Electronic Supplementary Information

Amphiphilic Peptoid Transporters – Synthesis and Evaluation

Sidonie B. L. Vollrath,[a] Daniel Fürniss,[a] Ute Schepers[b] and Stefan Bräse[a,b]*

[a]*Karlsruhe Institute of Technology, Institute of Organic Chemistry, Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany. Fax: +49 721 608-48581; Tel: +49 721 608-42903; E-mail: braese@kit.edu*

[b]*Karlsruhe Institute of Technology, Institute of Toxicology and Genetics (ITG), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen,Germany*

Contents

1 General Experimental Details

Preparative HPLC was performed on a C18 VYDAC 218 TP 1022 column (Grace Davision Discovery Sciences, 22 cm x 250 mm) with a linear gradient of 30-95% acetonitrile/water (0.1% TFA) over 40 min with a flow rate of 15 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 (3-5 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 mL/min.

Amphiphilic peptoids were purified on a preparative RPC4 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).

Analytical HPLC was 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).

MALDI-TOF mass spectra were measured on a Bruker Biflex IV spectrometer using dihydroxybenzoic acid (DHB) 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.

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 Experimental Procedures

General. 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).

General Synthesis Protocols for peptoid oligomers

Synthesis of the linear peptoid was performed as previously reported[1] *via* solid phase peptoid synthesis on 2-chlorotrityl chloride resin (Agilent, 1.2 mmol/g) or Rink amide resin (NovaBiochem, 0.67 mmol/g) with modified reaction times. Solid phase reactions were performed using fritted 5 mL plastic syringes (Multisynthec GmbH) filled with the resin. Yields were calculated according to the resin-loading value given by the manufacturer.

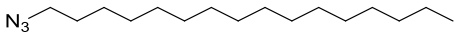
Propargylamine and TIPS-protected propargylamine were used as submonomers during solid phase peptoid synthesis. 3-Azidopropan-1-amine was synthesized as previously described[2] and used for the functionalization reactions as well as 1-azidohexadecane.

Synthesis of 3-azidopropan-1-amine: The synthesis of 3-azidopropan-1-amine was conducted as previously described[2] and used without further purification. 3-Chloropropylamine hydrochloride (1.93 g, 14.8 mmol, 1.00 equiv.) was dissolved in 50 mL water. Sodium azide (2.89 g, 44.4 mmol, 3.00 equiv.) was added and the reaction mixture stirred at 80 °C for 23 h. About 2/3 of the solvent was removed under reduced pressure. The residual mixture was cooled using an ice bath and 20 mL diethyl ether were added. KOH (1.89 g, 33.6 mmol, 2.27 equiv.) was added slowly to keep the temperature below 10 °C. The organic phase was separated and the aqueous phase was extracted with 20 mL diethyl ether twice. The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The colorless liquid could be obtained in 67% yield (993 mg, 9.92 mmol).

^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 1.19 (s, 2 H, NH_2), 1.51 (quin., ^3J = 6.8 Hz, 2 H, $CH_2CH_2CH_2$), 2.57 (t, ^3J =6.8 Hz, 2 H, CH_2N_3), 3.16 (t, ^3J =6.7 Hz, 2 H, CH_2NH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]: 31.9 (–,$CH_2CH_2CH_2$), 38.7 (–,CH_2NH_2), 48.6 (–,CH_2N_3); – IR

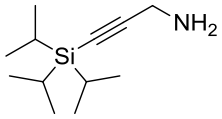
(KBr): $\tilde{\nu}$ [cm^{-1}] = 33656 (m), 2940 (m), 2872 (m), 2099 (s,), 1599 (w), 1458 (w), 1260 (m), 1069 (w), 849 (w), 672 (w), 558 (vw); MS (70 eV, EI), *m/z* (%): 101 (15) $[M+H]^+$, 84 (30) $[C_3H_6N_3]^+$, 70 (36), 57 (80), 44 (100) $[C_2H_6N]^+$; HR-MS (70 eV, EI), $C_3H_{10}N_4$: calc.: 101.0827, found: 101.0828.

Synthesis of 1-Azidohexadecane: 1-Hexadecyl bromide (4.56 g, 15.0 mmol, 1.00 equiv.) was dissolved in 300 mL DMF. Sodium azide (1.00 g, 15.0 mmol, 1.00 equiv.) was added and the reaction mixture was stirred for 24 h at 60 °C. After cooling to room temperature, 150 mL of water were added and the product was extracted with 3 × 150 mL dichloromethane. The combined organic layers were washed with water (3 × 150 mL), 150 mL saturated sodium chloride solution and were dried over Na_2SO_4. The solvent was evaporated under reduced pressure to yield 3.78 g (14.1 mmol, 94%) of a colorless oil. The product was used in solid phase peptoid synthesis without further purification.



^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) = 0.86−0.90 (m, 3 H, C*H*$_3$), 1.26−1.30 (m, 24 H, C*H*$_2$), 1.37−1.44 (m, 2 H, C*H*$_2$), 1.81−1.89 (m, 2 H, C*H*$_2$), 3.41 (t, ^3J = 8.9 Hz, 2 H, NC*H*$_2$). – ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) = 14.1 (+, *C*H$_3$), 22.7 (−, *C*H$_2$), 26.7 (−, *C*H$_2$), 28.2 (−, *C*H$_2$), 28.8 (−, *C*H$_2$), 29.2 (−, *C*H$_2$), 29.36 (−, *C*H$_2$), 29.44 (−, *C*H$_2$), 29.5 (−, *C*H$_2$), 29.6 (−, *C*H$_2$), 29.7 (−, *C*H$_2$), 31.9 (−, *C*H$_2$), 32.9 (−, *C*H$_2$), 34.0 (−, *C*H$_2$), 51.5 (−, *C*H$_2$). Other analytical data are in accordance with the literature.[3]

3-(Triisopropylsilyl)prop-2-yn-1-amine: In a flask equipped with a septum propargylamine (864 mg, 15.7 mmol, 1.00 equiv.) were dissolved in 40 mL dry THF and were cooled down to −78 °C. Under stirring, *n*-butyllithium (6.40 mL 2.5 M in hexane, 16.0 mmol, 1.02 equiv.) was added dropwise and the mixture was kept at that temperature for another 15 minutes. Afterwards, the solution was slowly warmed up to 0 °C and triisopropylsilyl chloride (4.00 mL, 18.7 mmol, 1.20 equiv.) was added drop wise. The reaction mixture was stirred for another 2 h and was subsequently quenched with 15 mL saturated $NaHCO_3$ solution. Afterwards, the aqueous phase was extracted with 3 × 20 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. For purification, the resulting crude product was subjected to column chromatography (cyclohexane/ethyl acetate 1:1, 1%

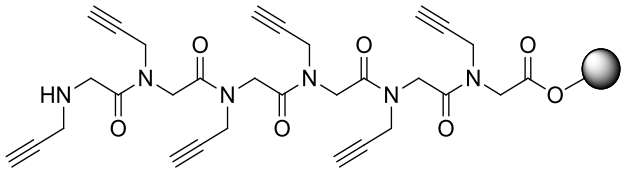


triethylamine) which yielded 1.74 g (52% yield, 8.24 mmol) of the product as a slight yellow oil.

R_f (CH/EE 1:1, 1% TEA): 0.37. –^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 0.98 – 1.09 (m, 21H, SiC*H*$_2$C*H*$_3$), 1.49 (bs, 2H, N*H*$_2$), 3.43 (s, 2H, C*H*$_2$NH$_2$). –^{13}C NMR (100 MHz, $CDCl_3$): δ [ppm] = 11.18 (+, Si*C*H), 18.56 (+, CH*C*H$_3$), 32.41 (–, *C*H$_2$NH$_2$), 82.52 (C_{quart}, *C*CH$_2$), 109.07 (C_{quart}, Si*C*). –IR (KBr): ῡ [cm^{-1}] = 3375 (w), 2943 (m), 2892 (m), 2866 (m), 2724 (vw), 2165 (w), 1632 (w), 1463 (m), 1383 (w), 1366 (w), 1330 (w), 1243 (vw), 1072 (w), 998 (m), 919 (w), 883 (m), 732 (w), 677 (m), 662 (m), 630 (w), 518 (vw), 455 (vw), 414 (vw) cm^{-1}. –MS (EI, 70 eV): m/z (%) = 211 (25) [M]$^+$, 210 (58) [M–H]$^+$, 196 (71) [M–CH$_3$]$^+$, 182 (25) [M+H–CH$_3$–CH$_3$]$^+$, 168 (85) [M–^{i}Pr]$^+$, 154 (11), 140 (16), 126 (16), 112 (12), 98 (15), 74 (18). –HRMS ($C_{12}H_{24}NSi$): calcd. 210.1678, found 210.1681.

Peptoid Synthesis:

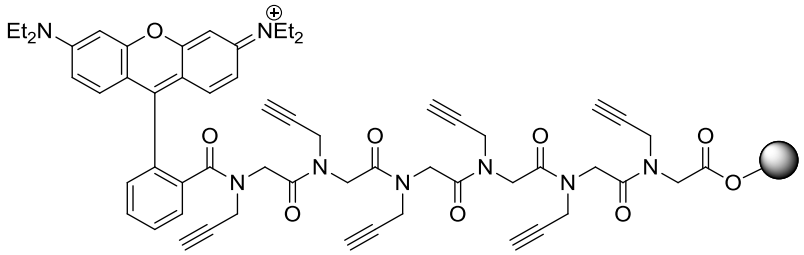
Unfunctionalized basic peptoid on 2-Chlorotrityl chloride resin (12): Synthesis of the



linear hexamer and the trimer were performed with 200 mg 2-chlorotrityl chloride resin (0.412 mmol, 1.00 equiv.) according to the general synthesis protocols for peptoid oligomers. The first coupling of bromoacetic acid was conducted with a mixture of bromoacetic acid (309 mg, 2.22 mmol, 5.40 equiv.) and DIPEA (290 mg, 400 µL, 2.22 mmol, 5.40 equiv.) in 3 mL DCM in a reaction time of 40 min. Amination steps were performed with propargylamine (220 µL, 3.44 mmol, 8.34 equiv.) in 3.4 mL DMF and acylation steps were achieved with a solution of bromoacetic acid (573 mg, 4.12 mmol, 10.0 equiv.) and DIC (640 µL, 520 mg, 4.12 mmol, 10.0 equiv.) in 3.4 mL DMF. Reaction time for both steps was 30 min. the reaction mixture was discarded and the resin was extensively washed with DMF.

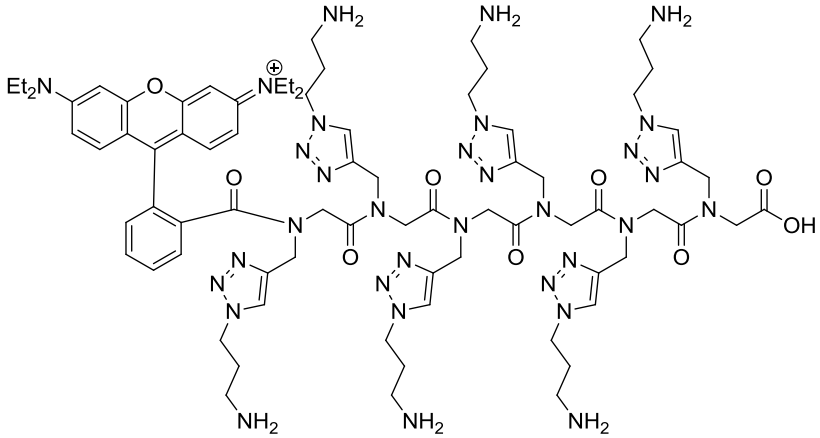
MS (MALDI, *matrix*: DHB) *m/z*: 611 [M+Na]$^+$, 589 [M+H]$^+$.

Reaction with rhodamine B (peptoid 14): The resin bound hexamer (92.4 mg, 50.0 µmol, 1.00 equiv.) was swollen in 3 mL DMF. A mixture of rhodamine B (71.9 mg, 0.150 mmol, 3.00 equiv.), HOBt (20.3 mg, 0.150 mmol, 3.00 equiv.) and DIC (38.0 µL, 30.8 mg, 0.149 mmol, 2.99 equiv.) in 3 mL DMF was added to the resin and the resin was shaken for 60 h at room temperature. Afterwards the reaction solution was discarded and the resin was extensively washed with DMF and DCM until the washing solution remained colorless.



MS (MALDI, *matrix*: DHB), *m/z*: 1013 $[M]^+$

Hydrophilic peptoid 2: The rhodamine B-labelled resin (29.4 mg, 50.0 µmol, 1.00 equiv.) was swollen in 2 mL dry THF. CuI (47.6 mg, 0.250 mmol, 5.00 equiv.) was added as well as a solution of 3-azidopropylamine (60.1 mg, 0.600 mmol, 12.0 equiv.) and DIPEA (446 µL, 323 mg, 2.50 mmol, 50.0 equiv.) in 3 mL dry THF. The reaction mixture was stirred for 20 h at room temperature. Afterwards, the reaction mixture was drained and the resin was washed with THF, sodium ascorbate solution, water, THF and DCM, using 3 × 2 mL for each washing step. The peptoid was cleaved from resin using 30% HFIP in DCM for 30 min. It was repeated once more with fresh cleaving solution and the resin was washed with DCM. All filtrates were combined and the solvent was evaporated under reduced pressure. HPLC purification gave 0.57 mg (0.35 µmol, 0.7%) of a pink powder after lyophilization.



MS (MALDI, *Matrix*: DHB) *m/z*: 1615 $[M]^+$. – Analytical HPLC (5–95% ACN + 0.1% TFA in 12 min, t_{Ret} 3.49 min detection at 245 nm), Purity: 88%.

Peptoid Synthesis on Rink amide-resin (13): For the synthesis of the basic peptoids on Rink amide resin, the Fmoc-protected resin (200 mg, 0.134 mmol, 1.00 equiv.) was swollen in DMF, deprotected with 20% piperidine in DMF (2 × 10 min) and washed with DMF. Synthesis of the peptoid was performed according to the general synthesis protocols for peptoid oligomers using bromoacetic acid (186 mg, 1.34 mmol, 10.0 equiv.) and DIC (201 µL, 169 mg, 1.34 mmol, 10.0 equiv.) in 1.50 mL DMF for the acylation steps and propargylamine (71 µL, 61.3 mg, 1.11 mmol, 8.30 equiv.) for amination steps. Reaction time for each step was 30 min. After each step, the reaction mixture was discarded and the resin was extensively washed with DMF. The resin bound hexamer was then subjected to coupling with rhodamine B. Therefore, a mixture of rhodamine B (193 mg, 0.402 mmol, 3.00 equiv.), DIC (102 µL, 82.9 mg, 0.402 mmol, 3.00 equiv.) and HOBt (54.3 mg, 0.402 mmol, 3.00 equiv.) dissolved in 3.0 mL DMF was added to the resin. It was shaken for 16 h at room temperature. Afterwards, the reaction mixture was discarded and the resin was extensively washed with DMF and DCM until the filtrate remained colorless.

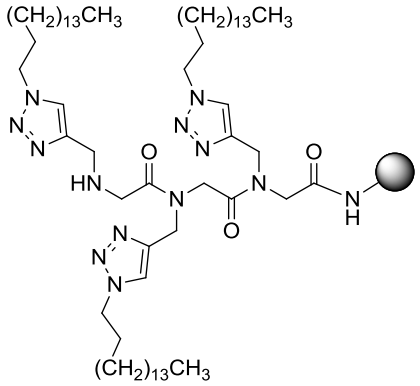
MS (MALDI, *matrix*: DHB), *m/z*: 1012 $[M]^+$.

Functionalization with 3-azidopropylamine (peptoid 1): The CuAAC reaction was performed as described above. To the swollen resin, a solution of 3-azidopropylamine (161 mg, 1.61 mmol, 12.0 equiv.) and DIPEA (1.20 mL, 869 mg, 6.72 mmol, 50.2 equiv.) in 3 mL abs. THF and CuI (128 mg, 0.670 mmol, 5.00 equiv.) was added and the resin was shaken for 60 h at room temperature. Afterwards, the reaction mixture was discarded and the resin was washed with THF, sodium ascorbate solution, water, THF and DCM, using 3 × 3 mL for each washing step. The peptoid was cleaved from resin using 95% TFA in DCM for 30 min. It was

repeated once more with fresh cleaving solution and the resin was washed with DCM. All filtrates were combined and the solvent was evaporated under reduced pressure. HPLC-purification yielded 1.75 mg (1.08 µmol, 0.8%) of a pink powder after lyophilization.

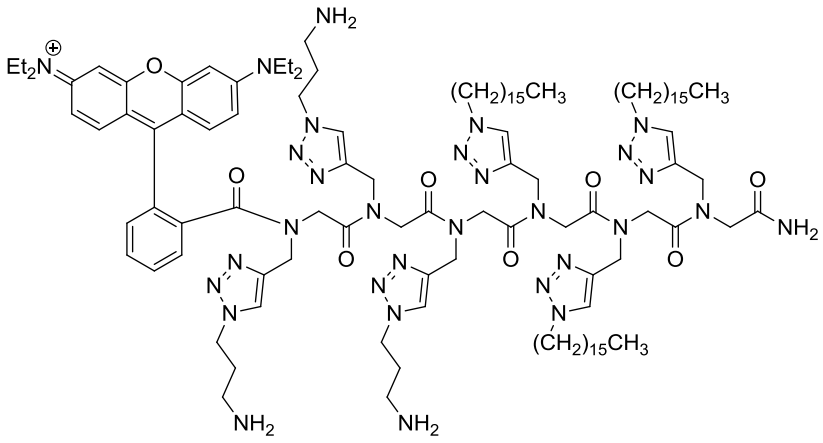
MS (MALDI, *matrix*: DHB), *m/z*: 1613 $[M]^+$; analytical HPLC (5−95% ACN + 0.1% TFA in 30 min, t_{Ret} = 11.6 min detection at 218 nm), purity: 93%.

Functionalized trimer 18: For the synthesis on Rink amide resin, the Fmoc-protected resin



(200 mg, 0.134 mmol, 1.00 equiv.) was swollen in DMF, deprotected with 20% piperidine in DMF (2 × 10 min) and washed with DMF. Synthesis of the peptoid was performed according to the general synthesis protocols for peptoid oligomers using bromoacetic acid (186 mg, 1.34 mmol, 10.0 equiv.) and DIC (201 µL, 169 mg, 1.34 mmol, 10.0 equiv.) in 1.50 mL DMF for the acylation steps and propargylamine (71 µL, 61.3 mg, 1.11 mmol, 8.30 equiv.) for amination steps. Reaction time for each step was 30 min. After each step, the reaction mixture was discarded and the resin was extensively washed with DMF. For the reaction with 1-azidohexadecane, the resin was swollen in THF and 128 mg CuI (0.670 mmol, 5.00 equiv.) as well as a solution of 358 mg 1-azidohexadecane (1.34 mmol, 10.0 equiv.) and 1.20 mL DIPEA (866 mg, 6.70 mmol, 50.0 equiv.) in 3.0 mL abs. THF was added to the resin and the resin was shaken at room temperature for 16 h. Afterwards, the reaction mixture was discarded and the resin washed with THF, sodium ascorbate solution, water, THF and DCM, using 3 × 3 mL for each washing step.

MS (MALDI, *matrix*: DHB), *m/z*: 1167 $[M+Cu]^+$.

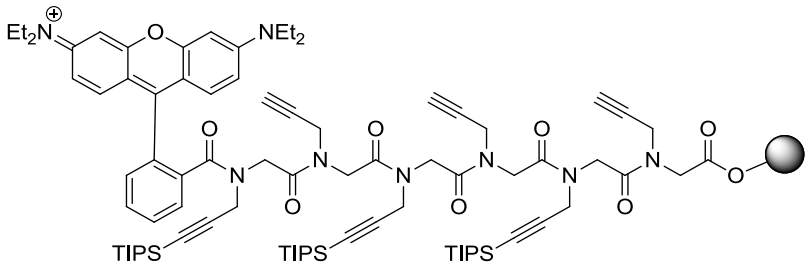


Synthesis of amphiphilic peptoid 3: For the synthesis, the resin-bound trimer **18** was swollen in DMF and the peptoid hexamer synthesized as described above. For the bromo acetylations, 186 mg bromoacetic acid

(1.34 mmol, 10.0 equiv.) and 201 µL DIC (169 mg, 1.34 mmol, 10.0 equiv.) in 1.0 mL DMF were used. For the amination steps, 71 µL propargylamine (61.3 mg, 1.11 mmol, 8.30 equiv.), dissolved in 1.0 mL DMF were used. The reaction time was 30 min at room temperature for all steps. After each step, the reaction mixture was discarded and the resin was extensively washed with DMF. For the coupling of rhodamine B, a solution of 193 mg rhodamine B (0.402 mmol, 3.00 equiv.), 54.3 mg HOBt (0.402 mmol, 3.00 equiv.) and 102 µL DIC (82.9 mg, 0.402 mmol, 3.00 equiv.) dissolved in 3.0 mL DMF and added to the resin. Afterwards, the reaction mixture was discarded and the resin was washed with DMF until the washing solution remained colorless. For the final coupling of 3-azidopropylamine, the resin was swollen in THF and 128 mg CuI (0.670 mmol, 5.00 equiv.) were added. A solution of 124 mg 3-azidopropylamine (1.24 mmol, 9.24 equiv.) and 1.20 mL DIPEA (866 mg, 6.70 mmol, 50.0 equiv.) dissolved in 2.0 mL abs. THF was added. The resin was shaken at room temperature for 24 h. Afterwards, the reaction mixture was discarded and the resin was washed with THF, sodium ascorbate solution, water, THF and DCM using 3 × 3 mL for each washing step. The peptoid was cleaved from resin using 95% TFA in DCM for 30 min. It was repeated once more with fresh cleaving solution and the resin was washed with DCM. All filtrates were combined and the solvent was evaporated under reduced pressure. HPLC-purification gave 2.57 mg (1.22 µmol, 0.9%) of a pink powder after lyophilization.

MS (MALDI, *matrix*: DHB), *m/z*: 2115 $[M]^+$; analytical HPLC (5−95% ACN + 0.1% TFA in 12 min, t_{Ret} 4,01 min detection at 245 nm), purity: 93%.

TIPS-protected peptoid 21: Synthesis of the linear hexamer and the trimer were performed



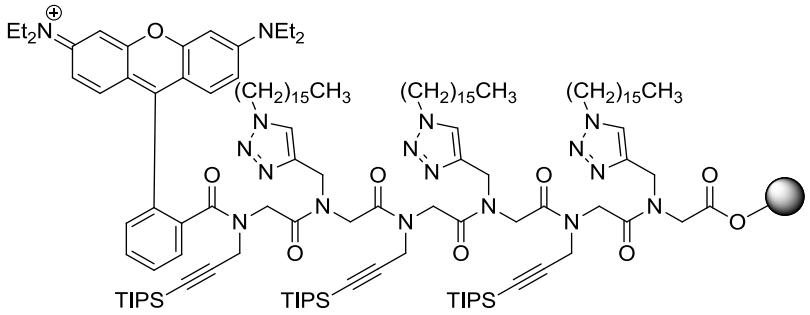
with 49 mg 2-chlorotrityl chloride resin (0.100 mmol, 1.00 equiv.) according to the general synthesis protocols for peptoid oligomers. The first coupling of bromoacetic acid was conducted with a mixture of bromoacetic acid (75 mg, 0.54 mmol, 5.40 equiv.) and DIPEA (97 µL, 0.54 mmol, 5.40 equiv.) in 3 mL DCM in a reaction time of 40 min. The reaction mixture was removed and the resin was washed with 1.5 mL dichloromethane as well as with 5 × 1.5 mL DMF. Amination steps were performed with propargylamine (43.5 mg, 0.79 mmol, 7.90 equiv.) in 0.40 mL DMF, or TIPS-protected propargylamine (167 mg, 0.79 mmol, 7.90 equiv.) in a reaction time of 120 min. Acylation steps were achieved with a solution of bromo acetic acid (110 mg, 0.79 mmol, 7.90 equiv.)

and DIC (122 µL, 0.79 mmol, 7.90 equiv.) in 0.66 mL DMF and the reaction time was 30 min after addition of propargylamine and 120 min after addition of the TIPS-protected submonomer. After each step, the reaction mixture was discarded and the resin was extensively washed with DMF.

Conjugation with rhodamine B: The resin (49 mg, 0.100 mmol, 1.00 equiv.) was swollen in 2 mL DMF and reacted with a mixture of rhodamine B, HOBt and DIC in DMF as described above. The reaction time was 18 h at room temperature. Afterwards, the reaction solution was discarded and the resin was washed with DMF and DCM until the washing solution remained colorless.

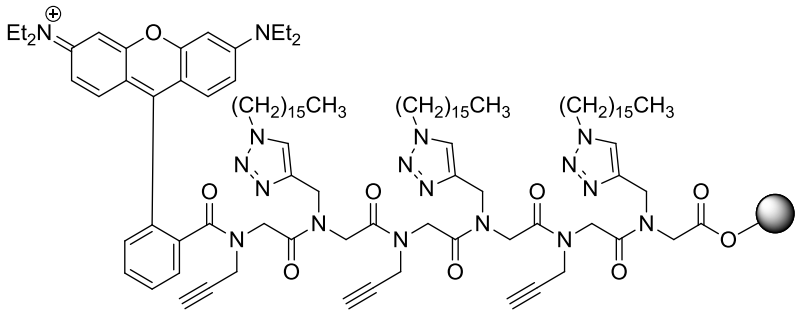
MS (MALDI, *matrix*: DHB), *m/z* 1483 $[M]^+$.

Reaction with 1-azidohexadecane (peptoid 22): The resin (49 mg, 0.100 mmol, 1.00 equiv.) was swollen in 2 mL THF. It was reacted with CuI and a solution of 1-azidohexadecane and DIPEA in THF as described for the CuAAC reaction with 3-azidopropylamine above. The reaction time was 60 h at room temperature.



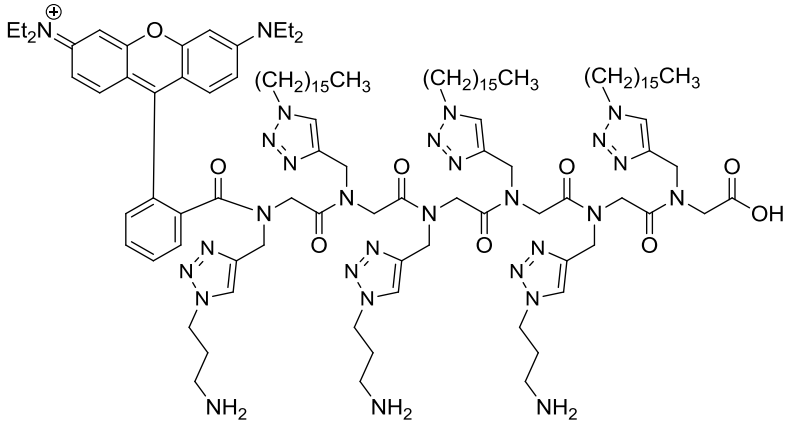
MS (MALDI, *matrix*: DHB), *m/z*: 2285 $[M]^+$.

Deprotection of TIPS (peptoid 23): For the deprotection, the resin (49 mg, 0.100 mmol, 1.00 equiv.) was swollen in 2 mL THF and reacted with 4 mL 1 M TBAF solution in THF for 18 h at room temperature. The reaction mixture was discarded and the resin was extensively washed with THF.



MS (MALDI, *matrix*: DHB), *m/z*: 1816 $[M]^+$.

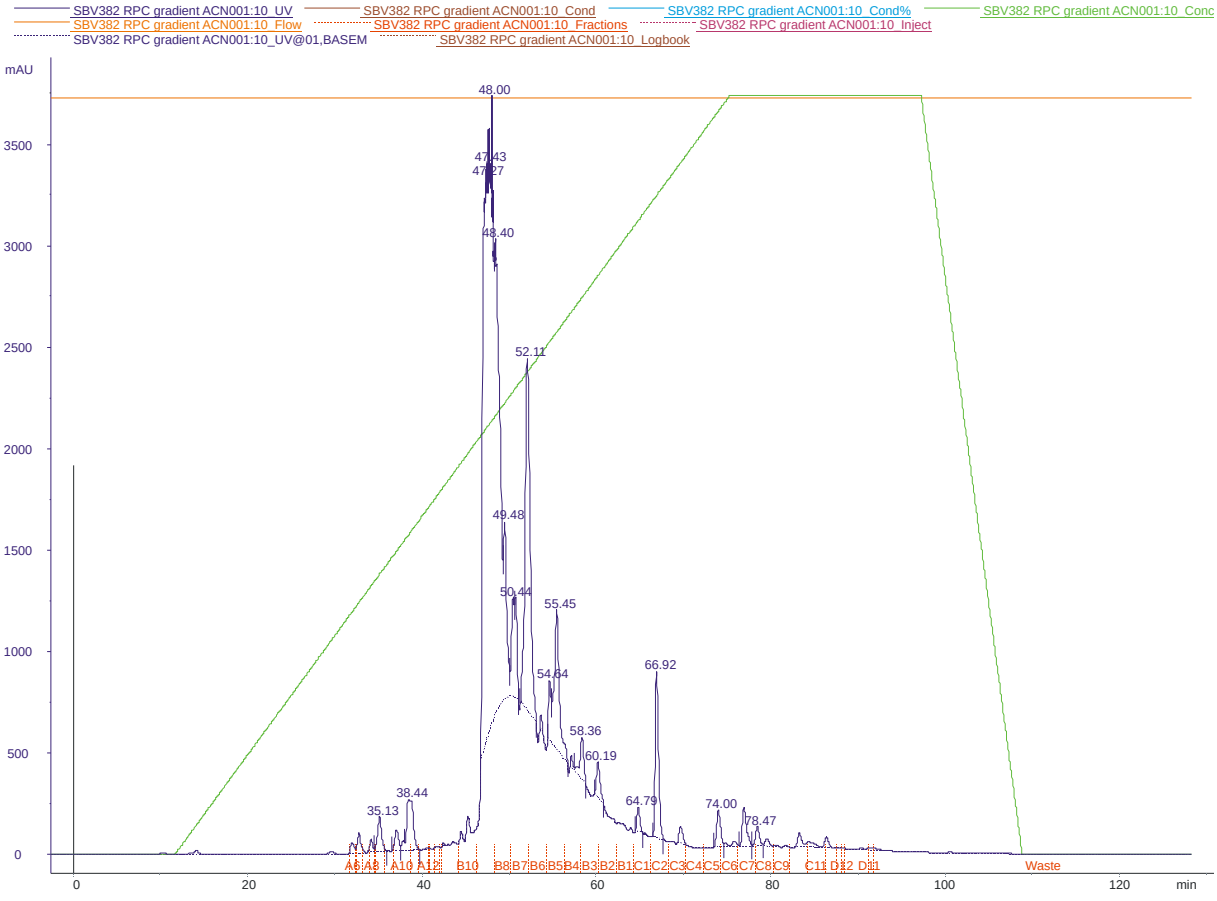
Reaction with 3-azidopropylamine (peptoid 4): The resin (49 mg, 0.100 mmol, 1.00 equiv.) was swollen in 2 mL THF. The reaction with 3-azidopropylamine was performed as described above. Reaction time was 18 h at room temperature. After the reaction, the peptoid was cleaved from the resin using 30% HFIP in DCM 2 × 30 min. The resin was washed with DCM and the filtrates and cleaving solutions were combined. The solvent was evaporated under reduced pressure. HPLC purification (RPC4, 5−95% Methanol + 0.1% TFA in 100 min, t_{Ret} = 45.4 min) and lyophilization gave 6.86 mg (3.24 µmol, 3.2%) of a pink powder.



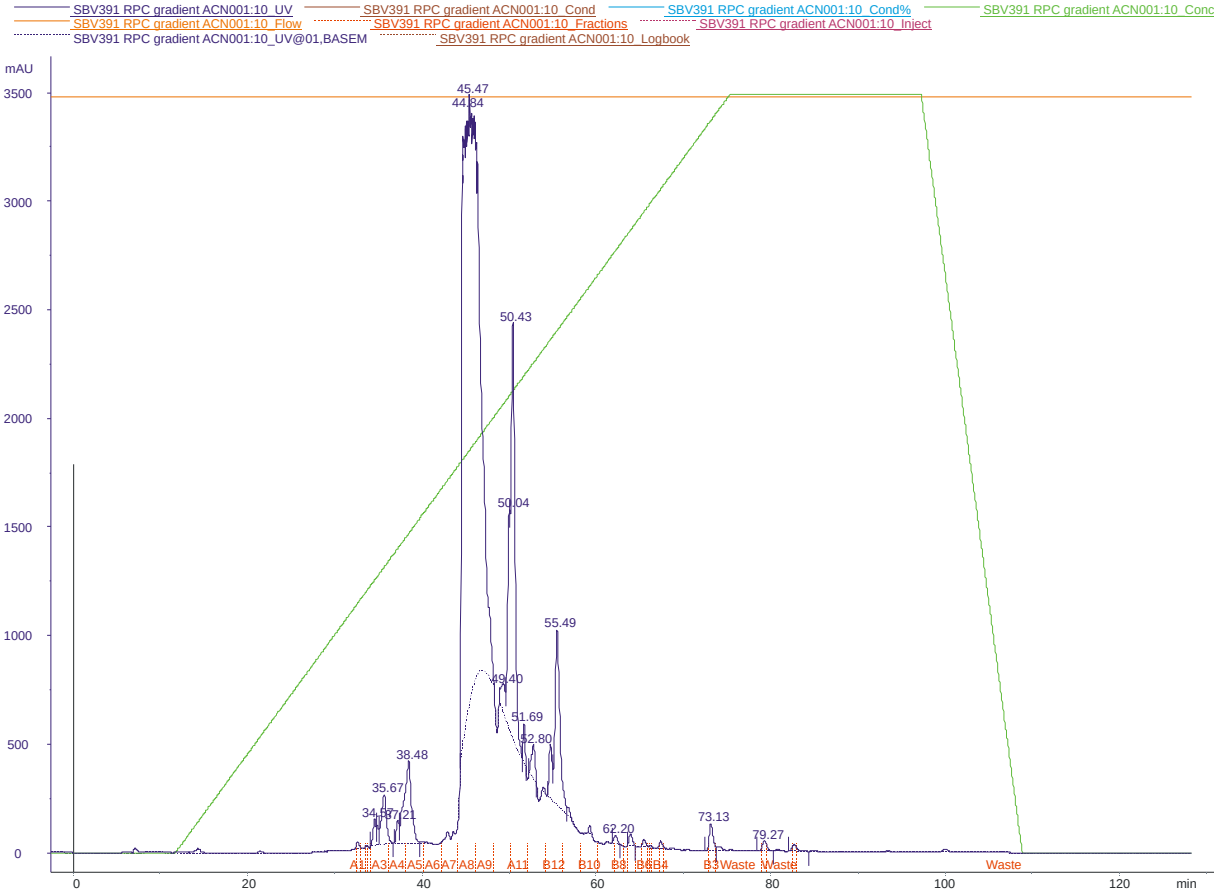
MS (MALDI, *Matrix*: DHB) *m/z*: 2117 $[M]^+$. –Analytical HPLC (5−95% ACN + 0.1% TFA in 12 min, t_{Ret} 3,59 min detection at 245 nm): Purity: 98%.

3 Preparative HPLC of peptoid 2 and 4

Peptoid 2

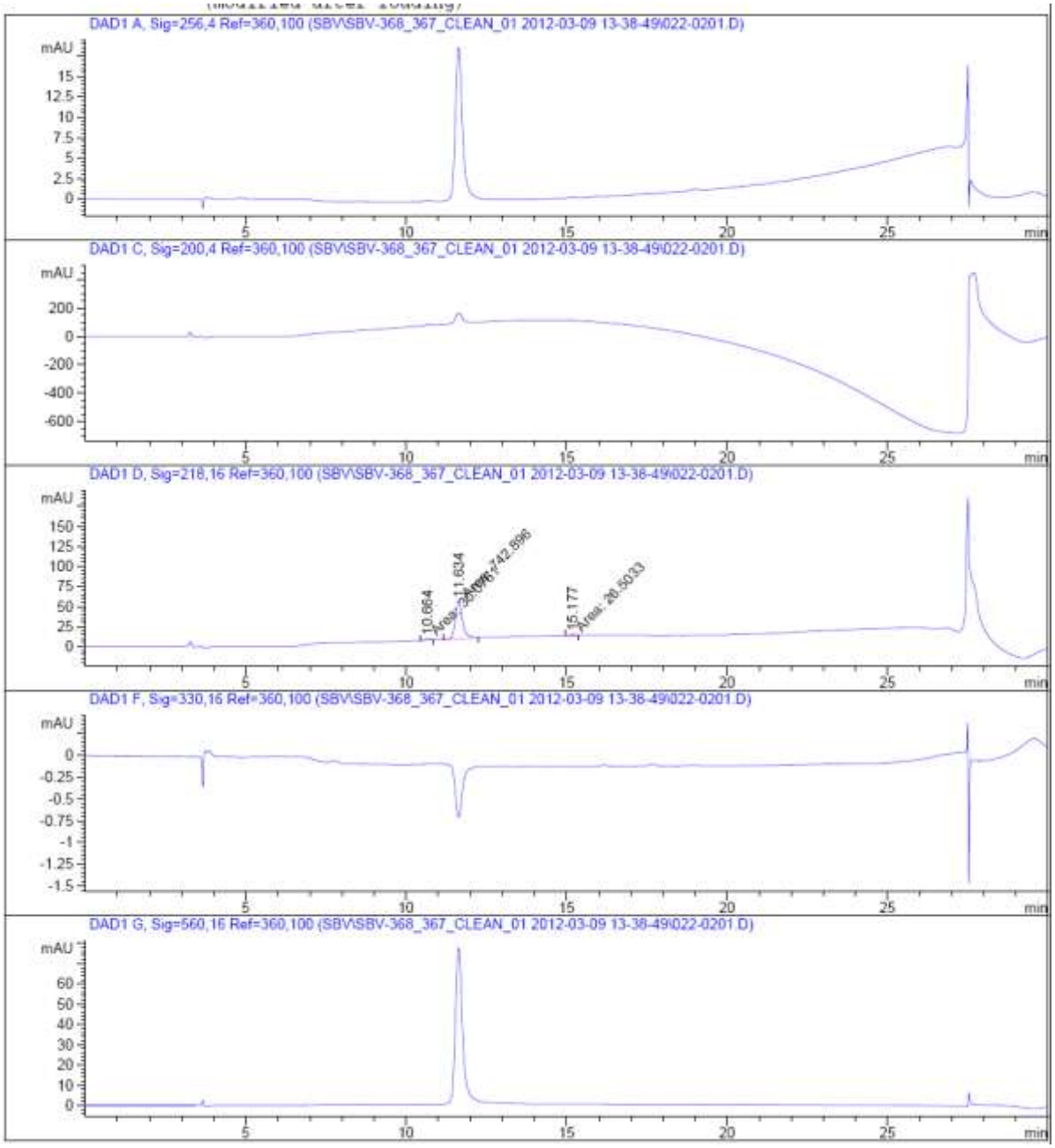


Peptoid 4



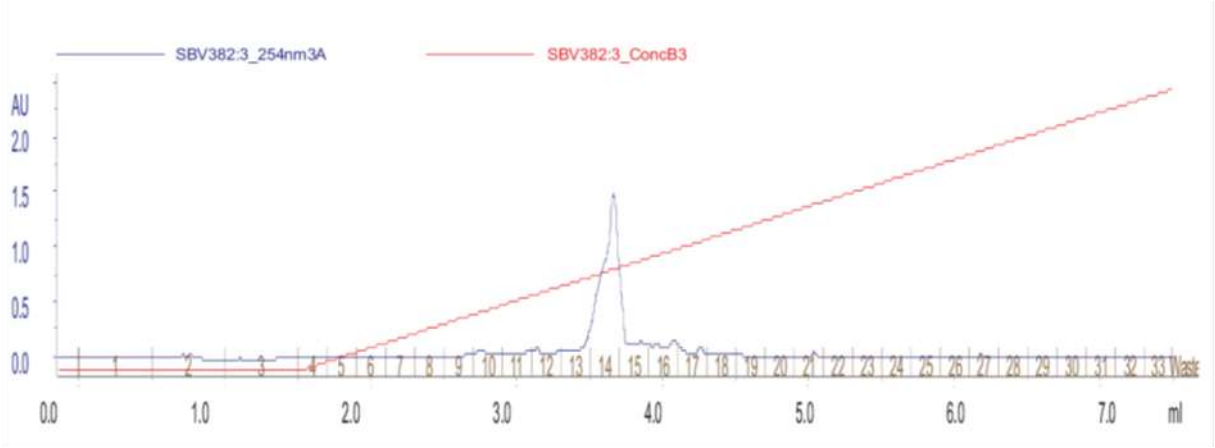
4 Analytical HPLC traces

Peptoid **1**



Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.664	MM	0.2371	30.07614	2.11426	3.7620
2	11.634	MM	0.2633	742.89600	47.01682	92.9229
3	15.177	MM	0.3022	26.50333	1.46150	3.3151

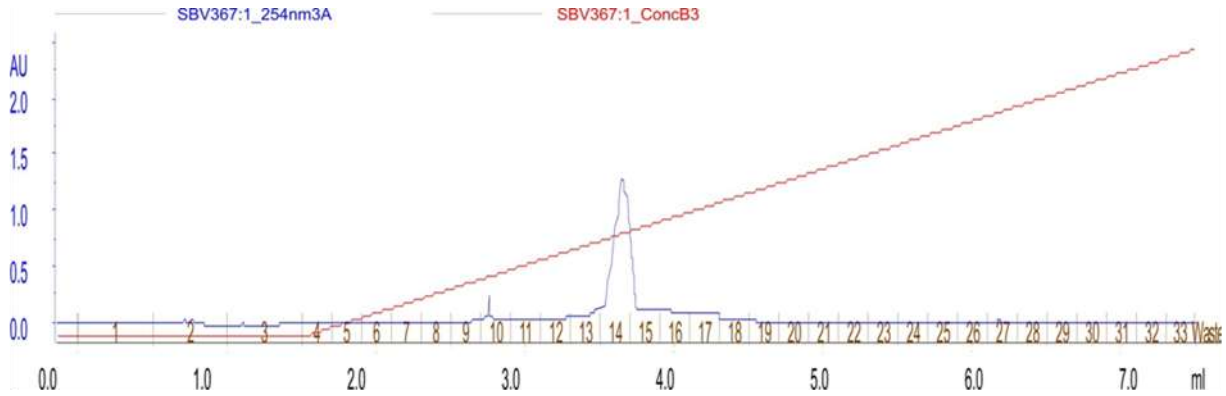
Peptoid **2**



RT [ml]	Area [AU*min]	Height [AU]
2.85	9.3658	0.0858
3.24	12.7334	0.0694
3.60	222.8039	0.8146
3.69	512.1902	1,4965
3.85	20.9609	0.1226
4.09	36.3297	0.1938
4.31	19.8256	0.0813

Total number of detected peaks	7
Total area (AU*min)	644.5972
Area in evaluated peaks (AU*min)	834.2095
Ratio peak area / total area	0.987701
Calculated from	SBV382 RPC gradient ACN001:10_UV
Baseline	SBV382 RPC gradient ACN001:10_UV@01,BASEM
Peak rejection on	
Maximum number of peaks ()	20

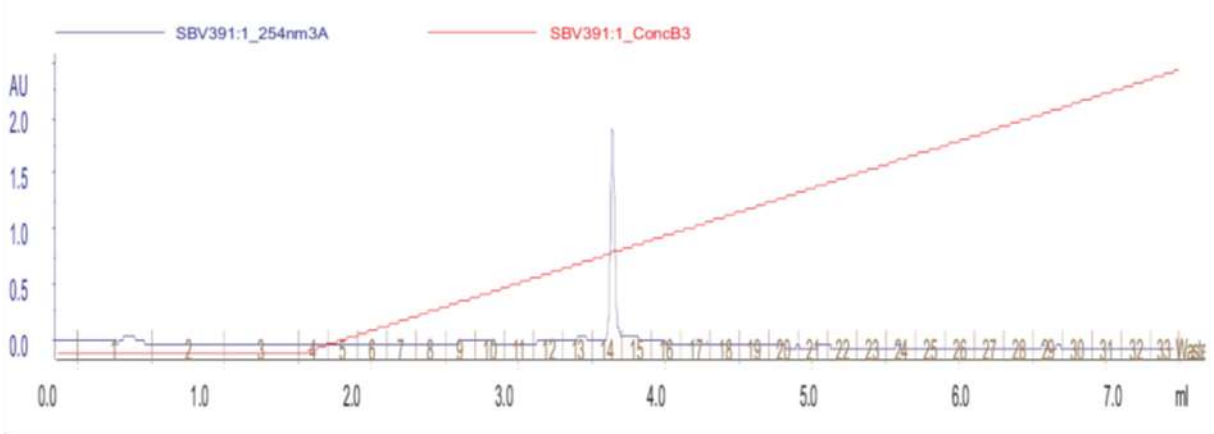
Peptoid **3**



RT [ml]	Area [AU*min]	Height [AU]
2.77	14.1439	0.2157
2.83	7.7843	0.0794
3.60	24.9427	0.4743
3.62	119.4750	0.8331
3.69	377.5469	1.2519
3.71	136.7642	1.1031

Total number of detected peaks	6
Total area (AU*min)	682.2733
Area in evaluated peaks (AU*min)	680.6570
Ratio peak area / total area	0.997631
Calculated from	SBV367 RPC gradient ACN001:10_UV
Baseline	SBV367 RPC gradient ACN001:10_UV@01,BASEM
Peak rejection on	
Maximum number of peaks ()	20

Peptoid **4**



RT [ml]	Area [AU*min]	Height [AU]
0.54	8.3817	0.0419
2.82	1.9655	0.0243
3.52	1.2181	0.0262
3.66	630.9540	1.8435

Total number of detected peaks	4
Total area (AU*min)	654.4079
Area in evaluated peaks (AU*min)	642.5193
Ratio peak area / total area	0.981833
Calculated from	SBV391 RPC gradient ACN001:10_UV
Baseline	SBV391 RPC gradient ACN001:10_UV@01,BASEM
Peak rejection on	
Maximum number of peaks ()	20

The main peaks of the chromatograms of the amphiphilic peptoids **2** and **3** do contain shoulders, which are due to rotamers of the same structure, which could not be separated on a RPC4-column.

5 Cellular uptake

5.1 Experimental

Cell culture techniques for mammalian cells

1×10^4 HeLa (human cervix carcinoma) cells were plated into each well of a 8-well Ibitreat µslide (IBIDI, Germany) and cultured in 200 µL Dulbecco's modified Eagle's medium, high glucose, (DMEM, Taufkirchen) supplemented with 10% fetal calf serum (FCS, PAA) and 1 u/mL Penicillin/Streptomycin at 37 °C and 5% CO_2. The purified peptoids were dissolved in 50% ethanol to yield a 2 mM stock solution and were further diluted with 10% DMEM to yield the respective incubation media. The cells cultured as described above were incubated with the different peptoids at final concentrations of 1−10 µM. Eventually, the cells were treated with 10 nM Mitotracker green (Invitrogen) for 15 min to label the mitochondria. After washing the cells to remove free peptoids and the Mitotracker, the nuclei were costained with Hoechst 33342 (2 µg/mL) in DMEM at 37 °C for 5 min.

Live imaging by confocal microscopy

Visualization of the peptoids was achieved by confocal microscopy using a Leica TCS-SP5 II, equipped with a DMI6000 microscope. The peptoids were excited at 561 nm using a DPSS laser. The Mitotracker Green (Invitrogen) for the detection of the mitochondria was excited at 488 nm using an argon laser and the Hoechst 33342 for the detection of the nuclei was excited at 364 nm using a UV laser. The objective was a HCX PL APO CS 63.0x1.2 Water UV. The exposure was set to minimize oversaturated pixels in the final images. Fluorescence emission was measured at 417 – 468 nm for the detection of the nuclei, at 499 – 552 nm for the detection of the mitochondria, and 593 – 696 nm for the detection of the rhodamine B labelled peptoids. Image acquisition was conducted at a lateral resolution of 1024 × 1024 pixels and 8 bit depth using LAS-AF 2.0.2.4647 acquisition software.

5.2 Rendering with Imaris software

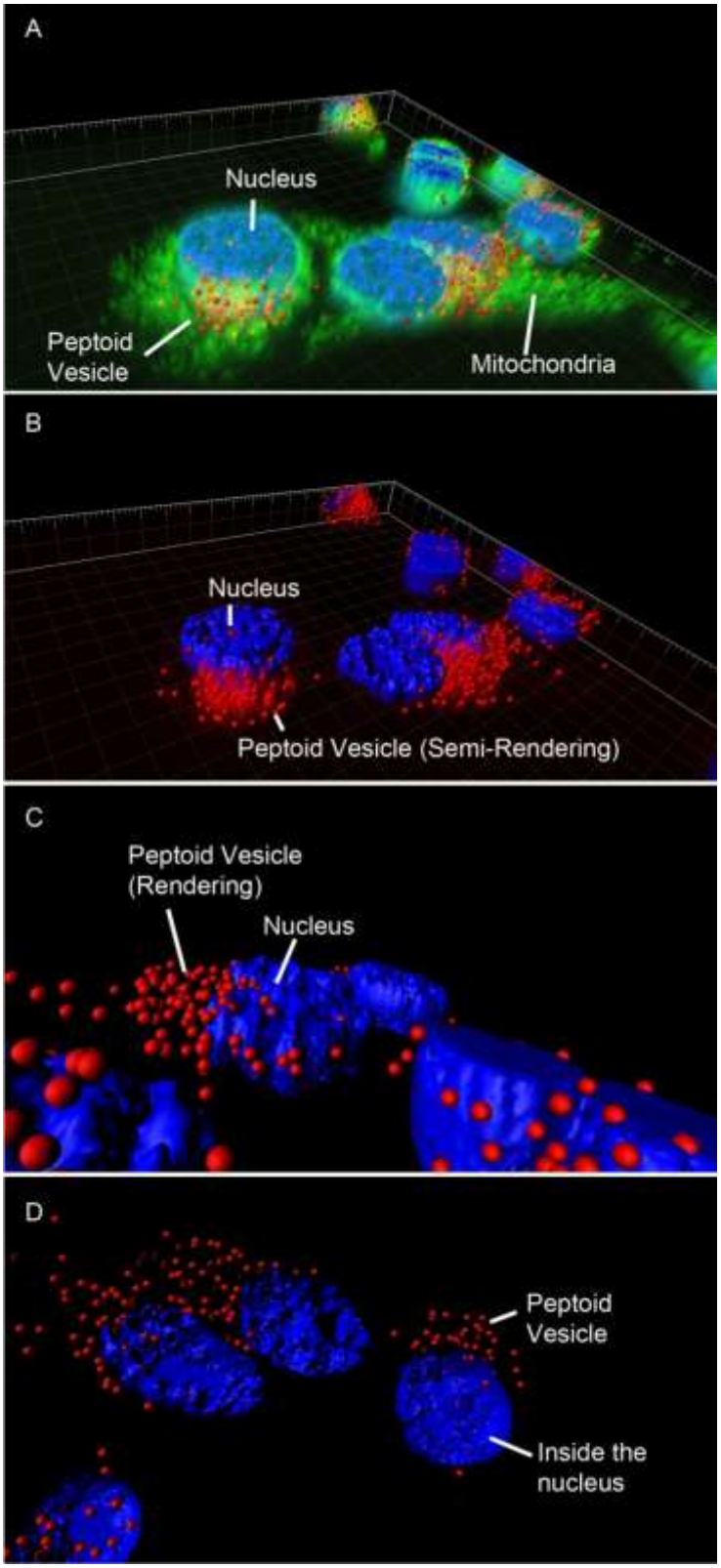


Figure S1. Three dimensional representation and rendering of HeLa cells for the exact localization of peptoid **2** co-localizing vesicles. A: Overall 3D-projection with nuclei (blue), mitochondria (green) and peptoid vesicles (red). B: Rendered nuclei and semi rendered

peptoid vesicles. C: Zoom of the nuclear region to show peptoid localization close to the nuclei. D: Dissection of the nuclei to prove no co-localization of the peptoids with the nuclei.

For the three dimensional rendering of peptoid **2** containing vesicles, 1×10^4 HeLa cells were incubated with a 1 µM solution of peptoid **2** for 24 h at 37 °C. For the visualization of the peptoid distribution, the cells were co-stained with Mitotracker Green™ (Invitrogen) for labelling the mitochondria and Hoechst 33342 (Sigma) for labelling the nuclei. The images show the 3D-projection of a confocal z-stack (30 frames × 200 nm, HCX PL APO CS 63.0 × 1.2 Water UV objective). For a more detailed localization of the peptoids, the 3D-projection was rendered with Imaris × 64 6.4.2 (Bitplane) software (Figure S1). The overall 3D-projection with rendered nuclei (blue), mitochondria (green) and peptoid vesicles (red) is shown (Figure S1-A). Image B shows only the rendered nuclei in blue and semi rendered peptoid vesicles in red. Image C shows an enlarged image of the rendered structures, where it becomes clear that the peptoid vesicles are localized very close to the nucleus and to some extent directly on the nuclear membrane. In D, a dissection through the rendered structure is shown, which clearly shows that there is no localization of the peptoid vesicles in the nucleus.

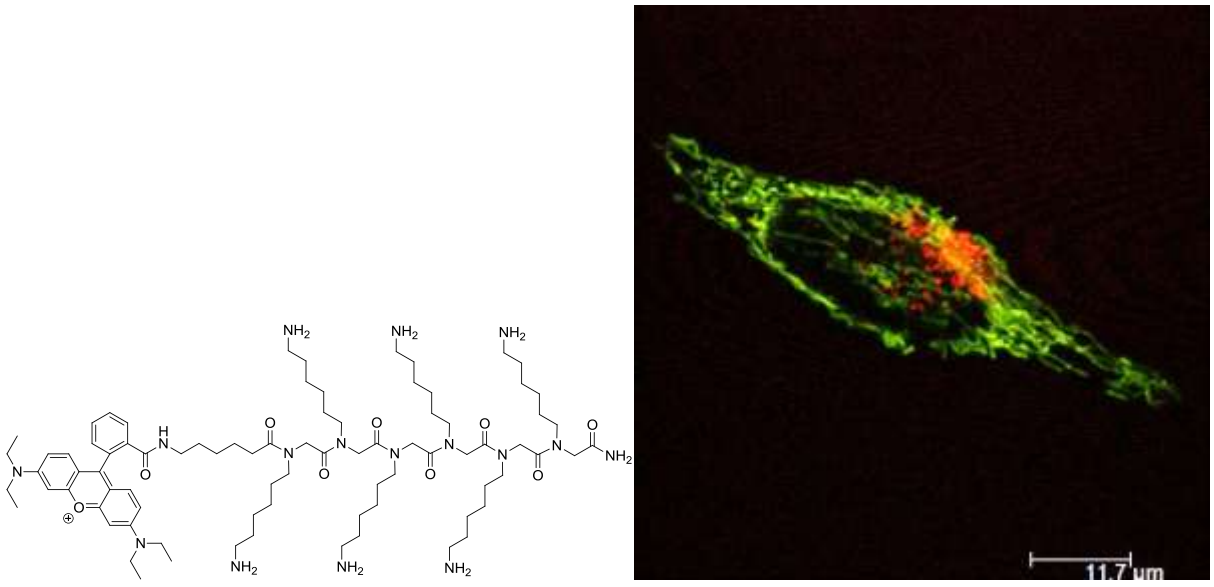


Figure S2: Cellular uptake of a control peptoid [RhoBSpiro-Ahx]-$[But]_{6A}NH_2$ in HeLa cells (see also Sternberg et al.). 1×10^4 HeLa cells were treated with 1 µM of the peptoid for 24 h at 37 °C. For co-staining of the mitochondria the cells were treated with 100 nm Mitotracker

Green (green) and excited at 488 nm with an Argon laser and at 561 nm using a DPSS laser. Eventually, the cells were analysed by fluorescence confocal imaging. The image shows the merges of the respective emission channels of each line by using the PMTs for the emission: 516 nm – 552 nm for the detection of the mitochondria (green), and 600 nm – 700 nm for the detection of the rhodamine B labelled peptoid (red). Scale bar= 11.7 µm.

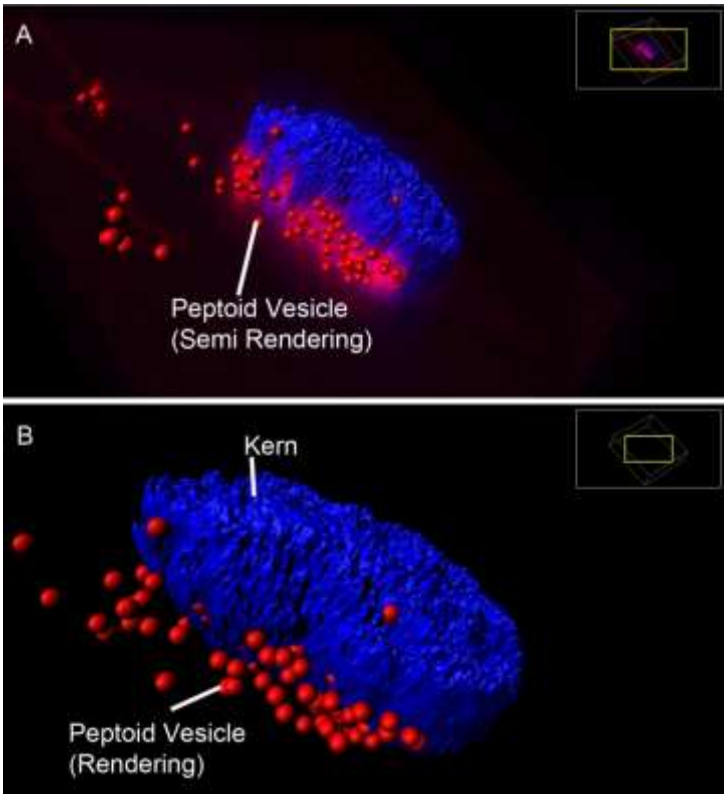


Figure S2. Rendering of the cellular uptake of peptoid **4** in HeLa cells. Enlarged region of one nucleus (blue) and rendered peptoid vesicles (red). The amphiphilic peptoids are localized closely at the membrane of the nucleus.

Also, the rendering of peptoid **4** colocalizing vesicles has been performed as described above and enlarged regions of the nucleus are shown in Figure S2. Figure S2-A shows the nucleus in blue with semi rendered peptoid vesicles. Figure S2-B shows the enlarged region of one nucleus with rendered peptoid vesicles. It becomes clear again that the peptoids are localized at the membrane of the nucleus and not in the nucleus.

5.3 Determination of the cytotoxicity

The toxicology of the peptoids on cell growth was investigated in HeLa cells. 1×10^4 HeLa cells/ well were seeded in 96-well microtiter plates (Becton Dickinson)(100 µL/well). The

peptoids **1**−**4** (0.1−100 µM) were added and the cells were incubated at 37°C for 72 h. After exposure the cell viability was tested by applying an MTT assay following the manufacturer's instructions (Promega). Cell growth was determined by measuring formazan formation from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium by using a Varioskan microplate reader (Thermofisher Scientific) at 540 nm. Mean and standard deviation (S.D.) of a total number of analysed samples (n=4) were calculated. Significance of the effects of various treatments as compared to untreated control was evaluated by paired t-test at the 95% confidence level using Origin 8.

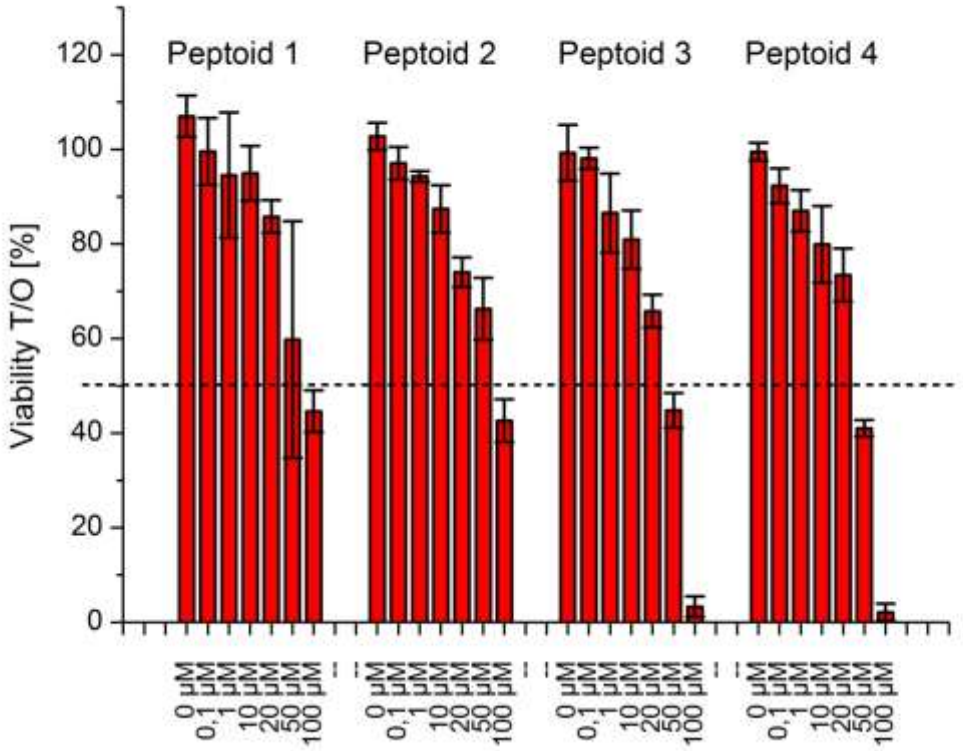


Figure S3: Viability assays of peptoids 1-4 using the MTT-assay. The viability of the cells was determined by the MTT assay and the S.D. was calculated. The toxicity was measured after 72 h upon incubation with varying concentrations of peptoids 1-4 and compared to the untreated control. The viability of the cells was measured using the Cell Titer 96 assay from Promega. T / C [%] = test over control (value for the viability of cells). S.D. was calculated from triplicates of n = 4.

6 Literature

[1] S. B. Y. Shin, B. Yoo, L. J. Todaro and K. Kirshenbaum, *J. Am. Chem. Soc.*, 2007, **129**, 3218−3225.

[2] B. Carboni, A. Benalil, and M. Vaultier, *J. Org. Chem.*, 1993, **58**, 3736−3741.

[3] M. Lamani, and K. R. Prabhu, Angew. Chem. 2010, 122, 6772−6775; *Angew. Chem. Int. Ed*. 2010, **49**, 6622−6625.

[4] U. Sternberg, E. Birtalan, I. Jakovkin, B. Luy, U. Schepers, S. Bräse, C. Muhle-Goll, Org. Biomol. Chem. 2013, 11, 640-647