Supplementary Information

Functionalized Triazolopeptoids – a class for mitochondrial targeted delivery

Daniela Althuon[a†], Franziska Rönicke,[b] Daniel Fürniss,[a†] Jasmin Quan,[a] Isabelle Wellhöfer,[b] Nicole Jung,[a,b] Ute Schepers[b*] and Stefan Bräse[a,b*]

1. General Experimental Details

Preparative HPLC was performed on a C18 VYDAC 218 TP 1022 column (Grace Davison Discovery Sciences, 22.0 cm × 250 mm) with a linear gradient of 30-95% acetonitrile/water (0.1% TFA) over 40 min with a flow rate of 15.0 mL/min using a Jasco instrument equipped with a *Jasco*CO-2060 Plus thermostat, two *Jasco*PU 2087 Plus pumps, a *Jasco*MD-2010 Plus diode array detector and a CHF 122SC fractioncollector from *Advantec*.

Analytical HPLC was performed on a C18 reversed-phase analytical RP-HPLC column at room temperature (3.00-5.00 μm, 4.0 mm × 250 mm) using an Agilent 1200 HPLC equipped with a G1322A degasser, a G1311A pump, G1313A autosampler, a G1316A column oven and a G1315B diode array detector. A linear gradient of 5-95% acetonitrile/water (0.1% TFA) over 20 min was used with a flow rate of 1.00 mL/min. Analytical HPLC was also performed on a Gemini RP C4 column (3 µm, 30 mm × 2 mm, Phenomenex) with a linear gradient of 5-95% acetonitrile/water (0.1% TFA) in 12 min using a *„reversed phase liquid chromatography-electrospray ionization-tandem mass spectrometry system"* (RP-LC-ESI-MS/MS) with a tandem quadrupol mass spectrometer (API 4000™, electrospray ionisation, LC/MS/MS System, Applied Biosystems/MDS SCIEX).

Amphiphilic triazolopeptoids were purified on a preparative RP C4 column (Macherey Nagel VP 250/10 Nucleosil 120-5 C4) with a linear gradient of 5-95% acetonitrile (0.1% TFA) over 100 min with a flow rate of 1.5 mL/min by using the Äkta Explorer system (GE Biosciences).

MALDI-TOF mass spectra were measured on a Bruker Biflex IV spectrometer using dihydroxybenzoic acid (DHB) and α-cyano-4-hydroxy-cinnamic acid (1:1) as matrix. The abbreviation used for the protonated molecule ion is $[M+H]^+$.

NMR spectra were measured on a Bruker AM 400 spectrometer. Chemical shifts are expressed in parts per million (ppm, δ) downfield from tetramethylsilane (TMS) and are referenced to $CHCl_3$ (7.26 ppm) or CH_3CN (1.94 ppm) as internal standard. All coupling constants are absolute values and J values are expressed in Hertz (Hz). For assigning signal separation of ^1H NMR spectra the following abbreviations

were used: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quin. = quintet, m = multiplet, dd = doublet of doublets, H_{ar} = aromatic proton.

In most cases, the molecules exhibit hindered rotation due to the amide functionality: C_{rot} refers to carbon atoms being split due to hindered rotation.

IR (infrared spectroscopy) data were recorded on a FT-IR Bruker Alpha-T as thin films on KBr and are reported as follows: wave number of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, vw = very weak).

EI-MS (electron ionization mass spectrometry) was performed by using a Finnigan MAT 90 (70 eV). The molecular fragments are reported as the ratio between mass and charge (*m/z*), the intensities are reported as a percentage value relative to the intensity of the base signal (100%).

2. General experimental Procedures

Solvents and reagents purchased from commercial sources were used without further purification. Abbreviations for reagents are as follows: trifluoroacetic acid (TFA); 1,1,1,3,3,3-hexafluoroisopropyl alcohol (HFIP); dichloromethane (DCM); *N*,*N*-dimethylformamide (DMF); *N*,*N*′-diisopropylcarbodiimide (DIC); *N,N*-diisopropylethylamine (DIPEA); acetonitrile (ACN); 1-hydroxybenzotriazole (HOBt); tetrahydrofuran (THF); tetrabutylammonium fluoride (TBAF).

2.1. General Synthesis Protocols for triazolopeptoids

Syntheses of the linear triazolopeptoid were performed as previously reported ([1] Z. H. Ke, H. F. Chow, M. C. Chan, Z. F. Liu, K. H. Sze, *Org Lett* **2012**, *14*, 394-397) *via* solid phase synthesis on Rink amide resin (NovaBiochem, 0.67 mmol/g) with modified reaction times. Solid phase reactions were performed using fritted 5.00 mL plastic syringes (Multisyntech GmbH) filled with the resin. Yields were calculated according to the resin-loading value given by the manufacturer.

Azidoacetic acid (**3a**):

2.11 g (15.0 mmol, 1.00 equiv.) bromoacetic acid dissolved in 3.00 mL of water was slowly added to 1.95 g (30.0 mmol, 2.00 equiv.) NaN_3 dissolved in 18.0 mL water and stirred at room temperature for 48 h. Subsequently, the solution was adjusted to pH 1 with 1 M HCl, extracted with ethyl acetate (3 × 50.0 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the remaining liquid was dried on high-vacuum. The product was obtained as grey oil (1.24 g, 27.1 mmol, 82% yield).

^{1}H NMR (250 MHz, $CDCl_3$): δ = 10.71 (bs, 1 H, COO***H***), 3.99 (s, 2 H, CH_2) ppm. –^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 174.4 (C_{quat}, C=O), 45.0 (+, CH_2) ppm.– IR (ATR): ν = 2922 (w), 2229 (w), 2104 (s), 1718

(m), 1417 (m), 1277 (m), 1190 (m), 999 (w), 943 (w), 872 (w), 721 (w), 621 (w), 550 (w), 463 (vw) cm^{-1}. – MS (EI, 70 eV, 20 °C): m/z (%): 101.0 (100) [M^+], 56.0 (8.95) [$(CH_2N_3)^+$]. – HRMS ($C_2H_3O_2N_3$): calc. 101.0221, found 101.0221.

N-Benzylprop-2-ynyl-1-amine (**4a-H**):

2.04 g of 80% propargyl bromide (13.8 mmol, 1.00 equiv.) in toluene were added dropwise to 3.60 mL (41.3 mmol, 3.00 equiv.) benzylamine. The reaction was stirred at RT for 15h and then 2 M NaOH solution (100 mL) and diethyl ether (100 mL) was added. The organic phase was separated and the aqueous phase extracted with diethyl ether (2 × 50.0mL). The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography on a silica gel (cyclohexane/ethyl acetate = 50:1 to ethyl acetate) and the product was obtained as a yellow liquid (940 mg, 6.47 mmol, 47% yield).

R_f (CH/EE, 2:1) = 0.25. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.46–7.20 (m, 5 H, $C_{Ar}\underline{\boldsymbol{H}}$), 3.89 (s, 2 H, $C\underline{\boldsymbol{H}}_2C_{Ar}$), 3.43 (d, 2 H, *J* = 2.4 Hz, $C\underline{\boldsymbol{H}}_2$), 2.27 (t, 1 H, *J* = 2.4 Hz, $C\underline{\boldsymbol{H}}$), 1.55 (s, 1 H, $N\underline{\boldsymbol{H}}$) ppm. – ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 139.3 (C_{quat}, $\underline{\boldsymbol{C}}_{Ar}$), 128.4 (+, 2 C, C_{Ar}), 127.1 (+, 2C, $\underline{\boldsymbol{C}}_{Ar}H$), 127.1 (+, $\underline{\boldsymbol{C}}_{Ar}H$), 82.0 ($C_{quat}$, $\underline{\boldsymbol{C}}$≡C), 71.5 (+, $\underline{\boldsymbol{C}}H$), 52.2 (–, $\underline{\boldsymbol{C}}H_2C_{Ar}$), 37.3 (–, $N\underline{\boldsymbol{C}}H_2$) ppm. – IR (ATR): ν = 3288 (w), 3061 (vw), 3027 (vw), 2837 (w), 1603 (w), 1494 (w), 1452 (w), 1360 (w), 1328 (w), 1201 (vw), 1103 (w), 1027 (w), 966.8 (vw), 906 (w), 819 (w), 733 (m), 697 (m), 563 (w), 541 (w), 469(w) cm^{-1}. – Raman: ν = 3057, 3005, 2929, 2838, 2107 (alkyne), 1605, 1586, 1448, 1363, 1205, 1177, 1157, 1030, 1004, 907, 823, 621, 333, 297, 224 cm^{-1}. – MS (EI, 70 eV, 40 °C): m/z (%) = 145 (35) [M^+], 144 (100) [$(M–H)^+$], 91 (57) [$C_7H_7^+$]. – HRMS ($C_{10}H_{10}N$): calcd. 144.0808, found 144.0809.

Fmoc-Benzylprop-2-ynyl-1-amine (**4a-Fmoc**):

840 mg (5.79 mmol, 1.00 equiv.) *N*-Benzylprop-2-ynyl-1-amine (**4a-H**) were dissolved in 40.0 mL dichloromethane at 0 °C. Then 1.95 g (5.79 mmol, 1.00 equiv.) FmocOSu, 70.7 mg (0.58 mmol, 0.10 equiv.) DMAP and 1.01 mL (748 mg, 5.79 mmol, 1.00 equiv.) DIPEA were added and stirred at room temperature for 4 h. The organic phase was washed with 1 M HCl solution, concentrated $NaHCO_3$ solution and concentrated NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure and the remaining liquid was dried on high-vacuum. The crude product was obtained as a colorless oil (2.00 g, 5.23 mmol, 91% yield) and was used without further purification.

R_f (CH:EE, 10:1) = 0.32. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.83–7.58 (m, 3 H, C_{Ar}***H***), 7.57–7.18 (m, 9 H, C_{Ar}***H***), 7.16 – 6.96 (m, 1 H, C_{Ar}***H***), 4.65 (s, 1 H, C***H***HC_{Ar}) 4.58–4.45 (m, 3 H, OC***H***$_2$, CH***H***C_{Ar}), 4.42 – 4.17 (m, 1 H, OCH_2C***H***), 4.07 (s, 1 H, C≡CC***H***H), 3.99 (s, 1 H, C≡CCH***H***), 2.27 (s, 1H, C***H***) ppm. – ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 155.8 (+, ***C***=O), 143.8 (C_{quat}, 2 C, ***C***$_{Ar}$), 141.3 (C_{quat}, 2 C, ***C***$_{Ar}$), 136.6 (C_{quat}, ***C***$_{Ar}$), 128.6 (+, 2 C, ***C***$_{Ar}$), 128.2 (+, ***C***Ar), 127.7 (+, 4 C, ***C***Ar), 127.0 (+, 2 C, ***C***Ar), 125.3 (+, ***C***Ar), 124.8 (+, ***C***Ar), 120.0 (+, 2 C, ***C***Ar), 78.3 (C_{quat}, ***C***≡CH), 72.3 (+, C≡***C***H), 67.7 (–, O***C***$_{rot}H_2$), 67.7 (–, O***C***$_{rot}H_2$), 49.5 (–, ***C***$_{rot}H_2C_{Ar}$), 49.0 (+, ***C***$_{rot}H_2$***C***Ar), 47.2 (+, OCH_2***C***H), 35.7 (–, C≡***C***$_{rot}$***C***H_2), 35.2 (–, C≡C_{rot}***C***H_2) ppm. –IR (ATR): ν = 3285 (w), 3063 (w), 292 (w), 1697 (s), 1494 (w), 1449 (m), 1414 (m), 1367 (w), 1229 (s), 1115 (m), 1029 (w), 997 (w), 944 (w), 879 (w), 752 (m), 738 (s), 698 (m), 620 (m), 536 (w), 457 (vw), 426 (w) cm^{-1}. – Raman: ῦ= 3055, 2955, 2248, 2123 (alkyne), 1581, 1483, 1449, 1297, 1233, 1221, 1156, 1104, 728, 416, 365, 300, 261 cm^{-1}. – MS (EI, 70 eV, 130 °C): m/z (%) = 367 (100) [M^+], 178 (100) [$(C_{14}H_{10})^+$]. – HRMS ($C_{25}H_{21}NO_2$): calc. 367.1567 found 367.1565.

N-(4-Chlorobenzyl)prop-2-yn-1-ylamine (**4b-H**):

1.56 mL of 80% propargyl bromide in toluene (16.4 mmol, 1.00equiv.) was added dropwise to 6.00 mL 4-chlorobenzylamine (49.3 mmol, 3.00 equiv.) in 10.0 mL DMF. The reaction was stirred at room temperature for 15 h and then 50.0 mL of 2 M NaOH solution and 50.0 mL of diethyl ether were added. The organic phase was separated and the aqueous phase extracted with diethyl ether (2 × 50.0 mL). The combined organic phases were dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was purified by column chromatography on a silica gel (cyclohexane / ethyl acetate = 2:1). The remaining liquid was dried on high-vacuum. The product was obtained as a yellow liquid (1.60 g, 9.04 mmol, 55% yield).

R_f (CH/EE, 4:1) = 0.25. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.28 (m, 4 H, C_{Ar}***H***), 3.85 (s, 2 H, C***H***$_2$-C_{Ar}), 3.41 (d, 2 H, *J* = 2.4 Hz, C***H***$_2$), 2.26 (t, 1 H, *J* = 2.4 Hz, C***H***), 1.60 (s, 1 H, N***H***) ppm. – ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 137.8 (C_{quat}, ***C***$_{Ar}$), 132.9 (C_{quat}, ***C***$_{Ar}$-Cl), 129.7 (+, 2 C, ***C***Ar), 128.5 (+, 2 C, ***C***Ar), 81.8 (C_{quat}, ***C***≡C), 71.8 (+, ***C***H≡C), 51.4 (–, ***C***H_2-NH), 37.2 (–, ***C***H_2-C) ppm. – IR (ATR): ν = 3295 (w), 2837 (w), 1596 (vw), 1490 (m), 1451 (w), 1407 (w), 1328 (w), 1087 (m), 1014 (m), 905 (w), 838 (w), 797(m), 756 (w), 707 (w), 632 (m), 566 (w), 513 (w), 489 (w), 430 (w) cm^{-1}. – Raman: ῦ = 3065, 2937, 2863, 2108 (alkyne), 1599, 1430, 1205, 1175, 1089, 799, 634, 362, 334, 303, 278 cm^{-1}. – MS (EI, 70 eV, 20 °C): m/z (%) = 179.1 (58) [M^+], 178.1 (100) [$(M–H)^+$], 91.1 (23) [$(C_7H_7)^+$]. – HRMS ($C_{10}H_9NCl$):

calcd. 178.0418, found 178.0419. – CHN analysis ($C_{10}H_{10}ClN$): calcd. N: 7.80, C: 66.86, H: 5.61; found N: 8.05, C: 66.97, H: 5,56.

9*H*-(Fluoren-9-yl)methylprop-2-yn-1-ylcarbamate (**4d-Fmoc**):

673 mg (12.2 mmol, 1.00 equiv.) propargylamine was dissolved in 37.0 mL dichloromethane. 4.12 g (12.2 mmol, 1.00 equiv.) Fmoc-OSu, 150 mg (1.22mmol, 0.10 equiv.) DMAP and 2.12 mL (12.2 mmol, 1.00 equiv.) DIPEA were added at 0 °C. The reaction solution was stirred at room temperature for 4 h. Then the organic phase was washed with 1 M HCl, concentrated $NaHCO_3$ solution and concentrated NaCl solution and dried over $MgSO_4$. The solvent was removed under reduced pressure and the remaining solid was dried on high-vacuum. The crude product was obtained as a colorless solid (2.57 g, 9.27 mmol, 76% yield).

R_f (CH/EE, 9:1) = 0.25. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.68 (d, *J* = 7.3 Hz, 2 H, CAr***H***), 7.51 (d, *J* = 7.2 Hz, 2 H, CAr***H***), 7.27 (m, 4 H, CAr***H***), 4.91 (bs, 1 H, N***H***), 4.35 (d, *J* = 6.9 Hz, 2 H, OC***H***$_2$), 4.14 (t, *J* = 6.7 Hz, 1 H, CH), 3.91 (m, 2 H, NC***H***$_2$), 2.26 (t, *J* = 2.5 Hz, 1 H, CH) ppm. − ^{13}C NMR (62.9 MHz, $CDCl_3$) δ = 155.9 ($C_{quat,}$ ***C***=O), 143.8 (***C***$_{Ar}$-CH_{Ar}-***C***$_{Ar}$), 141.3 (***C***$_{Ar}$-***C***$_{Ar}$), 127.7 (+, C_{Ar}H-C_{Ar}-C_{Ar}H-***C***$_{Ar}$H), 127.0 (+, ***C***$_{Ar}$H-C_{Ar}H-C_{Ar}-C_{Ar}), 124.0 (+, ***C***$_{Ar}$H-C_{Ar}-C_{Ar}H), 120.0 (+, ***C***$_{Ar}$H-C_{Ar}-C_{Ar}), 79.6 (C_{quat}, ***C***≡CH), 71.7 (+, ***C***H≡C), 67.0 (–, ***C***H_2-O), 47.1 (+, C-H***C***-C), 30.8 (–, ***C***H_2-NH) ppm. – IR (ATR): ν = 3291 (w), 2940 (w), 2884 (s), 1695.4 (m), 1532 (m), 1448 (m), 1431 (m), 1274 (w), 1257 (w), 1144 (w), 1100 (w), 1081 (w), 1044 (w), 988 (vw) , 925 (m), 902 (m), 778 (w), 758 (w), 742 (w), 730 (w), 671 (w), 629 (w), 586 (w), 544 (m), 461 (m), 441 (w), 425 (w) cm^{-1}. – Raman: ῦ= 3065, 3054, 2943, 2122 (alkyne), 1581, 1483, 1432, 1346, 1297, 1222, 1194, 1102, 990, 855, 732, 416, 266, 117, 100, 76 cm^{-1}. – MS (EI, 70 eV, 100 °C): m/z (%): 277.1 (100) [M^+]. – HRMS ($C_{18}H_{15}NO_2$): calcd. 277.1097, found 277.1095. – CHN analysis ($C_{18}H_{15}NO_2$): calcd. N: 5.05, C: 77.96, H: 5.45; found. N: 4.93, C: 77.77, H: 5.35.

Tert-butyl(6-aminohexyl)carbamate:

79.3 g 1,6-hexanediamine (682 mmol, 1.00 equiv.) was dissolved in 1.05 mL dioxane. A solution of 19.3 g of Boc-anhydride (88.7 mmol, 0.13 equiv.) in 225 mL dioxane was slowly added dropwise and the reaction mixture was stirred at room temperature overnight. Afterwards, the solvent was removed under reduced pressure. The residue was suspended in 225 mL water and filtered. The product was extracted

from the aqueous phase three times with ethyl acetate (225 mL) and the combined organic phases were washed with water (2 × 225 mL) and concentrated NaCl solution (225 mL). The solvent was removed under reduced pressure and the remaining liquid was dried on high vacuum. The product was obtained as a colorless oil (14.0 g, 64.7 mmol, 73% yield).

R_f (CH/EE, 2:1) = 0.72. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.64 (bs, 1 H, N***H***), 3.05 (m, 2 H, C***H***$_2$-NH), 2.63 (t, *J*= 6.8 Hz, 2 H, NH_2-C***H***$_2$), 1.59–1.39 (m, 13 H, C***H***$_3$, NH_2-CH_2-C***H***$_2$), 1.39–1.27 (m, 4 H, C***H***$_2$-C***H***$_2$) ppm. – ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 155.9 (C_{quat}, C=O), 78.6 (C_{quat}, ***C***CH_3), 41.9 (–, ***C***H_2-NH), 40.3 (–, ***C***H_2-NH_2), 33.5 (–, NH-CH_2-***C***H_2), 29.8 (–, NH_2-CH_2-***C***H_2), 28.2 (–, 3 C, ***C***H_3), 26.4 (–, NH_2-CH_2-CH_2-***C***H_2), 26.3 (–, ***C***H_2-CH_2-CH_2-NH_2) ppm. – IR (ATR): ν = 3366 (w), 2930 (w), 2860 (w), 1683 (w), 1518 (w), 1478 (w), 1463 (w), 1388 (w), 1364 (w), 1276 (w), 1249 (w), 1167 (w), 1050 (w), 993 (w), 868 (w), 820 (vw), 780 (w), 72 (vw), 590 (w), 499 (vw), 449. (vw) cm^{-1}. – Raman: ῡ = 2979, 2932, 2856, 2716, 1691, 1452, 1368, 1306, 1252, 1073, 922, 872, 765, 464, 330, 84 cm^{-1}. – MS (EI, 70 eV, 80 °C), m/z-(%) 216.2 [M^+], 160.1 [M^+-C_4H_7]. – HRMS ($C_{11}H_{24}N_2O_2$): calc. 216.1832; found 216.1830.

Tert-butyl(6-(pro-2-yn-1-ylamino)hexyl)carbamate (**4c-Fmoc**):

2.96 g of 80% propargyl bromide (20.1 mmol, 1.00 equiv.) in toluene was added dropwise to 13.0 g (60.3 mmol, 3.00 equiv.) *tert*-butyl(6-aminohexyl)carbamate in 10.0 mL DMF. The reaction was stirred at room temperature for 15 h. Then, 100 mL of 2 M NaOH solution and 100 mL of diethyl ether were added. The organic phase was separated and the aqueous phase extracted with diethyl ether (2 × 50.0 mL). The solvent was removed under reduced pressure. The crude product was purified by column chromatography on a silica gel (DCM/MeOH = 50:1 to MeOH). After drying on high-vacuum the product was obtained as a green oil (1.67 g, 6.56 mmol, 33%) yield.

R_f(CH/EE, 4:1) = 0.25. – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.53 (s, 1 H, N***H***) 3.41 (d, *J* = 2.4 Hz, 2 H, NH-C***H***$_2$-CH), 3.09 (m, 2 H, NH-C***H***$_2$), 2.66 (m, 2 H, C***H***$_2$-NH), 2.20 (d, *J* = 2. Hz, 1 H, C***H***≡C), 1.52–1.38 (m, 13 H, C***H***$_3$, NH_2-CH_2-C***H***$_2$), 1.38–1.28 (m, 4 H, C***H***$_2$-C***H***$_2$) ppm. – ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 160.0 (C_{quat}, ***C***=O), 82.2 (C_{quat}, ***C***≡CH), 79.0 (***C***(CH_3), 71.2 (+, C≡***C***H), 48.5 (–, ***C***H_2-NH), 40.5 (–, ***C***H_2-C), 38.1 (–, ***C***H_2-NH_2), 30.0 (–, NH-CH_2-***C***H_2), 29.7 (–, NH_2-CH_2-***C***H_2), 28.4 (3 C, ***C***H_3), 26.9 (–, NH_2-CH_2-CH_2-***C***H_2), 26.6 (–, ***C***H_2-CH_2-CH_2-NH_2) ppm. – IR (ATR): ν = 3304 (w), 2928 (w), 2855.8 (w), 1692 (m), 1513 (m), 1454 (w), 1390 (w), 1364 (m), 1270 (m), 1248 (m), 1167 (w), 1041

(vw), 980 (vw), 864 (vw), 640 (w) cm^{-1}. – Raman: ν = 2979, 2932, 2855, 2722, 2108 (alkyne), 1709, 1451, 1306, 1257, 1114, 1039, 922, 870, 764, 332, 83 – MS (EI, 70 eV, 110 °C): m/z (%) = 255.2 (100) $[(M+1)^+]$, 199.2 (100) $[M^+-C_4H_6]$. – HRMS ($C_{14}H_{26}N_2O_2$): calcd. 254.1989, found 254.1990 – CHN analysis ($C_{14}H_{26}O_2N_2$): calcd. N: 11.01, C: 66.11, H: 10.30; found N: 11.16, C: 66.07, H: 10.26.

Triazolopeptoid **14**:

100 mg (0.064 mmol, 1.00 equiv.) Rink amide resin was swollen in 2.00 mL of DMF (2.00 mL per 0.064 mmol of resin) in a syringe with a frit and cannula for 1 h. The solvent was removed, the resin was agitated with 1.50 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. The resin was washed five times with DMF. After the deprotection, the resin was agitated with 0.420 mL (0.506 mmol, 7.90 equiv.) of a 1.20 M bromoacetic acid solution in DMF and 78.0 µl (0.506 mmol, 7.90 equiv.) DIC for 1 h. The solvent was removed and the resin was washed three times with DMF. For the substitution of the bromine the resin was agitated with 0.510 mL (0.506 mmol, 7.90 equiv.) of a 1 M solution of 2-methoxyethylamine (**10**) in DMF for 30 min. After removal of the solvent the resin was washed three times with DMF. The resin was acylated by agitating with 0.420 mL (0.506 mmol, 7.90 equiv.) of a 1.2 M azidoacetic acid (**3a**) in DMF, and 78.0 µL (0.506 mmol, 7.90 equiv.) DIC for 1 h. After removal of the solvent, the resin was washed three times with DMF. Then, 18.0 mg (96.0 µmol, 1.50 equiv.) copper iodide, 63.1 mg (0.128 mmol, 2.00 equiv.) Fmoc-*N*-benzylprop-2-ynyl-1-amine (**4a-Fmoc**), 0.570 mL (3.20 mmol, 50.0 equiv.) DIPEA and 1.5 mL of abs. THF were given to the resin and agitated overnight. After removal of the solvent the resin was agitated with 1.50 mL of 20% piperidine in DMF at room temperature (deprotection) for 3 × 5 min. The resin was washed five times with DMF. The acylation, CuAAC and deprotection were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 92.0 mg (0.192 mmol, 3.00 equiv.) rhodamine B, 30.0 mg (0.192 mmol, 3.00 equiv. HOBt and 24.0 mg (0.192 mmol, 3.00 equiv.) DIC were added in 2.00 mL DMF (1.00 mL per 0.064 mmol resin). The suspension was agitated overnight. The solvent was removed and the resin was washed with DMF and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 1.50 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed five times with MeOH. Finally the solvent was removed at 40 °C under reduced pressure. The product was then purified by HPLC

Trimer **14c** (n = 3): 25.2 mg (2.03 µmol, 32% yield over 9 steps, purity: 85%) of a purple solid was obtained. – Analytical HPLC (5-95% acetonitrile +0.1% TFA in 30 min, detection at 218 nm).

MS (MALDI): m/z (%): 1241.4 [M^+].

Triazolopeptoid **15** (Variant A):

188 mg (0.12 mmol, 1.00 equiv.) Rink amide resin was swollen in 4.00 mL of DMF in a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. The resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was acylated by agitating with 35.5 mg (0.35 mmol, 3.00 equiv.) azidoacetic acid (**3a**) in 4.00 mL DMF, 7.15 mg (59.0 µmol, 0.50 equiv.) DMAP and 72.4 mg (0.35 mmol, 3.00 equiv.) DCC for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then, 33.4 mg (0.18 mmol, 1.50 equiv.) CuI, 63.1 mg (0.13 mmol, 3.00 equiv.) 4-(chlorobenzyl)prop-2-ynyl-1-amine (**4b-H**), 0.10 mL (0.56 mmol, 5.00 equiv.) DIPEA in 4.00 mL THF/DMF 1:1 were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH /DMF/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation and CuAAC were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 168 mg (0.35 mmol, 3.00 equiv.) of rhodamine B, 47.4 mg (0.35 mmol, 3.00 equiv.) of HOBt and 72.2 mg (0.35 mmol, 3.00 equiv.) of DCC were added in 4.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed three times with DMF, CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 2.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed with CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The crude product was pre-purified by column chromatography on silica gel (DCM to MeOH) and then purified by HPLC.

Monomer **15a** (n = 1): 6.00 mg (8.55 µmol, 7.2% yield over 9 steps, purity: 95%) of a purple solid was obtained. – MS (MALDI), $C_{40}H_{43}ClN_7O_3$: *m/z*: 703.5 $[M]^+$. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 23.3 min.

Dimer **15b** (n = 2): 4.7 mg (4.86 µmol, 4.1% yield over 9 steps, purity: 91%) of a purple solid was obtained. – MS (MALDI), $C_{52}H_{54}Cl_2N_{11}O_7$: *m/z*: 965.6 [$M^+$]. – MS (FAB), $C_{52}H_{54}Cl_2N_{11}O_7$: *m/z*: 966.4

[M^+]. – HRMS ($C_{52}H_{54}Cl_2N_{11}O_7$): calc. 966.3732, found 966.3733. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 26.5 min.

Trimer **15c** (n = 3): 0.50 mg (0.41 µmol, 0.35% yield over 9 steps, purity: 90%) of a purple solid was obtained. – MS (MALDI) $C_{64}H_{55}Cl_2N_{15}O_{12}$: *m/z*: 1229.8 [$M^+$]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 22.9 min.

Triazolopeptoid **15 (Variant B)**:

151 mg (96.0 µmol, 1.00 equiv.) of Rink amide resin were swollen in 4.00 mL of DMF in a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. The resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was acylated by agitating with 29.2 mg (0.29 mmol, 3.00 equiv.) azidoacetic acid (**3a**), 7.15 mg (59.0 µmol, 0.50 equiv.) DMAP and 59.6 mg (0.29mmol, 3.00 equiv.) DCC in 4.00 mL DMF for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then, 91.7 mg (0.48 mmol, 1.50 equiv.) CuI, 51.9 mg (0.29 mmol, 2.00 equiv.), 198 mg (0.48 mmol, 3.00 equiv.) 4-(chlorobenzyl)prop-2-ynyl-1-amine (**4b-H**) and 0.16 mL (0.98 mmol, 10.0 equiv.) DIPEA in 3.00 mL DMF/Piperidin (7:3) were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH/DMF/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation and CuAAC were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 55.0 mg (0.29 mmol, 3.00 equiv.) Rhodamine B, 39.0 mg (0.29 mmol, 3.00 equiv.) of HOBt and 59.6 mg (0.29 mmol, 3.00 equiv.) of DCC were added in 4.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed three times with DMF, CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 3.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed with CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The product was then purified by HPLC.

Monomer **15a** (n = 1): 19.3 mg (2.73 µmol, 29% yield over 9 steps, purity: 88%) of a purple solid were obtained. – MS (MALDI), $C_{40}H_{43}ClN_7O_3$: *m/z*: 703.5 [$M]^+$. – MS (FAB), $C_{40}H_{43}ClN_7O_3$: *m/z*: 704.3 [M^+].

– HRMS ($C_{40}H_{43}ClN_7O_3$): cal. 704.3110, found 704.3107. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 23.3 min.

Dimer **15b** (n = 2): 8.80 mg (9.09 µmmol, 1.6% yield over 9 steps, purity: 84%) of a purple solid was obtained. – MS (MALDI), $C_{52}H_{54}Cl_2N_{11}O_7$: *m/z*: 965.6[$M^+$] – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 26.5 min.

Trimer **15c** (n = 3): 2.20 mg (1.79 µmol, 2.2% yield over 9 steps, purity: 61%) of a purple solid was obtained. – MS (MALDI), $C_{64}H_{55}Cl_2N_{15}O_{12}$: *m/z*: 1229.8 [$M^+$]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 22.9 min.

Triazolopeptoid **16** (Variant A):

266 mg (0.17 mmol, 1.00 equiv.) of Fmoc-protected Rink amide resin (**7**) were swollen in 6.00 mL of DMF for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. After removal of the solvent the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was acylated by agitating with 50.3 mg (0.50 mmol, 3.00 equiv.) azidoacetic acid (**3a**) in 4.00 mL DMF and 103 mg (0.50 mmol, 3.00 equiv.) DCC for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. 47.4 mg (0.25 mmol, 1.50 equiv.) CuI, 106 mg (0.13 mmol, 3.00 equiv.) *tert*-butyl(6-(pro-2-yn-1-ylamino)hexyl)carbamate (**4c-H**) and 150 µL (830 µmol, 5.00 equiv.) DIPEA in 6.00 mL THF/DMF (1:1) were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH/DMF/MeOH/CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation, CuAAC and deprotection were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 94.8 mg (0.50 mmol, 3.00 equiv.) rhodamine B, 67.3 mg (0.50 mmol, 3.00 equiv.) HOBt and 103 mg (0.50 mmol, 3.00 equiv.) DCC were added in 6.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed with DMF (3 times), CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 3.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed with

CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The product was then purified by HPLC.

Trimer **16c** (n = 3): 4.79 mg (4.15 µmol, 2.4% yield over 9 steps, purity: 80%) of a purple solid was obtained. – MS (MALDI), $C_{61}H_{89}N_{18}O_5$: *m/z*: 1154.3 [M^+]. – MS (FAB), $C_{61}H_{89}N_{18}O_5$: *m/z*: 1154.2 [M^+]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 14.1 min.

Triazolopeptoid **16** (Variant B):

161 mg (0.10 mmol, 1.00 equiv.) of Fmoc-protected Rink amide resin (**7**) were swollen in 4.00 mL of DMF in a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. The resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was acylated by agitating with 31.3 mg (0.31 mmol, 3.00 equiv.) azidoacetic acid (**3a**) in 4.00 mL DMF and 63.9 mg (0.31 mmol, 3.00 equiv.) DCC for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then 98.3 mg (0.51 mmol, 5.00 equiv.) CuI, 65.6 mg (0.26 mmol, 2.50 equiv.) *tert*-butyl(6-(pro-2-yn-1-ylamino)hexyl)carbamate (**4c-H**), 102 mg (0.52 mmol, 5.00 equiv.) ascorbic acid and 0.28 mL (1.03 mmol, 10.0 equiv.) DIPEA in 4.00 mL DMF/piperidine (7:3) were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH/DMF/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation, CuAAC and deprotection were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 59.0 mg (0.31 mmol, 3.00 equiv.) Rhodamine B, 146 mg (0.31 mmol, 3.00 equiv.) HOBt and 63.0 mg (0.31 mmol, 3.00 equiv.) DCC were added in 4.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed with DMF (3 times), CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 2.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed with CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C on a rotary evaporator. The product was then purified by HPLC.

Monomer **16b** (n = 1): 1.35 mg (1.99 µmol, 1.98% yield over 9 steps, purity: 80%) of a purple solid was obtained. – MS (MALDI), $C_{39}H_{51}N_8O_3$: : 678.7 [M^+]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 15.9 min.

Dimer **16b** (n = 2): 6.00 mg (6.54 µmol, 6.3% yield over 9 steps, purity: 77%) of a purple solid was obtained. – MS (MALDI), $C_{50}H_{70}N_{13}O_4$: *m/z*: 917.0 [M^+] – Analytical HPLC (5–95% acetonitrile+0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 14.7 min.

Trimer **16c** (n = 3): 5.5 mg (4.33 µmol, 4.8% yield over 9 steps, purity: 61%) of a purple solid was obtained. – MS (MALDI), $C_{61}H_{89}N_{18}O_5$: *m/z*: 1154.3 [M^+]. – MS (FAB), $C_{61}H_{89}N_{18}O_5$: *m/z*: 1154.2 $[M]^+$. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 14.1 min.

Triazolopeptoid **16** (Variant C):

150 mg (96.0 µmol, 1.00 equiv.) of Fmoc-protected Rink amide resin (**7**) were swollen in 4.00 mL of DMF In a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20 % piperidine in DMF at room temperature for 3 × 5 min. The resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was acylated by agitating with 29.2 mg (0.29 mmol, 3.00 equiv.) azidoacetic acid (**3a**), 11.8 mg (9.60 µmol, 1.00 equiv.) DMAP und 59.6 mg (0.29 mmol, 3.00 equiv.) DCC in 4.00 mL DMF for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then 7.66 mg (48.0 µmol, 0.50 equiv.) $CuSO_4$, 61.1 mg (0.24 mmol, 2.50 equiv.) *tert*-butyl(6-(pro-2-yn-1-ylamino)hexyl)carbamate (**4c-H**) and 95.5 mg (0.48 mmol, 5.00 equiv.) ascorbic acid in 4.00 mL DMF/piperidine (7:3) were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH /DMF/MeOH/CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After removal of the solvent for 3 × 5 min with 3.00 mL of 20% piperidine in DMF was agitated at room temperature (deprotection). The resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation and CuAAC were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 54.9 mg (0.29 mmol, Rhodamine B 38.9 mg (0.30 mmol, 3.00 equiv.) HOBt and 59.4 mg (0.30 mmol, 3.00 equiv.) DCC were added in 4.00 mL DMF. The resin was agitated at 60 °C for 15 h. The solvent was removed and the resin was washed three times with DMF, CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 1.50 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a frit and the resin was washed with CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The product was detected by mass spectrometry. The crude product was then purified by HPLC.

Monomer **16a** (n = 1): – MS (MALDI), $C_{39}H_{51}N_8O_3$: *m/z*: 678.7 [M^+]. – Analytische HPLC (5–95% Acetonitril + 0.1% TFA in 30 min, Detection at 218 nm): t_{Ret} = 14.8 min.

Dimer **16b** (n = 2): – MS (MALDI), $C_{50}H_{70}N_{13}O_4$: *m/z*: 917.0 [M^+] – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, Detection at 218 nm): t_{Ret} = 13.8 min.

Trimer **16c** (n = 3): 1.6 mg (1.26 μmol, 1.4% yield over 9steps, purity: 94%) of a purple solid was obtained. – MS (MALDI), $C_{61}H_{89}N_{18}O_5$: *m/z*: 1154.3 [M^+]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 13.0 min.

Triazolopeptoid **17**:

156 mg (0.10 mmol, 1.00 equiv.) Rink amide resin was swollen in 4.00 mL of DMF in a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. After removal of the solvent the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was agitated with 83.37 mg (0.60 mmol, 6.00 equiv.) bromoacetic acid solution and 62.7 mg (0.30 mmol, 3.00 equiv.) DCC in 4.00 mL DMF for 1 h. The solvent was removed and the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. For the substitution of the bromine the resin was agitated with 79.91 mg (0.60 mmol, 6.00 equiv.) 2-methoxyethylamine (**10**) in 4.00 mL DMF for 30 min. After removal of the solvent the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The resin was acylated by agitating with 30.7 mg (0.30 mmol, 3.00 equiv.) azidoacetic acid (**3a**) in 4.00 mL DMF and 62.7 mg (0.30 mmol, 3.00 equiv.) DCC for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then, 28.9 mg (0.15 mmol, 1.50 equiv.) CuI, 54.4 mg (0.30 mmol, 3.00 equiv.) 4-(chlorobenzyl)prop-2-ynyl-1-amine (**4b-Fmoc**) and 90.0 μL (0.51 mmol, 5.00 equiv.) DIPEA in 4.00 mL THF/DCM (1:1) were given to the resin and agitated overnight. The resin was washed three times with THF and MeOH /DMF/MeOH/CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation and CuAAC were repeated two times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 144 mg (0.30 mmol, 3.00 equiv.) rhodamine B, 41.5 mg (0.30 mmol, 3.00 equiv.) HOBt and 62.4 mg (0.30 mmol, 3.00 equiv.) DCC were added in 4.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed three times with DMF, CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 2.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was filtered through a

frit and the resin was washed withCH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The product was detected by mass spectrometry. The crude product was purified by column chromatography on a silica gel (DCM to MeOH). The product was then purified by HPLC, because no purification is achieved.

Dimer **17b** (n = 2): 1.80 mg (1.66 µmol, 1.6% yield over 11 steps, purity: 89%) of a purple solid was obtained. – MS (MALDI), $C_{57}H_{63}Cl_2N_{12}O_8$: *m/z*: 1081.0 [$M^+$]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 22.6 min.

Trimer **17c** (n = 3): 2.40 mg (1.78 µmol, 1.8% yield over 11 steps, purity: 95%) of a purple solid was obtained – MS (MALDI, $C_{69}H_{74}Cl_3N_{16}O_7$): *m/z*: 1354.1 [$M^+$]. – Analytical HPLC (5–95% acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 24.0 min.

Triazolopeptoid **18**:

203 mg (0.13mmol, 1.00 equiv.) Rink amide resin was swollen in 4.00 mL of DMF in a syringe with a frit and cannula for 1 h. The solvent was removed and the resin was agitated with 3.00 mL of 20% piperidine in DMF at room temperature for 3 × 5 min. The solvent the resin was washed three times with DMF and CH_2Cl_2/MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. After the deprotection the resin was agitated with 108 mg (0.78 mmol, 6.00 equiv.) bromoacetic acid solution in 4.00 mL DMF and 82.5 mg (0.40 mmol, 3.00 equiv.) DCC for 1 h. The solvent was removed and the resin was washed 3 times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. For the substitution of the bromine the resin was agitated with 58.6 mg (0.78 mmol, 6.00 equiv.) 2-methoxyethylamine in 4.00 mL DMF for 30 min. After removal of the solvent the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The resin was acylated by agitating with 30.7 mg (0.30 mmol, 39.4 mg (0.39 mmol, 3.00 equiv.) azidoacetic acid in 4.00 mL DMF and 80.5 mg (0.39 mmol, 3.00 Äquiv.) DCC equiv.) DCC for 2 h. After removal of the solvent, the resin was washed three times with DMF and CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Then, 37.1 mg (0.20 mmol, 1.50 equiv.) CuI, 82.7 mg (0.33 mmol, 2.50 equiv.) *tert*-butyl(6-(pro-2-yn-1-ylamino)hexyl)carbamate and 0.11 mL (0.65 mmol, 5.00 equiv.) DIPEA in 4.00 mL THF/DCM (1:1) were given to the resin and agitated overnight. The resin was washed 3 times with THF and MeOH/DMF/MeOH/CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. The acylation and CuAAC were repeated 2 times to obtain the triazole-trimer. For the binding of the marker, the resin was given in a syringe with a frit and cannula and 74.3 mg (0.39 mmol, 3.00 equiv.) rhodamine

B, 52.7 mg (0.39 mmol, 3.00 equiv.) HOBt and 80.5 mg (0.39 mmol, 3.00 equiv.) DCC were added in 4.00 mL DMF. The resin was agitated at room temperature for 15 h. The solvent was removed and the resin was washed 3 times with DMF, CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2 and then washed with CH_2Cl_2 until the filtrate remained colorless. The triazol-trimer was cleaved from the resin by addition of 2.00 mL 95% TFA solution in CH_2Cl_2. The suspension was agitated overnight. The solution was extracted by a frit and the resin was washed with CH_2Cl_2/ MeOH/CH_2Cl_2/MeOH/CH_2Cl_2. Finally the solvent was removed at 40 °C under reduced pressure. The product was then purified by HPLC.

Trimer **18c** (n = 3): 11.6 mg (9.16 µmol, 7% yield over 11 steps, purity: 86%) of a pink oil. – MS (MALDI), $C_{66}H_{89}N_{19}O_7$: m/z: 1268.3 [M^+]. – MS (FAB), $C_{66}H_{89}N_{19}O_7$: m/z: 1268.0 [M^+]. – Analytical HPLC (5–95% Acetonitrile + 0.1% TFA in 30 min, detection at 218 nm): t_{Ret} = 14.2 min.

3. Methods

3.1. Cell penetrating properties and analysis of the intracellular location

Rhodamine B-labelled triazolopeptoids were tested for their ability to penetrate the cell membrane. In addition their intracellular distribution in the living cell was investigated by confocal fluorescent microscopy.

Cell culture: All cell culture of HeLa (human cervix carcinoma) cells were performed under sterile conditions. 1×10^4 HeLa cells were plated into each well of an 8 well µ-slides (ibidi, Germany) with 200 µl of Dulbecco's modified Eagle's medium (DMEM, high glucose, gibco) supplemented with 60 µg/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS, PAA) and incubated at 37 °C, 5% CO_2.

Rhodamine B-labeled triazolopeptoids treatment: All triazolopeptoids were added to HeLa cells to a final concentration of 10 µM (2 mM stock solution in DMSO, freshly diluted with DMEM) and subsequently incubated for 24 hours at 37 °C, 5% CO_2 and 95% humidity. Their cell penetrating ability was visualized by live cell imaging.

Intracellular distribution: To determine the subcellular localization of the triazolopeptoids, cells were additionally treated with MitoTracker® Green FM (Final concentration: 125 nM, Molecular Probe, Germany) for 45 min to stain their mitochondria. Cells were then washed three times with PBS

(Dulbecco's Phosphate Buffered Saline, gibco). Nuclei were counterstained with Hoechst 33342 dye (2 µg/mL). Cells were subjected to live fluorescent confocal microscopy at 37 °C.

Live fluorescent confocal microscopy: Simultaneous visualization of the triazolopeptoids, mitochondria and nuclei was performed using a Leica TCS-SP5 II (DMI6000) inverse microscope with an HCX PL APO CS 63.0x1.2 Water UV objective. Fluorophores were excited using an argon ion laser (488 nm for MitoTracker® Green FM), a DPSS laser (561 nm for rhodamine B-labeled triazolopeptoids) and a UV laser (364 nm for Hoechst 33342). The emission detection bandwidths were at 417–468 nm for Hoechst 33342, 499–552 nm for MitoTracker Green FM and 593–696 nm for the triazolopeptoids. Channels were detected simultaneously and the exposure was set to minimize oversaturated pixels in the final image. Using the acquisition software LAS-AF 2.0.2.4647, the picture ratio was adjusted to 1024 × 1024 pixels 8 bit depth.

3.2. Cytotoxicity of Triazolopeptoids in HeLa cells

To determine the toxic effect of triazolopeptoids towards HeLa cells the viability was tested using the CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega) according to the manufacturer`s instructions. This assay is based on the intracellular reduction of a tetrazolium salt (yellow) into a formazan product (blue), which only takes place in metabolic active cells. The generated formazan is detectable at wavelengths between 630–750 nm and is a direct measure for the viability of the cells.

For this assay, each well of a 96 well plate (Cstar 3596, 96 Well Cell Culture Cluster, sterile) was seeded with 1×10^4 HeLa cells in 100 µL Dulbecco's modified Eagle's medium (DMEM, high glucose, gibco) supplemented with 10% fetal calf serum (FCS, PAA), and 60 µg/ml penicillin, 100 µg/ml streptomycin at 37 °C, 5% CO_2. After 24 hours cells were incubated with a final concentration of 5 µM, 10 µM, 20 µM and 50 µM of the particular triazolopeptoids. For each concentration 8 wells were prepared and incubated for 72 hours. A set of positive (cells treated with 5 µl of 20% triton) and negative (untreated cells) control wells, as well as the test samples, were treated with 15 µl of the Dye Solution and incubated for 1,5 hours. 100 µL Solubilization Solution/Stop Mix is then added to each well to solubilize the formazan product, according to the manufacturer's instructions. After 1 hour incubation the absorbance was measured at 595 nm using a 96-well plate reader (Ultra Microplate Reader ELx808, BioTEK Instruments, INC). Mean values and standard deviation were calculated from n=8 experiments using Student´s T-Test

3.3. Zebrafish experiments

Zebrafish husbandary: Adult zebrafish (Danio rerio, Casper line (mitfaw2/w2;roya9/a9)) were bred and maintained according to standard methods (Westerfield 2007) in a 14h on/10h off light cycle. Fish were crossed pair-wise and eggs were collected within 3 h of laying and incubated in petridishes filled with E3 medium (5 mM NaCl, 0,17 mM KCl, 0,33 mM CaCl2) and 1 mg/ml of methylene blue. E3 medium was daily renewed until the beginning of the experiment.

Analysis of the 1,4-triazolopeptoids in zebrafish embryos: At 96 hpf, larvae were anesthetized by immersion in 0,02% tricaine, sorted into 8-well µ-slide (IBIDI, Ibitreat, Germany) and exposed to 5 µM triazolopeptoid solution for 2 h. After incubation, larvae were washed with E3 medium supplemented with 0,02% tricaine for three times and subjected to live confocal microscopy.

2.2. Cytotoxicity of triazolopeptoids towards HeLa cells

To analyse the toxic effect of the triazolopeptoids towards HeLa cells, a toxicity test was conducted. This test enables an indirect determination of the viable cell number after triazolopeptoid treatment by measuring the metabolic activity of cellular enzymes, which is detectable by the generation of the blue formazan salt. Concentrations of 5 µM, 10 µM, 20 µM and 50 µM were tested for each triazolopeptoid for 72 hours, data averaged and composed in Figure SI-2.

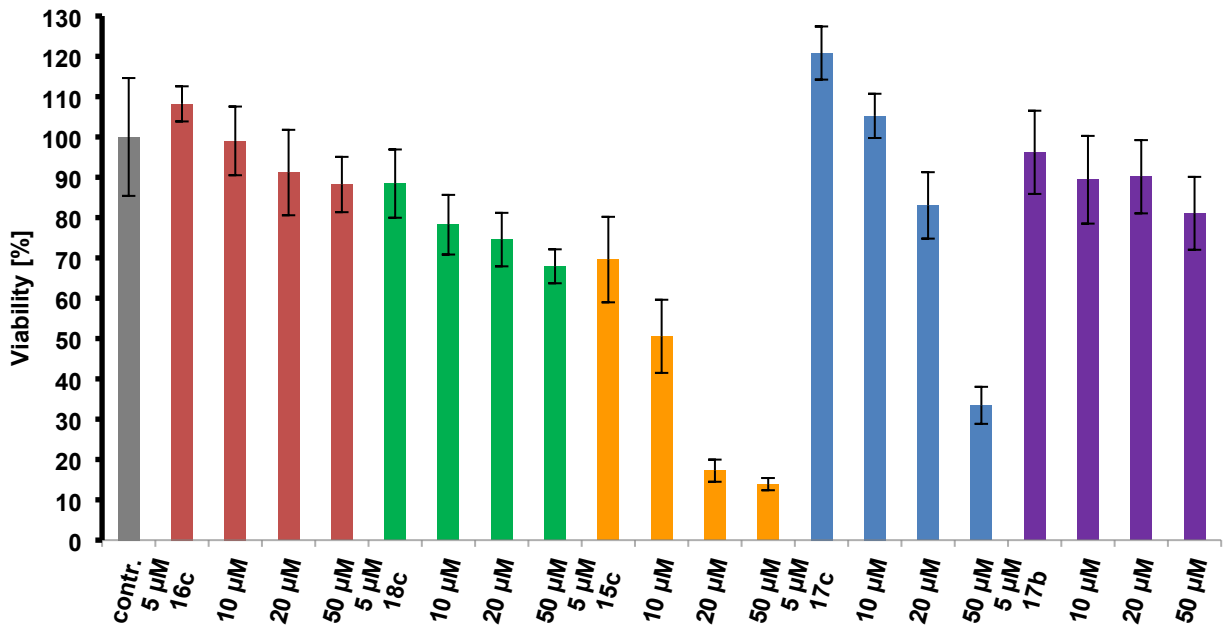

Figure S2. Effect of 1,4-triazolopeptoids on the viability of HeLa cells. 1 x 10^4 HeLa cells were treated with the indicated concentrations of the respective 1,4-triazolopeptoids. Especially **15c** and **17c** showed a stronger cytotoxic response than the other 1,4-triazolopeptoids. Both of them depicted a strong

mitochondrial localization (see Figure 1) which is most likely the reason for their toxic effect. Experiments were carried out with n=8 and SD was calculated by Student´s t-Test