Design and Synthesis of Unsymmetrical Peptidyl Urea Inhibitors of Aspartic Peptidases

Natalie A. Dales[1], Regine S. Bohacek[2], Kenneth A. Satyshur[3], Daniel H. Rich*[1,3]

General procedure A: Urea coupling using phosgene and $NaHCO_3$ for in situ formation of the isocyanate.

To a mixture of $NaHCO_3$ (20 eq) in CH_2Cl_2 or MeCN (0.2 M) at 0°C was added a solution of phosgene (1.9 M in toluene, 4-5 eq). A suspension of the amino acid methyl ester (first coupling partner, 1 eq) in CH_2Cl_2 or MeCN (0.2 M) was added dropwise. Upon complete conversion of the amine to the isocyanate (TLC, 0.5-1 hour), the reaction vessel was equipped with a NaOH bubbler and excess phosgene was purged from the system using a stream of argon (0°C). Complete removal of phosgene was detected using an ethanolic solution of 4-(dimethylamino) benzaldehyde (10%) and diphenyl amine (10%). Additional solvent (CH_2Cl_2 or MeCN) was added if necessary. The second coupling partner (1.1 eq) was added in CH_2Cl_2 or MeCN (or as a solid) and additional $NaHCO_3$ was added. The reaction was warmed to room temperature. Upon completion (2-12 hours) the reaction mixture was diluted with CH_2Cl_2 and water. The layers were separated and the organic layer was washed with 10% $KHSO_4$ then dried (Na_2SO_4) and concentrated. Flash chromatography (hexane-EtOAc or MeOH-CH_2Cl_2) afforded the purified urea product.

General procedure B: Urea coupling using triphosgene and DIEA for in situ formation of the isocyanate.

Triphosgene (0.4 eq) was dissolved in CH_2Cl_2 (0.2 M) and a solution of the amino acid methyl ester (first coupling partner, 1 eq) in CH_2Cl_2 (0.3 M) and DIEA (2.2 eq) was added slowly via a syringe pump (0.1 mL/min). Gas evolution was noticeable. The reaction was stirred an additional 5 minutes, then a solution of the second coupling partner (1.1 eq) in CH_2Cl_2 (0.6 M) and

DIEA (2.2 eq) was added in one portion. After 1 hour, the reaction mixture was concentrated completely. The oily residue was diluted in EtOAc (3x reaction volume), washed with 10% $KHSO_4$, 5% $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated to afford an oil which was purified by flash column chromatography (EtOAc-hexane or MeOH-CH_2Cl_2).

General procedure C: General procedure for palladium cross coupling of iodo-phenylalanine derivatives.

To a mixture of $PdCl_2(PPh_3)_2$ (0.1 eq) in THF (0.4 M) was added DIBAL (1.5 M solution in toluene, 0.22 eq) dropwise. The reaction mixture turned from yellow to dark orange to brown. After 5 minutes, a solution of the aryl iodide (1.0 eq) in THF (0.4 M) was added slowly. After 5 minutes, benzyl Grignard (0.2 M in THF, 2-4 eq depending on the number of acidic protons) was added. After 1-2 hours, the reaction was quenched with saturated citric acid, the layers were separated and the aqueous layer was further extracted with CH_2Cl_2 (4x). The combined organic layers were dried over $MgSO_4$ and concentrated to give a brown oil which was flash column purified and recrystallized (or triturated).

General procedure D: General procedure for tert-butyl carbamate (Boc) removal:

The Boc-protected amine was treated with 4N HCl in dioxane (prescored in vials from Pierce) (15-20 equivalents). The reaction mixture was stirred at room temperature until complete conversion was observed by TLC (< 1hr). Upon completion, the reaction mixture was concentrated completely. Trituration with Et_2O (3x) afforded the HCl salt. The HCl salt was then dried over NaOH on the vacuum pump. The residue was recrystallized from Et_2O-MeOH when appropriate.

*[retro-Cha-OMe]Ψ[NHCONH]Phe-ol (**2**).*

According to general procedure A, the title compound was prepared on a 0.34 mmol scale: HCl.Cha-OMe (0.34 mmol, 76 mg), phosgene (1.4 mmol, 0.74 mL), $NaHCO_3$ (570 mg, 6.8 mmol), HCl.Phe-ol (3.44 mmol, 645 mg, added as a suspension), CH_2Cl_2 (4 mL total). Flash column chromatography (2:1 EtOAc: hexane) afforded the urea (**2**) as white solid (112 mg, 91%): mp 97-99°C; TLC R_f= 0.30 (2:1 EtOAc:hexane); HPLC T_r = 22.4 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^1H NMR ($CDCl_3$) δ 7.11-7.36 (m, 5H), 5.12 (d, 1H, J=8.4 Hz), 4.94 (d, 1H, J=7.2 Hz), 4.42 (m, 1H), 3.92 (m, 1H), 3.69 (s,

3H), 3.50-3.72 (m, 2H), 2.70-2.93 (m, 2H), 1.51-1.92 (m, 6H), 1.03-1.51 (m, 5H), 0.77-1.04 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.35, 158.47, 138.08, 129.30, 128.52, 126.46, 64.72, 54.01, 52.24, 51.12, 40.24, 37.55, 34.64, 33,49, 32.52, 26.37, 26.17, 26.00; COSY; HetCor; MS (HR-EI) calc. for $C_{20}H_{30}N_2O_4$ $[M]^+$ 362.2205 found 362.2202.

*[retro-Cha-OMe]Ψ[NHCONH]-4-(benzyl)-phenylalanine alcohol (**3**).*

According to general procedure A, the title urea was prepared on a 0.15 mmol scale: HCl.Cha-OMe (0.15 mmol, 33 mg), phosgene (0.6 mmol, 0.32 mL), $NaHCO_3$ (252 mg, 3.0 mmol), HCl.(benzyl)Phe-ol (0.15 mmol, 42 mg, added as a suspension), CH_2Cl_2 (2 mL total). Flash chromatography (1:1 hexane-EtOAc) afforded urea **3** as a white solid (58 mg, 85%): mp 111-113 °C; TLC R_f = 0.30 (1:1 hexane-EtOAc); HPLC T_r = 27.0 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 6.94-7.36 (m, 14H), 5.40 (d, 1H, J=8.5 Hz), 5.22 (d, 1H, J=8.6 Hz), 4.35 4.56 (m, 3H), 4.06 (m, 1H), 3.89 (s, 2H), 3.59 (s, 3H), 3.30-3.43 (m, 2H), 2.70-2.93 (m, 2H), 1.50-1.85 (m, 6H), 1.04-1.50 (m, 5H), 0.79-1.01 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.32, 157.48, 141.23, 139.08, 138.11, 136.01, 129.62, 128.98, 128.49, 128.45, 127.86, 127.77, 126.08, 73.24, 70.67, 52.12, 51.52, 51.03, 41.62, 40.62, 37.67, 34.09, 33.55, 32.66, 26.48, 26.26, 26.10; MS (HR-EI) calc. for $C_{27}H_{36}N_2O_4$ $[M]^+$ 452.2675 found 452.2655.

*[retro-D-Cha-OMe]Ψ[NHCONH]Phe-ol urea (**8**).*

According to general procedure B, the dipeptidyl urea was prepared on a 0.45 mmol scale: triphosgene (54mg, 0.18 mmol) in CH_2Cl_2 (0.9 mL), HCl.D-Cha-OMe (100mg, 0.450 mmol) in CH_2Cl_2 (1.6 mL) and DIEA (0.172 mL, 0.99 mmol) HCl.Phe-ol (93 mg, 0.495 mmol) in CH_2Cl_2 (0.8 mL) and DIEA (0.172 mL, 0.99 mmol). Flash chromatography (2:1 hexane:EtOAc) furnished 136 mg (83%) of urea **8** as a white solid: mp 84-85 °C; TLC R_f = 0.30 (1:1 EtOAc:hexane); ^{1}H NMR ($CDCl_3$) δ 7.15-7.30 (m, 5H), 5.67 (d, J= 8.4 Hz, 1H), 5.31 (d, J= 8.1 Hz, 1H), 4.42-4.52 (m, 1H), 3.95 (bs, 1H), 3.57-3.87 (m, 1H), 3.70 (s, 3H), 3.30-3.40 (m, 1H), 2.71-2.88 (m, 2H), 1.53-1.80 (m, 6H), 1.36-1.49 (m, 1H), 1.06-1.36 (m, 4H), 0.81-1.01 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 177.01, 158.34, 138.25, 129.42, 128.35, 126.27, 63.96, 53.21, 52.47, 50.88, 40.30, 38.24, 34.06, 33.55, 32.43, 26.36, 26.19, 25.97; MS HR-EI calc. for $C_{20}H_{30}O_4N_2$ $[M+H]^+$ 363.2284 found 363.2287.

*[retro-Cha-OMe]Ψ[NHCONH]D-Phe-ol (**9**).*

According to general procedure B, the dipeptidyl urea was prepared on a 0.165 mmol scale: triphosgene (20 mg, 0.066 mmol) in CH_2Cl_2 (0.4 mL), HCl.Cha-OMe (36 mg, 0.165 mmol) in CH_2Cl_2 (0.6 mL) and DIEA (0.06 mL, 0.363 mmol) H-D-Phe-ol (25 mg, 0.165 mmol) in CH_2Cl_2 (0.3 mL) and DIEA (0.03 mL, 0.182 mmol). Flash chromatography (2:1 hexane:EtOAc) furnished 44 mg (73%) of urea **9** as a white solid: mp 65-67°C; TLC R_f = 0.30 (1:1 EtOAc-hexane); ^{1}H NMR ($CDCl_3$) δ 7.14-7.35 (m, 5H), 5.69 (d, 1H, J=8.1 Hz), 5.34 (d, 1H, J=7.9 Hz), 4.48 (m, 1H), 3.96 (bm, 1H), 3.70 (s, 3H), 3.67 (m, 1H), 3.38 (m, 1H), 2.71-2.95 (m, 2H), 1.05-1.85 (m, 11H), 0.78-1.05 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.95, 158.34, 138.25, 129.41, 128.35, 126.28, 63.94, 53.20, 52.46, 50.89, 40.27, 38.20, 34.07, 33.55, 32.43, 26.36, 26.19, 25.98; MS HR-EI calc. for $C_{20}H_{30}O_4N_2$ $[M+H]^+$ 363.2284 found 363.2286.

*[retro-D-Cha-OMe]Ψ[NHCONH]D-Phe-ol (**10**).*

According to general procedure B, the dipeptidyl urea was prepared on a 0.45 mmol scale: triphosgene (54mg, 0.18 mmol) in CH_2Cl_2 (0.9 mL), HCl.D-Cha-OMe (100mg, 0.450 mmol) in CH_2Cl_2 (1.6 mL) and DIEA (0.172 mL, 0.99 mmol) H-D-Phe-ol (93 mg, 0.495 mmol) in CH_2Cl_2 (0.8 mL) and DIEA (0.172 mL, 0.99 mmol). Flash chromatography (1:1 EtOAc:hexane) furnished 105 mg (64%) of urea **10** as a white solid: mp 95-98°C; TLC R_f = 0.30 (2:1 EtOAc:hexane); ^{1}H NMR ($CDCl_3$) δ 7.16-7.31 (m, 5H), 5.54 (d, J= 8.3 Hz, 1H), 5.38 (d, J= 7.6 Hz, 1H), 4.36-4.48 (m, 1H), 3.90 (bs, 1H), 3.68 (s, 3H), 3.68-3.76 (m, 1H), 3.42-3.56 (m, 1H), 2.73-2.91 (m, 2H), 1.56-1.81 (m, 6H), 1.37-1.49 (m, 1H), 1.25-1.37 (m, 1H), 1.04-1.25 (m, 3H), 0.80-1.00 (m, 2H); ^{1}H NMR ($CDCl_3$) δ 175.38, 158.46,138.09, 129.31, 128.52, 126.46, 64.71, 54.01, 52.24, 51.12, 40.25, 37.55, 34.04, 33.49, 32.52, 26.37, 26.17, 26.00; MS HR-EI calc. for $C_{20}H_{30}O_4N_2$ $[M+H]^+$ 363.2284 found 363.2296.

*[retro-Cha-OMe]Ψ[NHCONH]AHPPA-OMe (**11**).*

According to general procedure A, the urea statine was prepared on a 0.08 mmol scale: HCl.Cha-OMe (0.08 mmol, 18 mg), phosgene (0.31 mmol, 0.11 mL), $NaHCO_3$ (135 mg, 1.6 mmol), HCl.AHPPA-OMe (0.096 mmol, 25mg, added as a suspension), CH_2Cl_2 (1 mL total). Flash column chromatography (3% MeOH-CH_2Cl_2) provided urea **11** as a white solid (30 mg, 86%): mp 88-92°C; TLC R_f = 0.21 (1:1 hexane-EtOAc); HPLC T_r = 24.0 min (gradient: 100% H_2O (0.045 M

TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.17-7.32 (m, 5H), 5.15 (d, J= 9.2 Hz, 1H), 5.08 (d, J= 8.5 Hz, 1H), 4.44-4.54 (m, 1H), 4.00 (bd, J=9.2 Hz, 1H), 3.81-3.91 (m, 1H), 3.72 (s, 3H), 3.66 (s, 3H), 2.83-3.01 (m, 2H), 2.52-2.65 (m, 1H), 2.38-2.46 (m, 1H), 1.56-1.85 (m, 6H), 1.40-1.52 (m, 1H), 1.29-1.39 (m, 1H), 1.07-1.29 (m, 3H), 0.83-1.02 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.9, 174.5, 157.1, 138.6, 129.4, 128.5, 126.4, 67.5, 55.2, 52.2, 51.8, 51.1, 40.6, 38.7, 38.6, 34.1, 33.6, 32.5, 26.4, 26.2, 26.0; COSY; MS (HR-EI) calc. for $C_{23}H_{35}N_2O_6$ $[M+H]^+$ 435.2495 found 435.2505.

*[retro-D-Cha-OMe]Ψ[NHCONH]AHPPA-OMe (**12**).*

According to general procedure A, the urea statine was prepared on a 0.08 mmol scale: HCl.D-Cha-OMe (0.08 mmol, 18 mg), phosgene (0.31 mmol, 0.11 mL), $NaHCO_3$ (135 mg, 1.6 mmol), HCl.AHPPA-OMe (0.096 mmol, 25mg, added as a suspension), CH_2Cl_2 (1 mL total). Flash column chromatography (2:1, hexane-acetone) provided urea **12** as a white solid (33 mg, 95%): mp 83-86˚C; TLC R_f = 0.40 (1:1 hexane-EtOAc); HPLC T_r = 23.8 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.19-7.31 (m, 5H), 5.03 (d, J= 9.1 Hz, 1H), 4.98 (d, J= 8.8 Hz, 1H), 4.42-4.52 (m, 1H), 4.00 (bd, J= 9.0 Hz), 3.81-3.89 (m, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 2.82-3.00 (m, 2H), 2.57-2.65 (m, 1H), 2.35-2.45 (m, 1H), 1.58-1.82 (m, 6H), 1.39-1.50 (m, 1H), 1.07-1.38 (m, 4H), 0.80-1.01 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 175.5, 174.3, 157.4, 138.0, 129.4, 128.5, 126.4, 67.6, 55.0, 52.2, 51.8, 51.1, 41.4, 38.7, 38.5, 34.0, 33.6, 32.5, 26.4, 26.2, 26.0; COSY; MS (HR-EI) calc. for $C_{23}H_{35}N_2O_6$ $[M+H]^+$ 435.2495 found 435.2490.

*[retro-Phe-OMe]Ψ[NHCONH]AHCPA-OMe (**13**).*

According to general procedure A, the urea statine was prepared on a 0.095 mmol scale: HCl.Phe-OMe (0.095 mmol, 21 mg), phosgene (0.37 mmol, 0.14 mL), $NaHCO_3$ (160 mg, 1.9 mmol), HCl.AHCPA-OMe (0.11 mmol, 30 mg, added as a suspension), CH_2Cl_2 (1 mL total). Flash column chromatography (1-3% MeOH-CH_2Cl_2) provided the urea **14** as a white solid (31mg, 83%): mp 110-115˚C; TLC R_f = 0.5 (3% MeOH-CH_2Cl_2); HPLC T_r = 23.6 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.18-7.31 (m, 3H), 7.09-7.14 (m, 2H), 4.90 (bs, 1H), 4.75 (bs, 1H), 4.63 (bs, 1H), 3.95-4.02 (m, 1H), 3,72 (s, 3H), 3.71 (s, 3H), 3.00-3.19 (m, 2H), 2,45-2.50 (m, 2H), 1.77 (bd, J = 13.4 Hz), 1.56-

1.72 (m, 4H), 1.09-1.59 (m, 7H), 0.75-1.01 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 173.91, 173.11, 157.04, 136.27, 129.37, 128.53, 126.98, 69.91,54.07, 52.25, 51.87, 50.97, 40.27, 38.66, 38.52, 34.06, 33.72, 32.92, 26.54, 26.27, 26.14; MS (HR-EI) calc. for $C_{23}H_{35}N_2O_6$ $[M+H]^+$ 435.2495 found 435.2495.

*[retro-D-Phe-OMe]Ψ[NHCONH]AHCPA-OMe (**14**).*

According to general procedure A, the urea statine was prepared on a 0.20 mmol scale: HCl.D-Phe-OMe (0.20 mmol, 43 mg), phosgene (0.80 mmol, 0.4 mL), $NaHCO_3$ (336 mg, 4.0 mmol), HCl.AHCPA-OMe (0.21 mmol, 55 mg, added as a suspension), MeCN (1 mL total). Flash column chromatography (1-3% MeOH-CH_2Cl_2) provided urea **14** as a white solid (58 mg, 67%): mp 125-129°C; TLC R_f = 0.6 (10% MeOH-CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 7.18-7.31 (m, 3H), 7.07-7.13 (m, 2H), 5.04 (d, J= 7.8 Hz, 1H), 4.84 (d, J= 9.4 Hz, 1H), 4.71-4.79 (m, 1H), 3.91-4.02 (m, 1H), 3.77 (bs, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.01-3.17 (m, 2H), 2.41-2.56 (m, 2H), 1.80 (bd, J= 12.9 Hz), 1.55-1.74 (m, 4H), 1.03-1.52 (m, 6H), 0.78-1.04 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.00, 173.22, 157.07, 136.25, 129.39, 128.47, 126.95, 70.11, 54.04,52.19, 51.82, 50.82, 40.34, 38.62, 38.45, 34.24, 33.80, 32.91, 26.54, 26.37, 26.23; MS (HR-EI) calc. for $C_{23}H_{35}N_2O_6$ $[M+H]^+$ 435.2495 found 435.2505.

*[retro-Cha-OMe]Ψ[NHCONH]-4-(benzyl)phenylalanine statine-OMe (**15**).*

According to general procedure A, the urea statine was prepared on a 0.035 mmol scale: HCl.Cha-OMe (0.035 mmol, 8 mg), phosgene (0.14 mmol, 0.08 mL), $NaHCO_3$ (60 mg, 0.72 mmol), HCl.4-(benzyl)phenylalanine statine-OMe (0.036 mmol, 13 mg, as a suspension), MeCN (1 mL total). Flash column chromatography (1-3% MeOH-CH_2Cl_2) provided urea **15** as a white solid (16 mg, 87%): mp 107-110°C; TLC R_f = 0.33 (3% MeOH-CH_2Cl_2); HPLC T_r = 27.8 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.25-7.31 (m, 3H), 7.07-7.21 (m, 6H), 5.05 (d, J= 9.0 Hz, 1H), 4.99 (d, J= 8.6 Hz, 1H), 4.46-4.53 (m, 1H), 3.85-3.94 (m, 1H), 3.93 (s, 2H), 3.71-3.90 (m, 2H), 3.70 (s, 3H), 3.65 (s, 3H), 2.84-2.91 (m, 2H), 2.53-2.63 (m, 1H), 2.38-2.44 (m, 1H), 1.56-1.86 (m,7H), 1.40-1.51 (m, 1H), 1.28-1.40 (m, 1H), 1.09-1.28 (m, 2H), 0.83-1.04 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 174.91, 173.90, 157.44, 141.11, 135.22, 129.47, 129.04, 128.46, 126.05, 67.38, 55.20, 52.23, 51.81, 51.07, 41.58, 40.56, 38.65, 38.11, 34.13; MS FAB$^+$ $C_{30}H_{40}N_2O_6$ $[M+Na]^+$ 547.2, MS (HR-EI) $[M-(OCH_3)]^+$ calc. 492.2624 found 492.2597.

*[retro-Cha-OMe]Ψ[NHCONH]AHPPA-Ala-Iaa (**16**).*

According to general procedure A, the urea statine was prepared on a 0.10 mmol scale: HCl.Cha-OMe (0.10 mmol, 22 mg), phosgene (0..40 mmol, 0.20 mL), $NaHCO_3$ (168 mg, 2.0 mmol), HCl.AHPPA-Ala-Iaa (0.10 mmol, 40 mg, added as a suspension), MeCN (1 mL total). Flash column chromatography (3-10% MeOH-CH_2Cl_2) provided urea **16** as a white solid (39 mg, 70%): mp 82-85°C; TLC R_f = 0.57 (10% MeOH-CH_2Cl_2); HPLC T_r = 26.8 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.34 (d, J= 7.5 Hz, 1H), 7.15-7.27 (m, 5H), 6.35-6.42 (m, 1H), 5.72 (bs, 1H), 5.56-5.67 (m, 1H), 4.36-4.45 (m, 1H), 4.27-4.35 (m, 1H), 4.04 (bs, 1H), 3.73 (s, 3H), 3.53-3.67 (m, 1H), 3.14-3.26 (m, 2H), 2.89-3.07 (m, 2H), 2.56-2.68 (m, 1H), 2.23-2.34 (m, 1H), 1.43-1.88 (m, 9H), 1.27-1.40 (m, 2H), 1.32 (d, J= 7.1 Hz, 3H), 1.10-1.27 (m, 2H), 0.85-0.98 (m, 2H), 0.85-0.89 (overlapping doublets, 6H); ^{13}C NMR ($CDCl_3$) δ 173.25, 172.17, 159.45, 138.99, 129.36, 128.34, 126.19, 69.68, 55.93, 52.38, 51.30, 49.15, 41.28, 39.61, 38.18, 38.00, 34.06, 33.59, 32.36, 26.39, 26.19, 26.02, 25.75, 22.40, 22.36, 17.99; MS FAB$^+$ (3NBA) $C_{30}H_{48}N_4O_6$ $[M]^+$ 561.2; MS (HR-EI) $[M+H-(OCH_3)]^+$ calc. 530.3468 found 530.3497.

*[retro-D-Cha-OMe]Ψ[NHCONH]AHPPA-Ala-Iaa (**17**).*

According to general procedure G, the urea statine was prepared on a 0.27 mmol scale: HCl.D-Cha-OMe (0.27 mmol, 60mg), phosgene (1.06 mmol, 0.55 mL), $NaHCO_3$ (455 mg, 5.4 mmol), HCl.AHPPA-Ala-Iaa (0.27 mmol, 104 mg, added as a suspension), MeCN (3 mL total). Flash column chromatography (3-10% MeOH-CH_2Cl_2) provided urea **17** as a white solid (108 mg, 71%): mp 110-112 ˚C; TLC R_f = 0.42 (10% MeOH-CH_2Cl_2); HPLC T_r = 26.4 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.14-7.36 (m, 6H), 6.23 (bs, 1H), 5.64 (bs, 1H), 4.41 (bs, 1H), 4.25-4.32 (m, 1H), 4.01-4.11 (m, 1H), 3.74 (s, 3H), 3.56 (bs, 1H), 3.14-3.25 (m, 2H), 2.91-3.10 (m, 2H), 2.60-2.72 (m 1H), 2.21-2.33 (m, 1H), 1.44-1.84 (m, 8H), 1.27-1.40 (m, H), 1.31 (d, J= 7.2 Hz, 3H), 1.10-1.28 (m, H), 0.86-0.98 (m, 2H), 0.85-0.91 (overlapping doublets, 6H); COSY; ^{13}C NMR ($CDCl_3$) δ 176.20, 173.32, 172.16, 159.40, 139.04, 129.38, 128.34, 126.19, 69.71, 56.03, 52.40, 51.35, 49.18, 41.34, 39.58, 38.21, 38.03, 34.07, 33.59, 32.36, 26.39, 26.19, 26.02, 25.75, 22.41, 22.35, 17.97; MS FAB$^+$ (3NBA) $C_{30}H_{48}N_4O_6$ $[M]^+$ 561.3; MS (HR-EI) $[M-(OCH_3)]^+$ calc. 529.3390 found 529.3408.

*[retro-Phe-OMe]Ψ[NHCONH]AHCPA-Ala-Iaa (**18**).*

According to general procedure A, the urea statine was prepared on a 0.018 mmol scale: HCl.Phe-OMe (0.018 mmol, 3.9 mg), phosgene (0.10 mmol, 0.05 mL), $NaHCO_3$ (43 mg, 51 mmol), HCl.AHCPA-Ala-Iaa (0.018 mmol, 7 mg, added as a solid), MeCN (1 mL total). Flash column chromatography (3-10% MeOH-CH_2Cl_2) provided urea **18** as a white solid (5.2 mg, 51%): mp 141-144˚C; TLC R_f = 0.35 (10% MeOH-CH_2Cl_2) ^{1}H NMR ($CDCl_3$) δ 7.20-7.35 (m, 3H), 6.41-6.48 (bm, 1H), 5.42-5.60 (bm, 2H), 4.63-4.73 (m, 1H), 4.22-4.34 (m, 1H), 3.94-4.01 (bm, 1H), 3.75 (s, 3H), 3.42-3.51 (m, 1H), 3.29-3.37 (m, 2H), 3.15-3.22 (m, 2H), 2.45-2.56 (m, 1H), 2.14-2.23 (m, 1H), 1.52-1.79 (m, 8H), 1.29-1.44 (m, 2H), 1.09-1.29 (m, 1H), 1.19 (d, J= 7.0 Hz, 3H), 0.68-1.05 (m, 2H), 0.91 (d, J= 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 174.24, 173.05, 171.95, 158.80, 136.43, 129.38, 128.56, 126.97, 77.21, 71.05, 54.38, 52.39, 51.15, 49.15, 41.22,39.22, 38.24, 38.13, 38.03, 34.05, 33.74, 32.83, 26.57, 26.28, 26.14, 25.83, 22.45, 22.42, 17.93; MS FAB$^+$ (3NBA) $C_{30}H_{48}N_4O_6$ $[M+H]^+$ 561.5; MS (HR-EI) $[M-(OCH_3)-(OH)]^+$ calc. 510.3206 found 510.3172.

*[retro-Phe-OMe]Ψ[NHCONH]-4(benyl)-phenylalanine statine-Ala-Iaa (**19**).*

According to general procedure G, the urea statine was prepared on a 0.10 mmol scale: HCl.Cha-OMe (0.022 mmol, 5 mg), phosgene (0.09 mmol, 0.05 mL), $NaHCO_3$ (37 mg, 0.44 mmol), HCl.4-(benzyl)-phenylalanine statine-Ala-Iaa (0.022 mmol, 10 mg added as a suspension), MeCN (1 mL total). Flash column chromatography (3-10% MeOH-CH_2Cl_2) provided urea **19** as a white solid (8.8 mg, 62%): mp 151-154˚C; TLC R_f = 0.47 (10% MeOH-CH_2Cl_2); HPLC T_r = 29.8 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.05-7.20 (m, 5H), 7.22-7.30 (m, 4H); 6.08 (bs, 1H), 5.55 (bs, 2H), 4.31-4.43 (m, 1H), 4.21-4.30 (m, 1H), 4.01-4.08 (m, 1H), 3.93 (s, 2H), 3.73 (s, 3H), 3.47-3.53 (m, 1H), 3.14-3.30 (m, 3H), 2.91-3.07 (m, 2H), 2.61-2.69 (m, 1H), 2.23-2.39 (m, 2H), 1.48-1.80 (m, 8H), 1.07-1.47 (m, 12H), 0.84-0.95 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 174.19, 173.42, 172.16, 159.53, 141.19, 138.90, 136.76, 129.46, 129.09, 128.94, 128.88, 128.43, 128.33, 126.01, 124.52, 119.07, 69.61, 56.10, 52.43, 51.36,49.17, 41.56, 41.32, 39.55, 38.21, 38.03, 34.09, 33.57, 32.38, 26.38, 26.19, 26.02, 25.83, 25.70, 22.41, 22.33, 17.90; MS FAB$^+$ (3NBA) $C_{37}H_{54}N_4O_6$ $[M+H]^+$ 651.4; MS (HR-EI) $[M-(OCH_3)-(OH)]^+$ calc. 600.3675 found 600.3692.

*[retro-Phe-OMe]Ψ[NHCONH]Sta -Ala-Iaa (**20**).*

According to general procedure A, the urea statine was prepared on a 0.05 mmol scale: HCl.Phe-OMe (0.05 mmol, 11 mg), phosgene (0.20 mmol, 0.11 mL), $NaHCO_3$ (84 mg, 1.0 mmol), HCl.Sta-Ala-Iaa (0.057 mmol, 20 mg, added as a suspension), MeCN (1 mL total). Flash column chromatography (5% MeOH-CH_2Cl_2) provided the urea **20** as a white solid (16 mg, 64%): mp 73-75˚C; TLC R_f = 0.35 (10% MeOH-CH_2Cl_2); HPLC T_r = 22.8 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.20-7.31 (m, 3H), 7.12-7.19 (m, 2H), 6.77 (bs, 1H), 5.72 (bs, 1H), 5.62 (bs, 1H), 4.65-4.75 (m, 1H), 4.25-4.37 (m, 1H), 3.93-4.02 (m, 1H), 3.73 (s, 3H), 3.46 (bm, 1H), 3.17-3.28 (m, 2H), 2.96-3.14 (m, 2H), 2.40-2.52 (m, 1H), 2.15-2.28 (m, 1H), 1.52-1.67 (m, 3H), 1.24-1.43 (m, 3H), 1.21 (d, J=7.1 Hz, 3H), 0.82-0.94 (overlapping doublets, 12H); ^{13}C NMR ($CDCl_3$) δ 174.32, 173.16, 171.92,158.90, 136.33, 129.38, 128.58, 126.99, 71.05, 54.34, 52.43, 51.99, 49.17, 41.25, 38.27, 38.09, 29.71, 25.84, 24.73, 23.14, 22.44, 22.10, 17.84; MS FAB$^+$ (3NBA) $C_{27}H_{44}N_4O_6$ $[M]^+$ 521.3; MS (HR-EI) $[M-(OCH_3)]^+$ calc. 489.3077 found 489.3032.

*[retro-D-Phe-OMe]Ψ[NHCONH]Sta-Ala-Iaa (**21**).*

According to general procedure A, the urea statine was prepared on a 0.10 mmol scale: HCl.D-Phe-OMe (0.10 mmol, 22 mg), phosgene (0.40 mmol, 0.22 mL), $NaHCO_3$ (170 mg, 2.0 mmol), HCl.Sta-Ala-Iaa (0.11 mmol, 40 mg, added as a suspension), MeCN (1 mL total). Flash column chromatography (5-10% MeOH-CH_2Cl_2) provided the urea **21** as a white solid (31 mg, 61%): mp 164-166˚C; TLC R_f = 0.31 (10% MeOH-CH_2Cl_2); HPLC T_r = 24.0 min (gradient: 100% H_2O (0.045 M TFA), to 20% H_2O (0.045 M TFA), 80% MeCN (0.368 M TFA) over 30 min); ^{1}H NMR ($CDCl_3$) δ 7.20-7.32 (m, 3H), 7.15 (bd, J= 7.2 Hz, 2H), 6.84 (d, J= 7.9 Hz, 1H), 5.66 (d, J= 7.1 Hz, 1H), 5.33 (d, J= 9.6 Hz, 1H), 4.59-4.68 (m, 1H), 4.33-4.44 (m, 1H), 4.08 (bs, 1H), 4.00 (bs, 1H), 3.69 (s, 3H), 3.11-3.24 (m, 2H), 2.97-3.11 (m, 2H), 2.41 (d, J= 6.2 Hz, 2H), 1.49-1.66 (m, 3H), 1.20-1.42 (m, 3H), 1.33 (d, J= 7.2 Hz, 3H), 0.88 (overlapping doublets, J= 6.5 Hz, 12H); ^{13}C NMR ($CDCl_3$) δ 174.09, 172.49, 171.49, 158.20, 136.24, 129.22, 128.60, 127.07, 71.12, 54.43, 52.22, 50.90, 49.39,41.56, 40.89, 38.18, 37.96, 25.88, 24.82, 23.20, 22.48, 22.13, 18.30;MS FAB$^+$ (3NBA) $C_{27}H_{48}N_4O_6$ $[M]^+$ 521.3; MS (HR-EI) $[M-OCH_3]^+$ calc. 489.3077 found 489.3046.

*HCl.H-p(I)-Phe-ol (**6**):*

To a solution of Boc-p(I)-Phe-OMe (**5**, 1.72 g, 4.25 mmol) in THF (11 mL) and anhydrous EtOH (28 mL) was added $LiBH_4$ (2.0 M in THF, 2.4 eq, 5.1 mL, 10.2 mmol) slowly (10 minutes). After 2 hours the reaction mixture was diluted with water (50 mL) and EtOAc (100 mL). The layers were separated and the aqueous layer was further extracted with EtOAc (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Flash column chromatography (1:1 hexane:EtOAc) afforded 1.56 g (97%) of Boc-p(I)Phe-ol as a white powder: mp 126-130°C; TLC R_f = 0.27 (1:1 hexane:EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 2H, J= 8.2Hz), 6.88 (d, 2H, J=8.2 Hz), 4.75 (bm, 1H), 3.86, (bs, 1H), 3.53-3.77 (m, 2H), 2.78-2.85 (m, 2H), 2.20 (bs, 1H), 1.42 (s, 9H); MS (EI/HR) calc. for $C_{14}H_{20}NO_3I$ $[M]^+$ 377.0490 found 377.0487. According to general procedure C, Boc-p(I)-Phe-ol (1.56 g), $PdCl_2(PPh_3)_2$ (290 mg), DIBAL (0.91 mL) and BnMgBr (3.3 eq, 6.83 mL) afforded 1.29 g (91%) of coupled product, Boc-p(benzyl)Phe-ol, as a white solid after flash column chromatography (40% EtOAc-hexane) and recrystallization (hexane-ether): mp 82-83°C; TLC R_f = 0.27 (40% EtOAc-hexane); ^{1}H NMR ($CDCl_3$) δ 7.03-7.31 (m, 9H), 4.79 (bd, 1H, J=6.5 Hz), 3.94 (s, 2H), 3.84 (bm, 1H), 3.62 (bm, 1H), 3.54 (bm, 1H), 2.81 (d, 2H, J=7.1 Hz), 2.57 (bs, 1H), 1.40 (s, 9H); ^{13}C ($CDCl_3$) δ 156.19, 141.12, 139.38, 135.50, 129.42, 129.10, 128.92, 128.48, 126.09, 79.76, 64.30, 53.73, 41.56, 37.09, 28.56; MS (EI/HR) calc for $C_{21}H_{27}NO_3$ $[M+H]^+$ 341.1991 found 341.1991. According to general procedure D, Boc-p(benzyl)Phe-ol (210 mg, 0.62 mmol) and 10 mL of 4N HCl in dioxane afforded a white solid which was recrystallized from ether/hexane/methanol to give 138 mg of **6** (81%); mp 188 192°C.