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Supporting Information

Total Synthesis and Functional Evaluation of Fourteen Stereoisomers of Yaku'amide B. Importance of Stereochemistry for Hydrophobicity and Cytotoxicity

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General remarks

¹H and ¹³C NMR spectra were recorded on a JEOL ECX 500 (500 MHz for ¹H NMR, 125 MHz for ¹³C NMR) spectrometer, a JEOL ECA 500 (500 MHz for ¹H NMR, 125 MHz for ¹³C NMR) spectrometer or a JEOL ECS 400 (400 MHz for ¹H NMR, 100 MHz for ¹³C NMR). Chemical shifts are denoted in δ (ppm) relative to residual solvent peaks as internal standard (CDCl₃, ¹H & 7.26, ¹³C & 77.0; DMSO-*d*₆, ¹H & 2.50, ¹³C δ 39.5; C₆D₆, ¹H δ 7.16, ¹³C δ 128.0). IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. ESI-TOF MS spectra were recorded on a JEOL T100LP mass spectrometer. Optical rotations were recorded on a JASCO P-2200 polarimeter. High-performance liquid chromatography (HPLC) experiments were performed with a JASCO HPLC system equipped with a PU-2089 or PU-2086 Plus intelligent pump. Ultrahigh-performance liquid chromatography (UHPLC) experiments were performed with a JASCO X-LC system. All reactions sensitive to air or moisture were carried out under argon atmosphere in dry, freshly distilled solvents under conditions, unless otherwise noted. THF, CH₂Cl₂, toluene, DMF and Et₂O were purified by Glass Contour solvent dispensing system (Nikko Hansen & Co., Ltd., Osaka, Japan). All other reagents were used as supplied unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed using E. Merck Silica gel 60 F254 pre-coated plates. Flash column chromatography was performed using 40-50 µm Silica Gel 60N (Kanto Chemical Co., Inc.) or 32-53 µm Silica-gel BW-300 (Fuji Silysia Chemical Ltd.).

Experimental procedures and compound characterizations Synthesis of compound 4b



Compound S2: S1 was synthesized from L-serine according to the reported procedure for ent-S1.¹

To a solution of carboxylic acid **S1** (302 mg, 1.22 mmol) and *N*-methylmorpholine (0.14 mL, 1.3 mmol) in dimethoxyethane (5.7 mL) was added isobutyl chloroformate (0.17 mL, 1.3 mmol) at -10 °C. The reaction mixture was stirred at the same temperature for 10 min. To the reaction mixture was added aqueous NH₃ (0.62 mL, 9.7 mmol) at 0 °C. After being stirred at room temperature for 12 h, the reaction mixture was quenched with citric acid. The resulting solution was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was recrystallized from EtOAc/hexane to afford amide **S2** (206 mg, 0.836 mmol, 69%) as a white foam: $[\alpha]_D^{23} = -3.1$ (c = 0.94, MeOH); ¹H NMR spectrum was identical with that of *ent*-**S2** [CAS 1428850-11-6].²

ent-**S2**: $[\alpha]_D^{18} = 4.8$ (*c* = 1.8, MeOH)



Compound S4: To a solution of amide **S2** (202 mg, 0.820 mmol) and Z-alkenyl iodide **S3** [CAS 1428850-12-7]² (614 mg, 1.32 mmol) in dioxane (0.8 mL) were added Cs₂CO₃ (321 mg, 0.984 mmol), copper iodide (46.9 mg, 0.246 mmol) and *N*,*N'*-dimethylethylenediamine (0.18 mL, 1.6 mmol) at room temperature. After being stirred at 70 °C for 24 h, the reaction mixture was directly purified with flash column chromatography (EtOAc/hexane = 1/20 to 1/0) to afford starting material **S2** (77.0 mg, 0.312 mmol, 38%) and enamide **S4** (177 mg, 0.305 mmol, 37%) as a white foam: $[\alpha]_D^{21} = 29$ (*c* = 0.29 , MeOH); ¹H NMR spectrum was identical with that of *ent*-**S4** [CAS 1428850-18-3].²

ent-**S4**: $[\alpha]_D^{19} = -23$ (*c* = 0.79, MeOH)



Compound S5: To a solution of enamide **S4** (177 mg, 0.305 mmol) in THF (2.4 mL) was added TBAF (1 M in THF, 0.61 mL, 0.61 mmol) at 0 °C. After being stirred at 0 °C for 2 h and at room temperature for 15 min, the reaction mixture was quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash

column chromatography (EtOAc/hexane = 1/1 to 9/1) to afford alcohol **S5** (99.3 mg, 0.288 mmol, 95%) as a white solid: $[\alpha]_D^{21} = 23$ (c = 0.50, MeOH); ¹H NMR spectrum was identical with that of *ent*-**S5** [CAS 1428850-38-7].²

ent-**S5**: $[\alpha]_D^{19} = -18$ (*c* = 1.8, MeOH)



Compound S7: To a solution of alcohol **S5** (99.3 mg, 0.288 mmol) in CH₂Cl₂ (5.7 mL) was added Dess-Martin periodinane (147 mg, 0.346 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 30 min. To the reaction mixture was added saturated aqueous NaHCO₃ and Na₂S₂O₃. The resulting solution was extracted with CH₂Cl₂ (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was roughly purified with flash column chromatography (EtOAc/hexane = 3/7 to 1/1) to afford the crude **S6**, which was used in the next reaction without further purification.

To a solution of the above aldehyde **S6** in *t*-BuOH/H₂O (1/1, 5 mL) were added 2-methyl-2-butene (2.6 mL, 24 mmol), NaH₂PO₄·2H₂O (396 mg, 2.54 mmol) and NaClO₂ (191 mg, 1.69 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. To the reaction mixture were added saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (3 times). The combined organic layers were dried over anhydrous MgSO₄ and filtered. The solution was concentrated to afford carboxylic acid **S7** (82.6 mg, 0.230 mmol, 82% for 2 steps) as a white foam: $[\alpha]_D^{22} = 17$ (c = 4.1, MeOH); ¹H NMR spectrum was identical with that of *ent*-**S7** [CAS 1428850-19-4].²

ent-**S7**: $[\alpha]_D^{19} = -22$ (*c* = 1.4, MeOH)



Compound S8: To a solution of **S7** (82.6 mg, 0.230 mmol) in DMF (2.3 mL) were added K₂CO₃ (42.0 mg, 0.304 mmol) and allyl bromide (0.024 mL, 0.28 mmol) at 0 °C. After being stirred at 60 °C, the reaction mixture was quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/4 to 3/7) to afford allyl ester **S8** (80.6 mg, 0.202 mmol, 88%) as a white foam: $[\alpha]_D^{21} = 30$ (c = 4.3, MeOH); ¹H NMR spectrum was identical with that of *ent*-**S8** [CAS 1428850-39-8].² *ent*-**S8**: $[\alpha]_D^{19} = -35$ (c = 0.35, MeOH)



Compound S9: To a solution of allyl ester **S8** (80.6 mg, 0.202 mmol) in CH₂Cl₂ (2 mL) were added Et₃N (0.031 mL, 0.22 mmol), DMAP (7.4 mg, 0.061 mmol) and Boc₂O (0.056 mL, 0.24 mmol) at 0 °C. After being stirred at 0 °C for 10 min, to the reaction mixture was added Boc₂O (0.014 mL, 0.060 mmol). After being stirred at the same temperature for 1.5 h, the reaction mixture was quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄ and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/20 to 3/7) to afford allyl ester **S9** (82.7 mg, 0.166 mmol, 82%) as a white foam: $[\alpha]_D^{21} = 62$ (c = 4.1, MeOH); ¹H NMR spectrum was identical with that of *ent-***S9** [CAS 1428850-40-1].²

ent-**S9**: $[\alpha]_D^{20} = -60$ (*c* = 0.92, MeOH)



Compound 4b: To a solution of allyl ester **S9** (82.7 mg, 0.166 mmol) in THF (1.7 mL) were added morpholine (0.72 mL, 8.3 mmol), 2-methyl-2-butene (0.18 mL, 1.7 mmol) and Pd(PPh₃)₄ (9.6 mg, 0.0083 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1 h. The reaction mixture was treated with 1 M aqueous HCl, and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (CHCl₃/MeOH/AcOH = 200/1/1) to afford carboxylic acid **4b** (66.7 mg, 0.145 mmol, 88%) as a white foam: $[\alpha]_D^{21} = 35$ (c = 3.1, MeOH); ¹H NMR spectrum was identical with that of **4a** [CAS 1428850-06-9].²

4a: $[\alpha]_D^{20} = -37 \ (c = 0.54, \text{MeOH})$

Synthesis of compound 4c



Compound S11: To a solution of carboxylic acid **S10** [CAS 167102-75-2]¹ (844 mg, 3.43 mmol) and *N*-methylmorpholine (0.41 mL, 3.8 mmol) in dimethoxyethane (16 mL) was added isobutyl chloroformate (0.49 mL, 3.8 mmol) at -10 °C. The reaction mixture was stirred at the same temperature for 10 min. To the reaction mixture was added aqueous NH₃ (1.8 mL, 28 mmol) at 0 °C. After being stirred at room temperature for 8 h, the reaction mixture was quenched with citric acid. The resulting solution was extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous

MgSO₄, and filtered. The solution was concentrated, and the residue was recrystallized from EtOAc/hexane to afford amide **S11** (627 mg, 2.54 mmol, 74%) as a white foam: $[\alpha]_D^{23} = -53$ (c = 1.2, CHCl₃); IR (film) v 3342, 3205, 2977, 2937, 2884, 1671, 1507, 1167 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.96 (3H, t, J = 7.7 Hz), 1.14 (3H, s), 1.45 (9H, s), 1.52–1.72 (2H, m), 3.81 (1H, br), 3.97 (1H, d, J = 9.1 Hz), 5.49 (1H, d, J = 8.1 Hz), 5.54 (1H, br), 6.41 (1H, br); ¹³C NMR (125 MHz, CDCl₃) δ 7.9, 21.4, 28.2, 32.3, 58.5, 73.9, 80.1, 156.2, 174.9; HRMS (ESI) calcd for C₁₁H₂₂N₂NaO₄ [M+Na]⁺ 269.1472, found 269.1472.



Compound S12: To a solution of amide **S11** (600 mg, 2.44 mmol) and Z-alkenyl iodide **S3** (2.17 g, 4.67 mmol) in dioxane (2.4 mL) were added Cs₂CO₃ (954 mg, 2.93 mmol), copper iodide (139 mg, 0.732 mmol) and *N*,*N*'-dimethylethylenediamine (0.53 mL, 5.0 mmol) at room temperature. After being stirred at 70 °C for 8 h, the reaction mixture was directly purified with flash column chromatography (EtOAc/hexane = 0/1 to 1/4) to afford enamide **S12** (1.13 g, 1.94 mmol, 79%) as a white foam: $[\alpha]_D^{24} = -28$ (*c* = 1.5 , CHCl₃); IR (film) v 3435, 3300, 2969, 2933, 2885, 2858, 1965, 1658, 1501, 1169, 1111, 1057, 703 cm⁻¹, ¹H NMR (400 MHz, CDCl₃) δ 0.94 (3H, t, *J* = 7.7 Hz), 0.96 (3H, t, *J* = 7.7 Hz), 1.04 (9H, s), 1.15 (3H, s), 1.45 (3H, s), 1.45 (9H, s), 1.57 (1H, dq, *J* = 14.7, 7.4 Hz), 1.68 (1H, dq, *J* = 14.8, 7.4 Hz), 1.98 (2H, q, *J* = 7.7 Hz), 3.97 (1H, d, *J* = 9.4 Hz), 4.18 (1H, br), 4.31 (2H, s), 5.52 (1H, d, *J* = 9.1 Hz), 7.31 (1H, br), 7.35–7.45 (6H, m), 7.64–7.68 (4H, m); ¹³C NMR (125 MHz, CDCl₃) δ 8.16, 11.7, 16.2, 19.2, 21.8, 26.5, 26.8, 28.3, 32.6, 58.6, 61.4, 74.0, 80.1, 125.5, 127.7, 127.7, 129.7, 133.3, 135.5, 135.5, 136.3, 156.1, 171.3; HRMS (ESI) calcd for C₃₃H₅₀N₂NaO₅Si [M+Na]⁺ 605.3381, found 605.3360.



Compound S13: To a solution of enamide **S12** (1.10 g, 1.89 mmol) in THF (16 mL) was added TBAF (1 M in THF, 3.77 mL, 3.77 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h, and quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/1 to 9/1) to afford alcohol **S13** (640 mg, 1.86 mmol, 98%) as a white solid: $[\alpha]_D^{24} = -26$ (c = 0.68, CHCl₃); IR (film) v 3437, 3304, 2968, 2932, 2857, 1689, 1657, 1499, 1169, 1111, 1057 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.97 (3H, t, J = 7.4 Hz), 1.00 (3H, t, J = 7.7 Hz), 1.16 (3H, s), 1.46 (9H, s), 1.55–1.64 (1H, m), 1.69 (1H, dq, J = 14.4, 7.0 Hz), 1.83 (3H, s), 2.07 (1H, q, J = 7.7 Hz), 2.07 (1H, q, J = 7.7 Hz), 3.86 (2H, br), 3.96 (1H, d, J = 8.7 Hz), 4.10 (1H, d, J = 12.4 Hz), 4.22 (1H, dd, J = 11.8, 5.7 Hz), 5.56 (1H, d, J = 8.7 Hz), 7.67 (1H, br); ¹³C NMR (125 MHz, CDCl₃) δ 8.0, 11.9, 16.4, 21.6, 26.2, 28.2, 32.5, 58.9, 60.3, 74.0, 80.4, 127.7, 134.6, 156.4, 172.0; HRMS (ESI) calcd for C₁₇H₃₂N₂NaO₅ [M+Na]⁺ 367.2203, found



Compound S14: To a solution of alcohol **S13** (626 mg, 1.82 mmol) in CH₂Cl₂ (36 mL) was added Dess-Martin periodinane (867 mg, 2.04 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 20 min. To the reaction mixture was added saturated aqueous NaHCO₃ and Na₂S₂O₃. The resulting solution was extracted with CH₂Cl₂ (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 3/7 to 1/1) to afford **S14** (485 mg, 1.42 mmol, 78%) as a white foam: $[\alpha]_D^{25} = -45$ (c = 4.7, CHCl₃); IR (film) v 3437, 3304, 2978, 2938, 2881, 1693, 1665, 1504, 1168 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.00 (3H, t, J = 7.7 Hz), 1.10 (3H, t, J = 7.7 Hz), 1.17 (3H, s), 1.46 (9H, s), 1.55–1.70 (2H, m), 2.28 (3H, s), 2.31 (2H, q, J = 8.0 Hz), 3.91 (1H, br), 4.09 (1H, d, J = 8.7 Hz), 5.49 (1H, d, J = 8.0 Hz), 9.91 (1H, s); ¹³C NMR (125 MHz, CDCl₃) δ 8.0, 11.3, 16.2, 21.6, 28.3, 28.9, 32.3, 59.6, 74.1, 80.1, 129.7, 156.1, 161.1, 171.6, 186.3; HRMS (ESI) calcd for C₁₇H₃₀N₂NaO₅ [M+Na]⁺ 365.2047, found 365.2048.



Compound S16: To a solution of aldehyde **S14** (392 mg, 1.15 mmol) in *t*-BuOH/H₂O (1/1, 20 mL) were added 2-methyl-2-butene (10 mL, 94 mmol), NaH₂PO₄·2H₂O (1.61 g, 10.4 mmol) and NaClO₂ (780 mg, 6.90 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. To the reaction mixture were added saturated aqueous NH₄Cl and citric acid (5 g). The resulting solution was extracted with EtOAc (twice). The combined organic layers were dried over anhydrous MgSO₄ and filtered. The solution was concentrated to afford the crude carboxylic acid **S15**, which was used in the next reaction without further purification.

To a solution of the above **S15** in DMF (12 mL) were added K₂CO₃ (207 mg, 1.50 mmol) and allyl bromide (0.119 mL, 1.38 mmol) at 0 °C. After being stirred at 60 °C, the reaction mixture was quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/4 to 3/7) to afford allyl ester **S16** (355 mg, 0.892 mmol, 78% for 2 steps) as a white foam: $[\alpha]_D^{25} = -45$ (c = 1.1, CHCl₃); IR (film) v 3441, 3297, 2976, 2938, 2881, 1717, 1664, 1502, 1168 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 0.84 (3H, t, J = 7.2 Hz), 0.95 (3H, t, J = 7.6 Hz), 1.04 (3H, s), 1.39 (9H, s), 1.39–1.44 (1H, m), 1,49 (1H, dd, J = 14.3, 7.6 Hz), 1.96 (3H, s), 2.15 (2H, m), 4.05 (1H, d, J = 9.7 Hz), 4.47–4.55 (2H, m), 4.56 (1H, br), 5.16 (1H, dd, J = 10.5, 1.3 Hz), 5.29 (1H, dd, J = 17.7, 1.7 Hz), 5.87 (1H, ddt, J = 17.7, 10.5,

5.5 Hz), 6.51 (1H, d, J = 9.7 Hz), 9.02 (1H, br); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 7.6, 11.5, 17.7, 22.5, 27.2, 28.1, 31.5, 60.4, 64.5, 73.0, 78.2, 117.6, 121.6, 132.6, 145.1, 155.3, 164.2, 170.1; HRMS (ESI) calcd for C₂₀H₃₀N₂NaO₆ [M+Na]⁺ 421.2309, found 421.2309.



Compound S17: To a solution of allyl ester S16 (69.0 mg, 0.173 mmol) in CH₂Cl₂ (1.6 mL) were added Et₃N (0.021 mL, 0.15 mmol), DMAP (5.06 mg, 0.0414 mmol) and Boc₂O (0.035 mL, 0.15 mmol) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was quenched with saturated aqueous NH₄Cl. The resulting solution was extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous MgSO₄ and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/20 to 3/7) to afford allyl ester **S17** (57.8 mg, 0.116 mmol, 67%) as a white foam: $[\alpha]_D^{25} = -41$ (c = 0.52, CHCl₃); IR (film) v 3494, 3445, 2978, 2934, 2882, 1746, 1718, 1492, 1368, 1153 cm⁻¹, ¹H NMR (500 MHz, benzene-d₆) (2:5 mixture of rotamers) δ 0.87 (0.9H, t, J = 7.6 Hz), 0.90 (2.1 H, t, J = 7.6 Hz), 1.10 (3H, t, J = 7.6 Hz), 1.20 (0.9H, s), 1.23 (2.1H, s), 1.36 (9H, s), 1.39 (9H, s), 1.71–1.90 (2H, m), 2.01–2.20 (2H, m), 2.18 (2.1H, s), 2.23 (0.9H, s), 3.76 (0.3H, s), 3.82 (0.7H, s), 4.40 (1.4H, d, *J* = 5.9 Hz), 4.44 (0.3H, dd, *J* = 13.9, 4.6 Hz), 4.53 (0.3H, dd, J = 13.9, 5.1 Hz), 4.92 (0.7H, dd, J = 10.5, 1.3 Hz), 5.03 (0.3H, d, J = 11.7 Hz), 5.06 (0.7H, dd, J = 17.7, 1.7 Hz), 5.20 (0.3H, d, J = 17.2 Hz), 5.58 (0.3H, d, J = 10.1 Hz), 5.65 (1H, ddt, J = 17.2, 10.5, 5.9 Hz), 5.75 (0.7H, d, J = 7.6 Hz), 6.23 (0.3H, d, J = 11.8 Hz), 6.26 (0.7H, d, J = 8.4 Hz); ¹³C NMR (125 MHz, benzene- d_6 (mixture of rotamers) δ 7.6, 7.8, 11.2, 18.8, 19.0, 20.8, 21.5, 27.6, 27.8, 28.4, 29.2, 29.4, 30.2, 32.9, 34.1, 58.6, 58.9, 64.9, 65.7, 75.0, 75.6, 77.7, 79.3, 83.8, 84.2, 117.2, 118.1, 123.1, 123.4, 132.3, 132.6, 152.9, 155.8, 156.9, 163.1, 164.2, 176.8; HRMS (ESI) calcd for C₂₅H₄₂N₂NaO₈ [M+Na]⁺ 521.2833, found 521.2819.



Compound 4c: To a solution of allyl ester **S17** (50.9 mg, 0.102 mmol) in THF (1 mL) were added morpholine (0.45 mL, 5.2 mmol), 2-methyl-2-butene (0.11 mL, 1.0 mmol) and Pd(PPh₃)₄ (6.0 mg, 0.0052 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 2.5 h. The reaction mixture was treated with 1 M aqueous HCl, and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (1st: CHCl₃/MeOH/AcOH = 200/1/1, 2nd: CHCl₃/MeOH = 500/1) to afford carboxylic acid **4c** and **4c'** (3:1 mixture, 40.1 mg, 0.0875 mmol, 85%) as a white foam: HRMS (ESI) calcd for C₂₂H₃₈N₂NaO₈ [M+Na]⁺ 481.2520, found 481.2532.

Compounds 4c and 4c' were used in the next reaction as a mixture of isomers.

Synthesis of compound 5b



Compound S20: To a solution of amide **S18** (177 mg, 0.942 mmol) and *E*-vinyl iodide **S19** [CAS 1628569-55-0]³ (303 mg, 1.13 mmol) in dioxane (0.9 mL) were added Cs₂CO₃ (369 mg, 1.13 mmol), copper iodide (53.6 mg, 0.281 mmol) and *N*,*N'*-dimethylethylenediamine (0.20 mL, 1.9 mmol) at room temperature. After being stirred at 70 °C for 5 h, the reaction mixture was filtered through a pad of silica gel. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane= 3/7 to 3/2) to afford enamide **S20** (248 mg, 0.755 mmol, *E*:*Z* = 10:1, 80%) as a white solid: ¹H NMR spectrum was identical with that of *ent*-**S20** [CAS 1628569-56-1].³



Compound S22: To a stirred solution of **S20** (196 mg, 0.596 mmol) in EtOH (4.5 mL) was added LiOH·H₂O (250 mg, 5.96 mmol) in H₂O (1.5 mL) at room temperature. The resulting mixture was stirred at 40 °C for 6 h. The reaction mixture was diluted with EtOAc, acidified with 1 M aqueous HCl, extracted with EtOAc (3 times). The combined organic layers were washed with brine (3 times), dried over anhydrous MgSO₄ and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/9) to afford the crude peptide **S21**, which was used in the next reaction without further purification.

To a solution of the above **S21** in DMF (6 mL) were added K_2CO_3 (100 mg, 0.724 mmol) and allyl bromide (62 µL, 0.72 mmol) at room temperature. After being stirred at 60 °C for 1 h, the reaction mixture was quenched with saturated aqueous NH₄Cl at 0 °C. The resulting solution was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 1/9 to 1/4) to afford allyl ester **S22** (157 mg, 0.462 mmol, 77% for 2 steps) as a white foam: ¹H NMR spectrum was identical with that of *ent-***S22** [CAS 1801625-90-0].⁴



Compound S23: To a solution of allyl ester **S22** (157 mg, 0.462 mmol) in CH₂Cl₂ (4.6 mL) were added Et₃N (72 μ L, 0.52 mmol), DMAP (18.1 mg, 0.148 mmol), and Boc₂O (105 μ L, 0.457 mmol) at 0 °C. After being stirred at 0 °C for 2 h, the reaction mixture was quenched with saturated aqueous NH₄Cl. The

resulting solution was extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄ and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (EtOAc/hexane = 0/1 to 1/1) to afford allyl ester **S23** (146 mg, 0.331 mmol, 72%) as a white foam: ¹H NMR spectrum was identical with that of *ent*-**S23** [CAS 1801625-91-1].⁴



Compound 5b: To a solution of allyl ester **S23** (146 mg, 0.331 mmol) in THF (3.3 mL) were added morpholine (1.5 mL, 17 mmol), 2-methyl-2-butene (0.35 mL, 3.3 mmol) and Pd(PPh₃)₄ (18.4 mg, 0.0159 mmol) at 0 °C. The reaction mixture was stirred at the same temperature for 1.5 h. The reaction mixture was acidified with saturated aqueous NH₄Cl and 1 M aqueous HCl (pH ~3), and then extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated, and the residue was purified with flash column chromatography (1st: MeOH/CHCl₃ = 0/1 to 1/50, 2nd: MeOH/CHCl₃/toluene = 0/100/1 to 4/100/1) to afford carboxylic acid **5b** (77.7mg, 0.194 mmol, 59%) as a white foam: $[\alpha]_D^{24} = 30$ (c = 0.09, CHCl₃); ¹H NMR spectrum was identical with that of **5a** [CAS 1801625-39-7].⁴

5a: $[\alpha]_D^{17} = -25$ (*c* 0.16, CHCl₃).

Syntheses of compounds 2e-2r

2e:



Peptide **14a** was synthesized according to the reported procedure.²

To a solution of peptide **14a** (5.1 mg, 0.0044 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.3 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (4.4 mg, 0.011 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (4.6 μ L, 0.027 mmol), PyBOP (5.5 mg, 0.011 mmol) and HOAt (1.5 mg, 0.011 mmol) at 0 °C. After 3 h at room temperature, *i*-Pr₂NEt (4.6 μ L, 0.027 mmol) was added to the reaction mixture. After being stirred for 1.5 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated, and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/24) to afford the crude peptide **15a**, which was used in the next reaction without further purification.

To a solution of 15a in CH₂Cl₂ (0.5 mL) was added TFA (0.5 mL). After being stirred at room temperature

for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4b** (5.1 mg, 0.011 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (6.2 μ L, 0.035 mmol), PyBOP (5.6 mg, 0.011 mmol) and HOAt (1.5 mg, 0.011 mmol) at 0 °C. After being stirred for 13 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and azeotroped with toluene (twice). The residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 3/97) to afford the crude peptide **16b**, which was used in the next reaction without further purification.

To a solution of **16b** in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3b** (4.9 mg, 0.025 mmol) in DMF (0.15 mL) were added 2,4,6-collidine (7.6 μ L, 0.058 mmol) and COMU (10.5 mg, 0.025 mmol) at 0 °C. After being stirred for 3.5 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and azeotroped with toluene (twice). The residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/24) to afford the crude peptide **2e**.

The crude peptide **2e** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 40 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2e** ($t_R = 16.5$ min, 1.22 mg, 0.000737 mmol, 17% from **14a**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0390.

2f:



To a solution of **14a** (3.9 mg, 0.0034 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was cooled to 0 °C, diluted with CH_2Cl_2 and brine, quenched with 3 M aqueous NaOH, and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (2.5 mg, 0.0062 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (2.4 μ L, 0.014 mmol), PyBOP (4.6 mg, 0.0088 mmol) and HOAt (1.4 mg, 0.010 mmol) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was cooled to 0 °C, diluted with EtOAc, quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography

(MeOH/CHCl₃ = 1/199 to 1/33 containing 0.5% Et₃N) to afford the crude peptide **15a**, which was used in the next reaction without further purification.

To a solution of **15a** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 3 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4c** (4.3 mg, 0.0094 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (2.4 μ L, 0.014 mmol), PyBOP (3.7 mg, 0.0071 mmol) and HOAt (2.0 mg, 0.015 mmol) at 0 °C. After 2 h at room temperature, **4c** (1.5 mg, 0.0033 mmol), *i*-Pr₂NEt (0.6 μ L, 0.0035 mmol) and PyBOP (3.8 mg, 0.0073 mmol) were added to the reaction mixture at 0 °C. After being stirred for 11 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was purified with flash column chromatography (1st: MeOH/CHCl₃ = 1/124 to 1/49 containing 0.5% Et₃N, 2nd: MeOH/CHCl₃ = 1/199 to 1/49 containing 0.5% Et₃N) to afford peptide **16c** (2.1 mg, 0.0013 mmol, 38% from **14a**) as a white solid.

To a solution of **16c** (0.90 mg, 0.00054 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (1.0 mg, 0.0050 mmol) in DMF (0.1 mL) were added 2,4,6-collidine (1.2 μ L, 0.0091 mmol) and COMU (2.4 mg, 0.0056 mmol) at 0 °C. After being stirred for 11 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and azeotroped with toluene to afford the crude peptide **2f**.

The crude peptide **2f** was then purified by reversed-phase HPLC (column: 5-phenylhexyl 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 35% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford **2f** ($t_R = 20.6 \text{ min}$, 0.365 mg, 0.000221 mmol, 41% from **16c**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0390.

fragment 14b



Peptide **11a** was synthesized according to the reported procedure.²

To a solution of **11a** (69.0 mg, 0.0747 mmol) in CH_2Cl_2 (1 mL) was added TFA (1 mL). After completion of the reaction, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid 8a (52.3 mg, 0.224 mmol) in DMF (0.7 mL)

were added 2,4,6-collidine (86 μ L, 0.67 mmol) and COMU (95.9 mg, 0.224 mmol). After being stirred for 11 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/9 containing 0.3% Et₃N) to afford the crude peptide **12a**, which was used in the next reaction without further purification.

To a solution of 12a in CH₂Cl₂ (1 mL) was added TFA (1 mL). After being stirred for 1.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7a** (51.8 mg, 0.224 mmol) in DMF (0.7 mL) were added 2,4,6-collidine (86 μ L, 0.67 mmol) and COMU (95.9 mg, 0.224 mmol) at 0 °C. After being stirred at room temperature, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/33) to afford the crude peptide **13a**, which was used in the next reaction without further purification.

To a solution of **13a** in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6b** (48.7 mg, 0.224 mmol) in DMF (0.7 mL) were added 2,4,6-collidine (86 μ L, 0.65 mmol) and COMU (95.9 mg, 0.224 mmol) at 0 °C. After being stirred at room temperature for 14 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 0/1 to 3/197) to afford peptide **14b** (76.3 mg, 0.0663 mmol, 89% from **11a**) as a white solid.

2g:



To a solution of **14b** (5.0 mg, 0.0043 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL). After being stirred for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (3.5 mg, 0.0087 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (4.5 μ L, 0.026 mmol), PyBOP (4.4 mg, 0.0087 mmol) and HOAt (1.2 mg, 0.0087 mmol) at 0 ° C. After being stirred at room temperature for 3 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was

roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/24) to afford the crude peptide **15b**, which was used in the next reaction without further purification.

To a solution of **15b** in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (2.1 mg, 0.0046 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (2.4 μ L, 0.014 mmol), PyBOP (2.4 mg, 0.0046 mmol) and HOAt (0.6 mg, 0.0044 mmol) at 0 °C. After being stirred at room temperature for 4 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/141 to 1/33) to afford the crude peptide **16d**, which was used in the next reaction without further purification.

To a solution of a nine-tenth of above **16d** in CH_2Cl_2 (0.8 mL) was added TFA (0.8 mL) at 0 °C. After being stirred for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (3.8 mg, 0.019 mmol) in DMF (0.15 mL) was added 2,4,6-collidine (7.5 μ L, 0.057 mmol) and COMU (8.1 mg, 0.019 mmol) at 0 °C. After being stirred for 3 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/141 to 1/24) to afford the crude peptide **2g**.

The crude peptide **2g** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 40 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2g** (t_R = 34.5 min, 1.08 mg, 0.000652 mmol, 15% from **14b**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0409.

2h:



To a solution of **14b** (4.1 mg, 0.0036 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (2.7 mg, 0.0067 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (3.6 μ L, 0.021 mmol), PyBOP (4.2 mg, 0.0081 mmol) and HOAt (1.8 mg, 0.013 mmol) at 0 °C. After 2.5 h at room temperature, *i*-Pr₂NEt (3.2 μ L, 0.018 mmol) and PyBOP (2.0 mg, 0.0038 mmol) were added to the reaction mixture. After being stirred at room temperature for 0.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ and extracted with

t-BuOH/EtOAc (1/10). The organic layer was washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **15b**, which was used in the next reaction without further purification.

To a solution of **15b** in CH₂Cl₂ (0.2 mL) was added TFA (0.2 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4b** (6.0 mg, 0.013 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (5.0 μ L, 0.029 mmol), PyBOP (8.5 mg, 0.016 mmol) and HOAt (2.5 mg, 0.018 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16e**, which was used in the next reaction without further purification.

To a solution of **16e** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3b** (4.9 mg, 0.024 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (5.6 μ L, 0.042 mmol) and COMU (10.0 mg, 0.023 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous MgSO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/49) to afford the crude peptide **2h**.

The crude peptide **2h** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford **2h** ($t_R = 17.9$ min, 0.798 mg, 0.000482 mmol, 13% from **14b**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0402.

2i:



Peptide **10a** was synthesized according to the reported procedure.²

To a solution of **10a** (40.2 mg, 0.0674 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid 9^2 (18.1 mg, 0.0407 mmol) in DMF (0.4 mL) were added *i*-Pr₂NEt (15 µL, 0.086 mmol), PyBOP (24.4 mg, 0.0469 mmol) and HOAt (7.7 mg, 0.057 mmol) at 0 °C. After 2 h at room temperature, *i*-Pr₂NEt (15 µL, 0.086 mmol) was added to the reaction mixture. After 0.5 h, *i*-Pr₂NEt (15 µL, 0.086 mmol) was added to the reaction mixture. After being stirred for 22 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **11a**, which was used in the next reaction without further purification.

To the solution of **11a** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of a one-sixth of above crude amine and **8a** (5.7 mg, 0.024 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (14.0 μ L, 0.106 mmol) and COMU (9.9 mg, 0.023 mmol)) at 0 °C. After being stirred for 3.5 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **12a**, which was used in the next reaction without further purification.

To a solution of **12a** in CH₂Cl₂ (0.5 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7b** (4.5 mg, 0.019 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (7.0 μ L, 0.053 mmol) and COMU (7.0 mg, 0.016 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **13b**, which was used in the next reaction without further purification.

To a solution of **13b** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6a** (1.9 mg, 0.0087 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (3.0 μ L, 0.023 mmol) and COMU (3.8 mg, 0.0089 mmol) at 0 °C. After being stirred for 11 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/19) to afford the crude peptide **14c**, which was used in the next reaction without further purification.

To a solution of 14c in CH₂Cl₂ (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room

temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (1.6 mg, 0.0039 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (1.2 μ L, 0.0069 mmol), PyBOP (1.7 mg, 0.0033 mmol) and HOAt (1.8 mg, 0.013 mmol) at 0 °C. After 1 h at room temperature, *i*-Pr₂NEt (4.0 μ L, 0.023 mmol) was added to the reaction mixture. After being stirred for 1 h, the reaction mixture was treated with CH₂Cl₂ (0.5 mL) and TFA (3 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was cooled to 0 °C, diluted with CH₂Cl₂ and brine, quenched with 3 M aqueous NaOH (14 mL), and extracted with *t*-BuOH/ Et₂O (1/10, 3 times). The combined organic layers were dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/4) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (1.1 mg, 0.0024 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (5.0 μ L, 0.029 mmol), PyBOP (1.7 mg, 0.0033 mmol) and HOAt (1.7 mg, 0.012 mmol) at 0 °C. After being stirred for 13 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16f**, which was used in the next reaction without further purification.

To a solution of **16f** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (1.4 mg, 0.0070 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (2.5 μ L, 0.019 mmol) and COMU (4.2 mg, 0.0098 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated to afford the crude peptide **2i**.

The crude peptide **2i** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2i** ($t_R = 19.5$ min, 0.536 mg, 0.000324 mmol, 4.8% from **9**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0405.

fragment 14d:



To a solution of **10a** (282 mg, 0.473 mmol) in CH_2Cl_2 (6 mL) was added TFA (2 mL). After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc and brine, quenched with 3 M

aqueous NaOH, and extracted with EtOAc (10 times). The combined organic layers were dried over anhydrous Na_2SO_4 , and filtered. The solution was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **9** (200 mg, 0.450 mmol) in DMF (4.5 mL) were added *i*-Pr₂NEt (0.16 mL, 0.919 mmol), PyBOP (260 mg, 0.500 mmol) and HOAt (66.6 mg, 0.489 mmol)) at 0 °C. The reaction mixture was stirred at room temperature. After completion of the reaction, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/EtOAc = 1/199 containing 0.5% Et₃N) to afford the crude peptide **11a** (247 mg, 0.268 mmol, 60% from **9**).

To a solution of **11a** (24.0 mg, 0.0260 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **8b** (19.9 mg, 0.085 mmol) in DMF (1 mL) were added 2,4,6-collidine (30.0 μ L, 0.228 mmol) and COMU (34.5 mg, 0.0806 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **12b**, which was used in the next reaction without further purification.

To a solution of **12b** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7a** (17.1 mg, 0.0739 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (30.0 μ L, 0.228 mmol) and COMU (34.8 mg, 0.0813 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **13c**, which was used in the next reaction without further purification.

To a solution of **13c** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6a** (15.7 mg, 0.0723 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (30.0 μ L, 0.228 mmol) and COMU (31.1 mg, 0.0726 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **14d** (22.5 mg, 0.0196

mmol, 75% from 11a).



To a solution of **14d** (6.6 mg, 0.0057 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (6.3 mg, 0.016 mmol) in DMF (0.4 mL) were added *i*-Pr₂NEt (6.0 μ L, 0.035 mmol), PyBOP (8.4 mg, 0.016 mmol) and HOAt (2.3 mg, 0.017 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and then treated with CH₂Cl₂ (0.5 mL) and TFA (2.5 mL) at 0 °C. After being stirred at room temperature for 7 h, the reaction mixture was cooled to 0 °C, diluted with brine, quenched with 3 M aqueous NaOH (11 mL), and extracted with *t*-BuOH/Et₂O (1/10, 3 times). The combined organic layers were dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (5.5 mg, 0.012 mmol) in DMF (0.4 mL) were added *i*-Pr₂NEt (5.0 μ L, 0.029 mmol), PyBOP (7.5 mg, 0.014 mmol) and HOAt (2.1 mg, 0.015 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16g**, which was used in the next reaction without further purification.

To a solution of **16g** (4.8 mg, 0.0029 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (4.0 mg, 0.020 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (7.0 μ L, 0.053 mmol) and COMU (10.7 mg, 0.0250 mmol) at 0 °C. After being stirred for 13 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **2j**.

The crude peptide **2j** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford **2j** ($t_R = 16.7 \text{ min}$, 0.705 mg, 0.000426 mmol, 7.5% from **14d**) as

a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0418.



To a solution of **14d** (4.7 mg, 0.0041 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (2.6 mg, 0.0065 mmol) in DMF (0.5 mL) were added *i*-Pr₂NEt (3.0 μ L, 0.017 mmol), PyBOP (4.2 mg, 0.0081 mmol) and HOAt (0.8 mg, 0.0059 mmol) at 0 °C. After 1 h at room temperature, *i*-Pr₂NEt (2.8 μ L, 0.016 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 1 h, and then treated with CH₂Cl₂ (0.5 mL) and TFA (2.5 mL) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was cooled to 0 °C, diluted with CH₂Cl₂ and brine, quenched with 3 M aqueous NaOH (11 mL), and extracted with *t*-BuOH/Et₂O (1/10, 3 times). The combined organic layers were dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4b** (4.2 mg, 0.0092 mmol) in DMF (0.5 mL) were added *i*-Pr₂NEt (4.0 μ L, 0.023 mmol), PyBOP (5.6 mg, 0.011 mmol) and HOAt (1.8 mg, 0.013 mmol) at 0 °C. After being stirred for 11 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16h**, which was used in the next reaction without further purification.

To a solution of **16h** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3b** (2.9 mg, 0.014 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (4.0 μ L, 0.030 mmol) and COMU (6.4 mg, 0.015 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **2k**.

The crude peptide **2k** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2k** (t_R = 12.5 min, 0.907 mg, 0.000548 mmol, 13% from **14d**) as a white

solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0390.

fragment 14e:



Peptide **10b** was synthesized according to the reported procedure.⁴

To a solution of **10b** (147 mg, 0.246 mmol) in CH_2Cl_2 (2 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with CH_2Cl_2 and brine, quenched with 3 M aqueous NaOH, and extracted with *t*-BuOH/EtOAc (1/10, 5 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **9** (91.1 mg, 0.205 mmol) in DMF (1.3 mL) were added *i*-Pr₂NEt (101 μ L, 0.580 mmol), PyBOP (112 mg, 0.215 mmol), HOAt (29.3 mg, 0.215 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and azeotroped with toluene. The residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/332 to 1/49 containing 0.3% Et₃N) to afford the crude peptide **11b**, which was used in the next reaction without further purification.

To a solution of **11b** in CH_2Cl_2 (1 mL) was added TFA (1 mL). After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **8a** (124 mg, 0.531 mmol) in DMF (1 mL) were added 2,4,6-collidine (210 μ L, 1.59 mmol) and COMU (227 mg, 0.531 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/19 containing 0.3% Et₃N) to afford the crude peptide **12c**, which was used in the next reaction without further purification.

To a solution of 12c in CH₂Cl₂ (1 mL) was added TFA (1 mL). After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7a** (123 mg, 0.531 mmol) in DMF (1 mL) were added 2,4,6-collidine (210 μ L, 1.59 mmol) and COMU (227 mg, 0.531 mmol) at 0 °C. After being stirred at room temperature for 11 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 3/497 to 1/33 containing 0.5% Et₃N) to afford the crude

peptide 13d, which was used in the next reaction without further purification.

To a solution of **13d** in CH_2Cl_2 (1 mL) was added TFA (1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6a** (115 mg, 0.531 mmol) in DMF (1 mL) were added 2,4,6-collidine (210 μ L, 1.59 mmol) and COMU (227 mg, 0.531 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/58 containing 0.4% Et₃N) to afford the crude peptide **14e** (139 mg, 0.121 mmol, 59% from **9**).

2l:

To a solution of **14e** (5.0 mg, 0.0043 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was diluted with CH_2Cl_2 and brine, quenched with 3 M aqueous NaOH (1 mL) at 0 °C, and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (3.6 mg, 0.0090 mmol) in DMF (0.2 mL) was added *i*-Pr₂NEt (3.1 μ L, 0.018 mmol), PyBOP (4.6 mg, 0.0088 mmol) and HOAt (1.3 mg, 0.0096 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/24) to afford the crude peptide **15e**, which was used in the next reaction without further purification.

To a solution of **15e** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (3.4 mg, 0.0074 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (2.8 μ L, 0.016 mmol), PyBOP (5.1 mg, 0.0098 mmol) and HOAt (2.1 mg, 0.015 mmol) at 0 °C. After 3.5 h at room temperature, **4a** (3.7 mg, 0.0081 mmol), *i*-Pr₂NEt (1.3 μ L, 0.0075 mmol) and PyBOP (3.1 mg, 0.0060 mmol) were added to the reaction mixture. After being stirred for 12 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried

over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (1st: MeOH/EtOAc = 1/99 to 1/4 containing 0.5% Et₃N, 2nd: MeOH/CHCl₃ = 1/99 to 1/4 containing 0.5% Et₃N) to afford the crude peptide **16i**, which was used in the next reaction without further purification.

To a solution of **16i** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated, azeotroped with toluene (4 times) and dried *in vacuo* over night to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (3.8 mg, 0.019 mmol) in DMF (0.1 mL) was added 2,4,6-collidine (4.0 μ L, 0.030 mmol) and COMU (7.3 mg, 0.017 mmol) at 0 °C. After being stirred for 3 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (1st: MeOH/EtOAc = 1/199 to 1/4 containing 0.5% Et₃N, 2nd: MeOH/EtOAc = 0/1 to 1/9 containing 0.5% Et₃N) to afford the crude peptide **2l**.

The crude peptide **2l** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2l** ($t_R = 25.3 \text{ min}$, 0.513 mg, 0.000310 mmol, 7.2% from **14e**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅₂NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0427.

2m:

To a solution of **14e** (4.8 mg, 0.0042 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (3.3 mg, 0.0083 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (4.4 μ L, 0.025 mmol), PyBOP (4.2 mg, 0.0083 mmol) and HOAt (1.1 mg, 0.0083 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/124 to 1/24 containing 0.5% Et₃N) to afford the crude peptide **15e**, which was used in the next reaction without further purification.

To a solution of **15e** in CH₂Cl₂ (0.5 mL) was added TFA (0.5 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (twice) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4b** (4.5 mg, 0.0098 mmol) in DMF (0.15 mL) were added *i*-Pr₂NEt (6.2 μ L, 0.036 mmol), PyBOP (5.9 mg, 0.012 mmol) and HOAt (1.5 mg, 0.011 mmol)

at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with EtOAc (twice). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and azeotroped with toluene. The residue was purified with flash column chromatography (MeOH/CHCl₃ = 1/124 to 1/49 containing 0.5% Et₃N) to afford peptide **16j** (4.7 mg, 0.0028 mmol, 67% from **14e**).

To a solution of **16j** (2.7 mg, 0.0016 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3b** (2.4 mg, 0.012 mmol) in DMF (0.1 mL) were added 2,4,6-collidine (3.4 μ L, 0.026 mmol) and COMU (5.74 mg, 0.0134 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **2m**.

The crude peptide **2m** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 20 mm × 250 mm; column oven: 45 °C; flow rate: 4.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2m** ($t_R = 27.1 \text{ min}$, 0.685 mg, 0.000414 mmol, 26% from **16j**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅2NaO₁₈ [M+2Na]²⁺ 850.0417, found 850.0399.

2n:



To a solution of **14e** (4.5 mg, 0.0039 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5b** (4.4 mg, 0.011 mmol) in DMF (0.2 mL) was added *i*-Pr₂NEt (5.0 μ L, 0.029 mmol), PyBOP (6.2 mg, 0.012 mmol) and HOAt (2.2 mg, 0.016 mmol) at 0 °C. After being stirred for 3 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **15f**, which was used in the next reaction without further purification.

To a solution of **15f** in CH_2Cl_2 (0.2 mL) was added TFA (0.2 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid 4a (3.1 mg, 0.0068 mmol) in DMF (0.2 mL)

were added *i*-Pr₂NEt (4.0 μ L, 0.023 mmol), PyBOP (3.6 mg, 0.0069 mmol) and HOAt (1.4 mg, 0.010 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16k**, which was used in the next reaction without further purification.

To a solution of **16k** in CH₂Cl₂ (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (3.2 mg, 0.016 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (6.5 μ L, 0.049 mmol) and COMU (10.0 mg, 0.023 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **2n**.

The crude peptide **2n** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford the crude **2n**.

The above crude **2n** was further purified by reversed-phase HPLC (column: Cosmosil π -NAP 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 50% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford **2n** ($t_R = 13.9$ min, 1.33 mg, 0.000804 mmol, 21% from **14e**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0432.

fragment 14f:



To a solution of **10b** (79.9 mg, 0.134 mmol) in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was diluted with brine, quenched with 3 M aqueous NaOH (1.5 mL) at 0 °C, and extracted with *t*-BuOH/EtOAc (1/10, 5 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **9** (50.0 mg, 0.112 mmol) in DMF (0.8 mL) were added *i*-Pr₂NEt (40 μ L, 0.23 mmol), PyBOP (59.5 mg, 0.114 mmol) and HOAt (15.2 mg, 0.112 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was

roughly purified with flash column chromatography (1st: MeOH/CHCl₃ = 1/333 to 1/19, 2nd: MeOH/CHCl₃ = 1/99 to 1/24) to afford the crude peptide **11b**, which was used in the next reaction without further purification.

To a solution of **11b** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **8a** (55.7 mg, 0.239 mmol) in DMF (1 mL) were added 2,4,6-collidine (100 μ L, 0.759 mmol) and COMU (103 mg, 0.241 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 5 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/19) to afford the crude peptide **12c**, which was used in the next reaction without further purification.

To a solution of **12c** in CH₂Cl₂ (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7a** (42.2 mg, 0.182 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (70 μ L, 0.531 mmol) and COMU (75.7 mg, 0.177 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/19) to afford the crude peptide **13d**, which was used in the next reaction without further purification.

To a solution of **13d** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6b** (39.0 mg, 0.180 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (66 μ L, 0.501 mmol) and COMU (75.2 mg, 0.176 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/199 to 1/33) to afford the crude peptide **14f** (52.2 mg, 0.0454 mmol, 41% from **9**) as a white solid.

2o:

To a solution of **14f** (6.0 mg, 0.0052 mmol) in CH_2Cl_2 (0.5 mL) was added TFA (0.25 mL) at 0 °C. After

being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (4.2 mg, 0.010 mmol) in DMF (0.5 mL) were added *i*-Pr₂NEt (6.8 μ L, 0.039 mmol), PyBOP (6.3 mg, 0.012 mmol) and HOAt (1.4 mg, 0.010 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/19) to afford the crude peptide **15g**, which was used in the next reaction without further purification.

To a solution of **15g** in CH₂Cl₂ (0.4 mL) was added TFA (0.4 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (4.7 mg, 0.010 mmol) in DMF (0.5 mL) were added *i*-Pr₂NEt (5.6 μ L, 0.032 mmol), PyBOP (5.3 mg, 0.010 mmol) and HOAt (2.0 mg, 0.015 mmol) at 0 °C. After 1.5 h at room temperature, **4a** (3.8 mg, 0.0083 mmol) and PyBOP (4.8 mg, 0.0092 mmol) were added to the reaction mixture. After being stirred at room temperature for 0.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/19) to afford the crude peptide **16l**, which was used in the next reaction without further purification.

To a solution of **161** in CH_2Cl_2 (0.4 mL) was added TFA (0.4 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (5.4 mg, 0.027 mmol) in DMF (0.4 mL) were added 2,4,6-collidine (9.0 μ L, 0.068 mmol) and COMU (14.3 mg, 0.0334 mmol) at 0 °C. After being stirred at room temperature for 2 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/EtOAc = 0/1 to 1/4) to afford the crude peptide **2o**.

The crude peptide **20** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **20** ($t_R = 29.0$ min, 1.45 mg, 0.000876 mmol, 17% from **14f**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0390.

2p:

To a solution of **14f** (5.5 mg, 0.0048 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (3.6 mg, 0.0090 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (5.0 μ L, 0.029 mmol), PyBOP (8.2 mg, 0.016 mmol) and HOAt (1.3 mg, 0.0096 mmol) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **15g**, which was used in the next reaction without further purification.

To a solution of **15g** in CH_2Cl_2 (0.2 mL) was added TFA (0.2 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4b** (6.3 mg, 0.014 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (8.5 μ L, 0.049 mmol), PyBOP (7.2 mg, 0.014 mmol) and HOAt (2.1 mg, 0.015 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16m**, which was used in the next reaction without further purification.

To a solution of **16m** in CH_2Cl_2 (0.2 mL) was added TFA (0.2 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3b** (3.5 mg, 0.017 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (4.6 μ L, 0.035 mmol) and COMU (8.0 mg, 0.019 mmol) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (1st: MeOH/CHCl₃ = 0/1 to 1/4, 2nd: MeOH/CHCl₃ = 1/99 to 1/49) to afford the crude peptide **2p**.

The crude peptide **2p** was then purified by reversed-phase HPLC (column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2p** (t_R = 27.5 min, 2.76 mg, 0.00167 mmol, 35% from **14f**) as a white

solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0423.



To a solution of **14f** (6.9 mg, 0.0060 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (5.3 mg, 0.013 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (5.2 μ L, 0.030 mmol), PyBOP (8.5 mg, 0.016 mmol) and HOAt (2.9 mg, 0.021 mmol) at 0 °C. After 2 h at room temperature, **5a** (2.0 mg, 0.0050 mmol), *i*-Pr₂NEt (2.6 μ L, 0.015 mmol), PyBOP (3.0 mg, 0.0058 mmol), HOAt (1.6 mg, 0.012 mmol) were added to the reaction mixture. After being stirred for 0.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **15g**, which was used in the next reaction without further purification.

To a solution of **15g** in CH₂Cl₂ (0.2 mL) was added TFA (0.2 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4c** (7.5 mg, 0.016 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (6.4 μ L, 0.037 mmol), PyBOP (8.2 mg, 0.016 mmol) and HOAt (2.6 mg, 0.019 mmol) at 0 °C. After being stirred for 12 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **16n**, which was used in the next reaction without further purification.

To a solution of **16n** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (6.7 mg, 0.033 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (10 μ L, 0.075 mmol) and COMU (18.6 mg, 0.043 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (1st: MeOH/EtOAc = 0/1 to 1/9, 2nd: MeOH/CHCl₃ = 0/1 to 1/4) to afford the crude peptide **2q**.

The crude peptide 2q was then purified by reversed-phase HPLC (column: Phenomenex 5-phenylhexyl

4.6 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 35% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford **2q** ($t_R = 27.5$ min, 1.59 mg, 0.000961 mmol, 16% from **14f**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₇N₁₅Na₂O₁₈ [M+2Na]²⁺ 850.0417, found 850.0425.

2r:



To the solution of **10b** (52.0 mg, 0.0871 mmol) in CH_2Cl_2 (0.6 mL) was added TFA (0.15 mL) at 0 °C. After being stirred at room temperature for 1.5 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **9** (41.6 mg, 0.0936 mmol) in DMF (0.6 mL) were added *i*-Pr₂NEt (80 μ L, 0.46 mmol), PyBOP (47.2 mg, 0.091 mmol) and HOAt (12.8 mg, 0.094 mmol) at 0 °C. After being stirred for 4 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **11b**, which was used in the next reaction without further purification.

To a solution of **11b** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 2.5 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **8b** (65 mg, 0.28 mmol) in DMF (1 mL) were added 2,4,6-collidine (100 μ L, 0.76 mmol) and COMU (124 mg, 0.29 mmol) at 0 °C. After being stirred for 2 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **12d**, which was used in the next reaction without further purification.

To a solution of **12d** in CH₂Cl₂ (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **7a** (51.9 mg, 0.22 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (100 μ L, 0.76 mmol) and COMU (112 mg, 0.262 mmol) at 0 °C. After being stirred for 1.5 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/9) to afford the crude peptide **13e**, which was used in the next reaction without further purification.

To a solution of **13e** in CH_2Cl_2 (1 mL) was added TFA (0.25 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **6a** (60.4 mg, 0.28 mmol) in DMF (0.5 mL) were added 2,4,6-collidine (100 μ L, 0.76 mmol) and COMU (119 mg, 0.28 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was purified with flash column chromatography (1st: MeOH/CHCl₃ = 1/99 to 1/19, 2nd: MeOH/CHCl₃ = 1/49 to 1/19) to afford peptide **14g** (68.2 mg, 0.0593 mmol, 68% from **10b**) as a white solid.

To a solution of **14g** (4.9 mg, 0.0043 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 1 h, the reaction mixture was concentrated and azeotroped with toluene (3 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **5a** (3.6 mg, 0.0090 mmol) in DMF (0.5 mL) were added *i*-Pr₂NEt (4.0 μ L, 0.023 mmol), PyBOP (4.7 mg, 0.0090 mmol), HOAt (1.2 mg, 0.0088 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2 h, and then treated with CH₂Cl₂ (0.3 mL) and TFA (0.9 mL) at 0 °C. After 0.5 h at room temperature, TFA (2.4 mL) was added to the reaction mixture over 2 h. After being stirred at room temperature for 0.5 h, the reaction mixture was cooled to 0 °C, diluted with CH₂Cl₂ and brine, quenched with 3 M aqueous NaOH (11 mL), and extracted with *t*-BuOH/Et₂O (1/10, 3 times). The combined organic layers were dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/33 to 1/9 containing 1% Et₃N) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **4a** (3.9 mg, 0.0085 mmol) in DMF (0.2 mL) were added *i*-Pr₂NEt (3.9 μ L, 0.022 mmol), PyBOP (5.4 mg, 0.010 mmol) and HOAt (1.5 mg, 0.011 mmol) at 0 °C. After being stirred for 1 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/33) to afford the crude peptide **160**, which was used in the next reaction without further purification.

To a solution of **160** in CH_2Cl_2 (0.4 mL) was added TFA (0.1 mL) at 0 °C. After being stirred at room temperature for 0.5 h, the reaction mixture was concentrated and azeotroped with toluene (4 times) to afford the crude amine, which was used in the next reaction without further purification.

To a solution of the above crude amine and carboxylic acid **3a** (5.3 mg, 0.026 mmol) in DMF (0.2 mL) were added 2,4,6-collidine (7.0 μ L, 0.053 mmol) and COMU (12.9 mg, 0.030 mmol) at 0 °C. After 1.5 h at room temperature, 2,4,6-collidine (6.0 μ L, 0.045 mmol) and COMU (12.5 mg, 0.029 mmol) were added to the reaction mixture. After being stirred for 2.5 h, the reaction mixture was diluted with EtOAc, quenched with saturated aqueous NaHCO₃ at 0 °C and extracted with *t*-BuOH/EtOAc (1/10, 3 times). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and filtered. The solution was

concentrated and the residue was roughly purified with flash column chromatography (MeOH/CHCl₃ = 1/99 to 1/19) to afford the crude peptide **2r**

The crude peptide $2\mathbf{r}$ was then purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 or 254 nm) to afford the crude $2\mathbf{r}$.

The above crude **2r** was further purified by reversed-phase HPLC (column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm) to afford **2r** ($t_R = 26.7$ min, 0.372 mg, 0.000225 mmol, 5.2% from **14g**) as a white solid: HRMS (ESI) calcd for C₈₄H₁₄₈N₁₅NaO₁₈ [M+H+Na]²⁺ 839.0507, found 839.0498.

Cytotoxicity assay

Cell culture

P388 mouse leukemia cells were obtained from Institute of Development Aging and Cancer (Tohoku University). Cells were maintained in RPMI1640 growth medium [RPMI1640 with phenol red (Wako Pure Chemical Industries, Osaka, Japan), 10% fetal bovine serum, penicillin G (100 units/mL), streptomycin (100 μ g/mL)] under atmosphere of 5% CO₂ at 37°C.

Cytotoxicity assay against P388 cells

Compounds 2a-2r were diluted in RPMI1640 growth medium [RPMI1640 with phenol red (Wako Pure Chemical Industries, Osaka, Japan), 10% fetal bovine serum, penicillin G (100 units/mL), streptomycin (100 µg/mL)] with 2% DMSO to various concentrations (5-fold serial dilutions). P388 cells were harvested at 4 °C by centrifugation at 1000 rpm for 3 min. The collected cells were resuspended into the growth medium at 2×10^4 cells/mL. Aliquots of the former medium (2% DMSO, 100 µL) containing the peptides and the latter medium containing P388 cells (100 µL) were mixed in 96-well plates. The final concentration of P388 cells and DMSO were 1×10^4 cells/mL and 1%, respectively. After incubation for 92 h under atmosphere of 5% CO2 at 37 °C, the numbers of viable cells were determined by 3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro)benzene-sulfonic acid hydrate (XTT) assay. Phenazine methosulfate (PMS)/XTT solution in growth medium (PMS 1.53 mg/mL in Milli-Q/XTT 50 mg/mL in Milli-Q/growth medium = 1/2/47, 50 µL) were added to each well and further incubated for 4 h under atmosphere of 5% CO2 at 37 °C. The absorbance of each well at 490 nm was measured using a Bio-Rad Benchmark microplate reader (Bio-Rad, CA, USA). The cytotoxicities were evaluated as IC_{50} (nM) by means of 3 replicates, and the error values are shown in Figure S1. Sigmoidal curve fittings were performed on Graphpad Prism (Graphpad Software, CA, USA).



Figure S1. Dose-response curves of 2a–2r.

LogD evaluation

LogD values were evaluated by UHPLC (column: Accucore 150 C₁₈ 2.1 mm × 150 mm; column oven: 37 °C; flow rate: 0.2 mL/min; eluent: linear gradient from 35% to 75% *i*-PrOH/H₂O containing 0.05% TFA over 32 min; detection: 226, 254 nm) (Figure S2). The column dead time was determined by injection of NaNO₃ as a non-retained marker, and its retention time (t_0) was 1.28 min. The standard curve was obtained by plotting the capacity factor [$k = (t_R - t_0)/t_0$] of the four standard compounds [benzonitrile ($t_R = 2.23$ min), toluene ($t_R = 8.92$ min), biphenyl ($t_R = 15.61$ min), *n*-butylbenzene ($t_R = 19.32$ min)] versus the literature LogD values.⁵ The LogD values of **2a–2r** were calculated from the retention times.



Figure S2. Determination of LogD values of 2a-2r.

¹H and ¹³C NMR spectra















HPLC charts for purification of synthetic isomers Compound 2e



column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 40 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 10 mm \times 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2g

2



column: Inertsil ODS-3 4.6 mm \times 250 mm; column oven: 40 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 4.6 mm \times 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 10 mm \times 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 35% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm





column: Inertsil ODS-3 10 mm \times 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 10 mm × 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2m



column: Inertsil ODS-3 20 mm \times 250 mm; column oven: 45 °C; flow rate: 4.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Cosmosil π -NAP 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 50% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2o

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column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2p



column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2q



column: Inertsil ODS-3 4.6 mm × 250 mm; column oven: 45 °C; flow rate: 0.5 mL/min; eluent: 30% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm



column: Inertsil ODS-3 10 mm \times 250 mm; column oven: 45 °C; flow rate: 1.0 mL/min; eluent: 40% *n*-PrOH/H₂O containing 1% AcOH; detection: UV at 226 nm

Compound 2r

- 1. G. Ageno, L. Banfi, G. Cascio G. Guanti, E. Manghisi, R. Riva and V. Rocca, *Tetrahedron*, 1995, **51**, 8121.
- 2. T. Kuranaga, Y. Sesoko, K. Sakata, N. Maeda, A. Hayata and M. Inoue, *J. Am. Chem. Soc.*, 2013, **135**, 5467.
- 3. T. Yamashita, H. Matoba, T. Kuranaga and M. Inoue, Tetrahedron, 2014, 70, 7746.
- 4. T. Kuranaga, H. Mutoh, Y. Sesoko, T. Goto, S. Matsunaga and M. Inoue, *J. Am. Chem. Soc.*, 2015, **137**, 9443.
- 5. OECD. (2004), Test No. 117: Partition Coefficient (n-octanol/water), HPLC Method, OECD Guidelines

for the Testing of Chemicals, Section 1, OECD Publishing, Paris.