Supplementary Material (ESI) for Chemical Communications

A new class of arginine analogues with an improved anion binding site in the side chain

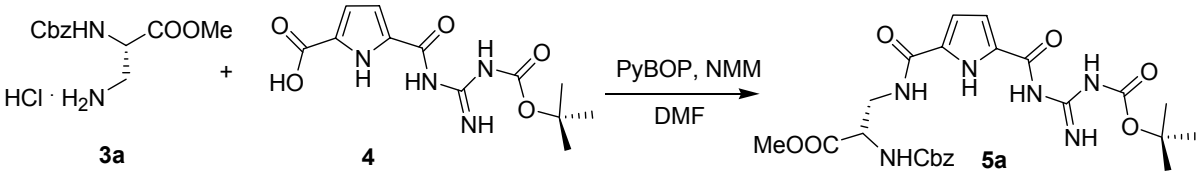
Carsten Schmuck* and Lars Geiger.

Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany.

Supporting Information

General Information: Reaction solvents were dried and distilled under argon before use. All other reagents were used as obtained from either Aldrich or Fluka. The ^{1}H NMR spectra were recorded at 400 MHz or 600 MHz, and the ^{13}C NMR spectra were recorded at 100 MHz or 150 MHz at room temperature, using [D6]DMSO or $CDCl_3$ as solvent. The chemical shifts are relative to the solvent signals. The apparent coupling constants are given in Hertz. The description of the fine structure means: s = singulett, br.s = broad singulett, d = dublett, t = triplett, q = quartett, m = multiplett, br = broad signal. Peak assignments are based on either DEPT, 2D NMR studies and/or comparison with literature data. All IR spectra were measured as KBr-pellets or thin films on a PerkinElmer FT-IR 1600 spectrophotometer. The maxima are classified in three intensities: s (strong), m (middle), w (weak) and are reported in cm^{-1}. Melting points were measured in open end glas capillary tubes and are uncorrected.

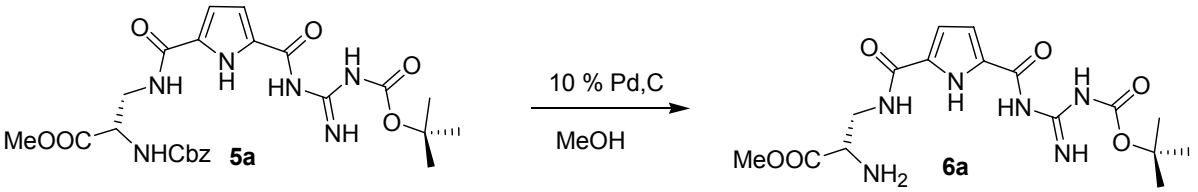
Arginine-analogue 2a (n = 1):



$C_{12}H_{17}ClN_2O_4$ 288.73 | $C_{12}H_{16}N_4O_5$ 296.28 | $C_{24}H_{30}N_6O_8$ 530.53

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **4** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **3a** (2.15 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with diethyl ether (3 x 100 ml). The combined organic phases were washed with water (100 ml), brine (100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 7/3) to yield a colorless solid (3.40 g, 95 %).

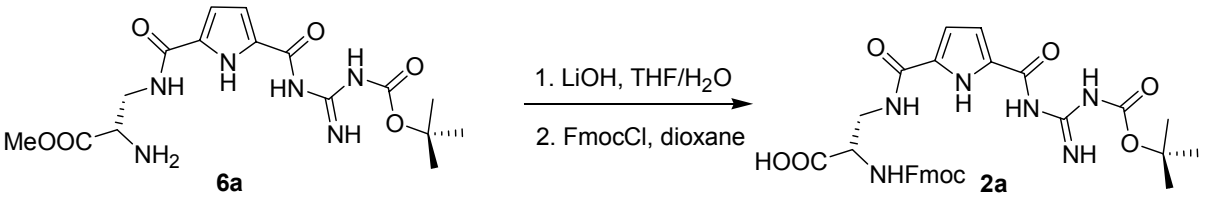
mp: 112 °C; R_f: 0.64 (SiO_2, dichloromethane/acetone = 7/3); ^{1}H NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, C*H*$_3$), 3.49-3.57 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 4.29 (q, 1H, C*H*), 5.03 (d, 2H, C*H*$_2$), 6.74-6.76 (m, 1H, pyrrole-C*H*), 6.81 (br.s, 1H, pyrrole-C*H*) 7.33 (br.s, 5H, benzyl-C*H*), 7.73 (d, J = 7.93 Hz, 1H, N*H*), 8.46-8.51 (m, 1H, N*H*), 8.55 (br.s, 1H, N*H*), 9.32 (br.s, 1H, N*H*), 10.84 (br.s, 1H, N*H*), 11.32 (br.s, 1H, N*H*); ^{13}C NMR (100MHz, [D_6]DMSO) δ = 27.9 (Boc-*C*H$_3$), 48.8 (*C*H$_2$), 52.2 (*C*H$_3$), 54.0 (*C*H), 65.8 (*C*H$_2$), 81.1 (*Cq*), 112.3 (pyrrole-*C*H), 113.8 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.2 (aryl-*Cq*), 128.4 (pyrrole-*Cq*), 128.5 (aryl-*C*H), 136.9 (pyrrole-*Cq*), 156.1 (*Cq*), 156.2 (*Cq*), 158.6 (*Cq*), 160.3 (*Cq*), 171.2 (*Cq*), (*Cq* of the guanidino group could not be detected); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3386 [m], 2978 [w], 1726 [s], 1636 [s], 1557 [s], 1470 [m], 1301 [s], 1241 [s], 1148 [s], 1051 [m], 842 [w], 780 [w], 755 [w], 697 [w]; HR-MS (ESI) m/z = 553.202 ± 0.005 (calculated for $^{12}C_{24}H_{30}N_6O_8$ + Na: 553.201)



$C_{24}H_{30}N_6O_8$
530.53

$C_{16}H_{24}N_6O_6$
396.40

A mixture of Cbz-protected amine **5a** (1.88 g, 3.55 mmol) and 10 % Pd/C (180 mg) in methanol (140 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **6a** as a colorless solid (1.33 g, 95%).



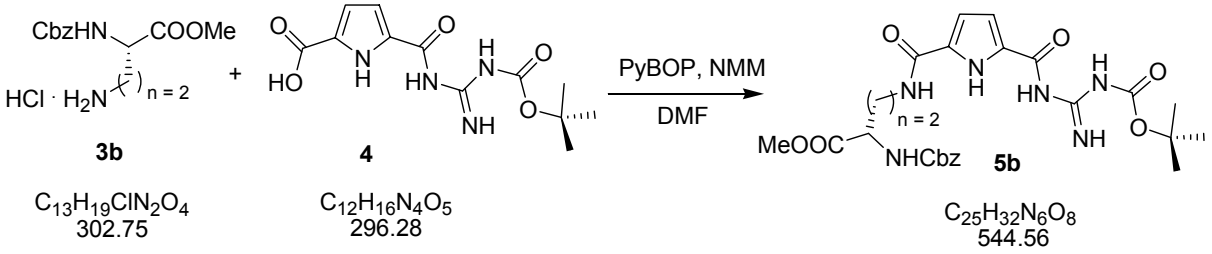
$C_{16}H_{24}N_6O_6$
396.4

$C_{30}H_{32}N_6O_8$
604.61

A mixture of the methyl ester **6a** (500 mg, 1.26 mmol, 1 eq.) and lithium hydroxide monohydrate (79.0 mg, 1.5 eq.) in THF/water (10 ml, 4/1) was stirred for 90 min until hydrolysis was complete (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylised. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and a solution of Fmoc-Cl (326 mg) in dioxane (10 ml) was added at 0 °C. The solution was stirred for additional 90 min at room temperature, the solution lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (472 mg, 62 %).

For analytical purposes a small amount was deprotected and converted into the picrate salt:
mp: 154 °C (picrate salt); R_f: 0.40 (SiO_2, dichlormethane/methanol = 9/1); ^{1}H NMR (400MHz, [D$_6$]DMSO, picrate salt) δ = 3.45-3.52 (m, 1H, C*H*$_2$), 3.64-3.75 (m, 1H, C*H*$_2$), 4.13-4.34 (m, 4H, fluorenyl-C*H*$_2$ and 2C*H*), 6.84-6.89 (m, 1H, pyrrole-C*H*), 7.00-7.05 (m, 1H, pyrrole-C*H*), 7.26-7.34 (m, 2H, fluorenyl-C*H*), 7.38-7.41 (m, 2H, fluorenyl-C*H*), 7.64-7.70 (m, 3H, 2 fluorenyl-C*H*´s and 1 N*H*), 7.87-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.58 (s, 3H, picrate-C*H* and N*H*), 10.93 (br.s, 1H, N*H*), 12.43 (br.s, 1H, pyrrole-N*H*), (the COO*H* could not be detected); ^{13}C NMR (100MHz, [D$_6$]DMSO, picrate-salt) δ = 40.4 (*C*H$_2$), 46.8 (fluorenyl-*C*H), 53.8 (amino acid-*C*H), 65.9 (fluorenyl-*C*H$_2$), 112.9 (pyrrole-*C*H), 115.5 (pyrrole-*C*H), 120.3 (fluorenyl-*CH*), 124.4 (picrate-*Cq*), 125.3 (picrate-*C*H), 125.6 (pyrrole-*Cq*), 127.2 (fluorenyl-*C*H), 127.3 (fluorenyl-*C*H), 127.8 (fluorenyl-*C*H), 132.6 (pyrrole-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 143.9 (fluorenyl-*Cq*), 154.9 (picrate *Cq*), 156.2 (*Cq*), 159.6 (*Cq*), 159.7 (*Cq*), 160.9 (*Cq*), 172.1 (*C*OOH); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] (picrate salt) = 3375 [br.m], 3202 [br.m], 1701 [s], 1632 [s], 1605 [s], 1550 [s], 1330 [m], 1273 [s], 742 [w]; HR-MS (ESI) m/z = 505.181 ± 0.005 (calculated for $^{12}C_{25}H_{25}N_6O_6$: 505.184) (guanidinium cation)

Arginine-analogue 2b (n = 2):



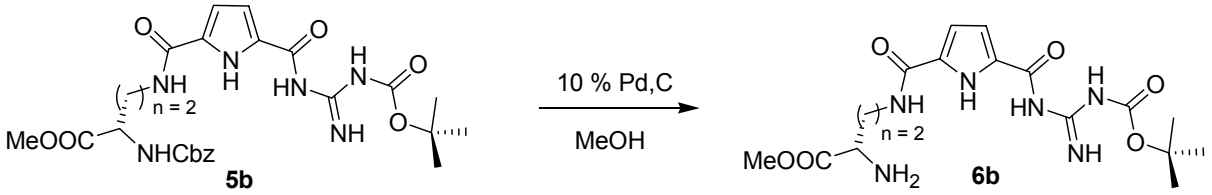
3b $C_{13}H_{19}ClN_2O_4$ 302.75

4 $C_{12}H_{16}N_4O_5$ 296.28

5b $C_{25}H_{32}N_6O_8$ 544.56

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **4** (1.50 g, 5.06 mmol, 1 eq.), PyBOP (2.63 g, 5.57 mmol, 1.1 eq.) and N-methyl morpholine (2 ml) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **3b** (1.69 g, 5.57 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with ethyl acetate (3 x 60 ml). The combined organic phases were washed with brine (100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 7/3) to yield a colorless solid (1.79 g, 3.30 mmol, 65 %).

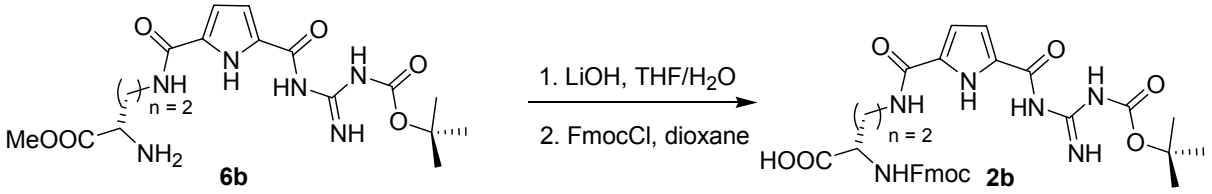
mp: 112 °C; R_f: 0.64 (SiO_2, dichlormethane/acetone = 7/3); ^{1}H NMR (400MHz, [D_6]DMSO) δ = 1.45 (s, 9H, C*H*$_3$), 1.77-1.87 (m, 1H, C*H*$_2$), 1.95-2.03 (m, 1H, C*H*$_2$), 3.25-3.32 (m, 2H, C*H*$_2$), 3.61 (s, 3H, C*H*$_3$), 4.09-4.15 (m, 1H, C*H*), 5.03 (d, J = 4.2 Hz, 2H, C*H*$_2$), 6.74-6.75 (m, 1H, pyrrole-C*H*), 6.76 (br.s, 1H, pyrrole-C*H*), 7.32-7.38 (m, 5H, benzyl-C*H*), 7.78 (d, J = 7.7 Hz, 1H, N*H*), 8.35 (t, J = 4.8 Hz, 1H, N*H*), 8.52 (br.s, 1H, N*H*), 9.30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.14 (br.s, 1H, N*H*); ^{13}C NMR (100MHz, [D_6]DMSO) δ = 27.9 (Boc-*C*H$_3$), 30.6 (C*H*$_2$), 35.8 (*C*H$_2$), 51.9 (*C*H), 52.1 (*C*H$_3$), 65.7 (*C*H$_2$), 79.3 (Boc-*Cq*), 112.0 (pyrrole-*C*H), 113.1 (pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.5 (aryl-*C*H), 128.6 (pyrrole-*Cq*), 128.9 (pyrrole-*Cq*), 137.0 (aryl-*Cq*), 156.1 (*Cq*), 156.3 (*Cq*), 159.7 (*Cq*), 159.8 (*Cq*), 171.9 (*Cq*), (*Cq* of the guanidino group could not be detected); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3380 [br.m], 3280 [br.m], 2928 [m], 1730 [s], 1635 [s], 1556 [s], 1468 [m], 1240 [s], 1149 [s], 842 [w], 755 [w]; HR-MS (ESI) m/z = 567.218 ± 0.005 (calculated for $^{12}C_{25}H_{32}N_6O_8 + Na^+$: 567.218)



5b $C_{25}H_{32}N_6O_8$ 544.56

6b $C_{17}H_{26}N_6O_6$ 410.42

A mixture of Cbz-protected amine **5b** (1.00 g, 1.84 mmol) and 10 % Pd/C (100 mg) in methanol (100 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **6b** as a colorless solid (680 mg, 90 %).



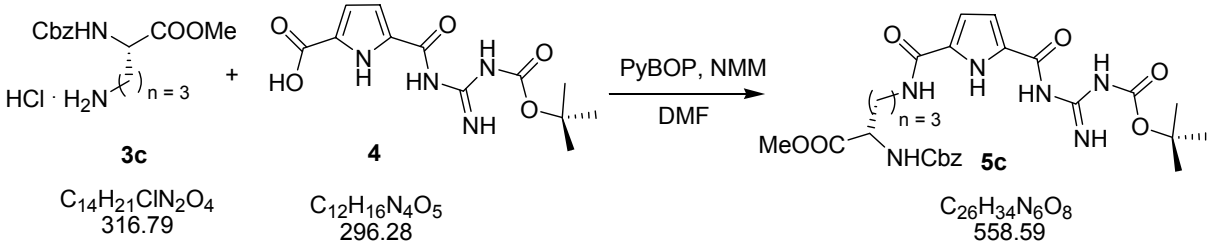
6b $C_{17}H_{24}N_6O_6$ 410.43

2b $C_{31}H_{34}N_6O_8$ 618.65

A mixture of the methyl ester **6b** (560 mg, 1.36 mmol, 1 eq.) and lithium hydroxide monohydrate (86.0 mg, 1.5 eq.) in THF/water (10 ml, 4/1) was stirred for 90 min until completion. The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (15 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (353 mg) in dioxane (15 ml) was added. The solution was stirred for additional 90 min at room temperature, the solution was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (563 mg; 67 %).

For analytical purposes a small amount was deprotected and converted into the picrate salt: mp: 200 °C (decomposition); ^{1}H NMR (400MHz, [D$_6$]DMSO, picrate salt) δ = 1.78-1.89 (m, 1H, C*H*$_2$), 1.98-2.07 (m, 1H, C*H*$_2$), 3.27-3.37 (m, 2H, C*H*$_2$), 4.00-4.06 (m, 1H, CH), 4.18-4.34 (m, 3H, fluorenyl-C*H*$_2$ and C*H*), 6.84-6.89 (m, 1H, pyrrole-C*H*), 6.99-7.03 (m, 1H, pyrrole-C*H*), 7.29-7.35 (m, 2H, fluorenyl-C*H*), 7.37-7.44 (m, 2H, fluorenyl-C*H*), 7.67-7.77 (m, 2H, fluorenyl-C*H*), 7.86-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.45 (br.s, 1H, C*H*), 8.58 (s, 3H, picrate-C*H* and N*H*), 10.93 (br.s, 1H, N*H*), 12.36 (br.s, 1H, pyrrole-N*H*), the COO*H* could not be detected; ^{13}C NMR (100MHz, [D$_6$]DMSO) δ = 30.6 (*C*H$_2$), 36.3 (*C*H$_2$), 46.9 (fluorenyl-*C*H), 51.9 (amino acid-*C*H), 65.9 (fluorenyl-*C*H$_2$), 112.6 (pyrrole-*C*H), 115.6 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.7 (picrate-*Cq*), 125.4 (picrate-*C*H), 125.5 (pyrrole-*Cq*), 127.3 (fluorenyl-*C*H), 127.8 (2 x fluorenyl-*C*H), 132.9 (pyrrole-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 154.9 (picrate-*Cq*), 156.4 (*Cq*), 159.4 (*Cq*), 159.6 (*Cq*), 160.8 (*Cq*), 173.9 (*C*OOH); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] (picrate salt) = 3360 [br.m], 3200 [br.m], 1701 [s], 1634 [s], 1600 [s], 1530 [s], 1330 [m], 1273 [s]; HR-MS (ESI) m/z = 519.198 ± 0.005 (calculated for $^{12}C_{26}H_{27}N_6O_6$: 519.199) (guanidinium cation)

Arginine-analogue 2c (n = 3):



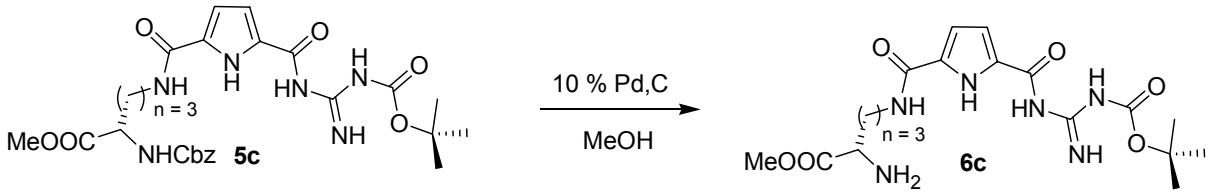
3c $C_{14}H_{21}ClN_2O_4$ 316.79

4 $C_{12}H_{16}N_4O_5$ 296.28

5c $C_{26}H_{34}N_6O_8$ 558.59

A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **4** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **3c** (2.46 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic phases were washed with water (100 ml), brine (2 x 100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 1/1) to yield a colorless solid (2.98 g, 79 %).

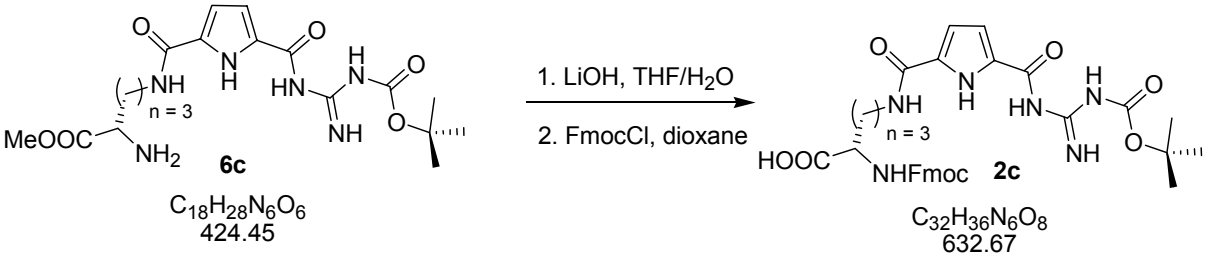
mp: 95-97 °C; R_f: 0.65 (SiO_2, dichloromethane/acetone = 1/1); ^{1}H NMR (400MHz, [D$_6$]DMSO) δ = 1.45 (s, 9H, C*H$_3$*), 1.57-1.67 (m, 4H, C*H$_2$*), 3.18-3.25 (m, 2H, C*H$_2$*), 3.62 (s, 3H, C*H$_3$*), 4.03-4.09 (m, 1H, C*H*), 5.02 (s, 2H, C*H$_2$*), 6.74-6.76 (m, 1H, pyrrole-C*H*), 6.81 (br.s, 1H, pyrrole-C*H*), 7.28-7.37 (m, 5H, benzyl-C*H*), 7.75 (d, J = 7.8 Hz, 1H, N*H*), 8.30 (t, J = 5.4 Hz, 1H, N*H*), 8.54 (br.s, 1H, N*H*), 9.30 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*); ^{13}C NMR (100MHz, [D$_6$]DMSO) δ = 27.9 (^{t}Boc-*C*H$_3$), 29.7 (*C*H$_2$), 32.3 (*C*H$_2$), 38.2 (*C*H$_2$), 52.0 (*C*H$_3$), 53.9 (*C*H), 65.7 (*C*H$_2$), 81.1 (*Cq*), 111.7 (pyrrole-*C*H), 113.1 (pyrrole-C*H*), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.1 (pyrrole-*Cq*), 128.5 (aryl-*C*H), 137.1 (pyrrole-*Cq*), 156.3 (*Cq*), 158.4 (*Cq*), 158.5 (*Cq*), 159.7 (*Cq*), 173.0 (*Cq*), (*Cq* of the guanidino group could not be detected); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3390 [br.m], 2955 [br.m], 1732 [s], 1635 [s], 1530 [s], 1470 [m], 1241 [s], 1155 [s], 842 [w]; HR-MS (ESI): m/z = 581.233 ± 0.005 (calculated for $^{12}C_{26}H_{35}N_6O_8$ + Na$^+$: 581.234)



5c $C_{26}H_{34}N_6O_8$ 558.58

6c $C_{18}H_{28}N_6O_6$ 422.45

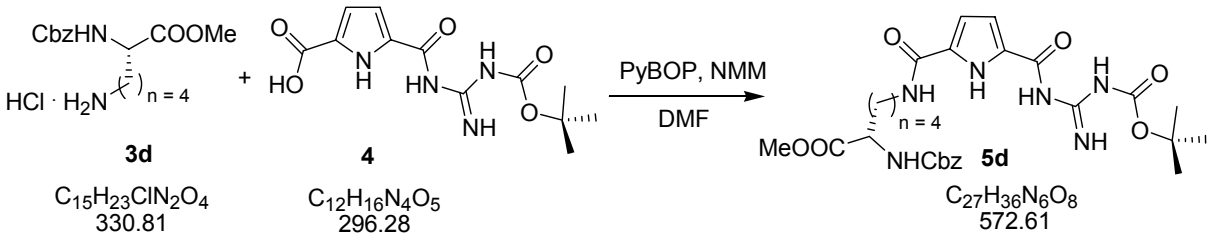
A mixture of Cbz-protected amine **5c** (600 mg, 1.07 mmol) and 10 %-Pd/C (60 mg) in methanol (100 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **6c** as a pale green solid (407 mg, 90 %).



A mixture of the methyl ester **6c** (500 mg, 1.18 mmol, 1 eq.) and lithium hydroxide monohydrate (74.0 mg, 1.5 eq.) in a THF/water mixture (10 ml, 4/1) was stirred for 90 min until complete hydrolysis (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (305 mg, 1.18 mmol, 1 eq.) in dioxane (10 ml) was added. The solution was stirred for additional 90 min at room temperature, the mixture was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (507 mg, 68 %).

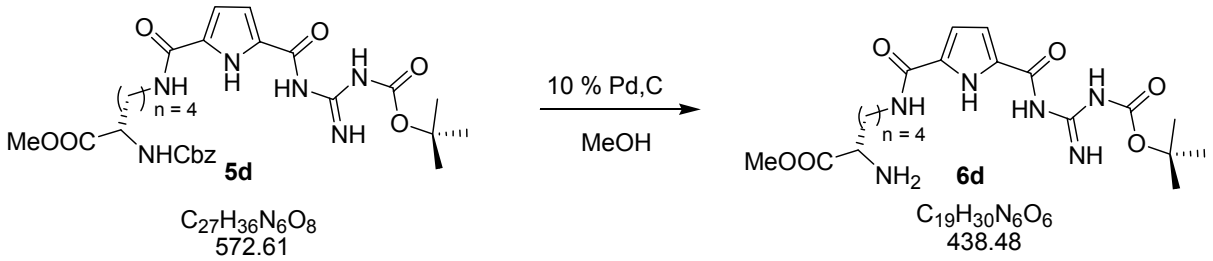
For analytical purposes a small amount was deprotected and converted into the picrate salt: mp: 210-220 °C (decomposition) (picrate salt); R_f: 0.50 (SiO_2, dichlormethane /methanol = 9/1); ^{1}H NMR (400MHz, [D$_6$]DMSO, picrate salt) δ = 1.49-1.82 (m, 4H, C*H*$_2$), 3.23-3.28 (m, 2H, C*H*$_2$), 3.94-4.00 (m, 1H, C*H*), 4.19-4.24 (m, 1H, C*H*) 4.27-4.28 (m, 2H, C*H*$_2$), 6.86-4.88 (m, 1H, pyrrole-C*H*), 7.02-7.03 (m, 1H, pyrrole-C*H*), 7.29-7.33 (m, 2H, fluorenyl-C*H*), 7.37-7.42 (m, 2H, fluorenyl-C*H*), 7.60-7.70 (m, 3H, fluorenyl-C*H* and N*H*), 7.86-7.88 (m, 2H, fluorenyl-C*H*), 8.15 (br.s, 4H, guanidinium-N*H*$_2$), 8.42 (t, J = 5.18 Hz, 1H, N*H*), 8.58 (s, 2H, picrate-C*H*), 10.93 (br.s, 1H, NH), 12.34 (br.s, 1H, pyrrole-NH), the COO*H* could not be detected; ^{13}C NMR (100MHz, [D$_6$]DMSO, picrate salt) δ = 26.1 (*C*H$_2$), 28.5 (*C*H$_2$), 38.5 (*C*H$_2$), 46.8 (fluorenyl-*C*H), 53.8 (amino acid-*C*H), 65.8 (fluorenyl-*C*H$_2$), 112.4 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.4 (picrate-*Cq*), 125.4 (picrate-*C*H), 125.4 (pyrrole-*Cq*), 127.2 (fluorenyl-*C*H), 127.8 (fluorenyl-*C*H), 133.0 (pyrrole-*Cq*), 140.8 (fluorenyl-*Cq*), 140.9 (fluorenyl-*Cq*), 142.0 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 144.1 (fluorenyl-*Cq*), 155.0 (picrate-*Cq*), 156.3 (*Cq*), 159.2 (*Cq*), 159.6 (*Cq*), 161.0 (*Cq*), 174.0 (*C*OOH); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3375 [br.m], 2965 [br.m], 1720 [s], 1620 [s], 1541 [s], 1271 [s], 742 [w]; HR-MS (ESI): /z = 533.216 ± 0.005 (calculated for $^{12}C_{27}H_{29}N_6O_6$: 533.215) (guanidinium cation)

Arginine-analogue 2d (n = 4):

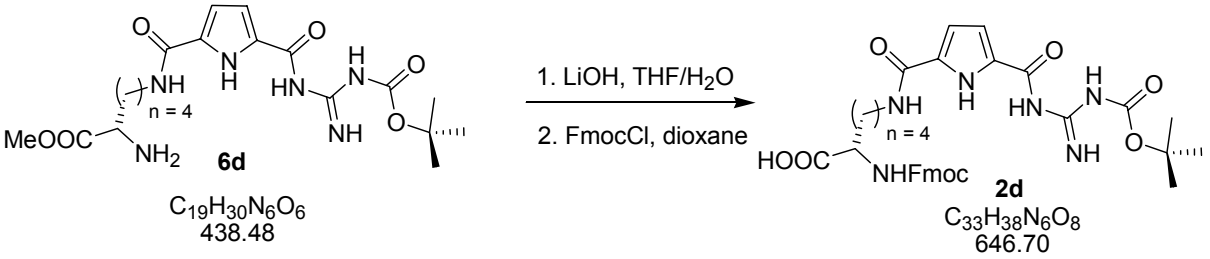


A mixture of the ^{t}Boc-protected guanidiniocarbonyl pyrrole compound **4** (2.00 g, 6.76 mmol, 1 eq.), PyBOP (3.52 g, 6.76 mmol, 1 eq.) and N-methyl morpholine (2 ml, 18.18 mmol) in DMF (40 ml) was stirred at room temperature for 15 min. The amino acid **3d** (2.46 g, 7.43 mmol, 1.1 eq.) was added and the solution was stirred over night. The mixture was hydrolyzed with water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic phases were washed with water (100 ml), brine (2 x 100 ml), dried (Na_2SO_4) and evaporated *in vacuo*. The yellow residue was purified by column chromatography (SiO_2, dichloromethane/acetone = 1/1) to yield a colorless solid (3.10 g, 80 %).

mp: 78 °C; R_f: 0.65 (SiO_2, dichloromethane/acetone = 1/1); ^{1}H NMR (400MHz, [D_6]DMSO) δ = 1.30-1.75 (m, 6H, C*H*$_2$), 1.44 (s, 9H, C*H*$_3$), 3.17-3.22 (m, 2H, C*H*$_2$), 3.62 (s, 3H, C*H*$_3$), 3.98-4.05 (m, 1H, C*H*), 5.02 (s, 2H, C*H*$_2$), 6.74-6.76 (m, 1H, pyrrole-N*H*), 6.81 (br.s, 1H, pyrrol-C*H*) 7.29-7.36 (m, 5H, benzyl-C*H*), 7.70 (d, J = 7.83 Hz, 1H, N*H*), 8.29 (t, J = 5.43 Hz, 1H, N*H*), 8.55 (br.s, 1H, N*H*), 9.31 (br.s, 1H, N*H*), 10.83 (br.s, 1H, N*H*), 11.16 (br.s, 1H, pyrrole-N*H*); ^{13}C NMR (100MHz, [D_6]DMSO) δ = 23.1 (CH_2), 27.9 (^{t}Boc-*C*H$_3$), 28.8 (*C*H$_2$), 30.5 (*C*H$_2$), 38.5 (*C*H$_2$), 51.9 (*C*H$_3$), 54.0 (*C*H), 65.6 (*C*H$_2$), 81.1 (*Cq*), 111.7 (2 x pyrrole-*C*H), 127.9 (aryl-*CH*), 128.0 (aryl-*C*H), 128.3 (pyrrole-*Cq*), 128.5 (aryl-*C*H), 137.1 (pyrrole-*Cq*), 156.1 (*Cq*), 158.5 (*Cq*), 158.6 (*Cq*), 159.7 (*Cq*), 173.1 (*Cq*), (*Cq* of the guanidino group could not be detected); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3384 [br.m], 3268 [br.m], 2928 [m], 1727 [s], 1635 [s], 1556 [s], 1467 [m], 1241 [s], 1149 [s], 842 [w], 754 [w], 697 [w]; HR-MS (ESI): m/z = 573.267 ± 0.005 (calculated for $^{12}C_{27}H_{36}N_6O_8 + H^+$: 573.267)



A mixture of Cbz-protected amine **5d** (2.00 g, 3.49 mmol) and 10 %-Pd/C (200 mg) in methanol (160 ml) was vigorously stirred for 6 h at 40 °C under hydrogen atmosphere. The catalyst was filtered off through a Celite pad and washed with methanol. The filtrate and washings were combined and evaporated to give the amine compound **5d** as a pale green solid (1.33 g, 95%).



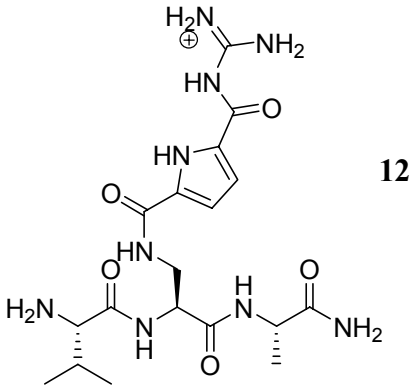
6d
$C_{19}H_{30}N_6O_6$
438.48

2d
$C_{33}H_{38}N_6O_8$
646.70

A mixture of the methyl ester **6d** (500 mg, 1.14 mmol, 1 eq.) and lithium hydroxide monohydrate (71.0 mg, 1.5 eq.) in a THF/water mixture (10 ml, 4/1) was stirred for 90 min until complete hydrolysis (tlc). The solution was neutralized with hydochloric acid (5 %) and lyophylized. The residue was suspended in an aqueous Na_2SO_4 solution (10 ml, 10 %) and at 0 °C a solution of Fmoc-Cl (295 mg, 1.14 mmol, 1 eq.) in dioxane (10 ml) was added. The solution was stirred for additional 90 min at room temperature, the mixture was lyophylized and the product purified by column chromatography (SiO_2, dichloromethane/methanol = 9/1) yielding a colorless powder (486 mg, 66 %).

For analytical purposes a small amount was deprotected and converted into the picrate salt:
mp: 220-230 °C (decomposition) (picrate salt); ^{1}H NMR (400MHz, [D$_6$]DMSO, picrate salt) δ = 1.28-1.78 (m, 6H, C*H*$_2$), 3.23-3.24 (m, 2H, C*H*$_2$), 3.89-3.96 (m, 1H, C*H*), 4.17-4.28 (m, 3H, C*H* and C*H*$_2$), 6.84-4.86 (m, 1H, pyrrole-C*H*), 7.01-7.02 (m, 1H, pyrrole-C*H*), 7.28-7.32 (m, 2H, fluorenyl-C*H*), 7.38-7.41 (m, 2H, fluorenyl-C*H*), 7.60-7.62 (m, 1H, fluorenyl-C*H*), 7.69-7.71 (m, 1H, fluorenyl-C*H*), 7.86-7.87 (m, 2H, fluorenyl-C*H*), 8.18 (br.s, 4H, guanidinium-N*H*$_2$), 8.42 (t, J = 5.18 Hz, 1H, N*H*), 8.57 (s, 2H, picrate-C*H*), 10.96 (br.s, 1H, NH), 12.32 (br.s, 1H, pyrrole-NH); ^{13}C NMR (100MHz, [D$_6$]DMSO, picrate salt) δ = 23.4 (*C*H$_2$), 28.8 (*C*H$_2$), 30.7 (*C*H$_2$), 38.8 (*C*H$_2$), 46.9 (fluorenyl-*C*H), 54.0 (amino acid-*C*H), 65.8 (fluorenyl-*C*H$_2$), 112.5 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 120.3 (fluorenyl-*C*H), 124.5 (picrate-*Cq*), 125.4 (picrate-*C*H), 125.5 (pyrrole-*Cq*), 127.3 (fluorenyl-*C*H), 127.9 (fluorenyl-*C*H), 133.1 (pyrrole-*Cq*), 140.8 (fluorenyl-*Cq*), 140.9 (fluorenyl-*Cq*), 142.1 (picrate-*Cq*), 144.0 (fluorenyl-*Cq*), 144.1 (fluorenyl-*Cq*), 155.1 (picrate-*Cq*), 156.4 (*Cq*), 159.3 (*Cq*), 159.7 (*Cq*), 161.0 (*Cq*), 174.2 (*C*OOH); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3385 [br.m], 2965 [br.m], 1700 [s], 1623 [s], 1541 [s], 1271 [s], 742 [w]; HR-MS (ESI): m/z = 647.283 ± 0.005 (calculated for $^{12}C_{33}H_{38}N_6O_8 + H^+$: 647.283) (guanidinium cation)

Solid-Phase Synthesis of the tripeptide H-Ala-2a-Val-NH2



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The synthesis of the tripeptide H-Ala-**2a**-Val-NH_2 (**12**) was synthesized on Rink amide MBHA resin by a standard protocol: Rink amide MBHA resin (300 mg, 0.67 mmol/g) was swollen in DMF for 1.5 h. The Fmoc protection group was removed by twice agitation with piperidine in DMF (20 %) for 20 min. The peptide was synthesized with the resulting free amine. Coupling conditions for the amino acids: 2.5 eq. of Fmoc amino acid, 2.5 eq. PyBOP, DMF containing NMM 3 % (10 ml). The mixture was shaken for 3.5 h to ensure quantitative coupling. The attachment of the unnatural amino acid AA_1 was performed under related conditions: 2.5 eq. PyBOP, DMF containing 5 % NMM. After every coupling step the completion of the reaction was checked by a Kaiser-test. The product was cleaved from the solid support by shaking the resin with CH_2Cl_2/TFA mixture (5:95). The solvent was evaporated up to a volume of 2 ml, and the remaining oil was treated with dry diethyl ether. The white precipitate was centrifuged and dried *in vacuo* yielding the desired tripeptide as a colorless solid (72.0 mg, 53 %).

mp: 186 °C; ^{1}H NMR (600MHz, [D_6]DMSO) δ = 0.90 (d, 3H, J = 6.9 Hz, C*H*$_3$), 0.92 (d, 3H, J = 6.9 Hz, C*H*$_3$), 1.22 (d, 3H, J = 7.1 Hz, C*H*$_3$), 2.03-2.08 (m, 1H, C*H*), 3.35- 3.39 (m, 1H, C*H*$_2$), 3.65 (br.s, 1H, C*H*-NH_3^+), 3.71-3.76 (m, 1H, C*H*$_2$), 4.18-4.23 (m, 1H, C*H*), 4.46-4.50 (m, 1H, C*H*), 6.89-6.90 (m, 1H, pyrrole-C*H*), 7.14-7.16 (m, 1H, pyrrole-C*H*), 7.19 (br.s, 1H, N*H*$_2$), 7.54 (br.s, 1H, N*H*$_2$), 8.27 (d, 1H, J = 6.8 Hz, N*H*), 8.12 (br.s, 3H, NH_3^+), 8.40-8.80 (br.s, 4H, guanidinium-N*H*$_2$), 8.60 (t, 1H, J = 5.5 Hz, N*H*), 8.71 (t, 1H, J = 7.6 Hz, N*H*), 11.51 (br.s, 1H, N*H*), 12.27 (br.s, 1H, pyrrole-N*H*); ^{13}C NMR (150MHz, [D_6]DMSO) δ = 17.8 (*C*H$_3$), 18.1 (*C*H$_3$), 18.5 (*C*H$_3$), 30.0 (*C*H), 40.5 (*C*H$_2$), 48.7 (*C*H), 52.6 (*C*H), 57.6 (*C*H), 112.6 (pyrrole-*C*H), 115.7 (pyrrole-*C*H), 125.8 (pyrrole-*Cq*), 132.6 (pyrrole-*Cq*), 158.7 (*Cq*), 159.0 (*Cq*), 159.7 (*Cq*), 159.9 (*Cq*), 168.0 (*Cq*), 169.9 (*Cq*); FT-IR $\tilde{\nu}$ (KBr-pellet) [cm^{-1}] = 3328 [br.s, m], 1672 [br.s, s], 1558 [s], 1286 [m], 1202 [s], 1138 [m], 839 [w], 759 [w], 723 [w]; HR-MS (ESI): m/z = 452.237 ± 0.005 (calculated for $^{12}C_{18}H_{29}N_9O_5 + H^+$: 452.237)

Supplementary Material (ESI) for Chemical Communications

List of Abbreviations:

DMSO	dimethylsulfoxide
Boc	*tert*-butyloxycarbonyl
Cbz	benzyloxycarbonyl (also called Z-group)
DMF	*N*,*N*-dimethylformamide
eq	equvalents
Fmoc	9-fluorenylmethyloxycarbonyl
NMM	*N*-methylmorpholin
PyBOP	benzotriazol-1-yloxy-tripyrrolidinophosphonium hexafluorophosphat
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TLC	thin layer chromatography