Supporting Information

Peptoidic amino- and guanidinyl-carrier systems: Drug delivery into the cell cytosol or the nulcei

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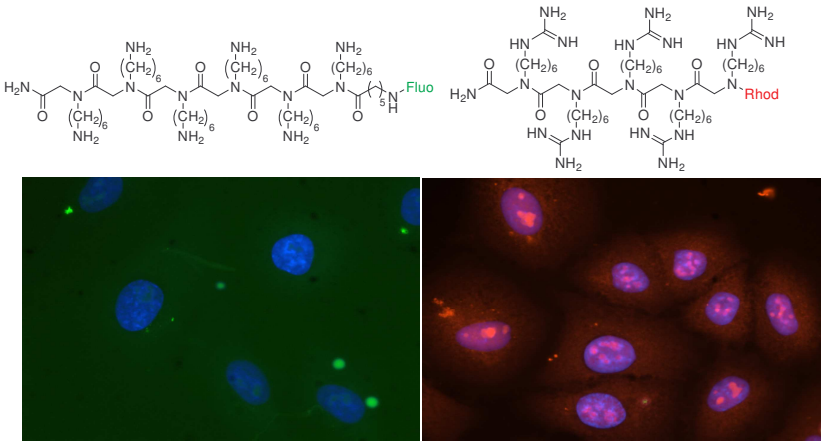

Contents Supporting information:

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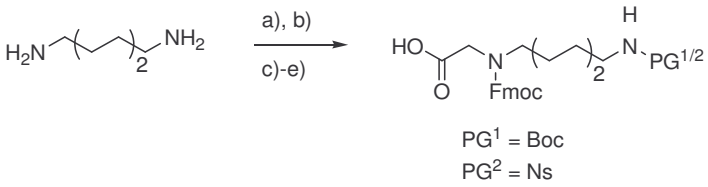
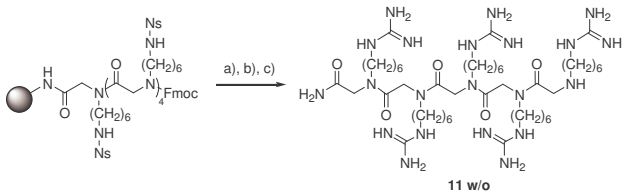
Supporting Information

Additional Schemes and Figures



Scheme 1. Synthesis of monomeric building blocks for peptoid synthesis. Reaction conditions: a) 0.13 eq Boc_2O, dioxane, rt, (argon) 22 h; b) 0.13 eq *o*-nosyl-chloride, dioxane, rt, (argon) 22 h; c) 3.00 eq of NEt_3, 1.00 eq ethyl bromoacetate, THF, rt, 16 h; d) 4N NaOH, MeOH, MeCN, rt, 30 min; e) 1.00 eq 9*H*-fluorene-9-ylmethoxycarbonyl-*N*-hydroxysuccinimide, H_2O/MeCN (1:1 (v/v)), rt, 30 min.



Scheme 5. Deprotection, formation of the guanidinyl groups and cleavage from the solid support of control-peptoid {6^G,6^G,6^G,6^G,6^G}-NH_2 (**11 w/o**). Reaction conditions a)-c) are identical to those described in Scheme 3 (manuscript).

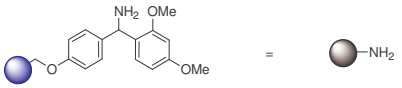


Figure 1. Rink amide resin **4** and its shortened representation.

General cell culture procedures

A549 and ECV304 cells were cultured in DMEM (Dulbecco´s modified Medium) respectively Medium M199 (both from GIBCO) supplemented with 10% fetal calf serum (FCS), 1 µg/ml streptomycin and penicillin at 37 °C in a 5% CO_2 atmosphere. Adherent monolayers of cells were harvested with trypsine solution (TrypLE Express, Invitrogen) after washing with PBS-/-, and the process was stopped by the addition of a fourfold excess of culture medium. After centrifugation, the medium was removed, and the pellet was resuspended in culture medium.

Preparation of mammalian cells for fluorescent microscopy on cover glass slides

For microscopy experiments, $10*10^4$ A549 cells were plated to each well of the 4-chambered coverglass (Lab-Tek, Nunc/Nalgene). The cells were allowed to attach to the culture dish overnight. The lyophilized peptoids were dissolved in water to yield a 1 mM stock solution. Various amounts of this peptoid solution were added directly to each well to give final concentrations of 1, 10 or/and 50 µM respectively. The culture medium was mixed by rocking the cover glass slide. After 10 minutes respectively 24 h, the cells were washed with an acid buffer (28 mM sodium acetate, 117 mM sodium chloride, 2 mM EDTA) and with PBS-/-. Then, the cells were fixed for 30 minutes in 4% para formaldehyde on ice. After a washing step with PBS the cells were treated with 0.2% Triton X 100 for 30 min to disrupt the cell membrane following another washing step with PBS-/- and an incubation of 1 h with Bisbenzimid (Hoechst) 1:100 diluted in PBS-/- for a nuclear counterstaining. After washing the cells with PBS-/- the cells were evaluated for peptoid uptake by fluorescence microscopy (Leica DMIRE2 Improvision, 630x, filter set 1 (FITC): λ_{excit} = 470 nm, λ_{em} = 525 nm; filter set 2 (Rhodamine):

$\lambda_{excit.}$ = 535 nm, λ_{em} = 610 nm; filter set 3 (Hoechst): $\lambda_{excit.}$ = 380 nm, λ_{em} = 425 nm with an OptiGrid confocal microscope with an EXFO X-CiteTM 120 120-watt lamp. Images were collected and visualized with the Improvision imaging software.

Preparation of mammalian cells for FACS-analysis

A549 cells were seeded in 24 well plates in a density of $10*10^4$ cells each well (NUNC, Wiesbaden, Germany) in triplicate. In Dulbecco´s modified Medium (DMEM) with 10% FCS, 1% glutamine and 1% penicillin/streptomycin. The cells were allowed to attach to the culture dish 2 days. The lyophilized peptoids were dissolved in water to yield a 1 mM stock solution. Various amounts of this peptoid solution were added directly to each well to give final concentrations of 50, 100, 200 µM respectively. The culture medium was mixed by rocking the well plate. After 10, 30, 60 minutes respectively the cells were washed with an acid buffer (28 mM sodium acetate, 117mM sodium chloride, 2 mM EDTA) and with PBS. Then, the adherent monolayers of cells were harvested with trypsine solution (TrypLE Express, Invitrogen) and the process was stopped by the addition of a tenfold excess of culture medium. After centrifugation, the medium was removed, and the pellet was resuspended in ice-cold PBS-/- and immediately measured via FACS-analysis: $\lambda_{excit.}$ = 488 nm (Argonlaser), FITC λ_{em} = 530/28 nm, Rhodamine λem = 575/26 nm. The results are given as the amount of fluorescent marked cells in percent. The experimental procedure was identical to that of human endothelial cells (ECV304).

Viability Tests

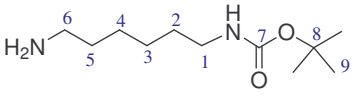
WST-1 assay: human epithelial lung cancer cells (A549) were grown in 96 well chambers overnight. For each set, $25*10^3$ cells were seeded into every well of a 96-well plate (NUNC, Wiesbaden, Germany) in quadruplicate in Dulbecco´s modified Medium (DMEM) with 10% FCS, 1% glutamine and 1% penicillin/streptomycin. The cells were prepared one day before the experiment and were treated following the standard procedure of the cell proliferation Kit I (WST-1) (Roche Diagnostics, Mannheim). The peptoid solution was added drop wise while constantly shaking the culture dish to ensure a homogenous distribution of the compound to the final concentrations: 20, 50, 100, 150 and 200 µM. After an incubation for 24 h and 48 h at 37 °C the cells were submitted to cell proliferation and viability assays. For every timepoint the test was carried out thrice. The reduced tetrazolium salt 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) is water-soluble; therefore, iso-2-propanol/HCl extraction is necessary. Photometric quantification was performed at 450 nm in a micro titer plate reader. The results are given as relative values to the negative control in percent, whereas untreated (negative) control is set to be 100% viable. The experimental procedure was identical to that of human endothelial cells (ECV304).

LDH assay: LDH can be released from dead cells into the medium and can be detected by measuring its catalytic activity by measuring the conversion of 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) to water-soluble formazane dye. The experimental procedure was identical to that of the two previous assays, using the cytotoxicity detection Kit (LDH) (Roche Diagnostics, Mannheim, Germany).

Experimental procedures

Synthesis of the monomer units

(6-Amino-hexyl)-carbamic acid *tert*-butylester[21]: 10.0 g (90.0 mmol, 1.00 eq) of 1,6-hexandiamine were dissolved in 150 ml of dioxane under argon and a solution of 2.44 g (11.7 mmol, 0.13 eq) of Boc-anhydride in 100 ml of dioxane was added drop wise over 1 h. The mixture was stirred at room temperature for 22.5 h. After concentration *in vacuo* and resuspension in water, the product was extracted with dichloromethane (3 x 100 ml). The combined organic layers were washed with water and then concentrated *in vacuo*. The product was obtained as a bright yellow oil, yield: 4.36 g (47%). – ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 4.64 (s, 1 H, NH); 3.01 (dt, $^3J1 \approx {}^3J2$ = 6.6 Hz, 2 H, CH_2(1)); 2.59 (t, 2 H, ^3J = 6.9 Hz, CH_2(6)); 1.42-1.21 (m, 17 H, CH_2(2, 3, 4, 5), CH_3(9)); 1.12 (s, 2H, NH_2)). – ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 155.9 (CO(7)); 78.8 ($C(CH_3)3$(8)); 42.0



(CH_2(1)); 33.6 (CH_2(6)); 29.9 (CH_2(5)); 28.3 (3C, CH_3(9)); 26.5 (CH_2(2)); 26.4 (2C, CH_2(3, 4)) – MS (FAB, matrix: 3-NBA), m/z (%): 217 (100) $[M+H]^+$, 161 (55) $[M - C_4H_7]$.

(6-(*tert*-Butoxycarbonylamino-hexylamino)-acetic acid ethyl ester[21]: 4.34 g (20.0 mmol, 1.00 eq) of (6-amino-hexyl)-carbamic acid *tert*-butyl ester and 8.36 ml (60.0 mmol, 3.00 eq) of NEt_3 were dissolved in 50 ml of THF. Then, 2.22 g (20.0 mmol, 1.00 eq) of ethyl bromo acetate dissolved in 50 ml of THF were added drop wise, and the mixture was stirred at rt overnight. The mixture was concentrated *in vacuo* and redissolved in ethyl ether. After filtration the solution was concentrated *in vacuo* to give yellow oil as product. Yield: 5.01 g (83%). – ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 4.58 (bs, 1 H, NHCO); 4.11 (q, ^{3}J = 7.2 Hz, 2 H, CH_2(2)); 3.31 (s, 2 H, CH_2(4)); 3.02 (dt, ^{3}J1 ≈ ^{3}J2 = 6.6 Hz, 2 H, CH_2(10)); 2.52 (t, ^{3}J = 7.1, 2 H, CH_2(5)); 1.67 (bs, 1 H, NH); 1.43-1.17 (m, 20 H, CH_3(1, 13), CH_2(6, 7, 8, 9)). – ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 172.4 (COO(3)); 155.9 (COO(11)); 78.8 ($C(CH_3)_3$(12)); 60.5 (CH_2(2)); 55.0 (CH_2(5)); 50.9 (CH_2(4)); 49.4 (CH_2(10)); 29.9 (CH_2(9)); 29.8 (CH_2(6)); 28.3 (3C, CH_3(13)); 26.7 (CH_2(7)); 26.5 (CH_2(8)); 14.1 (CH_3(1)). – MS (FAB, matrix: 3-NBA), m/z (%): 303 (100) $[M+H]^+$, 247 (47) $[M^+ - C_4H_7]$, 229 (10) $[M^+ - C_4H_9O]$, 173 (8) $[C_8H_{17}N_2O_2]$.

(6-*tert*-Butoxycarbonylamino-hexylamino)-acetic acid sodium salt[21]: 5.01 g (16.6 mmol, 1.00 eq) of (6-(tert-butoxycarbonyl-amino-hexyl-amino)-acetic acid ethyl ester were dissolved in 33 ml of dioxane and 12.4 ml methanol were added. After adding of 4.1 ml of 4N sodium hydroxide solution, 30 min stirring at room temperature, and concentration *in vacuo*, a white solid was obtained as product. Yield: 5.01 g (quant.). – ^{1}H NMR (300 MHz, D_2O), δ (ppm): 3.31 (s, 1 H, NH); 3.24 (s, 2 H, CH_2(7)); 3.13 (t, ^{3}J = 6.8 Hz, 2 H, CH_2(1)); 3.13 (t, ^{3}J = 7.2 Hz, 2 H, CH_2(6)); 1.57-1.51 (m, 13 H, CH_2(2, 5), CH_3(11)); 1.44-1.39 (m, 4 H, CH_2(3, 4)). – ^{13}C NMR (75 MHz, D_2O), δ (ppm): 181.8 (COOH(8)); 181.5 (CONH(9)); 83.3 ($C(CH3)_3$(10)); 69.2 (CH_2(7)); 54.6 (CH_2(6)); 50.9 (CH_2(1)); 31.0 (CH_2(2)); 30.4 (4C, CH_2(5), CH_3(11); 28.7 (CH_2(4)); 28.4 (CH_2(3)). – MS (FAB, matrix: 3-NBA), m/z (%): 319 (45) $[M+Na]^+$, 297 (7) $[M+H]^+$, 251 (10) [M–COOH]$^+$, 219 (100) [C9H18O4N2+H]$^+$, 176 (77) [Matrix+Na]$^+$. – IR (drift): ν = 3365 (m), 2977 (m), 2930 (s), 2858 (s), 2804 (m), 1682 (s), 1594 (s), 1403 (m), 1366 (m), 1392 (m), 1369 (m), 770 (m), 745 (m).

N-(6-*tert*-Butoxycarbonylaminohexyl)-N-(9 H-fluorene-9-ylmethoxycarbonyl)-amino acetic acid[21]: 3.45 g (11.6 mmol, 1.00 eq) of (6-tert-butoxycarbonylamino-hexylamino)-acetic acid sodium salt were dissolved in 20 ml H_2O and were reacted with 3.74 g (11.6 mmol, 1.00 eq) of 9H-fluorene-9-ylmethoxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu) dissolved in 40 ml acetonitrile for 30 min. The reaction mixture was concentrated *in vacuo* to remove acetonitrile and the residue was poured into 20% citric acid (120 ml). The aqueous layer was extracted with ethyl acetate (3x 75 ml). The combined organic layers were washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo* to obtain the solid which was recrystallized from ethyl acetate / hexane. A white solid was isolated with a yield of 3.28 g (57%). – ^{1}H NMR (400 MHz, [D]4-MeOH) δ (ppm): 7.84 (d, ^{3}J = 6.9 Hz, 1 H, Ar-H(19)); 7.82 (d, ^{3}J = 6.9 Hz, 1 H, Ar-H(19´)); 7.65 (d, ^{3}J = 7.8 Hz, 1 H, Ar-H(16)); 7.63 (d, ^{3}J = 7.8 Hz, 1 H, Ar-H(16´)); 7.45-7.40 (m, 2 H, Ar-H(18, 18´)); 7.37-7.32 (m, 2H, Ar-H(17, 17´)); 4.61 (d, 3J = 5.2 Hz, 2H, CH_2(13)); 4.25 (m, 1H, CH(14)); 3.91 (s, 2H, CH_2(7)); 3.35 (m, 2H, CH_2(6)); 3.05 (m, 2H, CH_2(1)); 1.58-1.03 (m, 17H, CH_2(2, 3, 4, 5), CH_3(11)). – ^{13}C NMR (100 MHz, [D]4-MeOH): 173.3 (q, COOH(8)); 158.5 (q, NCOO(12)); 158.2 (q, NHCOO(9)); 145.7 (q, Ar-C(15)); 145.5 (q, Ar-C(15´)); 143.1 (q, Ar-C(20)); 142.9 (q, Ar-C(20´)); 129.0 (+, 2C, Ar-CH(18, 18´)); 128.5 (+, 2C, Ar-CH(17, 17´)); 126.4 (+, Ar-CH(16)); 126.0 (+, Ar-CH(16´)); 121.2 (+, 2C, Ar-CH(19, 19´)); 80.1 (q, 1C, $C(CH_3)$3(10)); 69.2 (–, CH_2 (13)); 68.4 (–, CH_2 (7)); 47.1 (+, CH(14)); 41.6 (–, CH_2(6)); 31.3 (–, CH_2(1)); 29.4 (–, CH_2(5)); 29.1 (+, 3C, CH_3(11)); 29.1 (–, CH_2(2)); 27.9 (–, CH_2(3)); 27.7 (–, CH_2(4)). – MS (FAB, matrix: 3-NBA), m/z (%): 519 (17) $[M+Na]^+$, 497 (6) $[M+H]^+$, 397 (27) $[M+H\text{-Boc}]^+$, 307 (24) $[2Ma+H]^+$, 179 (100) [$C_{14}H_{11}$ (from Fmoc)]$^+$, 165 (22) [$C_{13}H_9$ (from Fmoc)]$^+$. – $C_{28}H_{36}N_2O_6$ (496.60): calc.: C 67.72, H 7.31,

N 5.64; found: C 67.90, H 7.37, N 5.46, found: C 67.93, H 7.30, N 5.51. – IR (drift): ν = 3349 (m), 3067 (m), 2934 (s), 2860 (m), 1705 (s), 1480 (m), 1451 (m), 1367 (m), 761 (m), 742 (m).

N-(6-Aminohexyl)-2´-nitrobenzenesulfonamide[21]: 10.0 g (90.0 mmol, 1.00 eq) of 1,6-hexandiamine were dissolved in 100 ml of dioxane and 2.60 g (12.0 mmol, 0.13 eq) of 2-nitrobenzenesulfonylchloride (o-Ns-Cl) dissolved in 75 ml of dioxane were added drop wise over 1 h. After a reaction time of 19.5 h, concentration *in vacuo*, and resuspension in water the product was extracted with dichloromethane (3 x 100 ml). The combined organic layers were washed with water and then concentrated *in vacuo*. Orange oil was obtained as product. Yield: 2.2 g (62%). – ^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.04-8.00 (m, 1 H, CH(3´)); 7.74-7.71 (m, 1 H, CH(6´)); 7.66-7.63 (m, 2 H, CH(5´), CH(4´)); 2.98 (t, ^{3}J = 7.0 Hz, 2 H, CH_2(1)); 2.81 (s, 3 H, NH_2, NH); 2.55 (t, ^{3}J = 6.9 Hz, 2 H, CH_2(6)); 1.45-1.17 (m, 8 H, CH_2(2, 3, 4, 5)). – ^{13}C NMR (75 MHz, $CDCl_3$), δ (ppm): 147.8 (q, CNO_2(2´)); 133.6 (q, CSO_2(1´)); 133.3 (+, CH(5´)); 132.4 (+, CH(4´)); 130.7 (+, CH(6´)); 124.9 (+, CH(3´)); 43.4 (–, CH_2(1)); 41.6 (–, CH_2(6)); 33.0 (–, CH_2(5)); 29.3 (–, CH_2(2)); 26.0 (–, 2C, CH_2(3, 4)). – MS (FAB, matrix: 3-NBA), m/z (%): 302 (100) $[M+H]^+$, 186 (10) $[C_6H_3NO_4S]^+$. – IR (drift): ν = 3290 (s), 2934 (s), 2860 (m), 1542 (s), 1441 (m), 1418 (m), 741 (m), 731 (m).

N-[6-(2-Nitrobenzenesulfonylamino)-hexyl]-amino acetic acid ethyl ester[21]: 2.20 g (7.30 mmol, 1.00 eq) of N-(6-aminohexyl)-2´-nitrobenzenesulfonamide and 3.07 ml (22.0 mmol, 3.00 eq) of NEt_3 were dissolved in 50 ml THF. Then, 0.81 ml (7.30 mmol, 1.00 eq) of ethyl bromo acetate dissolved in 50 ml THF was added drop wise over 1.5 h, and the reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated *in vacuo* and redissolved in diethyl ether. After filtration, the solution was concentrated *in vacuo* to yield a yellow oil as product in a yield of 0.950 g (34%). – ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.08 (m, 1 H, Ar-H(3´)); 7.81 (m, 1 H, Ar-H(4´)); 7.71-7.68 (m, 2 H, Ar-H(5´, 6´)); 4.15 (q, ^{3}J = 7.1 Hz, 2 H, CH_2(9)); 3.34 (s, 2 H, CH_2(7)); 3.05 (t, ^{3}J = 7.0 Hz, 2 H, CH_2(1)); 2.52 (t, ^{3}J = 7.0 Hz, 2 H, CH_2(6)); 1.50-1.20 (m, 11 H, CH_2(2, 3, 4, 5), CH_3(10)). – ^{13}C NMR (100 MHz, $CDCl_3$), δ (ppm): 172.5 (q, COO(8)); 148.0 (q, Ar-CNO_2(2´)); 133.8 (q, Ar-CSO_2(1´)); 133.4 (+, Ar-CH(5´)); 132.6 (+, Ar-CH(4´)); 131.0 (+, Ar-CH(6´)); 125.2 (+, Ar-CH(3´)); 60.6 (–, CH_2(9)); 50.9 (–, CH_2(6)); 49.2 (–, CH_2(7)); 43.6 (–, CH_2(1)); 29.6 (–, CH_2(5)); 29.4 (–, CH_2(2)); 26.5 (–, CH_2(3)); 26.2 (–, CH_2(4)); 14.2 (+, CH_3(10)). – MS (FAB, matrix: 3-NBA), m/z (%): 388 (100) $[M+H]^+$, 186 (12) $[C_6H_3NO_4S]^+$. – IR (film on KBr): ν = 3326 (w), 3095 (w), 2933 (m), 2858 (m), 1736 (s), 1543 (s), 1466 (m), 1442 (m), 742 (m), 730 (m).

N-{6-(2´-Nitrobenzenesulfonylamino)-hexyl}-amino acetic acid sodium salt[21]: 0.950 g (2.50 mmol, 1.00 eq) of N-[6-(2-nitrobenzenesulfonylamino)-hexyl]-amino acetic acid ethyl ester was dissolved in 5.3 ml of dioxane. Then 2 ml of methanol and 0.70 ml of a 4N sodium hydroxide solution were added. After 30 min of stirring at room temperature and concentration *in vacuo*, a yellow-orange solid was obtained in a yield of 0.720 g (76%). – ^{1}H NMR (300 MHz, D_2O), δ (ppm): 7.85 (m, 1 H, Ar-CH(3´)); 7.70-7.63 (m, 3 H, Ar-CH(4´, 5´, 6´)); 3.06 (s, 2 H, CH_2(7)); 2.72 (t, ^{3}J = 7.3 Hz, 2 H, CH_2(1)); 2.40 (t, ^{3}J = 7.4 Hz, 2 H, CH_2(6)); 1.39-1.31 (dt, ^{3}J = 7.4 Hz, 4 H, $_{CH2}$(2, 5)); 1.18 – 1.15 (m, 4 H, CH_2(3, 4)). – ^{13}C NMR (75 MHz, D2O), δ (ppm): 182.2 (q, COOH(8)); 150.9 (q, Ar-CNO_2(2´)); 138.2 (q, Ar-CSO_2(1´)); 134.9 (+, 1H, Ar-CH(5´)); 134.7 (+, 1H, Ar-CH(4´)); 132.2 (+, Ar-CH(6´)); 126.4 (+, Ar-CH(3´)); 54.7 (–, CH_2(7)); 50.9 (–, CH_2(6)); 47.8 (–, CH_2(1)); 33.4 (–, CH_2(5)); 31.1 (–, CH_2(2)); 28.9 (–, C`$H_2$(3)); 28.8 (–, C`H_2(4)). – MS (FAB, matrix: 3-NBA), m/z (%): 382 (6) $[M+Na+H]^+$, 329 (18) $[2Matrix+Na]^+$, 307 (5) $[2Ma+H]^+$, 176 (100) $[Ma+Na]^+$. – IR (drift): ν = 3289 (m, br), 2932 (m), 2859 (m), 2452 (w), 1995 (vw), 1594 (m), 1541 (m), 1424 (m), 1164 (m), 740 (m).

N-(9H-Fluorene-9-ylmethoxycarbonyl)-N-{6-(2´-nitrobenzenesulfonylamino)-hexyl}-aminoacetic acid[21]: 1.65 g (4.00 mmol, 1.00 eq) of N-[6-(2´-nitrobenzenesulfonylamino)-hexyl]-amino acetic acid sodium salt was dissolved in 10 ml of water and reacted with 1.29 g (4.00 mmol, 1.00 eq) of 9H-fluorene-9-ylmethoxycarbonyl-N-hydroxysuccinimide (Fmoc-OSu), dissolved in 20 ml of acetonitrile, for 30 min. The reaction mixture was concentrated *in vacuo* to remove acetonitrile, and the residue was poured into 20% citric acid (120 ml). The aqueous layer was extracted with ethyl acetate (3 x 75 ml). The combined organic layers were washed with water and brine, dried over Na2SO4, and concentrated *in vacuo* to yield the crude product. After processing, the product was cleaned by chromatography (ethyl acetatle / cyclohexane (1:1 (v/v)), then methanol). 1.12 g (48%) of an orange solid was obtained. – Rf = 0.22 (ethyl acetate). – ^{1}H NMR (400 MHz, [D]4-MeOH): 8.10 (m, 1 H, Ar-H(6´)); 7.85-7.79 (m, 5 H, Ar-H(4´, 5´, 13, 16, 16´)); 7.65-7.63 (m, 2 H, Ar-H(3´, 13´)); 7.41 (t, ^{3}J = 7.3 Hz, 2 H, Ar*-H(14, 14´)); 7.33 (td, ^{3}J = 7.4 Hz, ^{4}J = 1.0 Hz, 2 H, Ar*-H(15, 15´)); 4.58 (d, ^{3}J = 5.2 Hz, 2 H, CH´$_2$(7)); 4.36 (d, ^{3}J = 6.8, 2 H, CH´$_2$(10)); 3.71 (s, 1 H, CH(11)); 3.09-3.03 (m, 4 H, CH$_2$(1, 6)); 1.53-1.44 (m, 4 H, CH$_2$(2, 5)); 1.20-0.94 (m, 4 H, CH$_2$(3, 4)). – ^{13}C NMR (100 MHz, [D]4-MeOH): 173.3 (q, COOH(8)); 158.6 (q, NCOO(9)); 150.0 (q, Ar-CNO2(2´)); 145.8 (q, Ar*-C(12)); 145.6 (q, Ar*-C(12´)); 143.1 (q, Ar´-C(17)); 142.9 (q, Ar´-C(17´)); 135.3 (q, Ar-CSO2(1´)); 135.2 (+, Ar-CH(5´)); 133.8 (+, Ar-CH(4´)); 131.8 (+, Ar-CH(6´)); 129.0 (+, 2C, Ar-CH(15, 15´)); 128.5 (+, 2C, Ar-CH(14, 14´)); 126.6 (+, Ar-CH(3´)); 126.1 (+, 2C, Ar-CH(13, 13´)); 121.2 (+, 2C, Ar-CH(16, 16´)); 69.2 (–, C$^{\#}$H$_2$(10)); 68.2 (–, C$^{\#}$H$_2$(7)); 52.3 (+, CH(11)); 44.6 (–, 2C, CH$_2$(1, 6)); 31.0 (–, CH$_2$(5)); 29.1 (–, CH$_2$(2)); 27.5 (–, 2C, CH$_2$(3, 4)). – MS (FAB, matrix: 3-NBA), m/z (%): 626 (7) [M+2Na-H]$^+$, 604 (22) [M+Na]$^+$, 482 (4) [3Ma+Na]$^+$, 360 (5) [M+H-Fmoc]$^+$, 307 (82) [2Ma+H]$^+$, 176 (100) [Matrix+Na]$^+$. – $C_{29}H_{31}N_3O_8S$ (581.64): calc.: C 59.88, H 5.37, N 7.22, S 5.51; found: C 58.08, H 5.04, N 7.09, S 5.28. – IR (drift): ν = 3348 (w), 3067 (w), 2938 (m), 2860 (w), 1687 (m), 1617 (m), 1450 (m), 761 (m), 742 (m).

Solid-phase synthesis

Solid-phase reactions greatly facilitated the synthesis as the growing oligomer attached to a solid support can easily be purified from excess reactants by washing the resin with the appropriate solvents and subsequent filtration. The protection of free amino groups with Fmoc has been well established as this protecting group can be quantitatively removed under mild conditions in a short reaction time. Rink-Amide-resin was chosen as a solid support due to its stability at ambient conditions, the ease of the first coupling step, and mild cleavage conditions. Furthermore, the reaction conditions are the same for attaching the first building block to the resin and the following coupling cycles.

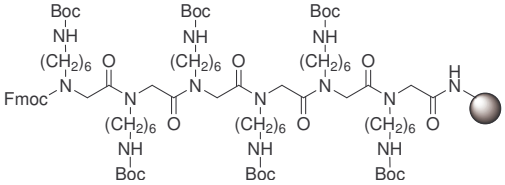
After removal of the Fmoc group that protects the amino-functionalized resin (with 20 % piperidine in DMF), an activated Fmoc-protected monomer was coupled to the solid phase via a peptide bond. In this reaction, bromo-tris(pyrrolidino)phosphonium-hexafluoro-phosphate (PyBrOP) was used to generate an activated ester and N,N-diisopropylethylamine (DIPEA) was added to enhance the rate of ester formation. The Fmoc group was removed with piperidine-solution yielding the coupled monomer for the attachment of the next building block. Coupling of the monomers to the growing peptoid chain proceeded under the same conditions as the attachment of the first building block to the solid support. All reaction procedures were succeeded by repetitive washing, ending with a solvent, in which the resin was swelled to expose its reactive sites to the next reagents. The cycles of coupling and deprotection were repeated until a peptoid of the desired length was obtained.

General procedure A (Synthesis of the peptoids): The amino-functionalized Rink-Amide-resin (0.51 mmol, 1.00 eq) was covered with five times its volume of dichloromethane and swelled for 30 min. After removal of the solvent, the Fmoc protection group of the linker-amine was removed by incubation with 6 ml of piperidine-solution (20% in DMF) for 2 min. This procedure was repeated twice. For the coupling step, the crystalline building block (1.53 mmol, 3.00 eq) was added to the resin, followed by PyBrOP (1.02 mmol, 2.00 eq) and DIPEA (2.04 mmol, 4.00 eq). The solid was suspended in 6 ml of dichloromethane and shaken for 24 h. Then, the solution was removed and the resin was washed according to a standard procedure using a sequence of MeOH/DMF/MeOH (2 times), THF/MeOH/THF/MeOH/THF/pentane, dichloromethane/n-pentane (3 x) and pentane. Finally, the resin was dried *in vacuo* (10-2 mbar) for 48 h. Coupling of the respective monomers

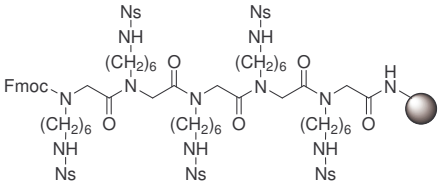
to the peptoid chain proceeded under identical conditions as the attachment of the first building block to the solid support. All reaction procedures were carried out by repetitive cycles of coupling and deprotection until a peptoid of the desired length was obtained. The procedure is outlined in Scheme 2.

Attachment to the solid phase and construction of the peptoids

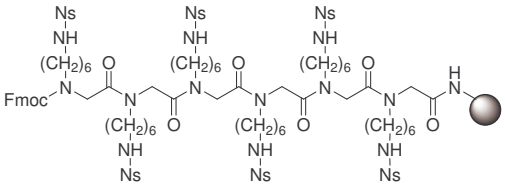
Hexamer at Rink-Amide-Linker[21]: As described in General Procedure A the Rink-Amide-resin was reacted with N-(6-tert-butoxycarbonylaminohexyl)-N-(9H-fluorene-9-ylmethoxycarbonyl)-amino acetic acid. This reaction was repeated five times to obtain the resin bound hexamer as beige resin in a yield of 0.19 g. – Loading of the hexamer: 0.31 mmol/g.



Nosyl-protected pentamer at Rink-Amide-Linker: As described in General Procedure A the Rink-Amide-resin was reacted with N-(9H-fluorene-9-ylmethoxy-carbonyl)-N-{6-(2´-nitrobenzenesulfonyl-amino)-hexyl} amino acetic acid. This reaction was repeated four times to obtain the resin bound pentamer as yellow resin in a yield of 0.54 g. – Loading of the pentamer: 0.30 mmol/g.



Nosyl-protected hexamer at Rink-Amide-Linker: As described in General Procedure A the Rink-Amide-resin was reacted with N-(9H-fluorene-9-ylmethoxy-carbonyl)-N-{6-(2´-nitrobenzenesulfonyl-amino)-hexyl} amino acetic acid. This reaction was repeated five times to obtain the resin bound hexamer as yellow resin in a yield of 0.17 g. – Loading of the hexamer: 0.28 mmol/g.



General procedure B (Labeling): Prior to attaching the fluorophore to peptoids, N-Fmoc-aminohexanoic acid was coupled as a spacer to limit steric hindrance between marker and transporter.

The immobilized peptoid was first deprotected as described in general procedure A. This step was repeated twice. For the coupling step of the spacer, N-Fmoc-aminohexanoic acid (0.25 mmol, 3.00 eq) was added to the resin. PyBrOP (0.16 mmol, 2.00 eq) and DIPEA (0.33 mmol, 4.00 eq) were added as activating reagents and the suspension was gently agitated for 24 h in 6 ml of dichloromethane. Then the solvents were removed and the resin was washed according to the standard procedure (see general procedure A). Finally, the resin was dried in vacuo for 48 h. After reswelling of the resin and deprotection with piperidine-solution (as described in general procedure A) the fluorophores 5(6)-carboxyfluoresceine (**9a**) and rhodamine B (**9b**) respectively, (0.25 mmol, 3.00 eq) were attached as described in general procedure B1.

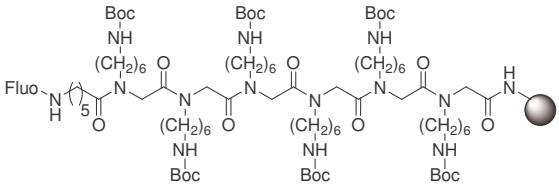
General procedure B1: To activate 5(6)-carboxyfluoresceine (**9a**) and rhodamine B (**9b**) respectively, the fluorophore (0.25 mmol, 3.00 eq) was mixed with HOBt (0.25 mmol, 3.00 eq) in a 50 ml flask, followed by addition of 2 ml of dichloromethane/DMF (1:1 (v/v)). Then, (0.25 mmol, 3.00 eq) of DIC were added and the mixture was shaken for 20 min at rt and was then added to the prepared resin derived from general procedure B. The suspension was agitated for 5 h.

Labeling with fluorophores of the immobilized peptoids

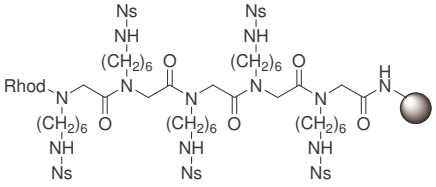
Prior to attaching the label to peptoids, N-Fmoc-aminohexanoic acid was coupled as a spacer to inhibit steric hindrance between label and transporter.

Supporting Information

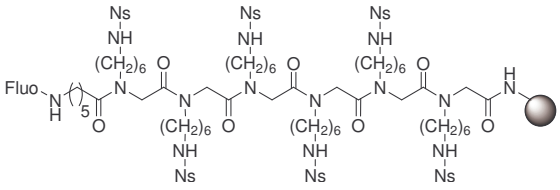
Fluorophore labeled hexamer[21]: As in general procedure B and B1 the hexapeptoid was reacted with 5(6)-carboxyfluoresceine (**9a**) for 5 h and afterwards washed and dried as in General Procedure B. The labeled peptoid was obtained in a yield of 0.210 g. – Loading: 0.28 mmol/g.



Rhodamine labeled pentamer: The pentamer was reacted as in general procedure B. To obatin carrier **11**, no spacer was attached prior to conjugation to the marker. As marker rhodamine-B-isothiocyanate (**9b**) was added and 5 ml of a mixture of CH_2Cl_2/DMF (1:1 (v/v)). The suspension was reacted in the shaker for 5 h and afterwards the resin was washed and dried as in General Procedure B. The labelled peptoid was obtained in a yield of 0.42 g . – Loading: 0.28 mmol/g.



Fluorophore labeled hexamer: As in General Procedure B and B1 the Nosyl-protected hexapeptoid was reacted with 5(6)-carboxyfluoresceine (**9a**) for 5 h and afterwards washed and dried as in General Procedure B. The labeled peptoid was obtained in a yield of 0.09 g. – Loading: 0.27 mmol/g.

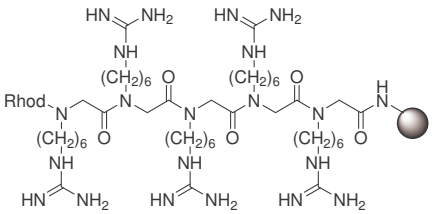


General Procedure C (Nosyl deprotection): For the deprotection of the nosyl-functionalized amino groups, the resin (0.09 mmol, 1.00 eq) was covered with dichloromethane (3 vol. according to the initial resin volume) and swelled for 30 min. After removing the solvent by filtration, the resin was deprotected by treatment with 3 ml of a solution of 2-mercaptoethanol/DBU (diaza-[5.4.0]-bicycloundecene (0.3 M) in DMF. The suspension was shaken for 45 min, the solvents were removed and the deprotection-step was repeated twice. The resin was washed and dried, as outlined in General Procedure A.

General Procedure D (Guanidinylation of the side-chain amines): After the Nosyl deprotection of the side-chain amines as outlined in General Procedure C, the resin was covered with five times its volume of dichloromethane and swelled for 30 min. After removal of the solvent, 1H-pyrazol-1-carboxamidine (1.60 mmol, 10.0 eq) was added to the resin, followed by DIPEA (1.60 mmol, 10.0 eq) and 5.00 ml of DMF. The suspension was shaken for 24 h. The solvents were removed and the resin was washed and dried, as outlined in General Procedure A (Scheme 4).

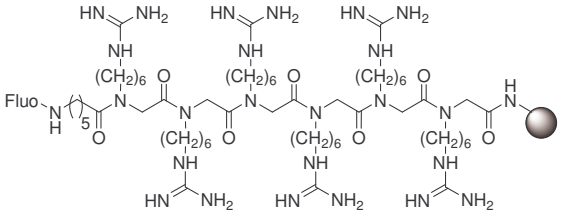
Deprotection of the nosyl-protected side-chains and reaction to guanidine groups

Rhodamine labeled pentamer: The pentamer was deprotected as in General Procedure C. Afterwards the free amino groups were converted to guanidine groups, following General Procedure D. The peptoid was obtained in a yield of 0.34 g . – Loading: 0.33 mmol/g.

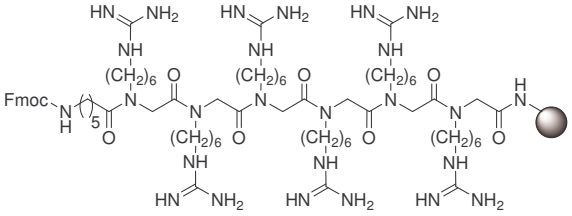


Supporting Information

Fluorophore labeled hexamer: The Nosyl-protected hexapeptoid was deprotected as in General Procedure C, followed by reaction of the free amines to give the resin bound guanidine peptoid in a yield of 0.07 g. – Loading: 0.32 mmol/g.



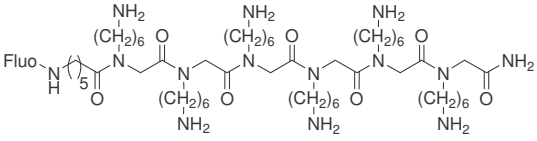
Nosyl-protected hexamer at Rink-Amide-Linker: To receive a "control peptoid" without fluorophore, a nosyl-protected hexamer was reacted as described in General Procedure C and D.



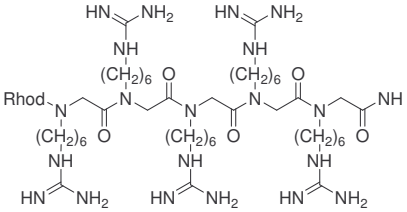
General Procedure E (Cleavage and isolation): To cleave the peptoid from solid support, the resin was transferred into a 25 ml flask and covered with 1 ml of a solution of TFA/TIS (95:5 (v/v)). The suspension was gently agitated at rt for 3 h under argon atmosphere. Then, the solution was filtered, and the resin was rinsed with TFA (2x 3 ml). In order to isolate the product, 50 ml of cold diethyl ether (–78 °C) were added to the solution. After gently agitating, the peptoid precipitated. It was filtered off and dried *in vacuo*.

Cleavage and isolation of the carriers

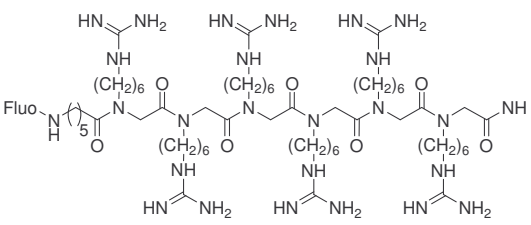
Fluo-{6,6,6,6,6,6}-NH_2 **10** [21]: As described in General Procedures A, B, B1 and E, the product was obtained as a brown solid. Yield: 66 mg (60%). – TLC: chloroform/methanol (5/1, v/v). Rf = 0.03; strong, green fluorescence in UV-light (366 nm). – MS (MALDI, matrix: DHB), m/z (%): 1463.891 (52) $[M + K]^+$, 1447.914 (100) $[M + Na]^+$, 1425.923 (62) $[M + H]^+$, 799 (45) [pentamer + H]$^+$, 486 (6) [trimer + H]$^+$. – IR (drift): ν = 2940 (m, br), 2100 (vw), 1677 (m), 1466 (w), 1203 (w), 1135 (w).



Rhod-{6^G,6^G,6^G,6^G,6^G}-NH_2 **11**: As described in General Procedures A–E, the product was obtained as a dark red solid. Yield: 135 mg (57%). – MS (FAB, matrix: glycerol), m/z (%): 1432 (3) $[M+H]^+$, 795 (2) [tetramer + H]$^+$, 597 (4) [trimer + H]$^+$, 397 (94) [dimer + H]$^+$, 326 (100) [NH_2 – dimer – CH_3N_2 (2 x)]$^+$. – IR (drift): ν = 3377 (m), 2949 (m), 2870 (w), 1673 (m), 1468 (w), 1440 (w), 1199 (m), 1148 (m), 843 (w), 795 (w), 725 (w). – UV/VIS (CH_3OH): λmax (log ε) = 193 (4.7), 256 (4.0), 357 (3.7), 561 (4.3).

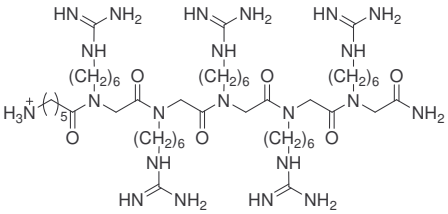


Fluo-{6^G,6^G,6^G,6^G,6^G,6^G}-NH_2 **12**: As described in General Procedures A–E, the product was obtained as an orange solid. Yield: 32 mg (59%). – MS (FAB, matrix: 3-NBA), m/z (%): 1677 (1) $[M]^+$, 1004 (9) $[NH_2$+pentamer–H$]^+$, 852 (26) $[NH_2$+tetramer+$CH_2N_2]^+$, 810 (18) $[NH_2$+tetramer+H$]^+$, 612 (10) $[NH_2$+trimer+H$]^+$, 456 (20) $[NH_2$+dimer+$CH_2N_2]^+$, 414 (6) $[NH_2$+dimer+H$]^+$, 154 (100) [Matrix+H]$^+$. – IR (drift): ν = 3347 (w), 3199 (w), 2945 (w), 2866 (w), 1668 (w), 1471 (w), 1180 (w), 1137 (w), 837 (w), 802 (w), 722 (w). – UV/VIS (CH3OH): λmax (log ε) = 193 (4.9), 486 (3.5).

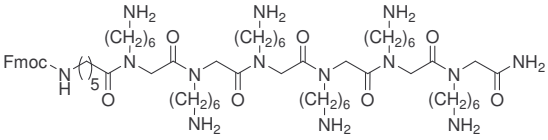


Supporting Information

Fmoc-{6^G,6^G,6^G,6^G,6^G}-NH_2 **11 w/o**: As described in general procedures A, C, D and E, the product was obtained as slightly brown viscous oil. Yield: 13 mg. – MS (MALDI, matrix: DHB), m/z (%): 1122 (1490 ai) $[M+H]^+$, 1008 (1100 ai) $[M\text{-}Spac+H]^+$. – IR (drift): ν = 3355 (m), 3189 (m), 2943 (m), 2866 (m), 1670 (m), 1469 (m), 1433 (m), 1204 (m), 1139 (m), 837 (m), 802 (m), 723 (m). – UV/VIS (CH_3OH): λmax (log ε) = 193.



{6,6,6,6,6,6}-NH_2 **10 w/o**: As described in general procedures A, and E, the product was obtained as a colorless highly viscous oil. Yield: 37 mg (quant). – MS (MALDI, matrix: DHB), m/z (%): 1234 (220 ai) $[M]^+$.



Additional Cell Pictures

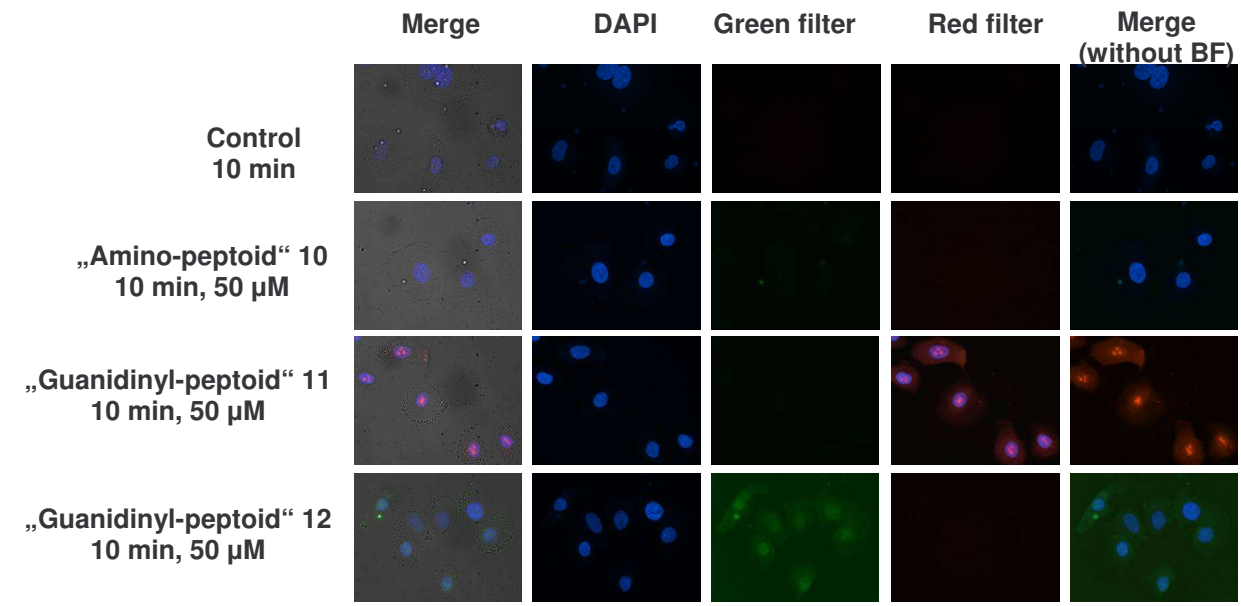


Figure 2. Grid confocal pictures showing the uptake of the carriers **10**, **11** and **12**. The uptake is demonstrated at a concentration of 50 µM and a incubation time of 10 min. The guanidinyl peptoids **11** and **12** show a higher uptake rate with accumulation in the cell nuclei and nucleoli (BF = bright field).

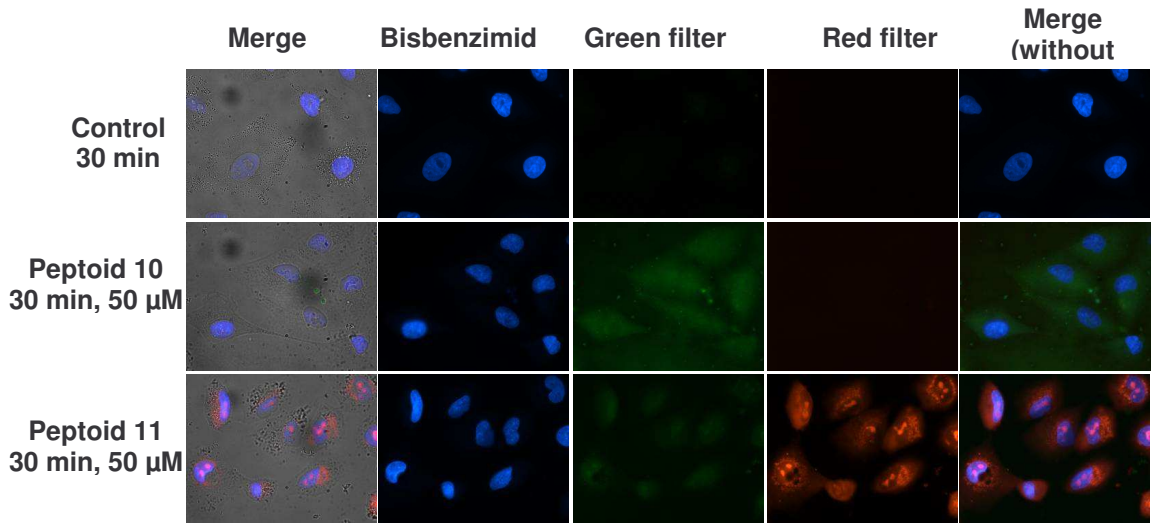


Figure 3. Grid confocal pictures of A549 cells, showing the uptake of the purified carriers **10** and **11**. The uptake is demonstrated at a concentration of 50 µM and an incubation time of 30 min. The guanidinyl-peptoid **11** shows a higher uptake rate with accumulation in the cell nuclei and nucleoli (BF = bright field).

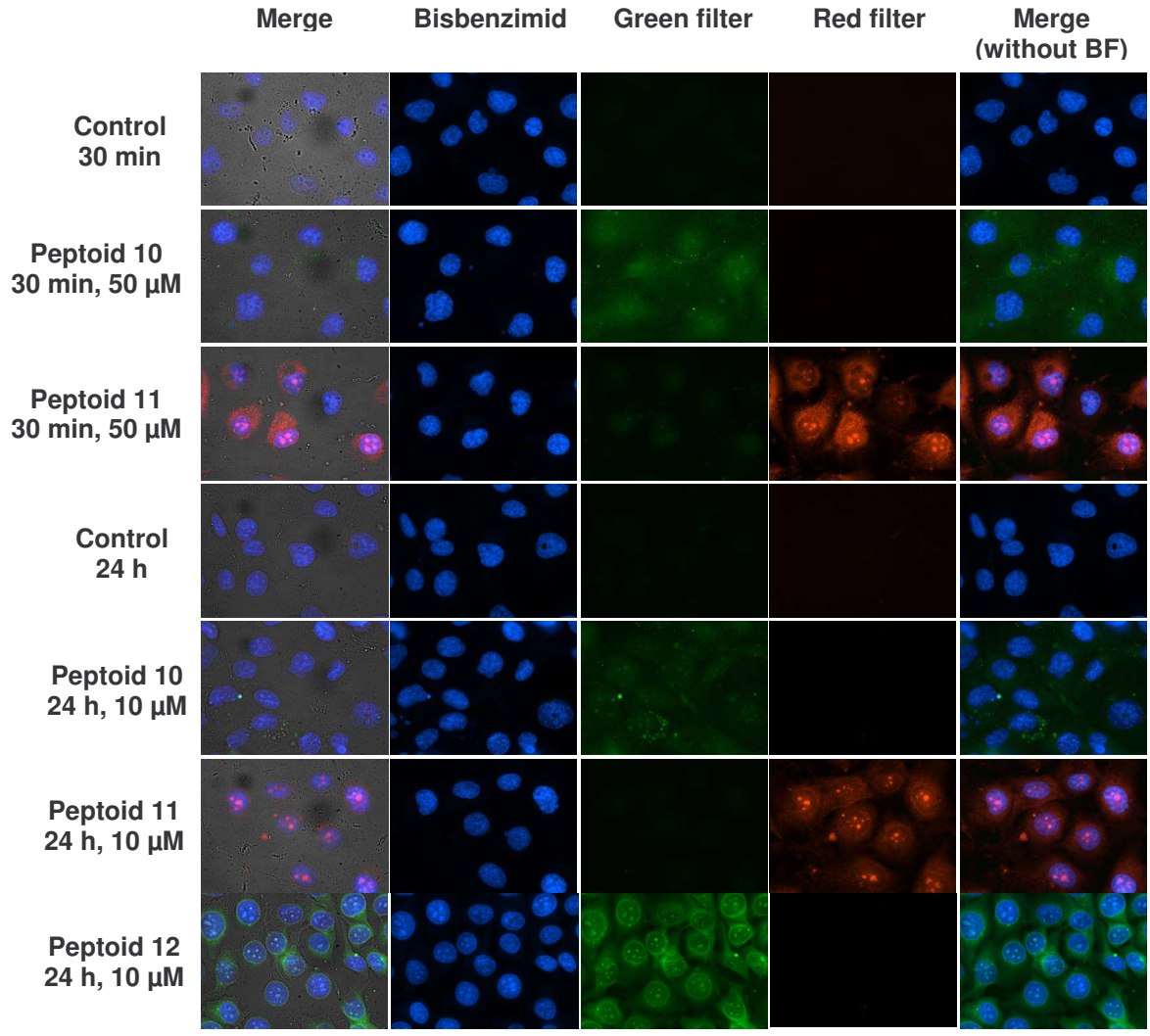


Figure 4. Grid confocal pictures of ECV304 cells showing the uptake of the purified carriers **10**, **11** and **12** (Purity > 95%). The uptake is demonstrated at a concentration of 50 µM (incubation 30 min) and 10 µM (incubation 24 h). Guanidinyl peptoids **11** and **12** show a higher uptake rate with accumulation in the cell nuclei and nucleoli (BF = bright field).

Additional Uptake Diagrams

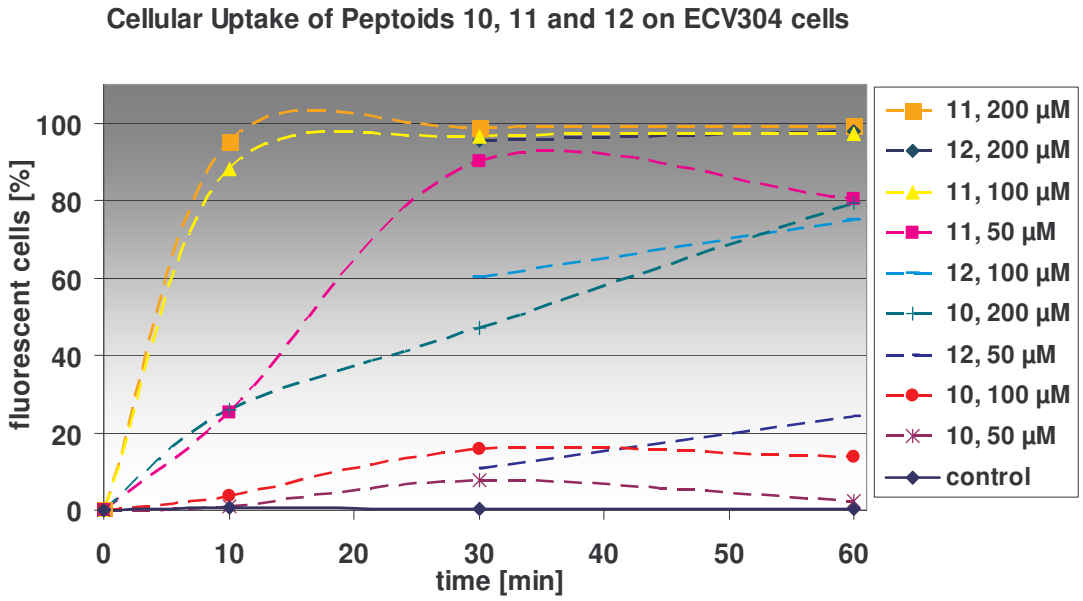


Figure 5. Cell penetration rate of carriers **10**, **11** and **12**. The uptake was tested on ECV304 cells, depending on peptoid concentration and on the incubation time. For guanidinyl carrier **12** the uptake rate was measured at 30 and 60 min.

Table 1. Uptake rate of the three peptoids **10**, **11** and **12** (detected by FACS), measured on A549 cells. The differences of carriers **11** and **12** could be because of the different fluorphores (Fluoresceine shows a weaker light emission compared to rhodamine). Nevertheless the difference in uptake between amino- and guanidinyl-peptoids is significant.

Peptoid	10 min	30 min	60 min
Amino peptoid **10**, 50 µM	3% ± 0.3%	6% ± 1.6%	72% ± 3.9%
Guanidinyl peptoid **11**, 50 µM	27% ± 6.1%	72% ± 5.4%	90% ± 2.6%
Guanidinyl peptoid **12**, 50 µM		25% ± 4.5%	58% ± 5.1%
Amino peptoid **10**, 100 µM	5% ± 3.3%	36% ± 5.3%	89% ± 0.7%
Guanidinyl peptoid **11**, 100 µM	56% ± 0.7%	94% ± 0.7%	94% ± 1.3%
Guanidinyl peptoid **12**, 100 µM		85% ± 4.2%	97% ± 0.4%
Amino peptoid **10**, 200 µM	32% ± 1.2%	88% ± 1.5%	93% ± 0.4%
Guanidinyl peptoid **11**, 200 µM	90% ± 3.2%	97% ± 0.4%	97% ± 0.7%
Guanidinyl peptoid **12**, 200 µM		96% ± 0.8%	98% ± 0.5%

Table 2. Uptake rate of the three peptoids **10**, **11** and **12** (detected by FACS), measured on ECV304 cells.

Peptoid	10 min	30 min	60 min
Amino peptoid **10**, 100 µM	4% ± 0.6%	16% ± 6.4%	14% ± 2.8%
Guanidinyl peptoid **11**, 100 µM	88% ± 10.6%	97% ± 0.2%	97% ± 0.1%
Guanidinyl peptoid **12**, 100 µM		60% ± 5.6%	75% ± 5.2%
Amino peptoid **10**, 200 µM	26% ± 1.9%	47% ± 4.0%	79% ± 2.1%
Guanidinyl peptoid **11**, 200 µM	95% ± 1.8%	99% ± 0.7%	99% ± 4.0%
Guanidinyl peptoid **12**, 200 µM		96% ± 0.7%	98% ± 0.3%

Additional Cell Viability Assays

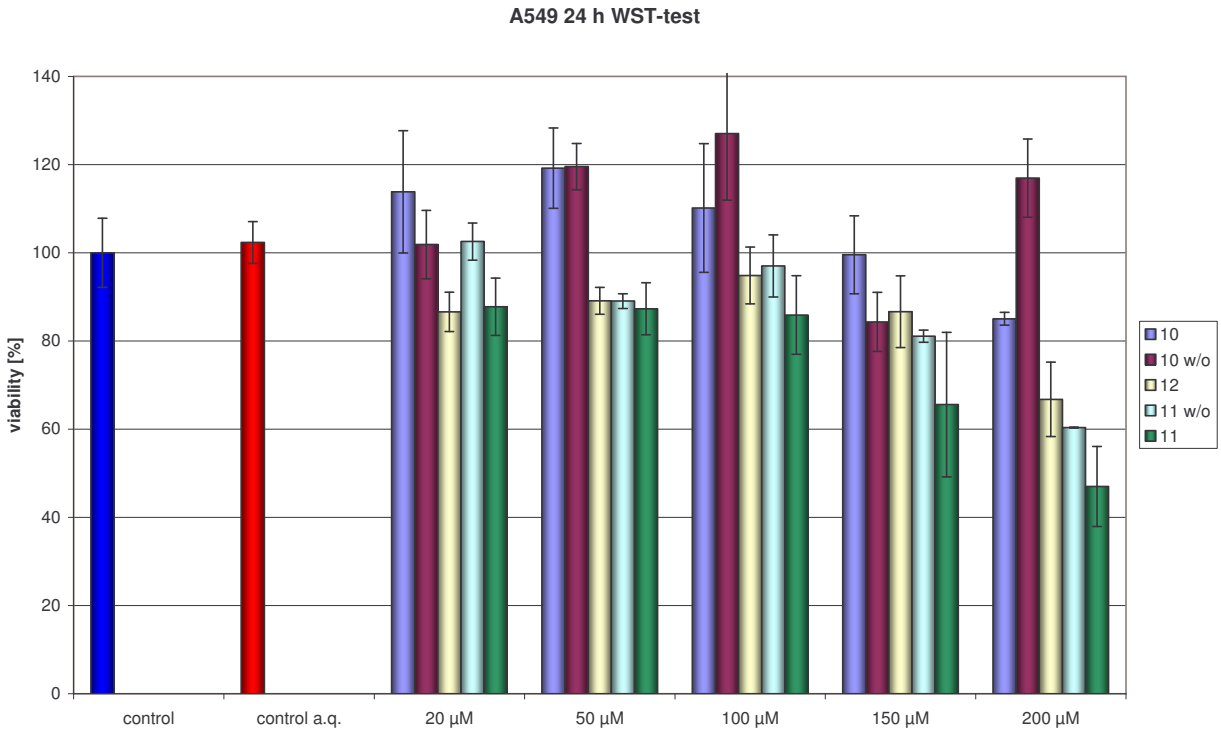


Figure 6. Viability test (WST-1) of carriers **10**, **11** and **12** as well as **10** without dye (**10 w/o**) and **11** without dye (**11 w/o**) in A549 cells; incubation time: 24 h.

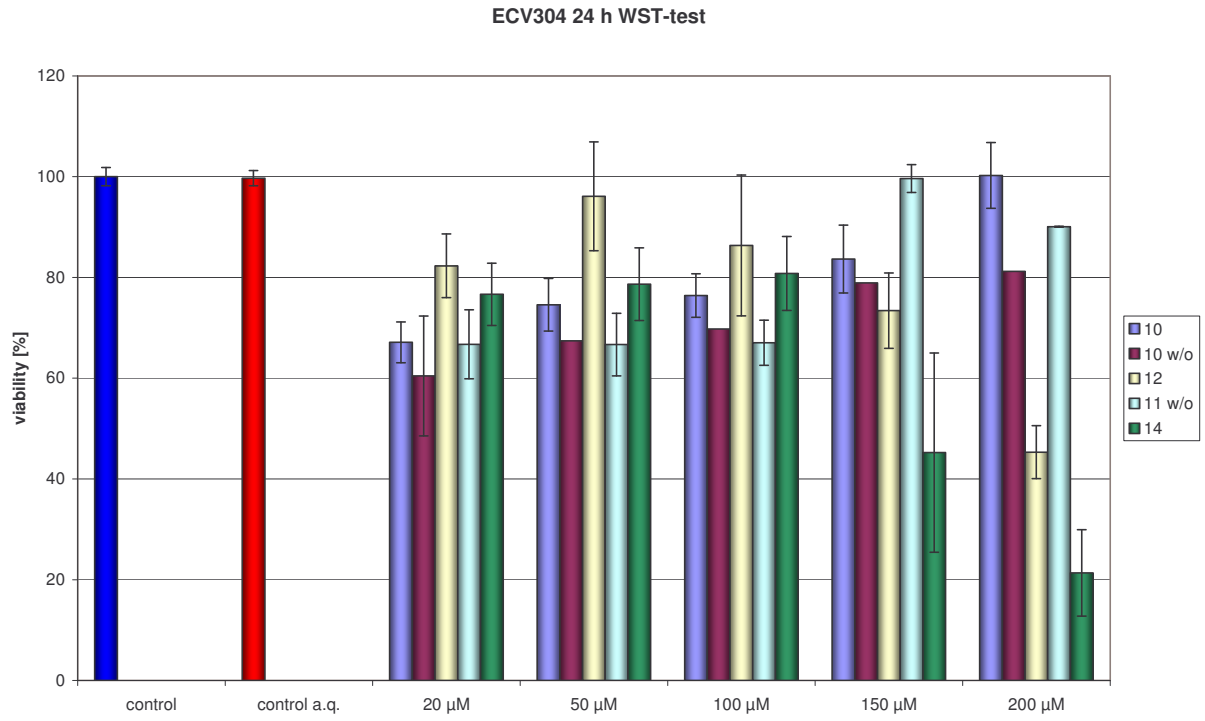


Figure 7. Viability test (WST-1) of carriers **10**, **11** and **12** as well as **10** without dye (**10 w/o**) and **11** without dye (**11 w/o**) in ECV304 cells; incubation time: 24 h.

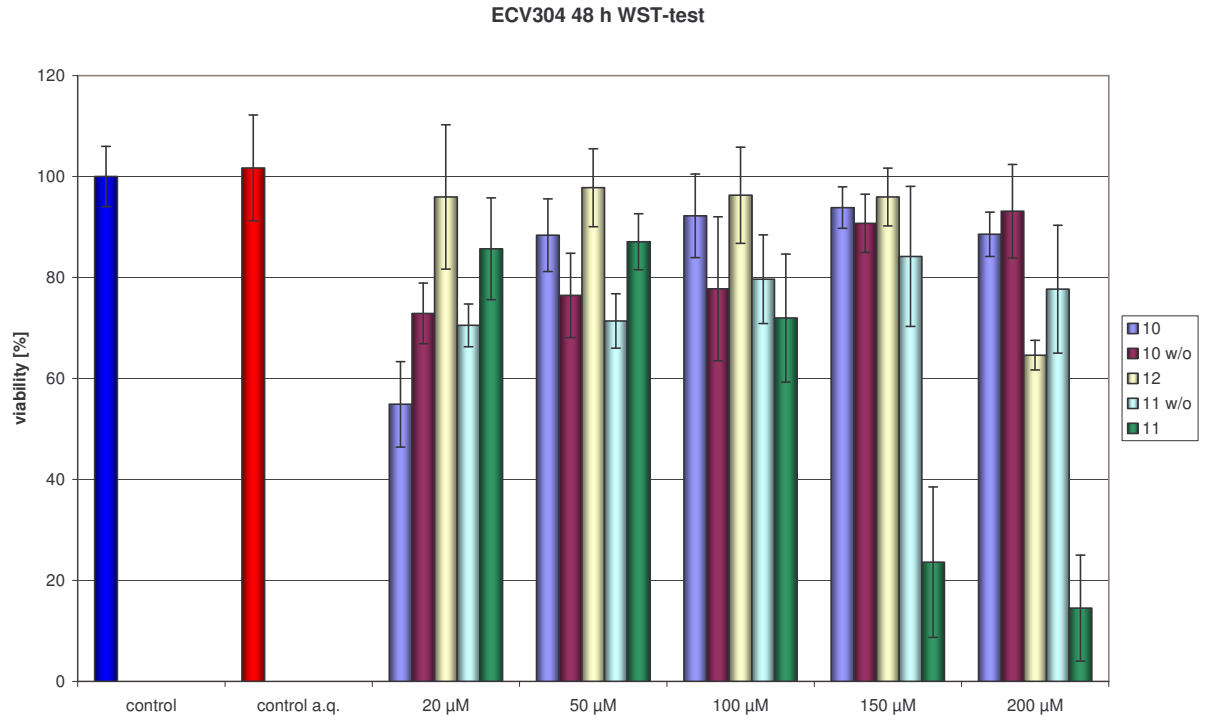


Figure 8. Viability test (WST-1) of carriers **10**, **11** and **12** as well as **10** without dye (**10 w/o**) and **11** without dye (**11 w/o**) in ECV304 cells; incubation time: 48 h.

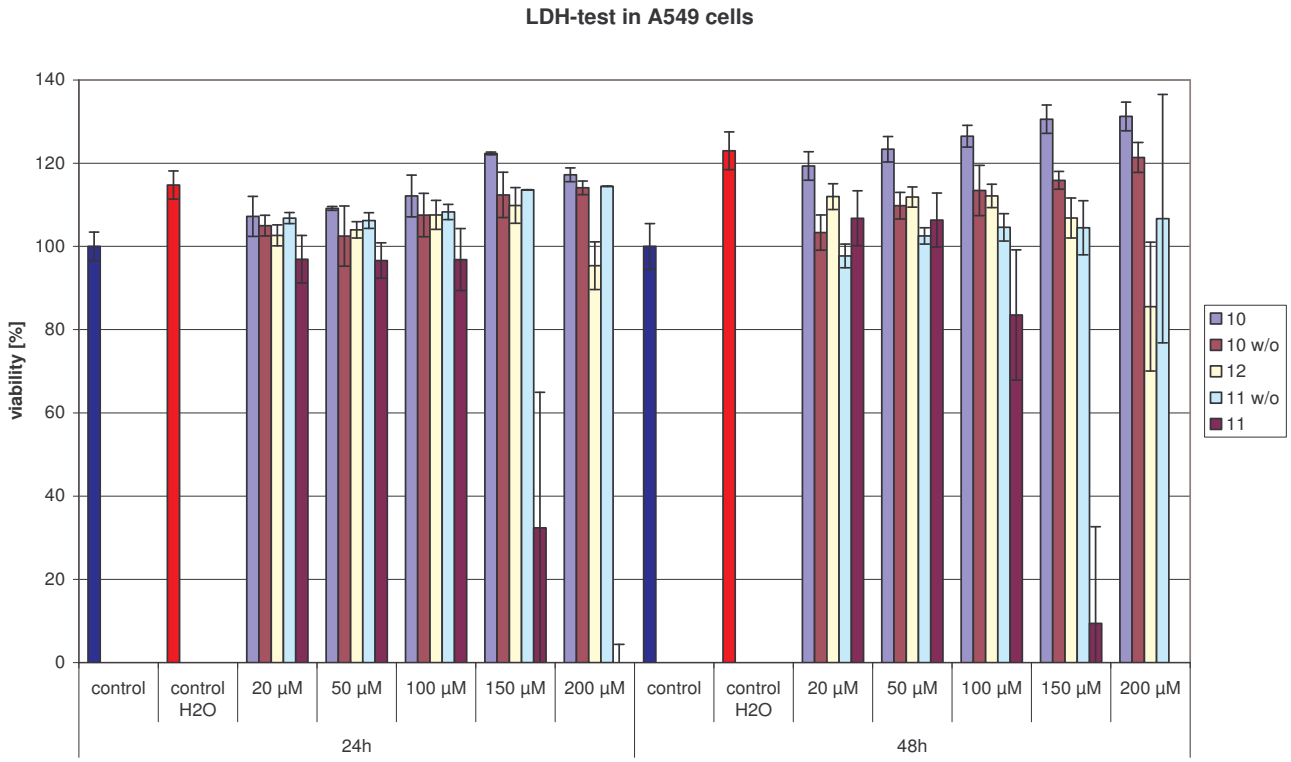


Figure 9. Viability test (LDH) of carriers **10**, **11** and **12** as well as **10** without dye (**10 w/o**) and **11** without dye (**11 w/o**) in A549 cells; incubation time: 24 h and 48 h. The results shown in the diagram confirm the assumption that the found toxic effects are due to the coupled fluorophores and not to the carriers themselves.

Table 3. Yields, and mass spectral data of the synthesized and tested peptoids.

Compound	Yield [mg]	Yield [%]	Purity*	$[M+H]^+$ calculated	$[M+H]^+$ found
Amino peptoid **10** (Fluo-{6,6,6,6,6,6}-NH_2)	56.0	51%	> 95%	1425.92	1425.9
Amino peptoid **10 w/o** ({6,6,6,6,6,6}-NH_2)	30.0	81%	> 95%	1010.81	1010.2
Guanidinyl peptoid **11** (Rhod-{6^G,6^G,6^G,6^G,6^G,6^G}-NH_2)	111	47%	> 95%	1432.99	1432.7
Guanidinyl peptoid **11 w/o** ({6^G,6^G,6^G,6^G,6^G,6^G}-NH_2)	10.0	28%	Not detected	1121.89	1122.0
Guanidinyl peptoid **12** (Fluo-{6^G,6^G,6^G,6^G,6^G,6^G}-NH_2)	23.0	42%	> 95%	1678.1	1678.5

* the purity was analyzed by reverse-phase HPLC.

HPLC-purification of the peptoids

Method:

Analytical and preparative high performance liquid chromatography (HPLC) was performed on a chromatographic system from Jasco (Tokyo, Japan) equipped with a diode-array detector. Reverse phase C18 analytical (4.6 x 250 mm, 5 µm) or semi-preparative (10 x 250 mm, 10 µm) columns from Grace (Grace, Deerfield, IL) were employed for purity assessment and purification respectively. For chromatographic separation of the peptoids, individually adjusted linear gradients were run at a constant temperature of 40°C. Solvent A: 0.1% trifluoroacetic acid (TFA); B: 90% acetonitrile in 0.1% TFA. The separation was monitored with UV-detection in the range 200 - 650 nm and UV spectra along with MALDI-mass spectrometry were used to identify the product peaks. Manually collected fractions of the semi-preparative runs were freeze-dried and immediately used in the biological assays. Prior to lyophilisation, fraction aliquots were directly re-injected onto the analytical column to quantify purity, which was determined by integration of the respective single peak area from the chromatograms at 220 nm.