Supporting Information MD-CAR-07-2014-000327

Synthesis and Antibacterial Activity of Trivalent Ultrashort Arg-Trp-based Antimicrobial Peptides (AMPs)

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Experimental Part

General information

ESI mass spectra were measured on an Esquire 6000 from Bruker Daltonics. MALDI-TOF mass spectra were measured on a Ultraflex III from Bruker.
NMR data were collected on a DPX 200 instrument from Bruker; chemical shift δ is given in ppm relative to TMS as an external standard.
Analytical HPLC was performed on a Knauer instrument. To elute the compounds a gradient of 100% buffer A (95% water, 5% ACN, 0.1% TFA) to 100% buffer B (95% ACN, 5% water, 0.1% TFA) and a flow rate of 1 mL/min over 40 min was used.
Preparative HPLC was performed on a Varian Star using the same gradient as above but now with a flow rate of 20 mL/min.
Column chromatography was performed on silica gel 60 (particle size 0.036-0.2 mm) purchased from Merck.
Only L-amino acids werde used in this work. They were purchased from IRIS Biotec (Germany). The chemicals were purchased from IRIS Biotech, Aldrich and Fluka and were used without further purification. All reactions were carried out using commercial-grade solvents purchased from Roth, Baker, Fischer and Biosolve.

General experimental procedures

Fmoc-deprotection in solution: Stirring the starting material in a mixture of 20% diethylamine in DCM over 3h.
Pbf-deprotection in solution: A mixture of 79% TFA, 1% TIS, 5% H_2O, 5% thioanisol, 5% mercaptoethanol and 5% phenol was added to the substance and stirred for 3h.
Peptide-couplings in solution: The protected amino acid containing a free carboxyl-group was dissolved in THF. 1 eq. isobutylchlorformiat and 1 eq. N-methylmorpholin were added. Then, 1 eq. of the amino acid containing the free amine group was added and the solution was stirred overnight at room temperature. Insoluble salts were filtered off and the solution was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and subsequently washed with water, $KHSO_4$ (3x), water, 5% $NaHCO_3$ (2x), water and brine. The organic phase was dried over Na_2SO_4, filtered and the solvent was removed *in vacuo*.

Minimal inhibitory concentration
The biological data were obtained in the group of Jun.-Prof Dr. Julia Bandow in Bochum following published procedures from this group (ACS Chem Biol. 2013).

Synthesis of Fmoc-Trp(Boc)-N(H)CH_2CCH ***1***
Fmoc-Trp(Boc)-OH (2 g, 3.8 mmol) was coupled to 2-propargylamine (260 µL, 3.8 mmol) as described in the general procedure. The product was purified by column chromatography (eluent: gradient 1-5% MeOH in CH_2Cl_2). Affording after condensation a yield of 0.6 g (47%).
^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 8.07 (s, 1H), 7.69 (s, 2H, NHC(O)), 7.49 (s, 4H, C^{Ar}H Trp), 7.27 (m, 8H, C^{Ar}H Fmoc), 5.92 (s, 1H, C^{Ar}H Trp), 4.60 (s, 1H, C^{α}H Trp), 4.29 (s, 2H, CH_2

Fmoc), 4.12 (s, 1H, CH Fmoc) 3.88 (s, 2H, CH_2 alkyne), 3.17 (s, 2H, $C^{\beta}H$ Trp), 2.02 (s, 1H, CH alkyne), 1.60 (s, 9H, $C(CH_3)_3$ Boc).
^{13}C-NMR ($CDCl_3$, 50 MHz, δ): 172.3, 150.0, 143.7, 136.2, 130.2, 127.5, 126.5, 125.1, 122.4, 120.9, 119.7, 115.6, 83.7, 78.8, 71.2, 67.1, 64.0, 57.4, 29.2, 28.5.
MS (ESI^+, m/z): 634.2, 586.1 (calc. 586.6 for $[M+Na]^+$), 478.2, 404.2, 304.1, 249.1.

Synthesis of Fmoc-Arg(Pbf)-N(H)CH_2CCH ***2***
Fmoc-Arg(Pbf)-OH (2 g, 3.1 mmol) was coupled to 2-propargylamine (212 µL, 3.1 mmol) as described in the general procedure. The product was purified by column chromatography (eluent: gradient 1-10% methanol in DCM). After condensation a white solid was obtained with a yield of 1.1 g (54%).
^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 7.59 (s, 2H, NHC(O)), 7.42 (s, 3H, $C^{Ar}H$ Fmoc), 7.13 (s, 5H, $C^{Ar}H$ Fmoc), 6.27 (s, 1H, CH Fmoc), 6.19 (s, 2H, CH_2 Fmoc), 4.02 (s, 1H, $C^{\alpha}H$ Arg), 3.86 (s, 2H, CH_2 alkyne), 3.12 (s, 2H, CH_2 Pbf), 2.79 (s, 2H, $C^{\delta}H_2$ Arg), 2.48 (s, 3H, CH_3 Pbf), 2.40 (s, 3H, CH_3 Pbf), 2.07 (s, 2H, $C^{\beta}H_2$ Arg), 1.96 (s, 3H, CH_3 Pbf), 1.70 (s, 1H, CH alkyne), 1.50 (s, 2H, $C^{\gamma}H_2$ Arg), 1.31 (s, 6H, CH_3 Pbf).
^{13}C-NMR ($CDCl_3$, 50 MHz, *δ*): 172.3, 158.8, 156.6, 143.8, 141.0, 138.1, 132.2, 127.8, 127.1, 125.0, 119.9, 117.4, 86.4, 79.4, 71.3, 67.1, 50.2, 46.8, 43.0, 28.5, 25.4, 19.4, 18.1, 12.4.

Synthesis of Fmoc-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH ***3***
Removal of the Fmoc-group on Fmoc-Trp(Boc)-N(H)CH_2CCH **1** was performed according to the described general procedure. For the work up, the solvent was removed *in vacuo* and the residue was dissolved in DCM (1 mL). The product was precipitated with Et_2O, filtered off and dried *in vacuo*. Fmoc-Arg(Pbf)-OH (0.7 g, 1.1 mmol) was coupled to deprotected **1** (0.4 g, 1.1 mmol) as described in the general procedure. The product was purified with column chromatography (eluent: gradient 1-4% methanol in DCM).
^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 7.66 (s, 2H, NHC(O)), 7.44 (s, 4H, $C^{Ar}H$ Trp), 7.18 (s, 8H, $C^{Ar}H$ Fmoc), 6.40 (s, 1H, $C^{Ar}H$ Trp), 6.13 (s, 2H, CH_2 Fmoc), 4.75 (s, 1H, $C^{\alpha}H$ Trp), 4.43 (s, 1H, CH Fmoc), 4.23 (s, 2H, CH_2 alkyne), 4.06 (s, 1H, $C^{\alpha}H$ Arg), 3.78 (s, 2H, $C^{\beta}H_2$ Trp), 3.30 (s, 2H $C^{\delta}H_2$ Arg), 3.13 (s, 2H, $C^{\beta}H_2$ Arg), 2.87 (s, 2H, CH_2 Pbf), 2.55 (d, J = 16.5Hz, 6H, CH_3 Pbf), 2.13 (s, 1H, CH alkyne), 2.04 (s, 3H, CH_3 Pbf), 1.82 (m, 2H $C^{\gamma}H_2$Arg), 1.56 (s, 9H, $C(CH_3)_3$ Boc), 1.39 (s, 3H CH_3 Pbf).
^{13}C-NMR ($CDCl_3$, 50 MHz, δ): 172.8, 171.5, 158.8, 156.7, 149.5, 143.7, 141.2, 138.4, 135.4, 132.7, 132.1, 130.1, 127.6, 127.0, 125.1, 124.4, 119.9, 118.3, 117.5, 115.5, 86.3, 83.6, 79.0, 71.4, 67.0, 54.1, 46.9, 43.2, 28.6, 28.1, 19.3, 18.0, 12.4.
MS (ESI^+, m/z): 994.3 (calc. 995.2 for $[M+Na]^+$), 972.3 (calc. 973.2 for $[M+H]^+$), 704.3, 595.3, 490.3, 427.3.

Synthesis of Fmoc-Trp(Boc)-Arg(Pbf)-N(H)CH_2CCH ***4***

Removal of the Fmoc-group on Fmoc-Arg(Pbf)-N(H)CH_2CCH was performed as described in the general procedure. For work up of the product, the solvent was removed *in vacuo* and the residue was dissolved in DCM (1 mL). The product was precipitated with Et_2O, filtered off and dried *in vacuo*.

Fmoc-Trp(Boc)-OH (0.9 g, 1.8 mmol) was coupled to H-Arg(Pbf)-N(H)CH_2CCH (1.1 g, 1.8 mmol) as described in the general procedure. The product was purified with column chromatography (eluent: EtOAc).

Characterization:

^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 7.92 (s, 2H, $C^{Ar}H$ Fmoc and Trp), 7.54-7.04 (m, 10H, $C^{Ar}H$ Fmoc and Trp), 6.24 (s, 4H, EtOAc), 5.94 (s, 1H, $C^{Ar}H$ Trp), 4.52 (d, J = 20.8 Hz, 1H, $C^{\alpha}H$ Trp), 4.14 (s, 2H, CH_2 Fmoc), 3.83 (s, 2H, CH_2 alkyne), 3.66 (s, 1H, $C^{\alpha}H$ Arg), 3.11 (s, 2H, $C^{\beta}H_2$ Trp), 2.76 (s, 3H, CH_3 Pbf), 2.46 (s, 3H, CH_3 Pbf), 2.39 (s, 3H, CH_3 Pbf), 2.05 (s, 1H, EtOAc), 2.00 (s, 1H, CH alkyne), 1.96 (s, 2H, $C^{\delta}H_2$ Arg), 1.94 (s, 2H, $C^{\beta}H_2$ Arg), 1.72 (s, 2H, $C^{\gamma}H_2$ Arg), 1.48 (s, 9H, $C(CH_3)_3$ Boc), 1.31 (s, 6H, CH_3 Pbf), 0.76 (s, 3H, EtOAc).

^{13}C-NMR ($CDCl_3$, 50 MHz, δ): 172.4, 171.4, 158.9, 156.7, 149.4, 143.6, 141.0, 138.4, 135.3, 132.2, 130.3, 127.4, 126.8, 124.9, 124.7, 119.8, 117.7, 115.5, 86.4, 83.8, 79.4, 71.3, 46.8, 43.3, 28.5, 27.9, 19.3, 18.8, 18.1, 12.4.

MS (ESI^+, m/z): 1011.6, 995.6, 973.7 (calc. 973.2 for $[M+H]^+$).

Synthesis of H-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH ***5***

Removal of the Fmoc-group on Fmoc-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH **2** was carried out as described in the general procedure. For the work up, the solvent was reduced *in vacuo* and the peptide was precipitated with Et_2O, filtered off and dried *in vacuo.* The product was purified by combi-flash chromatography with a yield of 400 mg (45%).

^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 8.00 (s, (s, 2H, NHC(O)), 7.50 (s, 1H, $C^{Ar}H$ Trp), 7.42 (s, 1H, $C^{Ar}H$ Trp), 7.14 (s, 2H, $C^{Ar}H$ Trp), 6.99 (s, 1H, $C^{Ar}H$ Trp), 6.32 (s, 2H, $C^{\beta}H_2$ Trp), 6.11 (s, 1H, $C^{\alpha}H$ Trp), 4.72 (s, 2H, $C^{\delta}H_2$ Arg), 3.87 (s, 2H, CH_2 alkyne), 3.37 (s, 1H, $C^{\alpha}H$ Arg), 3.13 (s, 3H, CH_3 Pbf), 2.91 (s, 2H, CH_2 Pbf), 2.54 (s, 3H, CH_3 Pbf), 2.47 (s, 3H, CH_3 Pbf), 2.12 (s, 1H, CH alkyne), 2.06 (s, 2H, NH_2), 1.97 (s, 2H, $C^{\beta}H_2$ Arg), 1.64 (s, 2H, $C^{\gamma}H_2$ Arg), 1.60 (s, 9H, $C(CH_3)_3$ Boc), 1.42 (s, 6H, CH_3 Pbf).

^{13}C-NMR ($CDCl_3$, 50 MHz, δ): 175.8, 171.2, 158.8, 156.4, 149.7, 138.4, 135.1, 132.8, 132.2, 130.3, 124.6, 122.5, 117.4, 115.2, 86.3, 83.8, 79.1, 71.3, 54.3, 53.0, 43.3, 28.5, 28.2, 25.1, 19.1, 17.8, 12.2, 10.5.

Synthesis of H-Trp(Boc)-Arg(Pbf)-N(H)CH_2CCH ***6***

Removal of the Fmoc-group on Fmoc-Trp(Boc)-Arg(Pbf)-N(H)CH_2CCH was performed as described in the general procedure. For the work up, the solvent was removed *in vacuo* and the residue was dissolved in DCM (1 mL). The product was precipitated with diethylether, filtered off and dried *in vacuo*, affording the desired compound in a yield of 32 mg (2.2%).

^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 8.05 (s, 2H, NHC(O)), 7.56-7.15 (s, 5H, $C^{Ar}H$ Trp), 6.37 (s, 2H), 6.27 (s, 1H, $C^{\alpha}H$ Arg), 4.52 (s, 1H, $C^{\alpha}H$ Trp), 3.96 (s, 2H, CH_2 alkyne), 3.72 (s, 1H, $C^{\beta}H$ Trp), 3.20 (s, 2H, CH_2 Pbf), 2.89 (s, 2H, $C^{\delta}H_2$ Arg), 2.81 (s, 1H, $C^{\beta}H$ Trp), 2.55 (s, 3H, CH_3 Pbf), 2.47 (s, 3H, CH_3 Pbf), 2.14 (s, 1H, CH alkyne), 2.05 (s, 3H, CH_3 Pbf), 1.98 (s, 2H, NH_2), 1.70 (s, 2H, $C^{\gamma}H_2$ Arg), 1.63 (s, 9H, $C(CH_3)_3$ Boc), 1.42 (s, 6H, CH_3 Pbf).

^{13}C-NMR ($CDCl_3$, 50 MHz, δ): 175.2, 171.4, 158.9, 156.4, 149.4, 138.1, 135.3, 132.2, 130.0, 124.6, 122.5, 119.3, 117.7, 116.5, 115.2, 86.4, 83.8, 79.4, 71.3, 54.9, 52.0, 43.3, 28.5, 28.2, 25.4, 19.4, 17.8, 12.4.

*Synthesis of H-Trp-Arg-Trp-N(H)CH_2CCH **7***

A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected peptide Boc-Trp(Boc)-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH **12** (100 mg, 79µmol). and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 41 mg (93%).

Characterization:

MS (ESI^+, *m*/z): 684.11, 584.10 (berechnet 584.3 für $[M+H]^+$), 292.45 (berechnet 292.65 für $[M+2H]^{2+}$).

HPLC (λ = 254 nm, tR): 17.6 min.

*Synthesis of H-Arg-Trp-Arg-N(H)CH_2CCH **8***

A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected peptide Boc-Arg(Pbf)-Trp(Boc)-Arg(Pbf)-N(H)CH_2CCH **13** (100 mg, 79µmol) and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 41 mg (93%).

Characterization:

MS (ESI^+, *m*/z): 554.1 (berechnet 554.3 für $[M+H]^+$), 277.5 (berechnet 277.7 für $[M+2H]^{2+}$).

HPLC (λ = 254 nm, t_R): 14.7 min.

1H NMR (DMSO, 200 MHz, δ): 10.87 (s, 1H), 8.52 (s, 1H), 8.33 (s, 2H), 8.09 (s, 3H), 7.66 (s, 1H), 7.56 (s, 2H), 7.35 (s, 1H), 7.06 (s, 8H), 4.68 (s, 1H), 4.27 (s, 1H), 3.89 (s, 2H), 3.84 – 3.66 (m, 1H), 3.32 (s, 78H), 3.14 (s, 7H), 2.87 (s, 1H), 2.52 (s, 105H), 2.17 (s, 1H), 1.61 (d, J = 35.7 Hz, 9H), 1.27 (s, 2H).

Synthesis of (H-Arg(Pbf)-Trp(Boc)-triazole)$_3$-benzene

1,3,5-tris(azidomethyl)benzene (4.1 mg, 20 µmol, 1eq.), copper iodide (0.9 mg, 5.1 µmol 0.3 eq.), H-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH (50 mg, 80 µmol, 4eq.) und D*i*PEA (1.7 µL, 10 µmol 0.6 eq.) were dissolved in THF (1 mL) and the mixture was stirred at room temperature for two days. After this, an additional amount of copper iodide (0.9 mg, 5.1 µmol) was added to the solution since analytical HPLC showed incomplete reaction. During the reaction the color of the solution changed from colorless to intense blue. The solvent was evaporated and the product was purified by HPLC with a yield of 23 mg (45%).

Characterization:

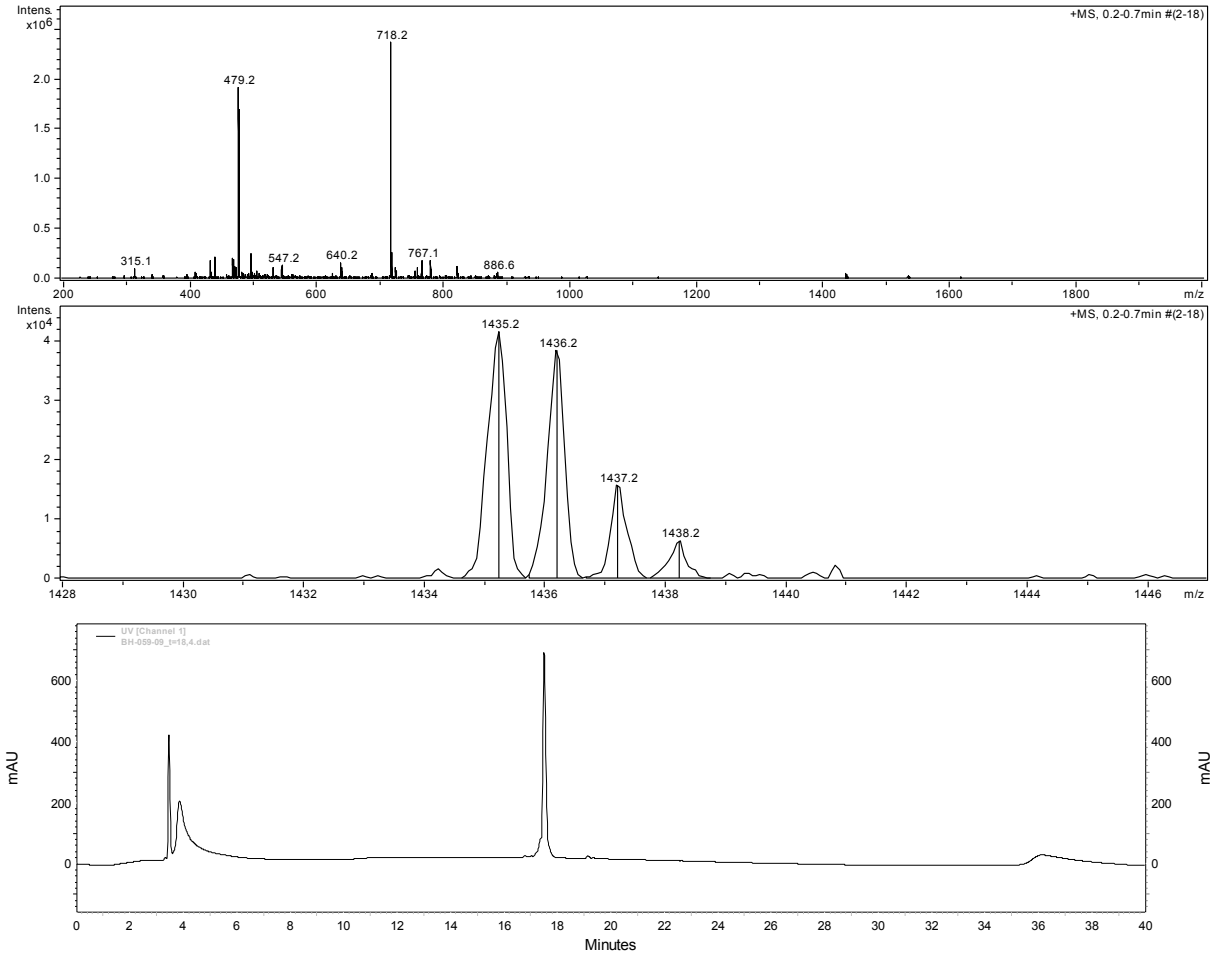
MS (ESI^+, *m*/*z*): 2492.4 (calc. 2493.4 for $[M+H]^+$), 1247.4 (calc. 1248.2 for $[M+2H]^{2+}$).

Synthesis of (H-Arg(Pbf)-Trp(Boc)-triazole)$_3$-(ethyl)$_3$-benzene
1,3,5-tris(azidomethyl)-2,4,6-triethylbenzene (5.7 mg, 20 µmol), copper iodide (0.9 mg, 5.1 µmol), H-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH (50 mg, 80 µmol) and D*i*PEA (1.7 µL, 10 µmol) were dissolved in THF (1 mL) and stirred at room temperature for two days. After this, an additional amount of copper iodide (0.9 mg, 5.1·µmol) was added to the solution. During the reaction the color of the solution changed from colorless to intense blue. The solvent was evaporated and the product was purified by HPLC with a yield of 9 mg (15%).
Characterization:
MS (ESI$^+$, *m*/*z*): 2576.6 (calc. 2578.1 for $[M+H]^+$).

Synthesis of (H-Trp(Boc)-Arg(Pbf)-triazole)$_3$-benzene
1,3,5-tris(azidomethyl)benzene (7.9 mg, 33 µmol), copper iodide (1.9 mg, 10 µmol), H-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH (100 mg, 130 µmol) und D*i*PEA (3.4 µL, 20 µmol) were dissolved in THF (1.5 mL) and the mixture was stirred at room temperature for one days. After this, an additional amount of copper iodide (1.9 mg, 10 µmol) was added. During the reaction the color of the solution changed from colorless to intense blue. The solvent was evaporated and the product was purified by HPLC with a yield of 74 mg (90%).
Characterization:
MS (ESI$^+$, *m*/*z*): 2492.9 (calc. 2492.2 for $[M+H]^+$).

Synthesis of (H- Trp(Boc)-Arg(Pbf)-triazole)$_3$-(ethyl)$_3$-benzene
1,3,5-tris(azidomethyl)-2,4,6-triethylbenzene (7.9 mg, 33 µmol), copper iodide (1.9 mg, 10 µmol), H-Arg(Pbf)-Trp(Boc)-N(H)CH_2CCH (100 mg, 130 µmol) and D*i*PEA (3.4 µL, 20 µmol) were dissolved in THF (1.5 mL) and stirred at room temperature for one days. After this, an additional amount of copper iodide (1.9 mg, 10·µmol) was added to the solution. During the reaction the color of the solution changed from colorless to intense blue. The solvent was evaporated and the product was purified by HPLC with a yield of 78 mg (91%).
Characterization:
MS (ESI$^+$, *m*/*z*): 2576.8 (calc. 2576.3 for $[M+H]^+$).

Synthesis of (H-Arg-Trp-triazole)$_3$-benzene ***9a***
A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected H-Arg(Pbf)-Trp(Boc)-triazol-benzene **4** (19.1 mg, 7.7 µmol) and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 6.4 mg (54%).
Characterization:
MS (ESI$^+$, *m*/*z*): 1435.2 (calc. 1435.2 for $[M+H]^+$), 718.2 (calc. 718.4 for $[M+2H]^{2+}$), 479.2 (calc. 479.3 for $[M+3H]^{3+}$).
HPLC (λ = 254 nm, t_R): 15.7 min.



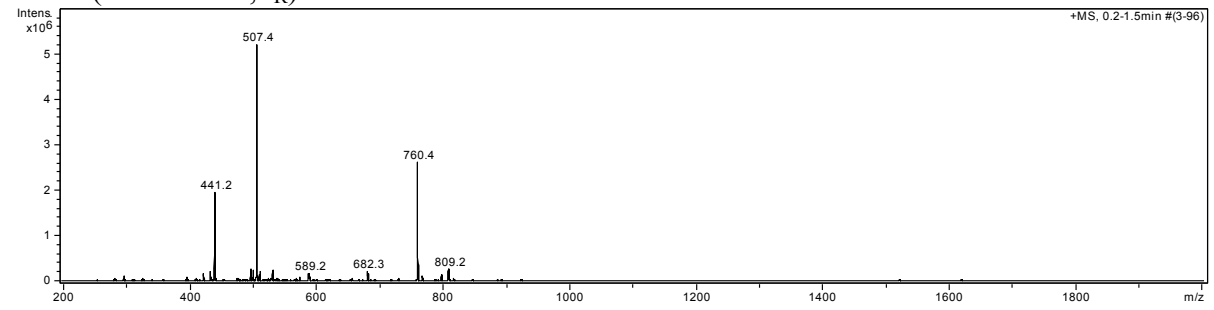
Synthesis of (H-Arg-Trp-triazole)$_3$-(ethyl)$_3$-benzene ***9b***

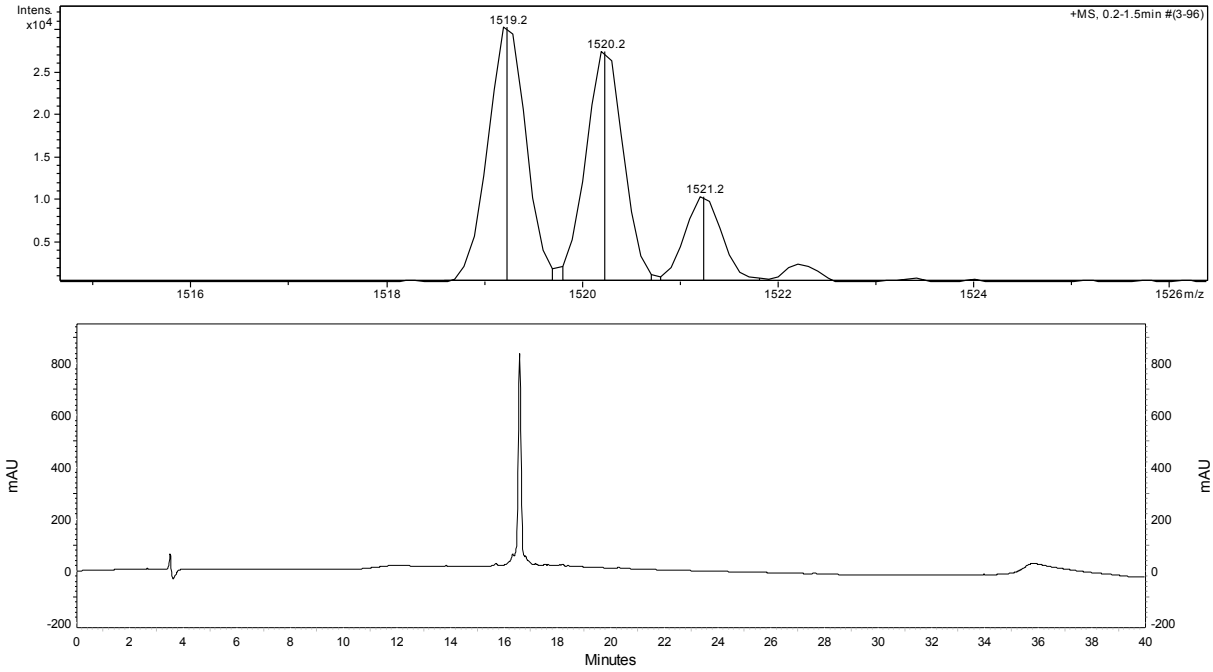
A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected H-Arg(Pbf)-Trp(Boc)-triazolethyl-benzene **5** (13 mg, 5.1 µmol) and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 8 mg (97%).

Characterization:

MS (ESI$^+$, *m/z*): 1519.2 (calc. 1519.8 for $[M+H]^+$, 760.4 (calc. 760.4 for $[M+2H]^{2+}$), 507.4 (calc. 507.3 for $[M+3H]^{3+}$), 441.2.

HPLC (λ = 254 nm, t_R): 16.6 min.





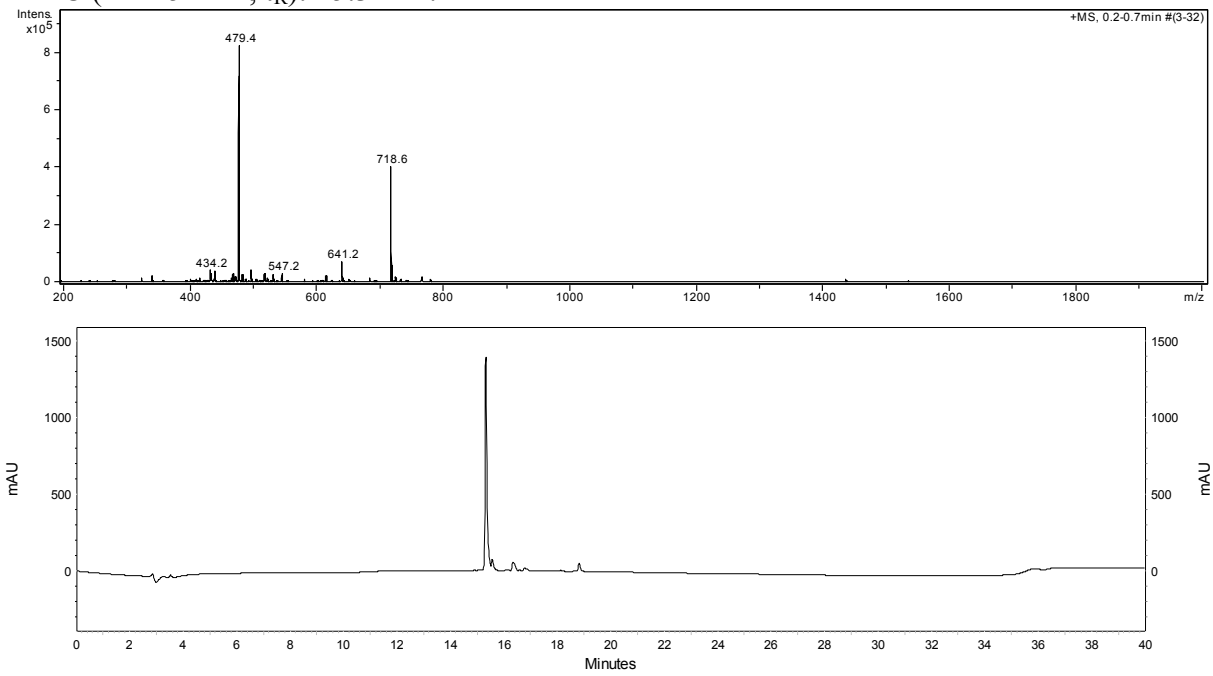
Synthesis of (H-Trp-Arg-triazole)$_3$-benzene ***10a***

A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected H-Trp(Boc)-Arg(Pbf)-triazol-benzene **11** (74.4 mg, 30 µmol) and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 31.5 mg (60%).

Characterization:

MS (ESI^+, *m*/*z*): 718.6 (calc. 718.4 for $[M+2H]^{2+}$), 479.4 (calc. 479.3 for $[M+3H]^{3+}$).

HPLC (λ = 254 nm, t_R): 15.3 min.



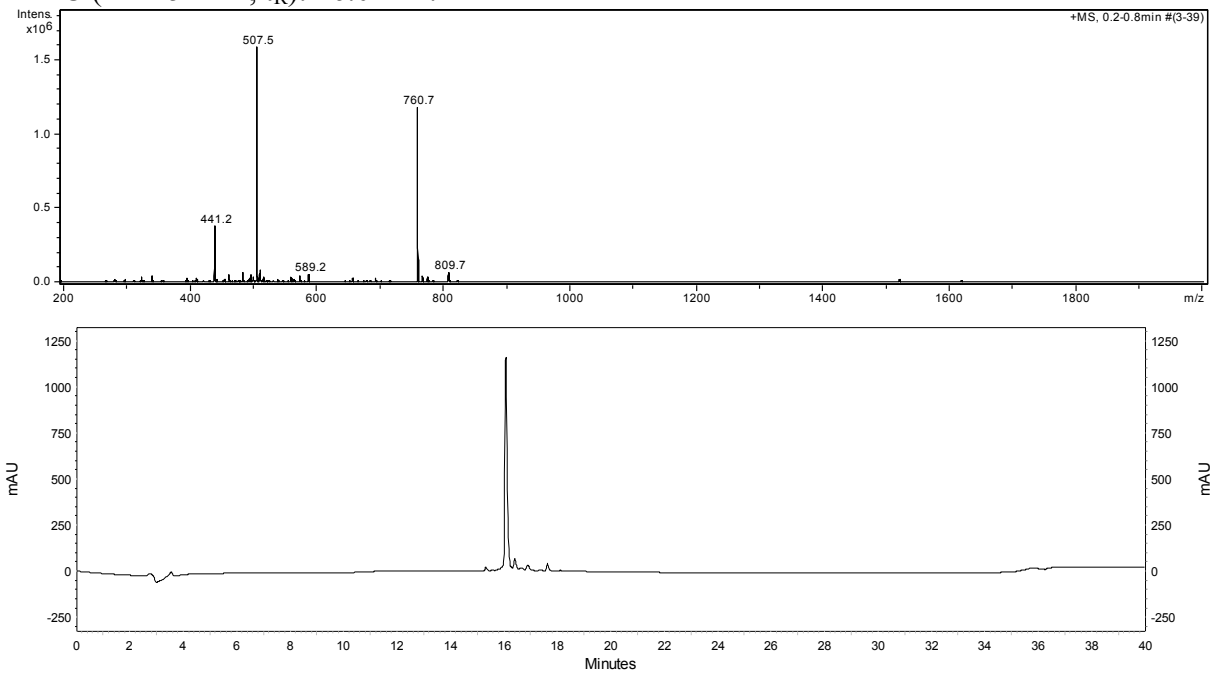
Synthesis of (H-Trp-Arg-triazole)$_3$-(ethyl)$_3$-benzene ***10b***

A mixture of trifluoroacetic acid (1580 µL, 39.5% (1/2 of 79%)), triisopropylsilane (40 µL, 1%), water (200 µL, 5%), thioanisole (200 µL, 5%), phenol (214 mg, 5%), 2-mercaptoethanol (200 µL, 5%) was added to the protected H-Trp(Boc)-Arg(Pbf)-triazolethyl-benzene (80 mg, 31 µmol) and stirred at room temperature for one hour. Trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred for further 20 minutes. Again, trifluoroacetic acid (790 µL, 19.8%) was added and the mixture was stirred another hour. The deprotected product was precipitated in cold diethylether/hexane, washed and lyophilized. Yield: 42.9 mg (90%).

Characterization:

MS (ESI$^+$, *m*/*z*): 760.7 (calc. 760.4 for $[M+2H]^{2+}$), 507.5 (calc. 507.3 for $[M+3H]^{3+}$), 441.2.

HPLC (λ = 254 nm, t_R): 16.0 min.



Synthesis of Boc-Trp(Boc)-Arg(Pbf)-Trp(Boc)-OH

The peptide was synthesized via solid phase peptide synthesis (SPPS) on a tritylchlorid resin as described in the general procedure. After cleavage from the resin the solvent was removed and the product was lyophilized. Yield: 0.9 g (90%).

Characterization:

^{1}H NMR (DMSO, 200 MHz) δ 8.32 (s, 1H, NHC(O)), 8.00 (s, 4H, C^{ar}H Trp), 7.64 (s, 2H, NHC(O)), 7.53 (s, 2H, C^{ar}H Trp), 7.28 (s, 4H, C^{ar}H Trp), 4.55 (s, 1H, C$^{\alpha}$H Trp), 4.38 (s, 1H, C$^{\alpha}$H Trp), 4.25 (s, 1H, C$^{\alpha}$H Arg), 3.91 (s, 9H), 3.03 (s, 6H, DMF), 2.90 (s, 4H, C$^{\beta}$H Trp), 2.74 (s, 2H, C$^{\delta}$H Arg), 2.49 (s, 11H, CH_3 Pbf, CH_2 Pbf), 2.01 (s, 3H, NH Arg), 1.59 (s, 27H, CH_3 Pbf), 1.40 (s, 10H, CH_3 Pbf, C$^{\beta}$H Arg, C$^{\gamma}$H Arg), 1.26 (s, 9H).

MS (ESI$^+$, *m*/z): 1099.7 (calc. 1099.5 for $[M+H]^+$).

Synthesis of Boc-Arg(Pbf)-Trp(Boc)-Arg(Pbf)-OH

The peptide was synthesized via solid phase peptide synthesis (SPPS) on a tritylchlorid resin as described in the general procedure. After cleavage from the resin the solvent was removed and the product was lyophilized. Yield: 0.9 g (97%).

Characterization:

^{1}H-NMR (DMSO, 200 MHz, δ): 7.99 (s, 1H, DMF), 7.80 (s, 1H, NHC(O)), 7.69 (s, 1H, NHC(O)), 7.52 (s, 1H, NHC(O)), 7.25 (s, 4H, $C^{ar}H$ Trp), 4.67 (s, 1H, $C^{\alpha}H$ Trp), 4.19 (s, 1H, $C^{\alpha}H$ Arg), 3.83 (s, 1H, $C^{\alpha}H$ Arg), 3.05 (s, 3H, DMF), 2.95 (s, 3H, DMF), 2.89 (s, 6H, $C^{\beta}H$ Trp, CH_2 Pbf), 2.74 (s, 4H, $C^{\delta}H_2$ Arg), 2.46 (d, J = 11.6 Hz, 18H, CH_3 Pbf), 2.01 (s, 2H, ACN), 1.60 (s, 12H, NH Arg, $C^{\beta}H$ Arg, $C^{\gamma}H$ Arg), 1.41 (s, 18H, CH_3 Boc), 1.34 (s, 12H, CH_3 Pbf).

MS (ESI^+, *m*/z): 1221.7 (calc. 1221.6 for [M+H]+), 613.2 (calc. 611.3 for [M+H]2+).

Synthesis of Boc-Trp(Boc)-Arg(Pbf)-Trp(Boc)-N(H)CH$_2$CCH

Propargylamine (0.18 mL, 2.7 mmol, 3 eq.) and PyBOP (0.57 g, 1.0 mmol, 1.2 eq) was added to a solution of Peptide Boc-Trp(Boc)-Arg(Pbf)-Trp(Boc)-OH (0.9 g, 0.7 mmol) in DCM. The solution was stirred at room temperature over night. The solvent was removed and the residue was dissolved in ethylacetat. The precipitate was filtered and the filtrate was washed with water, $NaHSO_4$-solution (3x), water, 5% $NaHCO_3$-solution (2x), water and brine. The solution was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product was purified via column chromatography (ethylacetat/hexane 9:1). Yield: 0.2 g (30%).

MS (ESI^+, *m*/z): 1174.20 (calc. 1174.54 for $[M+K]^+$), 1158.39 (calc. 1158.54 for $[M+Na]^+$), 1136.38 (calc. 1136.54 for $[M+H]^+$).

^{1}H-NMR ($CDCl_3$, 200 MHz, *δ*): 9.68 (s, 1H), 8.28 (m, 3H, NHC(O)), 7.53 (m, 8H, $C^{ar}H$ Trp), 7.10 (m, 1H, CH Trp), 6.84 (m, 1H, CH Trp), 6.04 (m, 1H, $C^{\alpha}H$ Trp), 5.84 (m, 1H, $C^{\alpha}H$ Trp), 5.50 (m, 3H), 5.34 (s, 1H, $C^{\alpha}H$ Arg), 4.40 (m, 2H, CH_2 Alkin), 4.25 (m, 7H), 4.14 (d, 8H), 3.46 (m, 2H, $C^{\beta}H$ Trp), 3.27 (m, 5H), 3.10 (s, 2H, CH_2 Pbf), 2.54 (m, 2H, CH Alkin), 2.46 (m, 2H, $C^{\delta}H$ Trp), 2.31 (s, 2H, NH Arg), 2.18 (m, 4H), 1.93 (m, 8H, CH_3 Pbf), 1.81 (m, 4H), 1.76 (m, 3H, $C^{\beta}H_2$ Arg), 1.71 (m, 6H, CH_3 Pbf), 1.51 (m, 2H, $C^{\gamma}H_2$ Arg), 1.19 (s, 27H, $C(CH_3)_3$ Boc).

^{13}C-NMR ($CDCl_3$, 50 MHz, *δ*): 156.36, 135.99, 125.48, 86.14, 83.03, 79.99, 71.39, 60.36, 34.23, 30.33, 30.33, 28.61, 27.97, 27.87, 20.97, 18.98, 14.16.

Synthesis of Boc-Arg(Pbf)-Trp(Boc)-Arg(Pbf)-N(H)CH$_2$CCH

Propargylamine (0.15 mL, 2.1 mmol, 3 eq.) and PyBOP (0.46 g, 0.84 mmol, 1.2 eq) was added to a solution of Peptide Boc-Arg(Pbf)-Trp(Boc)-Arg(Pbf)-OH (0.9 g, 0.7 mmol) in DCM. The solution was stirred at room temperature over night. The solvent was removed and the residue was dissolved in ethylacetat. The precipitate was filtered and the filtrate was washed with water, $NaHSO_4$-solution (3x), water, 5% $NaHCO_3$-solution (2x), water and brine. The solution was dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product was purified via column chromatography (ethylacetat/hexane 9:1). Yield: 0.23 g (25%).

Characterization:

MS (ESI^+, *m*/z): 1296.2 (calc. 1296.6 for $[M+K]^+$), 1280.8 (calc. 1280.6 for $[M+Na]^+$), 1259.7 (calc. 1258.6 for $[M+H]^+$).

^{1}H-NMR ($CDCl_3$, 200 MHz, δ): 7.99 (s, 1H), 7.46 (s, 4H, NHC(O)), 7.11 (s, 4H, $C^{ar}H$ Trp), 6.75 (s, 1H, Trp), 6.29 (s, 6H, EtOAc), 5.64 (s, 1H, $C^{\beta}H$ Trp), 4.75 (s, 1H, $C^{\alpha}H$ Trp), 4.30 (d, 2H, CH_2 Alkin), 4.08 (s, 2H, $C^{\alpha}H$ Arg), 3.86 (s, 1H, $C^{\beta}H$ Trp), 3.14 (s, 5H, CH_2 Pbf), 2.91 (s,

4H, $C^{\delta}H_2$ Arg), 2.49 (d, 18H, CH_3 Pbf), 2.27 (s, 1H, CH Alkin), 2.05 (s, 4H, NH Arg), 2.02 (s, 4H, $C^{\beta}H_2$ Arg), 1.74 (s, 4H, $C^{\gamma}H_2$ Arg), 1.59 (s, 13H, CH_3 Pbf), 1.43 (s, 18H, $C(CH_3)_3$ Boc), 1.31 (s, 9H), 1.27 (s, 3H, EtOAc),1.24 (s, 5H).

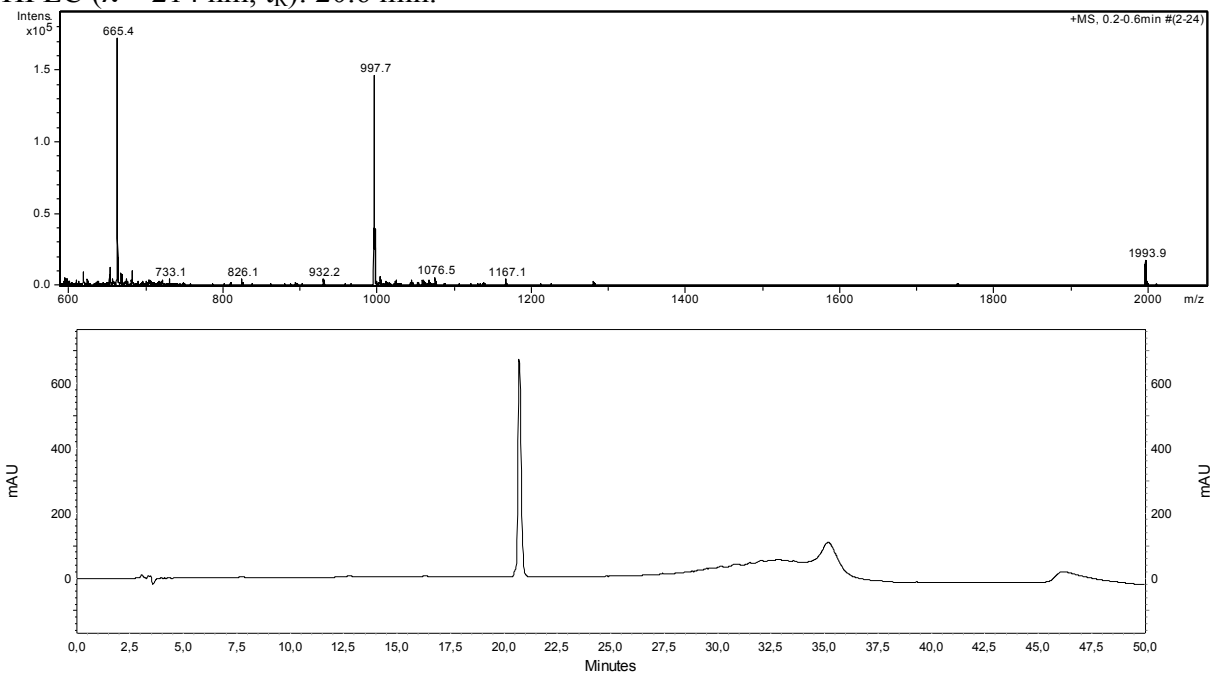
Synthesis of (H-Trp-Arg-Trp-triazole)$_3$-benzene ***11a***

The peptide H-Trp-Arg-Trp-N(H)CH_2CCH (20 mg, 34 µmol, 4 eq.) was dissolved in DMF. One third of this solution was added to a solution of 1,3,5-triazidomethylbenzene (1.67 µL, 9 µmol, 1 eq.), $(EtO)_3$PCuI (1.5 mg, 4.5 µmol, 0.5 eq.) and DiPEA (5.8 µL, 34 µmol, 4 eq.) in DMF. The reaction mixture was heated in the microwave for 90 min at 60 °C. One third of the peptide solution was added again to the reaction mixture. It was again heated in the microwave for another 90 min at 60 °C. The last third of the peptid solution was added as well as $(EtO)_3$PCuI (0.6 mg, 1.5 µmol, 0.5 eq.) and the reaction in the microwave was repeated. The solvent was removed in vacuo and the mixture was purified via HPLC. Yield: 4.7 mg (25%).

Characterization:

MS (ESI^+, *m*/z): 1993.9 (calc. 1994.0 for $[M+H]^+$), 997.7 (calc. 997.5 for $[M+2H]^{2+}$), 665.4 (calc. 665.3 for $[M+3H]^{3+}$).

HPLC (λ = 214 nm, t_R): 20.6 min.



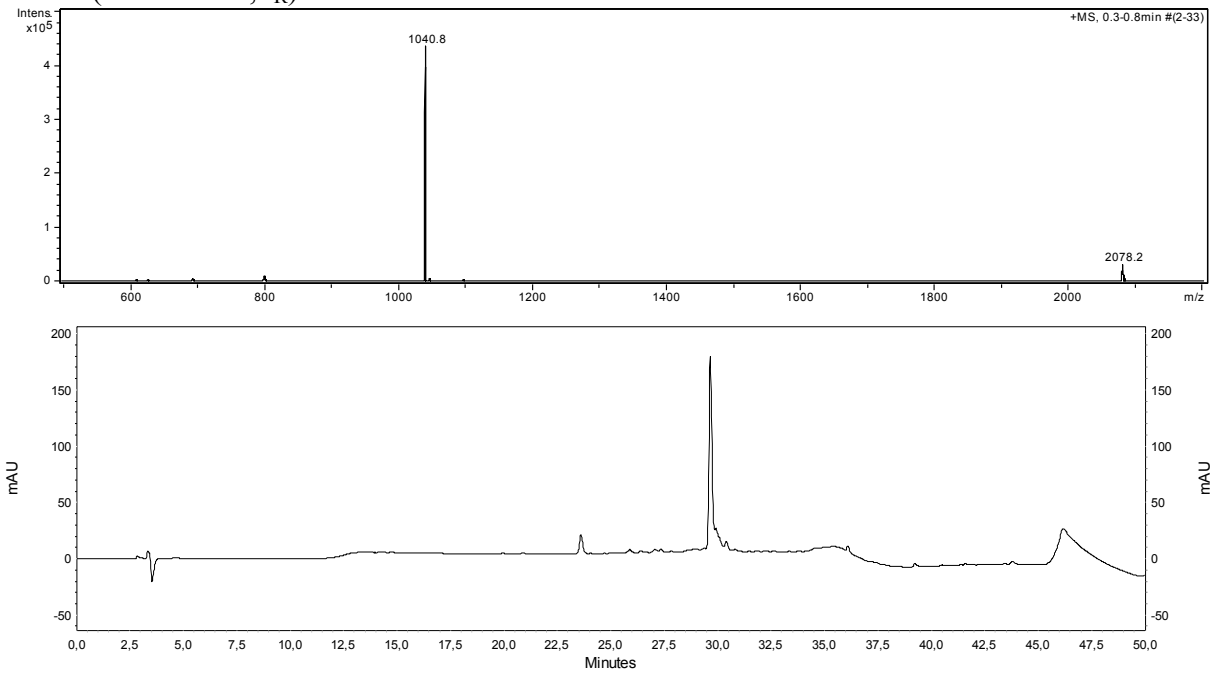
Synthesis of (H-Trp-Arg-Trp-triazole)$_3$-(ethyl)$_3$-benzene ***11b***

The peptide H-Trp-Arg-Trp-N(H)CH_2CCH (20 mg, 34 µmol, 4 eq.) was dissolved in DMF. One third of this solution was added to a solution of 1,3,5-triazidomethyl-2,4,6-ethyl-benzene (2.9 mg, 9 µmol, 1 eq.), $(EtO)_3$PCuI (1.5 mg, 4.5 µmol, 0.5 eq.) and DiPEA (5.8 µL, 34 µmol, 4 eq.) in DMF. The reaction mixture was heated in the microwave for 90 min at 60 °C. One third of the peptide solution was added again to the reaction mixture. It was again heated in the microwave for another 90 min at 60 °C. The last third of the peptid solution was added as well as $(EtO)_3$PCuI (0.6 mg, 1.5 µmol, 0.5 eq.) and the reaction in the microwave was repeated. The solvent was removed in vacuo and the mixture was purified via HPLC. Yield: 5.2 mg (27%).

Characterization:

MS (ESI^+, *m*/z): 2078.2 (calc. 2078.1 for $[M + H]^+$), 1040.8 (calc. 1039.6 for $[M + 2H]^{2+}$).

HPLC (λ = 214 nm, t_R): 29.6 min.



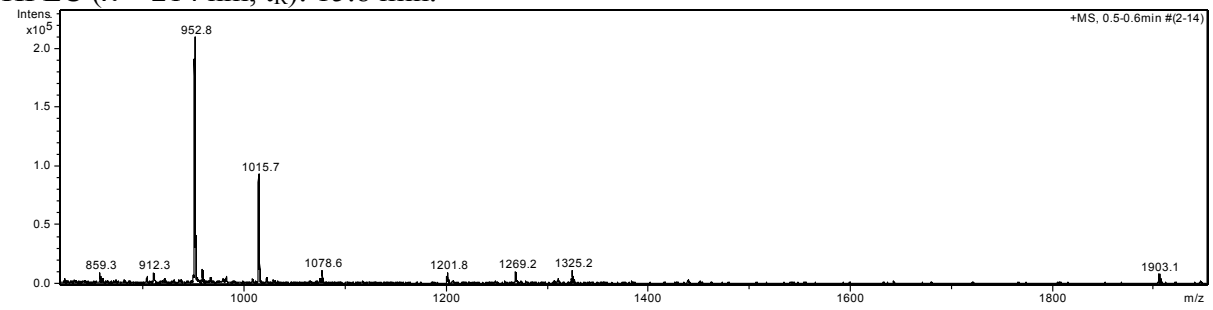
Synthesis of (H-Arg-Trp-Arg-triazole)$_3$-benzene ***12a***

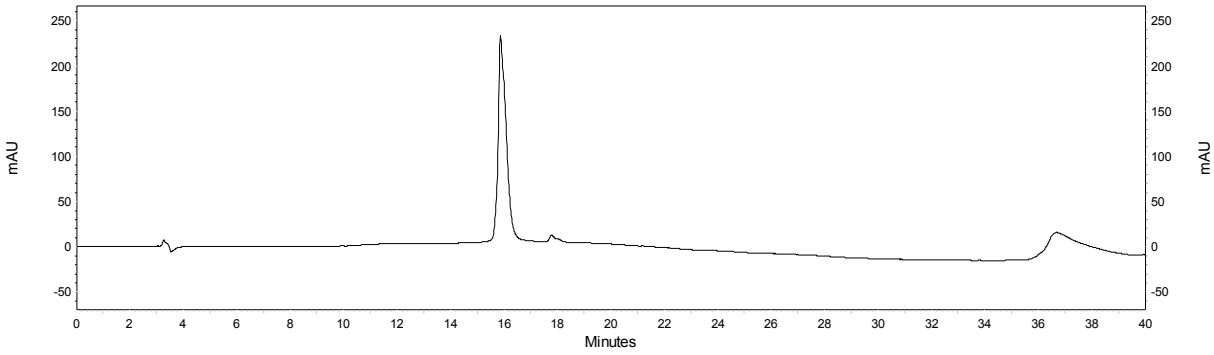
The peptide H-Arg-Trp-Arg-N(H)CH_2CCH (7.2 mg, 13 µmol, 4 eq.) was dissolved in DMF. One third of this solution was added to a solution of 1,3,5-triazidomethylbenzene (0.64 µL, 3 µmol, 1 eq.), $(EtO)_3$PCuI (0.6 mg, 1.5 µmol, 0.5 eq.) and DiPEA (2.2 µL, 13 µmol, 4 eq.) in DMF. The reaction mixture was heated in the microwave for 90 min at 60 °C. One third of the peptide solution was added again to the reaction mixture. It was again heated in the microwave for another 90 min at 60 °C. The last third of the peptid solution was added as well as $(EtO)_3$PCuI (0.6 mg, 1.5 µmol, 0.5 eq.) and the reaction in the microwave was repeated. The solvent was removed in vacuo and the mixture was purified via HPLC. Yield: 2.2 mg (36%).

Characterization:

MS (ESI$^+$, *m*/z): 1903.1 (calc. 1904.1 for $[M+H]^+$), 1015.7, 952.8 (calc. 952.6 for $[M+2H]^{2+}$).

HPLC (λ = 214 nm, t_R): 15.8 min.





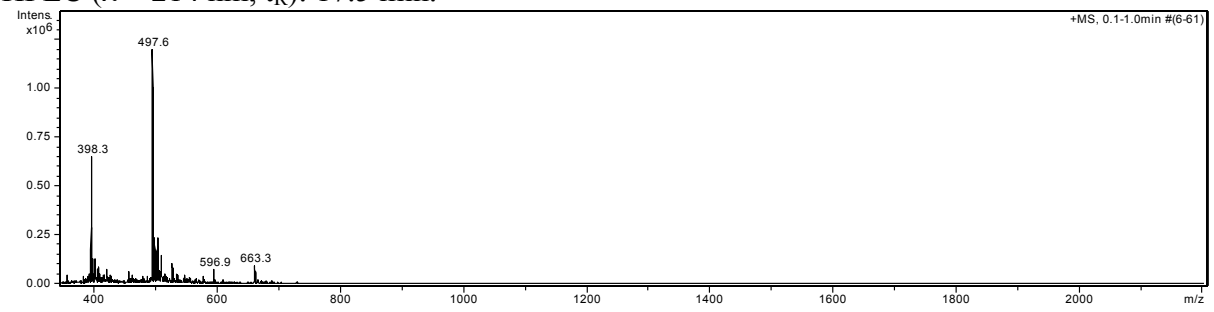
Synthesis of (H-Arg-Trp-Arg-triazole)$_3$-(ethyl)$_3$-benzene ***12b***

The peptide H-Arg-Trp-Arg-N(H)CH_2CCH (7.2 mg, 13 µmol, 4 eq.) was dissolved in DMF. One third of this solution was added to a solution of 1,3,5-triazidomethyl-2,4,6-ethyl-benzene (1 mg, 3 µmol, 1 eq.), $(EtO)_3PCuI$ (0.6 mg, 1.5 µmol, 0.5 eq.) and DiPEA (2.2 µL, 13 µmol, 4 eq.) in DMF. The reaction mixture was heated in the microwave for 90 min at 60 °C. One third of the peptide solution was added again to the reaction mixture. It was again heated in the microwave for another 90 min at 60 °C. The last third of the peptid solution was added as well as $(EtO)_3PCuI$ (0.6 mg, 1.5 µmol, 0.5 eq.) and the reaction in the microwave was repeated. The solvent was removed in vacuo and the mixture was purified via HPLC. Yield: 2.1 mg (33%).

Characterization:

MS (ESI^+, *m*/z): 663.3 (calc. 663.4 for $[M+3H]^{3+}$), 497.6 (calc. 497.8 for $[M+4H]^{4+}$), 398.3 (calc. 398.4 for $[M+5H]^{5+}$).

HPLC (λ = 214 nm, t_R): 17.5 min.



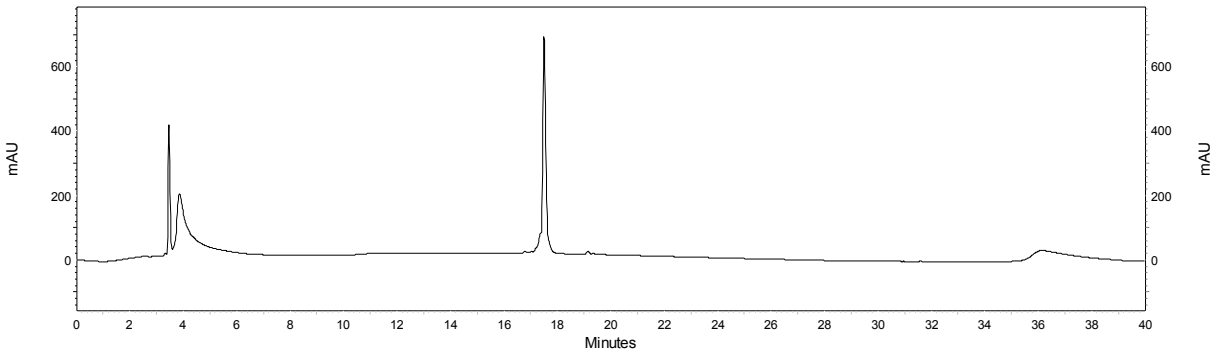


Table 1: Crystallographic data of scaffold **b**

Scaffold **b**: 1,3,5-tris(azidomethyl)-2,4,6-triethylbenzene	
Empirical formula	$C_{15}H_{21}N_9$
Formula weight	327.41 g / mol
Temperature	223 K
Crystal system, space group	Monoclinic, P 21/c
Unit cell dimension	a = 8.429(5) Å, α = 90°
	b = 15.689(9) Å, β = 101.668(13)°
	c = 13.238(8) Å, γ = 90°
Volume	1714.5(18)
Z	4
Theta range of data collection	2.04 – 25.00°
Reflections collected/unique	13278/3002 (R_{int} = 0.1480)
Completeness of theta = 24.99	99.4%
Data/ restraints/ parameters	3002/0/218
Goodness of fit on F^2	1.140
Final R indides ($I > 2\sigma_I$)	R1 = 0.0986, wR2 = 0.2218
R indices (all data)	R1 = 0.1880, wR2 = 0.2671
Largest diff. peak and hole	0.331 and -0.291 e.Å^{-3}