-dioxane; (ax) [^{125}I]NaI, 1 M HCl, 3% H_2O_2; (ay) TFA, CH_2Cl_2

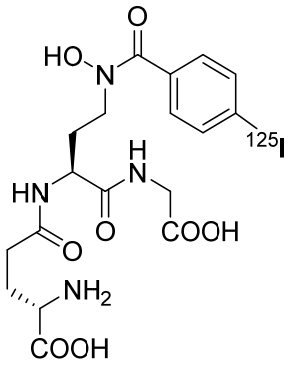
Boc-γ-Glu[-Dab(*N*-(*p*-bromobenzoyl)-*N*-hydroxy)-Gly-O*t*Bu]-O*t*Bu (35)

Compound **35** was synthesized according to the method of More *et al*.[1)]



Boc-γ-Glu[-Dab(*N*-(*p*-(trimethylstannyl)benzoyl)-*N*-hydroxy)-Gly-O*t*Bu]-O*t*Bu (36)

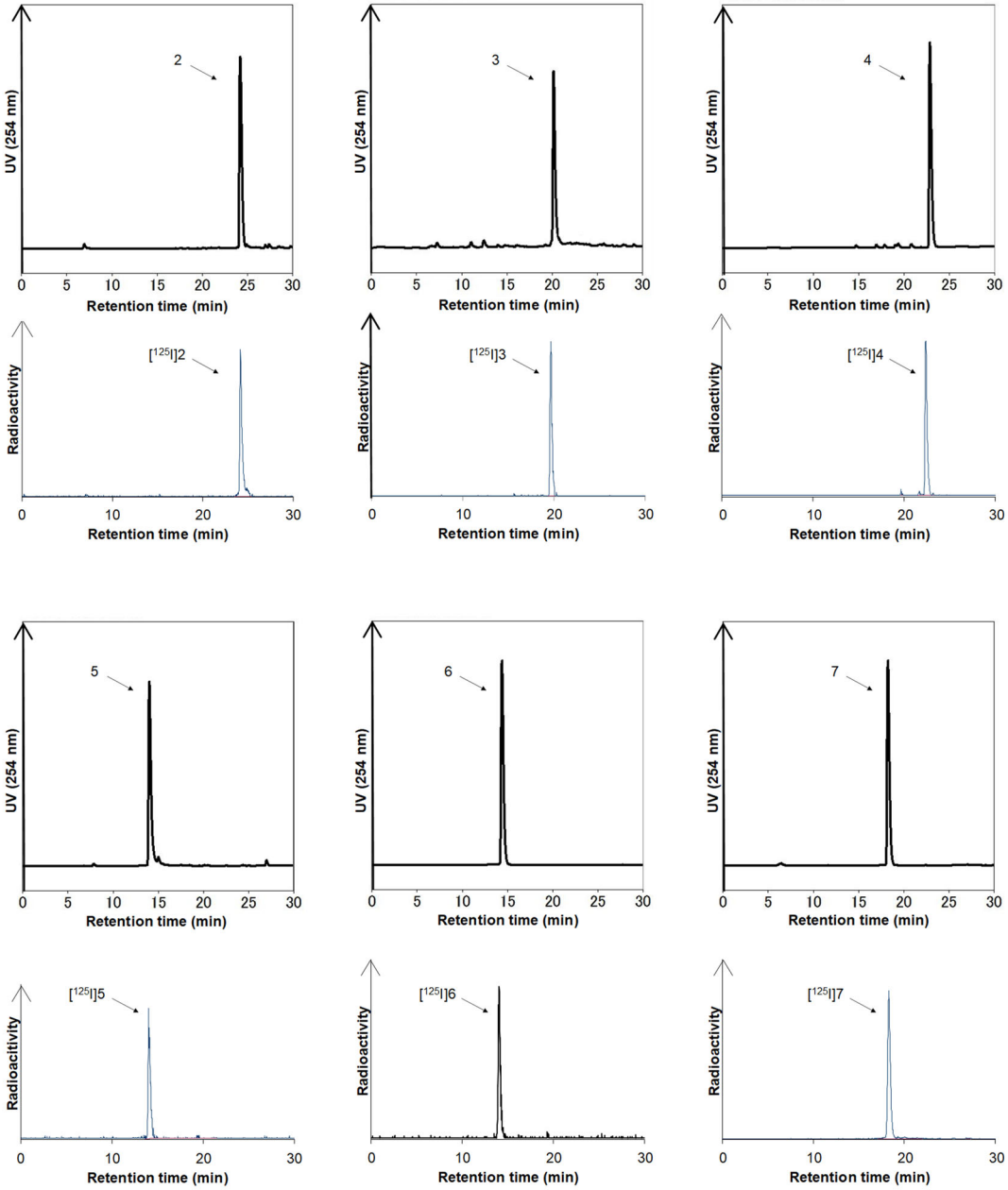
Hexamethylditin (11.2 μL, 54.0 μmol) and $Pd(TPP)_2Cl_2$ (1.86 mg, 2.65 μmol) were added to a solution of **35** (19 mg, 26.6 μmol) in 1,4-dioxane (186 μL). The resulting mixture was stirred at 95°C for 1 min. The mixture was then filtered, and the filtrate was purified by PTLC (60% EtOAc / hexane) to obtain **36** as a yellow oil (2.80 mg, 13.2%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.78 (1H, brs), 7.77–7.65 (3H, m), 7.52–7.43 (3H, m), 5.21 (1H, d, J = 7.2 Hz), 4.64–4.54 (2H, m), 4.23–4.16 (1H, m), 3.97–3.69 (2H, m), 3.53–3.47 (1H, m), 2.51–2.14 (4H, m), 1.97–1.87 (2H, m), 1.46, 1.45, 1.43 (27H, 3s), 0.30 (9H, s); ESI-MS *m/z*: 823.26 (Calcd for $C_{34}H_{56}N_4NaO_{10}Sn$: 823.29).



NH_2-γ-Glu[-Dab(*N*-(*p*-[^{125}I]iodobenzoyl)-*N*-hydroxy)-Gly-OH]-OH ([^{125}I]5)

[^{125}I]NaI (185 kBq), 1 M HCl (50 μL) and 3% H_2O_2 (50 μL) were added to a solution of **36** in ethanol (1 mg/mL, 50 μL), and stirred at room temperature for 30 min. After terminating the reaction by adding saturated aqueous $NaHSO_3$ (50 μL), saturated aqueous $NaHCO_3$ (50 μL) was added, and the mixture was extracted three times by EtOAc (100 μL). The solvent was removed under vacuum. To the residue, 95% TFA / CH_2Cl_2 (100 μL) was added at 4°C and vortexed at room temperature for 30 min. After removing the solvent under vacuum, the residue was purified by RP-HPLC to obtain [^{125}I]**5** (70.1 kBq, 37.9%). The mobile phase of RP-HPLC consisted of 0.1% TFA/H_2O-methanol, with the percentage of methanol increasing in a linear gradient from 40% to 100% over 30 min at a flow rate of 2.0 mL/min. The eluate was monitored using an in-line NaI(Tl) radio-detector.

2. Supplementary Figures



Supplementary Figure 1. HPLC analyses of [^{125}I]**2**–**7** and their corresponding non-radioactive compounds.

[^{125}I]**2**–**7** were characterized by simultaneous HPLC analyses with the corresponding non-radioactive **2**–**7**, respectively.

3. Cell Culture

U251MG cells were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Eagle's minimal essential medium (EMEM) (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biowest, Nuaillé, France) and 1% penicillin and streptomycin (FUJIFILM Wako Pure Chemical Co., Tokyo, Japan). The cells were maintained in a humidified atmosphere with 5% CO_2 at 37°C.

4. Reference

1) More SS, Vince R: Inhibition of glyoxalase I: the first low-nanomolar tight-binding inhibitors. *J Med Chem,* **52**, 4650-4656 (2009).