Electronic Supplementary Material (ESI) for ChemComm.

Supporting information for:

Transfecting tissue models with CRISPR/Cas9 plasmid DNA using peptide dendrimers

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1. Materials and reagents

All reagents, salts and buffers were used as purchased from Sigma Aldrich, Fluorochem Ltd, Iris Biotech Gmbh, TCI (Tokyo Chemical Company), GL Biochem. Amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-Ser(tBu)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Leu-OH, Fmoc-D-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-D-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-D-Lys(Fmoc)-OH, Fmoc-Lys(Alloc)-OH, Fmoc-D-Lys(Alloc)-OH, Fmoc-Arg(Pbf)-OH and Fmoc-His(Trt)-OH were purchased by Iris Biotech GmbH or GL Biochem. Rink Amide AM resin LL was purchased from Novabiochem. Peptide dendrimers synthesis was performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper or automatically by CEM Liberty Blue Automated Microwave Peptide Synthesizer. Analytical RP-HPLC was performed with an Ultimate 3000 Rapid Separation LC-MS System (DAD-3000RS diode array detector) using an Acclaim RSLC 120 C18 column (2.2 µm, 120 Å, 3×50 mm, flow 1.2 mL/min) from Dionex. Data recording and processing was done with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). All RP-HPLC were using HPLC-grade acetonitrile and Milli-Q deionized water. The elution solutions were: A Milli-Q deionized water containing 0.05% TFA; D Milli-Q deionized water/acetonitrile (10:90, v/v) containing 0.05% TFA. Preparative RP-HPLC was performed with a Waters automatic Prep LC Controller System containing the four following modules: Waters2489 UV/Vis detector, Waters2545 pump, Waters Fraction Collector III and Waters 2707 Autosampler. A Dr. Maisch GmbH Reprospher column (C18-DE, 100×30 mm, particle size 5 µm, pore size 100 Å, flow rate 40 mL/min) was used. Compounds were detected by UV absorption at 214 nm using a Waters 248 Tunable Absorbance Detector. Data recording and processing was performed with Waters ChromScope version 1.40 from Waters Corporation. All RP-HPLC were using HPLC-grade acetonitrile and Milli-Q deionized water. The elution solutions were: A: Milli-Q deionized water containing 0.1% TFA; D: Milli-Q deionized water/acetonitrile (10:90, v/v) containing 0.1% TFA. MS spectra were recorded on a Thermo Scientific LTQ OrbitrapXL. MS spectra were provided by the MS analytical service of the S4 Department of Chemistry and Biochemistry at the University of Bern (group PD Dr. Stefan Schürch).

2. Solid phase synthesis of peptide dendrimers

Manual solid phase synthesis of peptide dendrimers. Peptide dendrimers were synthesized by placing 300 mg Rink Amide AM resin LL (0.22-0.25 mmol/g) in a 10 mL polypropylene syringe equipped as described previously. Stirring of the reaction mixture at any given step described below was performed by attaching the closed syringe to a rotating axis. The resin was swollen in DCM for 60 min. Then, the following conditions were used:

Removal of the Fmoc protecting group – At each step the Fmoc protecting group was removed with 8 mL of piperidine/DMF (1:4, v/v) for 2 x 10 min. After filtration the resin was washed with NMP (3 x 6 mL), MeOH (3 x 6 mL) and DCM (3 x 6 mL).

Coupling of the Fmoc-protected amino acids – 5 eq. of Fmoc-protected amino acid, 5 eq. of Oxyma Pure **(**Ethyl cyano(hydroxyimino)acetate) and 5 eq. of DIPEA (*N*,*N*-Diisopropylethylamine) per reaction site in 8 mL of NMP/DCM (80:20, v/v) were added to the resin and the reaction was stirred for 60 min. Reactions were carried out according to the dendrimer generations with 1 h for the 0^{th} generation, 2 h for the 1^{st} generation, 3 h for the 2^{nd} generation and 4 h for the 3^{rd} generation. The resin was then washed with NMP (3 x 6 mL), MeOH (3 x 6 mL) and DCM (3 x 6 mL).

Solid phase synthesis of peptide dendrimers by CEM Liberty Blue. Peptide dendrimers were synthesized by CEM Liberty Blue (scale 0.10 mmol) using 300 mg of Rink Amide AM resin LL (0.22-0.25 mmol/g). Stirring of the reaction mixture at any given step described below was performed by bubbling of N_2 in the vial. The resin was swollen in DMF/DCM 50:50 for 15 min at R.T. Then, the following conditions were used:

Removal of the Fmoc protecting group – At each step the Fmoc protecting group was removed with 5 mL of piperidine/DMF (1:4, v/v) for 2 min at 75 °C. After filtration, the resin was washed 5 times with 5 mL DMF.

Coupling of the Fmoc-protected amino acids – 5 eq. of Fmoc-protected amino acid, 5 eq. of Oxyma and 5 eq. of DIC all at a concentration of 0.2 M, were used as coupling reagents in 4 mL of DMF. The reaction was stirred for 5 minutes at 50 or 75 °C. The resin was then washed with 4 mL DMF 4 times. The couplings were repeated according to the generations and performed once for the zero generation, twice for the first generation, four times for the second generation. Only the first two peptide dendrimer generations were synthetized by CEM Liberty Blue, and the third generation synthesis was performed manually.

Deprotection of Lys(Alloc) and coupling – The resin was dried *in vacuo* and bubbled twice in dry DCM (8 ml) for 5 minutes with nitrogen. Solutions of $Pd(PPh_3)_4$ (0.1 eq., 10 mg) in dry DCM (3 mL) and $(CH_3)_2NH \cdot BH_3$ (25 eq., 100 mg) in dry DCM (3 mL) were added to the resin and bubbled with nitrogen for 1h. The resin was washed with dry DCM (3x8 mL) and reaction repeated once for 2 h. The resin was washed with sodium diethyldithiocarbamate (0.02 M in DMF, 10 mL) for 20 min and NMP, MeOH and DCM (2x10 mL each). Then, the carboxylic acids were coupled according to the manual procedure.

Cleavage and purification – The cleavage was carried out by treating the resins with 7 mL of a TFA/DODT/TIS/H_2O (94:2.5:2.5:1, v/v/v/v) solution for 5 h. The peptide solutions were precipitated with 40 mL of TBME, centrifuged for 10 min at 3500 rpm (twice), evaporated and dried under high vacuum for 60 min. The crude was then dissolved in a H_2O/CH_3CN mixture with 0.1% TFA, some drops of MeOH added when needed and purified by preparative RP-HPLC. The fractions of the crudes were then lyophilized. Yields are given as SPPS total yields. In all cases, yields are calculated for the corresponding TFA salts.

Homodimerisation. The monomeric linear peptide (~4-5 mg, 1.0 equivalent) was dissolved in 200 µL degassed mQ-deionized H_2O and degassed for 5 min with argon. Then, Aldrithiol (2,2-dithiodipyridine) (45.5 mM in MeOH, 0.45 equivalents) was added to the reaction solution. The thiol activation was followed by analytical RP-HPL. When the reaction was completed, the pH was adjusted to 8-9 with $(NH_4)HCO_3$ buffer solution (400 mM). A second portion of the monomeric peptide dendrimer X (~5 mg, 1.0 equivalent) was added to the reaction mixture. The reaction mixture was stirred for several hours under argon at room temperature and followed by analytical RP-HPL. After completion of the reaction, the mixture was acidified with 3 mL of aqueous TFA (0.1%). The homodimer was purified by preparative RP-HPLC.

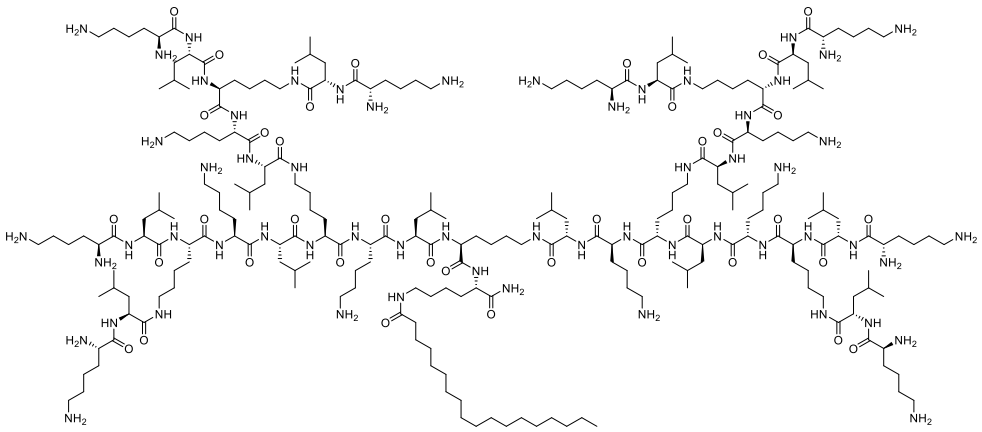
3. Peptide Dendrimers characterization

Table S1. Synthesis and pDNA transfection activity of peptide dendrimers

no.	Sequence[a)]	Yield[b)] mg (%)	MS[c)] calc/obs	% GFP pos. HEK cells[d)]	% GFP pos. HeLa cells[d)]	%DNA free[e)]
Z1	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})$	8.8 (2)	4684.55/4684.57	10.7±3.2	2.4±2.2	7.3±1.7
Z2	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})K(C_{18})$	1.1 (1)	5078.91/5078.92	11.7±3.6	2.0±1.4	12.4±2.8
Z3	$(KL)_8(KKL)_4(KKL)_2KLLLLL$	11.5 (2)	4855.62/4855.63	7.2±2.8	3.8±2.5	6.8±3.5
Z4	$(KL)_8(KKL)_4(KKL)_2KK(C_{16})$	5.4 (1)	4656.52/4656.53	7.1±1.8	1.91 ± 0.4	11.3±0.3
Z5	$(KL)_8(KKL)_4(KKL)_2KK(C_{16})K(C_{16})$	39.1 (8)	5022.85/5022.86	7.1 ±2.3	2.02 ± 0.1	8.3±3.0
Z6	$(KL)_8(KKL)_4(KKL)_2KK(C_{22})$	11.5 (2)	4740.62/4740.63	3.5 ± 0.3	3.09 ± 0.1	7.1±1.1
Z7	$(KL)_8(KKL)_4(KKL)_2KK(C_{12})K(C_{12})$	8.5 (1)	4910.72/4910.74	3.1 ±0.4	2.1 ± 0.4	7.9±6.5
Z8	$(KL)_8(KKL)_4(KKL)_2KK(C_{10})K(C_{10})$	7.5 (1)	4854.66/4854.67	0.9 ± 0.1	1.79 ± 0.3	30.7±4.5
Z9	$(KL)_8(KKL)_4(KKL)_2KK(C_8)K(C_8)$	4.7 (1)	4798.60/4798.61	0.1 ±0.4	2.28 ± 0.4	61±2.1
Z10	$(KL)_8(KKL)_4(KKL)_2KHHHH$	11 (2)	4838.43/4838.45	0.12 ±0.1	1.34 ± 0.1	16.0±7.71
Z11	$(KH)_8(KKH)_4(KKL)_2KK(C_{18})K(C_{18})$	2.6 (3)	5366.61/ 5366.62	0.01 ± 0.2	0.99 ± 0.1	11.7±3.2
Z12	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})C$	1.5 (2)	4787.56/4787.57	12.0±3.0	3.8±2.5	5.4±1.5
Z13	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})K(C_{18})C$	4.4 (3)	5181.92/5181.93	12.8±2.1	1.4±0.5	7.5±6.9
Z14	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})S$	3.6 (3)	4771.59/4771.60	7.4±1.6	2.1±1.9	6.7±3.5
Z15	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})A$	8.3 (5)	4755.59/4755.60	10.1±2.4	1.0±0.9	5.8±5.0
Z16	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})CC$	5.1 (3)	4890.57/4890.58	9.5±2.6	1.7±1.0	7.1±1.3
Z17	$((KL)_8(KKL)_4(KKL)_2KK(C_{18})C)_2$	1 (1)	9573.11/9573.10	15.0±5.7	3.5±1.0	11.7±8.6
Z18	$(kl)_8(KKL)_4(KKL)_2KK(C_{18})C$	5.3 (5)	4787.56/4787.57	16.7±3.9	2.9±1.1	4.2±2.7
Z19	$((kl)_8(KKL)_4(KKL)_2KK(C_{18})C)_2$	1.5 (5)	9573.11/9574.13	15.4±5.6	4.2±2.0	13.7±2.1
Z20	$(kl)_8(kkl)_4(kkl)_2kk(C_{18})c$	3.1 (5)	4787.56/4787.57	51.6±12.5	34.2±13.1	10.6±2.1
Z21	$((kl)8(kkl)4(kkl)2kk(C_{18})c)_2$	3.2 (5)	9573.11/9573.13	55.0±10.6	37.2±12.6	10.1±4.0
Z22	$(rl)_8(krl)_4(krl)_2kk(C_{18})c$	7.8 (4)	5179.65/5179.66	62.5±9.5	47.3±9.3	6.5±1.8

a) One-letter code amino acids are used, *K* is the branching lysine residue, C-termini are carboxamide $CONH_2$, and all N-termini are free. Alkyl chains in the structure are represented by "C" followed by their number of carbon atoms. b) Isolated yields as trifluoroacetate salt after preparative HPLC purification. c) ESI-MS, see also the Supporting Information below. d) Transfection efficiency in HEK and HeLa cells measured as percentage of GFP positive cells after 48h transfection. Experiments were carried out in triplicate in three independent experiments (N/P 5, 175 pmol of peptide dendrimers in 100 µL Optimem per well, 1.7µM, 250 ng pDNA). e) Fluorescence from intercalation in pDNA/peptide dendrimer complex at N/P 5 (200 µL, final concentration of 0.085 nM pDNA and 0.35 µM or 1 µg/mL L2000) by Quant-It PicoGreen and normalized to the value of pDNA alone.

Z1 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (8.8 mg, 1.9 µmol, 2%). Analytical RP-HPLC: t_R= 3.81 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{234}H_{455}N_{59}O_{37}$ calc./obs. 4684.55/4684.57



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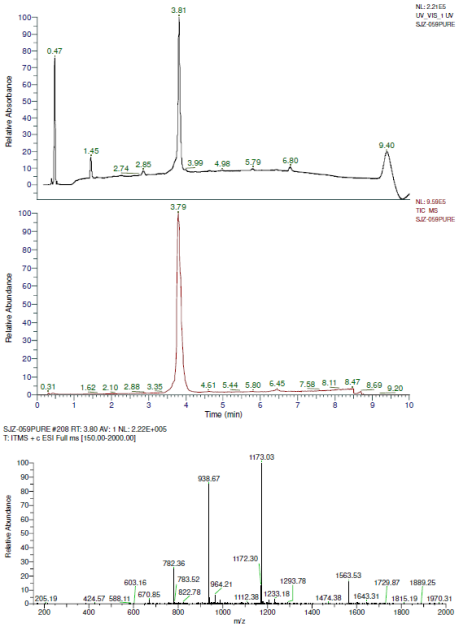

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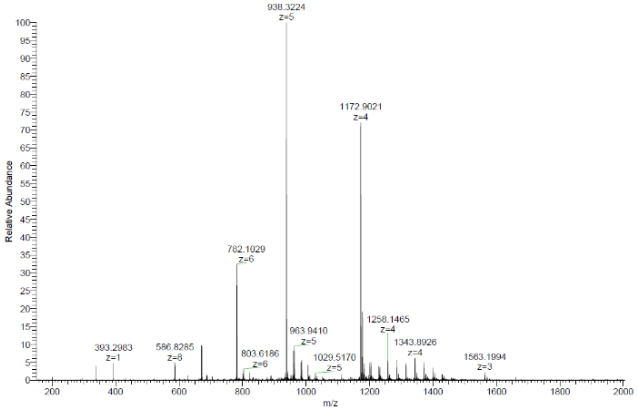
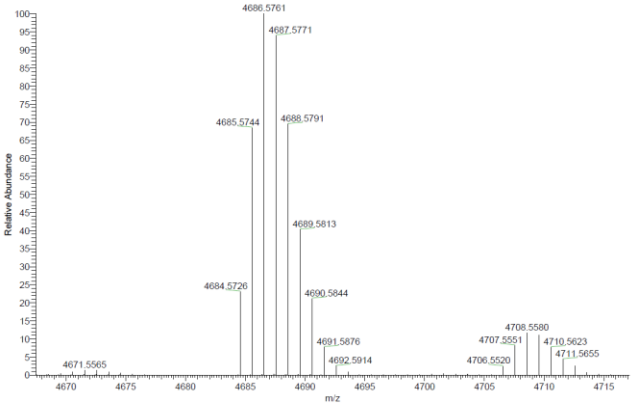
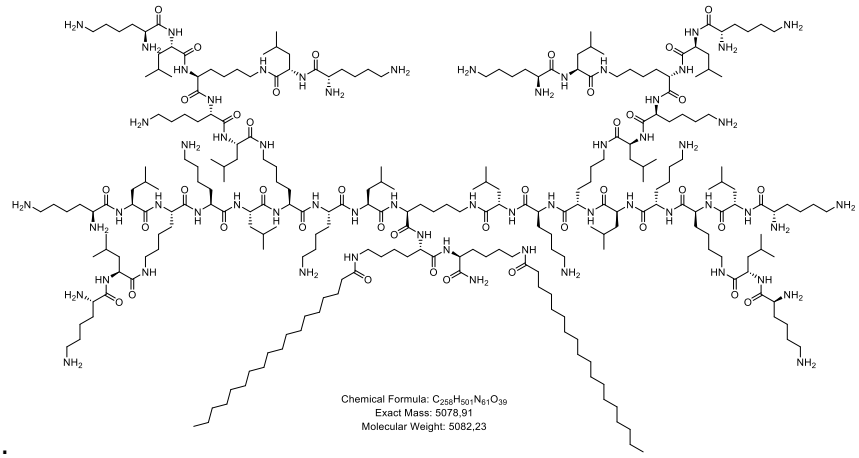




Z2 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)K(C$_{18}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (1.1 mg, 0.2 µmol, 1%). Analytical RP-HPLC: t_R= 5.06 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{258}H_{501}N_{61}O_{39}$ calc./obs. 5078.91/5078.92



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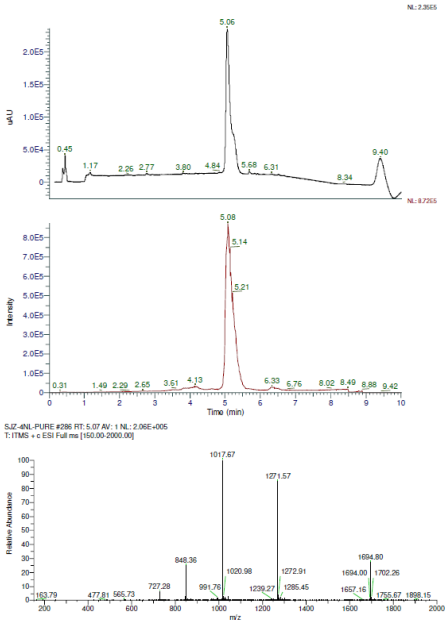
LCMS



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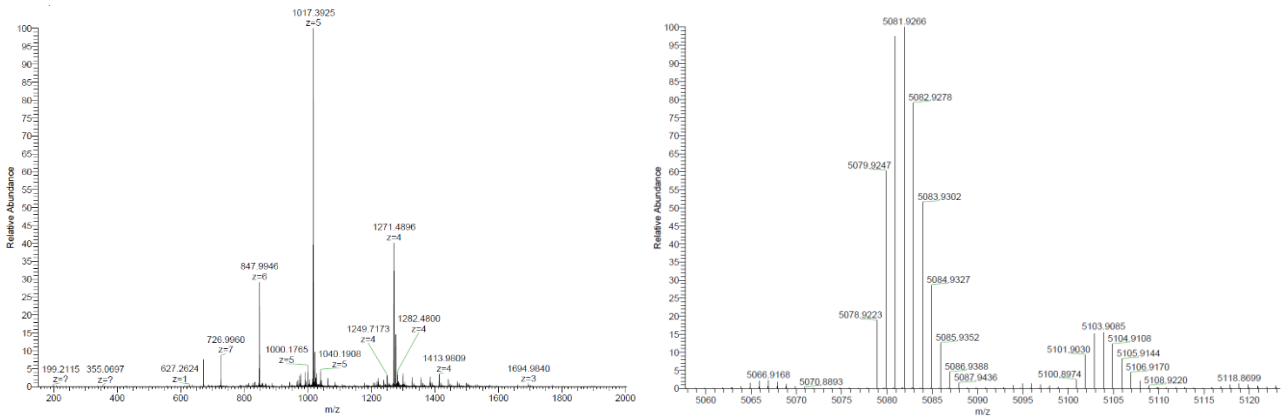
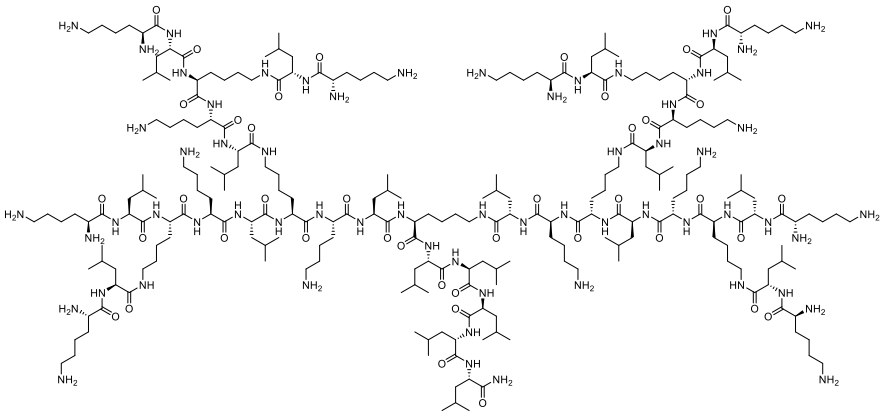


Z3 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*LLLLL was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (11.5 mg, 2.4 µmol, 2%). Analytical RP-HPLC: t_R = 3.26 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{240}H_{464}N_{62}O_{40}$ calc./obs. 4855.62/4855.63



Chemical Formula: C240H464N62O40
Exact Mass: 4855,62
Molecular Weight: 4858,75

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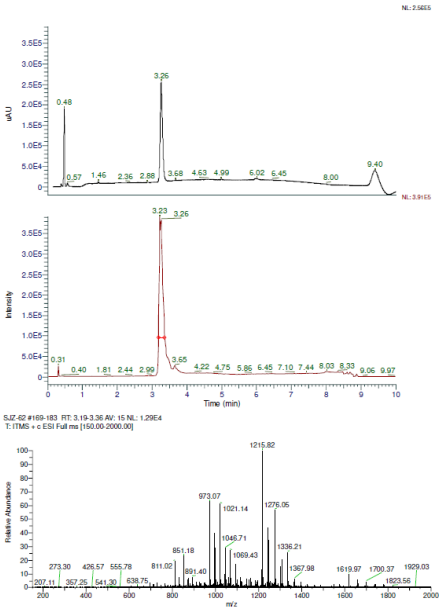

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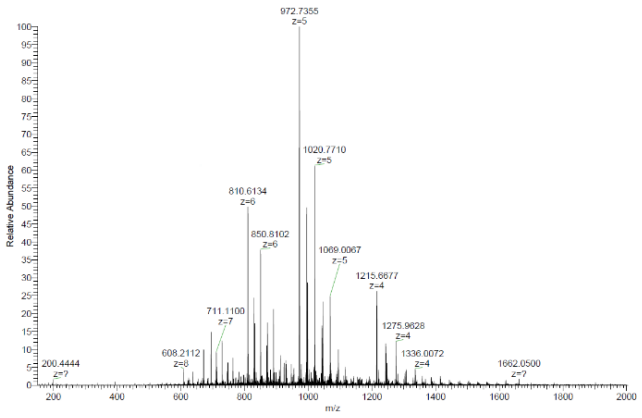
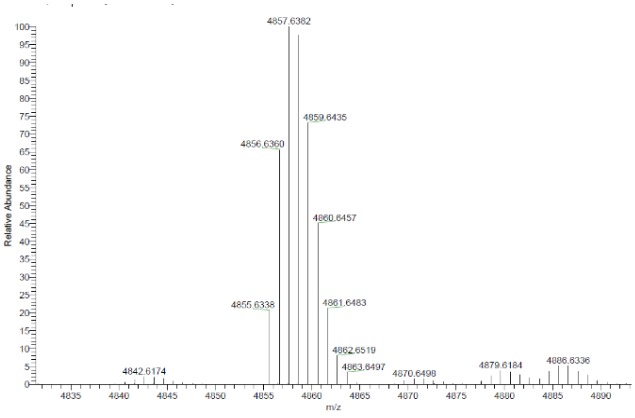
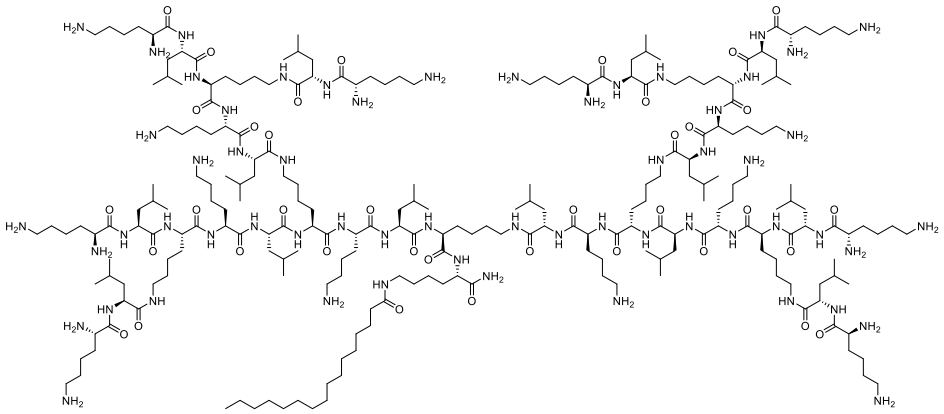




Z4 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{16}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (5.4 mg, 1.2 µmol, 0.8%). Analytical RP-HPLC: t_R= 3.65 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{232}H_{451}N_{59}O_{37}$ calc./obs. 4656.52/4656.53



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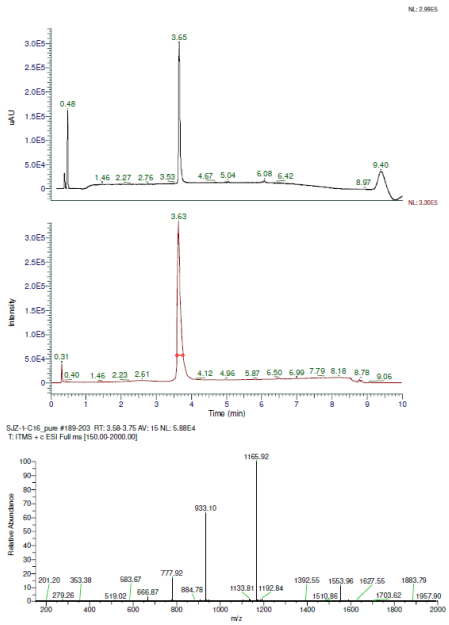

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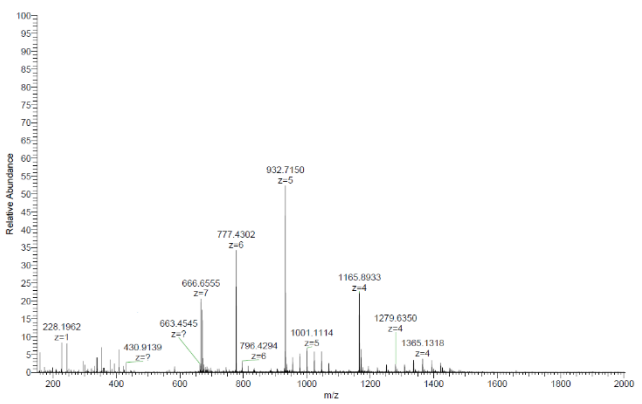
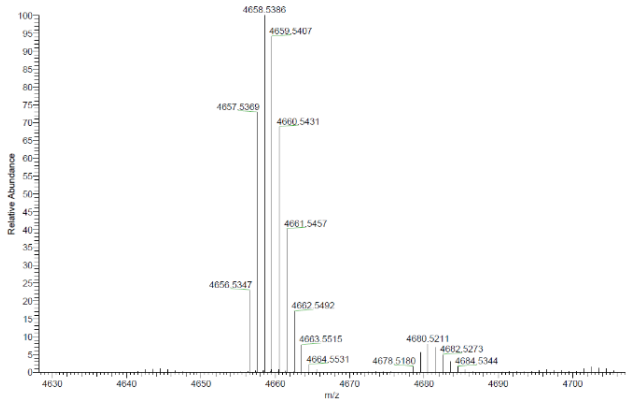
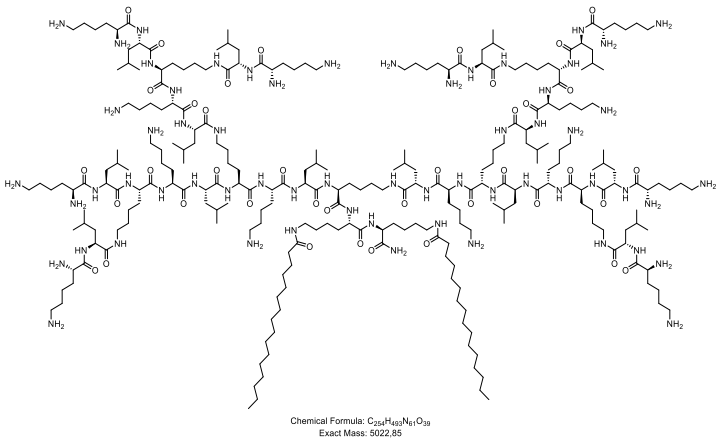




Z5 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{16}$)K(C$_{16}$) was obtained as foamy colorless solid after preparative RP-HPLC (39.1 mg, 5.18 µmol, 8 %). Analytical RP-HPLC: t_R = 4.35 min (100 % A to 100 % D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{254}H_{493}N_{61}O_{39}$ calc/found 5022.85/5022.86



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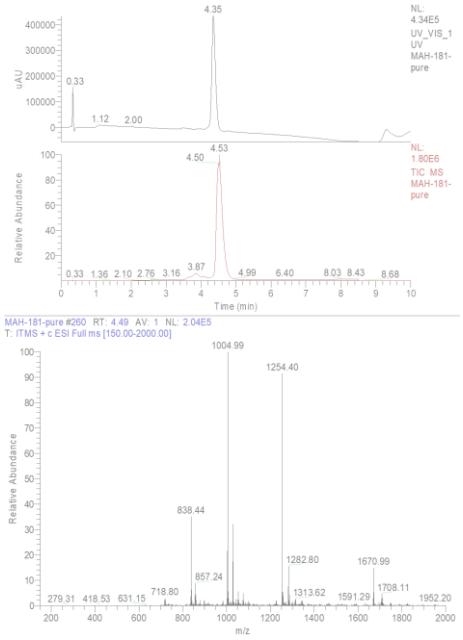
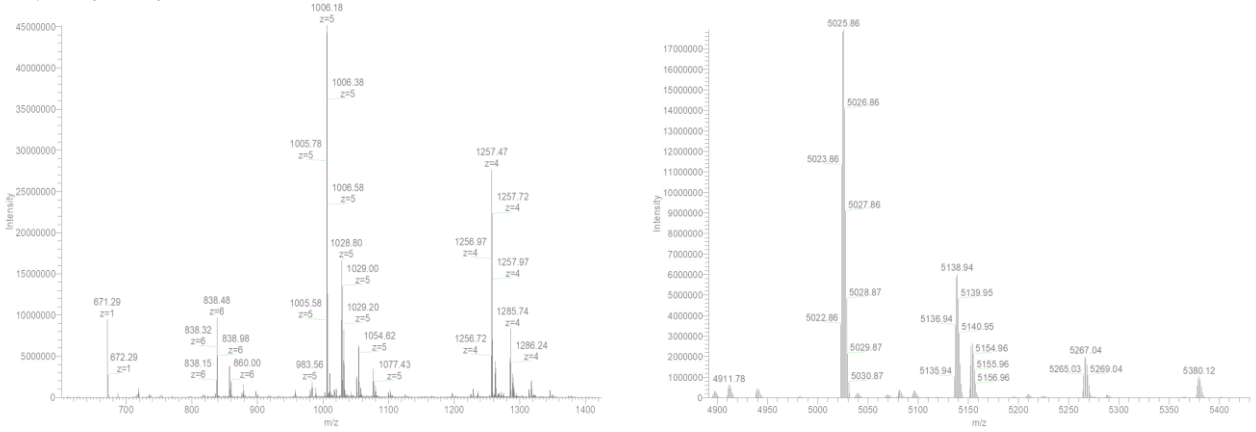
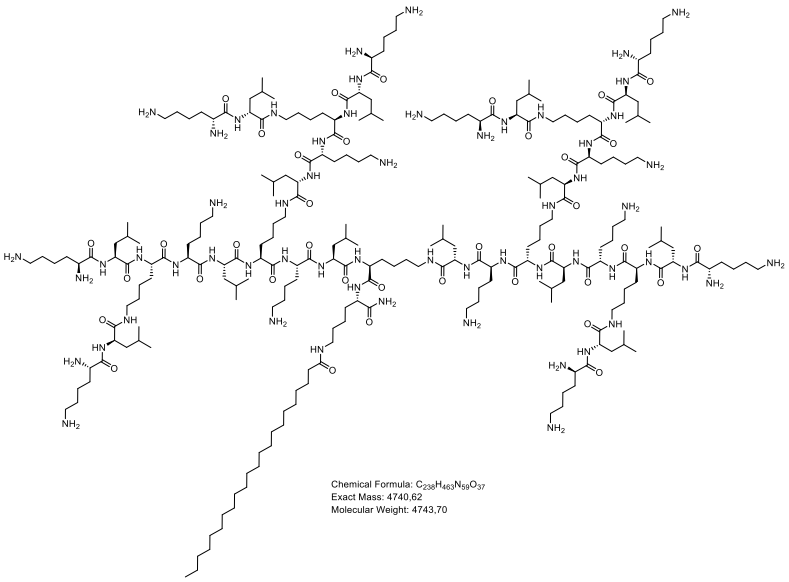


MASS SPECTRUM, HRMS (NSI+)



Z6 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{22}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (11.5 mg, 2.4 µmol, 1.8%). Analytical RP-HPLC: t_R= 4.18 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{238}H_{463}N_{59}O_{37}$ calc./obs. 4740.62/4740.63 [M+H$^+$].



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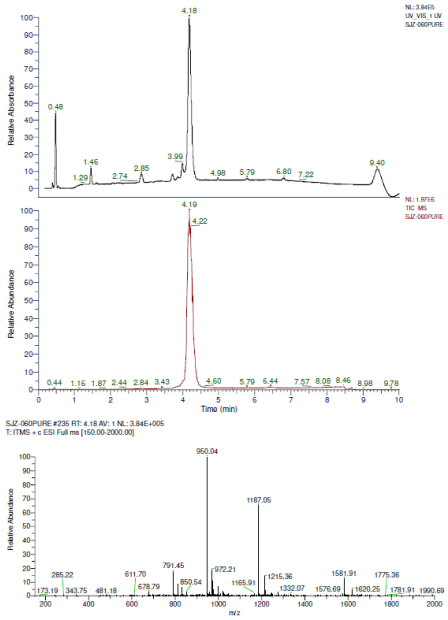

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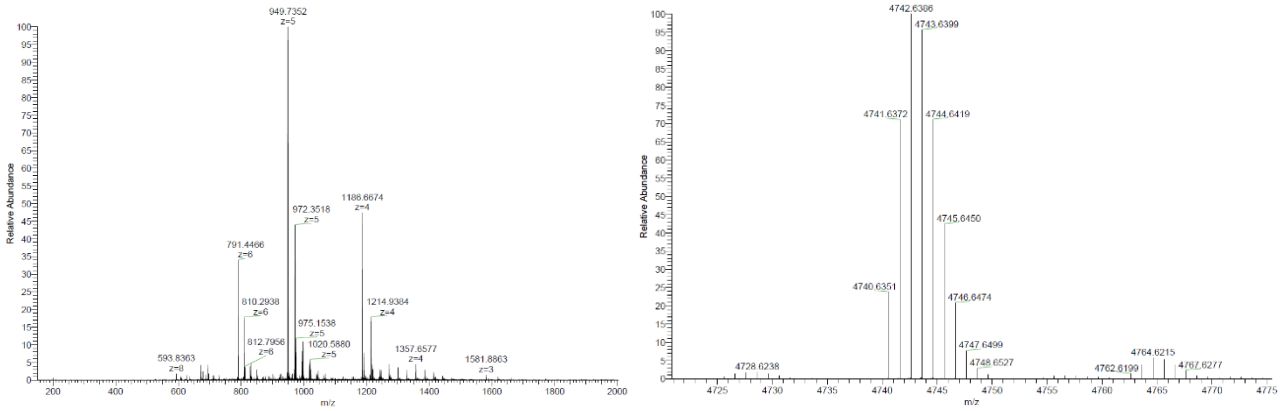
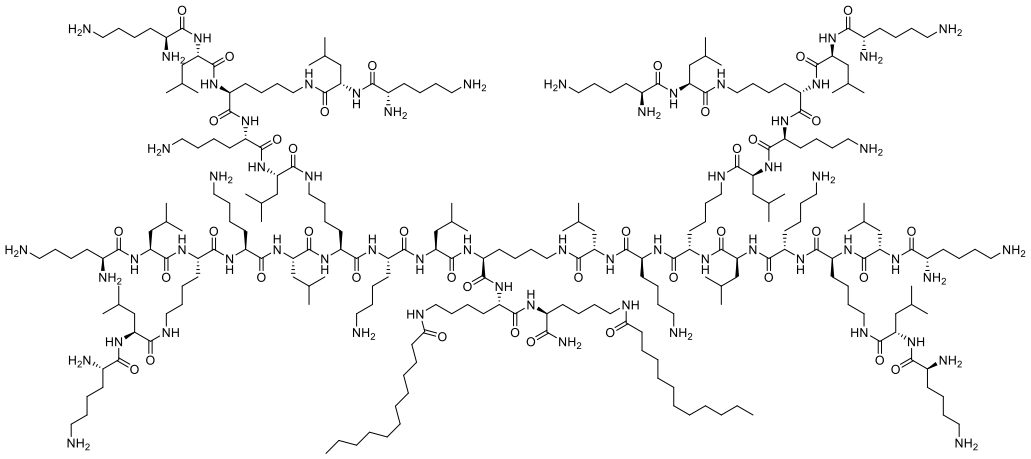
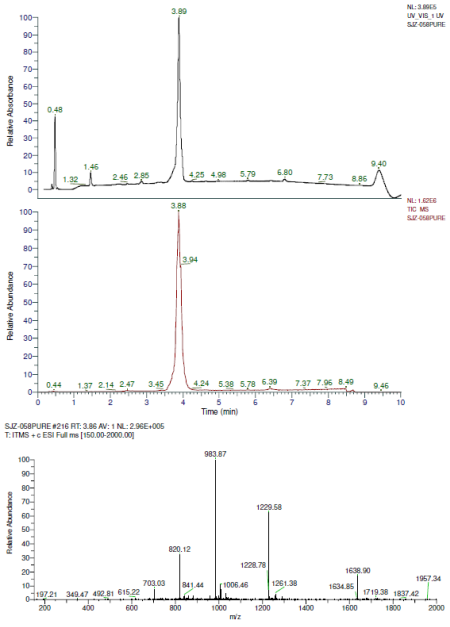


Z7 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{12}$)K(C$_{12}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (8.5 mg, 1.7 µmol, 1.3%). Analytical RP-HPLC: t_R= 3.89 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{246}H_{477}N_{61}O_{39}$ calc./obs. 4910.72/4910.74

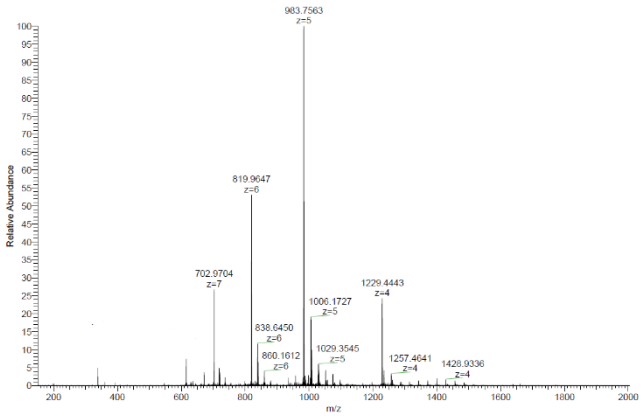


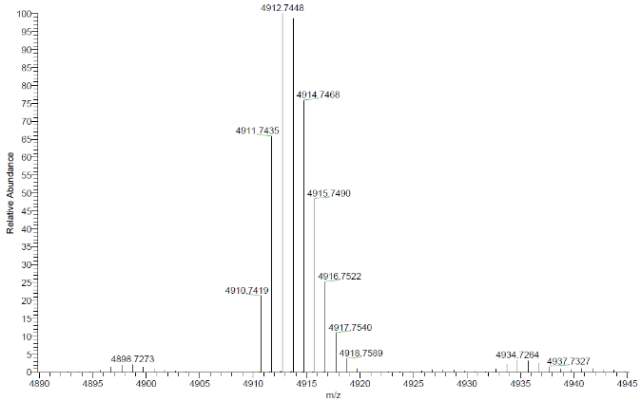
Chemical Formula: $C_{246}H_{477}N_{61}O_{39}$
Exact Mass: 4910,72
Molecular Weight: 4913,91

LCMS

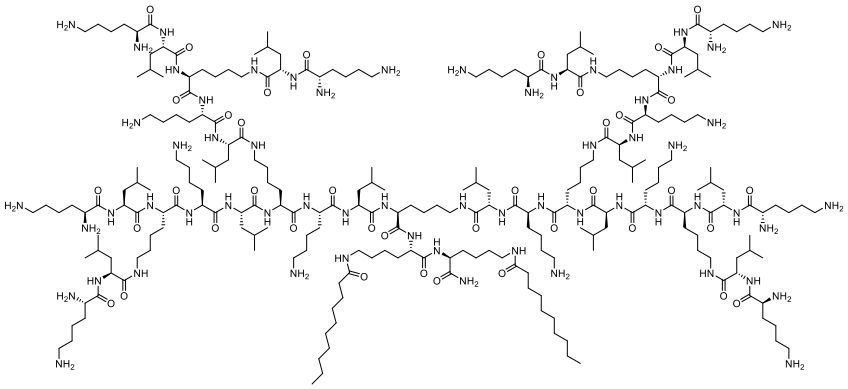


MASS SPECTRUM, HRMS (NSI+):

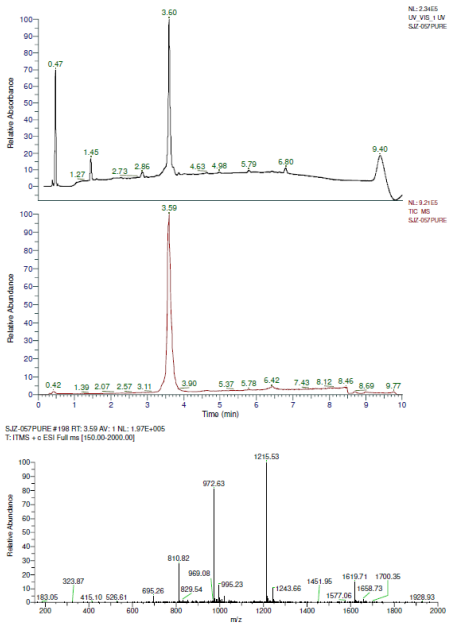




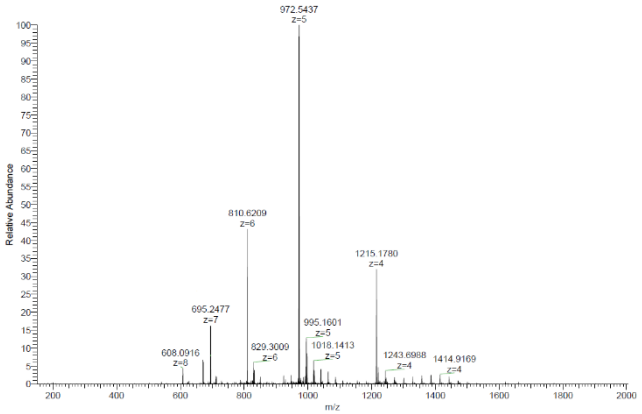
Z8 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{10}$)K(C$_{10}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (7.5 mg, 1.5 µmol, 1.2%). Analytical RP-HPLC: t_R= 3.60 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{242}H_{469}N_{61}O_{39}$ calc./obs. 4854.66/4854.68

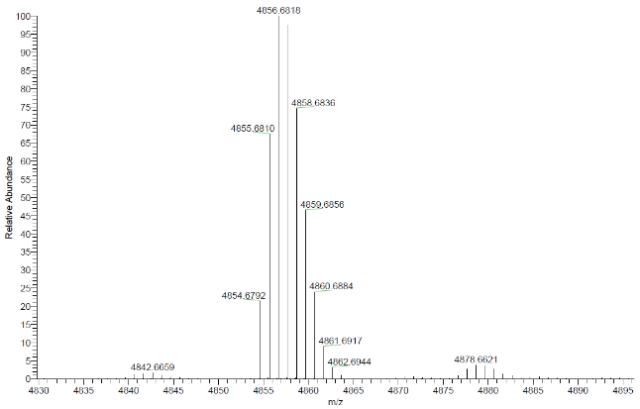


LCMS

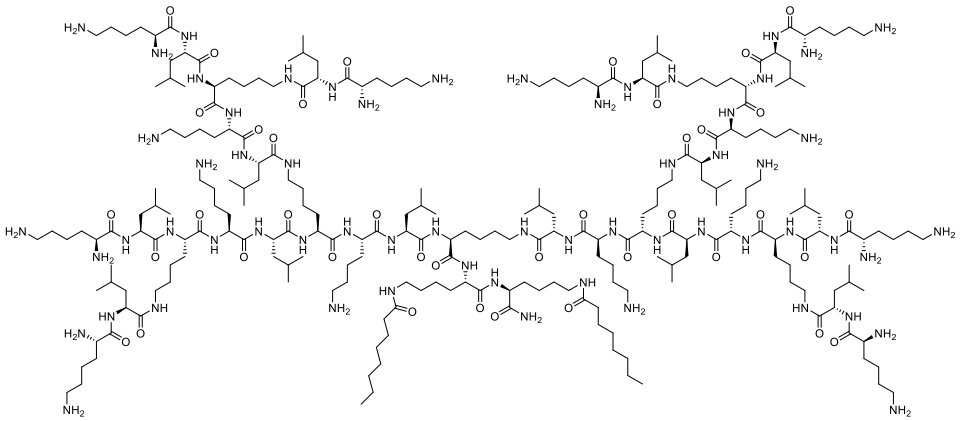


MASS SPECTRUM, HRMS (NSI+):

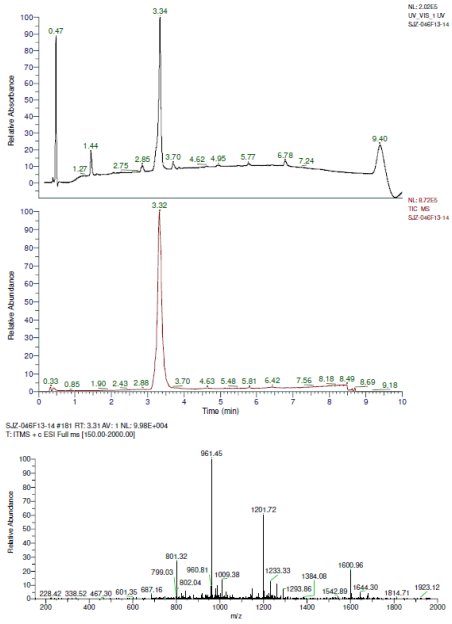




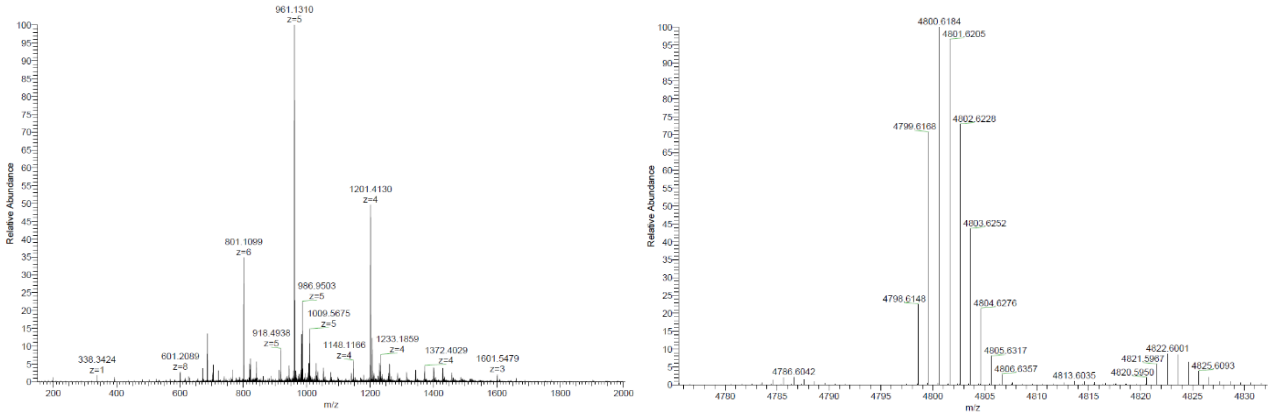
Z9 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_8$)K(C$_8$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (4.7 mg, 1 µmol, 0.8%). Analytical RP-HPLC: t_R= 3.34 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{238}H_{461}N_{61}O_{39}$ calc./obs. 4798.60/4798.61



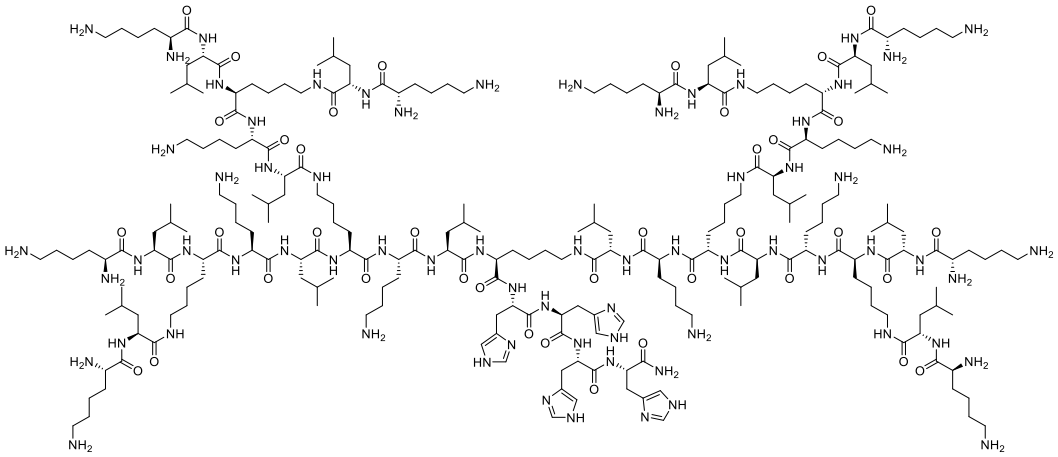
LCMS



MASS SPECTRUM, HRMS (NSI+):

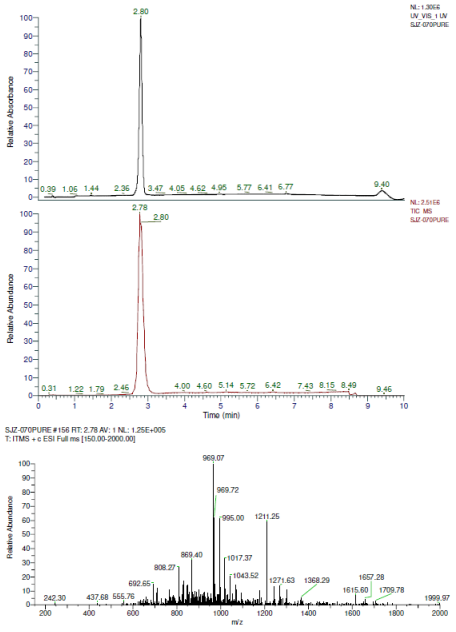


Z10 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*HHHH was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (11 mg, 2.3 µmol, 1.7%). Analytical RP-HPLC: t_R= 2.80 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{437}N_{69}O_{39}$ calc./obs. 4838.43/4838.45

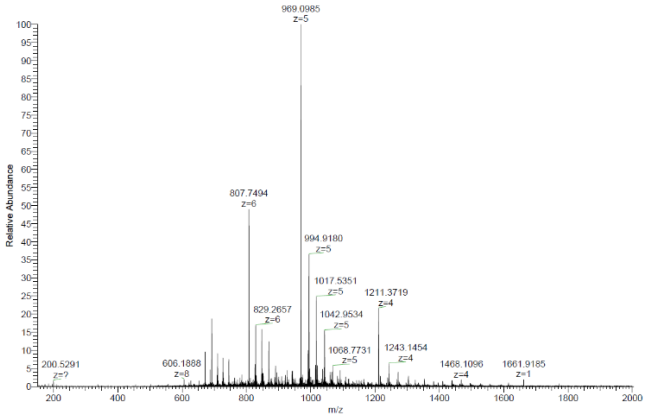


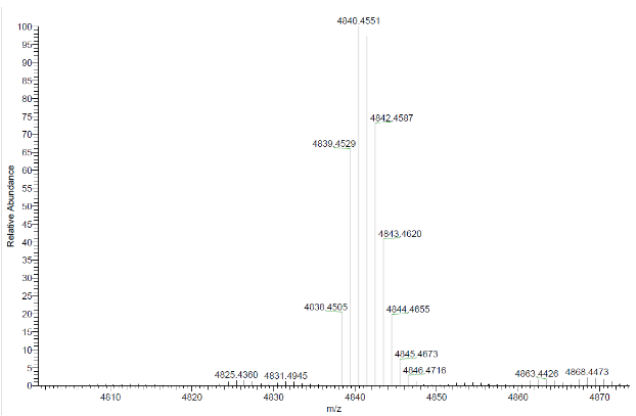
Chemical Formula: $C_{234}H_{437}N_{69}O_{39}$
Exact Mass: 4838,43
Molecular Weight: 4841,51

LCMS

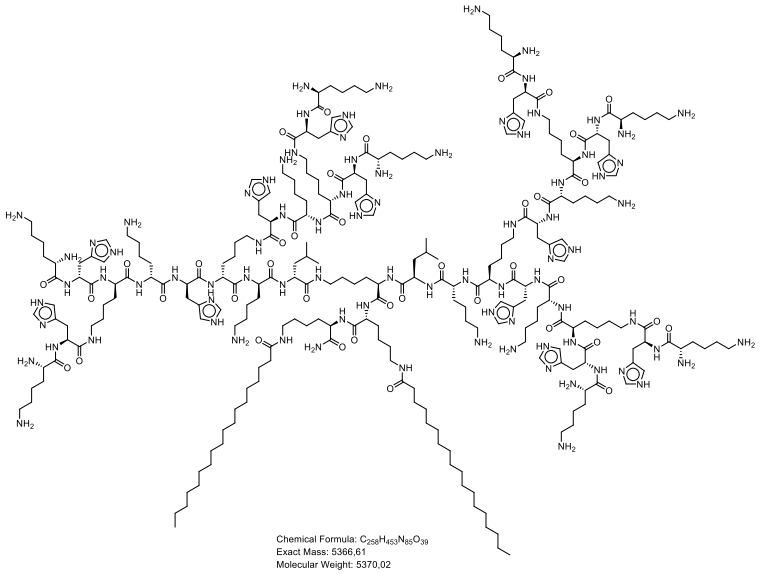


MASS SPECTRUM, HRMS (NSI+):

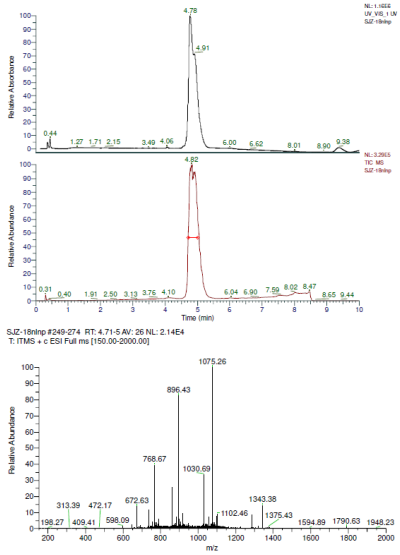




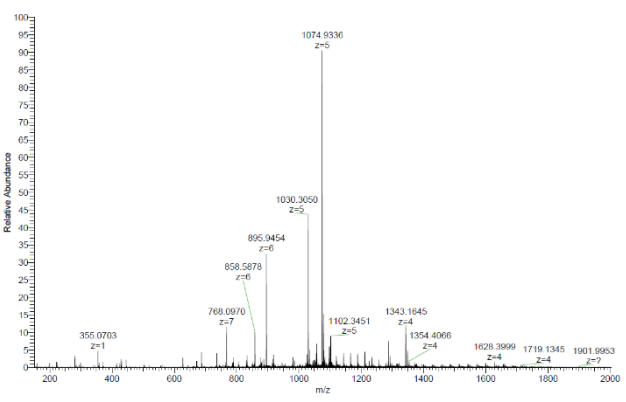
Z11 (KH)$_8$(*K*KH)$_4$(*K*KL)$_2$*K*K(C$_{18}$)K(C$_{18}$) was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (2.6 mg, 0.5 µmol, 2.6%). Analytical RP-HPLC: t_R= 4.78 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{258}H_{453}N_{85}O_{39}$ calc./obs. 5366.61/ 5366.62

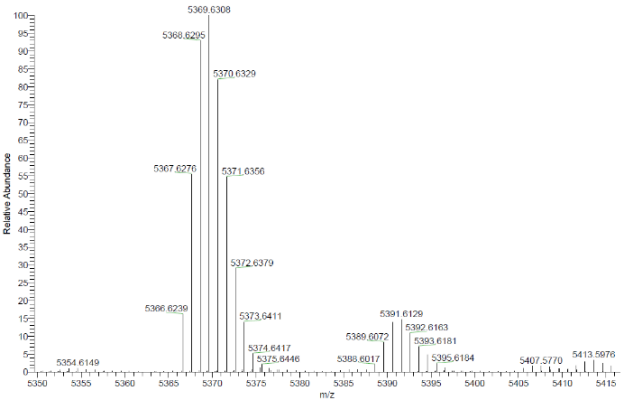


LCMS

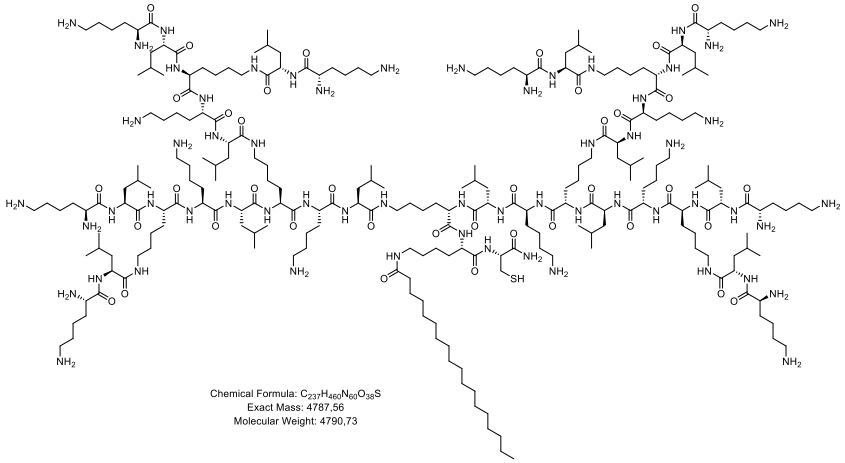


MASS SPECTRUM, HRMS (NSI+):

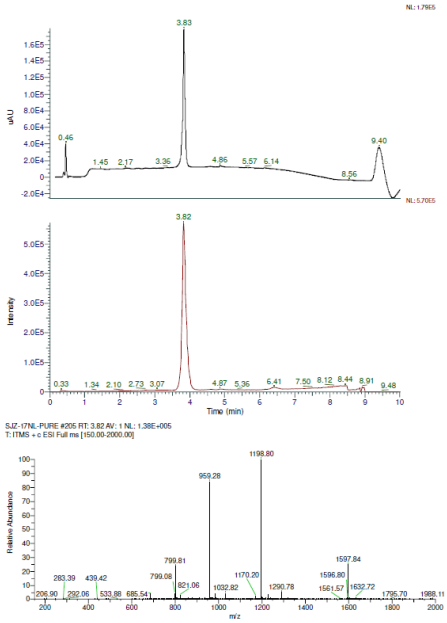




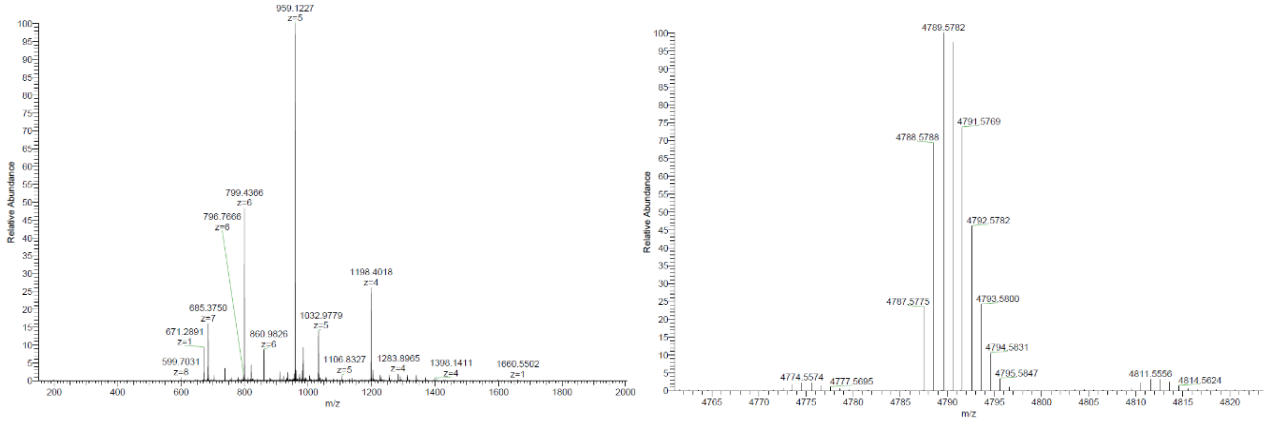
Z12 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)C was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (1.5 mg, 0.3 µmol, 2%). Analytical RP-HPLC: t_R= 3.83 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{237}H_{460}N_{60}O_{38}S$ calc./obs. 4787.56/4787.58



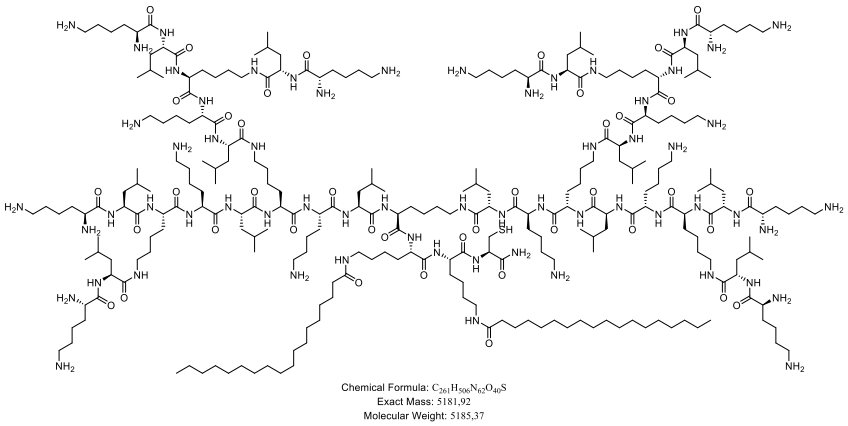
LCMS



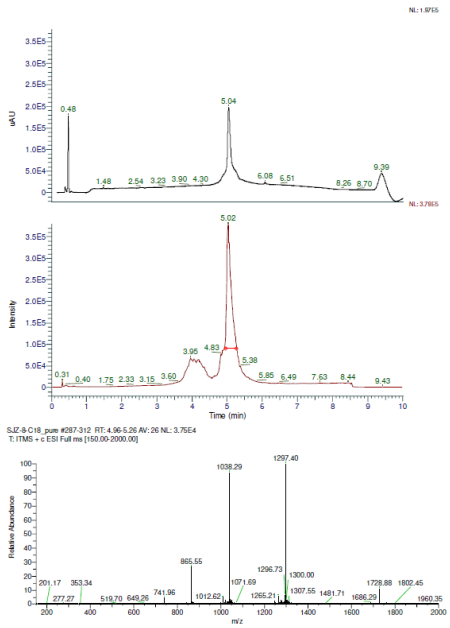
MASS SPECTRUM, HRMS (NSI+):



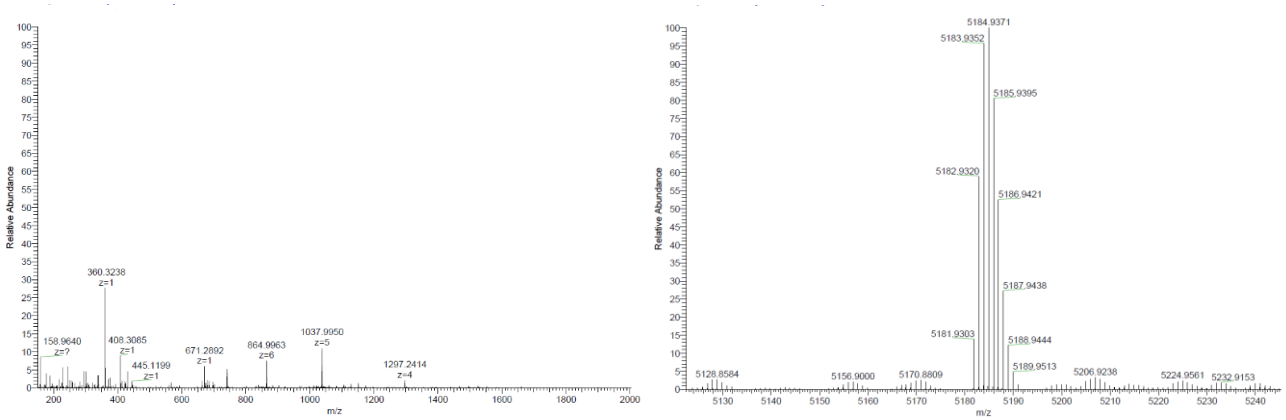
Z13 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*KK*(C$_{18}$)K(C$_{18}$)C was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (4.4 mg, 0.9 µmol, 3%). Analytical RP-HPLC: t_R= 5.04 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{261}H_{506}N_{62}O_{40}S$ calc./obs. 5181.92/5181.93



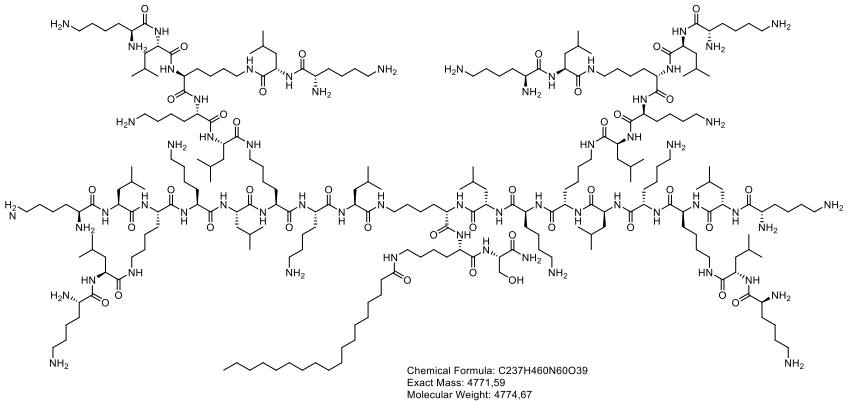
LCMS



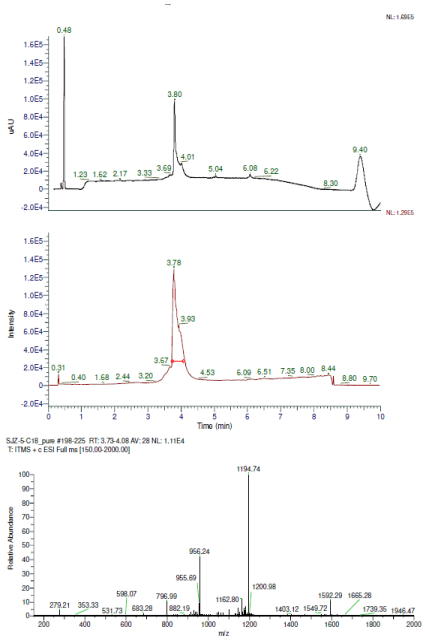
MASS SPECTRUM, HRMS (NSI+):



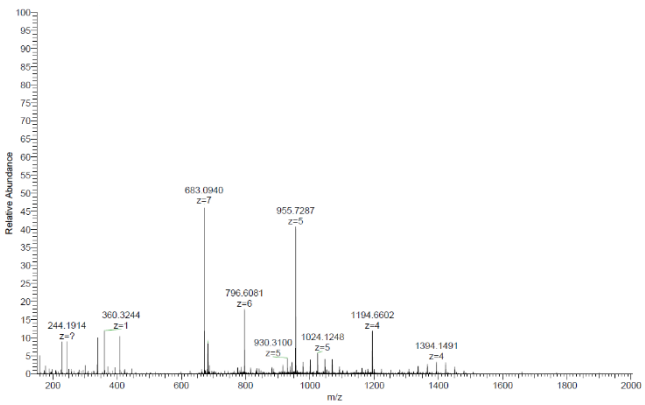
Z14 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)S was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (3.6 mg, 0.8 µmol, 3%). Analytical RP-HPLC: t_R= 3.80 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{237}H_{460}N_{60}O_{39}$ calc./obs. 4771.59/4771.60

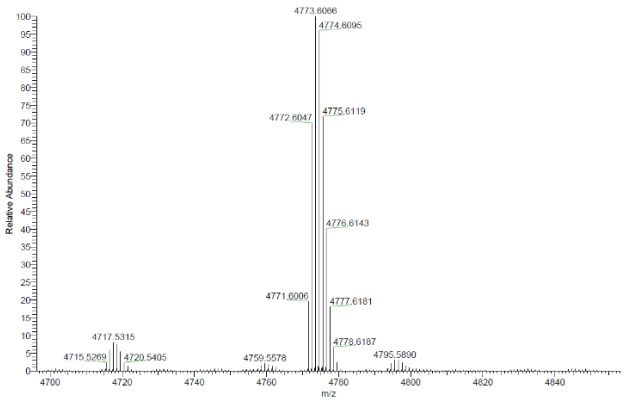


LCMS

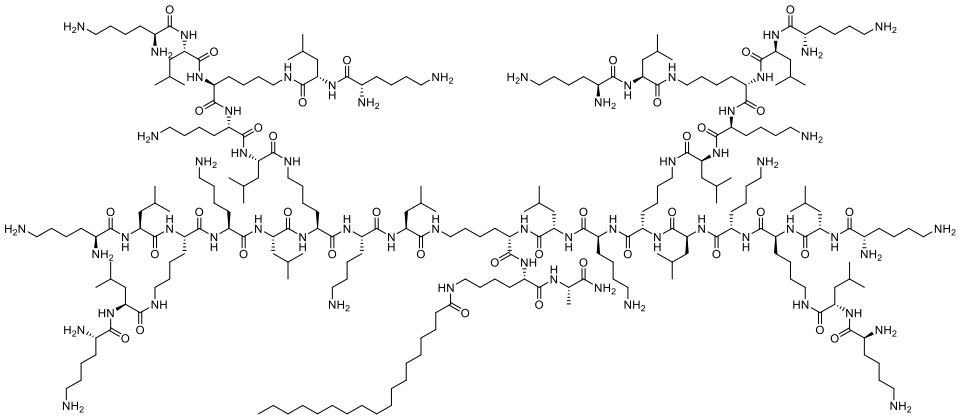


MASS SPECTRUM, HRMS (NSI+):

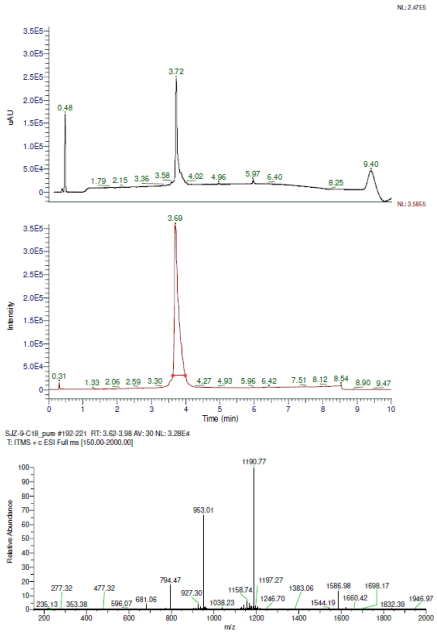




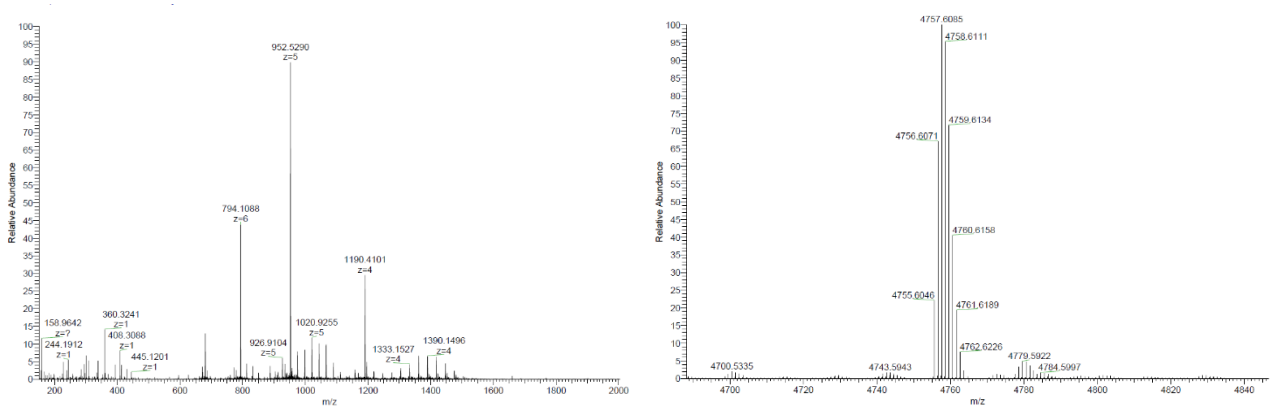
Z15 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)A was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (8.3 mg, 1.7 µmol, 5%). Analytical RP-HPLC: t_R= 3.72 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{237}H_{460}N_{60}O_{38}$ calc./obs.4755.59/4755.60



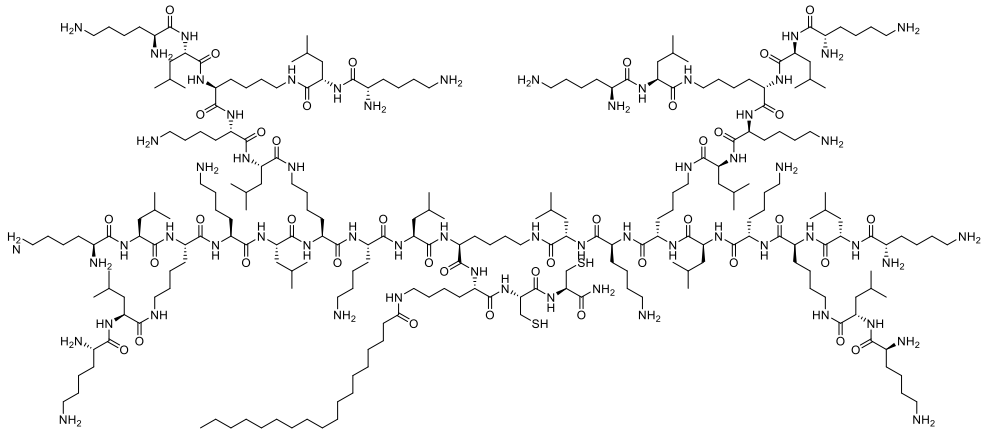
LCMS



MASS SPECTRUM, HRMS (NSI+):

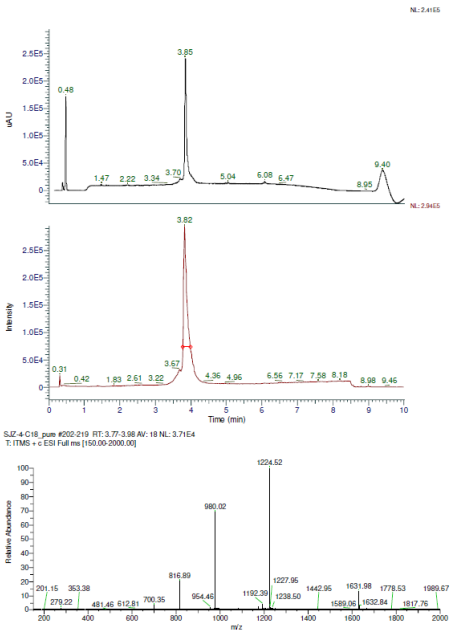


Z16 (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)CC was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (5.1 mg, 1.1 µmol, 3%). Analytical RP-HPLC: t_R= 3.85 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{240}H_{465}N_{61}O_{39}S_2$ calc./obs. 4890.57/4890.58

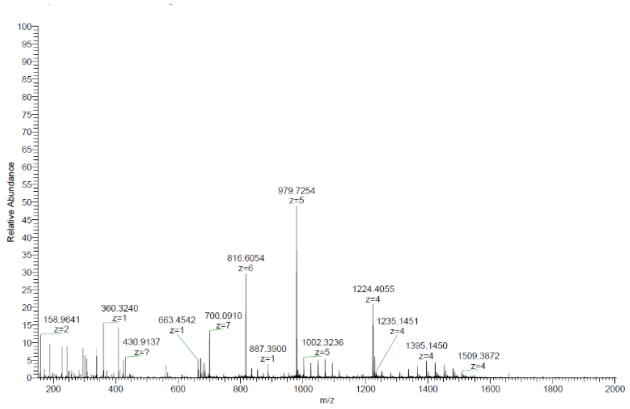


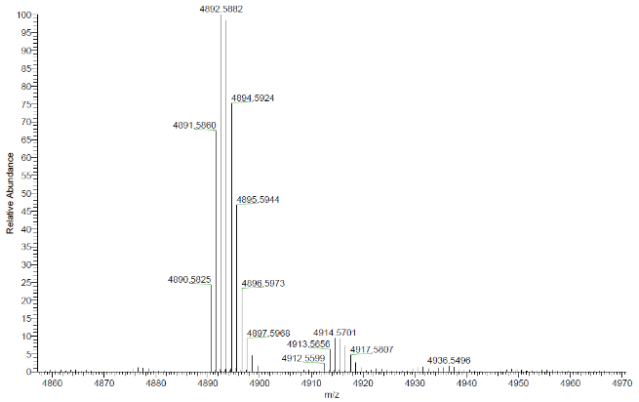
Chemical Formula: $C_{240}H_{465}N_{61}O_{39}S_2$
Exact Mass: 4890,57
Molecular Weight: 4893,87

LCMS

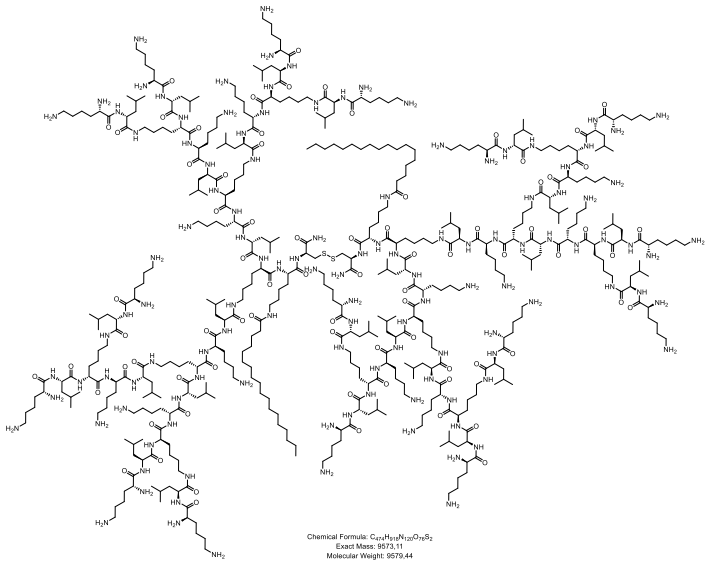


MASS SPECTRUM, HRMS (NSI+):

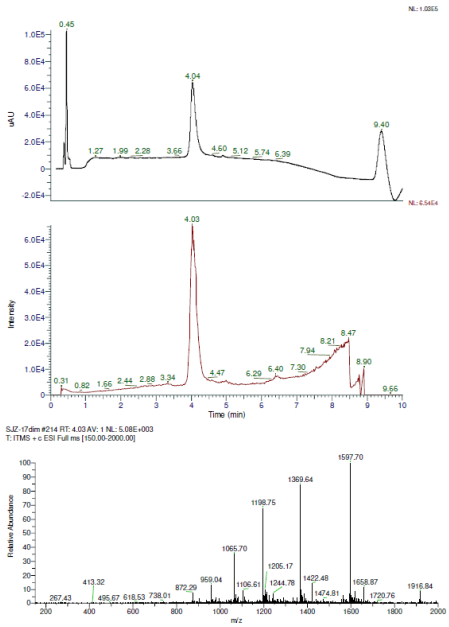




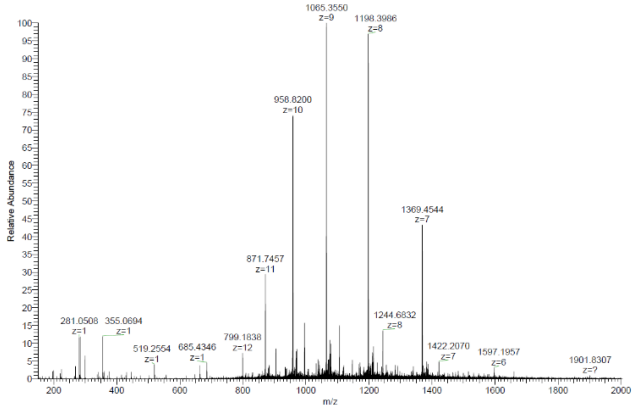
Z17 ((KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*KK*(C$_{18}$)C)$_2$ was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (1 mg, 0.2 µmol, 1%). Analytical RP-HPLC: t_R= 4.04 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{474}H_{916}N_{120}O_{76}S_2$ calc./obs. 9573.11/9573.11

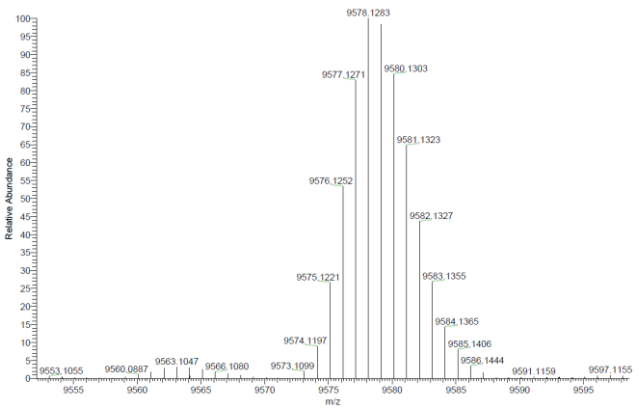


LCMS

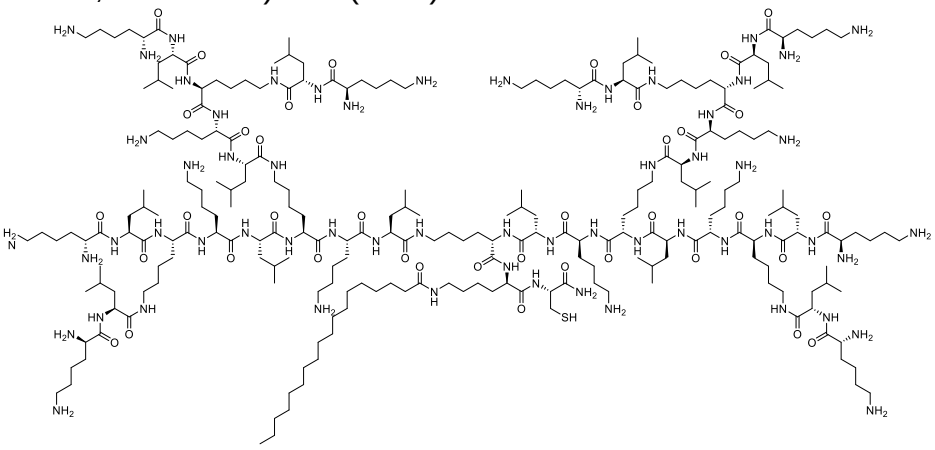


MASS SPECTRUM, HRMS (NSI+):



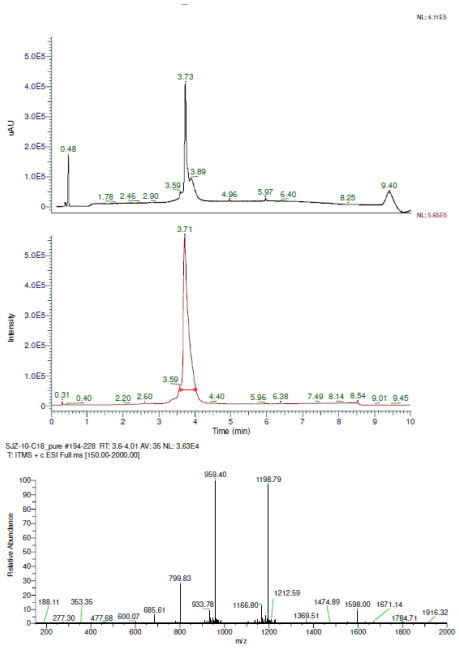


Z18 (kl)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*K(C$_{18}$)C was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (5.3 mg, 1.1 µmol, 5%). Analytical RP-HPLC: t_R= 3.73 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{237}H_{460}N_{60}O_{38}S$ calc./obs. 4787.56/4787.58

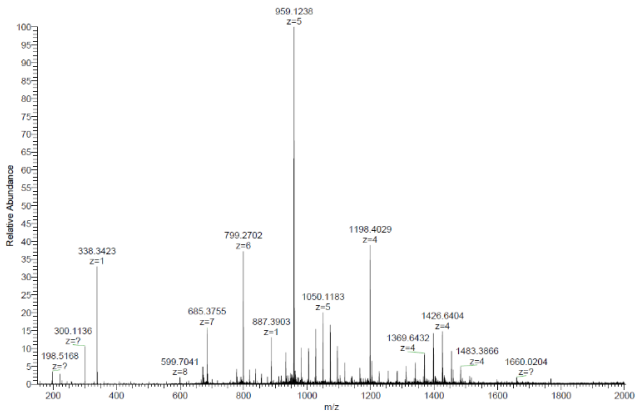


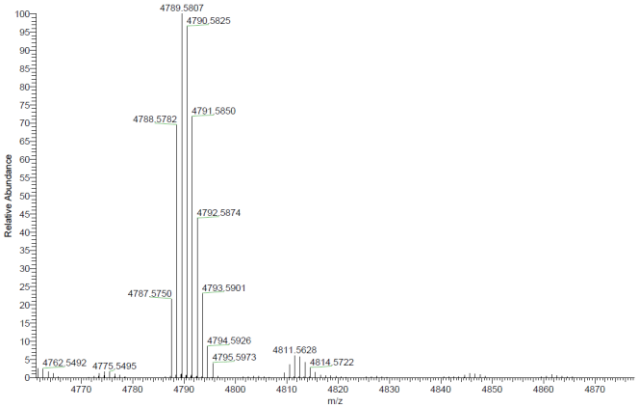
Chemical Formula: C237H460N60O38S
Exact Mass: 4787,56
Molecular Weight: 4790,73

LCMS

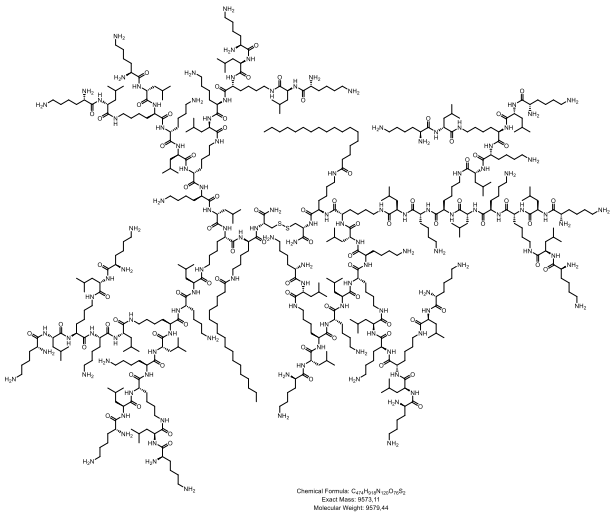


MASS SPECTRUM, HRMS (NSI+):

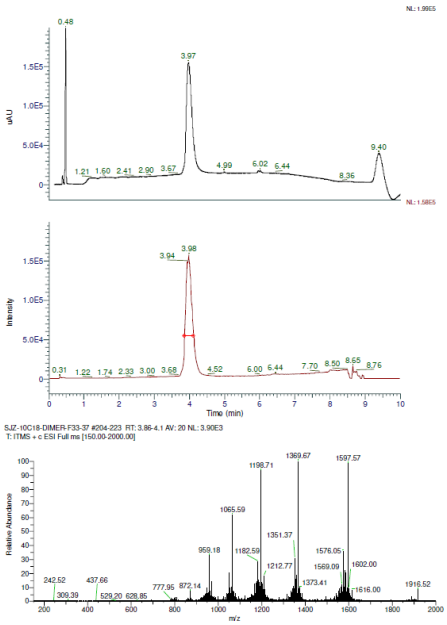




Z19 ((kl)$_8$(*K*KL)$_4$(*K*KL)$_2$*KK*(C$_{18}$)C)$_2$ was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (1.5 mg, 0.3 µmol, 5%). Analytical RP-HPLC: t_R= 3.97 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{474}H_{918}N_{120}O_{76}S_2$ calc./obs. 9573.11/9574.14



LCMS



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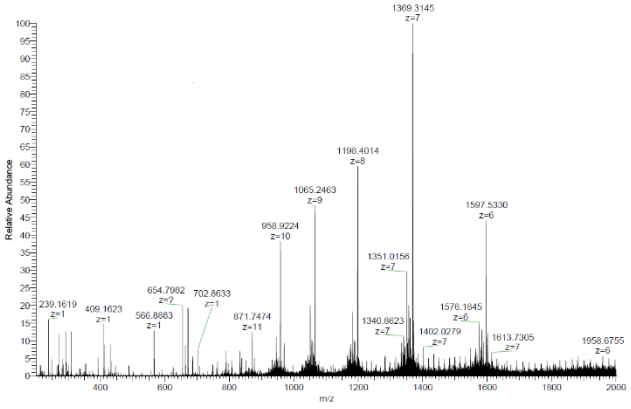
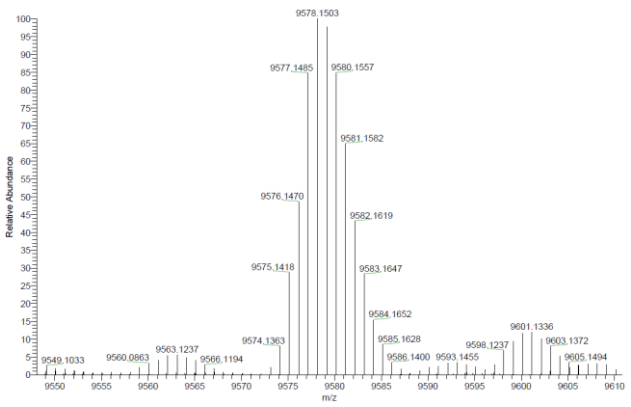
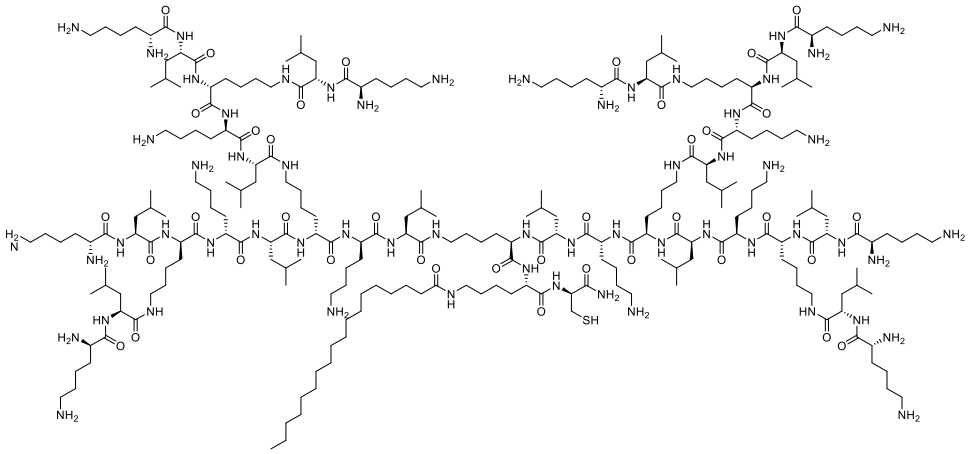


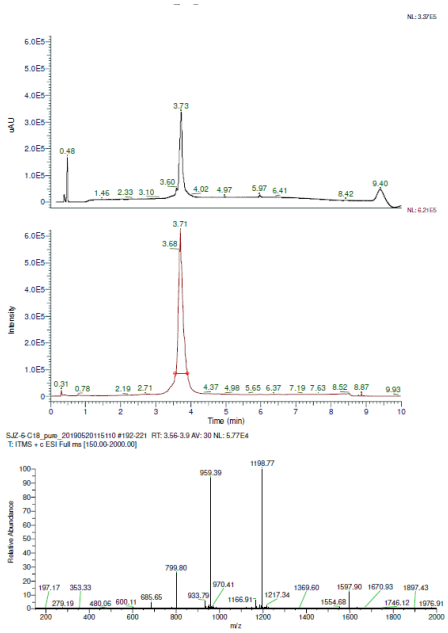


Z20 (kl)$_8$(*k*kl)$_4$(*k*kl)$_2$*k*k(C$_{18}$)c was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (3.1 mg, 6.5 µmol, 5%). Analytical RP-HPLC: t_R= 3.73 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{237}H_{460}N_{60}O_{38}S$ calc./obs. 4787.56/4787.57



Chemical Formula: C237H460N60O38S
Exact Mass: 4787,56
Molecular Weight: 4790,73

LCMS



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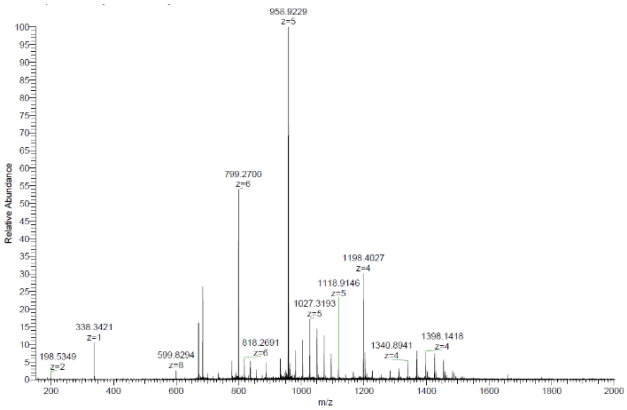
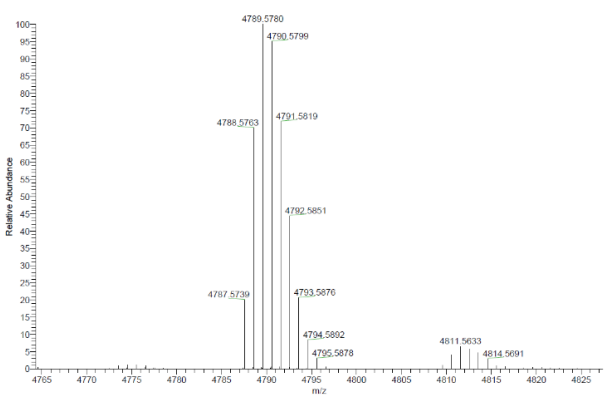
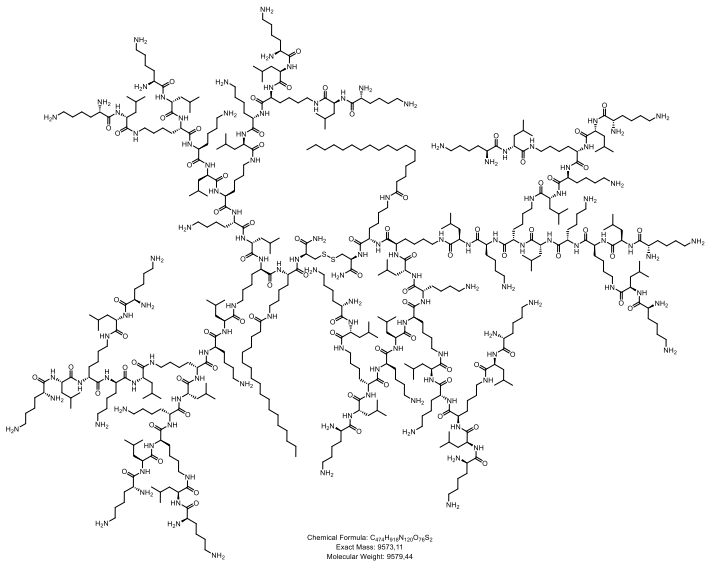




Z21 ((kl)$_8$(*k*kl)$_4$(*k*kl)$_2$*k*k(C$_{18}$)k(C$_{18}$)c)$_2$ was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (3.2 mg, 0.2 µmol, 5%). Analytical RP-HPLC: t_R= 3.91 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{474}H_{918}N_{120}O_{76}S_2$ calc./obs. 9573.11/9573.14



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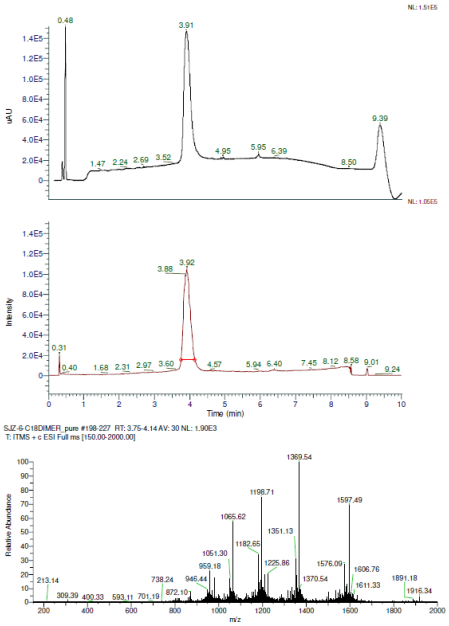

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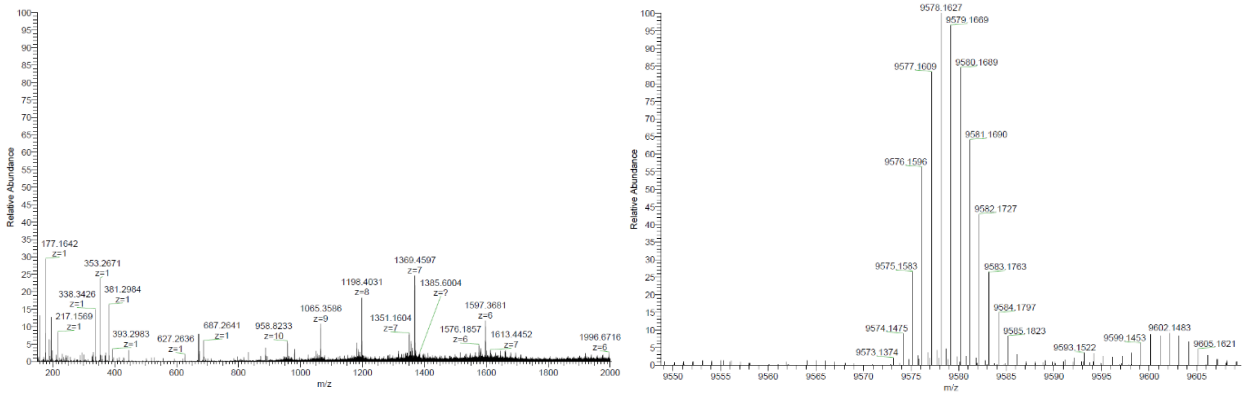
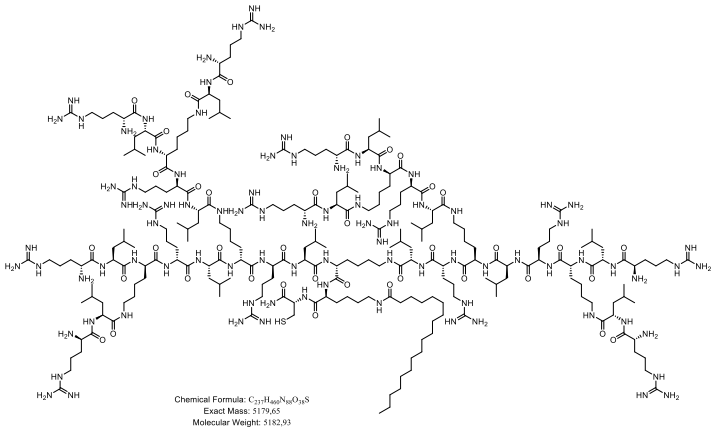


Z22 (rl)$_8$(*k*rl)$_4$(*k*rl)$_2$*k*k(C$_{18}$)c was obtained after manual synthesis as foamy colorless solid after preparative RP-HPLC (7.8 mg, 1.5 µmol, 4%). Analytical RP-HPLC: t_R= 3.99 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+):$C_{237}H_{460}N_{88}O_{38}S$ calc./obs. 5179.65/5179.66



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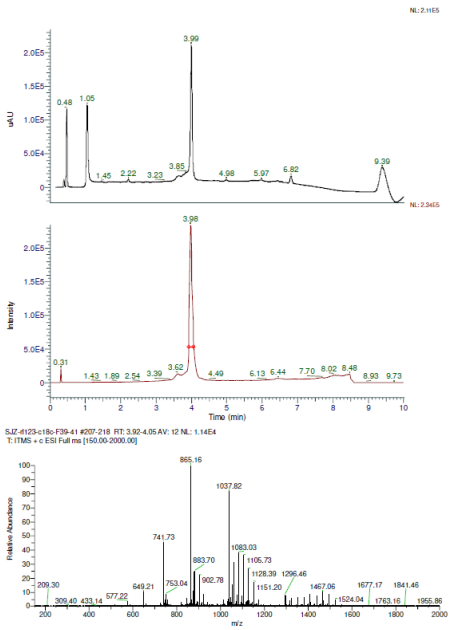

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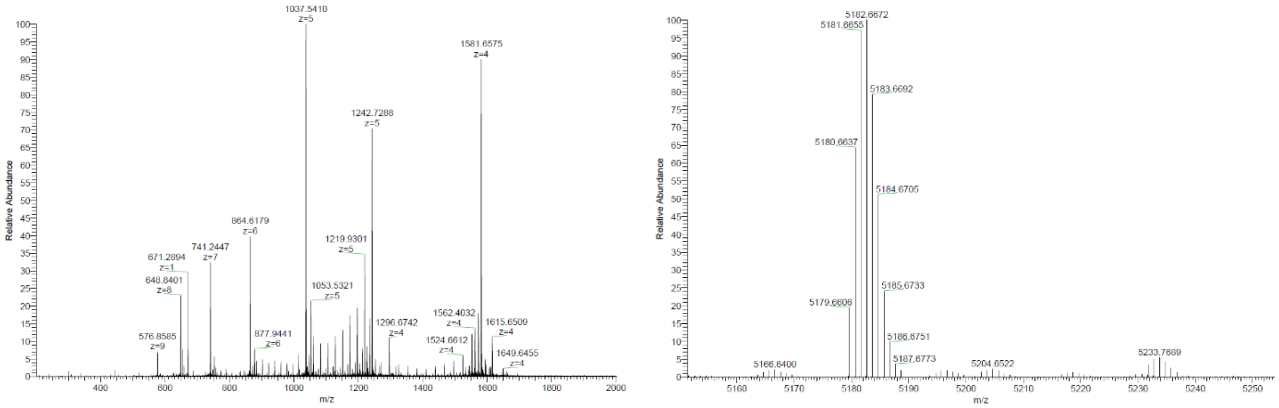


4. Cell Culture and Transfection Reagents

HeLa and HEK-293 cells (ATCC, Manassas, USA) were maintained in DMEM (Thermo Fisher Scientific, Reinach, CH) supplemented with 10% fetal calf serum (FCS, Thermo fisher Scientific) at 37 °C in a humidified atmosphere in 5% carbon dioxide. THP-1 cells (ATCC, Manassas, USA) were maintained in RPMI 1640 Media (Thermo Fisher Scientific, Reinach, CH) supplemented with 10% fetal calf serum (FCS, Thermo fisher Scientific) at 37 °C in a humidified atmosphere in 5% carbon dioxide. The plasmid DNA encoding both for CRISPR/Cas9 and GFP proteins (CRISPR-Cas9-2A-GFP, 9062 bp) was purchased from DNA2.0. Lipofectamine® 2000 (L2000) and Lipofectin™ (DOTMA:DOPE, 1:1 (w/w)) were obtained from Sigma-Aldrich. L2000 and KL-1,2,3-GSC/Lipofectin™ were used as positive control transfection agents, in accordance with the manufacturer's instructions and the published data[1].

5. pDNA Transfection

The day before transfection, HEK and HeLa cells were seeded in TPP 96-well plates (Faust Laborbedarf AG, Schaffhausen), respectively at $45 \cdot 10^3$ and $25 \cdot 10^3$ cells per well, in order to reach 70-90 % confluence. Peptide dendrimers/DNA complexes were formed by mixing the dendrimers (N/P 2-10, 0.07- 35 nmol, 0.5-2.6 µl from a 1 mg/mL Milli-Q water solution in 6.25 µL OptiMEM, 11-56 µM) with plasmid DNA (250ng in 6.25 µL OptiMEM, 6.8 nM). Transfection control complexes, L2000 or KL-1,2,3-GSC/Lipofectin™, were mixed with plasmid DNA (250ng in 6.25 µL OptiMEM, 6.8 nM) at the respective published recommended concentrations[1] (2:1, v/w, L2000:DNA, 0.5 µL from the 1 mg/mL commercial solution in 6.25 µL OptiMEM). These mixtures were incubated in OptiMEM for 30 min at 25°C (12.5 µL, concentration of 3.4 nM pDNA and 5.6-27.9 µM or 40 µg/mL L2000). The complexes were then diluted in OptiMEM or in OptiMEM plus 10% FCS to a final volume of 100 µL per well (final concentration of 0.42 nM pDNA and 0.7-3.4 µM peptide dendrimers or 5 µg/mL L2000). After removing complete media from the cells, the complexes were added to the plates. The plates were incubated for 4 hours at 37 °C in a humidified atmosphere in 5% carbon dioxide. Then, the transfection solutions were replaced by full growth media for 48 hours before transfection efficiency was assayed.

Transgene expression assay. The cells were washed twice with PBS and incubated with trypsin for 5 min at 37 °C. Then 100µL of PBS were added to each well and the transfection efficiency was assessed by FACS analysis (CytoFLEX Flow Cytometer, Beckman Coulter), counting 10K events per well.

6. Cell cytotoxicity by Pierce™ LDH Assay

Cells were transfected in TPP 96-well plates for 4h with peptide dendrimers/pDNA complexes, as previously described. Then, cell cytotoxicity was determined via Pierce™ LDH Cytotoxicity Assay Kit (Thermo Fisher Scientific, Reinach, CH), according to manufacturer's recommendations. During this experiment, three different LDH Activity Control conditions were used: a set of triplicate wells was incubated with 10 µL of sterile milliQ water, a second set of wells was untreated and the last set of wells was incubated with 10 µL of Lysis Buffer (10X) for 45 min, according to the manufacturer's instructions. Following transfections, 50µL of supernatant from each well (including LDH Activity Controls) were transferred to a new TPP 96-well flat-bottom plate. Then, 50µL of Reaction Mixture were added to each well and the plate was incubated at room temperature for 30 minutes protected from light. Successively, 50µL of Stop Solution were added to each sample well and the absorbance was measured on a Tecan Infinite M1000 Pro plate reader at 490 nm and 680 nm. The percentage of cytotoxicity is obtained with the following formula:

$$\%\ \text{Cytotoxicity} = \frac{(\text{Compound treated well}) - (\text{Water well}) * 100}{(\text{Untreated well}) - (\text{Water well})}$$

7. Cell Viability by PrestoBlue™ Assay

2D Cell culture. Cells were transfected in TPP 96-well plates as previously described. Following transfections, the medium was removed and replaced with 10% PrestoBlue™ (Thermo Fisher Scientific, Reinach, CH) in DMEM supplemented with 10% FCS. Cells were incubated for 30 min at 37° C in a humidified atmosphere in 5% carbon dioxide. Then, plates were measured on a Tecan Infinite M1000 Pro plate reader at λ_{ex}= 560 nm and λ_{em}= 590 nm and value normalized to the one of untreated cells.

Spheroid culture. HEK Spheroids were formed and transfected as described below. Following 4h transfection, the medium was removed and replaced with 10% PrestoBlue™ (Thermo Fisher Scientific, Reinach, CH) in DMEM supplemented with 10% FCS. Spheroids were incubated overnight at 37° C in a humidified atmosphere in 5% carbon dioxide. Then, plates were measured on a Tecan Infinite M1000 Pro plate reader at λ_{ex}= 560 nm and λ_{em}=590 nm and value normalized to the one of untreated spheroids.

Table S2. Peptide Dendrimers Cytotoxicity and Viability on HeLa cells.

no.	Sequence[a)]	% cytotox. HEK cells[b)]	% viability HEK cells [b)]	% cytotox. HeLa cells [b)]	% viiability HeLa cells [b)]
L2000	L2000	6.6 ± 8.5	85.1 ± 6.4	2 ± 1.1	56.8 ± 11.3
DMH13	$(kl)_8(kkl)_4(kll)_2k(C_{16})k(C_{16})$	1 ± 1.3	95.5 ± 5.6	0.3 ± 0.25	96.7 ± 6.2
DMH18	$(kl)_8(kkl)_4(kll)_2klllll$	1.8 ± 1.1	91.7 ± 13.2	1.2 ± 0.2	74.9 ± 10.4
G123KL	$(KL)_8(KKL)_4(KKL)_2KGSC$ [c)]	2.3 ± 1.3	51.6 ± 8.6	2.2 ± 0.7	74.5 ± 21.2
Z1	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})$	11.3 ± 1.1	64.0 ± 7.0	18.9 ± 4.6	50.5 ± 5.8
Z2	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})K(C_{18})$	2.3 ± 0.6	78.6 ± 11.2	2.2 ± 0.8	46.5 ± 3.5
Z3	$(KL)_8(KKL)_4(KKL)_2KLLLLL$	7.0 ± 0.9	75.6 ± 6.6	7 ± 3.6	50.7 ± 4.6
Z12	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})C$	3.8 ± 0.7	80.2 ± 5.3	15.7 ± 4.3	111.5 ± 9.5
Z13	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})K(C_{18})C$	2.9 ± 0.9	60.3 ± 10.0	3.2 ± 2.3	80.1 ± 7.5
Z14	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})S$	5 ± 0.9	62.2 ± 6.5	5.7 ± 1.5	85.5 ± 13.2
Z15	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})A$	3 ±0.2	71 ± 6.9	8.6 ± 2.1	107.4 ± 10
Z16	$(KL)_8(KKL)_4(KKL)_2KK(C_{18})CC$	1.1 ± 0.7	61.6 ± 10.8	3.3 ± 0.5	87 ± 8.3
Z17	$((KL)_8(KKL)_4(KKL)_2KK(C_{18})C)_2$	3.7 ±0.8	105.5 ± 12.0	2.3 ± 0.2	69.8 ± 9.6
Z18	$(kl)_8(KKL)_4(KKL)_2KK(C_{18})C$	1.7 ± 0.7	69.6 ± 7.0	4.1 ± 1.8	106.6 ± 9.9
Z19	$((kl)_8(KKL)_4(KKL)_2KK(C_{18})C)_2$	3.3 ±0.6	61.7 ± 5.6	2.9 ± 2.6	72.6 ± 9.0
Z20	$(kl)_8(kkl)_4(kkl)_2kk(C_{18})c$	3.7 ±1.4	84.8 ± 8.7	2.1 ± 0.9	84.8 ± 9.1
Z21	$((kl)_8(kkl)_4(kkl)_2kk(C_{18})c)_2$	5 ± 2.8	63.1 ± 9.6	4.9 ± 4.5	86.8 ± 4.1
Z22	$(rl)_8(krl)_4(krl)_2kk(C_{18})c$	4.7 ± 2.5	83.1 ± 8.5	3.6 ± 2.1	64.6 ± 5.1

a) One-letter code amino acids are used, *K* is the branching lysine residue, C-termini are carboxamide $CONH_2$, and all N-termini are free. Alkyl chains in the structure are represented by "C" followed by their number of carbon atoms. b) Viability and cytotoxicity assays were performed after 4h incubation of HEK or HeLa cells with peptide dendrimers/pDNA complexes at N/P 5. Cytotoxicity was then determined by Pierce LDH Cytotoxicity Assay Kit according manufacturer's instructions, cell viability was assessed by PrestoBlue reagent. c) Previously published peptide dendrimer as co-transfection reagent with Lipofectin displaying good pDNA transfection efficiency.

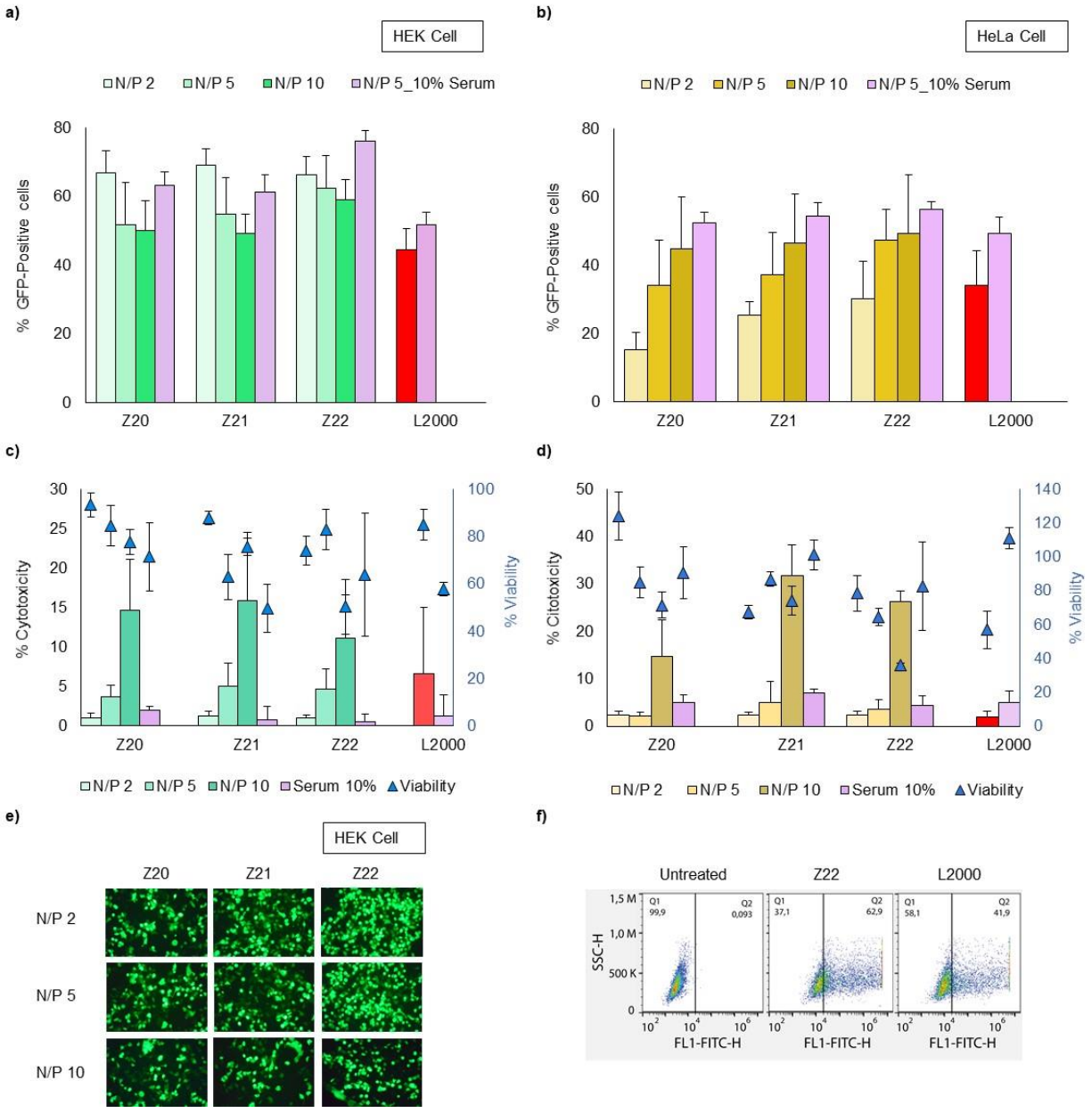


Figure S1. Transfection efficiency on **a)** HEK and **b)** HeLa cells of best-performing peptide dendrimers at different N/P ratios and in presence of 10% serum (N/P 2,5,10, 70-175-350 pmol of peptide dendrimers in 100 µL Optimem per well, 0.7-1.7-3.5 µM). Transfection efficiency was detected by FACS and expressed in percentage of transfected cells relative to the whole cell population (10k events per well). **c**, **d)** Cytotoxicity and viability assays of best-performing peptide dendrimers/pDNA complexes on HEK and HeLa cells. Cells were incubated for 4h with peptide dendrimers/pDNA complexes, as described above. Cytotoxicity was then determined by Pierce™ LDH Cytotoxicity Assay Kit according manufacturer's instructions. Cell viability was assessed by PrestoBlue™ reagent. **e)** Fluorescent microscope images of HEK cells transfected by the best-performing compounds at different N/P ratios. Pictures taken by Nikon Eclipse TS100 (20X objective) 48h after transfection. **f)** FACS raw data for transfection efficiency on HEK cells of Z22 (N/P 5) and L2000; untreated cells are showed as control.

8. Free pDNA assay by Quant-iT™ PicoGreen®

The complexes were formed in OptiMEM by mixing pDNA (250 ng in 6.25 µL OptiMEM, 6.8 nM) with peptide dendrimers (N/P ratio of 1-20, 35-670 pmol, 0.25-5.1 µL from a 1 mg/mL Milli-Q water solution in 6.25 µL OptiMEM, 5.6-112 µM) or L2000 (2:1, v/w, L2000:DNA, 0.5 µL from the 1 mg/mL commercial solution in 6.25 µL OptiMEM) for 30 min at room temperature (12.5 µL, concentration of 3.4 nM pDNA and 2.8 - 56 µM peptide dendrimers or 40 µg/mL L2000). Then, the Quant-iT™ PicoGreen® dsDNA Reagent Kit (Thermo Fisher Scientific, Reinach, CH) was used following the manufacturer's protocol. Briefly, 1 µl of reagent was diluted in 200 µl of TE buffer and 195 µL added to the well of a TPP 96-well plate. Then, 5 µl of the complexes were added to the wells (200 µL, final concentration of 0.085 nM pDNA and 0.07-1.4 µM of peptide dendrimer or 1 µg/mL L2000) and fluorescence measured at λ_{ex}= 480 nm and λ_{em}= 520 nm after 10 min on a Tecan Infinite M1000 Pro plate reader. The Quant-iT™ PicoGreen® signal from the complexes were normalized against a « pDNA alone » control to yield the percentage of the signal detected.

9. Immunogenicity ELISA Assay

THP-1 cells were seeded into 24-well plates at $50 \cdot 10^3$ cells/well the day before the experiment. Cells were treated with peptide dendrimers alone or in complex with pDNA for 4 h, following the same procedure for transfection described above. LPS 100 ng/mL (LPS25, Sigma Aldrich, Buch, CH), DMSO 10% in volume and Phorbol 12-myristate 13-acetate 8nM (PMA, Sigma Aldrich, Buch CH) were used as positive controls. After 4 h incubation at 37°C and 5% CO_2, 100 µL of new fresh media were added on top of each well and after 24 h incubation time IL-1β and TNF-α release was assessed by IL-1 beta Human Uncoated Elisa Kit (Thermo Fisher Scientific, Reinach, CH) and by Human TNF-α Standard ELISA Development Kit (Peprotech), following the manufacturer's instructions. Briefly, after coating the Corning™ Costar™ ELISA plate with capture antibody, 100 µL of cell supernatant was added to each well. After overnight incubation at 4°C and several washes, 100 µL of detection antibody were added to each well. Successfully, Avidin-HRP solution was added to each well and, after 1h incubation time and the addition of TMB and STOP solution, the absorbance was measured on a Tecan Infinite M1000 Pro plate at 440 nm and 570 nm. To define the quantity of TNF-alpha and IL-1 beta released, absorbance values at 570 nm were subtracted from the one at 440 nm for IL-1 beta, while absorbance values at 620 nm were subtracted from the one at 405 nm for TNF-alpha and data were then analyzed.

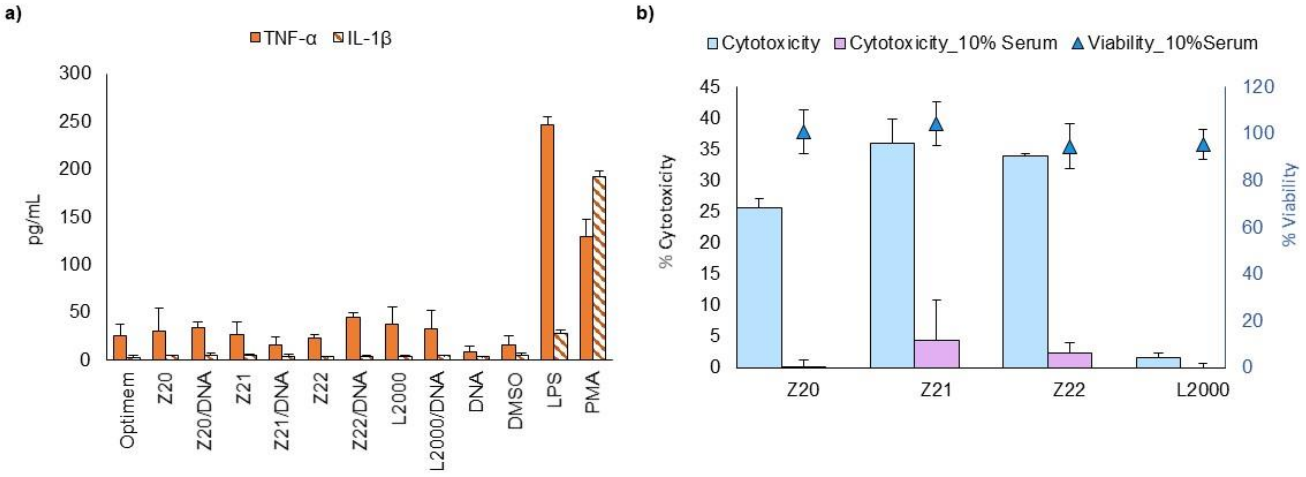


Figure S2. a) TNF-α and IL-1β release from THP-1 after 24h incubation of best-performing peptide dendrimers alone or in complex with pDNA at N/P 5 (175 pmol of peptide dendrimers in 100 µL Optimem per well, 1.7µM; 250ng of pDNA per well in 100 µL OptiMEM, 0.42 nM). PMA (8nM) and LPS (100ng/mL) are used as positive controls. Cytokines release was measured by ELISA assay. **b)** Cytotoxicity and viability of peptide dendrimers/pDNA best-performing complexes at N/P 5, in serum free condition and in presence of 10% FCS. Cytotoxicity was measured by Pierce™ LDH Cytotoxicity Assay Kit after 4h cell incubation with peptide dendrimers/pDNA complexes. Cell viability was assessed by PrestoBlue™ reagent after 4h cell incubation with peptide dendrimers/pDNA complexes.

10. Spheroid Formation and Transfection

HEK cells were seeded in 96-well ultra-low attachment U-bottom plate (Nunclon™ Sphera™ Microplates, Thermo Fisher Scientific, Reinach, CH) at 500 cells/well and the plate was then centrifuged at 250 g for 5 min. Two days after seeding, spheroids were formed and they were transfected as described above. 48h after transfection, HEK spheroids had a size between 200 and 300 µm and the transfection efficiency was assed quantitatively upon spheroid disruption by FACS analysis (CytoFLEX Flow Cytometer, Beckman Coulter) or qualitatively by Confocal Imaging (Leica SP8 confocal microscope).

11. Confocal Microscopy

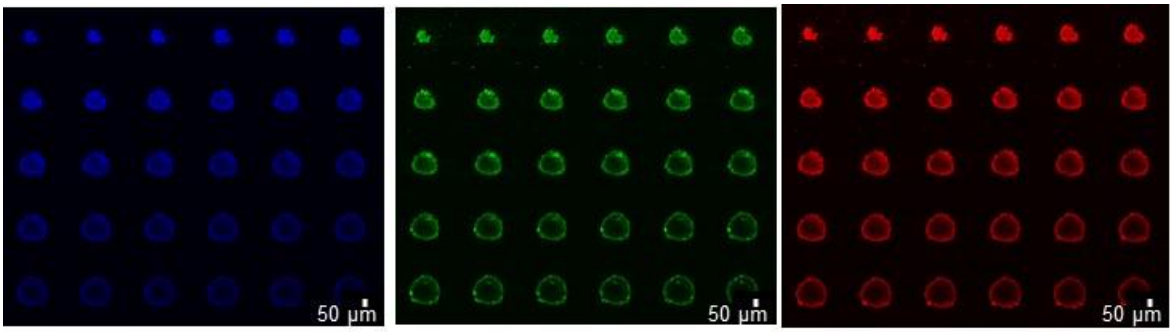
Fixation/permeabilization and Immunostaining. Fixation and permeabilization procedures were performed following Dangles-Marie publication[2]**.** 48 h after transfection, spheroids were fixed and permeabilized for 3 h at 4°C in phosphate buffered saline (PBS) containing 4% PFA (Sigma Aldrich, Buchs, CH) and 1% Triton X-100 (Sigma Aldrich, Buchs, CH) and washed in PBS (3 × 10 min). Spheroids were then dehydrated in an ascending series of methanol at 4°C in PBS (25%, 50%, 75%, 15 min each and 100% for 2 h), rehydrated in the same descending series and washed in PBS (3 × 10 min). After blocking

in PBST (0.1% Triton X-100 in PBS) containing 3% Bovine Serum Albumin (Sigma Aldrich, Buchs, CH) overnight at 4°C and washing in PBST (2 × 15 min), spheroids were incubated with primary antibodies (Anti-GFP Goat IgG, R&D System; Anti-Collagen I Rabbit IgG, Abcam,UK) diluted in PBST at 4°C for 24 h and rinsed in PBST (4 × 10 min). Then, spheroids were then incubated in appropriate AlexaFluor conjugated secondary antibodies for 24 h (Alexa Fluor® 488 Bovine Anti-Goat IgG, Jackson ImmunoResearch; Alexa Fluor® 594 Goat anti-Rabbit IgG, Invitrogen). Cell nuclei were counterstained by DAPI (Thermo Fisher Scientific, Reinach, CH) diluted to 200nM solution in PBS for 30 min at room temperature.

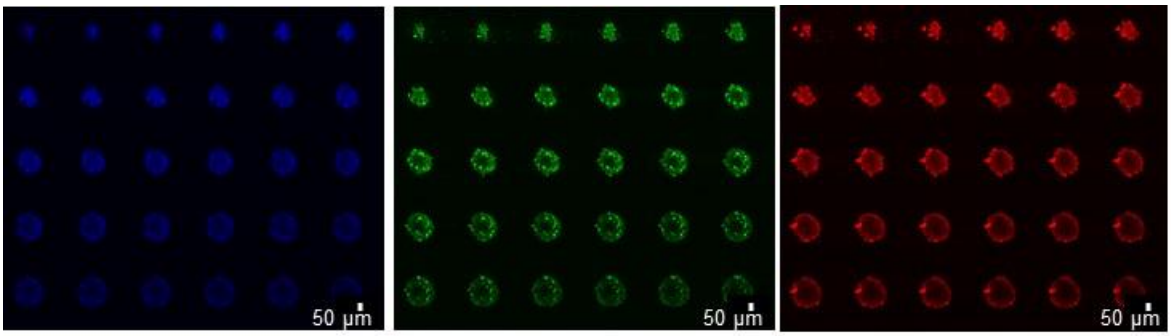
Imaging. Stained spheroids were transferred in 8-well chambered coverglass plates (In Vitro Scientific, Cellvis) in Glycergel Mounting Medium (Dako, Ca). Images were recorded on a Leica SP8 confocal microscope with lens x10. The three channels were acquired sequentially and were merged using Image J software. For images reconstruction, a stack of confocal images was collected through the spheroids with step size of 1-2 µm between adjacent optical planes, starting from one pole of the spheroids. Afterwards, this stack was used to generate pictures by using the Z-Stack project routine in Image J.

Confocal Stack-Montage of HEK spheroids.

a) Z20



b) Z21



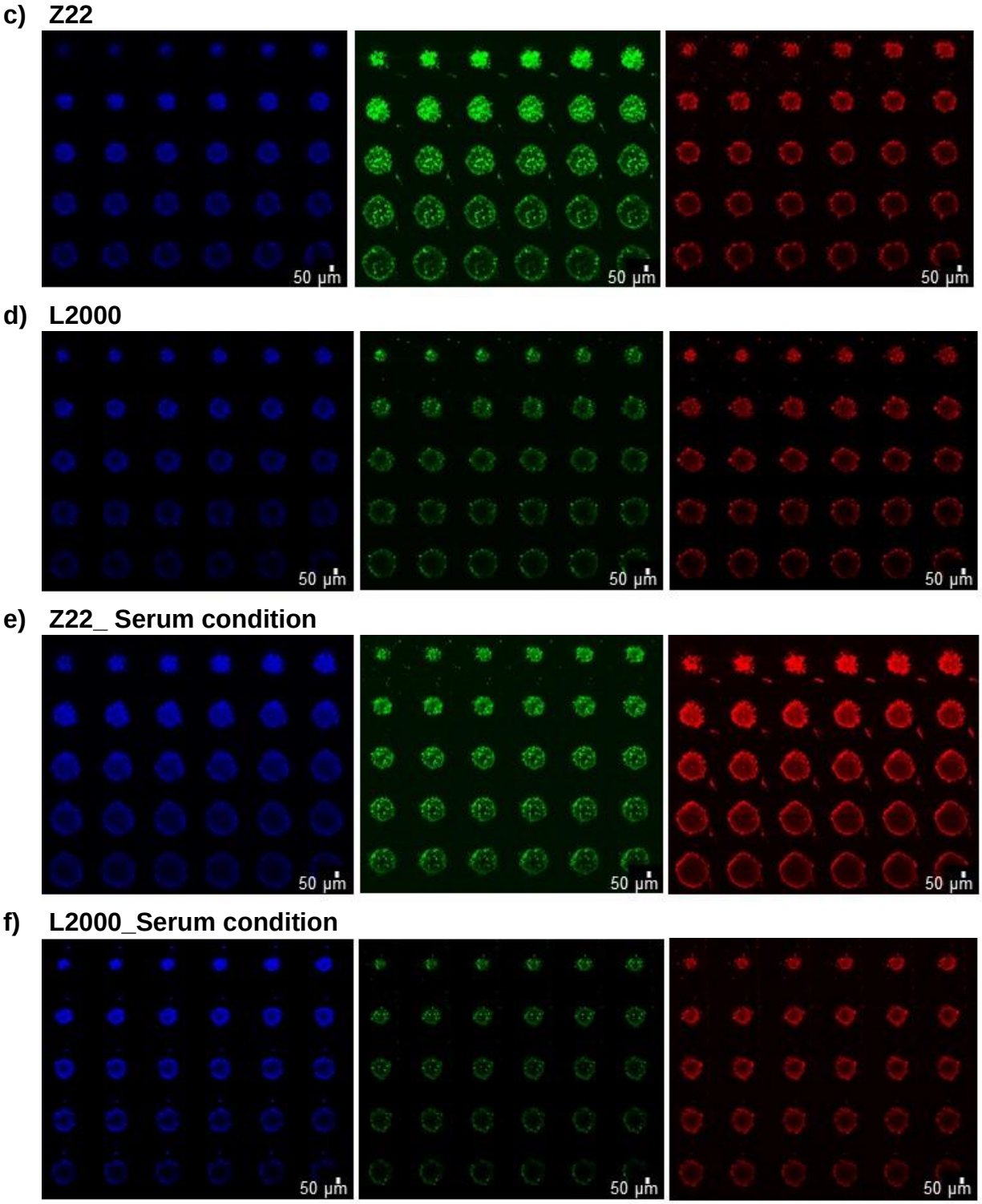


Figure S3. Stack montage of confocal images recorded on a Leica SP8 confocal microscope with lens x10. HEK spheroid were transfected by peptide dendrimers and L2000, as previously described. Three channels were acquired sequentially: DAPI for nuclei staining in blue, GFP protein in green, Collagen I in red. Scale bar 50µm.Every stack is 2µM thick. Acquisitions were taken with 70-80 µm depth within the spheroid.

12. Dynamic Light Scattering (DLS) and Zeta potential

Complexes were formed in phosphate buffer (PB) at pH 7.4 with pDNA (1µg, in 25 µL PB at pH 7.4, 6.8 nM) and peptide dendrimers (N/P ratio of 5, 690 pmol, 5-5.4 µL from a 1 mg/mL Milli-Q water solution in 25 µL PB at pH 7.4, 27.9 µM) and incubated at room temperature for 30 minutes (50 µL, final concentration of 3.4 nM pDNA and 13.9 µM of peptide dendrimer). Then, 50 µL was transferred to a low-volume Univette (Sigma aldrich, Buchs, CH). The dynamic light scattering and Zeta potential were then measured on an Anton Paar Litesizer 500 (Buchs, CH) and the data processed by the software provided by the manufacturer (Kalliope) using the “number of particles” parameter.

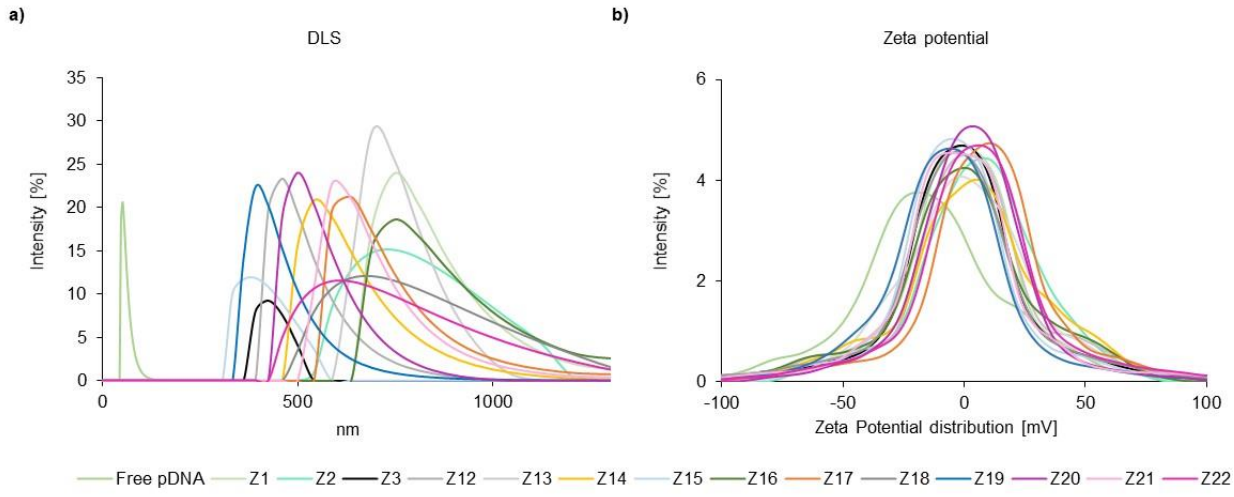


Figure S4. a) DLS and **b)** Zeta Potential measurement of peptide dendrimers/pDNA complexes at N/P 5 in PB buffer pH 7.4 (50 µL total volume, final concentration of 3.4 nM pDNA and 13.9 µM of peptide dendrimer).

13. Competition assay by Quant- iT ™ PicoGreen®

The complexes were formed as in the free pDNA assay and 5 µL of the complexes were diluted in 190 µL of assay buffer as described in the part above. Then, 5 µL of heparin at different concentrations were added to the wells of a TPP 96-well plate and incubated for 30 min at room temperature (200 µL, final concentration of heparin 0-2 U/mL, final concentration of 0.085 nM pDNA and 0.35 µM or 1 µg/mL L2000). Fluorescence measured at λ_{ex}= 480 nm and λ_{em}= 520 nm was performed on a Tecan Infinite M1000 Pro plate reader. The Quant-iT™ PicoGreen® signal from the complexes were normalized against a control (pDNA only) to yield the percentage of the signal detected.

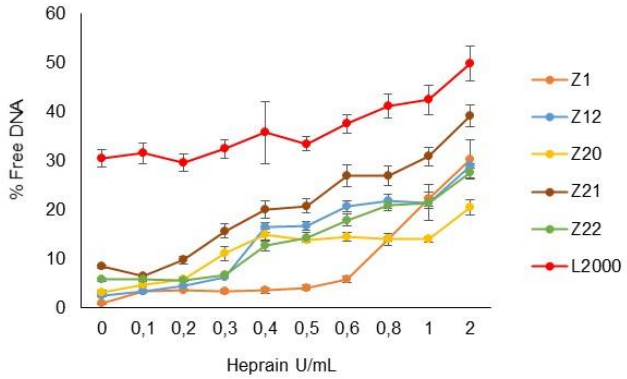


Figure S5. Heparin displacement of pDNA from complexes formed of pDNA and peptide dendrimers (N/P 5, 250 ng pDNA) or L2000 (2:1, v/w, L2000 : DNA) by addition of heparin (0−2 U/mL) measured by tracking free pDNA by Quant-it PicoGreen® assay. Fluorescence normalized to pDNA alone set as 100%. Fluorescence measured at λ_{ex}= 480 nm and λ_{em}= 520 nm.

14. Critical Micellar Concentration (CMC)

Nile red (Sigma Aldrich, Buchs, CH) was diluted in methanol at a concentration of 2 µM and 5 µL were added to each well of a TPP 96-well plate (Faust Laborbedarf AG, Schaffhausen) and dry under the fumehood air flow at room temperature for 1 h. Serial dilution of the peptide dendrimers, were performed in 10 mM phosphate buffer (pH 5 or pH 7.4) starting from 1.25 mg/mL to 2 µg/mL and 50 µL was added to the plate containing the dried Nile red fluorophore (final concentration 0.2 µM). The plates were incubated for 2 h before measurement of fluorescence at λ_{ex}= 540 nm and λ_{em}= 615 nm on a Tecan Infinite M1000 Pro plate reader.

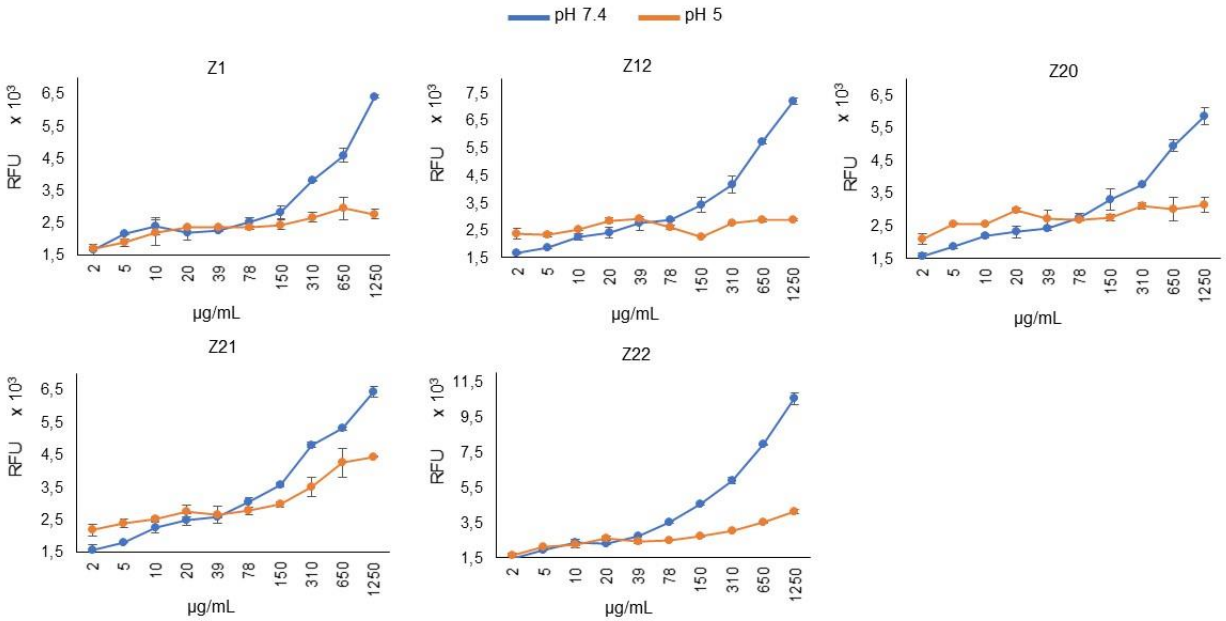
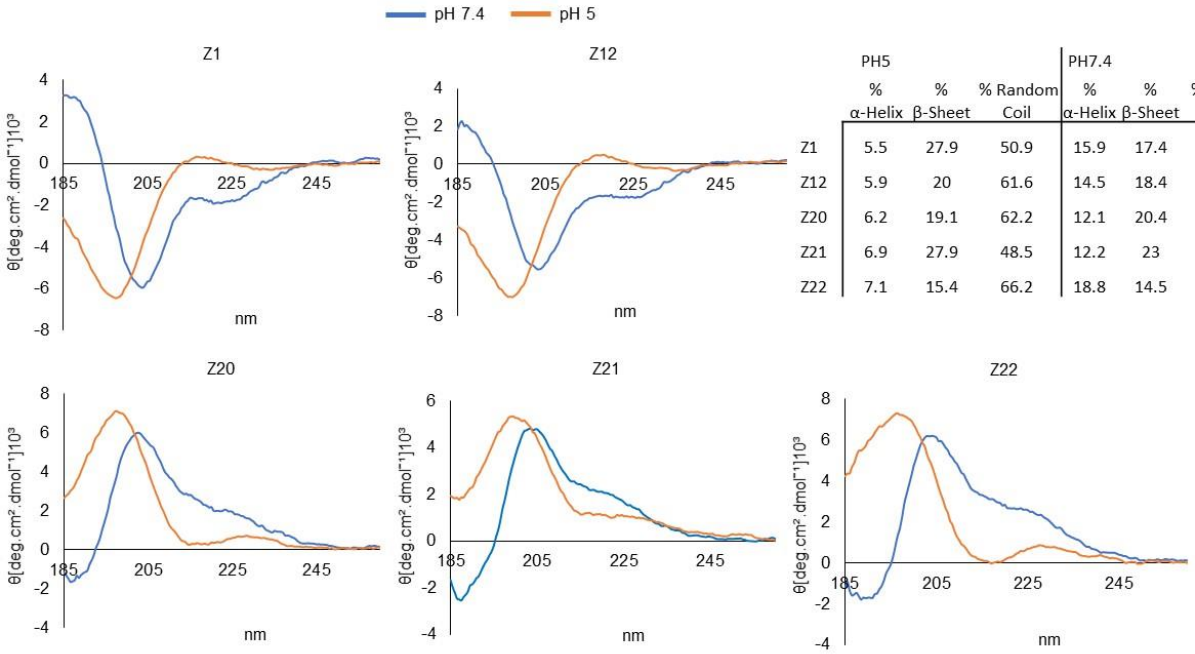


Figure S6. Critical aggregation concentration determination in PB buffer pH 7.4 and pH 5, performed by serially diluting compounds starting from 1.25 mg/mL concentration to 2 µg/mL, added on dried Nile red (final concentration of 0.2 µM). Fluorescence measured at λ_{ex} = 540 nm and λ_{em} = 615 nm. RFU = relative fluorescence unit. The fluorescence intensity of Nile Red is the same at pH 7.4 (1962 RFU ± 144 and pH 5.0 (2067 RFU ± 490).

15. Circular Dichroism (CD) Spectroscopy

The CD spectra were recorded using a Jasco J-715 spectrometer equipped with a PFD-350S temperature controller and a PS-150J power supply. All experiments were measured using a Hellma Suprasil R 100-QS 0.1 cm cuvette. Stock solution (1 mg/mL) of peptides dendrimers were freshly prepared in Milli-Q water. For the measurement, the peptides were diluted to a final concentration of 100 µg/mL with PB buffer (pH = 7.4 or pH = 5, 10 mM final concentration). The range of measurement was 185-260 nm, scan rate was 20 nm/min, pitch 0.5 nm, response 16 sec. and band 1.0 nm. The nitrogen flow was kept above 10 L/min. The baseline (buffer solution) was recorded under the same conditions and subtracted manually. The cuvettes were washed with 1M HCl, mQ deionized H_2O and PB buffer before each measurement. The percentage of α-helix, β-sheet and random coil at pH 7.4 and pH 5.0 were processed by Dichroweb using the CONTIN analysis program and the reference set 7 using the spectra between 190 and 240 nm.



	PH5			PH7.4		
	% α-Helix	% β-Sheet	% Random Coil	% α-Helix	% β-Sheet	% Random Coil
Z1	5.5	27.9	50.9	15.9	17.4	53.5
Z12	5.9	20	61.6	14.5	18.4	53.6
Z20	6.2	19.1	62.2	12.1	20.4	51.9
Z21	6.9	27.9	48.5	12.2	23	48.5
Z22	7.1	15.4	66.2	18.8	14.5	52.3

Figure S7. Circular dichroism spectra of peptide dendrimers (100 µg/mL) in 10 mM PB buffer at pH 7.4 and pH 5. Percentage of α-helix, β-sheet and random coil at pH 7.4 and pH 5.0 processed by Dichroweb using the CONTIN analysis program and reference set 7 and the recorded data between 190 and 240 nm.

16. pDNA Transfection in Presence of Bafilomycin

HEK cells were treated with Bafilomycin A1 (200 nM, Alfa Aesar, Karlsruhe, DE) in DMEM supplemented with 10% FCS for 1 h before transfection, in 96-well TPP plates described above. Peptide dendrimers/pDNA complexes were added to cells and incubated for 4 h at 37°C in a humidified atmosphere in 5% carbon dioxide. Then, complexes were removed and replaced with 100 µL of DMEM supplemented with 10% FCS containing 200 nM Bafilomycin A1 and incubated for 48 h. Transfection efficiency was assessed as described above after a total of 48 h.

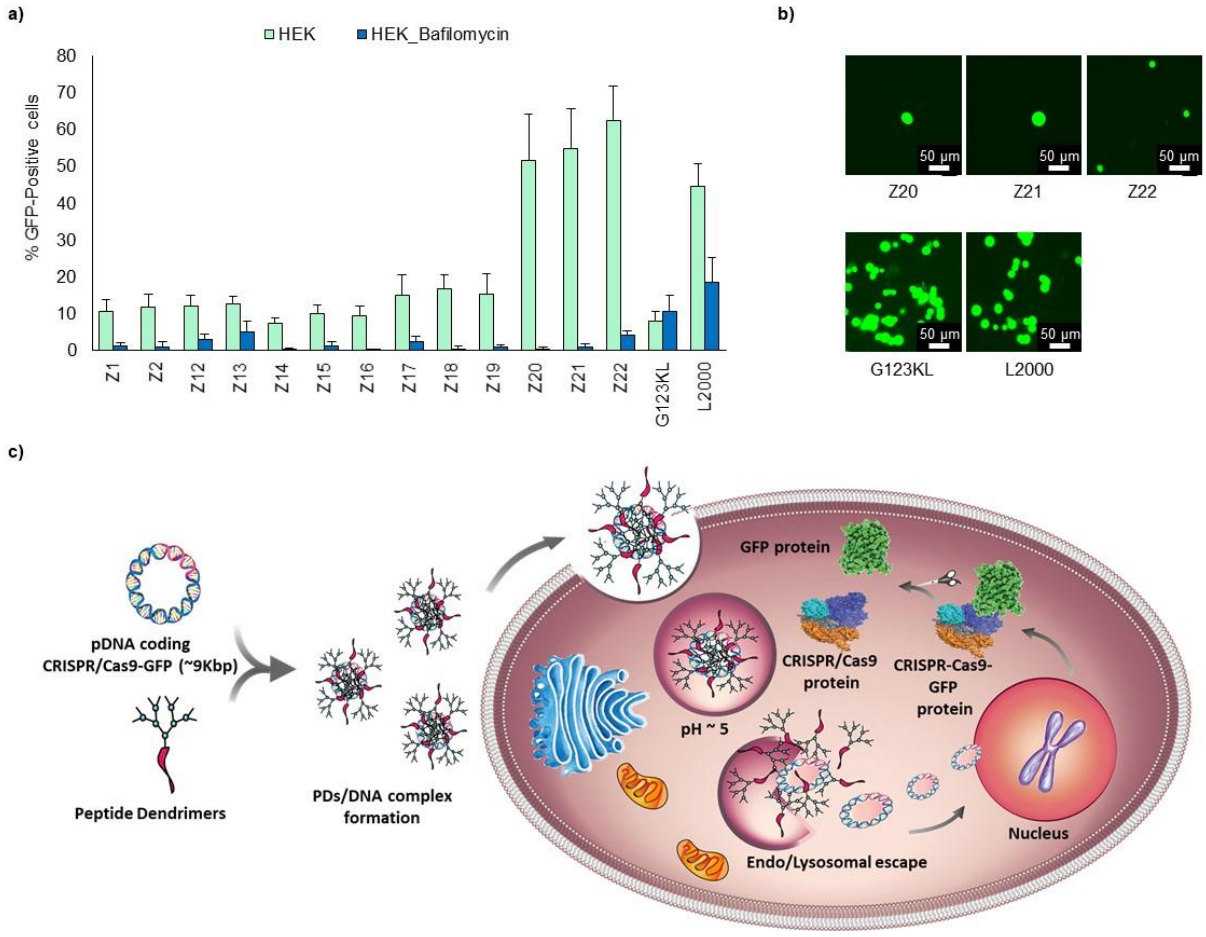


Figure S8. a) Transfection on HEK cells with and without Bafilomycin A1 treatment (200nM final concentration per well, 100µL volume). Cells were incubated with Bafilomycin for 1h before transfection and for 48h after cell incubation with peptide dendrimers/pDNA complexes. **b)** Fluorescent microscope images of HEK cells transfected by best performing compounds under Bafilomicyn A1 treatment. Pictures taken by Nikon Eclipse TS100 (40X objective), 48h after transfection. Scale bar size is 50 µm. **c)** Schematic representation of the proposed transfection mechanism. Peptide dendrimers (PDs) are incubated at N/P 5 with pDNA to formulate polyplexes, which can penetrate the cell membrane. Through an endo/lysosomal escape mechanism, peptide dendrimers can release the pDNA into the cytosol. The transcription and translation of CRISPR-Cas9-GFP protein is followed by the cleavage of GFP form CRISPR-Cas9 and the efficiency of gene transfection is assessed by the fluorescence of GFP.

[1] A. Kwok, G. A. Eggimann, J.-L. Reymond, T. Darbre, F. Hollfelder, *ACS Nano* **2013**, *7*, 4668–4682.
[2] L.-B. Weiswald, J.-M. Guinebretière, S. Richon, D. Bellet, B. Saubaméa, V. Dangles-Marie, *BMC Cancer* **2010**, *10*, 106.