

Supporting Information

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SI Text

Compound Characterization. ^1H NMR and ^{13}C NMR were measured on a Bruker AV300 spectrometer. Chemical shifts are expressed in ppm downfield from internal standard (tetramethylsilane, 0.00 ppm), and coupling constants are reported as hertz. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. High-resolution mass spectra (HRMS) were obtained with Thermo Exactive spectrometer.

General Procedure for the Synthesis of Amino Acids with 3,5-Dinitrobenzyl Ester (Procedure A). (*S*)-3,5-Dinitrobenzyl 2-Amino-3-Cyclopropylpropanoate Hydrochloride (for the Introduction of Three-Membered-Ring Side Chain). The following is a representative experimental procedure of procedure A: To a solution of 2-amino-3-cyclopropylpropanoic acid (30 mg, 0.23 mmol) and Boc_2O (61 mg, 0.28 mmol) in dioxane (0.4 mL) and aqueous 1 M NaOH (0.4 mL), NaHCO_3 (48 mg, 0.57 mmol) was added at room temperature. After stirring for 5 h, the reaction mixture was diluted by adding ethyl ethanoate (EtOAc). The mixture was washed by saturated aqueous KHSO_4 and extracted with EtOAc (x3). The combined organic extracts were washed with brine, dried (MgSO_4), and concentrated in vacuo. To a solution of this crude mixture in dimethylformamide (DMF) (0.8 mL), 2, 4-dinitrobenzylchloride (65 mg, 0.30 mmol), and diisopropylethylamine (42 mg, 0.057 mL, 0.42 mmol) were added at room temperature. After stirring for 16 h, the reaction was quenched by adding saturated aqueous KHSO_4 , and mixture was extracted with Et_2O (x2). The combined organic extracts were washed with water (x2), saturated NaHCO_3 (x2) and brine, dried (MgSO_4), and concentrated in vacuo. The residue was purified by preparative thin-layer chromatography (PTLC) (hexane/acetone = 4/1) to afford 3,5-dinitrobenzylester (DNB) derivative (40 mg, 44%). To a solution of DNB derivative (20 mg, 0.051 mmol) in EtOAc (0.5 mL), 4 M HCl in EtOAc (2 mL) was added at 0 °C. After stirring for 10 h, starting material was disappeared and the reaction mixture was concentrated in vacuo (16 mg, 95%). ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.11–0.18 (m, 2H), 0.40–0.53 (m, 2H), 0.82–0.95 (m, 1H), 1.85–1.96 (m, 2H), 4.38 (t, 1H, $J = 6.3$ Hz), 5.58 (d, 1H, $J = 14.1$ Hz), 5.62 (d, 1H, $J = 14.1$ Hz), 8.75 (d, 2H, $J = 2.4$ Hz), 8.87 (t, 1H, $J = 2.4$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 4.88, 5.02, 7.24, 35.8, 54.3, 66.3, 119.2, 129.4, 140.9, 149.5, 170.2; HRMS (ESI) calculated for $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 310.1034, found: m/z 310.1032

(*S*)-3,5-Dinitrobenzyl 2-Amino-3-Cyclopentylpropanoate Hydrochloride (for the Introduction of Five-Membered-Ring Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 1.06–1.22 (m, 2H), 1.41–1.63 (m, 4H), 1.75–1.89 (m, 2H), 1.96–2.08 (m, 1H), 4.22 (t, 2H, $J = 7.5$ Hz), 5.56 (d, 1H, $J = 14.1$ Hz), 5.61 (d, 1H, $J = 14.1$ Hz), 8.73 (d, 2H, $J = 2.1$ Hz), 8.89 (t, 1H, $J = 2.1$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 25.4, 25.5, 32.8, 33.1, 36.8, 37.8, 53.5, 66.2, 119.2, 129.3, 140.9, 149.5, 170.9; HRMS (ESI) calculated for $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 338.1347, found: m/z 338.1344

(*S*)-3,5-Dinitrobenzyl 2-Amino-3-Cyclohexylpropanoate Hydrochloride (for the Introduction of Six-Membered-Ring Side Chain). The compound was obtained according to procedure A described above.

^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.85–1.03 (m, 2H), 1.05–1.32 (m, 3H), 1.52–1.69 (m, 4H), 1.72–1.85 (m, 2H), 1.85–2.03 (m, 2H), 4.30 (t, 1H, $J = 7.2$ Hz), 5.57 (d, 1H, $J = 13.8$ Hz), 5.62 (d, 1H, $J = 13.8$ Hz), 8.77 (d, 2H, $J = 2.1$ Hz), 8.90 (t, 1H, $J = 2.1$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 26.5, 26.6, 26.9, 33.3, 33.6, 34.3, 51.7, 66.2, 119.2, 129.4, 141.0, 149.5, 170.8; RMS (ESI) calculated for $\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 352.1503, found: m/z 352.1497

(*S*)-3,5-Dinitrobenzyl 2-Aminobutanoate Hydrochloride (for the Introduction of Two-Carbon Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 1.09 (t, 3H, $J = 7.2$ Hz), 2.05–2.22 (m, 2H), 4.35 (t, 1H, $J = 6.3$ Hz), 5.62 (d, 1H, $J = 13.8$ Hz), 5.67 (d, 1H, $J = 13.8$ Hz), 8.78 (d, 2H, $J = 1.8$ Hz), 8.94 (t, 1H, $J = 1.8$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 9.62, 24.3, 54.8, 66.4, 119.2, 129.4, 140.8, 149.4, 170.0; HRMS (ESI) calculated for $\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 284.0877, found: m/z 284.0877

(*S*)-3,5-Dinitrobenzyl 2-Aminopentanoate Hydrochloride (for the Introduction of Three-Carbon Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.97 (t, 3H, $J = 7.2$ Hz), 1.39–1.69 (m, 2H), 1.98–2.16 (m, 2H), 4.38 (t, 1H, $J = 6.3$ Hz), 5.62 (d, 1H, $J = 13.5$ Hz), 5.67 (t, 1H, $J = 13.5$ Hz), 8.78 (d, 2H, $J = 2.1$ Hz), 8.93 (t, 1H, $J = 2.1$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 13.8, 18.8, 32.9, 53.6, 66.4, 119.1, 129.3, 140.9, 149.5, 170.1; HRMS (ESI) calculated for $\text{C}_{12}\text{H}_{15}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 298.1034, found: m/z 298.1031

(*S*)-3,5-Dinitrobenzyl 2-Aminohexanoate Hydrochloride (for the Introduction of Four-Carbon Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.88 (t, 3H, $J = 7.2$ Hz), 1.30–1.63 (m, 4H), 2.09–2.14 (m, 2H), 4.31 (t, 1H, $J = 6.3$ Hz), 5.63 (s, 2H), 8.81 (d, 2H, $J = 2.4$ Hz), 8.92 (t, 1H, $J = 2.4$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 13.3, 22.8, 27.7, 30.7, 53.7, 66.2, 119.2, 129.5, 141.0, 148.6, 169.1; HRMS (ESI) calculated for $\text{C}_{13}\text{H}_{17}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 312.1190, found: m/z 312.1189

(*S*)-3,5-Dinitrobenzyl 2-Aminoheptanoate Hydrochloride (for the Introduction of Five-Carbon Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.84 (t, 3H, $J = 6.6$ Hz), 1.19–1.56 (m, 6H), 2.01–2.16 (m, 2H), 4.38 (t, 1H, $J = 6.3$ Hz), 5.61 (d, 1H, $J = 13.2$ Hz), 5.69 (d, 1H, $J = 13.2$ Hz), 8.78 (s, 2H), 8.94 (s, 1H); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 14.3, 23.0, 25.4, 29.3, 30.9, 32.0, 53.7, 66.4, 119.2, 129.4, 140.9, 149.4, 170.1; HRMS (ESI) calculated for $\text{C}_{16}\text{H}_{23}\text{N}_3\text{O}_6$: $([\text{M} + \text{H}]^+)$ m/z 326.1347, found: m/z 326.1342

(*S*)-3,5-Dinitrobenzyl 2-Amino-octanoate Hydrochloride (for the Introduction of Six-Carbon Side Chain). The compound was obtained according to procedure A described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 0.83 (t, 3H, $J = 6.9$ Hz), 1.13–1.63 (m, 8H), 2.02–2.15 (m, 2H), 4.38 (t, 1H, $J = 6.3$ Hz), 5.61 (d, 1H, $J = 13.5$ Hz), 5.70 (d, 1H, $J = 13.5$ Hz), 8.78 (d, 2H, $J = 2.4$ Hz), 8.94 (t, 1H, $J = 2.4$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 14.3, 23.0, 25.4, 29.3, 30.9, 32.0,

53.7, 66.4, 119.2, 129.4, 140.9, 149.4, 170.1; HRMS (ESI) calculated for $C_{16}H_{23}N_3O_6$: $([M + H]^+) m/z$ 340.1503, found: m/z 340.1495

(S)-3,5-Dinitrobenzyl 2-Aminononanoate Hydrochloride (for the Introduction of Seven-Carbon Side Chain). The compound was obtained according to procedure A described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 0.80 (t, 3H, $J = 6.9$ Hz), 1.19–1.32 (m, 12H), 4.10 (t, 1H, $J = 6.3$ Hz), 5.48 (d, 1H, $J = 12.9$ Hz), 5.52 (d, 1H, $J = 12.9$ Hz), 8.71 (s, 2H), 8.89 (s, 1H); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 14.3, 23.2, 25.8, 29.7, 29.9, 32.4, 32.8, 54.2, 65.7, 119.1, 128.5, 129.2, 141.4, 149.5; HRMS (ESI) calculated for $C_{16}H_{23}N_3O_6$: $([M + H]^+) m/z$ 354.1660, found: m/z 354.1657

(S)-3,5-Dinitrobenzyl 2-Aminodecanoate Hydrochloride (for the Introduction of Eight-Carbon Side Chain). The compound was obtained according to procedure A described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 0.688 (t, 3H, $J = 7.2$ Hz), 0.95–1.33 (m, 12H), 1.90 (dt, 2H, $J = 9.3, 6.0$ Hz), 4.21 (t, 1H, $J = 6.0$ Hz), 5.39 (d, 1H, $J = 13.2$ Hz), 5.54 (d, 1H, $J = 13.2$ Hz), 8.65 (d, 2H, $J = 1.8$ Hz), 8.96 (t, 1H, $J = 1.8$ Hz); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 13.4, 22.0, 23.9, 28.1, 28.28, 28.35, 29.7, 31.1, 52.7, 65.7, 119.0, 128.8, 139.2, 148.4, 169.8; HRMS (ESI) calculated for $C_{17}H_{25}N_3O_6$: $([M + H]^+) m/z$ 368.1816, found: m/z 368.1812

General Procedure for the Synthesis of Amino Acids with Cyanomethyl Ester (CME) (Procedure B). (S)-Cyanomethyl 2-Amino-3-(1-Methyl-1H-indol-3-yl) Propanoate Trifluoroacetate (for the Introduction of Me-Trp). The following is a representative experimental procedure of Procedure B: To a solution of 2-amino-3-(1-methyl-1H-indol-3-yl) propanoic acid (50 mg, 0.23 mmol) and *N*-[2-(trimethylsilyl)ethoxycarbonyl-oxy]succinimide (79 mg, 0.31 mmol) in H_2O (0.4 mL) and THF (0.4 mL), $NaHCO_3$ (48 mg, 0.57 mmol) was added at room temperature. After stirring for 5 h, the reaction mixture was diluted by adding EtOAc. The mixture was washed by saturated aqueous $KHSO_4$ and extracted with EtOAc (x3). The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. To a solution of this crude mixture in DMF (0.8 mL), chloroacetonitrile (25 mg, 0.021 mL, 0.33 mmol) and triethylamine (76 mg, 0.10 mL, 0.75 mmol) were added at room temperature. After stirring for 16 h, the reaction was quenched by adding saturated aqueous $KHSO_4$, and mixture was extracted with Et_2O (x2). The combined organic extracts were washed with water (x2), saturated $NaHCO_3$ (x2) and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by PTLC (hexane/EtOAc = 2/1) to afford CME derivative (72 mg, 78%). To a solution of DNB derivative (20 mg, 0.051 mmol) in EtOAc (0.5 mL), CME derivative (72 mg, 78%). To a solution of CME derivative (13 mg, 0.032 mmol) in CH_2Cl_2 (1 mL) was added trifluoroacetic acid (2 mL) at 0°C. After stirring for 10 h, starting material was disappeared. The reaction mixture was concentrated in vacuo (13 mg, quantitative). 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 3.50 (dd, 1H, $J = 6.6, 2.4$ Hz), 3.79 (s, 3H), 4.60 (t, 1H, $J = 6.6$ Hz), 4.97 (d, 1H, $J = 15.9$ Hz), 5.57 (d, 1H, $J = 15.9$ Hz), 7.18 (ddd, 1H, $J = 8.1, 7.2, 0.9$ Hz), 7.24 (s, 1H), 7.30 (ddd, 1H, $J = 8.1, 7.2, 0.9$ Hz), 7.46 (d, 1H, $J = 8.1$ Hz), 7.62 (d, 1H, $J = 8.1$ Hz); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 25.7, 32.2, 50.2, 53.2, 104.7, 110.1, 114.7, 116.6 (q, 1C, $J = 288$ Hz), 118.2, 119.6, 122.2, 127.0, 129.5, 137.1, 161.9 (q, 1C, $J = 35$ Hz), 168.5; HRMS (ESI) calculated for $C_{14}H_{15}N_3O_2$: $([M + H]^+) m/z$ 258.1237, found: m/z 258.1227

(S)-Cyanomethyl 2-Amino-3-(1H-Indol-3-yl) Propanoate Trifluoroacetate (for the Introduction of Trp). The compound was obtained

according to procedure B described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 2.78 (s, 1H), 3.54 (dd, 1H, $J = 15.3, 6.6$ Hz), 3.59 (dd, 1H, $J = 15.3, 6.6$ Hz), 4.63 (t, 1H, $J = 6.6$ Hz), 5.01 (d, 1H, $J = 15.9$ Hz), 5.07 (d, 1H, $J = 15.9$ Hz), 7.22 (ddd, 1H, $J = 8.1, 6.9, 1.5$ Hz), 7.31 (ddd, 1H, $J = 8.1, 6.9, 0.9$ Hz), 7.36 (s, 1H), 7.56 (dd, 1H, $J = 6.9, 1.5$ Hz), 7.65 (dd, 1H, $J = 6.9, 1.5$ Hz); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 25.6, 50.3, 53.2, 105.7, 112.1, 116.4 (q, 1C, $J = 290$ Hz), 114.4, 117.9, 119.7, 122.3, 125.4, 126.4, 136.3, 168.6; HRMS (ESI) calculated for $C_{13}H_{13}N_3O_2$: $([M + H]^+) m/z$ 244.1081, found: m/z 244.1080

(S)-Cyanomethyl 2-Amino-3-(4-Methoxyphenyl) Propanoate Trifluoroacetate (for the Introduction of Me-Tyr). The compound was obtained according to procedure B described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 3.16 (d, 1H, $J = 6.6$ Hz), 3.70 (s, 3H), 4.41 (t, 1H, $J = 6.6$ Hz), 4.90 (d, 1H, $J = 15.9$ Hz), 4.96 (d, 1H, $J = 15.9$ Hz), 6.86–6.89 (m, 2H), 7.10–7.14 (m, 2H); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 34.7, 50.3, 53.9, 55.3, 114.7, 116.4 (q, 1C, $J = 290$ Hz), 125.6, 130.8, 158.7, 162.7 (q, 1C, $J = 35$ Hz), 168.3; HRMS (ESI) calculated for $C_{12}H_{14}N_2O_3$: $([M + H]^+) m/z$ 235.1077, found: m/z 235.1076

(S)-Cyanomethyl 2-Amino-3-(4-Hydroxyphenyl) Propanoate Trifluoroacetate (for the Introduction of Tyr). The compound was obtained according to procedure B described above. 1H NMR (300 MHz, D_2O , the minor rotamer were marked with an asterisk) δ 3.03–3.25 (m, 2H), 4.27–4.44 (m, 1H), 4.48* (d, 0.3H, $J = 14.4$ Hz), 4.54* (d, 0.3H, $J = 14.4$ Hz), 4.91 (d, 0.7H, $J = 15.9$ Hz), 4.97 (d, 0.7H, $J = 15.9$ Hz), 6.75–6.82 (m, 2H), 7.03–7.11 (m, 2H); ^{13}C NMR (75 MHz, D_2O) δ 34.6, 50.3, 53.9, 114.7, 115.89, 115.97, 116.2 (q, 1C, $J = 290$ Hz), 124.9, 130.77, 130.82, 155.3, 162.7 (q, 1C, $J = 35$ Hz), 168.3; HRMS (ESI) calculated for $C_{12}H_{14}N_2O_3$: $([M + H]^+) m/z$ 221.0921, found: m/z 221.0933

(S)-Cyanomethyl 2-Amino-3-(Benzo[b]thiophen-3-yl) Propanoate Trifluoroacetate (for the Introduction of Benzothiophene Side Chain). The compound was obtained according to procedure B described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 3.60 (dd, 1H, $J = 14.4, 7.2$ Hz), 3.67 (dd, 1H, $J = 14.4, 7.2$ Hz), 4.67 (t, 1H, $J = 7.2$ Hz), 4.96 (d, 1H, $J = 15.9$ Hz), 5.02 (d, 1H, $J = 15.9$ Hz), 7.45–7.56 (m, 2H), 7.57 (s, 1H), 7.85 (dd, 1H, $J = 6.6, 1.2$ Hz), 8.01 (dd, 1H, $J = 6.9, 1.2$ Hz); ^{13}C NMR (75 MHz, acetone- d_6/D_2O , 10/1, vol/vol) δ 28.4, 50.4, 52.4, 114.5, 116.4 (q, 1C, $J = 290$ Hz), 121.1, 123.3, 124.7, 125.0, 126.8, 127.6, 137.7, 140.3, 162.7 (q, 1C, $J = 35$ Hz), 168.2; HRMS (ESI) calculated for $C_{13}H_{12}N_2O_2S$: $([M + H]^+) m/z$ 261.0692, found: m/z 261.0684

(S)-Cyanomethyl 2-Amino-3-(4-Aminophenyl) Propanoate Trifluoroacetate (for the Introduction of Aniline Side Chain). The compound was obtained according to procedure B described above. 1H NMR (300 MHz, DMSO- d_6/D_2O , 10/1, vol/vol, the minor rotamer was marked with an asterisk) δ 3.14–3.34 (m, 2H), 4.35–4.48 (m, 1H), 4.53* (d, 0.4H, $J = 15.3$ Hz), 4.60* (d, 0.4H, $J = 15.3$ Hz), 5.07 (s, 1.2H), 7.33–7.51 (m, 4H); ^{13}C NMR (75 MHz, DMSO- d_6/D_2O , 10/1, vol/vol) δ 34.9, 50.3, 53.4, 114.6, 116.4 (q, 1C, $J = 288$ Hz), 118.3, 131.0, 131.2, 134.7, 168.1; HRMS (ESI) calculated for $C_{13}H_{12}N_2O_2S$: $([M + H]^+) m/z$ 220.1081, found: m/z 220.1081

(S)-Cyanomethyl 2-Amino-3-(Biphenyl-4-yl) Propanoate Trifluoroacetate (for the Introduction of Biphenyl). The compound was obtained according to procedure B described above. 1H NMR (300 MHz, acetone- d_6/D_2O , 10/1, vol/vol, the minor rotamer was marked with an asterisk) δ 3.44–3.65 (m, 2H), 4.76* (br, 0.25H), 5.02–5.15

(m, 2H), 5.35 (dd, 0.75H, $J = 9.9, 4.5$ Hz), 7.33–7.41 (m, 1H), 7.44–7.52 (m, 4H), 7.61–7.70 (m, 4H); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 36.9, 50.8, 62.7, 115.3, 127.7, 127.99, 128.05, 128.2, 128.3, 129.71, 129.75, 129.77, 131.6, 135.8, 140.9, 141.2, 169.2; HRMS (ESI) calculated for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$: $([\text{M} + \text{H}]^+)$ m/z 281.1285, found: m/z 281.1285

(*S*)-Cyanomethyl 2-Amino-3-(Naphthalen-1-yl)Propanoate Trifluoroacetate (for the Introduction of 1-Naphthyl Side Chain). The compound was obtained according to procedure B described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol, the minor rotamer was marked with an asterisk) δ 3.70–3.87 (m, 1H), 3.96–4.11 (m, 1H), 4.70 (t, 0.7H, $J = 7.5$ Hz), 4.89 (d, 0.7H, $J = 16.2$ Hz), 4.95 (d, 0.7H, $J = 16.2$ Hz), 5.05* (d, 0.3H, $J = 16.5$ Hz), 5.11* (d, 0.3H, $J = 16.5$ Hz), 5.22* (dd, 0.3H, $J = 10.2, 3.9$ Hz), 7.43–7.64 (m, 4H), 7.89 (d, 1H, $J = 8.1$ Hz), 7.96 (dd, 1H, $J = 8.1, 1.2$ Hz), 8.33 (dd, 1H, $J = 7.5, 7.2$ Hz); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 34.6, 50.6, 54.3, 115.1, 124.1, 126.4, 126.9, 127.6, 129.4, 129.4, 129.9, 131.5, 132.7, 134.9, 169.3; HRMS (ESI) calculated for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2$: $([\text{M} + \text{H}]^+)$ m/z 255.1128, found: m/z 255.1119

(*S*)-Cyanomethyl 2-Amino-3-(Naphthalen-6-yl) Propanoate Trifluoroacetate (for the Introduction of 2-Naphthyl Side Chain). The compound

was obtained according to procedure B described above. ^1H NMR (300 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 3.43 (dd, 1H, $J = 15.3, 6.9$ Hz), 3.48 (dd, 1H, $J = 15.3, 6.9$ Hz), 4.67 (t, 1H, $J = 6.9$ Hz), 4.94 (d, 1H, $J = 16.2$ Hz), 5.03 (d, 1H, $J = 16.2$ Hz), 7.37 (dd, 1H, $J = 8.7, 1.8$ Hz), 7.46–7.54 (m, 2H), 7.74 (s, 1H), 7.78–7.92 (m, 3H); ^{13}C NMR (75 MHz, acetone- $d_6/\text{D}_2\text{O}$, 10/1, vol/vol) δ 35.8, 50.2, 53.8, 114.6, 116.5 (q, 1C, $J = 290$ Hz), 126.6, 126.8, 126.9, 127.75, 127.78, 128.5, 129.1, 130.9, 132.7, 133.2, 162.5 (q, 1C, $J = 35$ Hz), 168.2; HRMS (ESI) calculated for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_2$: $([\text{M} + \text{H}]^+)$ m/z 255.1128, found: m/z 255.1117

(*S*)-Cyanomethyl 2-Amino-3-Phenylpropanoate Hydrochloride (for the Introduction of Phe). The compound was obtained according to procedure B described above and also reported in ref. 1. ^1H NMR (300 MHz, DMSO- d_6 , the minor rotamers was marked with asterisks) δ 3.08–3.35 (m, 2H), 4.28–4.44 (m, 1H), 4.45* (d, 0.2H, $J = 15.6$ Hz), 4.54* (d, 0.2H, $J = 15.6$ Hz), 4.76** (d, 0.2H, $J = 14.7$ Hz), 4.82** (d, 0.2H, $J = 14.7$ Hz), 5.02 (d, 0.6H, $J = 16.5$ Hz), 7.20–7.48 (m, 5H), 8.88 (s, 3H); ^{13}C NMR (75 MHz, DMSO- d_6) δ 35.6, 50.0, 53.0, 115.1, 127.3, 128.5, 128.6, 129.4, 129.5, 168.1; HRMS (ESI) calculated for $\text{C}_{11}\text{H}_{12}\text{N}_2\text{O}_2$: $([\text{M} + \text{H}]^+)$ m/z 284.0877, found: m/z 284.0877

1. Saito H, Kourouklis D, Suga H (2001) An in vitro evolved precursor tRNA with aminoacylation activity. *EMBO J* 20:1797–1806.

Table S1. Measured ΔG_{app} values and standard deviations (three or four measurements) in kcal/mol for all sequences and amino acids in the study

H segments with G flanks (helix length 19)

ID	Amino acid	Sequence of test helix H3	$\Delta G_{\text{app}}^{\text{meas}}$	SD
(S)-2-aminobutanoic acid				
1		GGPG*AAALAAAAAAAAALAAAAGPGG	0.72	0.05
2		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.36	0.04
3		GGPGAAAAAL*AAAAAAAAALAAAAGPGG	0.52	0.03
4		GGPGAAAAALAAAA*AAAAALAAAAGPGG	0.55	0.12
5		GGPGAAAAALAAAAAAAA*LAAAAGPGG	0.49	0.03
6		GGPGAAAAALAAAAAAAAALAAAGPGG	0.58	0.04
7		GGPGAAAAALAAAAAAAAALAAA*GPGG	0.53	0.11
(S)-2-aminopentanoic acid (L-norvaline)				
8		GGPG*AAALAAAAAAAAALAAAAGPGG	0.36	0.05
9		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.31	0.03
10		GGPGAAAAAL*AAAAAAAAALAAAAGPGG	0.27	0.07
11		GGPGAAAAALAAAA*AAAAALAAAAGPGG	0.28	0.03
12		GGPGAAAAALAAAAAAAA*LAAAAGPGG	0.36	0.03
13		GGPGAAAAALAAAAAAAAALAAAGPGG	0.36	0.03
14		GGPGAAAAALAAAAAAAAALAAA*GPGG	0.41	0.06
(S)-2-aminohexanoic acid (L-norleucine)				
15		GGPG*AAALAAAAAAAAALAAAAGPGG	0.12	0.01
16		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.05	0.05
17		GGPGAAAAAL*AAAAAAAAALAAAAGPGG	-0.02	0.03
18		GGPGAAAAALAAAA*AAAAALAAAAGPGG	0.11	0.04
19		GGPGAAAAALAAAAAAAA*LAAAAGPGG	0.30	0.06
20		GGPGAAAAALAAAAAAAAALAAAGPGG	0.21	0.07
21		GGPGAAAAALAAAAAAAAALAAA*GPGG	0.23	0.03
(S)-2-aminoheptanoic acid				
22		GGPG*AAALAAAAAAAAALAAAAGPGG	-0.02	0.09
23		GGPGAA*ALAAAAAAAAALAAAAGPGG	-0.28	0.07
24		GGPGAAAAAL*AAAAAAAAALAAAAGPGG	-0.34	0.04
25		GGPGAAAAALAAAA*AAAAALAAAAGPGG	-0.28	0.10
26		GGPGAAAAALAAAAAAAA*LAAAAGPGG	-0.07	0.05
27		GGPGAAAAALAAAAAAAAALAAAGPGG	0.06	0.05
28		GGPGAAAAALAAAAAAAAALAAA*GPGG	0.03	0.09

H segments with G flanks (helix length 19)

ID	Amino acid	Sequence of test helix H3	$\Delta G^{\text{meas}}_{\text{app}}$	SD
(S)-2-amino-3-(4-aminophenyl)propanoic acid				
85		GGPG*AAALALAAAAALALAAAAGPGG	-0.71	0.01
86		GGPGAA*ALALAAAAALALAAAAGPGG	-0.41	0.04
87		GGPGAAAAL*LAAAAALALAAAAGPGG	-0.06	0.09
88		GGPGAAAALALAA*ALALAAAAGPGG	0.15	0.07
89		GGPGAAAALALAAAAAL*LAAAAGPGG	-0.39	0.01
90		GGPGAAAALALAAAAALALA*AAGPGG	-0.94	0.13
91		GGPGAAAALALAAAAALALAAA*GP GG	-0.76	0.08
L-tyrosine				
92		GGPG*AAALAAAAAAAAALAAAAGPGG	0.60	0.12
93		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.78	0.07
94		GGPGAAAAL*AAAAAAAAALAAAAGPGG	0.88	0.11
95		GGPGAAAALAAAA*AAAAALAAAAGPGG	1.04	0.11
96		GGPGAAAALAAAAAAAAA*LAAAAGPGG	0.90	0.03
97		GGPGAAAALAAAAAAAAALA*AAGPGG	0.43	0.05
98		GGPGAAAALAAAAAAAAALAAA*GP GG	0.77	0.05
L-tyrosine				
99		GGPG*AAALALAAAAALALAAAAGPGG	-0.82	0.12
100		GGPGAA*ALALAAAAALALAAAAGPGG	-0.62	0.01
101		GGPGAAAAL*LAAAAALALAAAAGPGG	-0.49	0.03
102		GGPGAAAALALAA*ALALAAAAGPGG	0.18	0.02
103		GGPGAAAALALAAAAAL*LAAAAGPGG	-0.62	0.08
104		GGPGAAAALALAAAAALALA*AAGPGG	-1.11	0.09
105		GGPGAAAALALAAAAALALAAA*GP GG	-0.90	0.07
L-tryptophan				
106		GGPG*AAALAAAAAAAAALAAAAGPGG	0.07	0.04
107		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.23	0.05
108		GGPGAAAAL*AAAAAAAAALAAAAGPGG	0.66	0.05
109		GGPGAAAALAAAA*AAAAALAAAAGPGG	0.77	0.05
110		GGPGAAAALAAAAAAAAA*LAAAAGPGG	0.66	0.08
111		GGPGAAAALAAAAAAAAALA*AAGPGG	0.25	0.09
112		GGPGAAAALAAAAAAAAALAAA*GP GG	0.43	0.12
L-tryptophan				
113		GGPG*AAALALAAAAALALAAAAGPGG	-1.41	0.17
114		GGPGAA*ALALAAAAALALAAAAGPGG	-1.04	0.08
115		GGPGAAAAL*LAAAAALALAAAAGPGG	-0.69	0.07
116		GGPGAAAALALAA*ALALAAAAGPGG	-0.37	0.09
117		GGPGAAAALALAAAAAL*LAAAAGPGG	-0.92	0.03
118		GGPGAAAALALAAAAALALA*AAGPGG	-1.47	0.18
119		GGPGAAAALALAAAAALALAAA*GP GG	-1.19	0.07
(S)-2-amino-3-(1-methyl-1H-indol-3-yl)propanoic acid				
120		GGPG*AAALAAAAAAAAALAAAAGPGG	0.06	0.06
121		GGPGAA*ALAAAAAAAAALAAAAGPGG	0.15	0.03
122		GGPGAAAAL*AAAAAAAAALAAAAGPGG	0.17	0.10
123		GGPGAAAALAAAA*AAAAALAAAAGPGG	0.15	0.09
124		GGPGAAAALAAAAAAAAA*LAAAAGPGG	0.41	0.09
125		GGPGAAAALAAAAAAAAALA*AAGPGG	0.09	0.02
126		GGPGAAAALAAAAAAAAALAAA*GP GG	0.17	0.08
(S)-2-amino-3-(benzo[b]thiophen-3-yl)propanoic acid				
127		GGPG*AAALAAAAAAAAALAAAAGPGG	-0.05	0.09
128		GGPGAA*ALAAAAAAAAALAAAAGPGG	-0.12	0.03
129		GGPGAAAAL*AAAAAAAAALAAAAGPGG	-0.18	0.01
130		GGPGAAAALAAAA*AAAAALAAAAGPGG	-0.20	0.01
131		GGPGAAAALAAAAAAAAA*LAAAAGPGG	0.09	0.11
132		GGPGAAAALAAAAAAAAALA*AAGPGG	-0.02	0.03
133		GGPGAAAALAAAAAAAAALAAA*GP GG	-0.19	0.02
(S)-2-amino-3-(naphthalen-1-yl)propanoic acid				
134		GGPG*AAALAAAAAAAAALAAAAGPGG	-0.11	0.00
135		GGPGAA*ALAAAAAAAAALAAAAGPGG	-0.14	0.02
136		GGPGAAAAL*AAAAAAAAALAAAAGPGG	-0.20	0.04
137		GGPGAAAALAAAA*AAAAALAAAAGPGG	-0.10	0.05
138		GGPGAAAALAAAAAAAAA*LAAAAGPGG	0.03	0.01
139		GGPGAAAALAAAAAAAAALA*AAGPGG	-0.11	0.02

H segments with G flanks (helix length 19)

ID	Amino acid	Sequence of test helix H3	ΔG_{app}^{meas}	SD
140		GGPGAAAALAAAAAAAAALAAA*GPGG	-0.06	0.00
	(S)-2-amino-3-(naphthalen-6-yl) propanoic acid			
141		GGPG*AAALAAAAAAAAALAAAAGPGG	-0.03	0.08
142		GGPGAA*ALAAAAAAAAALAAAAGPGG	-0.04	0.04
143		GGPGAAAAL*AAAAAAAAALAAAAGPGG	-0.15	0.00
144		GGPGAAAALAAAA*AAAAALAAAAGPGG	-0.24	0.04
145		GGPGAAAALAAAAAAAA*LAAAAGPGG	0.01	0.05
146		GGPGAAAALAAAAAAAAALA*AAGPGG	-0.07	0.03
147		GGPGAAAALAAAAAAAAALAAA*GPGG	-0.03	0.06
	(S)-2-amino-3-(biphenyl-4-yl) propanoic acid			
148		GGPG*AAALAAAAAAAAALAAAAGPGG	-0.06	0.02
149		GGPGAA*ALAAAAAAAAALAAAAGPGG	-0.15	0.05
150		GGPGAAAAL*AAAAAAAAALAAAAGPGG	-0.43	0.02
151		GGPGAAAALAAAA*AAAAALAAAAGPGG	-0.37	0.07
152		GGPGAAAALAAAAAAAA*LAAAAGPGG	-0.07	0.02
153		GGPGAAAALAAAAAAAAALA*AAGPGG	-0.07	0.00
154		GGPGAAAALAAAAAAAAALAAA*GPGG	-0.21	0.07
	L-alanine			
155		GGPGAAAALAAAAAAAAALAAAAGPGG	0.66	0.06
	L-valine			
156		GGPGAAAALAAAAVAAAAALAAAAGPGG	0.18	0.03
	L-leucine			
157		GGPGAAAALAAAAALAAAALAAAAGPGG	0.10	0.02
	L-isoleucine			
158		GGPGAAAALAAAAIAAAAALAAAAGPGG	-0.07	0.05
	<i>amino acid</i>	<i>sequence of test helix H4</i>	ΔG_{app}^{meas}	SD
	(S)-2-aminooctanoic acid			
159		GGPG*AAAAAAAAAAAAAAAAAAGPGG	0.52	0.09
160		GGPGAA*AAAAAAAAAAAAAAAAAAGPGG	0.49	0.05
161		GGPGAAAAA*AAAAAAAAAAAAAAAAAAGPGG	0.34	0.08
162		GGPGAAAAAAAAA*AAAAAAAAAAGPGG	0.29	0.05
163		GGPGAAAAAAAAA*AAAAAAGPGG	0.18	0.03
164		GGPGAAAAAAAAA*AAAGPGG	0.36	0.10
165		GGPGAAAAAAAAA*GPGG	0.29	0.10
	(S)-2-aminodecanoic acid			
166		GGPG*AAAAAAAAAAAAAAAAAAGPGG	0.16	0.07
167		GGPGAA*AAAAAAAAAAAAAAAAAAGPGG	0.31	0.05
168		GGPGAAAAA*AAAAAAAAAAAAAAAAAAGPGG	0.19	0.05
169		GGPGAAAAAAAAA*AAAAAAAAAAGPGG	0.04	0.04
170		GGPGAAAAAAAAA*AAAAAAGPGG	-0.03	0.11
171		GGPGAAAAAAAAA*AAAGPGG	-0.02	0.15
172		GGPGAAAAAAAAA*GPGG	0.12	0.08
	L-alanine			
173		GGPGAAAAAAAAAAAAAAAAAAGPGG	1.20	0.10