Solution and solid supported synthesis of 3,4,5-trisubstituted 1,2,4-triazole based peptidomimetics

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RP-HPLC monitoring: The monitoring was performed on a Beckman instrument (NewGold software) equipped with a 126 solvent module, a 168 detector and a 507[e] autosampler. Analysis were performed on a Chromolith (VWR) column (4.6 x 50 mm) with a flowrate of 5 ml/min in a gradient mode from water (0.1% TFA) to acetonitrile (0.1 % TFA) in 3 min.

Flash chromatographies: Purifications were performed with Silicagel Si 60 (40-63 µm) (VWR) with hexane/AcOEt as solvent. The desired compounds were eluted with pure AcOEt.

Compound 3a

^{1}H NMR (DMSO-d^6, 400 MHz, 305 K) δ (ppm) 1.32 (9H, s, CH_3 Boc); 3.30 (2H, m, CH_2β Trp); 3.34 (2H, m, -C$\underline{H}_2$-C_6H_5); 3.79 (1H, m, -N-CH_2-C$\underline{H}$(C_6H_5)$_2$); 4.08 (1H, dd, 14.3 Hz, 8.2 Hz, 1H -N-C$\underline{H}_2$-CH(C_6H_5)$_2$); 4.45 (1H, dd, 14.3 Hz, 7.8 Hz, 1H -N-C$\underline{H}_2$-CH(C_6H_5)$_2$); 4.79 (1H, m, CH_2α Trp); 6.72 (2H, m, CH ar); 6.86 (2H, m, CH ar); 6.94 (2H, m, CH ar); 6.95 (1H, t, 7.8 Hz, CH In); 7.03 (1H, bs, CH In); 7.10 (1H, t, 7.8 Hz, CH In); 7.15-7.30 (9H, m, CH ar); 7.36 (1H, d, 7.8 Hz, CH In); 7.50 (1H, d, 7.8 Hz, CH In); 7.57 (1H, d, 8.4 Hz, NH urethane); 10.88 (1H, bs, NH In).

^{13}C NMR (DMSO-d^6, 100 MHz, 305 K) δ (ppm) 28.0 (CH_3 Boc); 29.3 (CH_2β Trp); 29.7 (-$\underline{C}H_2$-C_6H_5); 46.7 (CH_2α Trp); 46.9 (-N-$\underline{C}H_2$-CH(C_6H_5)$_2$); 50.4 (-N-CH_2-$\underline{C}$H(C_6H_5)$_2$); 78.2 (Cquat Boc); 109.9 (Cquat In); 111.4 (CH In); 118.2 (CH In); 118.4 (CH In); 120.8 (CH In); 123.9 (CH In); 126.5 (CH Ar); 126.8 (CH Ar); 127.2 (Cquat In); 127.7 (CH Ar); 127.8 (CH Ar); 128.1 (CH Ar); 128.3 (CH Ar); 128.4 (CH Ar); 136.0 and 136.1 (Cquat $CH_2C_6H_5$ and Cquat In); 140.6 (Cquat Ar); 140.7 (Cquat Ar); 152.6 (Cquat triazole); 155.3 and 155.4 (Cquat triazole and CO urethane Boc).

SM (ES) m/z 598.3 $[M+H]^+$. **R_f** = 0.38 (AcOEt/H, 9/1). α^{D}_{24} (**3a(S)**)= +63.5 (c = 0.99, MeOH). **Yield** = 64 %.

Compound 3b

^{1}H NMR (DMSO-d^6, 400 MHz, 305 K) δ (ppm) = 0.70 (3H, t, 7.3 Hz, CH_3); 0.99 (2H, m, -CH_2-C$\underline{H}_2$-CH_3); 0.85 (4H, m, -C$\underline{H}_2$-CH_2-CH_3 and -N-CH_2-CH_2-C$\underline{H}_2$-); 1.06-1.22 (2H, m, -N-CH_2-C$\underline{H}_2$-CH_2-); 3.38 (1H, dd, 14.2 Hz, 7.0 Hz, 1H CH_2β Trp); 3.51 (1H, dd, 14.2 Hz, 7.9 Hz, 1H CH_2β Trp); 3.66-3.81 (2H, m, -N-C$\underline{H}_2$-CH_2-CH_2-); 4.99 (1H, d, 12.08 Hz, 1H CH_2 Z); 5.04 (1H, d, 12.08 Hz, 1H CH_2 Z); 5.06 (1H, m, CHα Trp); 6.96 (1H, t, 7.4 Hz, CH In); 7.07 (1H, t, 7.4 Hz, CH In); 7.11 (1H, bs, CH In); 7.25-7.60 (12 H, m, CH Ar and CH In); 8.21 (1H, d, 8.4 Hz, NH urethane); 10.84 (1H, bs, NH In).

^{13}C NMR (DMSO-d^6, 100 MHz, 305 K) δ (ppm) = 13.5 (CH_3); 21.5 (-CH_2-$\underline{C}H_2$-CH_3); 25.1 (-N-CH_2-CH_2-$\underline{C}H_2$-); 29.2 (-N-CH_2-$\underline{C}H_2$-CH_2-); 29.7 (CH_2β Trp); 30.1 (-$\underline{C}H_2$-CH_2-CH_3); 42.9 (-N-$\underline{C}H_2$-CH_2-CH_2-); 47.4 (CHα Trp); 65.4 (CH_2 Z); 109.9 (Cquat In); 111.3 (CH In); 117.9

(CH In); 118.3 (CH In); 120.8 (CH In); 124.0 (CH In); 127.1 (Cquat In); 2 x 127.4 (CH Ar); 127.6 (CH Ar); 2 x 128.2 (CH Ar); 2 x 128.4 (CH Ar); 2 x 128.7 (CH Ar); 129.6 (CH Ar); 2 x 136.0 (Cquat Ar and Cquat In); 136.9 (Cquat Z); 153.5 (Cquat triazole); 155.4 (Cquat triazole); 155.8 (CO Z).
SM (ES) m/z 522.2 $[M+H]^+$. $\mathbf{R_f}$ = 0.27 (AcOEt/Hexane, 8/2). α^{D}_{24} (**3b(S)**)= +36.4 (c = 1.18, MeOH). **Yield** = 47 %.

Compound 3c
^{1}H NMR (DMSO-d^6, 400 MHz, 305 K) δ (ppm) = 0.80 (3H, t, 7.1 Hz, -CH_2-C$\underline{H}_3$); 1.06 (2H, m, -N-CH_2-CH_2-C$\underline{H}_2$-); 1.13 (2H, m, -CH_2-C$\underline{H}_2$-CH_3); 1.19-1.35 (2H, m, -N-CH_2-C$\underline{H}_2$-CH_2-); 1.43 (9H, s, CH_3 Boc); 1.62-1.75 (6H, m, 2 x CH_2 pip and 2H -C$\underline{H}_2$-CH_2-CH_3); 2.83 (1H, m, CH pip); 2.97 (2H, m, CH_2 pip); 3.31 (1H, m, 1H CH_2β Trp); 3.42 (1H, dd, 13.9 Hz, 7.8 Hz, 1H CH_2β Trp); 3.56 – 3.72 (2H, m, N-C$\underline{H}_2$-CH_2-CH_2-); 3.97 (2H, m, CH_2 Pip); 4.95 (1H, m, CHα Trp); 4.96 (1H, d, 12.8 Hz, 1H CH_2 Z); 5.02 (1H, d, 12.8 Hz, 1H CH_2 Z); 6.94 (1H, t, 7.8 Hz, CH In); 7.01 (1H, bs, CH In); 7.05 (1H, t, 7.8 Hz, CH In); 7.20-7.40 (6 H, m, 5 CH Ar and 1 CH In); 7.47 (1H, d, 7.8 Hz, CH In); 8.10 (1H, d, 8.4 Hz, NH urethane); 10.80 (1H, bs, NH In).
^{13}C NMR (DMSO-d^6, 100 MHz, 305 K) δ (ppm) = 13.7 (CH_3); 21.8 (-CH_2-$\underline{C}H_2$-CH_3); 25.5 (-N-CH_2-CH_2-$\underline{C}H_2$-); 3 x 28.0 (CH_3 Boc); 29.7 (CH_2β Trp); 30.1 (-N-CH_2-$\underline{C}H_2$-CH_2-); 3 x 30.4 (-$\underline{C}H_2$-CH_2-CH_3 and 2 x $\underline{C}H_2$ Pip); 31.0 (CH pip); 41.9 (-N-$\underline{C}H_2$-CH_2-CH_2-); 2 x 42.9 (2 x $\underline{C}H_2$ Pip);47.1 (CHα Trp); 65.3 (CH_2 Z); 78.5 (Cquat Boc); 109.9 (Cquat In); 111.3 (CH In); 117.9 (CH In); 118.3 (CH In); 120.7 (CH In); 123.9 (CH In); 127.1 (Cquat In); 2 x 127.3 (CH Ar); 127.6 (CH Ar); 2 x 128.2 (CH Ar); 135.9 (Cquat In); 136.9 (Cquat Ar); 153.8 (CO Boc); 154.2 (Cquat triazole); 155.7 (CO Z); 156.4 (Cquat triazole).
SM (ES) m/z 629.4 $[M+H]^+$. $\mathbf{R_f}$ = 0.2 (AcOEt/ Hexane, 9/1). α^{D}_{24} (**3c(S)**)= +34.0 (c = 0.97, MeOH). **Yield** = 31 %.

Compound 3d
^{1}H NMR (DMSO-d^6, 400 MHz, 305 K) δ (ppm) = 1.30 (9H, s, CH_3 tBu); 1.36 (3H, d, 7.2 Hz, CH_3 Ala); 3.41 (2H, m, CH_2β Trp); 4.02 (1H, m, 1H CH_2 Fmoc);4.10 (1H, t, 7.2 Hz, CH Fmoc); 4.22 (1H, m, 1H CH_2 Fmoc);4.83 (1H, q, 7.2 Hz, CHα Ala); 5.07 (1H, m, CHα Trp); 6.94 (1H, t, 7.8 Hz, CH In); 7.05 (1H, t, 7.8 Hz, CH In); 7.12 (1H, bs, CH In); 7.29 (2H, t, 7.6 Hz, 2 CH Ar Fmoc); 7.32 (1H, d, 7.8 Hz, CH In); 7.40 (2H, t, 7.6 Hz, 2 CH Ar Fmoc); 7.53 (1H, d, 7.8 Hz, CH In); 7.63 (2H, d, 7.6 Hz, 2 CH Ar Fmoc); 7.87 (2H, d, 7.6 Hz, 2 CH Ar Fmoc); 8.15 (1H, d, 8.4 Hz, NH urethane); 8.55 (1H, s, CH triazole); 10.80 (1H, bs, NH In).
^{13}C NMR (DMSO-d^6, 100 MHz, 305 K) δ (ppm) = 17.8 (CH_3 Ala); 3 x 27.2 (CH_3 tBu); 29.2 (CH_2β Trp); 46.5 (CH Fmoc); 46.7 (CHα Trp); 52.4 (CHα Ala); 65.8 (CH_2 Fmoc); 81.9 (Cquat tBu); 109.9 (Cquat In); 111.2 (CH In); 118.2 (CH In); 118.3 (CH In); 2 x 120.0 (CH Ar Fmoc); 120.8 (CH In); 123.8 (CH In); 125.1 (CH Ar Fmoc); 125.3 (CH Ar Fmoc); 2 x 127.0 (CH Ar Fmoc); 127.1 (Cquat In); 2 x 127.5 (CH Ar Fmoc); 136.0 (Cquat In); 2 x 140.5 (Cquat Ar Fmoc); 142.8 (CH triazole); 2 x 143.6 (Cquat Ar Fmoc); 153.7 (Cquat triazole); 155.6 (CO Fmoc); 168.7 (COOtBu).
SM (ES) m/z 578.1 $[M+H]^+$. $\mathbf{R_f}$ = 0.24 (AcOEt/ Hexane, 8/2). α^{D}_{24} (**3d(S,S)**)= +22.5 (c = 1.16, MeOH). **Yield** = 76 %.

Compound 3e
^{1}H NMR (DMSO-d^6, 400 MHz, 305 K) δ (ppm) = 1.25 (9H, s, CH_3 tBu); 1.26 (3H, d, 7.5 Hz, CH_3 Ala); 3.43 (2H, m, CH_2β Trp); 4.00-4.20 (5H, m, -C$\underline{H}_2$-C_6H_5, CH Fmoc, CH_2 Fmoc); 4.99 (1H, m, CHα Trp); 5.19 (1H, q, 7.5 Hz, CHα Ala); 6.93 (1H, t, 7.5 Hz, CH In); 7.04 (1H, t,

7.5 Hz, CH In); 7.13 (1H, bs, CH In); 7.16 (2H, m, CH Ar); 7.25-7.45 (8H, m, 4 CH Ar Fmoc, 3 CH Ar and 1H CH In); 7.61-7.65 (3H, m, 2 CH Ar Fmoc and 1CH In); 7.87 (2H, d, 7.5 Hz, CH Ar Fmoc); 8.17 (1H, d, 8.7 Hz, NH urethane); 10.78 (1H, bs, NH In).
^{13}C NMR (DMSO-d^6, 100 MHz, 305 K) δ (ppm) = 16.7 (CH_3 Ala); 3 x 27.2 (CH_3 tBu); 29.6 (CH_2β Trp); 30.9 (-$\underline{C}H_2$-C_6H_5) ; 46.5 (CH Fmoc); 47.2 (CHα Trp); 52.5 (CHα Ala); 65.7 (CH_2 Fmoc); 82.3 (Cquat tBu); 110.2 (Cquat In); 111.2 (CH In); 118.2 (CH In); 118.2 (CH In); 2 x 119.9 (CH Ar Fmoc); 120.7 (CH In); 123.7 (CH In); 125.2 (CH Ar Fmoc); 125.3 (CH Ar Fmoc); 126.5 (CH Ar); 2 x 126.9 (CH Ar Fmoc); 127.3 (Cquat In); 2 x 127.5 (CH Ar Fmoc); 2 x 128.3 (CH Ar); 2 x 128.4 (CH Ar); 136.0 (Cquat In); 136.3 (Cquat Ar); 2 x 140.5 (Cquat Ar Fmoc); 143.5 (Cquat Ar Fmoc); 143.7 (Cquat Ar Fmoc); 152.6 (Cquat triazole); 155.0 (Cquat triazole); 155.6 (CO Fmoc); 168.1 (C̲OOtBu).
SM (ES) m/z 668.2 $[M+H]^+$. **R_f** = 0.42 (AcOEt/ Hexane, 7/3). α^{D}_{24} (**3e(S,S)**)= +21.2 (c = 1.52, MeOH). **Yield** = 80 %.

Compound 3f
^{1}H NMR (DMSO-d^6, 400 MHz, 300 K) δ (ppm) = 1.28 (9H, s, Boc); 2.84 (2H, m, -CH_2-CH_2-In); 3.29 (2H, m, CH_2βTrp); 4.05 (2H, m, -CH_2-CH_2-In); 4.95 (1H, m, CHα); 6.83 (1H, sl, CH indole); 6.88-6.97 (2H, m, CH indole); 6.98-7.10 (3H, m, CH indole); 7.31 (1H, d, 8.0 Hz, CH indole); 7.32 (1H, d, 8.0Hz, CH indole); 7.40 (1H, d, 7.8 Hz); 7.48 (1H, d, 7.8 Hz, H indole); 7.63 (1H, d, 8.4 Hz, NH urethane); 8.21 (1H, sl, CH triazole); 10.80 (1H, sl, NH indole); 10.85 (1H, sl, NH indole).
SM (ES) m/z 415.0 $[M+H-56]^+$; 471.3 $[M+H]^+$; 941.3 $[2M+H]^+$. **R_f** = 0.2 (96/4 - AcOEt/MeOH). **Yield** = 51 %.

Compound 3g
^{1}H NMR (DMSO-d^6, 400 MHz, 300 K) δ (ppm) = 1.21 (9H, s, CH_3 Boc); 3.10-3.30 (3H, N-CH_3 et 2H CH_2β); 4.15 (2H, -CH_2-In); 4.90 (1H, m, CHα); 6.90-7.10 (m, 6H, CH In); 7.33 (1H, d, 8.0 Hz, CH In); 7.35 (1H, d, 7.9 Hz, CH In); 7.41-7.58 (3H, CH In et NH urethane); 10.75 (1H, s, NH indole); 10.90 (1H, s, NH indole).
SM (ES) m/z 471.4 $[M+H]^+$; 941.3 $[2M+H]^+$. **R_f** = 0.2 (96/4 - AcOEt/MeOH).
Chiral HPLC (column Chiralcel OD at 30°C, flow 1 mL/min, detection at 280 nm, mobile phase 80/20/0.1 (v/v/v) Hexane/2-propanol/Et_2NH) : **RT (3g(R))** 14.12 min ; **RT(3g(S))** 19.88 min. e.e. (**3g(R)**) ≥ 98 %. α^{D}_{24} (**3g(R)**)= -27.8 (c = 1.16, MeOH)**. Yield** = 50 %

Compound 3h
^{1}H NMR (DMSO-d^6, 400 MHz, 300 K) δ (ppm) = 1.32 (9H, s, CH_3 Boc); 2.85-3.10 (4H, m, -$C\underline{H_2}$-$C\underline{H_2}$-In); 3.20(3H, s, N-CH_3); 3.22-3.40 (2H, m, CH_2β Trp); 4.92 (1H, m, CHα Trp); 6.93-7.02 (2H, m, CH In); 7.04-7.10 (2H, m, CH In); 7.13 (1H, s, CH In); 7.16 (1H, s, CH indole); 7.32-7.38 (2H, m, CH indole); 7.42-7.52 (3H, m, 2 CH In and NH urethane); 10.85 (2H, sl, NH indole).
SM (ES) m/z 485.3 $[M+H]^+$. **R_f** = 0.2 (96/4 – AcOEt/MeOH).
Chiral HPLC (column Chiralcel OD at 30°C, flow 1 mL/min, detection at 280 nm, mobile phase 80/20/0.1 (v/v/v) Hexane/2-propanol/Et_2NH) : **RT (3h(R))** = 11.30 min ; **RT (3h(S))** = 16.03 min. e.e. (**3h(R)**) ≥ 98 %. α^{D}_{24} (**3h(R)**)= -11.6 (c = 1.11, MeOH). **Yield** = 51 %.

Compound 3i
^{1}H NMR (DMSO-d^6, 400 MHz, 300 K) δ (ppm) = 1,20 (9H, s, CH_3 Boc); 3,29 (2H, m, CH_2β Trp); 3,70 (3H, s, -O-CH_3); 4,01 (2H, m, -$C\underline{H_2}$-In); 4,83 (1H, m, CHα Trp); 5,04 (2H, s, CH_2 PMB); 6,62-6,78 (m, 4H, CH Ar); 6,86 (1H, t, J = 7,4 Hz, CH In); 6,93 (1H, t, J = 7,4 Hz, CH

In); 6,98-7,12 (4H, m, CH In); 7,21 (1H, d, J = 7,8 Hz, CH In); 7,24 (1H, d, J = 7,6 Hz, CH In); 7,26 (1H, d, J = 7,5 Hz, CH In); 7,39 (1H, d, J = 7,8 Hz, CH In); 7,56 (1H, d, J = 8,5 Hz, NH urethane); 10,75 (1H, s, NH indole); 10,85 (1H, s, NH indole).
^{13}C NMR (DMSO-d^6, 100 MHz, 300 K) δ (ppm) = 22,3 (-$\underline{C}H_2$-In); 3 x 28,9 (CH_3 Boc); 29,7 ($CH_2\beta$ Trp); 45,9 (CH_2 PMB); 47,1 ($CH\alpha$ Trp); 55,9 (-O-$\underline{C}H_3$); 78,9 (Cquat Boc); 109,2 (Cquat In); 111,1 (Cquat In); 112,1 (CH In); 112,2 (CH In); 2 x 114,7 (2 x CH PMB); 2 x 119,0 (2 x CH In); 119,3 (CH In); 119,4 (CH In); 121,6 (CH In); 122,0 (CH In); 124,3 (CH In); 124,8 (CH In); 127,6 (Cquat In); 128,0 (Cquat In); 2 x 128,1 (2 x CH Ar); 128,6 (Cquat PMB); 136,8 (Cquat In); 137,1 (Cquat In); 154,1 (Cquat PMB); 156,1 (Cquat triazole); 156,3 (Cquat triazole); 159,3 (Cquat CO urethane).
SM (ES) m/z 577,6 $[M+H]^+$. $\mathbf{R_f}$ = 0.19 (AcOEt). α^D_{24} (**3i(R)**)= -53.3 (c = 1.00, $CHCl_3$). **Yield** = 49 %.