SUPPORTING INFORMATION

Fluctuating β-sheet secondary structure in DS119 explains the small effects of backbone N-amination on thermal stability

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Figure S1. Far-UV CD wavescans of the backbone-substituted DS119 variants at each modified site compared to wild-type DS119. All samples were analysed at 0.2 mg/mL in 20 mM aq sodium phosphate, pH 7.3, 25 °C.

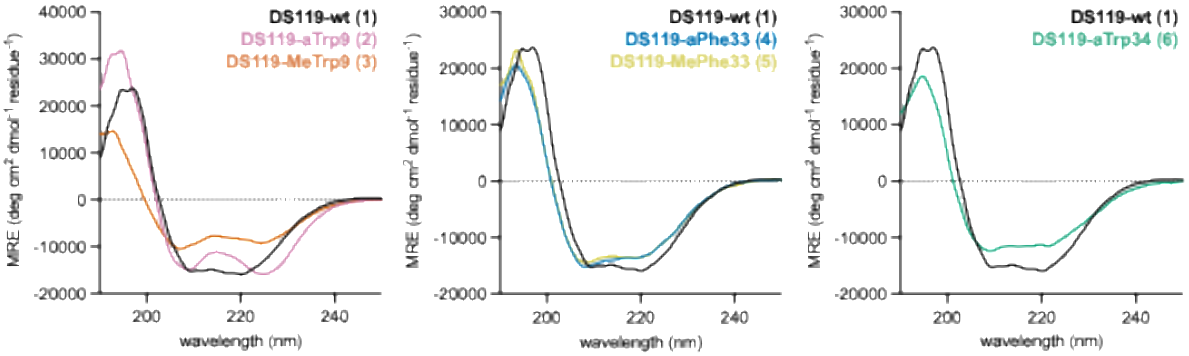


Figure S2. Secondary structure elements as a function of time calculated for the IRNOE_54A7 (upper panel) and TANOE_54A7 (lower panel) simulations of DS119. Red: α-helix; blue: β-strand or β-bridge; brown: turn.

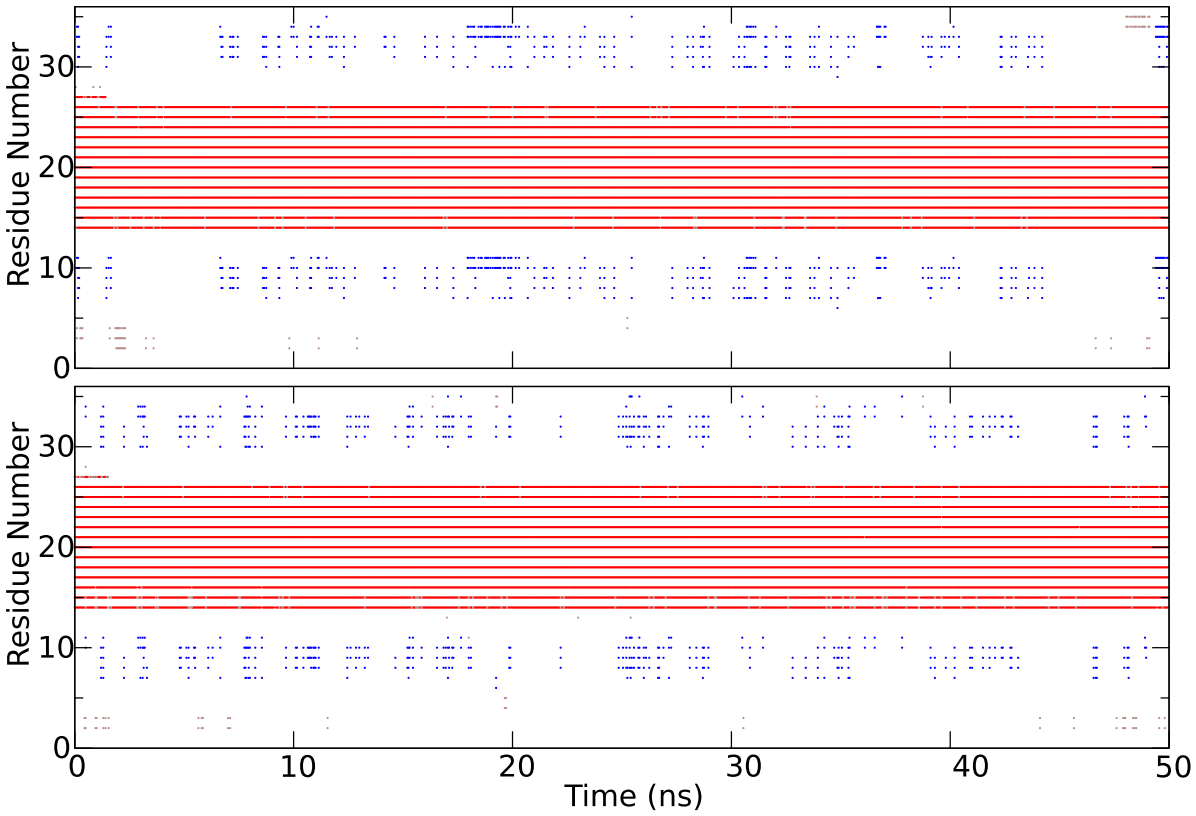
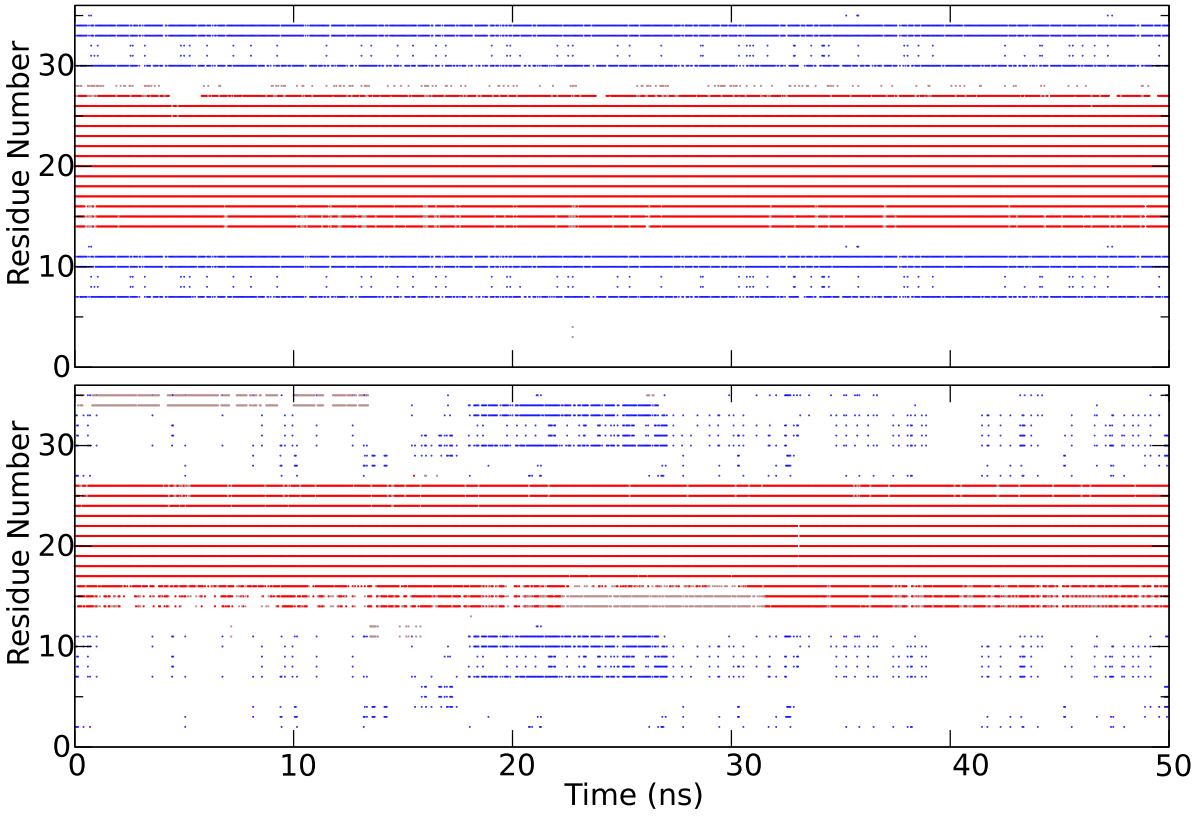


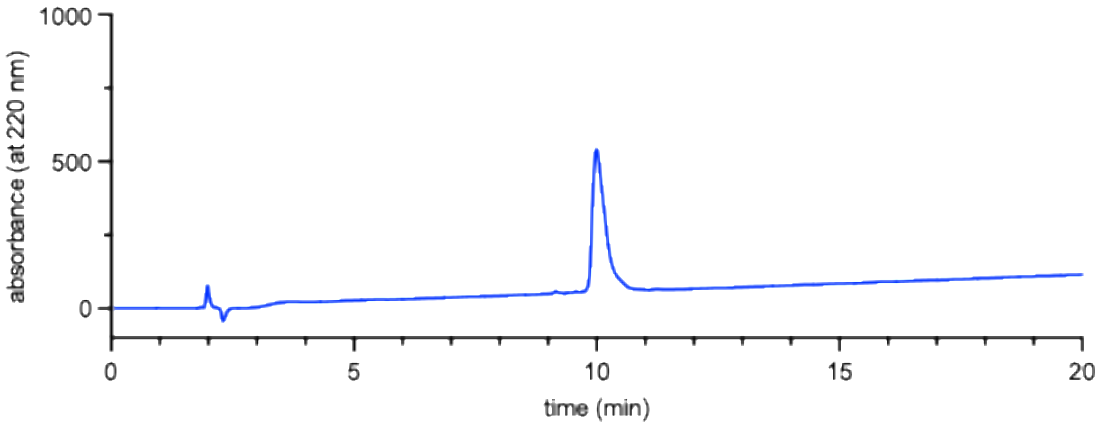
Figure S3. Secondary structure elements as a function of time calculated for the IRNOE_54B7 (upper panel) and TANOE_54B7 (lower panel) simulations of DS119 from 0-50ns. Red: α-helix; blue: β-strand or β-bridge; brown: turn.



CHARACTERIZATION DATA FOR ALL SYNTHESIZED COMPOUNDS

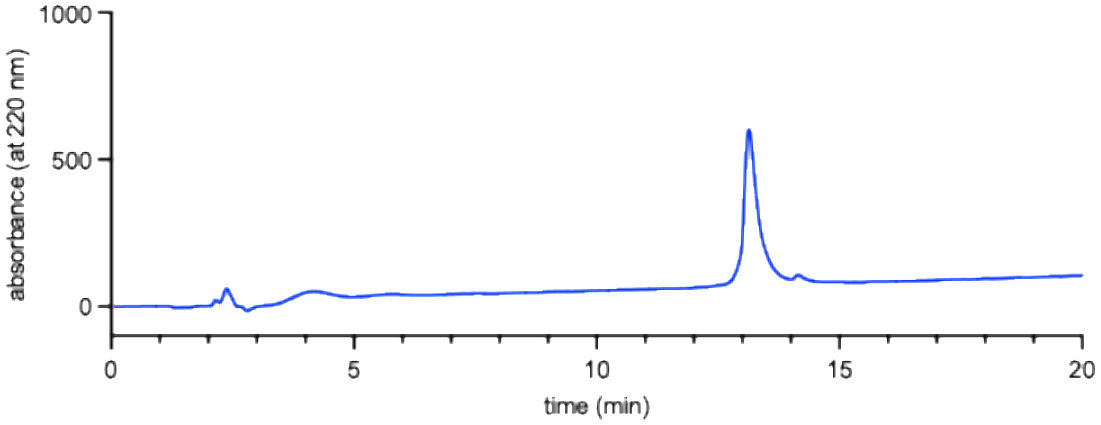
wt DS119 (1). The crude peptide was purified by preparative scale RP-HPLC using a 10–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 10.0 min. The pure peptide was obtained in 9% overall yield based on initial resin loading.

Analytical RP-HPLC 10–40% MeCN/H_2O gradient (with 0.1% formic acid) :



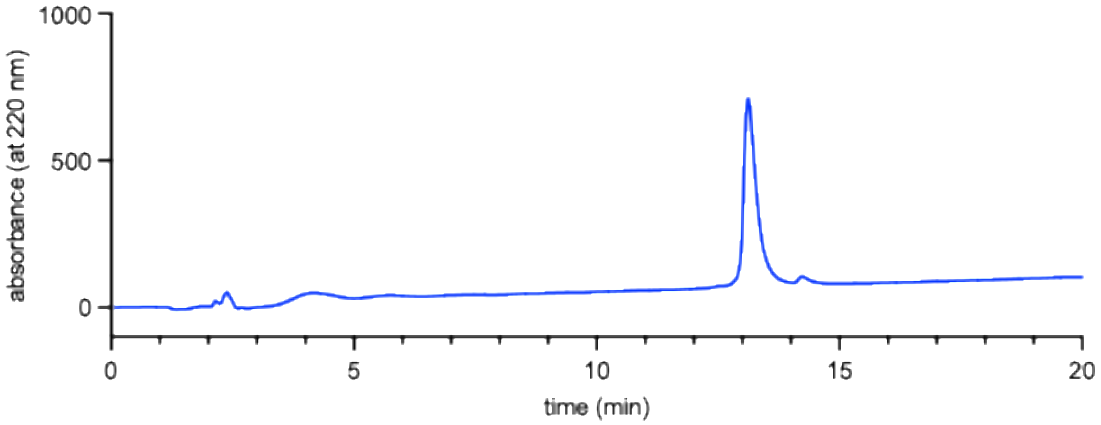
aTrp9 DS119 (2). The crude peptide was purified by preparative scale RP-HPLC using a 5–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 13.1 min. The pure peptide was obtained in 6% overall yield based on initial resin loading.

Analytical RP-HPLC 5–40% MeCN/H_2O gradient (with 0.1% formic acid):



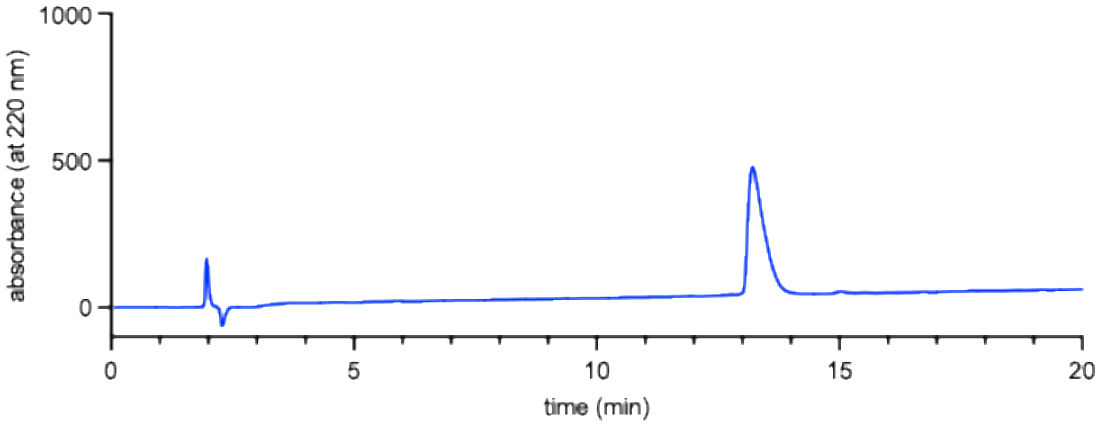
MeTrp9 DS119 (3). The crude peptide was purified by preparative scale RP-HPLC using a 5–50% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 13.2 min. The pure peptide was obtained in 14% overall yield based on initial resin loading.

Analytical RP-HPLC 5–50% MeCN/H_2O gradient (with 0.1% formic acid):



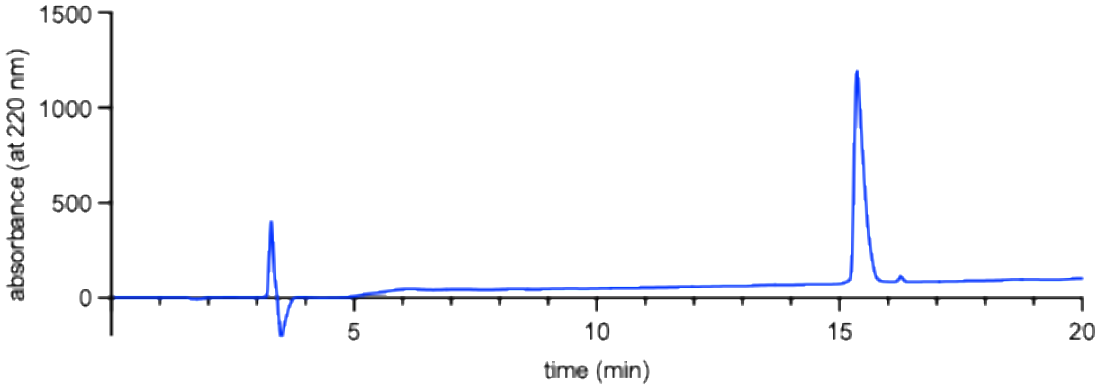
aPhe33 DS119 (4). The crude peptide was purified by preparative scale RP-HPLC using a 10–30% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 13.3 min. The pure peptide was obtained in 10% overall yield based on initial resin loading.

Analytical RP-HPLC 10–30% MeCN/H_2O gradient (with 0.1% formic acid):



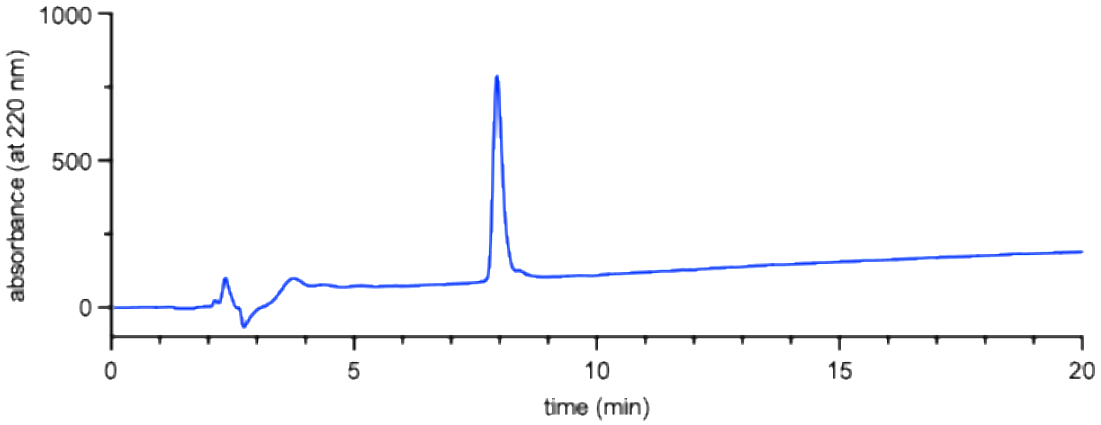
MePhe33 DS119 (5). The crude peptide was purified by preparative scale RP-HPLC using a 5–40% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 15.4 min. The pure peptide was obtained in 21% overall yield based on initial resin loading.

Analytical RP-HPLC 5–40% MeCN/H_2O gradient (with 0.1% formic acid):



aTrp34 DS119 (6). The crude peptide was purified by preparative scale RP-HPLC using a 5–80% MeCN/H_2O gradient (with 0.1% formic acid). t_R = 8.0 min. The pure peptide was obtained in 4% overall yield based on initial resin loading.

Analytical RP-HPLC 5–80% MeCN/H_2O gradient (with 0.1% formic acid):

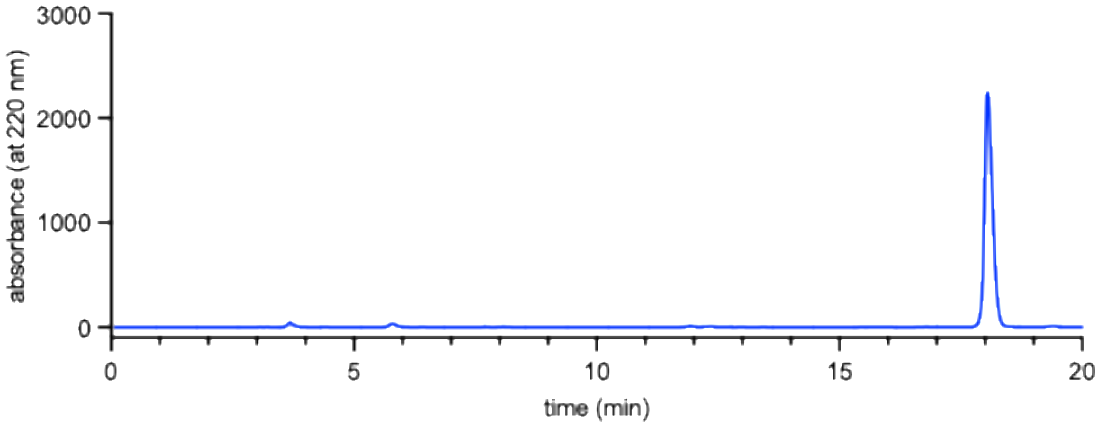


HRMS data for synthetic DS119 variants:

entry	Chemical formula	*m*	*z*	Observed mass (*m*/*z*)	Expected mass (*m*/*z*)	Error (ppm)
1	$C_{174}H_{280}N_{48}O_{49}$	$[M+3H]^{3+}$	3	1276.3749	1276.3704	3.5
2	$C_{174}H_{281}N_{49}O_{49}$	$[M+2H]^{2+}$	2	1921.5545	1921.5574	–1.5
3	$C_{174}H_{281}N_{49}O_{49}$	$[M+2H]^{2+}$	2	1921.5565	1921.5574	–0.5
4	$C_{174}H_{281}N_{49}O_{49}$	$[M+2H]^{2+}$	2	1921.5557	1921.5574	–0.9
5	$C_{175}H_{281}N_{48}O_{49}$	$[M+2H]^{2+}$	2	1920.5554	1920.5559	–0.3
6	$C_{175}H_{281}N_{48}O_{49}$	$[M+2H]^{2+}$	2	1920.5538	1920.5559	–1.1

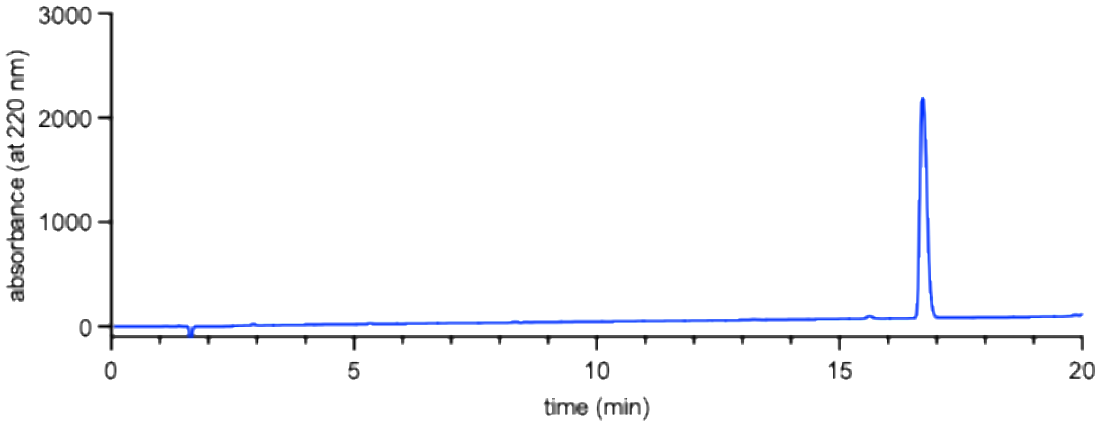
Fmoc-Ile-(*N*'-Boc)aTrp(Boc)-OH. The crude dipeptide was purified by flash column chromatography (30–100 EtOAc/hexanes, then 20% MeOH/EtOAc) to afford Fmoc-Ile-(*N*'-Boc)aTrp(Boc)-OH as a white crystalline solid (32% overall yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.75 (d, *J* = 7.7 Hz, 2H), 7.62 – 7.16 (m, 11H), 5.36 – 5.24 (m, 1H), 4.28 (s, 1H), 3.60 – 3.32 (m, 1H), 3.12 (dd, *J* = 16.1, 10.9 Hz, 1H), 1.96 – 1.79 (m, 1H), 1.72 – 1.35 (m, 18H), 1.13 (s, 2H), 0.94 – 0.77 (m, 6H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6, mixture of rotamers) δ 174.8, 171.0, 156.4, 155.4, 149.8, 143.7, 141.3, 134.5, 130.7, 127.7, 125.0, 124.5, 123.7, 122.5, 119.9, 119.6, 116.9, 115.3, 83.8, 80.3, 67.1, 57.2, 56.2, 47.1, 35.8, 28.3; HRMS (ESI-TOF) *m/z* [M + Na]$^{+}$ calcd for $C_{42}H_{50}N_4NaO_9$ 777.3470, found 777.3474.

RP-HPLC using a 50–95% MeCN/H_2O gradient (modified with 0.1% HCO_2H) over 20 min (C12, flow rate = 1 mL/min, λ = 220 nm) t_R = 18.01 min.



Fmoc-Thr(*t*Bu)-(*N*'-Boc)aPhe-OH. The crude dipeptide was purified by flash column chromatography (30–100 EtOAc/hexanes, then 20% MeOH/EtOAc) to afford Fmoc-Thr(*t*Bu)-(*N*'-Boc)aPhe-OH as a white crystalline solid (39% overall yield). ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers) δ 7.89 (d, *J* = 7.5 Hz, 2H), 7.74 (d, *J* = 7.2 Hz, 2H), 7.46 – 7.37 (m, 2H), 7.35 – 7.13 (m, 8H), 5.02 (s, 1H), 4.49 (d, *J* = 9.3 Hz, 1H), 4.24 (t, *J* = 8.1 Hz, 1H), 3.94 (s, 1H), 3.22 (s, 1H), 3.08 (s, 1H), 2.93 (s, 1H), 1.44 (s, 8H), 1.34 (s, 1H), 1.25 (s, 1H), 1.11 (s, 8H), 1.04 (s, 3H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6, mixture of rotamers) δ 172.7, 166.6, 164.3, 155.5, 155.1, 144.4, 144.2, 141.2, 138.2, 130.0, 129.5, 128.7, 128.7, 128.1, 127.6, 126.9, 126.8, 125.8, 120.6, 80.7, 74.2, 69.8, 66.4, 65.2, 60.9, 56.3, 55.4, 47.1, 38.5, 28.8, 28.4, 21.2, 20.7; HRMS (ESI-TOF) *m/z* [M + Na]$^{+}$ calcd for $C_{37}H_{45}N_3NaO_8$ 682.3099, found 682.3103.

RP-HPLC using a 50–95% MeCN/H_2O gradient (modified with 0.1% HCO_2H) over 20 min (C12, flow rate = 1 mL/min, λ = 220 nm) t_R = 16.7 min.



Fmoc-Phe-(*N*'-Boc)aTrp(Boc)-OH. The crude dipeptide was purified by flash column chromatography (30–100 EtOAc/hexanes, then 20% MeOH/EtOAc) to afford Fmoc-Phe-(*N*'-Boc)aTrp(Boc)-OH as a white crystalline solid (36% overall yield). ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.61 – 7.00 (m, 18H), 4.25 (s, 1H), 4.06 (s, 1H), 3.41 (m, 2H), 3.01 (m, 2H), 1.76 – 1.36 (m, 18H); ^{13}C{^{1}H} NMR (126 MHz, DMSO-d_6, mixture of rotamers) δ 170.9, 169.8, 155.8, 154.4, 149.4, 143.9, 141.4, 136.8, 135.0, 134.7, 130.3, 129.1, 128.6, 128.5, 128.1, 127.5, 127.2, 127.0, 125.3, 124.2, 123.1, 122.3, 120.0, 119.2, 118.4, 115.1, 84.7, 80.9, 67.4, 66.8, 56.9, 53.4, 47.5, 37.7, 28.1, 26.3, 23.4; HRMS (ESI-TOF) *m/z* [M + Na]$^{+}$ calcd for $C_{45}H_{48}N_4NaO_9$ 811.3313, found 811.3320.

RP-HPLC using a 70–99% MeCN/H_2O gradient (modified with 0.1% HCO_2H) over 20 min (C12, flow rate = 1 mL/min, λ = 220 nm) t_R = 11.1 min.