Supporting information for:

Stereoselective pH Responsive Peptide Dendrimers for siRNA Transfection

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Table of Contents

1 Experimental Part and Supplementary Figures

1.1 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-Gly-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-Lys(Palm)-OH, Fmoc-D-Lys(Palm)-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-Trp(Boc)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-His(Trt)-OH, Fmoc-12-Ado-OH and Fmoc-Nle-OH and purchased by Iris Biotech GmbH or GL Biochem. Tentagel S RAM resin was purchased from Rapp Polymere GmbH. Peptide dendrimers synthesis was performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper or automatically by Biotage Initiator+Alstra and 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

Department of Chemistry and Biochemistry at the University of Bern (group PD Dr. Stefan Schürch).

1.2 Solid phase synthesis of peptide dendrimers

1.2.1 Manual solid phase synthesis of peptide dendrimers

Peptide dendrimers were synthesized by placing 300 mg Tentagel S RAM resin (0.22–0.25 mmol/g) in a 10 mL polypropylene syringe equipped as described previously.[1] 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 × 6 mL), MeOH (3 × 6 mL) and DCM (3 × 6 mL).

Coupling of the Fmoc-protected amino acids – 3 eq. of Fmoc-protected amino acid, 3 eq. of PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) 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. Reaction 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 × 6 mL), MeOH (3 × 6 mL) and DCM (3 × 6 mL).

1.2.2 Solid phase synthesis of peptides dendrimers by Biotage Initiator+ Alstra

Peptide dendrimers were also synthesized by Biotage Initiator Alstra using 300 mg of Tentagel S RAM resin (0.22-0.25 mmol/g). Stirring of the reaction mixture at any given step described below was performed by vortexing the vial. The resin was swollen in DMF for 60 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 6 mL of piperidine/DMF (1:4, v/v) for 5 min at 75 °C. After filtration, the resin was washed for 8 min with 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 0.5 M were used as coupling reagents in 5 mL of DMF. The reaction was stirred for 8 minutes at 75 °C. The resin was then

washed with DMF for 8 min. 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 and seven times for the third generation.

1.2.3 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 Tentagel S RAM resin (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 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 and seven times for the third generation.

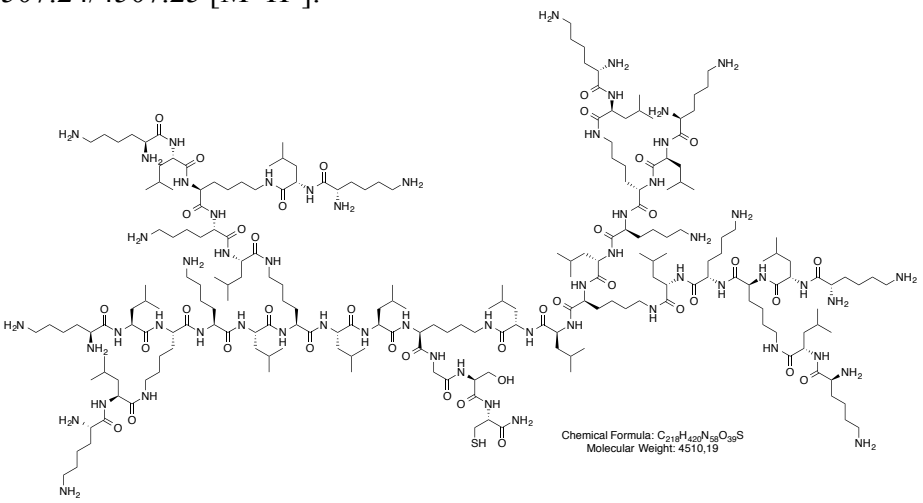
Orthogonal Boc protection of the N-terminus – The peptide N-terminus was Fmoc deprotected according to the previously written procedure, resin swelled in 8 mL THF for 10 minutes, then replaced with 8 mL of THF containing Di-*tert*-butyl dicarbonate (16 eq., 250 mg) and TEA (24 eq., 0.3 mL) and stirred 3 h. Reaction repeated once and stirred overnight, then the resin was washed with NMP (3 × 6 mL), MeOH (3 × 6 mL) and DCM (3 × 6 mL).

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 (3 × 8 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 (2 × 10 ml each). Then, the carboxylic acid or other amino acids were coupled according to the manual procedure.

Last Fmoc deprotection and Acetylation – After the last amino acid was coupled by the three different methods, and in some case the carboxylic acid coupled, Fmoc deprotection was performed manually with 8 mL of piperidine/DMF (1:4, v/v) for 20 min. After filtration, the resin was washed with NMP (3 × 6 mL), MeOH (3 × 6 mL) and DCM (3 × 6 mL). When necessary, the peptide was acetylated with a solution of acetic acid anhydride/DCM (1/1, v/v) for 2 times 10 min. The resin was washed with NMP (3 × 6 mL), MeOH (3 × 6 mL) and DCM (3 × 6 mL).

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.

G2,3-KL ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*GSC) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (33 mg, 4.9 µmol, 7%). Analytical RP-HPLC: t_R = 2.93 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{218}H_{420}N_{58}O_{39}S$ calc./obs. 4507.24/4507.25 [M+H$^+$].



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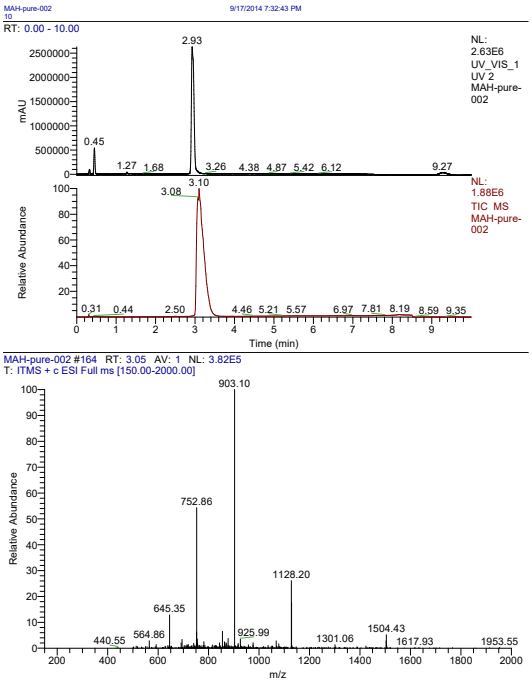

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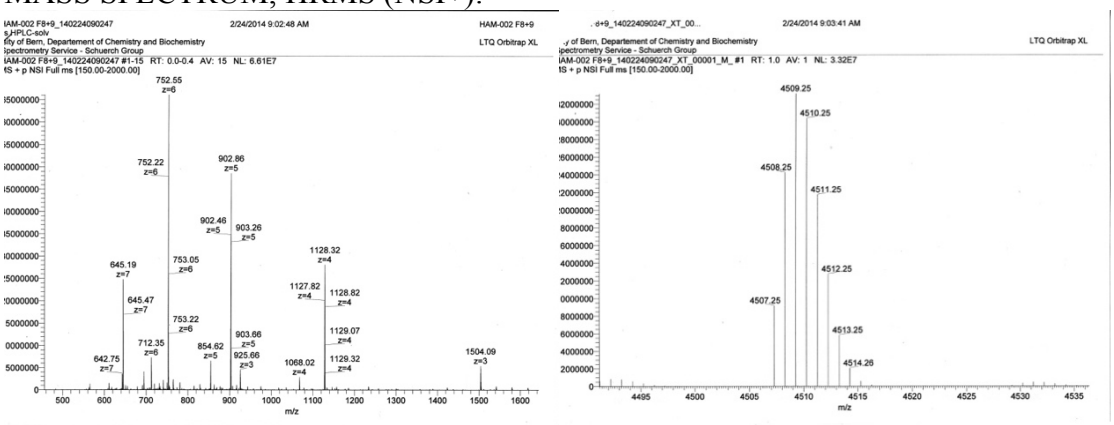
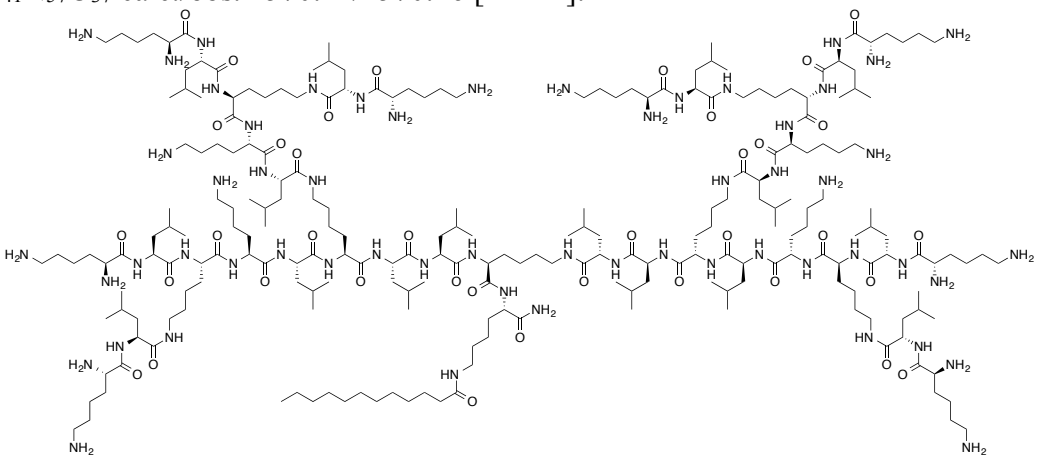


MH01 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{12}$)) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (6.9 mg, 1.0 µmol, 2%). Analytical RP-HPLC: t_R = 3.14 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{228}H_{441}N_{57}O_{37}$ calc./obs. 4570.44/4570.46 [M+H$^+$].



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Molecular Weight: 4573,40

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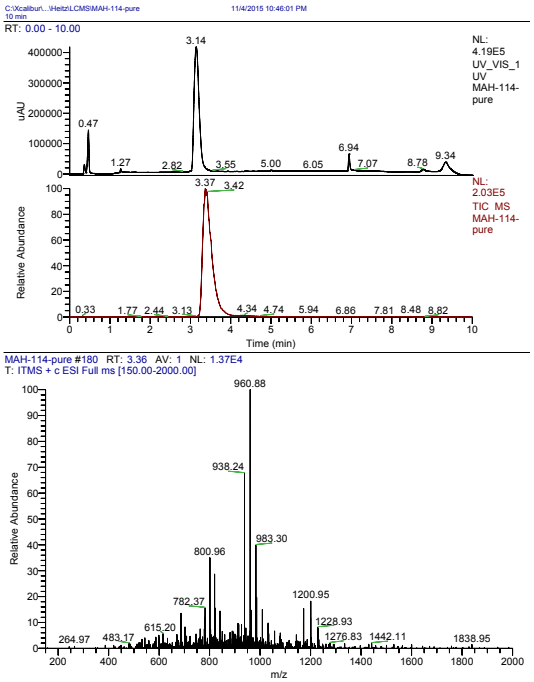

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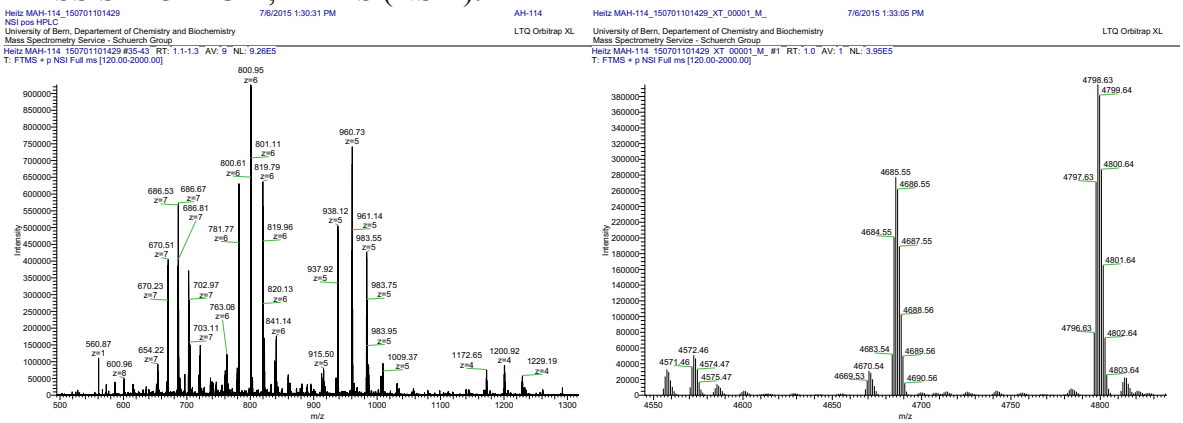
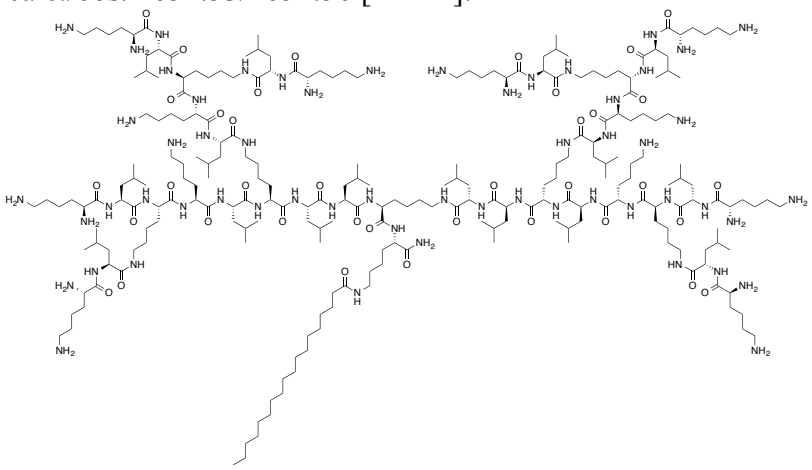


MH02 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{18}$)) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (6.0 mg, 0.86 µmol, 2%). Analytical RP-HPLC: t_R = 3.44 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{453}N_{57}O_{37}$ calc./obs. 4654.53/4654.56 [M+H$^+$].



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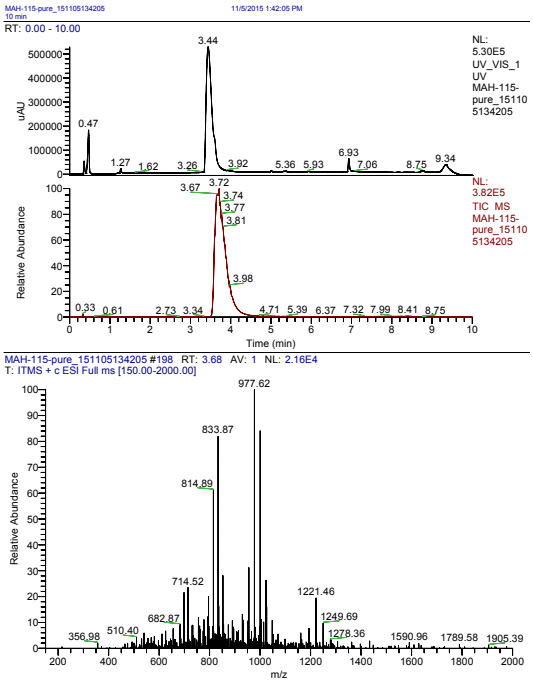

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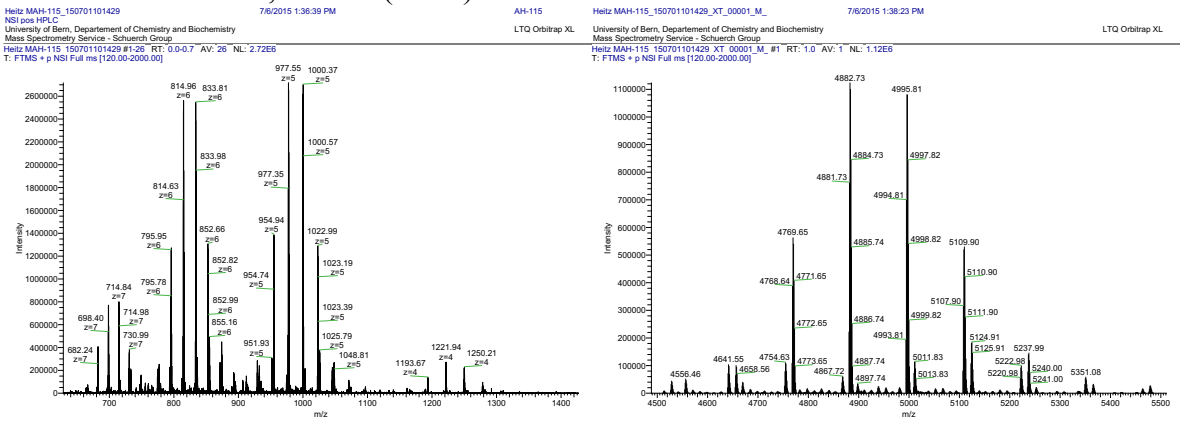
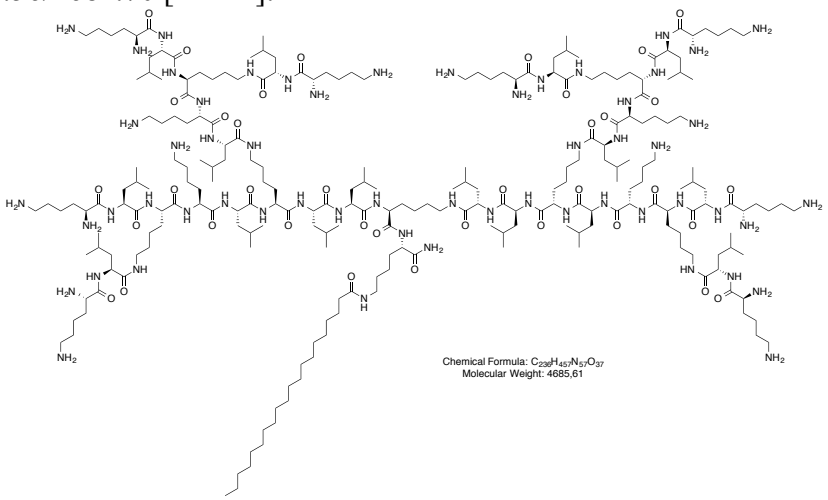


MH03 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{20}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (4.1 mg, 0.59 µmol, 1%). Analytical RP-HPLC: t_R = 4.01 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{236}H_{457}N_{57}O_{37}$ calc./obs. 4682.56/4682.70 [M+H$^+$].



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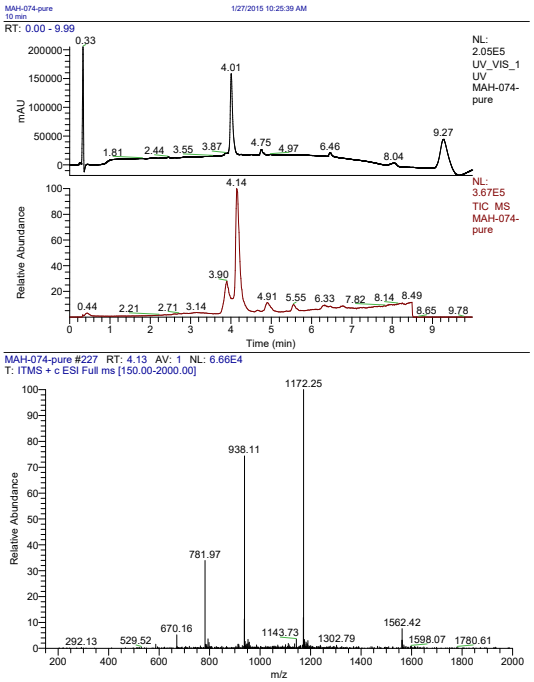

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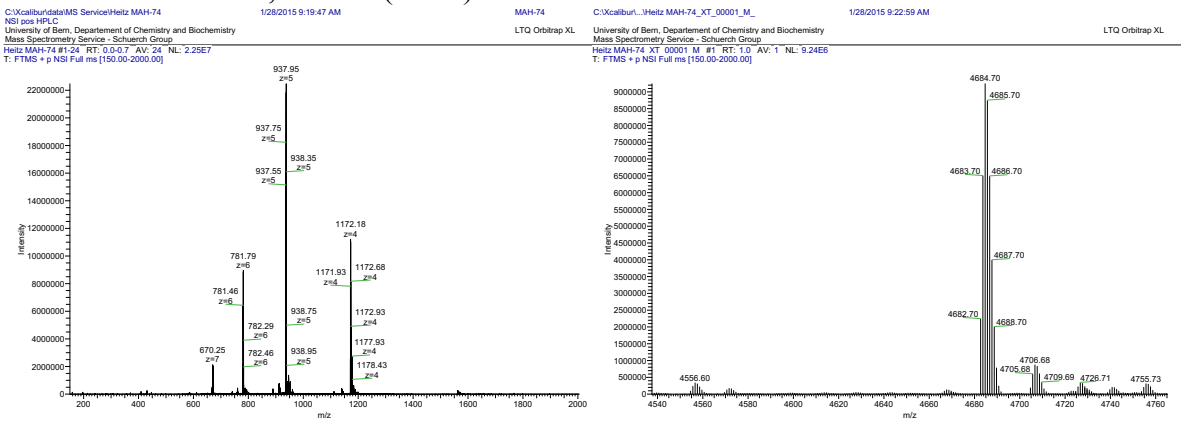
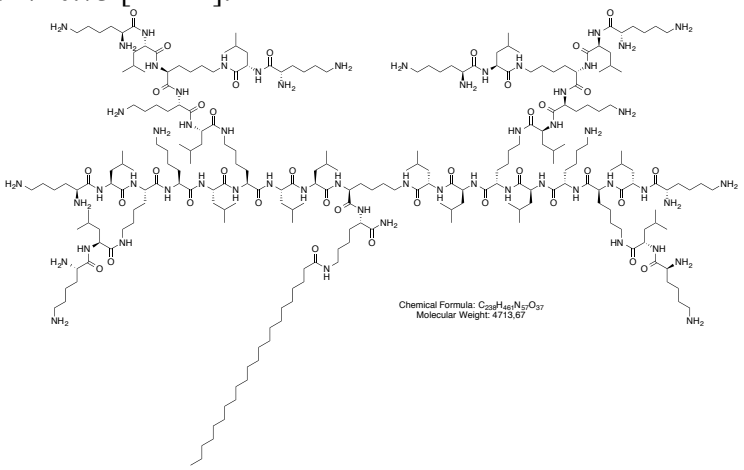


MH04 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{22}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (3.2 mg, 0.46 µmol, 1%). Analytical RP-HPLC: t_R = 4.20 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{238}H_{461}N_{57}O_{37}$ calc./obs. 4710.59/4710.73 [M+H$^+$].



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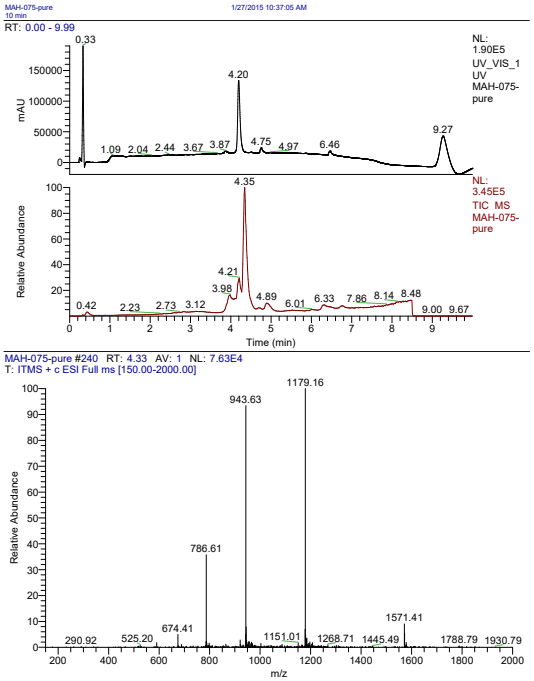

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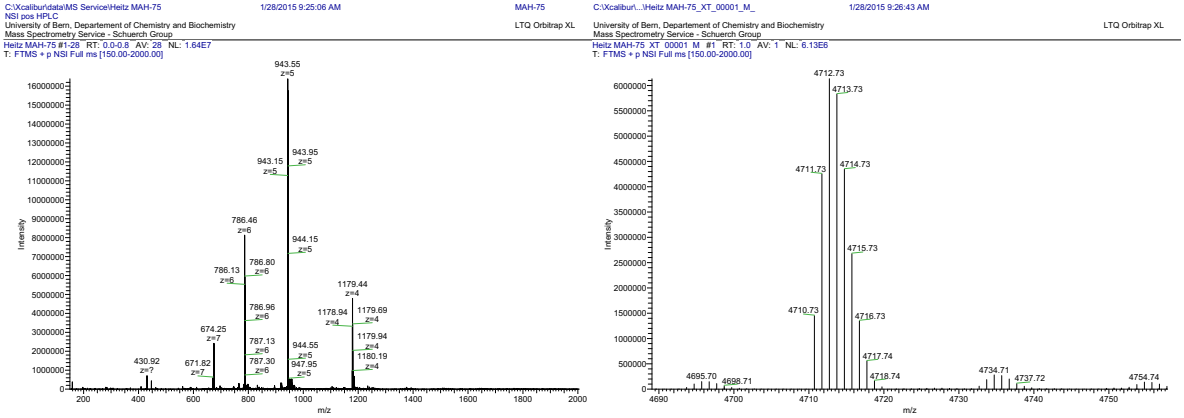
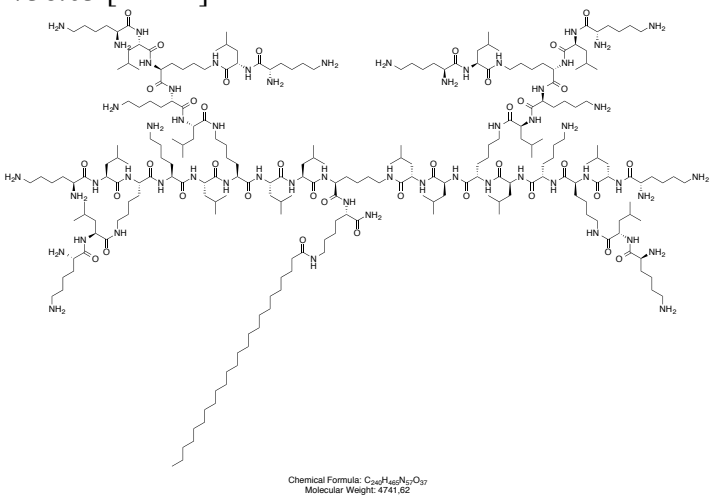


MH05 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{24}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (4.5 mg, 0.6 µmol, 1%). Analytical RP-HPLC: t_R = 4.40 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{240}H_{465}N_{57}O_{37}$ calc./obs. 4738.63/4738.63 [M+H$^+$]



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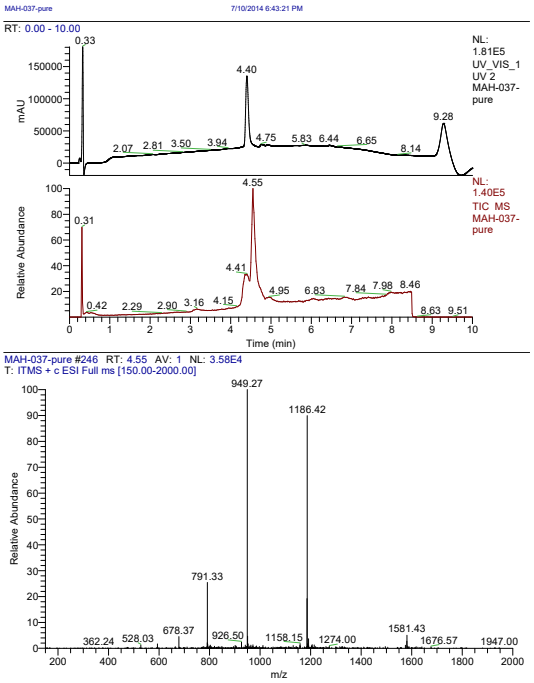

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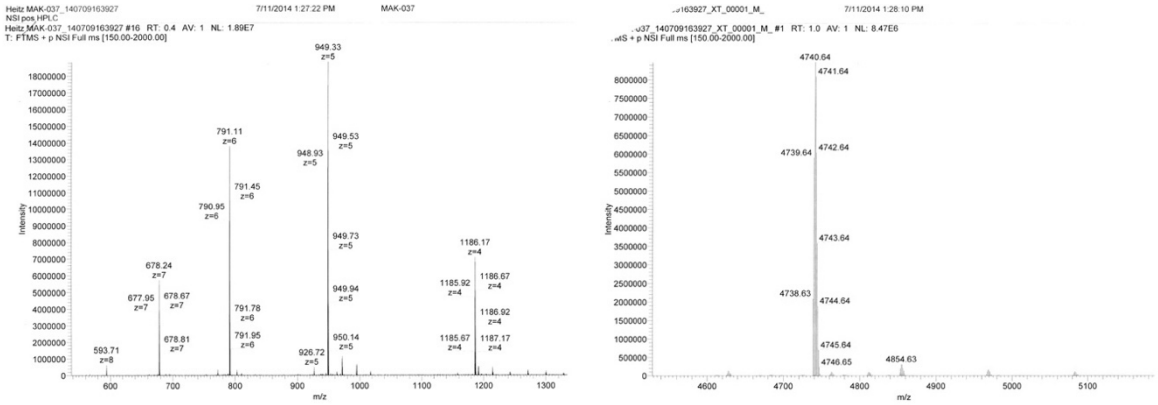
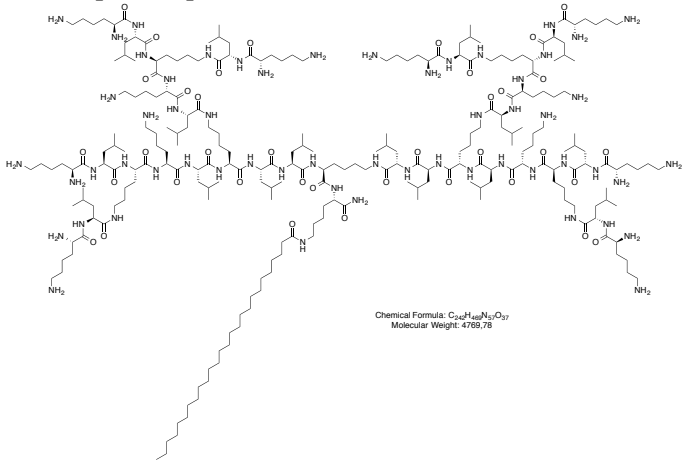


MH06 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{26}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (3.1 mg, 0.44 µmol, 1%). Analytical RP-HPLC: t_R = 4.67 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{242}H_{469}N_{57}O_{37}$ calc./obs. 4766.66/4766.65 [M+H$^+$].



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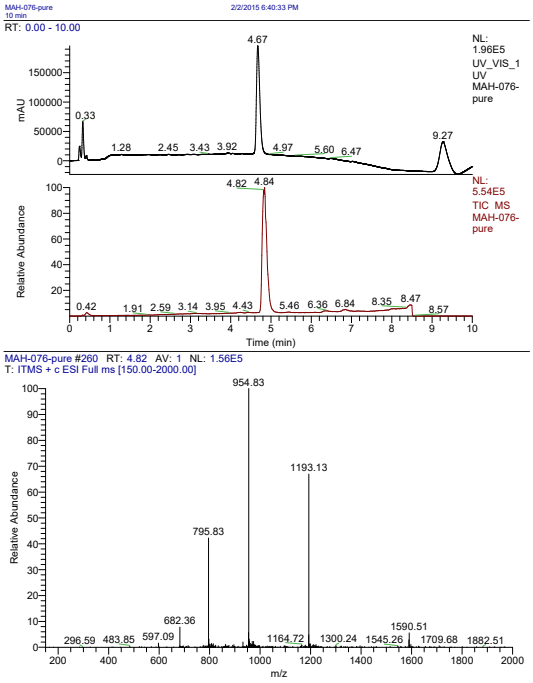

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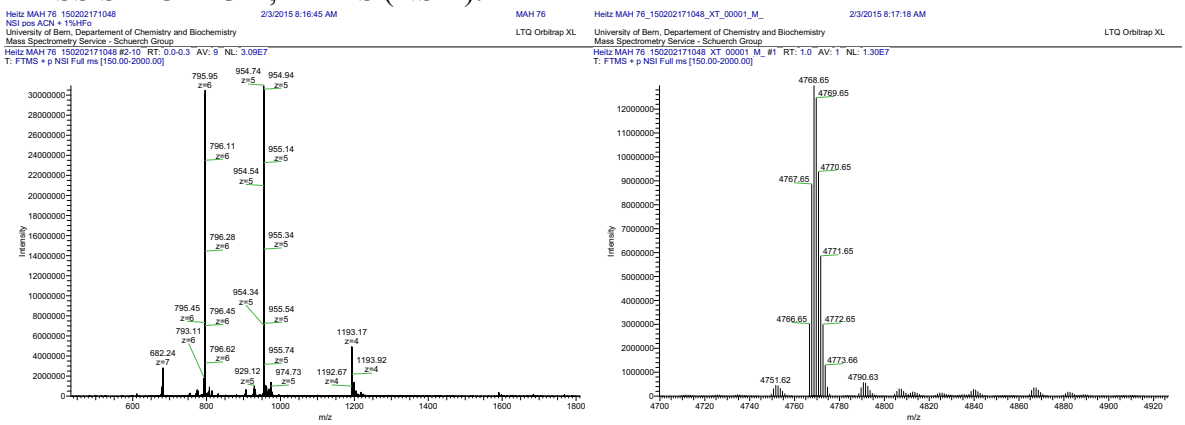
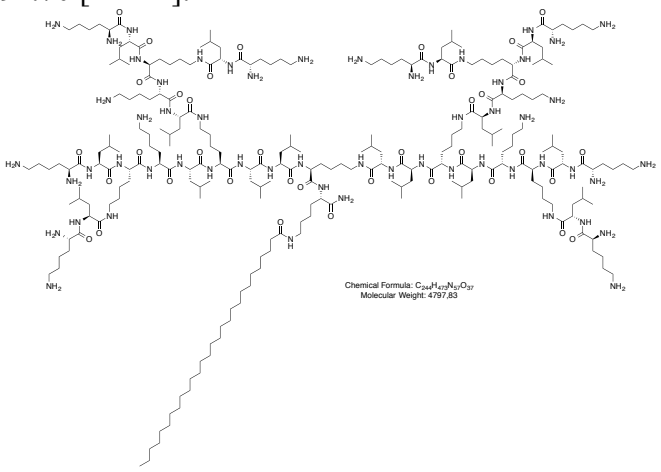


MH07 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{28}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (3.1 mg, 0.44 µmol, 1%). Analytical RP-HPLC: t_R = 4.81 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{244}H_{473}N_{57}O_{37}$ calc./obs. 4794.69/4794.70 [M+H$^+$].



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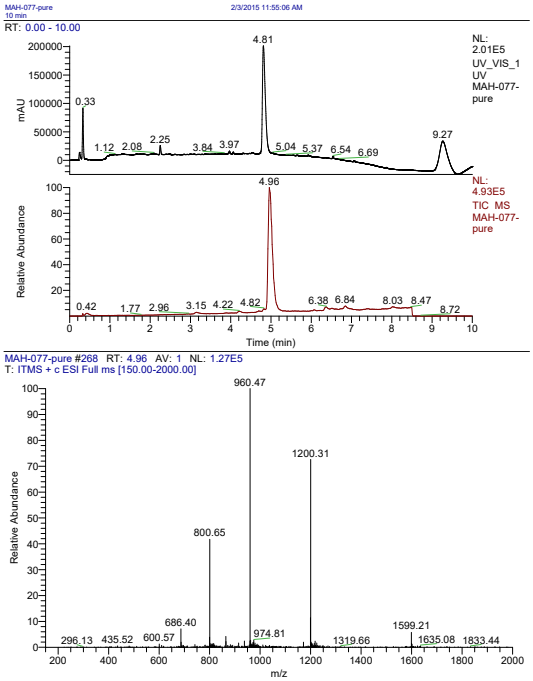

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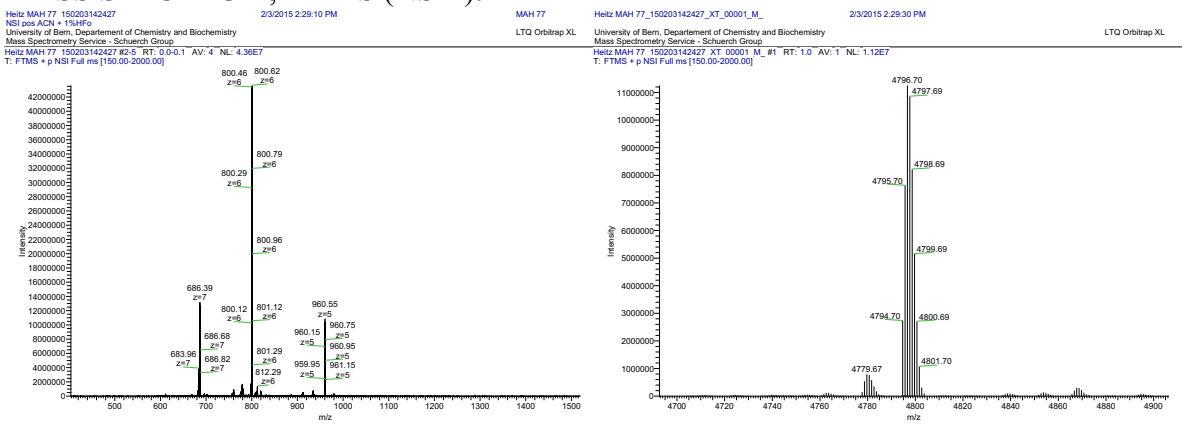
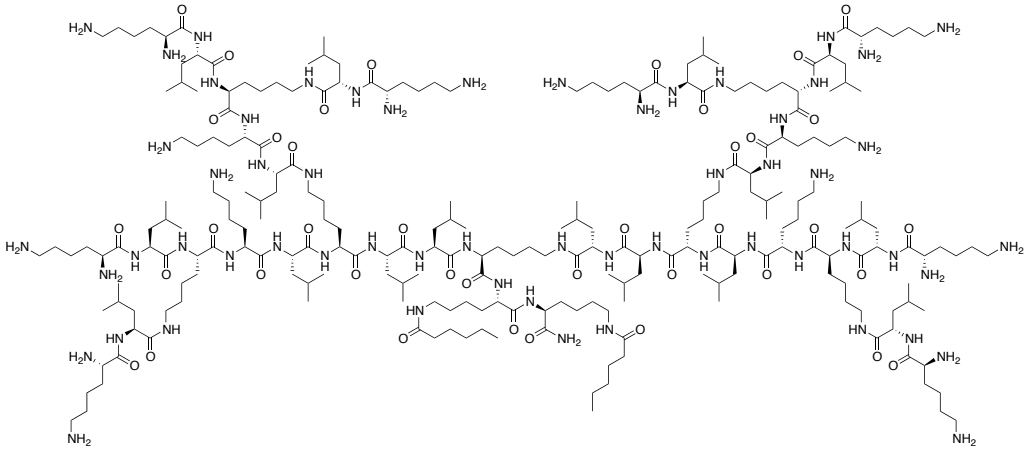
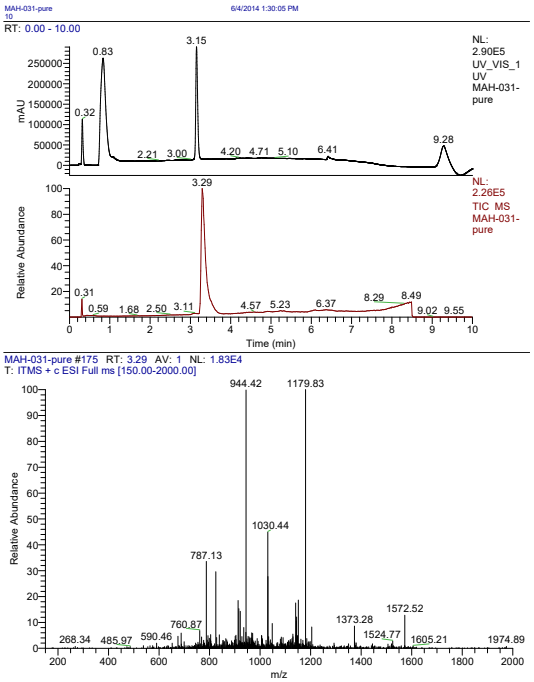


MH08 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_6$)K(C$_6$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (5.4 mg, 0.8 µmol, 1%). Analytical RP-HPLC: t_R = 3.15 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.52 [M+H$^+$].



Chemical Formula: $C_{234}H_{451}N_{59}O_{39}$
Molecular Weight: 4715,46

LCMS



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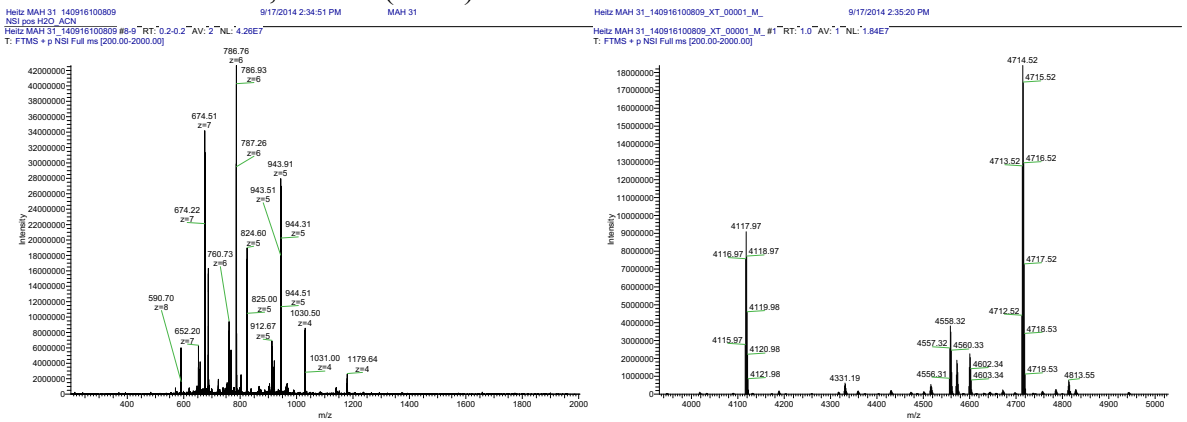

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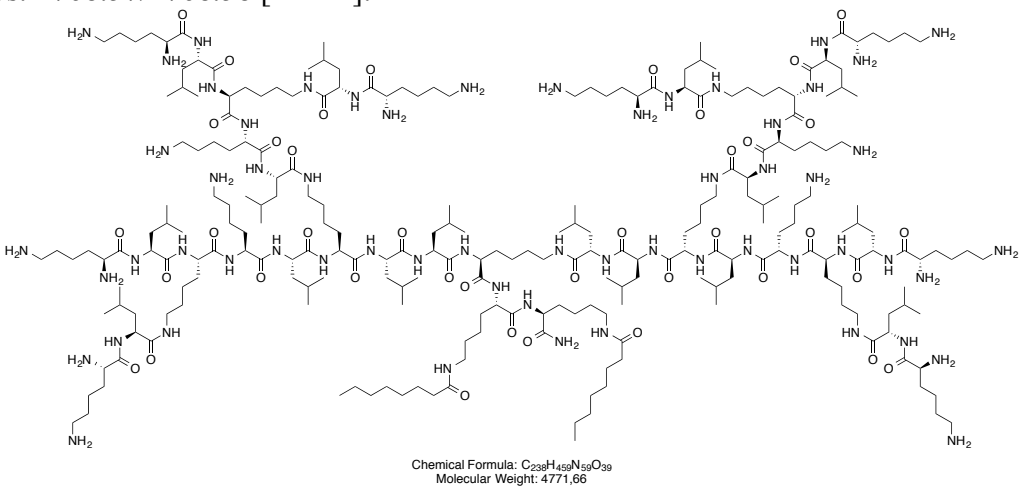

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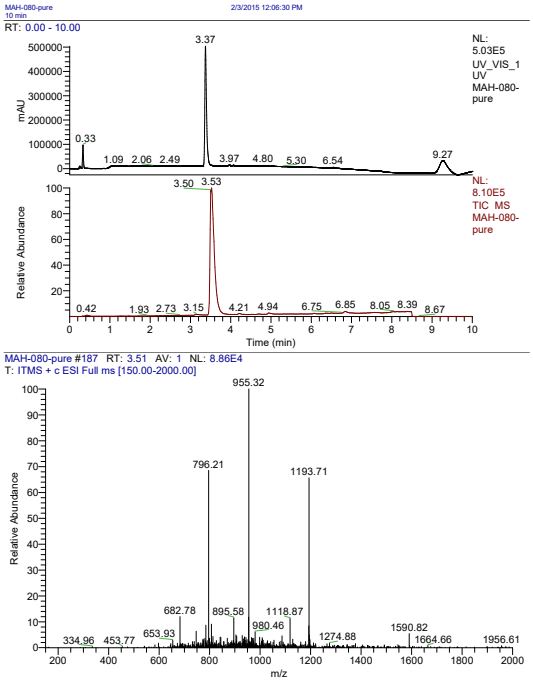
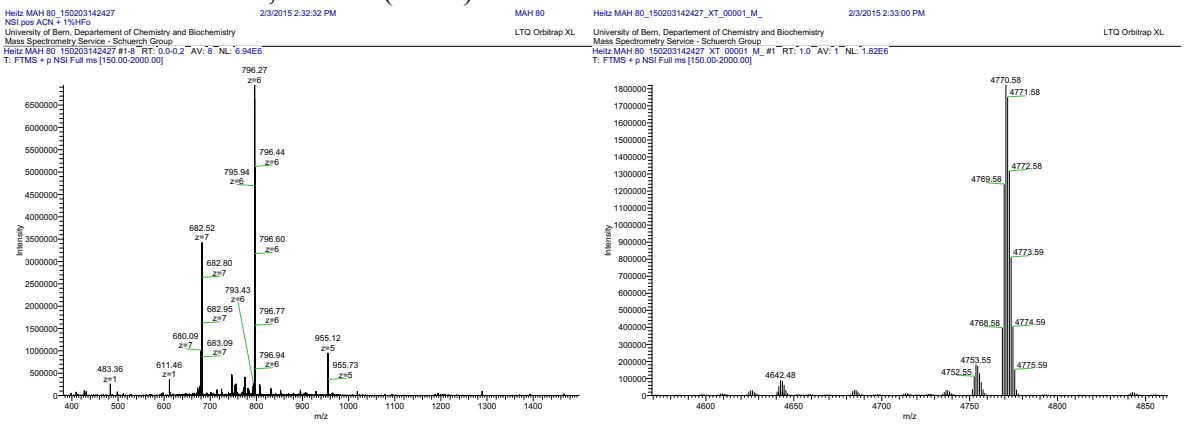
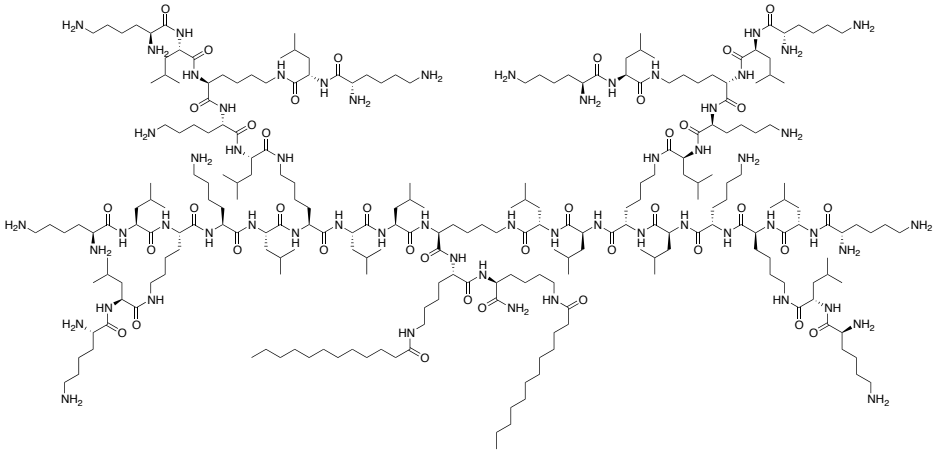
LCMS



MASS SPECTRUM, HRMS (NSI+):

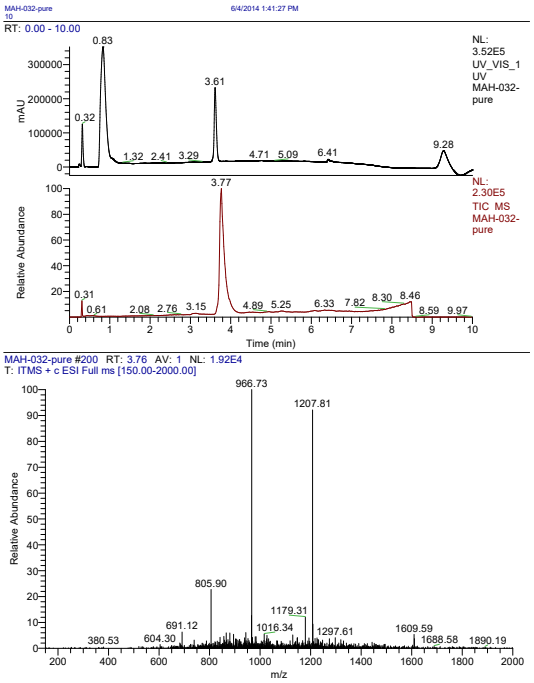


MH10 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{10}$)K(C$_{10}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (4.4 mg, 0.6 µmol, 1%). Analytical RP-HPLC: t_R = 3.61 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{242}H_{467}N_{59}O_{39}$ calc./obs. 4824.64/4824.64 [M+H$^+$].

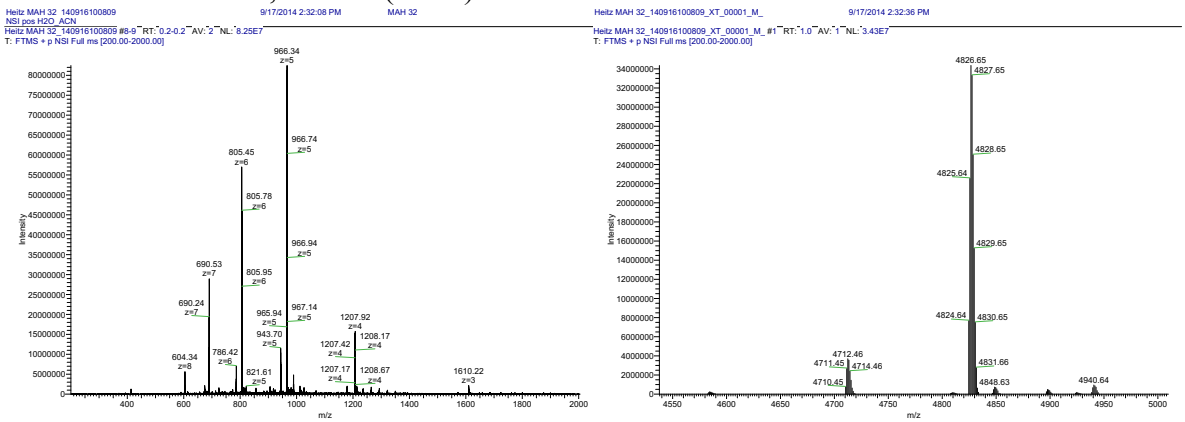


Chemical Formula: $C_{246}H_{475}N_{59}O_{39}$
Molecular Weight: 4883,78

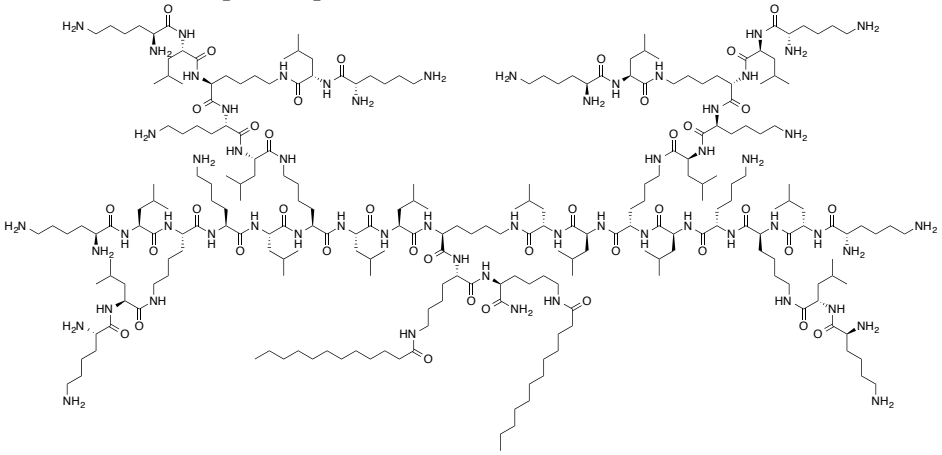
LCMS



MASS SPECTRUM, HRMS (NSI+):

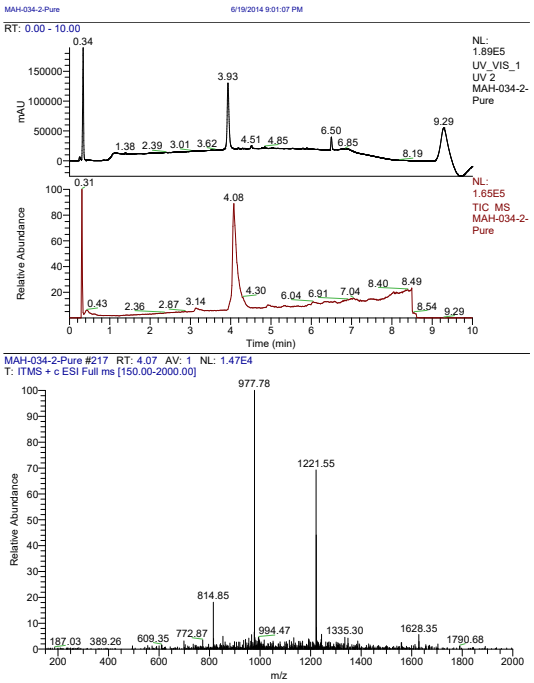


MH11 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{12}$)K(C$_{12}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (2.7 mg, 0.4 µmol, 0.5%). Analytical RP-HPLC: t_R = 3.93 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{246}H_{475}N_{59}O_{39}$ calc./obs. 4880.70/4880.70 [M+H$^+$].

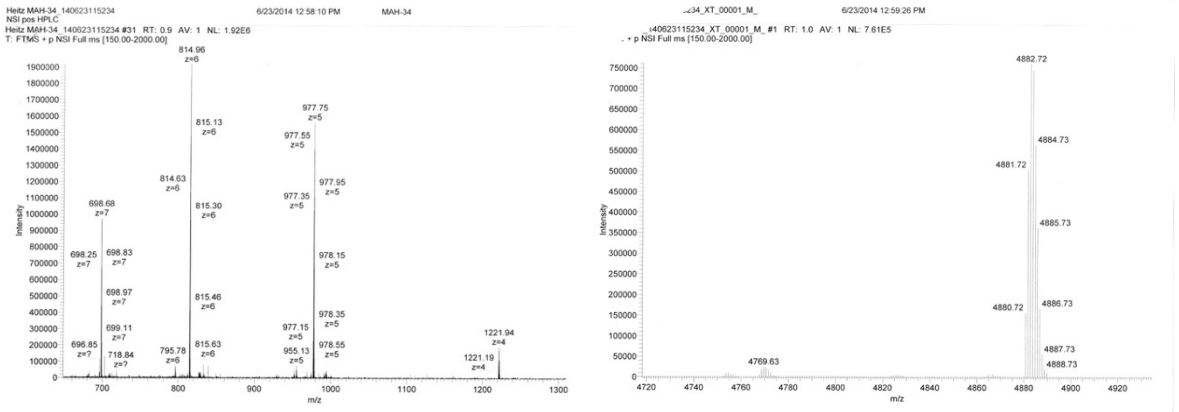


Chemical Formula: $C_{246}H_{475}N_{59}O_{39}$
Molecular Weight: 4883,78

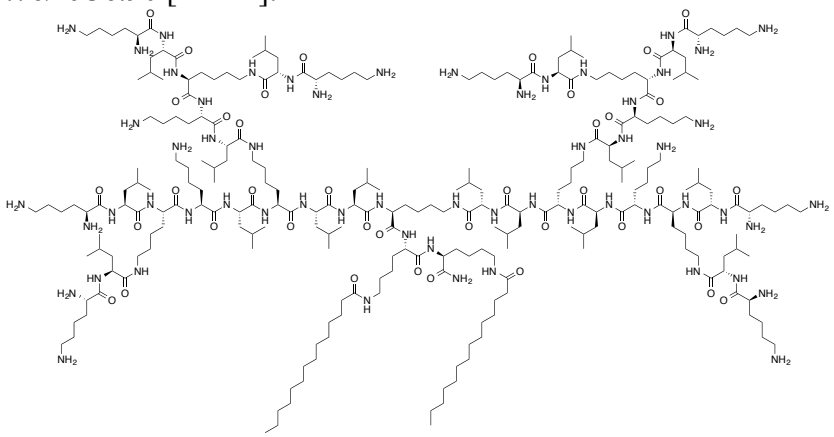
LCMS



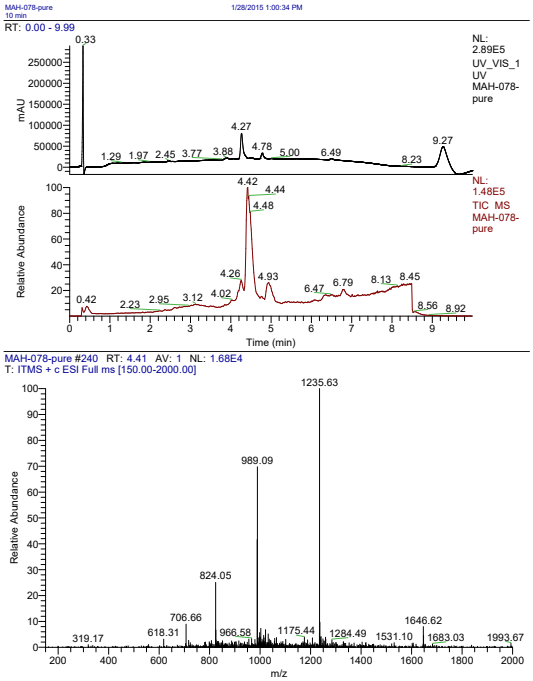
MASS SPECTRUM, HRMS (NSI+):



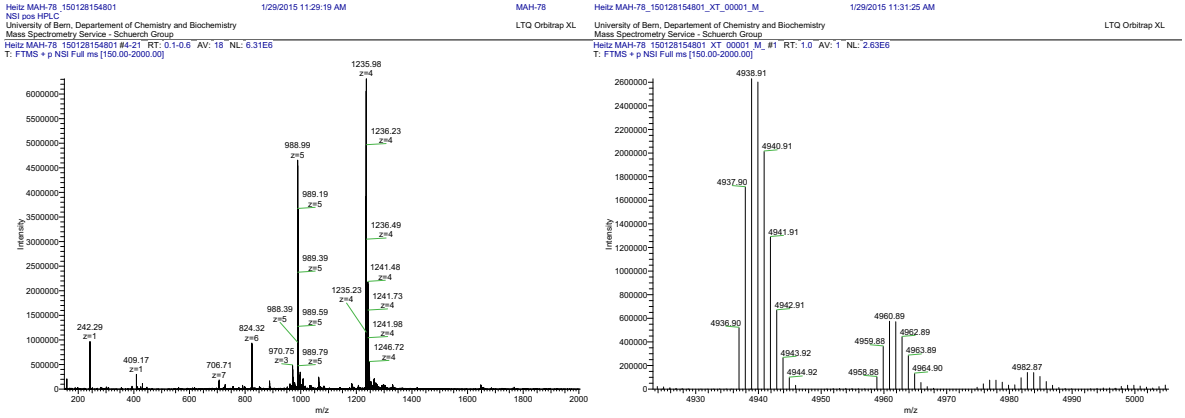
MH12 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{14}$)K(C$_{14}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (2.3 mg, 0.32 µmol, 1%). Analytical RP-HPLC: t_R = 4.27 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{250}H_{483}N_{59}O_{39}$ calc./obs. 4936.76/4936.90 [M+H$^+$].



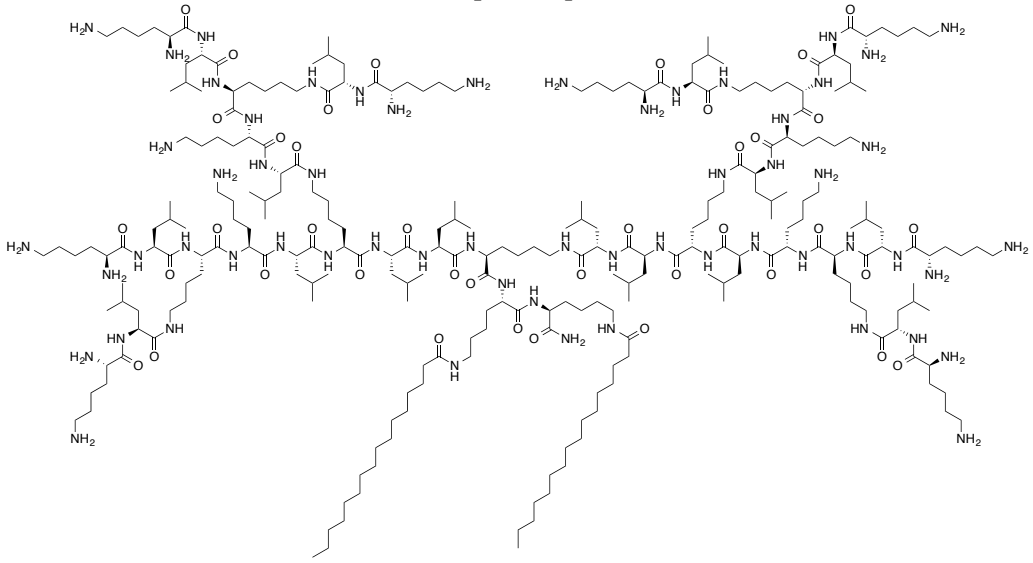
LCMS



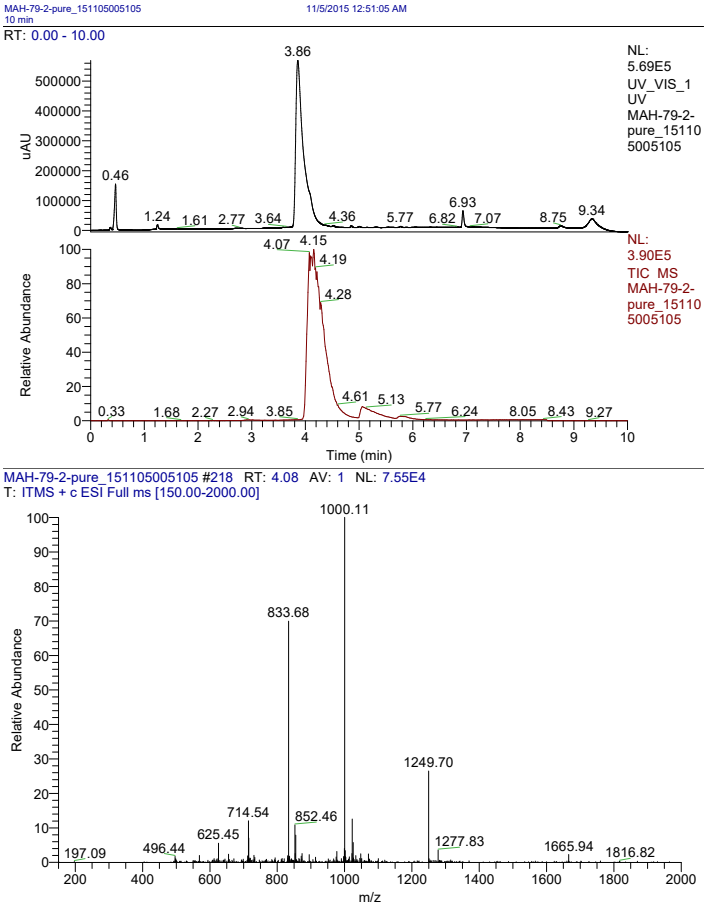
MASS SPECTRUM, HRMS (NSI+):



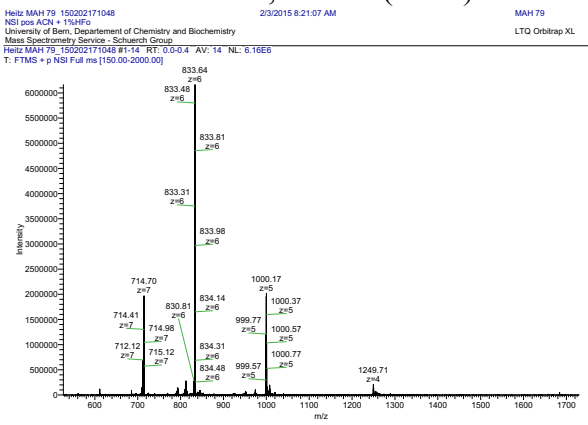
MH13 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{16}$)K(C$_{16}$)) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (26.8 mg, 3.68 µmol, 4%). Analytical RP-HPLC: t_R = 4.68 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{254}H_{491}N_{59}O_{39}$ calc./obs. 4992.83/4992.82 [M+H$^+$].

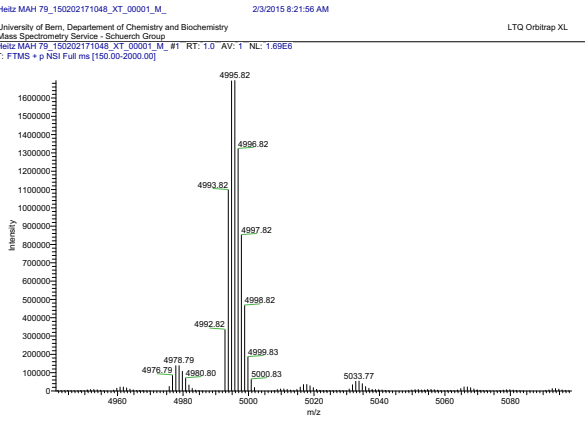


LCMS

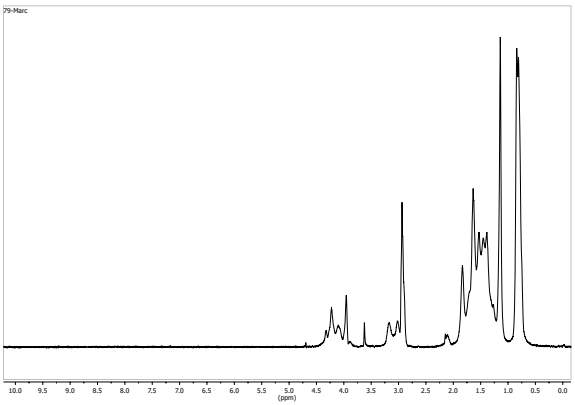


MASS SPECTRUM, HRMS (NSI+):

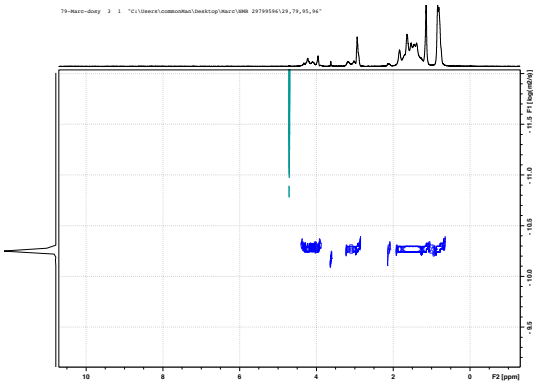


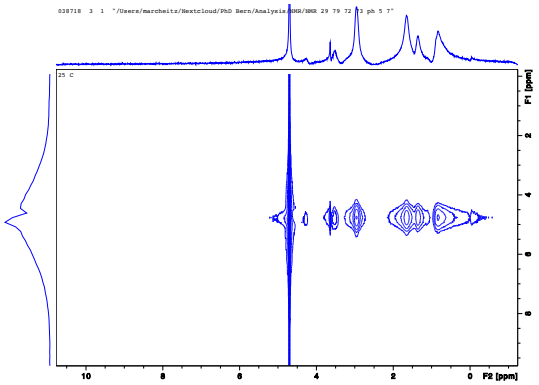


^{1}H NMR

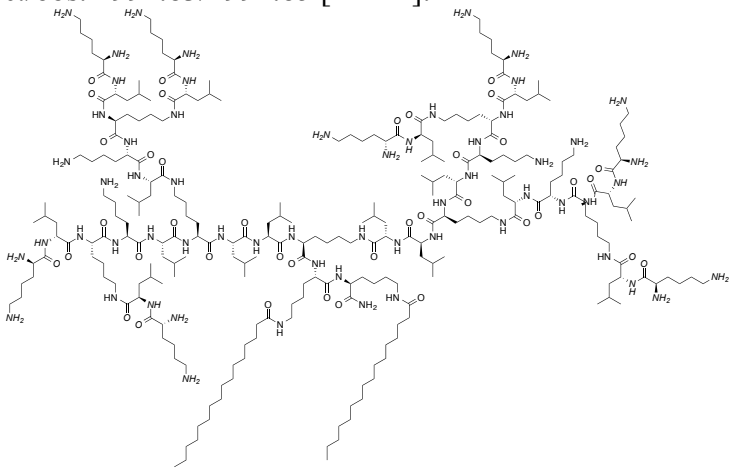


DOSY NMR pH 5 and pH 7.4

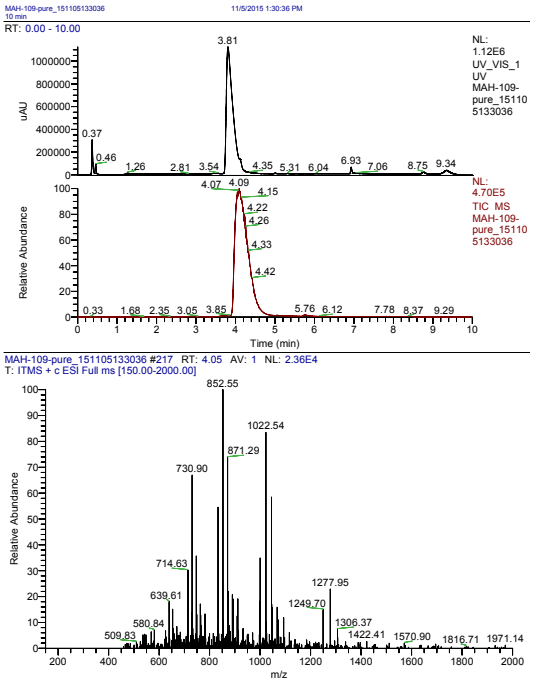




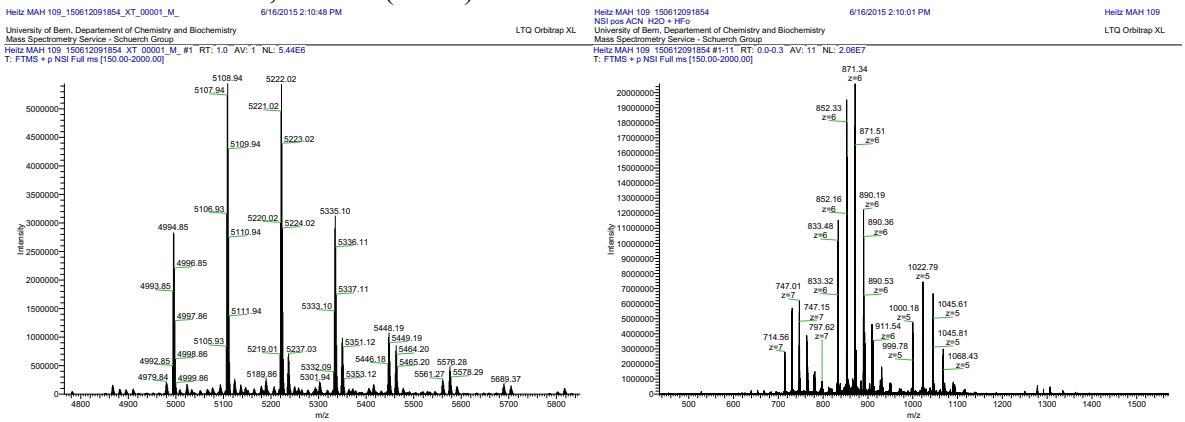
MH13D1 ((kl)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{16}$)K(C$_{16}$)) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (16.2 mg, 2.23 µmol, 2%). Analytical RP-HPLC: t_R = 3.81 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{254}H_{491}N_{59}O_{39}$ calc./obs. 4992.83/4992.85 [M+H$^+$].



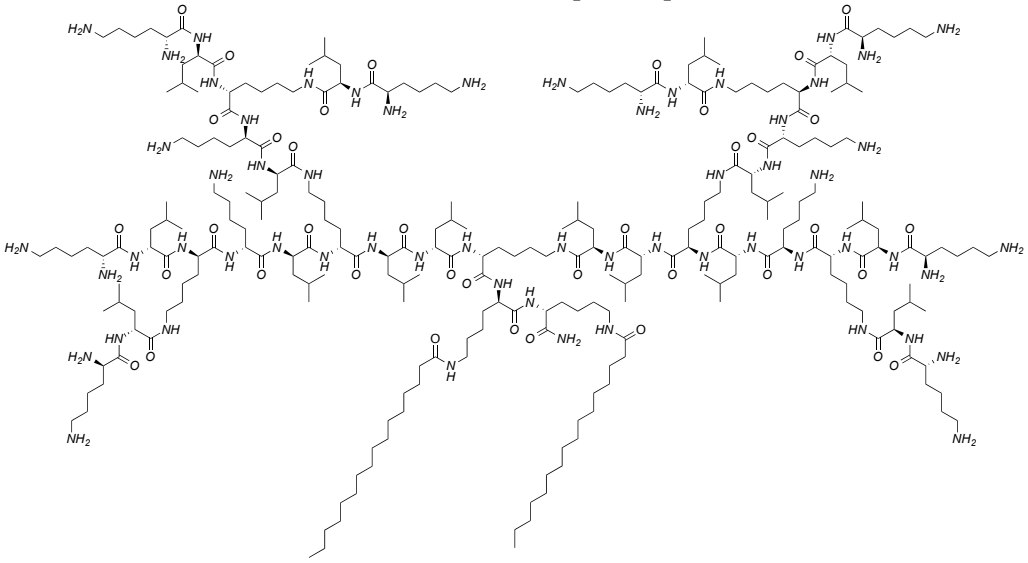
LCMS



MASS SPECTRUM, HRMS (NSI+):

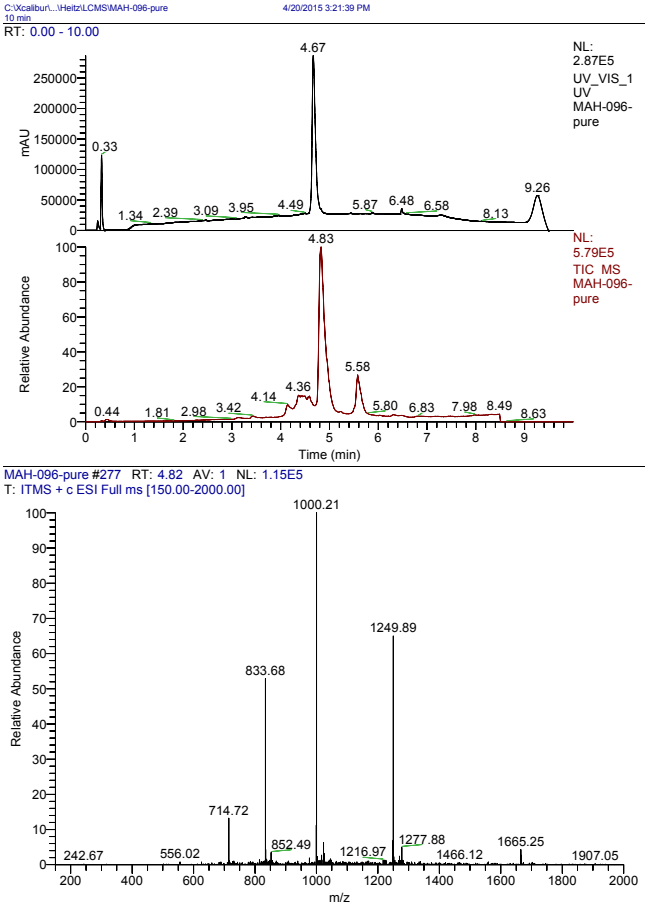


DMH13 **((kl)$_8$(*k*kl)$_4$(*k*ll)$_2$*k*k(C$_{16}$)k(C$_{16}$))** was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (128.4 mg, 17.65 µmol, 7%). Analytical RP-HPLC: t_R = 4.67 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{254}H_{491}N_{59}O_{39}$ calc./obs. 4992.83/4992.84 [M+H$^+$].

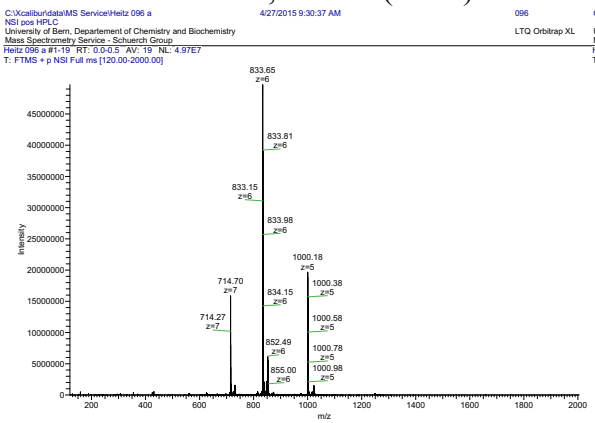


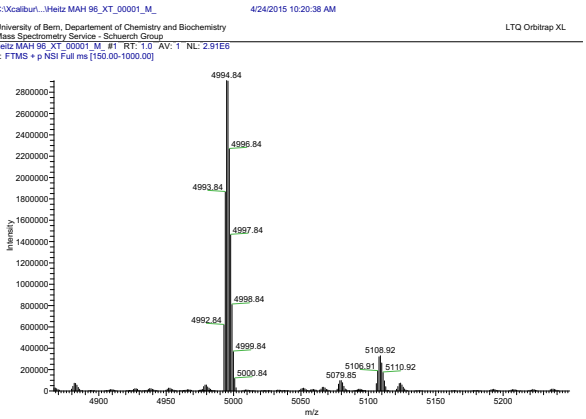
Chemical Formula: $C_{254}H_{491}N_{59}O_{39}$
Molecular Weight: 4996,10

LCMS

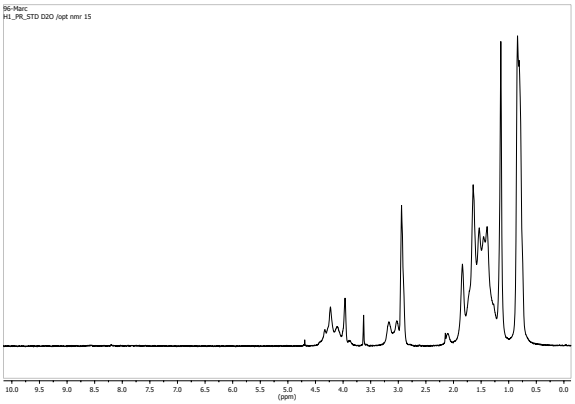


MASS SPECTRUM, HRMS (NSI+):

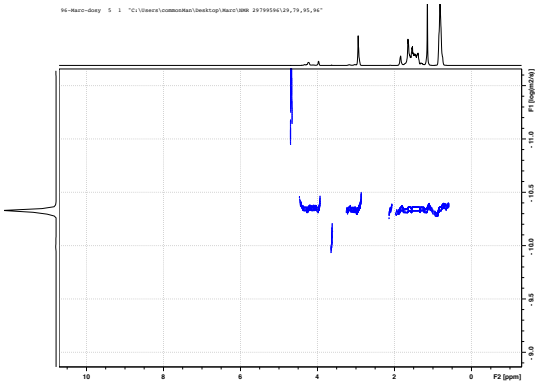


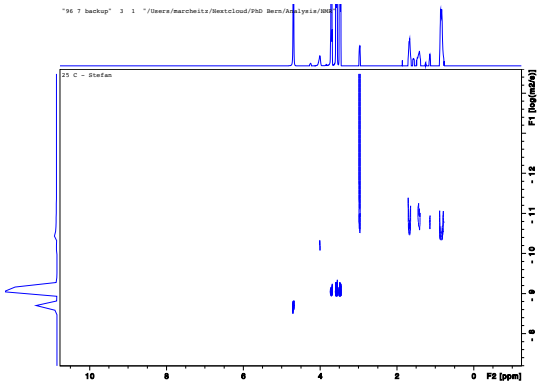


^{1}H NMR

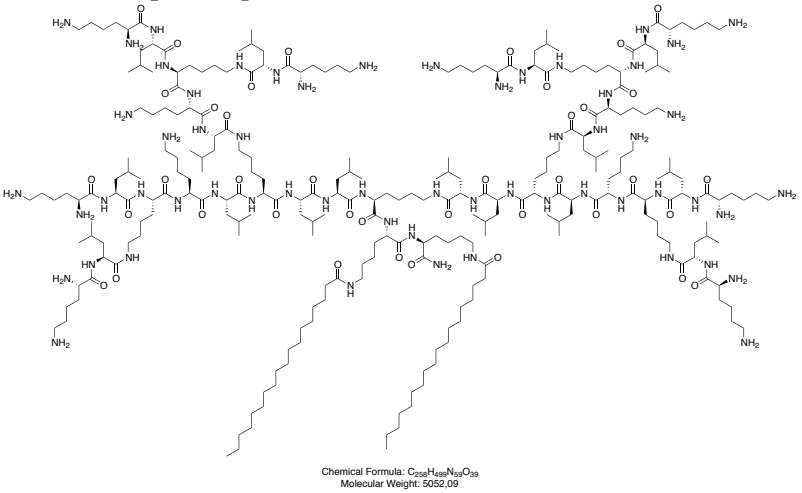


DOSY NMR pH 5 and pH 7.4

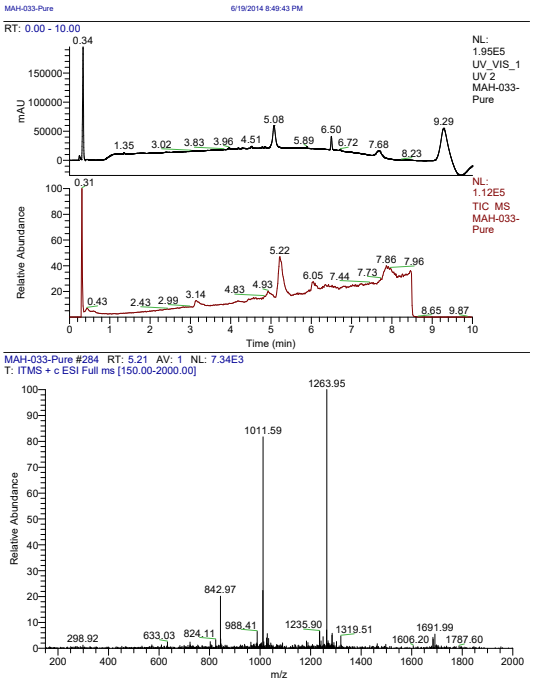




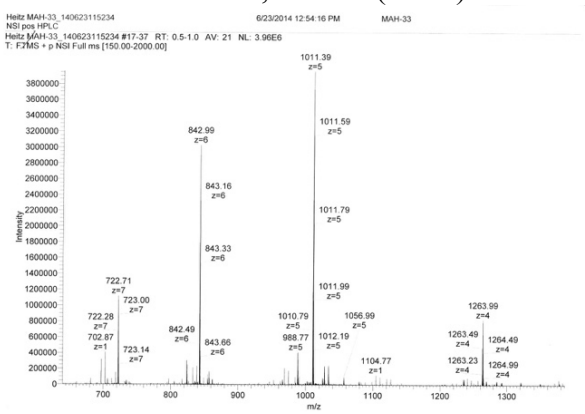
MH14 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{18}$)K(C$_{18}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (2.4 mg, 0.3 μmol, 1%). Analytical RP-HPLC: t_R = 5.08 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{258}H_{499}N_{59}O_{39}$ calc./obs. 5048.89/5048.91 [M+H$^+$].

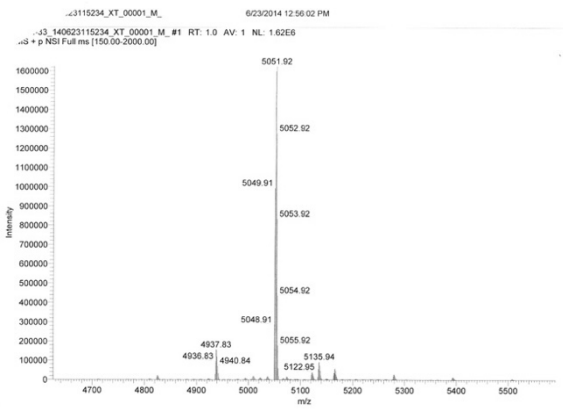


LCMS

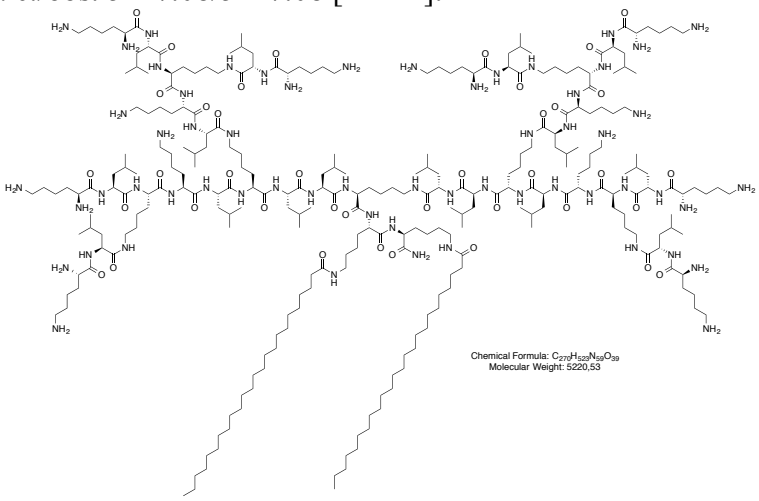


MASS SPECTRUM, HRMS (NSI+):

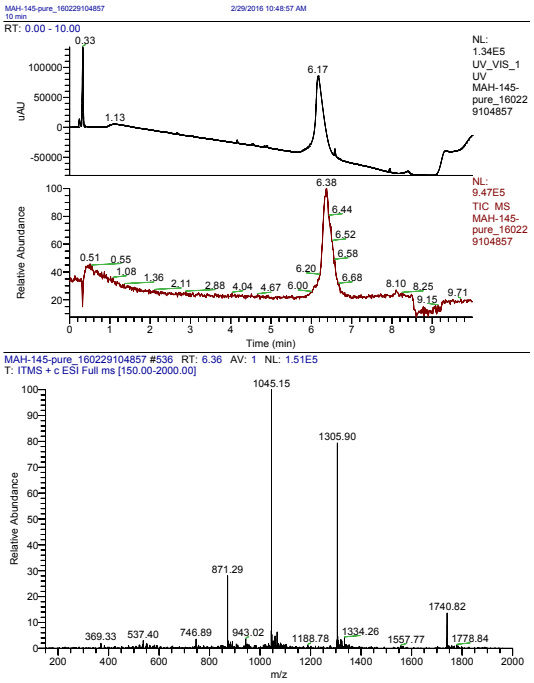




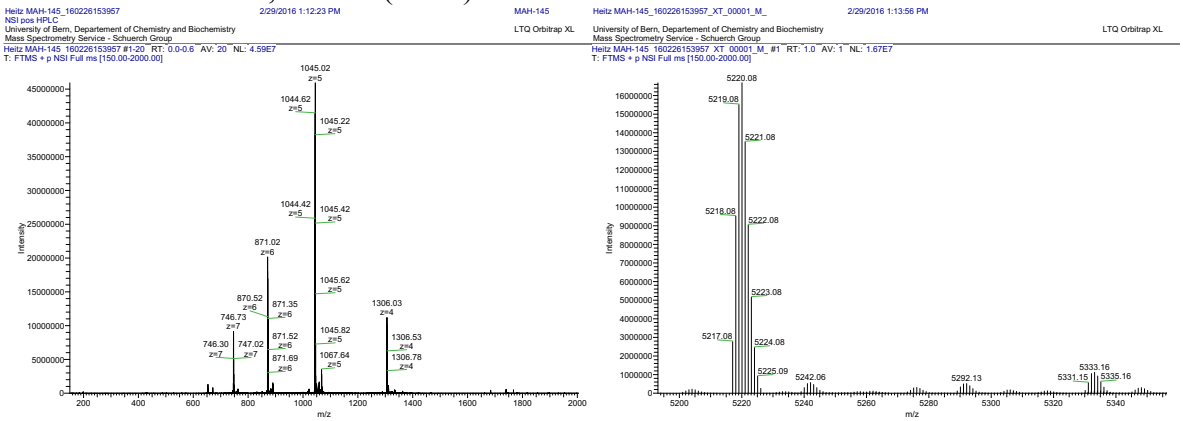
MH15 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_{24}$)K(C$_{24}$)) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (13.4 mg, 1.79 µmol, 3%). Analytical RP-HPLC: t_R = 6.17 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{270}H_{523}N_{59}O_{39}$ calc./obs. 5217.08/5217.08 [M+H$^+$].



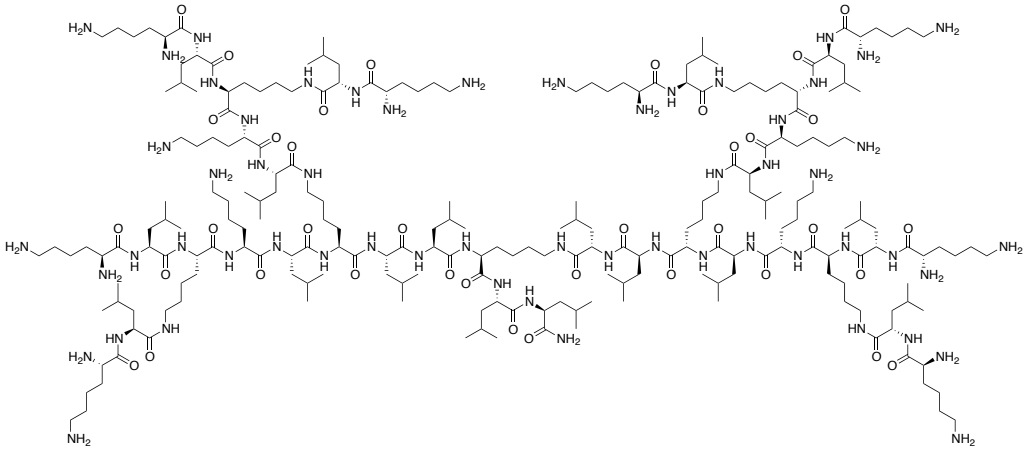
LCMS



MASS SPECTRUM, HRMS (NSI+):

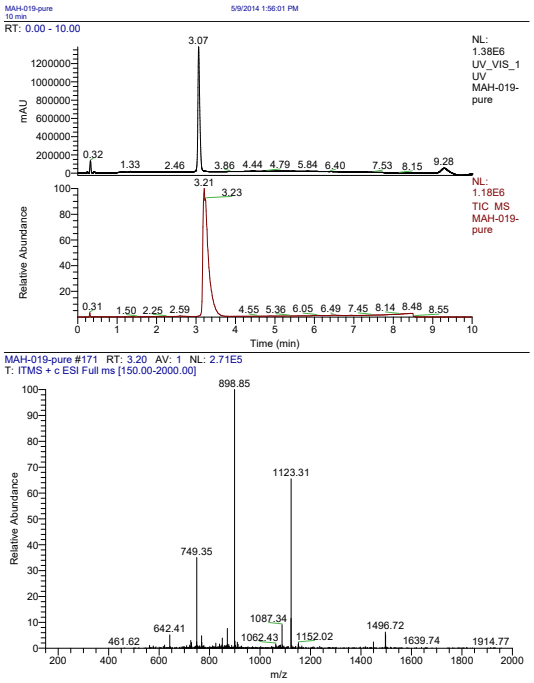


MH16 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (11 mg, 1.62 μmol, 2%). Analytical RP-HPLC: t_R = 3.07 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{222}H_{429}N_{57}O_{37}$ calc./obs. 4486.34/4486.35 [M+H$^+$].

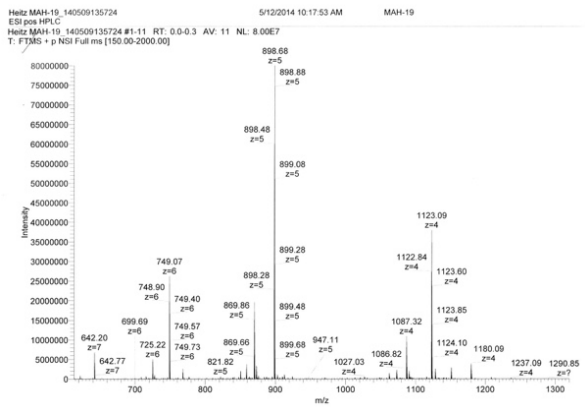


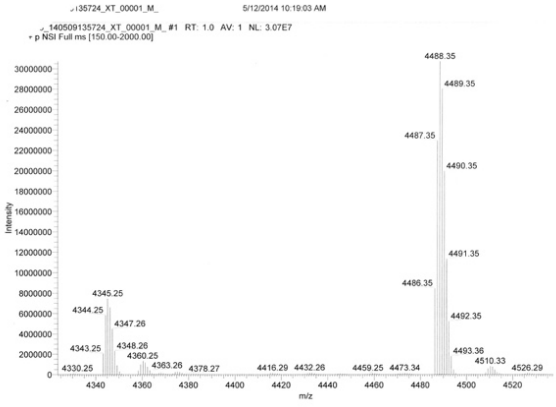
Chemical Formula: $C_{222}H_{429}N_{57}O_{37}$
Molecular Weight: 4489,14

LCMS

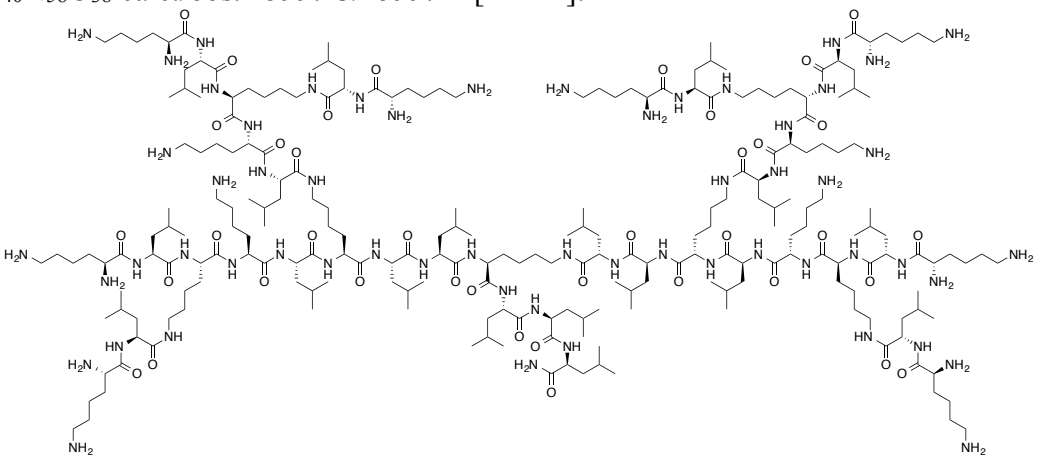


MASS SPECTRUM, HRMS (NSI+):



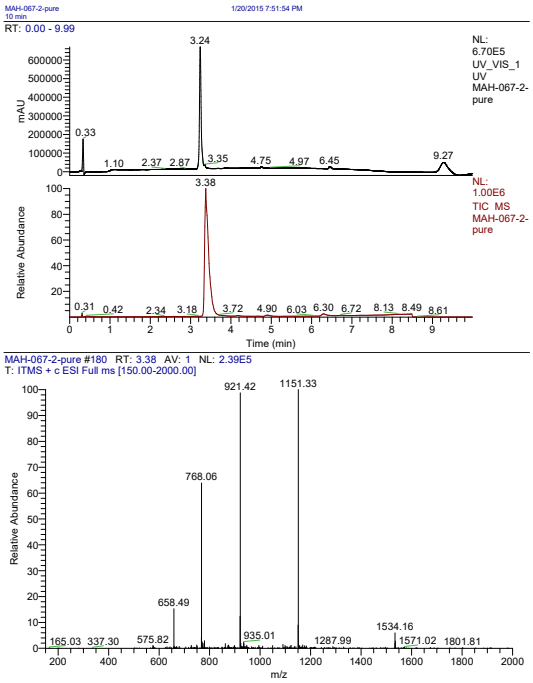


MH17 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLL) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (16.8 mg, 2.44 µmol, 3%). Analytical RP-HPLC: t_R = 3.24 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{228}H_{440}N_{58}O_{38}$ calc./obs. 4599.43/4599.44 [M+H$^+$].

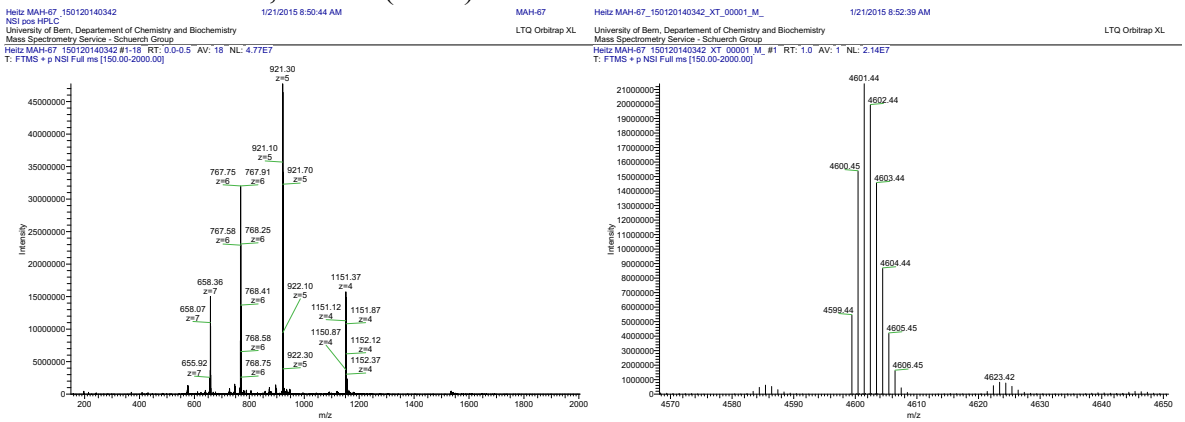


Chemical Formula: $C_{228}H_{440}N_{58}O_{38}$
Molecular Weight: 4602,40

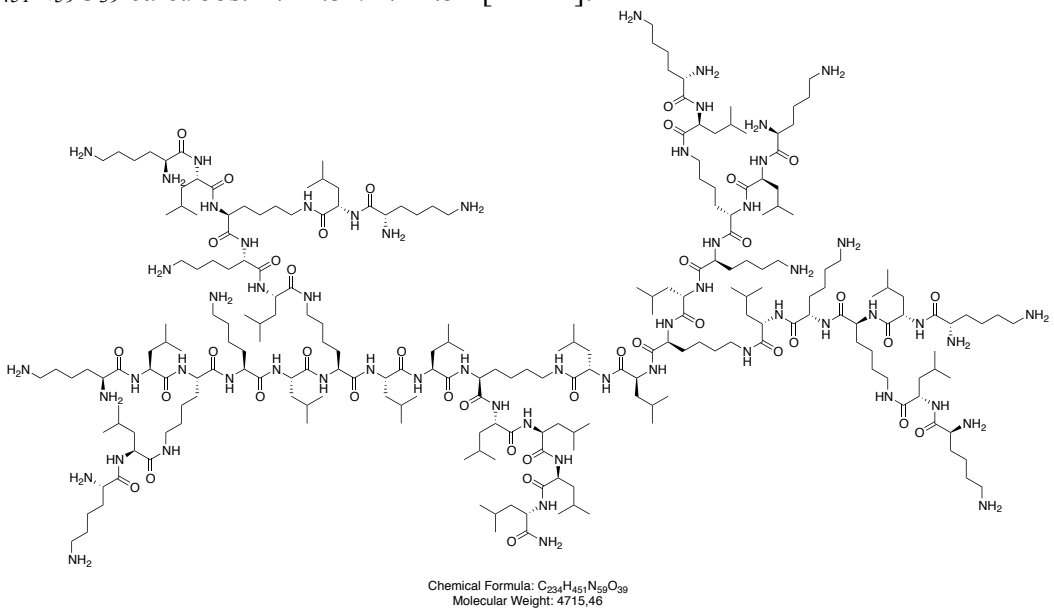
LCMS



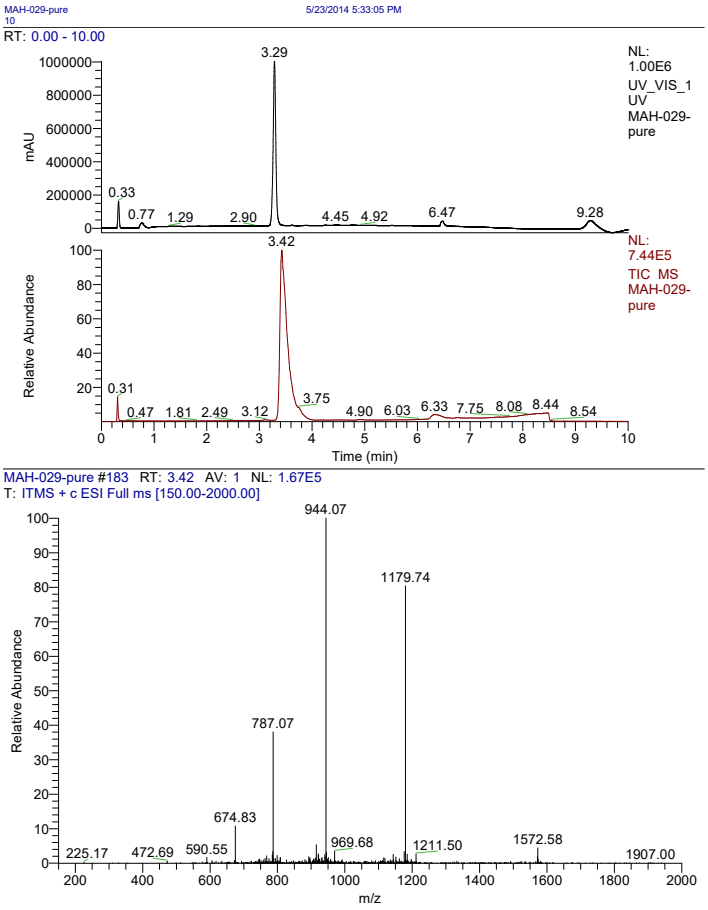
MASS SPECTRUM, HRMS (NSI+):



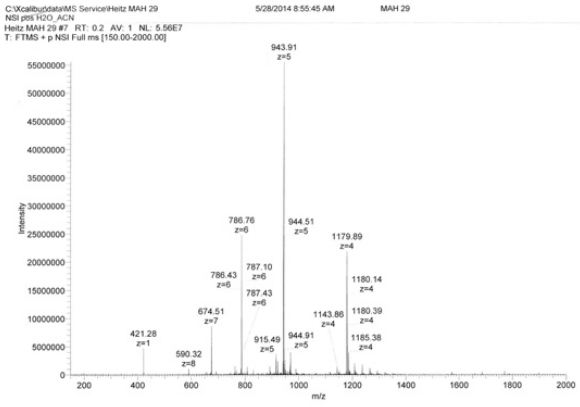
MH18 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (52.7 mg, 7.53 µmol, 8%). Analytical RP-HPLC: t_R = 3.29 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.52 [M+H$^+$].

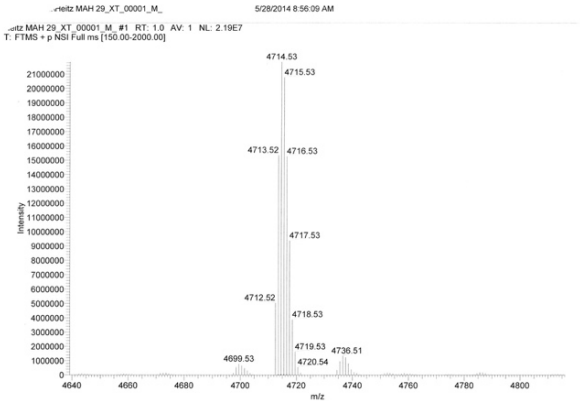


LCMS

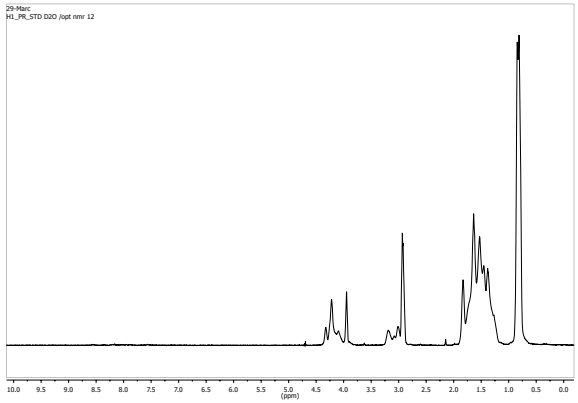


MASS SPECTRUM, HRMS (NSI+):

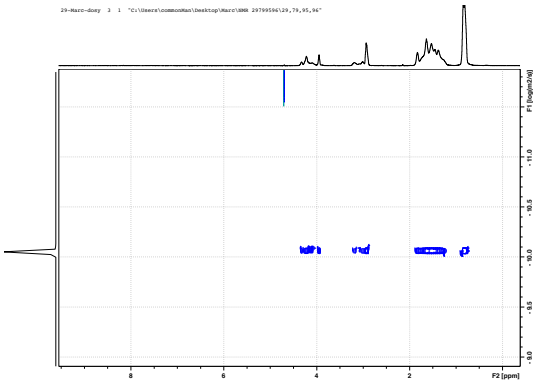


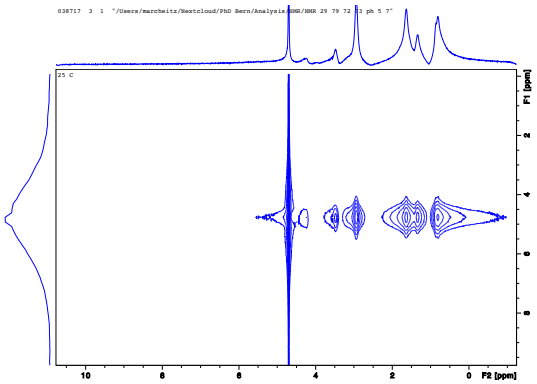


^{1}H NMR

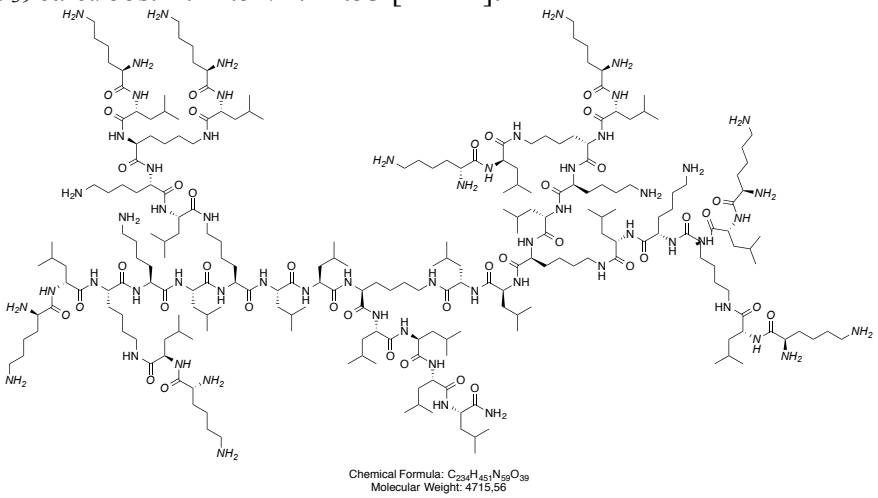


DOSY NMR pH 5 and pH 7.4

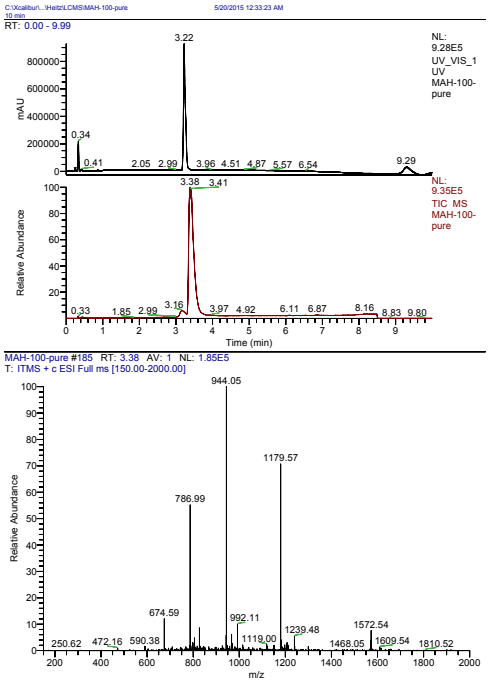




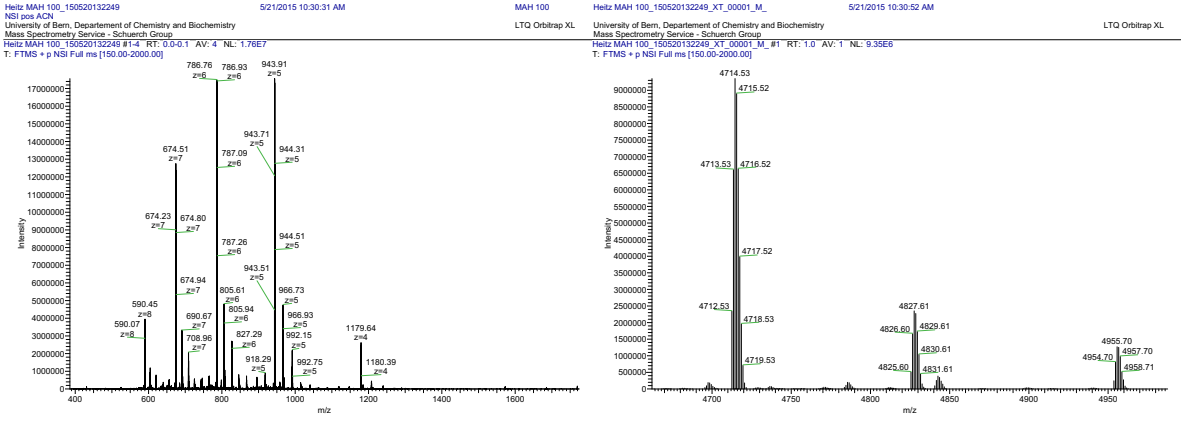
MH18D1 ((kl)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (25.1 mg, 3.59 µmol, 4%). Analytical RP-HPLC: t_R = 3.22 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.53 [M+H$^+$].



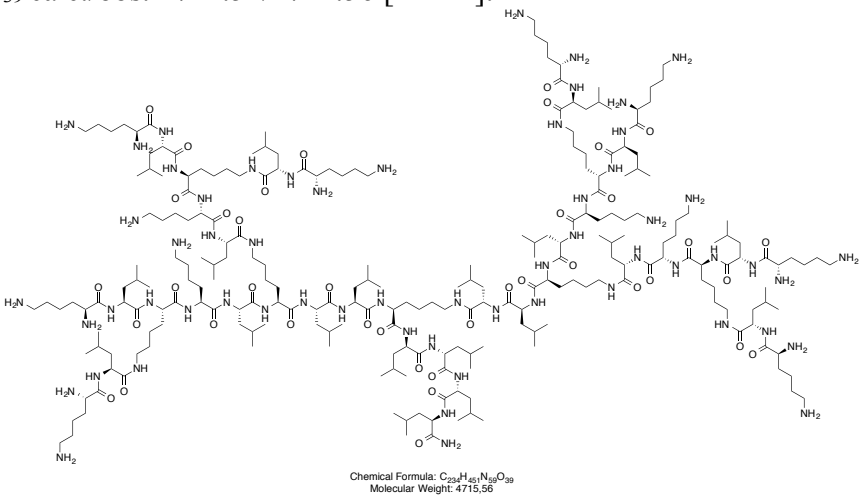
LCMS



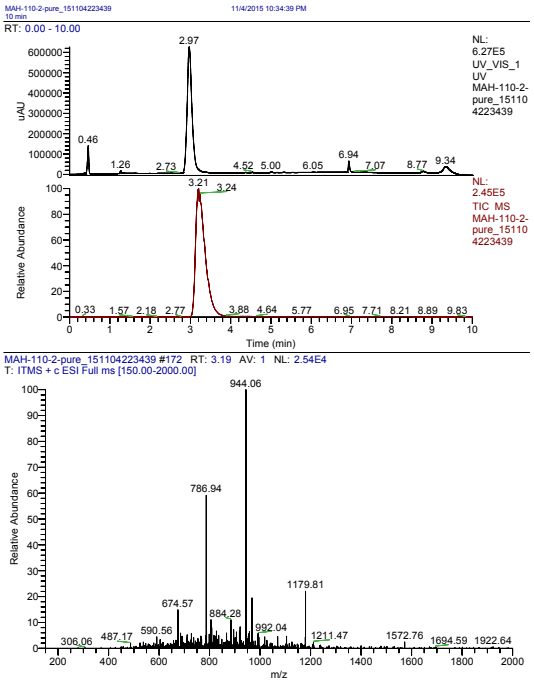
MASS SPECTRUM, HRMS (NSI+):



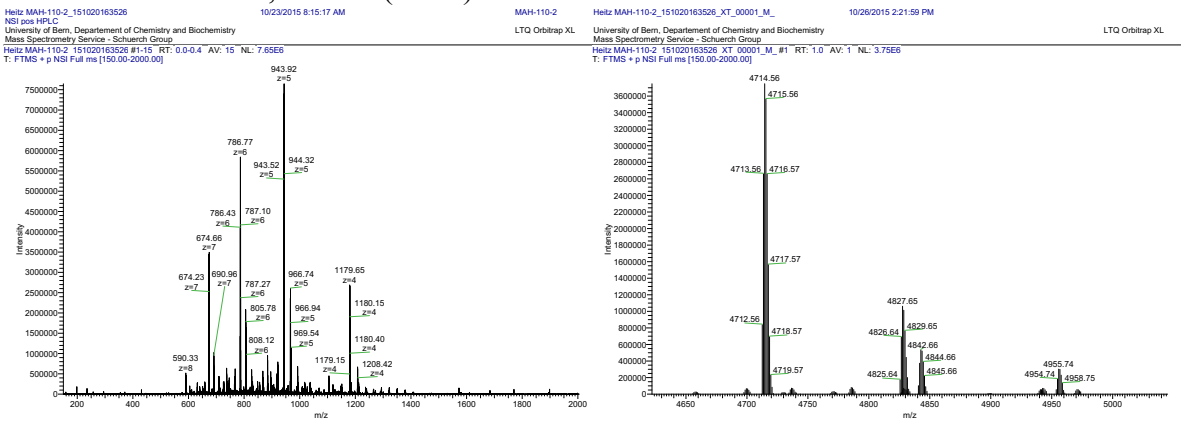
MH18D2 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*llll) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (24.0 mg, 3.43 µmol, 3%). Analytical RP-HPLC: t_R = 2.97 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.56 [M+H$^+$].



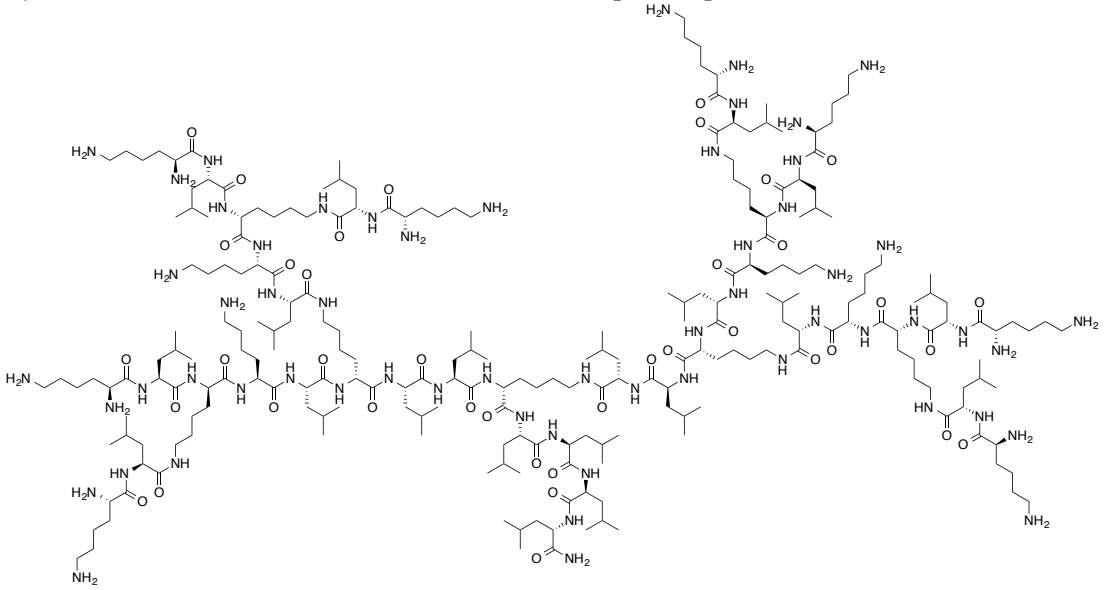
LCMS



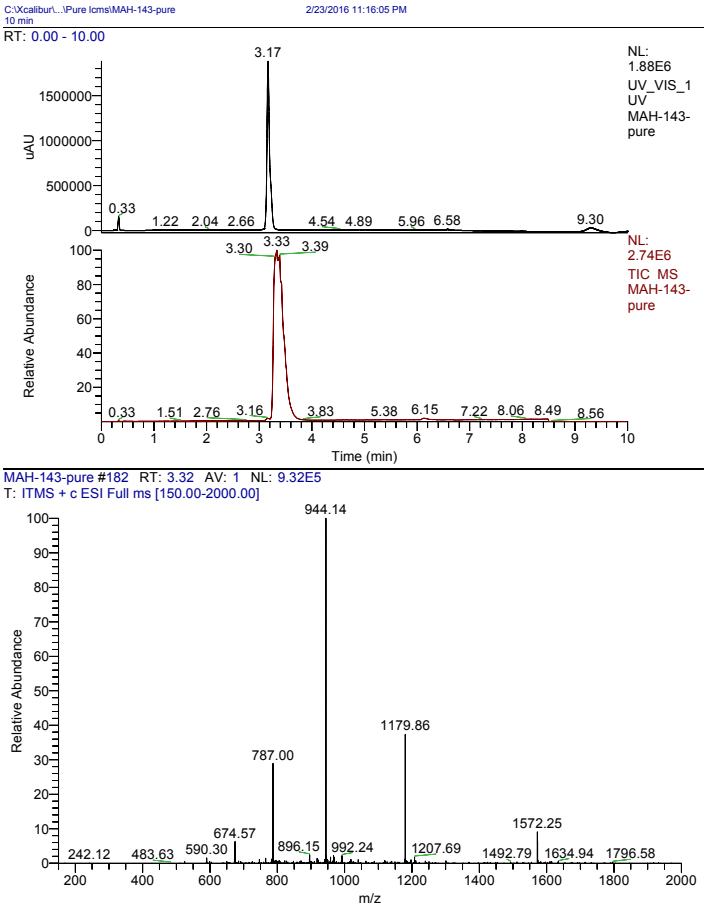
MASS SPECTRUM, HRMS (NSI+):



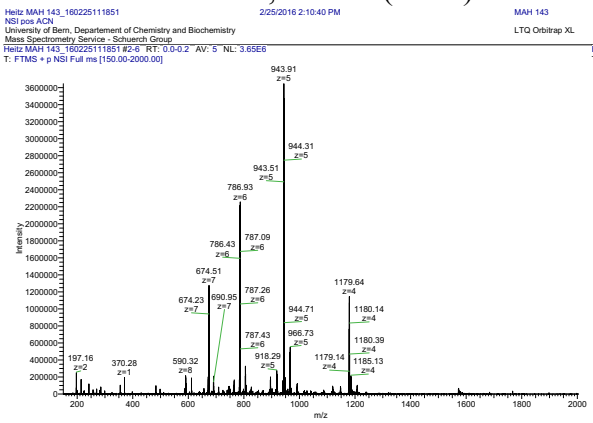
MH18D3 ((KL)$_8$(*k*KL)4(*k*LL)$_2$*k*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (79.3 mg, 11.34 µmol, 16%). Analytical RP-HPLC: t_R = 3.17 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.52 [M+H$^+$].

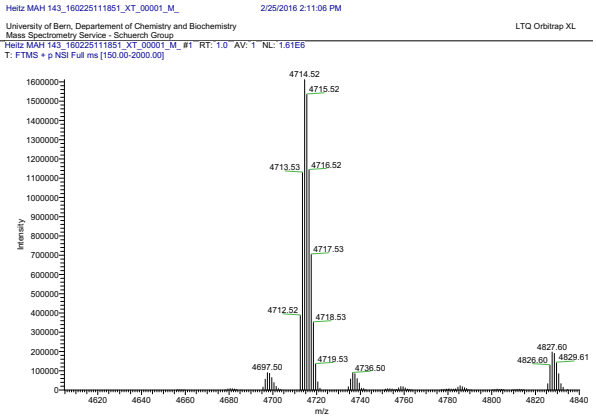


LCMS

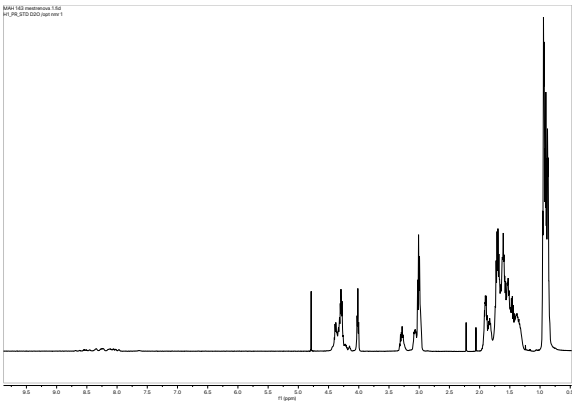


MASS SPECTRUM, HRMS (NSI+):

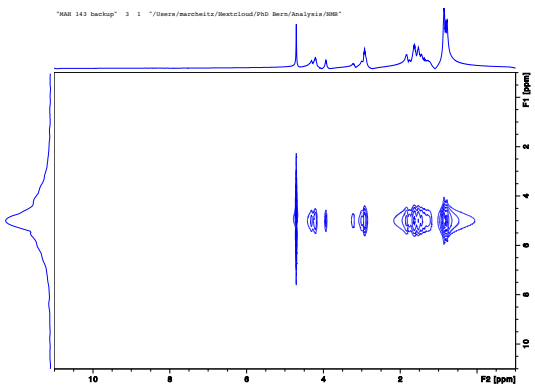


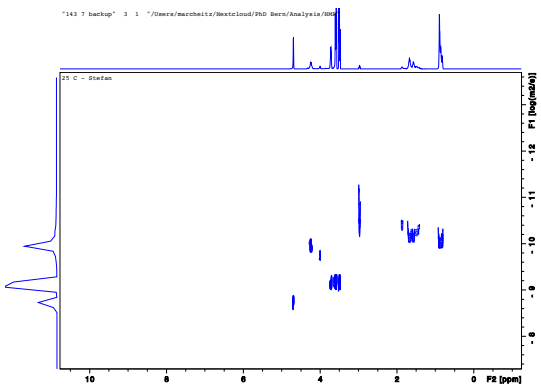


^{1}H NMR

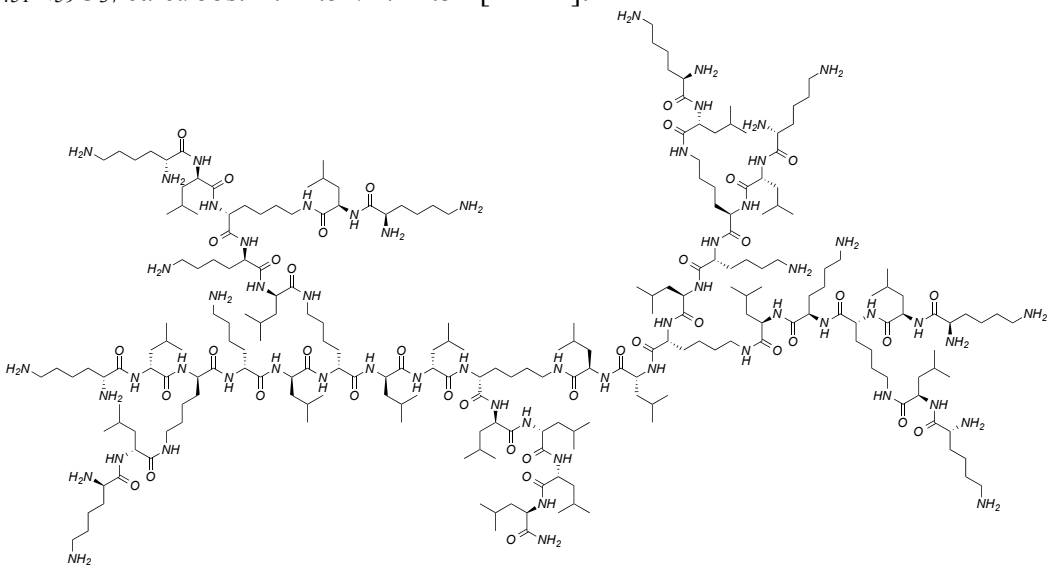


DOSY NMR pH 5 and 7.4



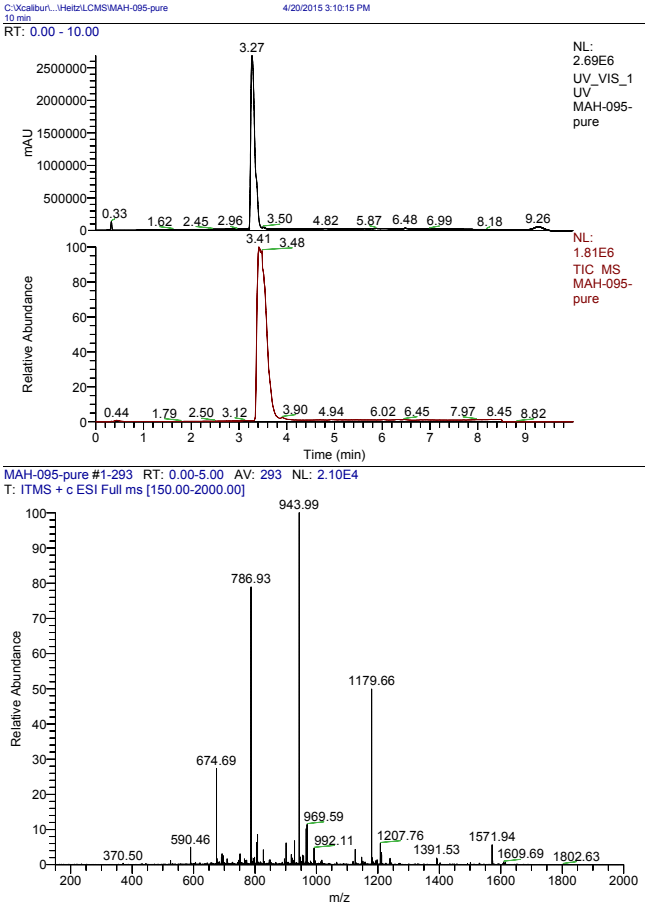


DMH18 ((kl)$_8$(*k*kl)$_4$(*k*ll)$_2$*k*llll) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (73.6 mg, 10.5 µmol, 11%). Analytical RP-HPLC: t_R = 3.27 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{37}$ calc./obs. 4712.51/4712.52 [M+H$^+$].

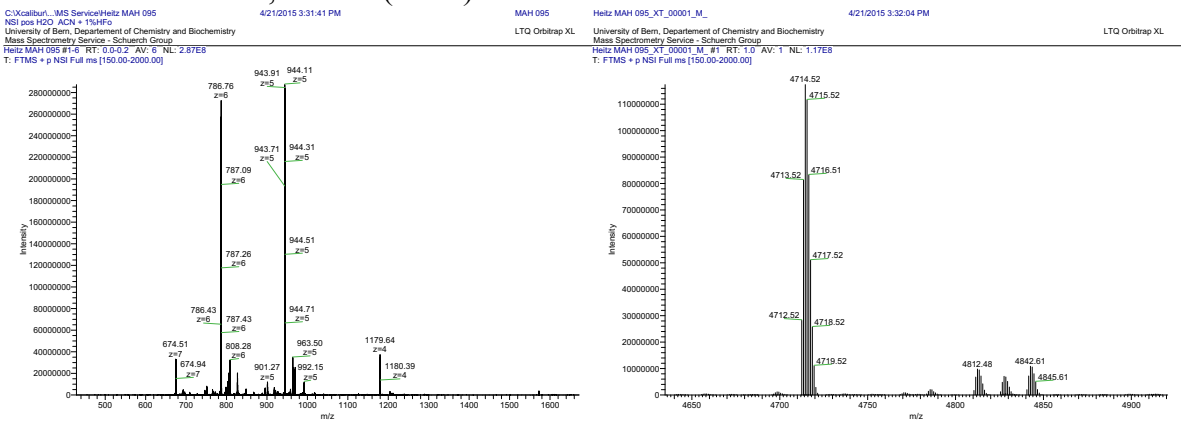


Chemical Formula: $C_{234}H_{451}N_{59}O_{39}$
Molecular Weight: 4715,56

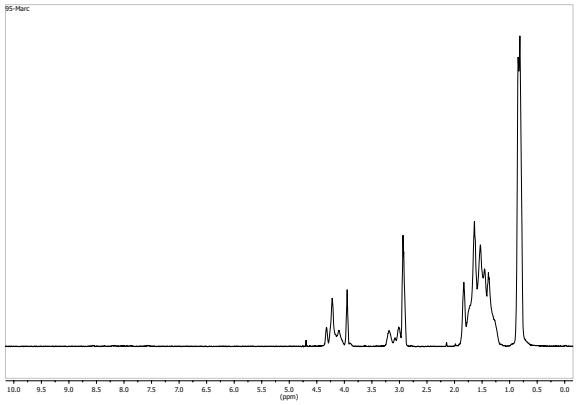
LCMS



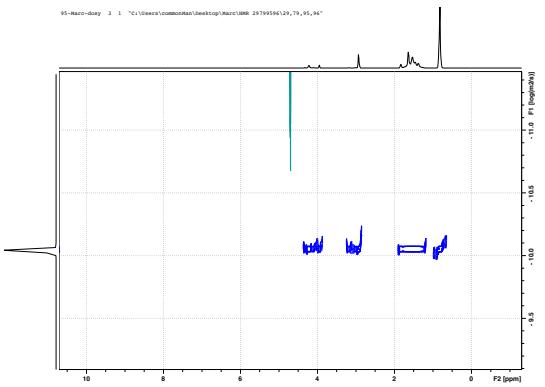
MASS SPECTRUM, HRMS (NSI+):

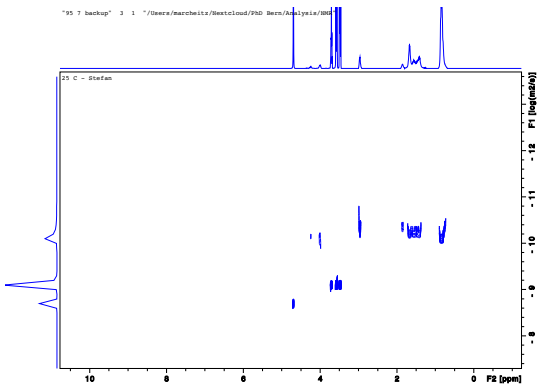


^{1}H NMR

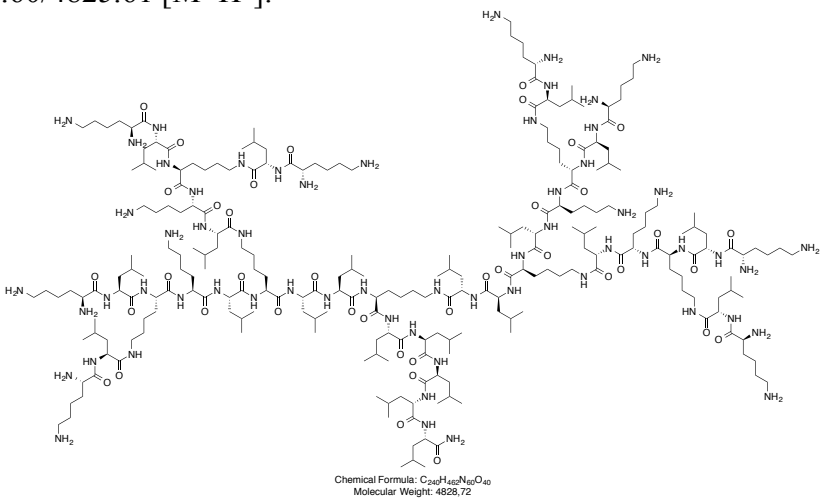


DOSY NMR pH 5 and pH 7.4

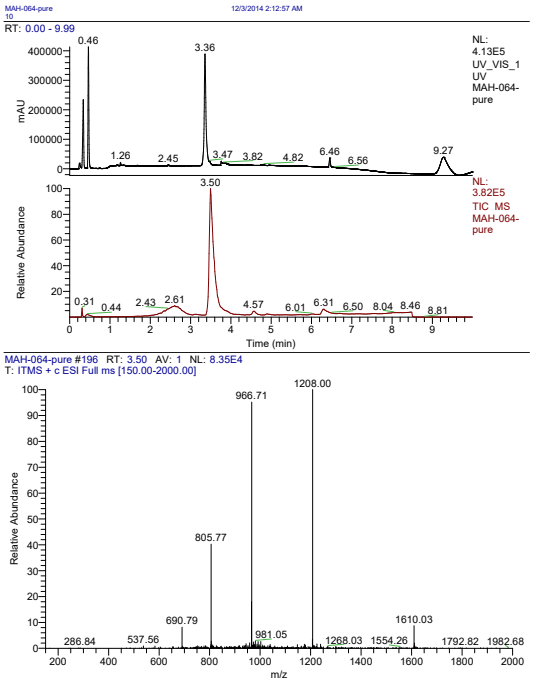




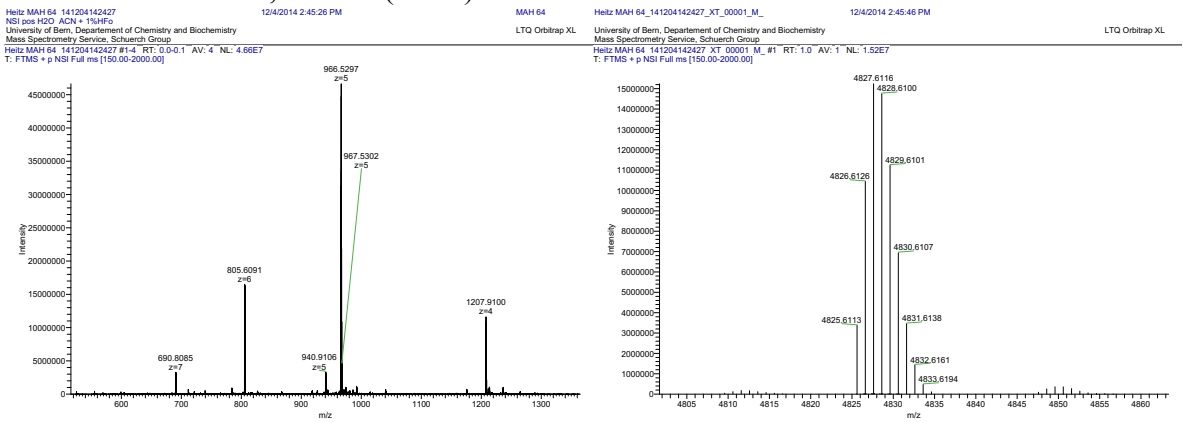
MH19 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (4.7 mg, 0.66 µmol, 1%). Analytical RP-HPLC: t_R = 3.36 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{240}H_{462}N_{60}O_{40}$ calc./obs. 4825.60/4825.61 [M+H$^+$].



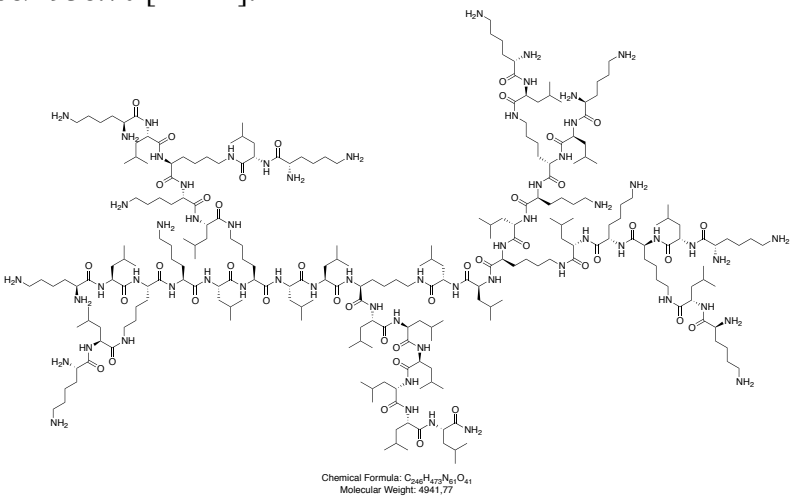
LCMS



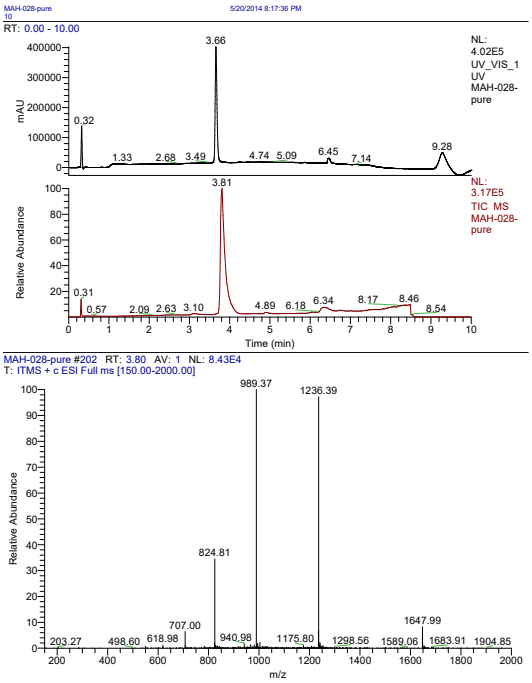
MASS SPECTRUM, HRMS (NSI+):



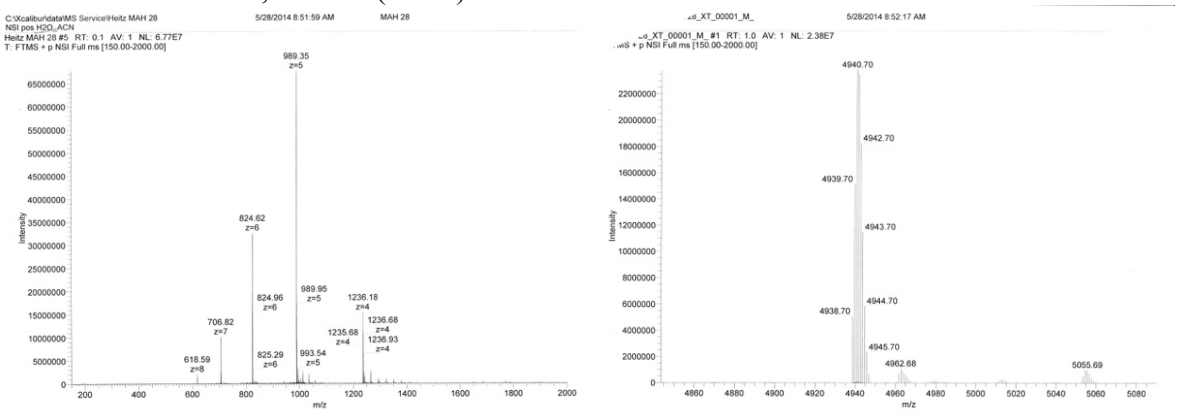
MH20 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (12.2 mg, 1.7 μmol, 2%). Analytical RP-HPLC: t_R = 3.66 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{246}H_{473}N_{61}O_{41}$ calc./obs. 4938.68/4938.70 [M+H$^+$].



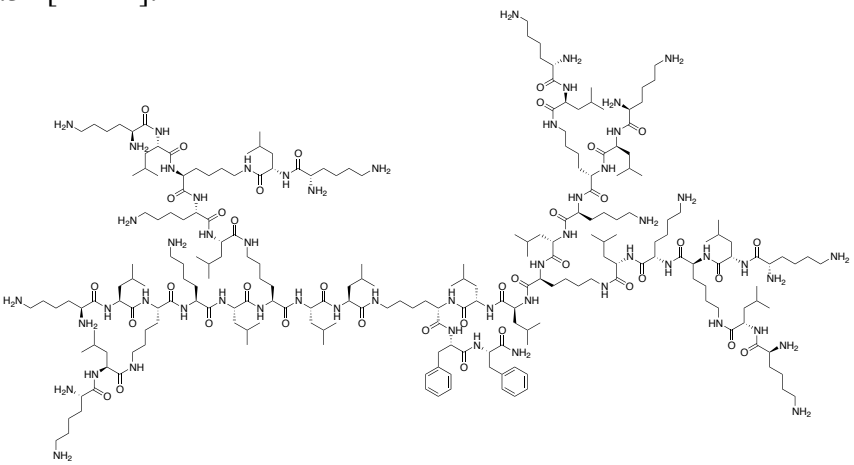
LCMS



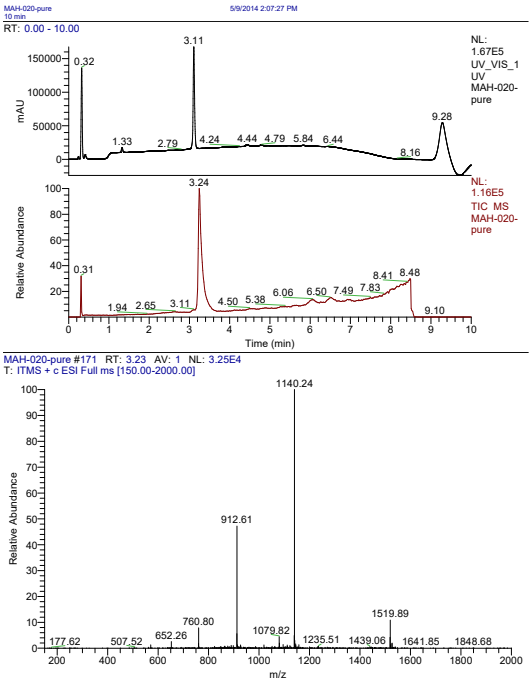
MASS SPECTRUM, HRMS (NSI+):



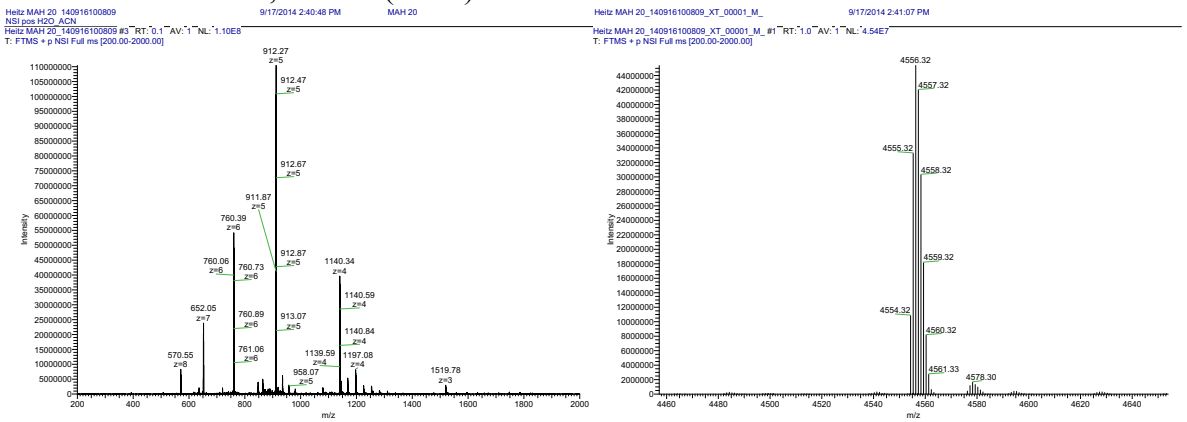
MH21 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*FF) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (16 mg, 2.34 μmol, 3%). Analytical RP-HPLC: t_R = 3.11 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{228}H_{425}N_{57}O_{37}$ calc./obs. 4554.31/4554.32 [M+H$^+$].



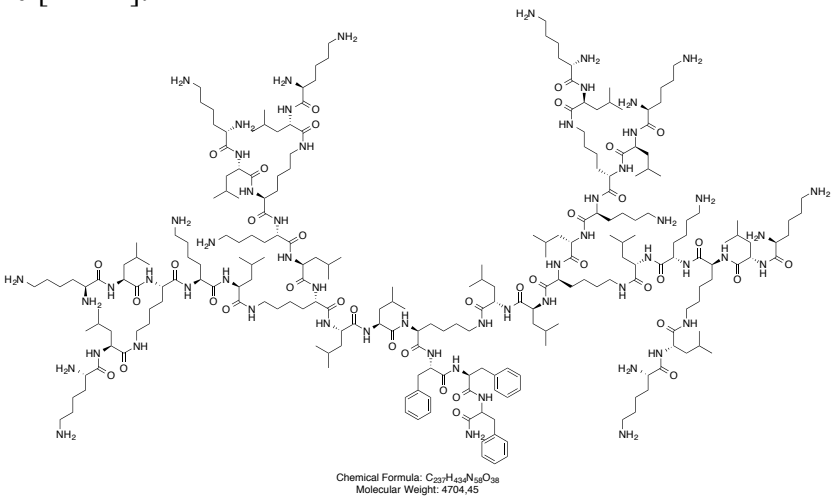
LCMS



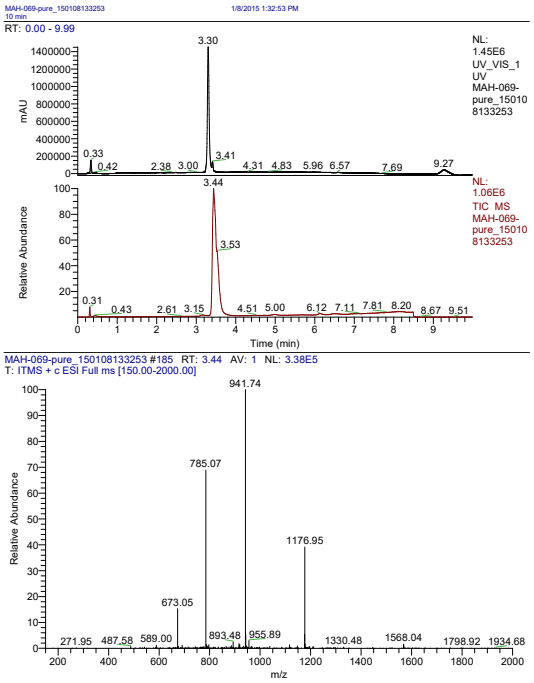
MASS SPECTRUM, HRMS (NSI+):



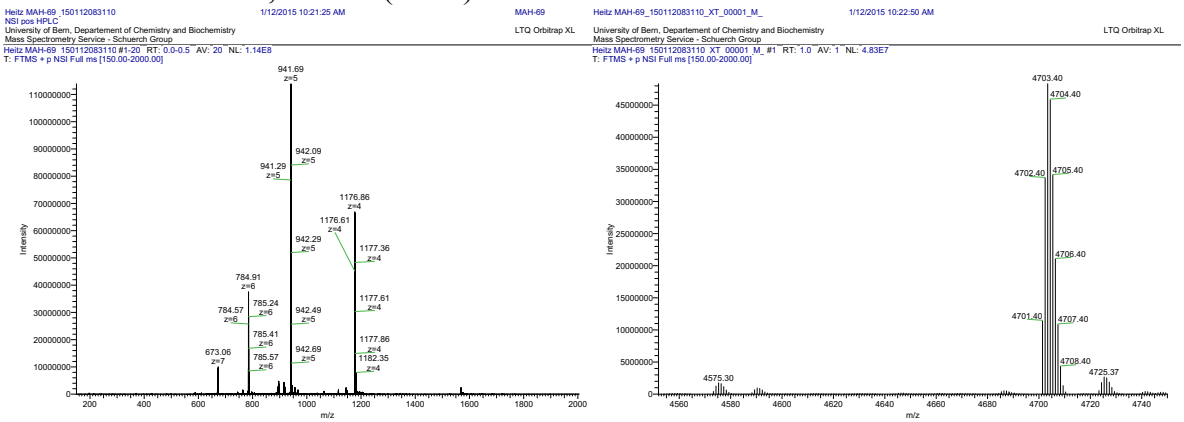
MH22 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*FFF) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (13.7 mg, 1.96 µmol, 3%). Analytical RP-HPLC: t_R = 3.30 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{237}H_{434}N_{58}O_{38}$ calc./obs. 4701.38/4701.40 [M+H$^+$].



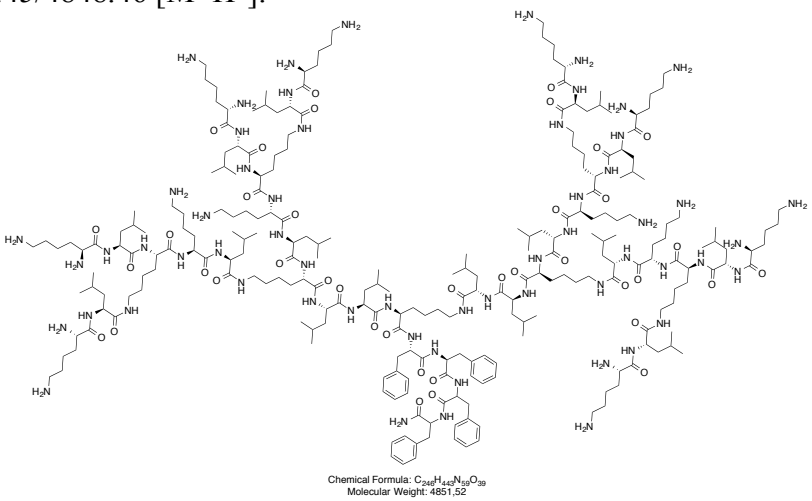
LCMS



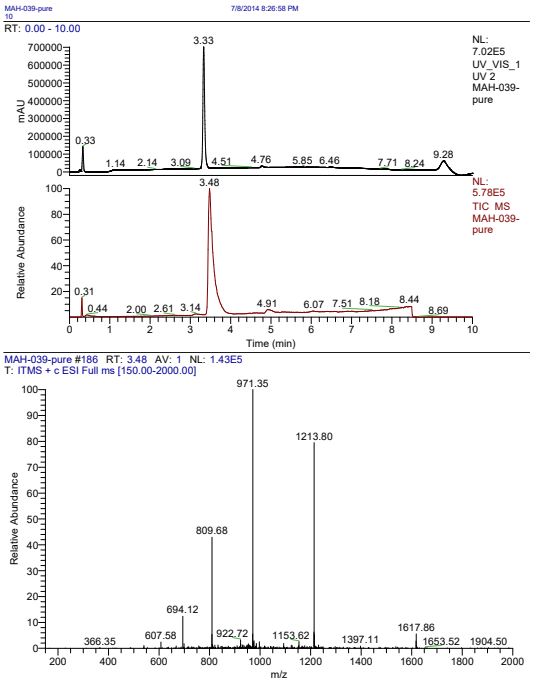
MASS SPECTRUM, HRMS (NSI+):



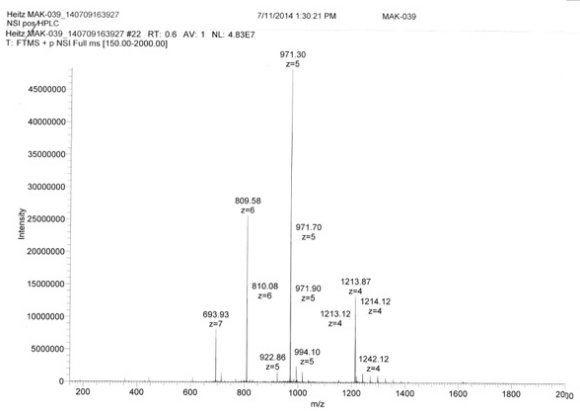
MH23 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*FFFF) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (6.0 mg, 0.8 μmol, 1%). Analytical RP-HPLC: t_R = 3.33 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{246}H_{443}N_{59}O_{39}$ calc./obs. 4848.45/4848.46 [M+H$^+$].

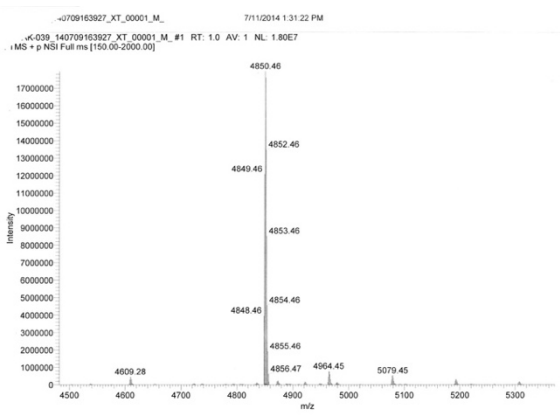


LCMS

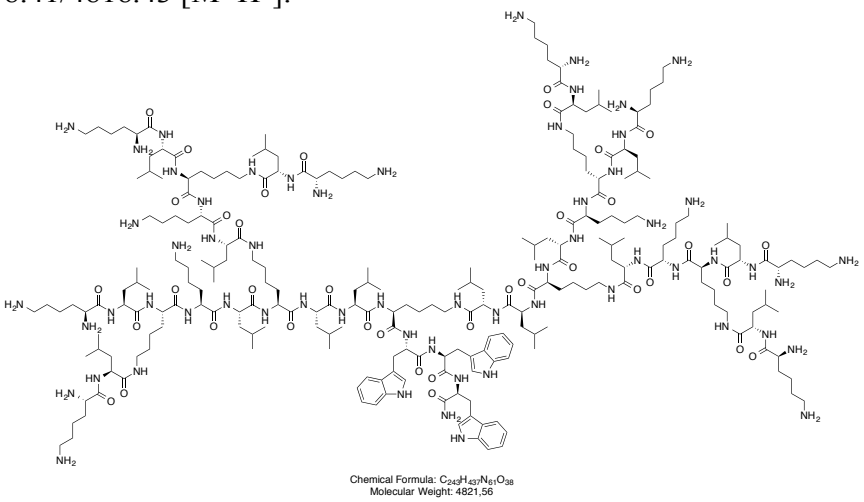


MASS SPECTRUM, HRMS (NSI+):

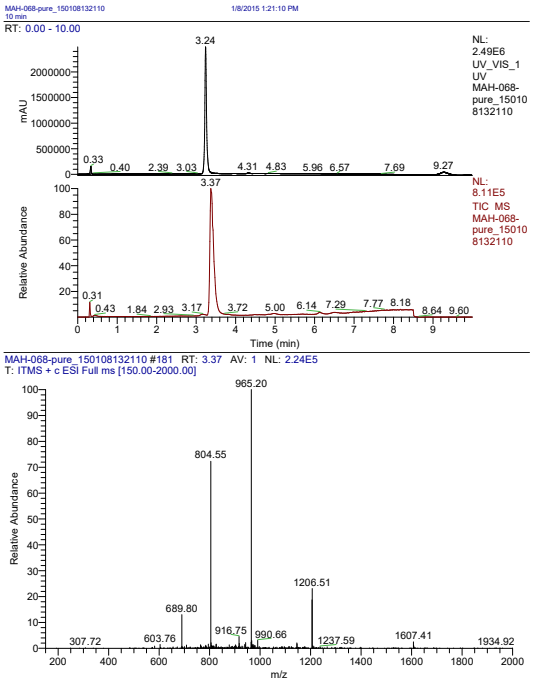




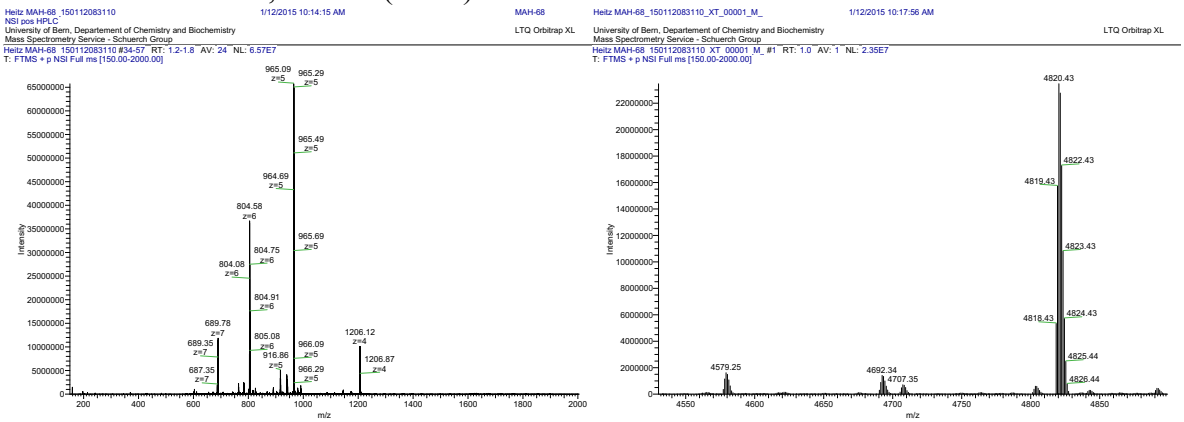
MH24 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*WWW) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (9.0 mg, 1.27 µmol, 2%). Analytical RP-HPLC: t_R = 3.24 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{243}H_{437}N_{61}O_{38}$ calc./obs. 4818.41/4818.43 [M+H$^+$].



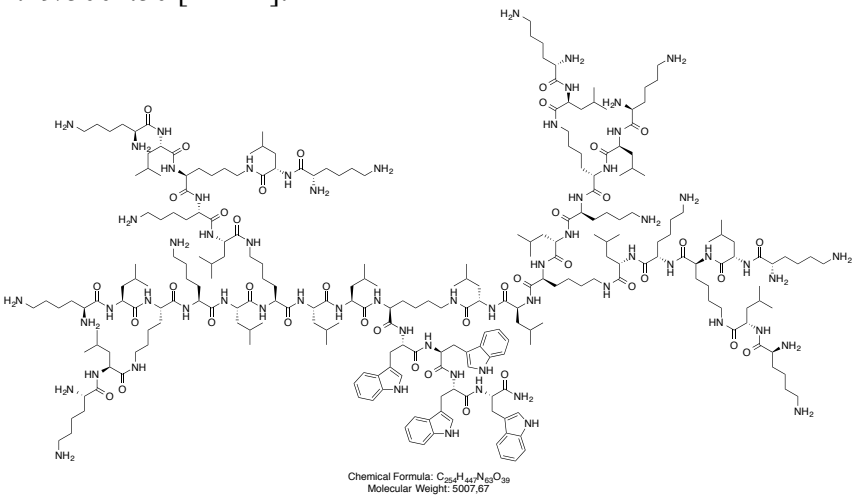
LCMS



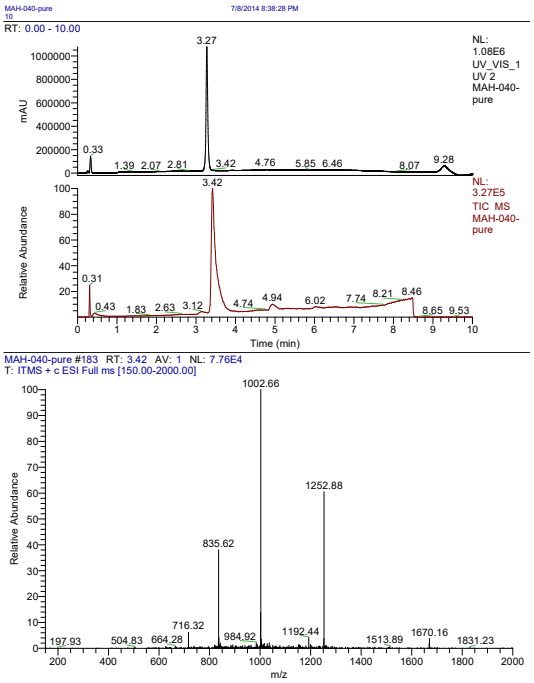
MASS SPECTRUM, HRMS (NSI+):



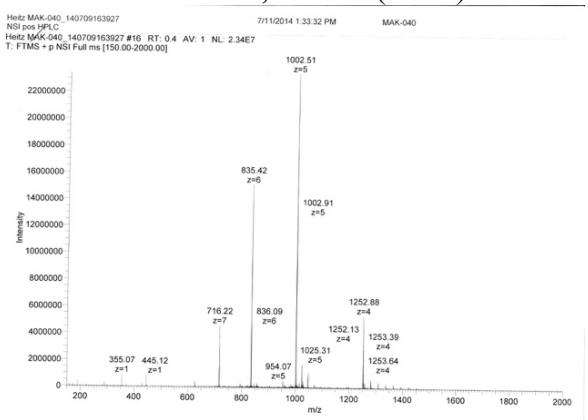
MH25 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*WWWW) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (4.5 mg, 0.6 µmol, 1%). Analytical RP-HPLC: t_R = 3.27 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{254}H_{446}N_{62}O_{40}$ calc./obs. 5004.49/5004.50 [M+H$^+$].

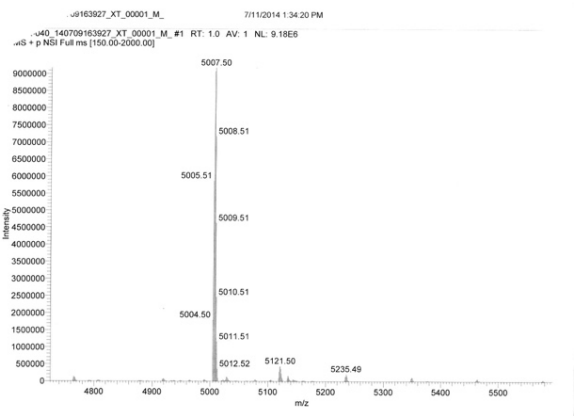


LCMS

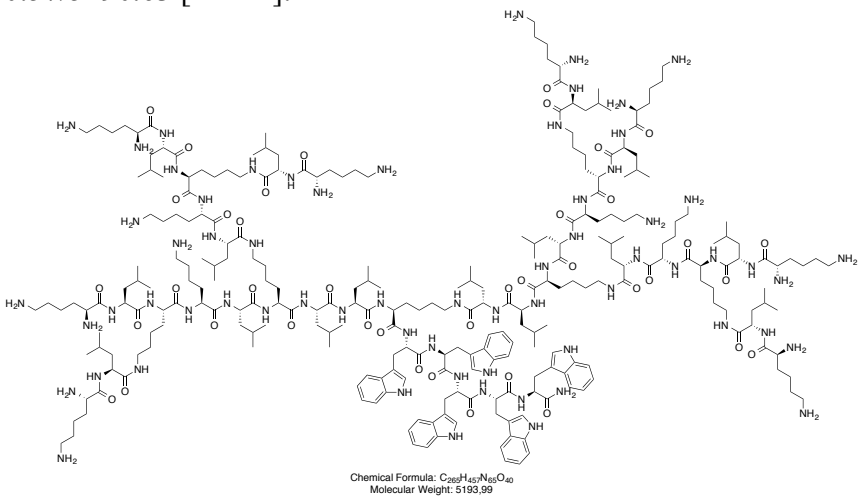


MASS SPECTRUM, HRMS (NSI+):

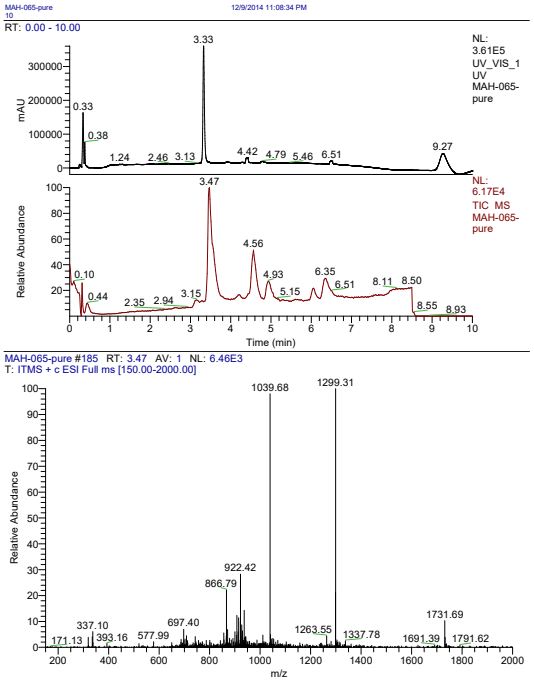




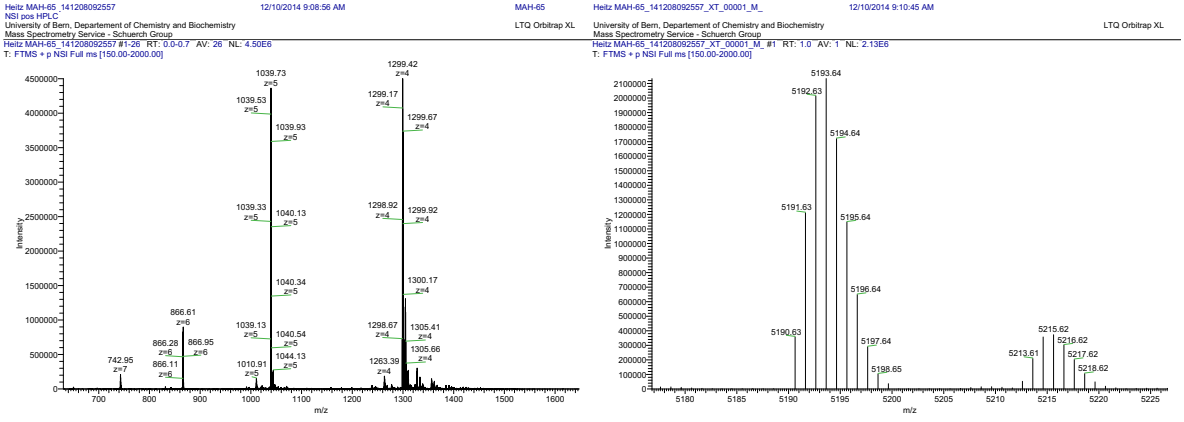
MH26 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*WWWWW) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (1.7 mg, 0.23 μmol, 1%). Analytical RP-HPLC: t_R = 3.33 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{265}H_{457}N_{65}O_{40}$ calc./obs. 5190.57/5190.63 [M+H$^+$].



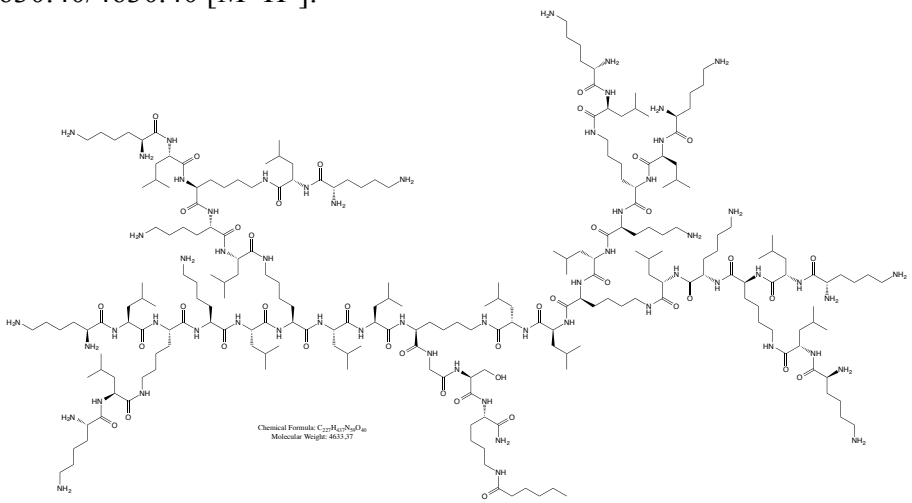
LCMS



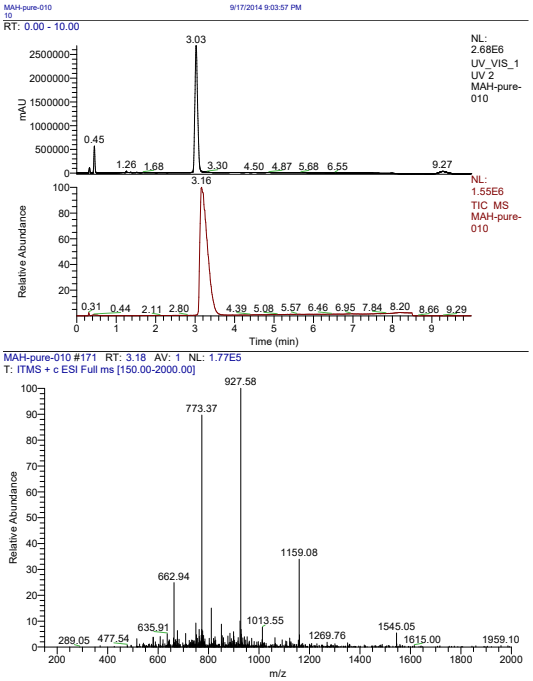
MASS SPECTRUM, HRMS (NSI+):



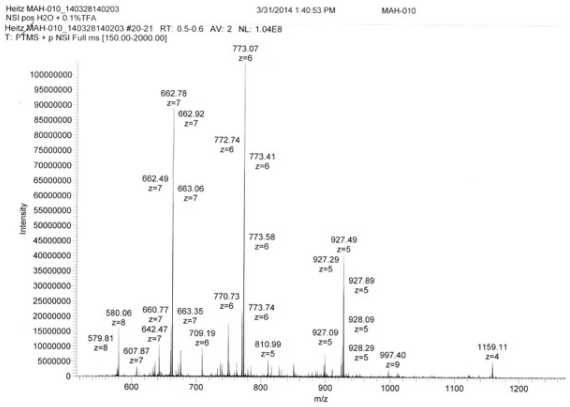
MH27 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*GSK(C$_6$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (25 mg, 3.6 µmol, 5%). Analytical RP-HPLC: t_R = 3.03 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{227}H_{437}N_{59}O_{40}$ calc./obs. 4630.40/4630.40 [M+H$^+$].

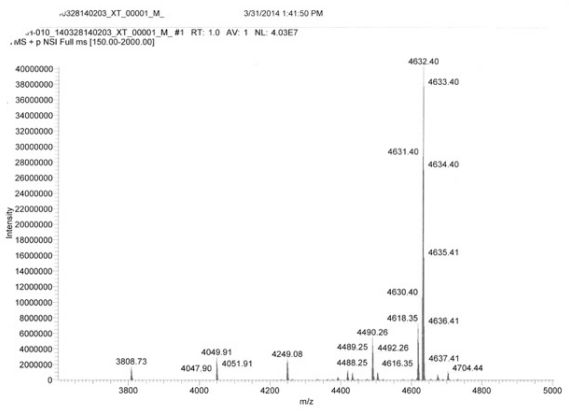


LCMS

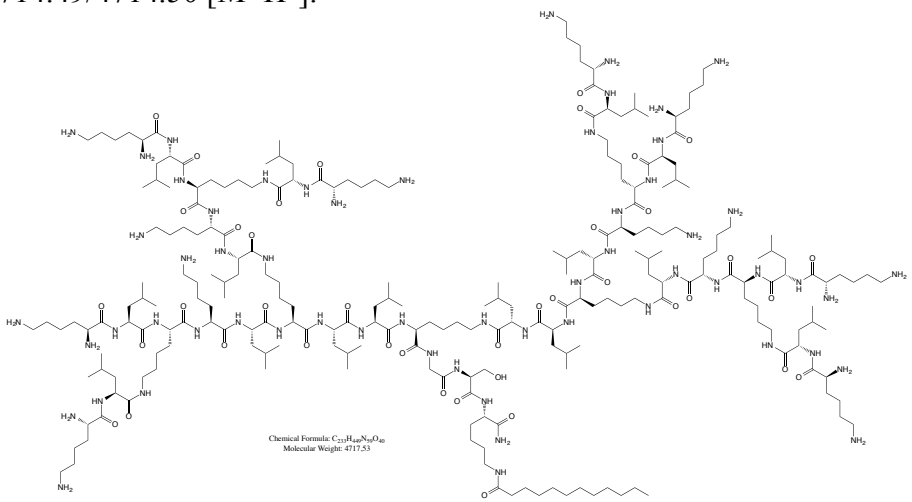


MASS SPECTRUM, HRMS (NSI+):

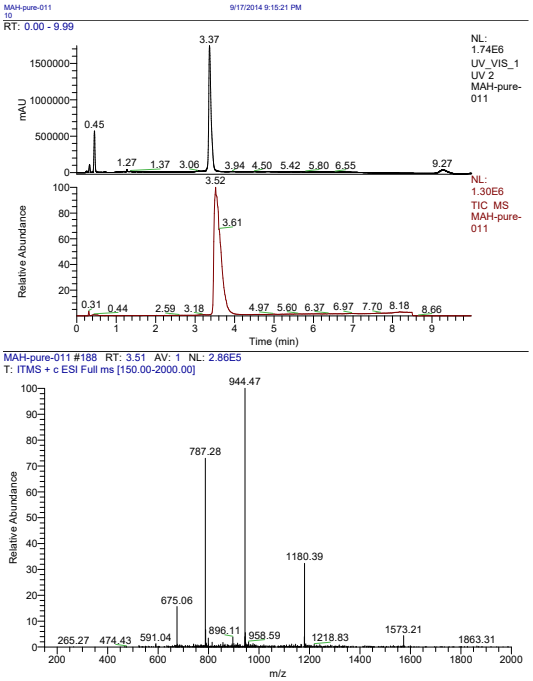




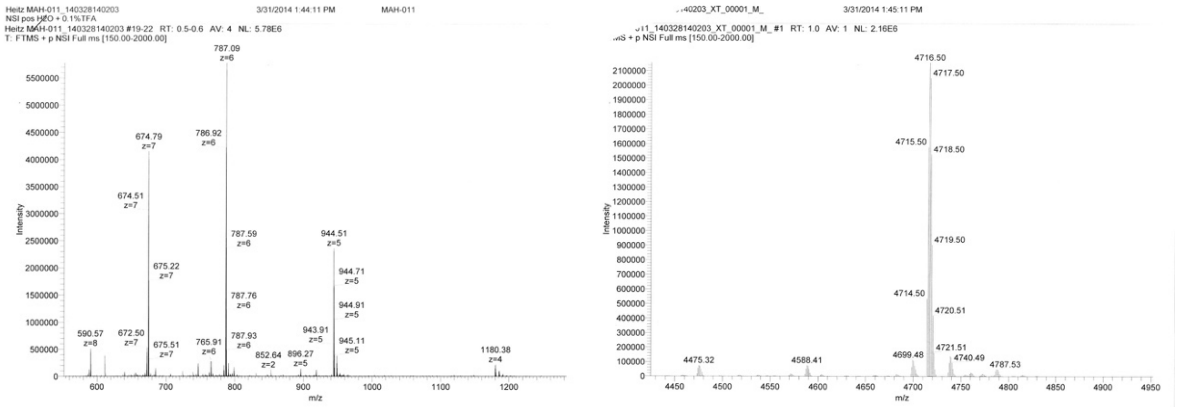
MH28 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*GSK(C$_{12}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (12 mg, 1.7 µmol, 3%). Analytical RP-HPLC: t_R = 3.37 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{233}H_{449}N_{59}O_{40}$ calc./obs. 4714.49/4714.50 [M+H$^+$].



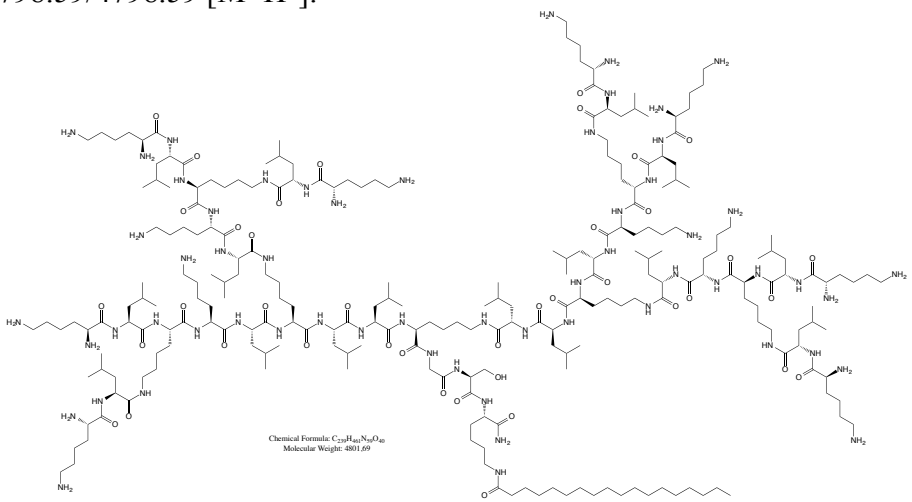
LCMS



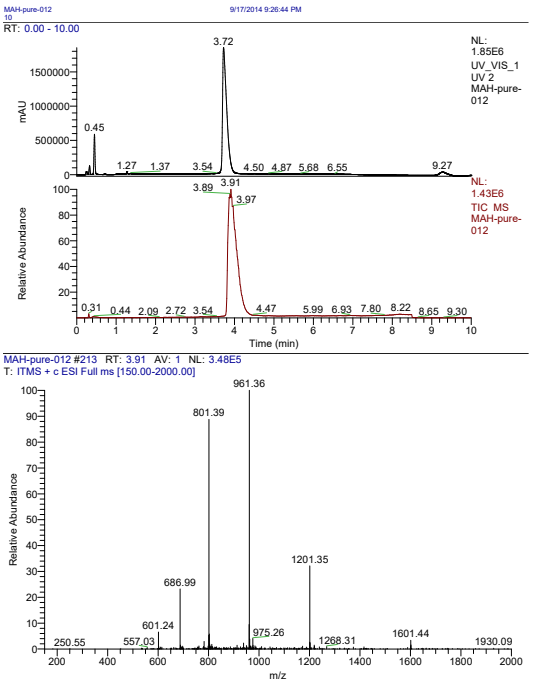
MASS SPECTRUM, HRMS (NSI+):



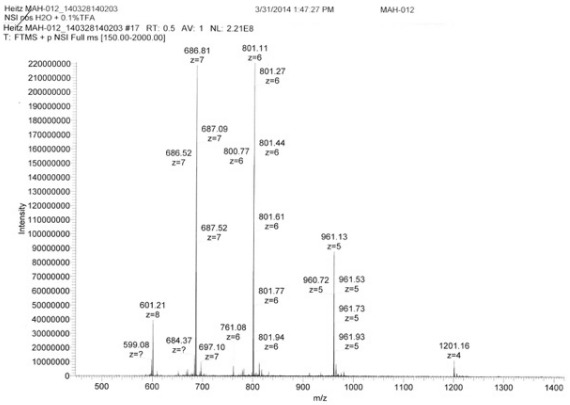
MH29 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*GSK(C$_{18}$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (7 mg, 1.0 µmol, 1%). Analytical RP-HPLC: t_R = 3.76 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{239}H_{461}N_{59}O_{40}$ calc./obs. 4798.59/4798.59 [M+H$^+$].

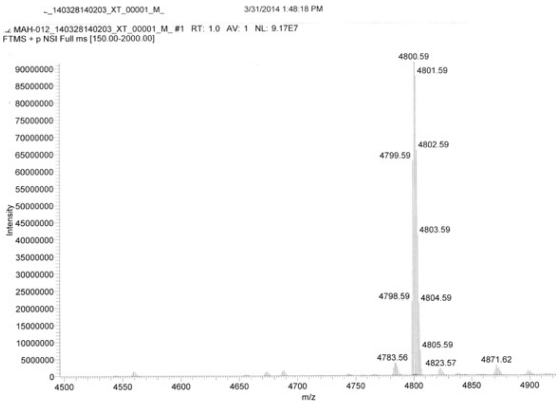


LCMS

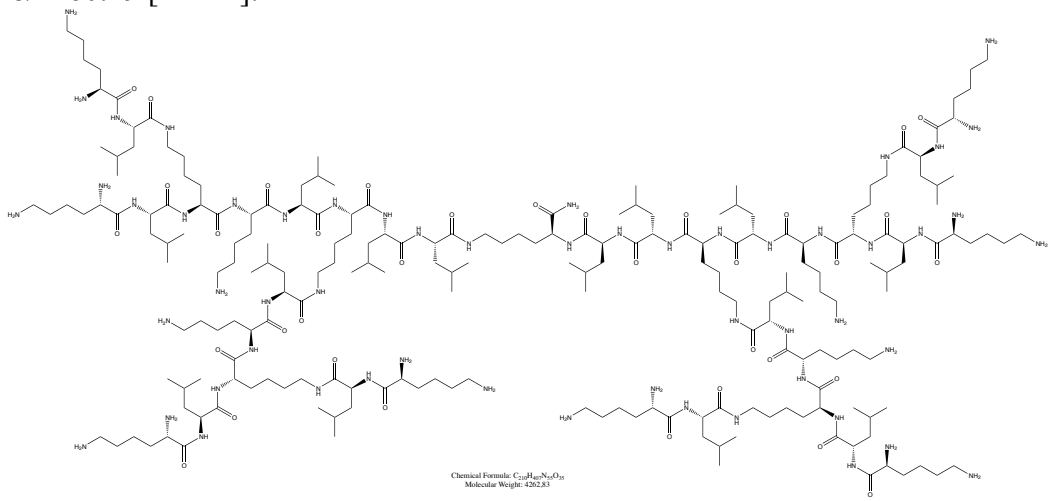


MASS SPECTRUM, HRMS (NSI+):

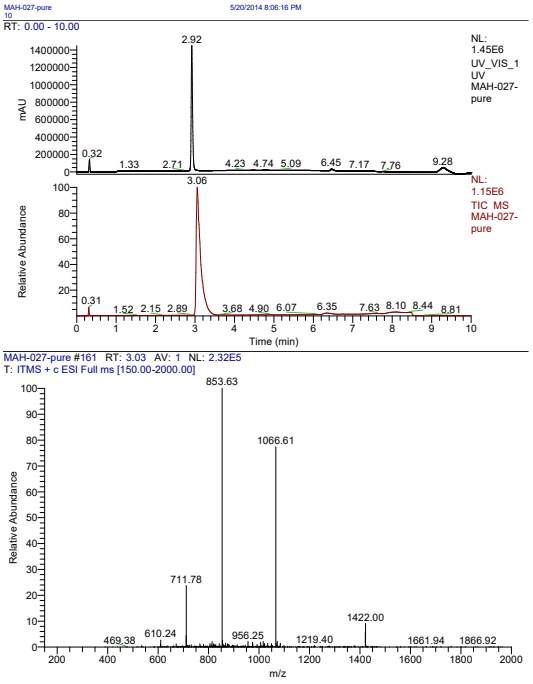




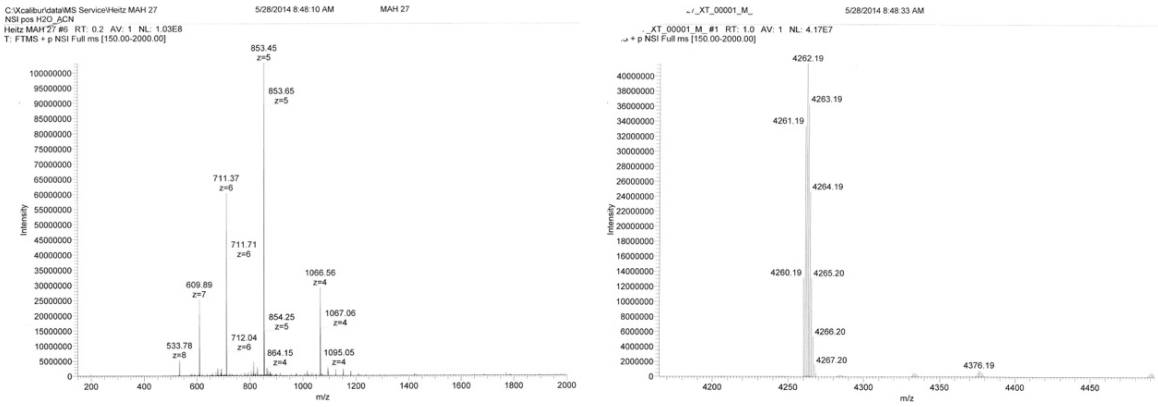
MH30 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (37.7 mg, 5.8 μmol, 8%). Analytical RP-HPLC: t_R = 2.92 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{210}H_{407}N_{55}O_{35}$ calc./obs. 4260.18/4260.19 [M+H$^+$].



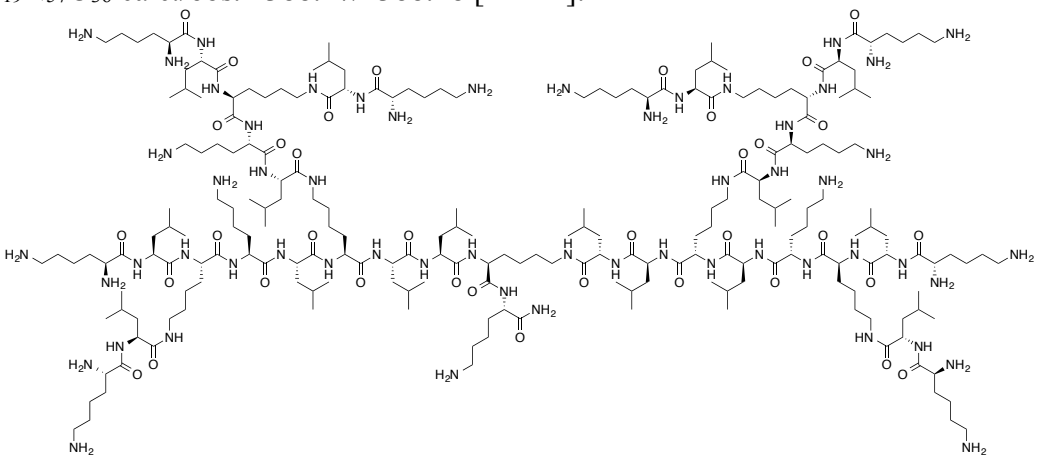
LCMS



MASS SPECTRUM, HRMS (NSI+):

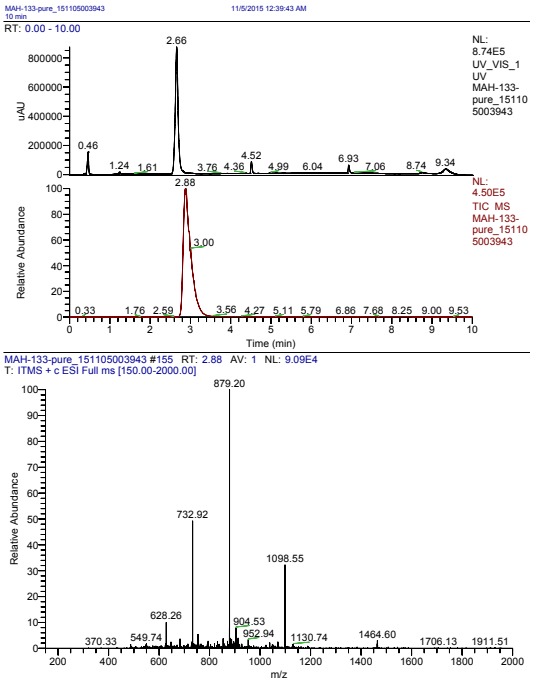


MH31 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (12.0 mg, 1.77 µmol, 3%). Analytical RP-HPLC: t_R = 2.66 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{216}H_{419}N_{57}O_{36}$ calc./obs. 4388.27/4388.28 [M+H$^+$].

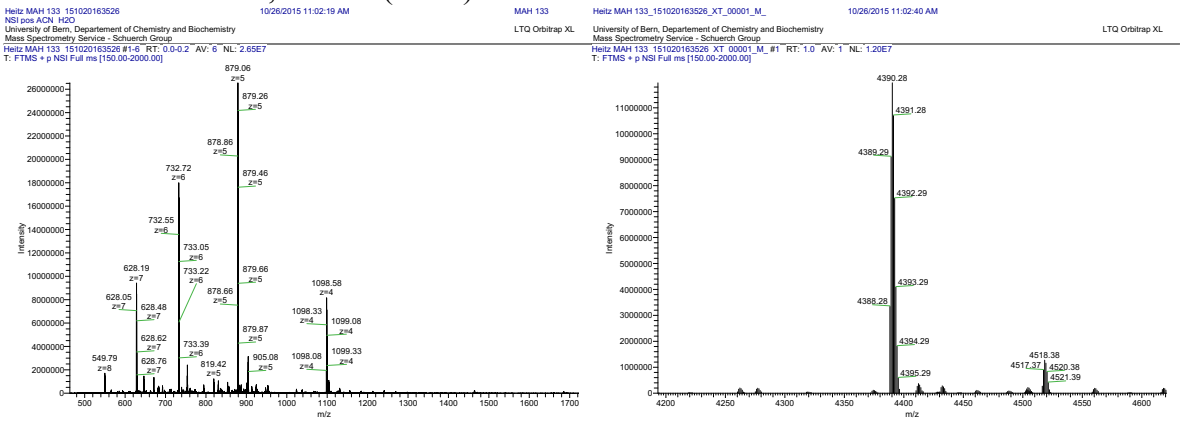


Chemical Formula: $C_{216}H_{419}N_{57}O_{36}$
Molecular Weight: 4391,09

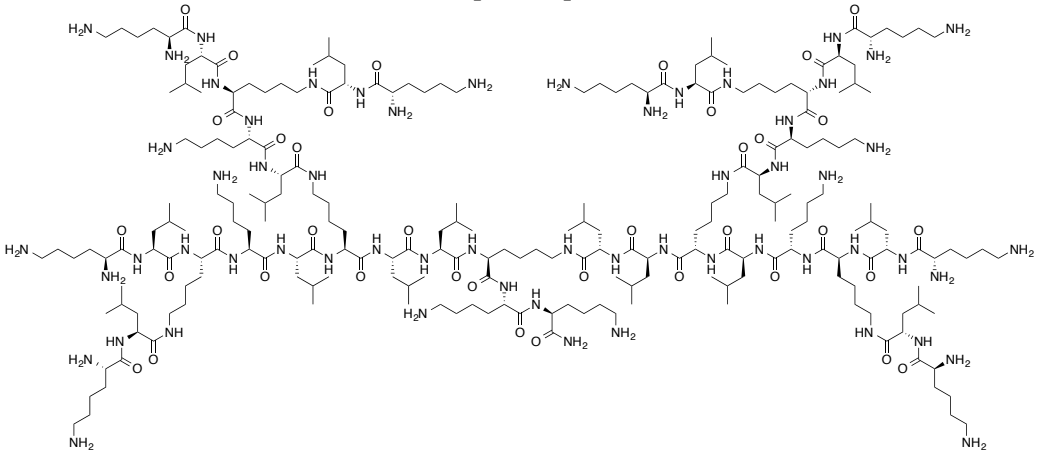
LCMS



MASS SPECTRUM, HRMS (NSI+):

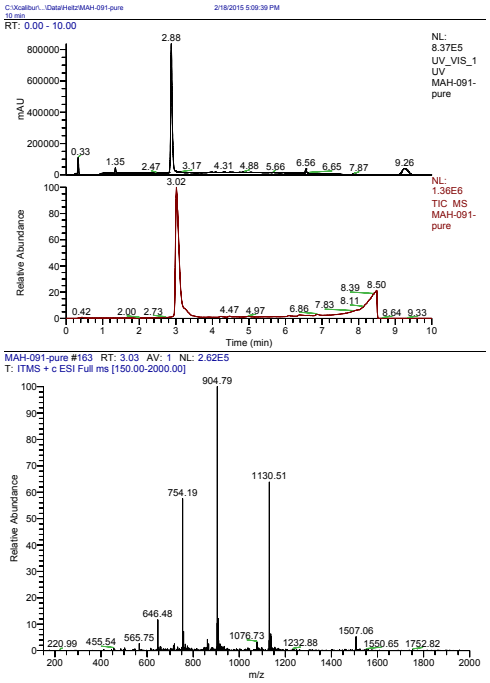


MH32 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*KK) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (6.6 mg, 0.91 µmol, 2%). Analytical RP-HPLC: t_R = 2.88 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{222}H_{431}N_{59}O_{37}$ calc./obs. 4516.37/4516.38 [M+H$^+$].

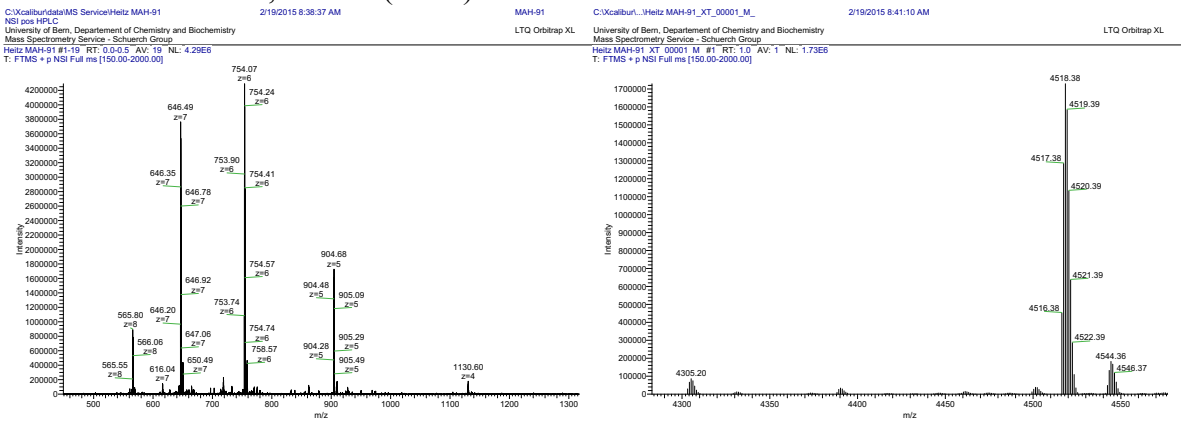


Chemical Formula: $C_{222}H_{431}N_{59}O_{37}$
Molecular Weight: 4519,27

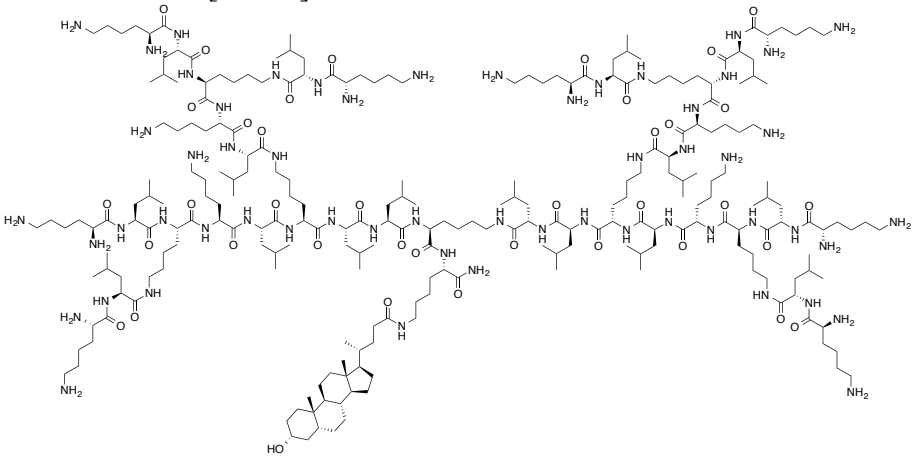
LCMS



MASS SPECTRUM, HRMS (NSI+):

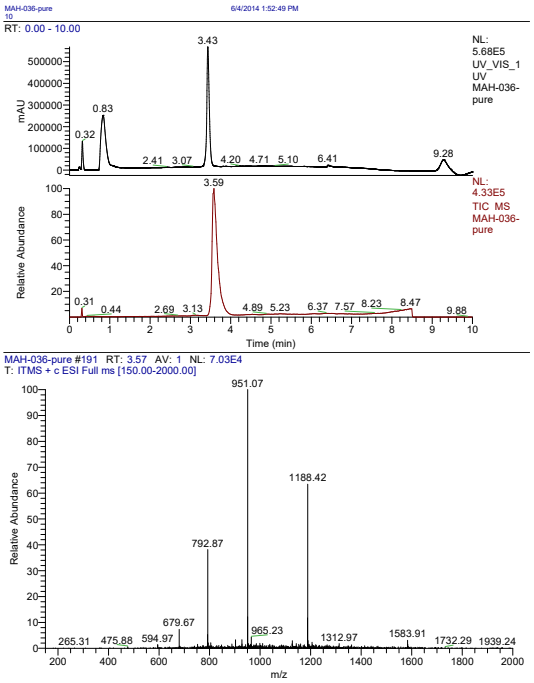


MH33 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(Lit)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (14.5 mg, 2.1 µmol, 3%). Analytical RP-HPLC: t_R = 3.43 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{240}H_{457}N_{57}O_{38}$ calc./obs. 4746.56/4746.56 [M+H$^+$].

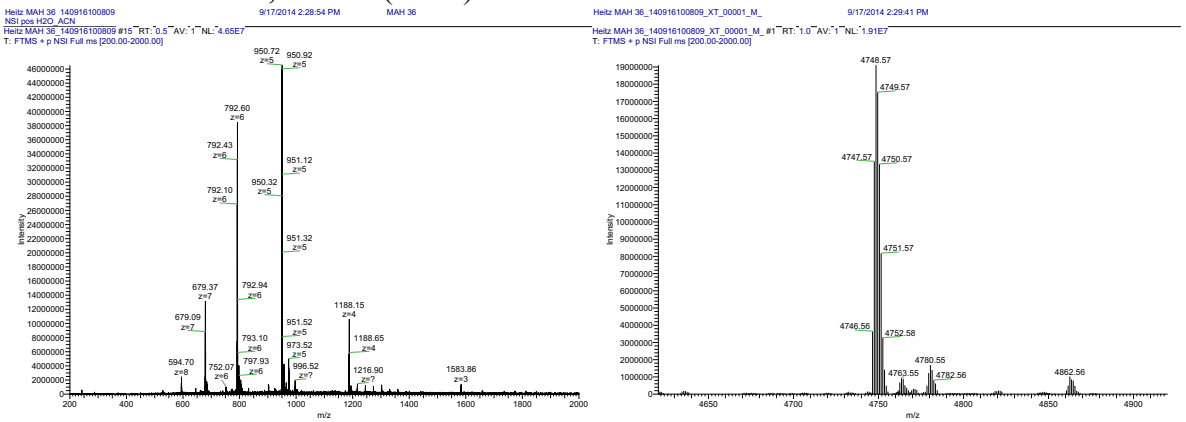


Chemical Formula: $C_{240}H_{457}N_{57}O_{38}$
Molecular Weight: 4749,56

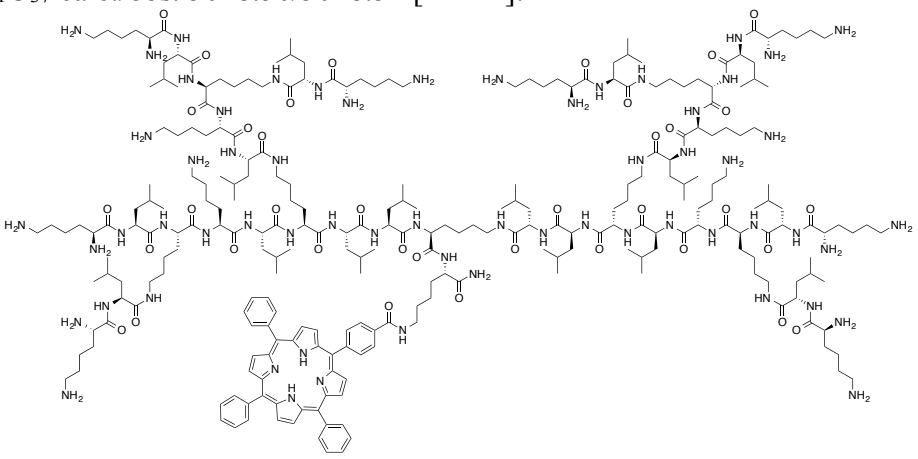
LCMS



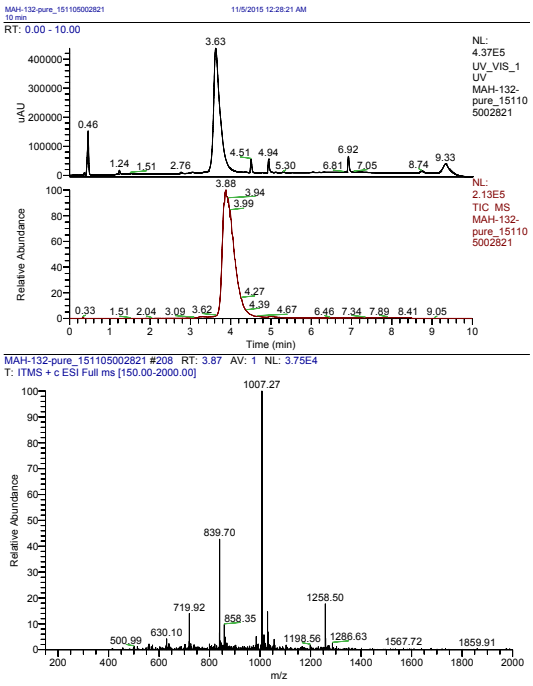
MASS SPECTRUM, HRMS (NSI+):



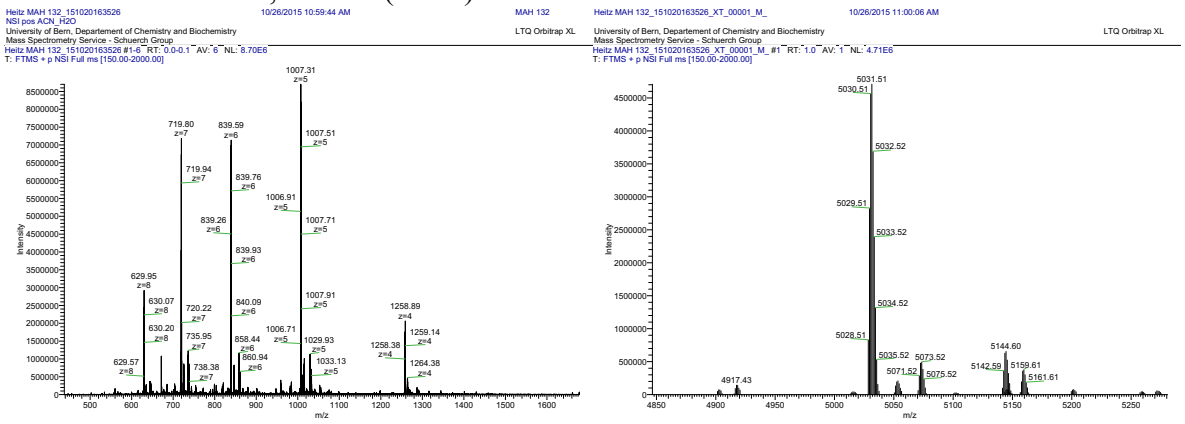
MH34 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(Por)) was obtained from the CEM Liberty Blue synthesizer as foamy green solid after preparative RP-HPLC (24.5 mg, 3.35 µmol, 6%). Analytical RP-HPLC: t_R = 3.63 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{261}H_{447}N_{61}O_{37}$ calc./obs. 5028.50/5028.51 [M+H$^+$].



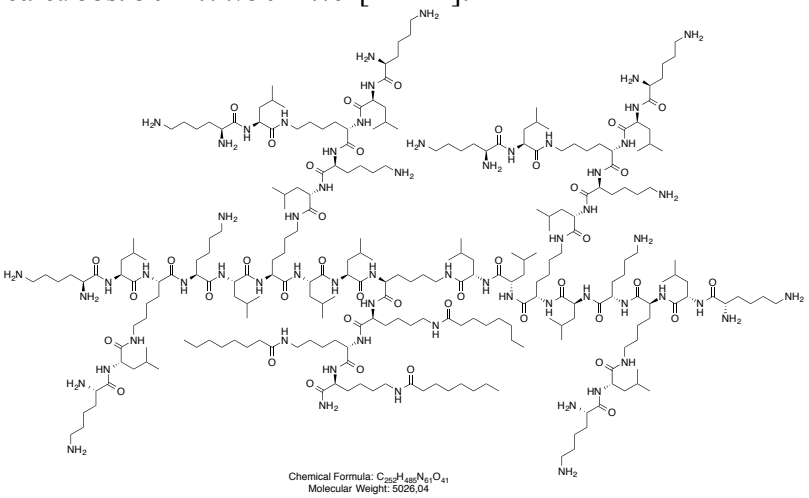
LCMS



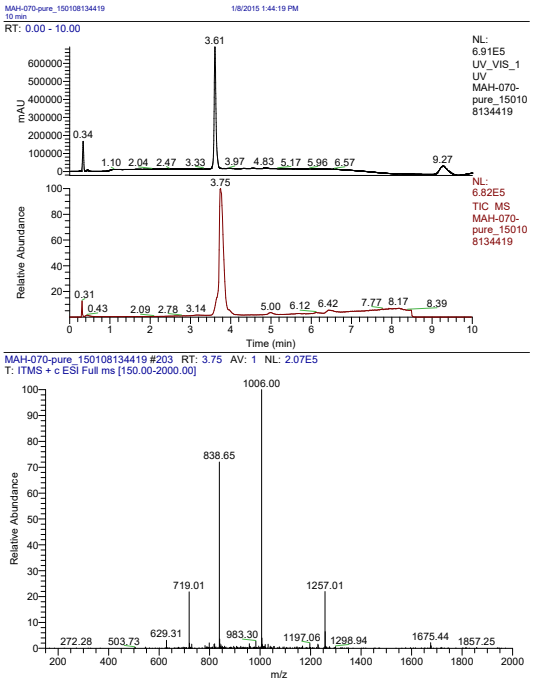
MASS SPECTRUM, HRMS (NSI+):



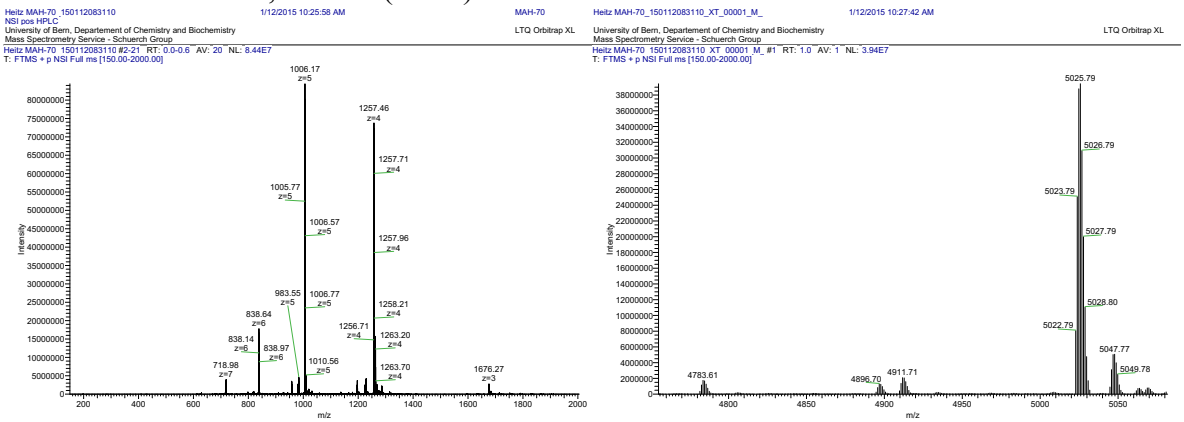
MH35 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(C$_8$)K(C$_8$)K(C$_8$)) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (10.9 mg, 1.49 µmol, 2%). Analytical RP-HPLC: t_R = 3.61 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{252}H_{485}N_{61}O_{41}$ calc./obs. 5022.77/5022.79 [M+H$^+$].



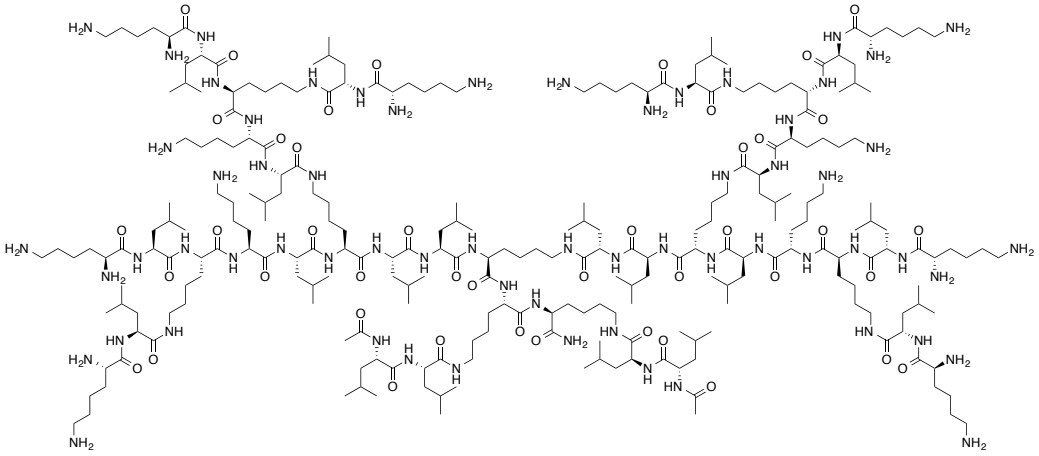
LCMS



MASS SPECTRUM, HRMS (NSI+):

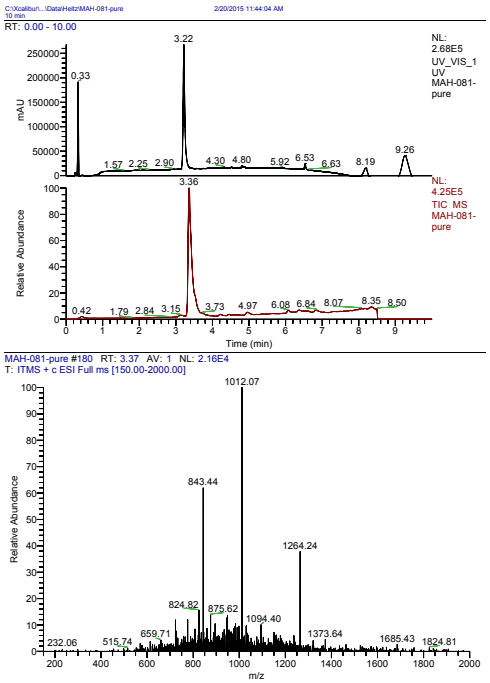


MH36 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(AcLL)K(AcLL)) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (5.0 mg, 0.68 µmol, 1%). Analytical RP-HPLC: t_R = 3.22 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{250}H_{479}N_{63}O_{43}$ calc./obs. 5052.72/5052.74 [M+H$^+$].

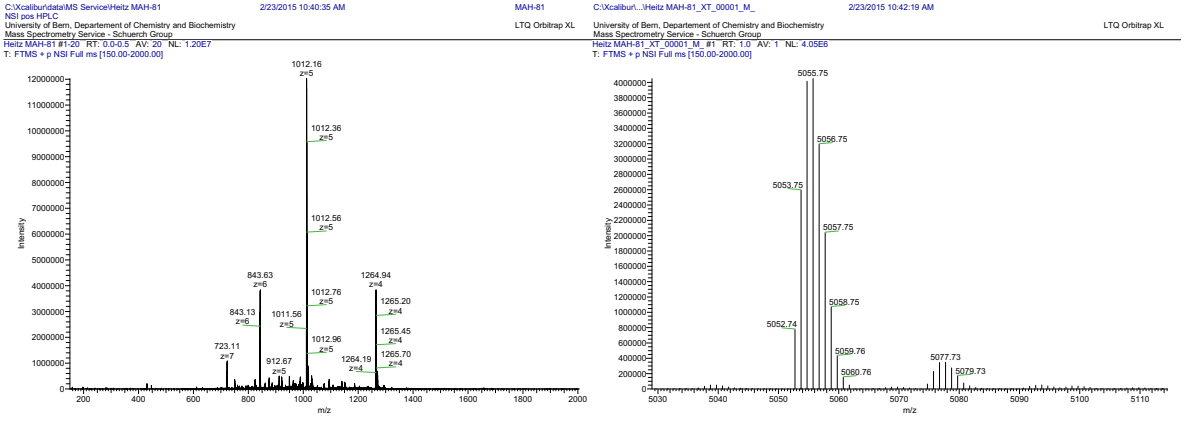


Chemical Formula: $C_{250}H_{479}N_{63}O_{43}$
Molecular Weight: 5055,98

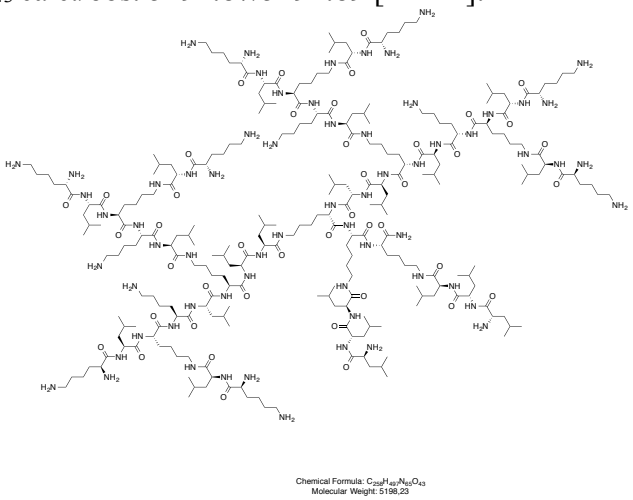
LCMS



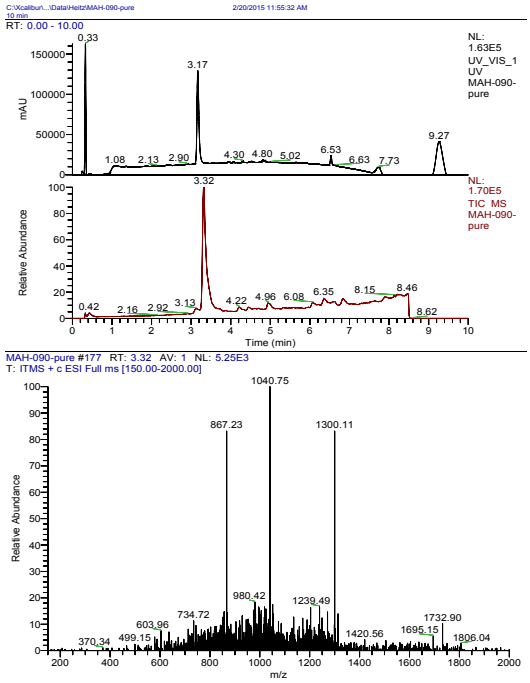
MASS SPECTRUM, HRMS (NSI+):



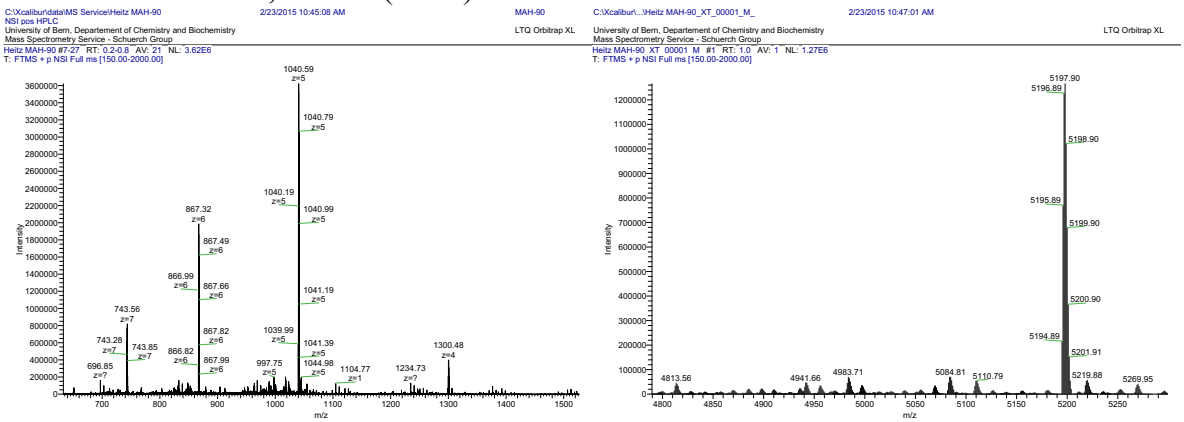
MH37 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*K(LLL)K(LLL)) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (2.1 mg, 0.272 µmol, 1%). Analytical RP-HPLC: t_R = 3.17 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{258}H_{497}N_{65}O_{43}$ calc./obs. 5194.87/5194.89 [M+H$^+$].



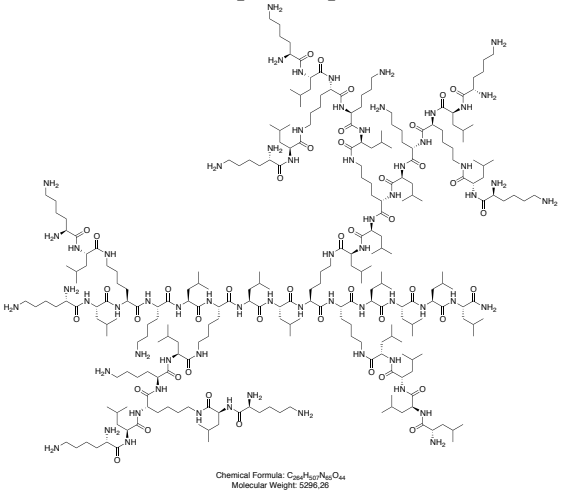
LCMS



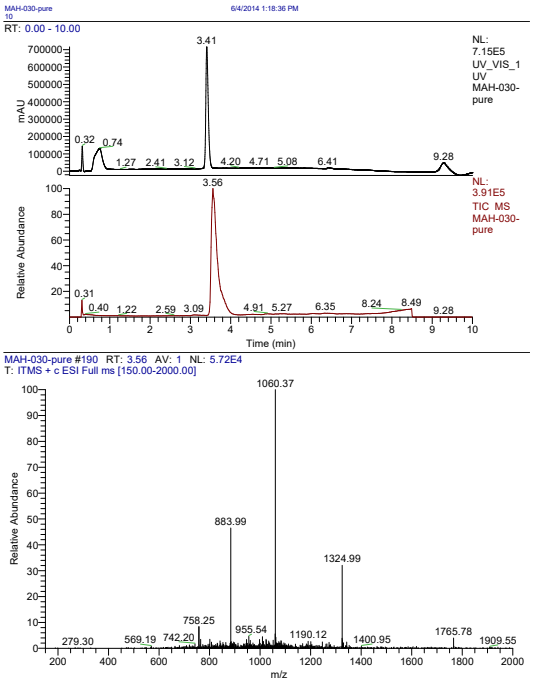
MASS SPECTRUM, HRMS (NSI+):



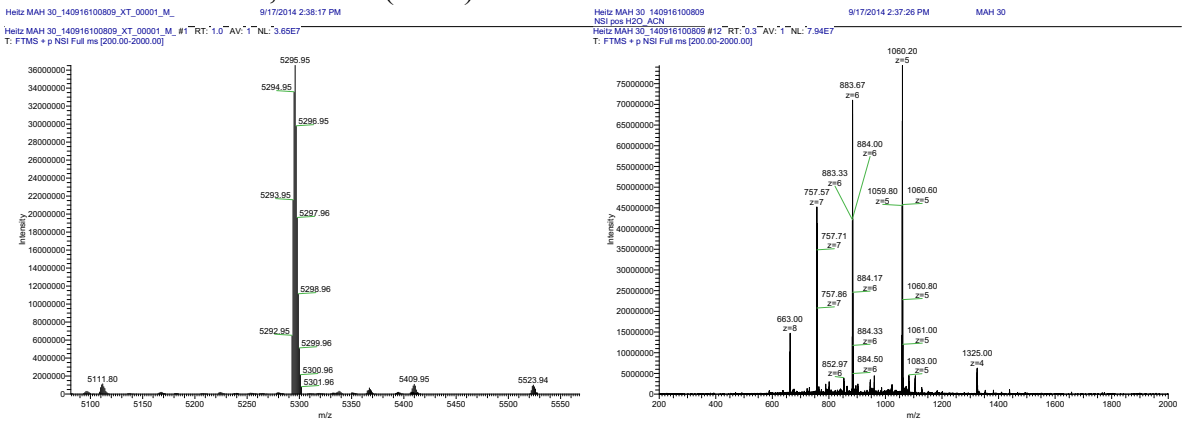
MH38 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*(*K*LLLL)LLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (13.3 mg, 1.8 μmol, 3%). Analytical RP-HPLC: t_R = 3.41 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{264}H_{507}N_{65}O_{44}$ calc./obs. 5292.94/5292.95 [M+H$^+$].



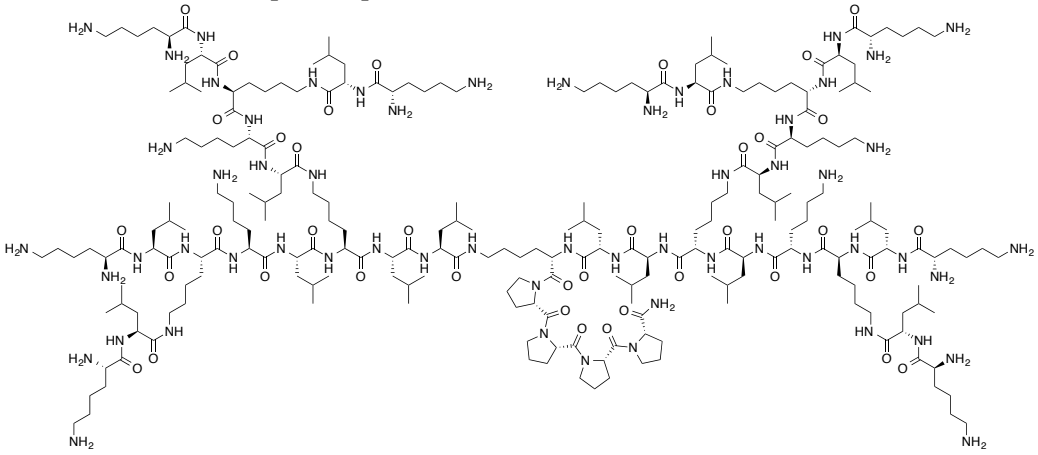
LCMS



MASS SPECTRUM, HRMS (NSI+):

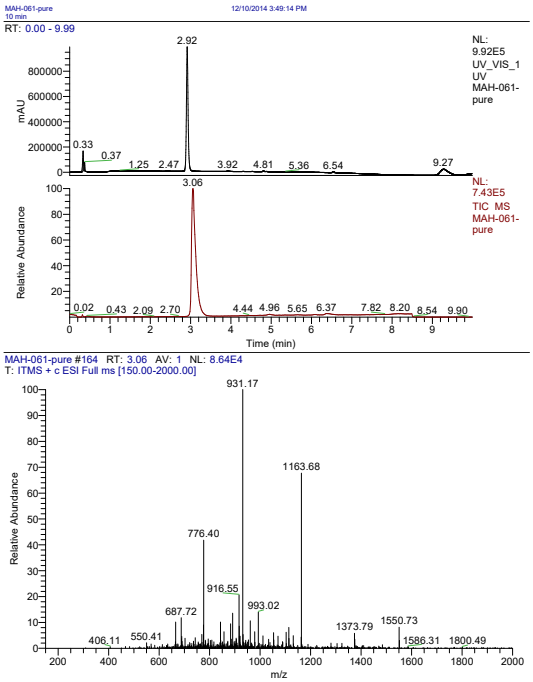


MH39 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*PPPP) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (7.2 mg, 1.04 μmol, 1%). Analytical RP-HPLC: t_R = 2.92 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{230}H_{435}N_{59}O_{39}$ calc./obs. 4648.39/4648.39 [M+H$^+$].

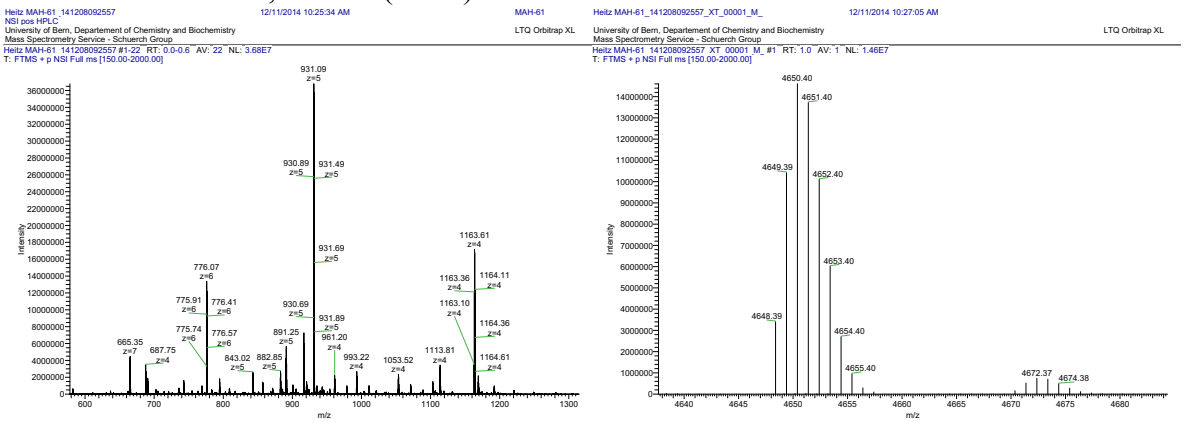


Chemical Formula: $C_{230}H_{435}N_{59}O_{39}$
Molecular Weight: 4651,38

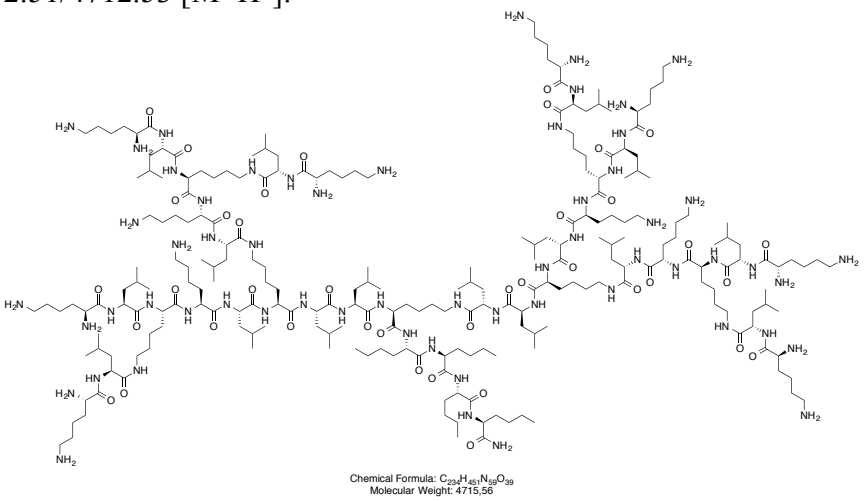
LCMS



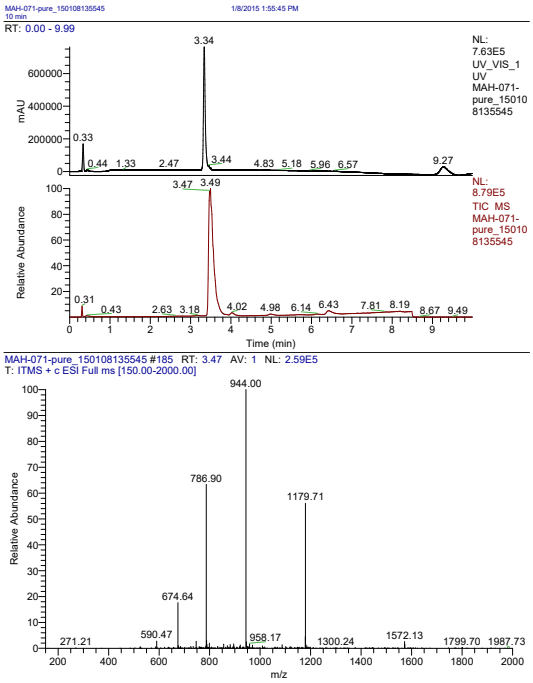
MASS SPECTRUM, HRMS (NSI+):



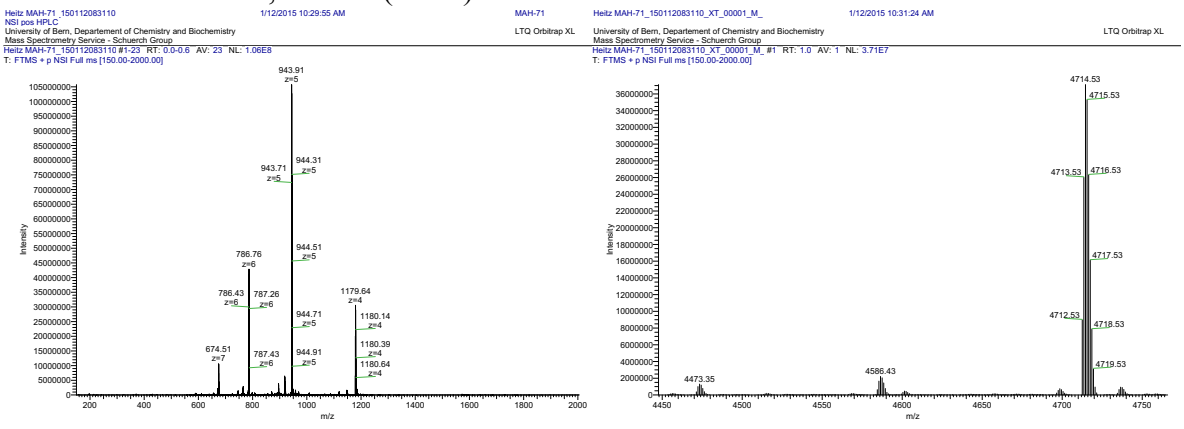
MH40 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*NleNleNleNle) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (5.1 mg, 0.73 µmol, 1%). Analytical RP-HPLC: t_R = 3.34 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.53 [M+H$^+$].



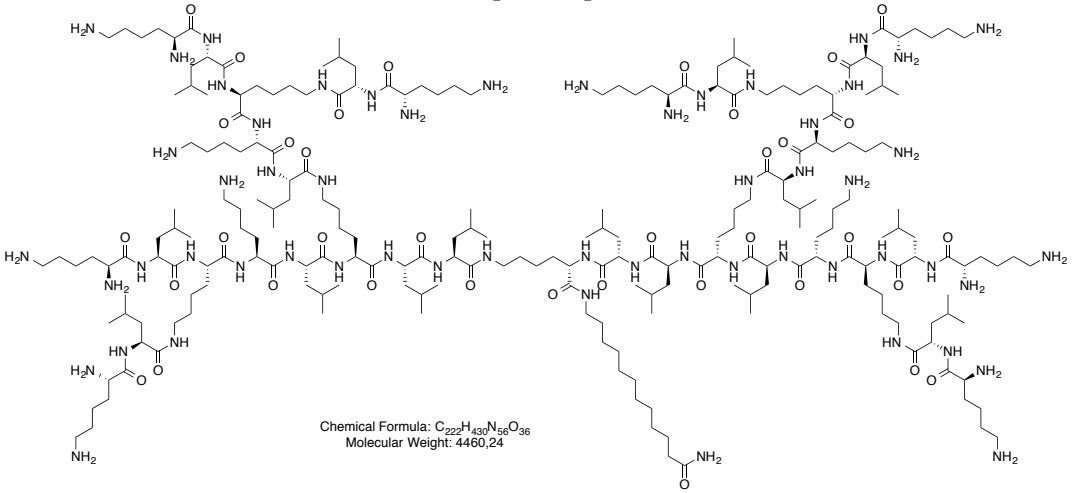
LCMS



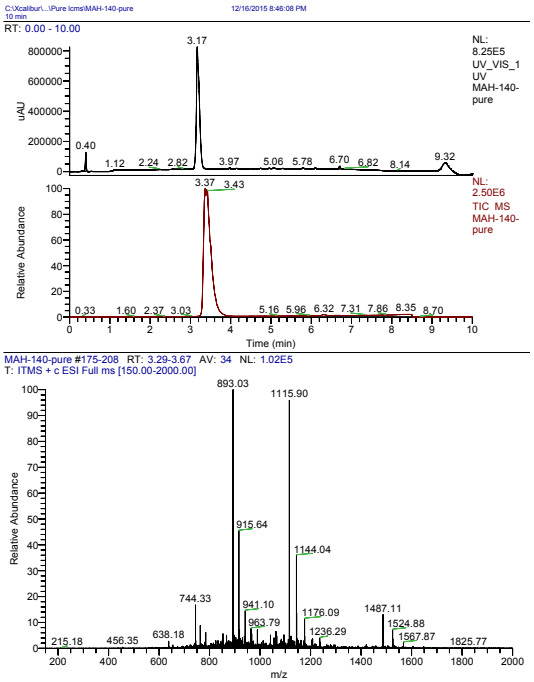
MASS SPECTRUM, HRMS (NSI+):



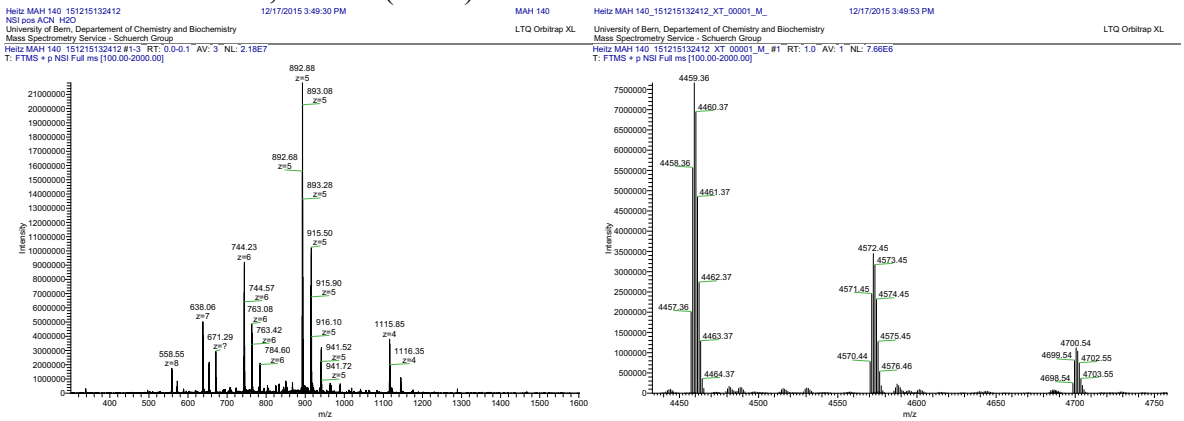
MH41 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*Ado) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (47.0 mg, 6.97 µmol, 13%). Analytical RP-HPLC: t_R = 3.17 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{222}H_{430}N_{56}O_{36}$ calc./obs. 4457.35/4457.36 [M+H$^+$].



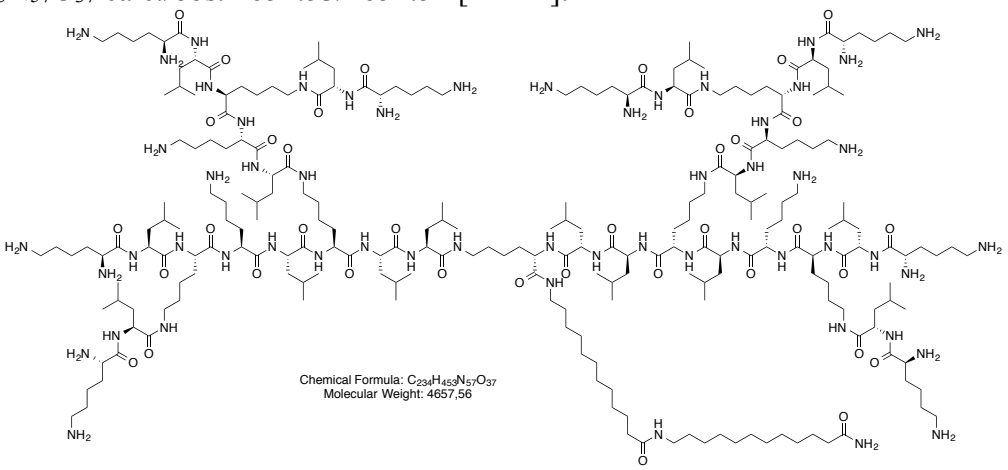
LCMS



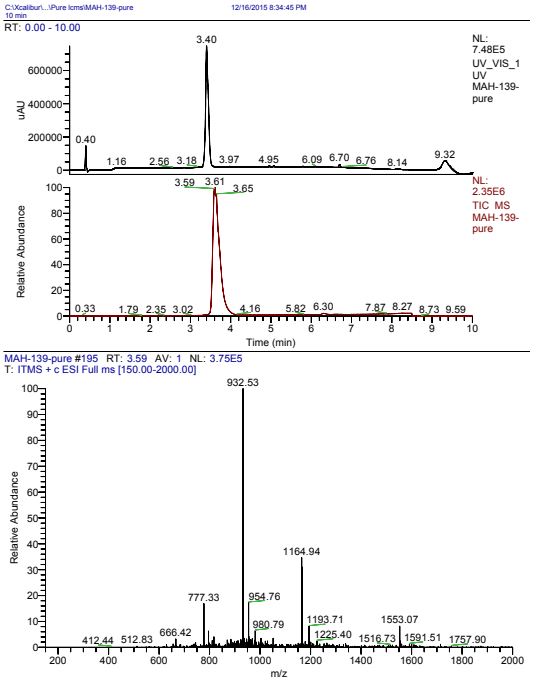
MASS SPECTRUM, HRMS (NSI+):



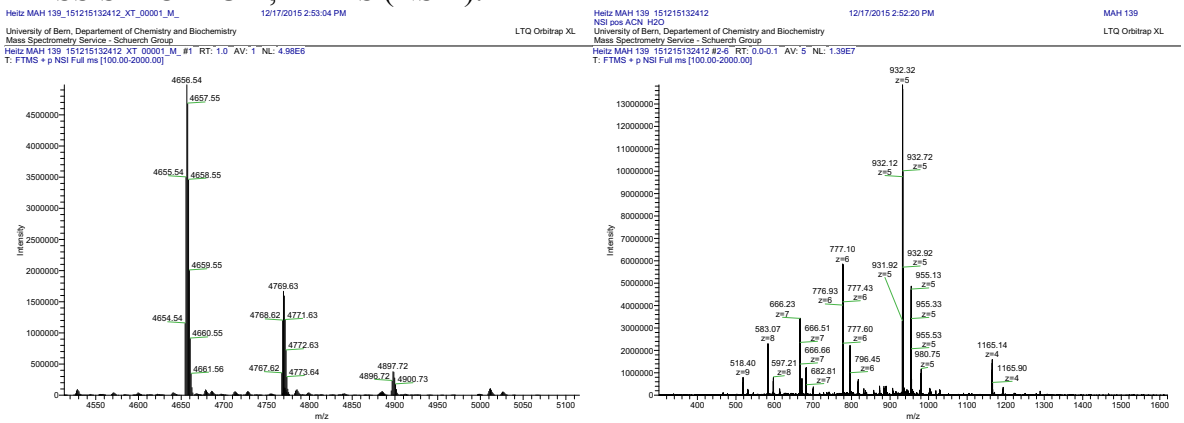
MH42 ((KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*AdoAdo) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (54.5 mg, 7.86 µmol, 15%). Analytical RP-HPLC: t_R = 3.40 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{453}N_{57}O_{37}$ calc./obs. 4654.53/4654.54 [M+H$^+$].



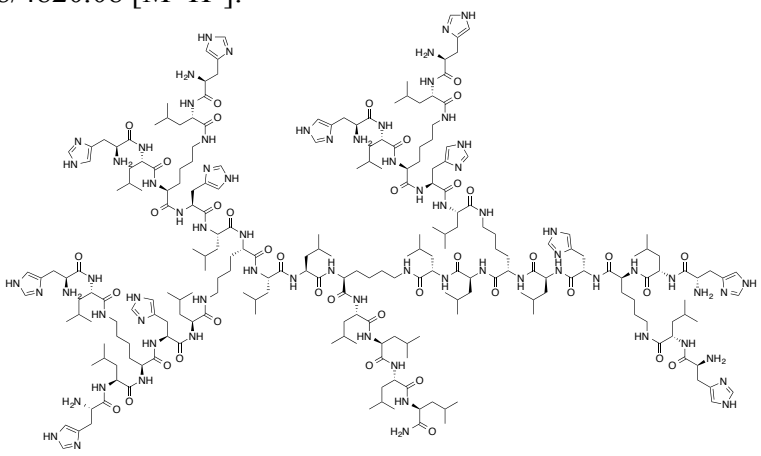
LCMS



MASS SPECTRUM, HRMS (NSI+):

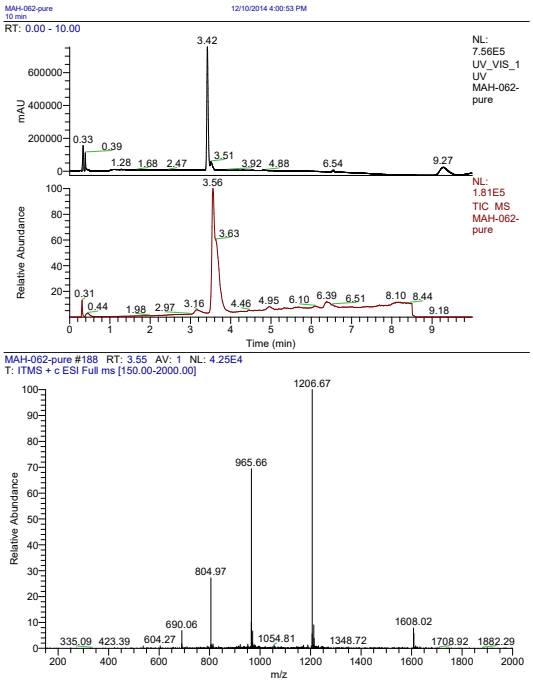


MH43 ((HL)$_8$(*K*HL)$_4$(*K*LL)$_2$*K*LLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (5.5 mg, 0.77 µmol, 1%). Analytical RP-HPLC: t_R = 3.42 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{390}N_{70}O_{40}$ calc./obs. 4820.08/4820.08 [M+H$^+$].

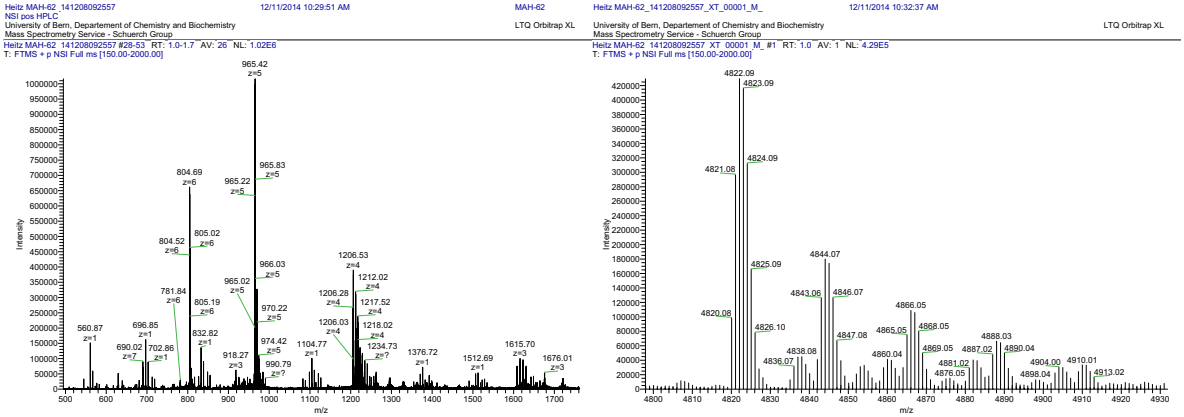


Chemical Formula: $C_{234}H_{391}N_{71}O_{39}$
Molecular Weight: 4823,16

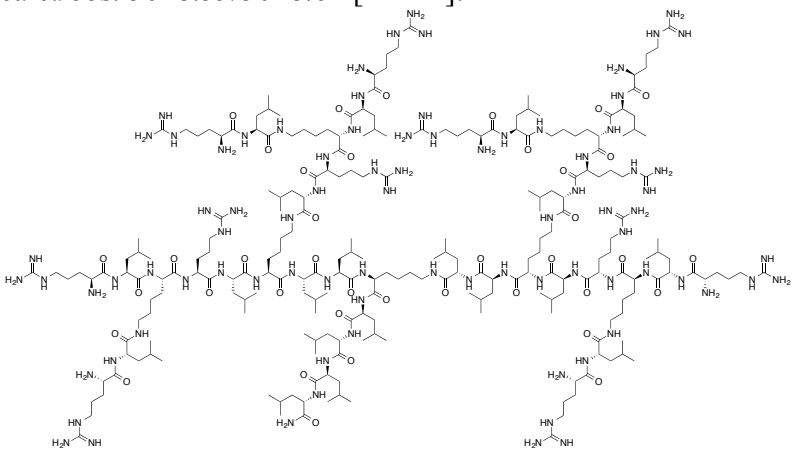
LCMS



MASS SPECTRUM, HRMS (NSI+):

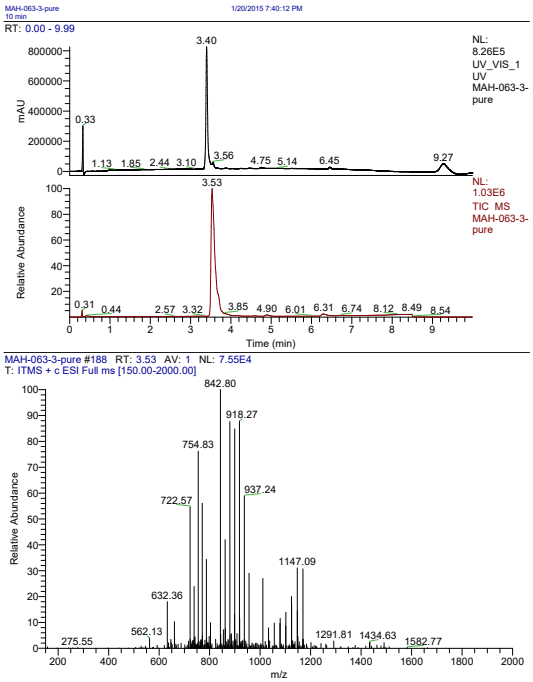


MH44 ((RL)$_8$(*K*RL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (25.9 mg, 3.53 µmol, 5%). Analytical RP-HPLC: t_R = 3.40 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{450}N_{82}O_{40}$ calc./obs. 5048.59/5048.61 [M+H$^+$].

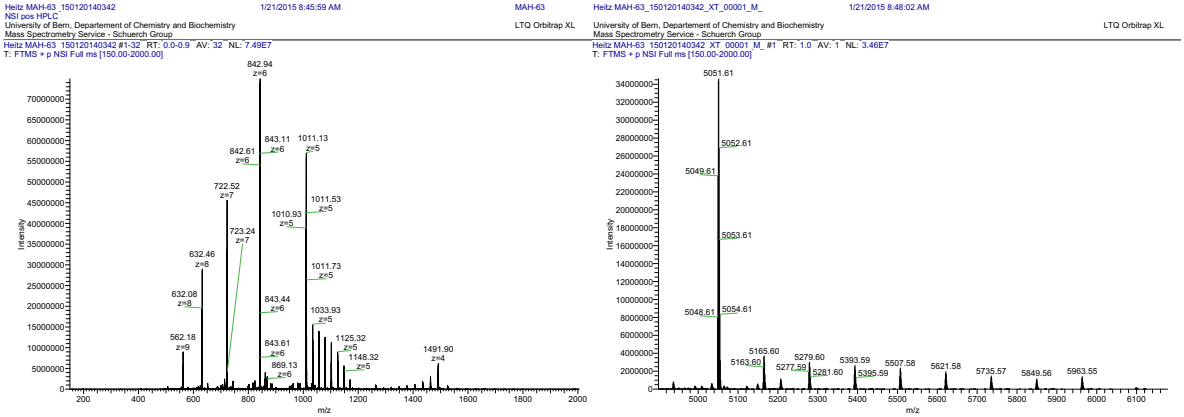


Chemical Formula: $C_{234}H_{451}N_{83}O_{39}$
Molecular Weight: 5051,72

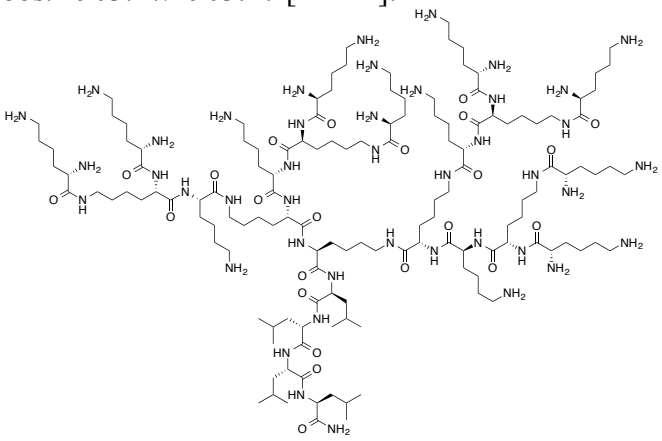
LCMS



MASS SPECTRUM, HRMS (NSI+):

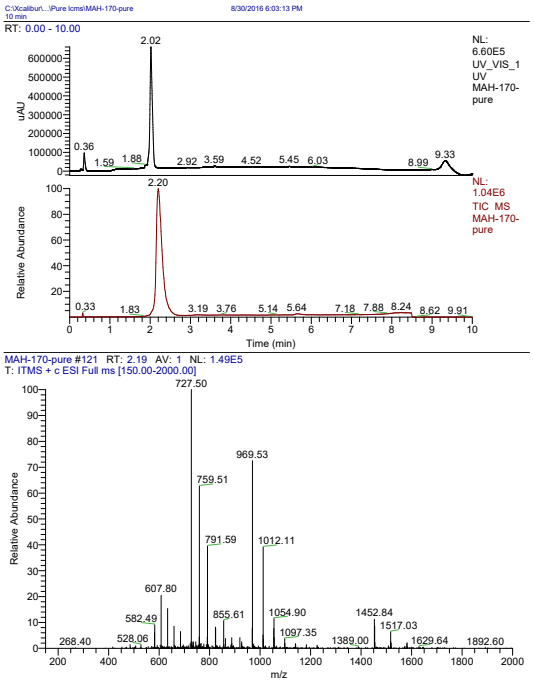


MH45 ((K)$_8$(*K*K)$_4$(*K*)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (45.1 mg, 8.70 µmol, 12%). Analytical RP-HPLC: t_R = 2.02 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{138}H_{275}N_{43}O_{23}$ calc./obs. 2903.17/2903.17 [M+H$^+$].

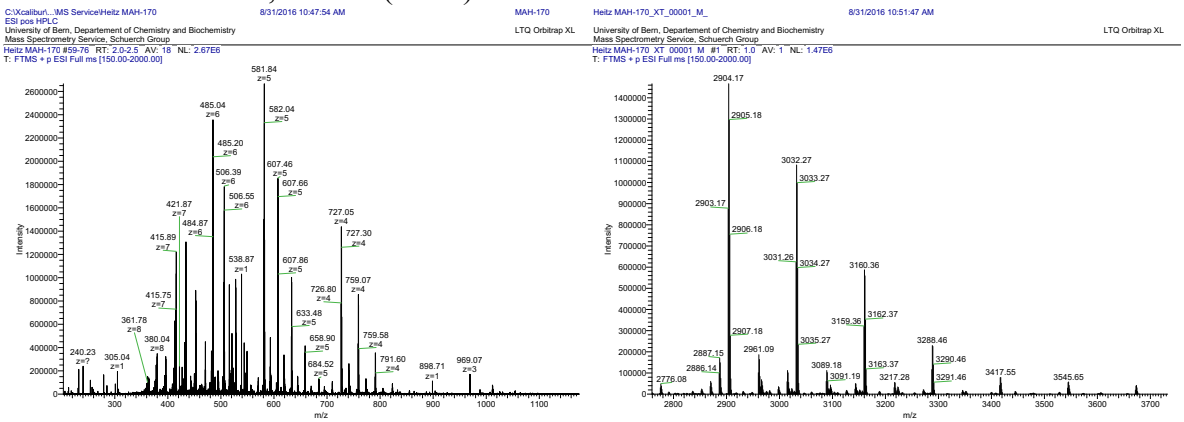


Chemical Formula: $C_{138}H_{275}N_{43}O_{23}$
Molecular Weight: 2905,00

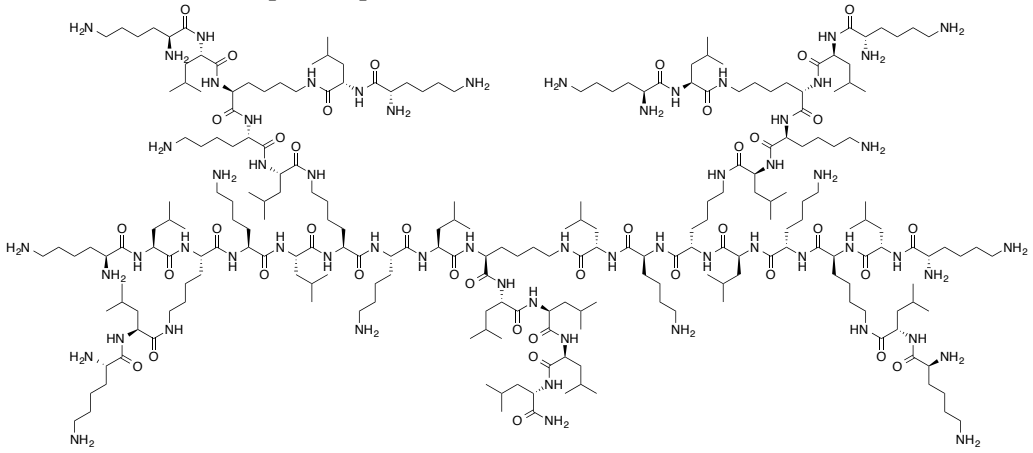
LCMS



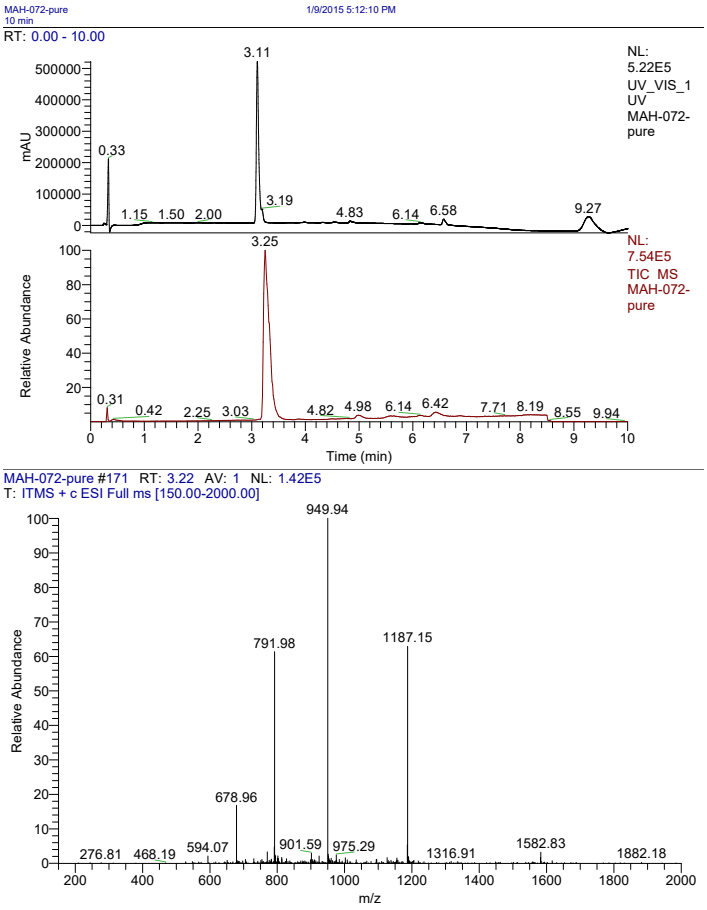
MASS SPECTRUM, HRMS (NSI+):



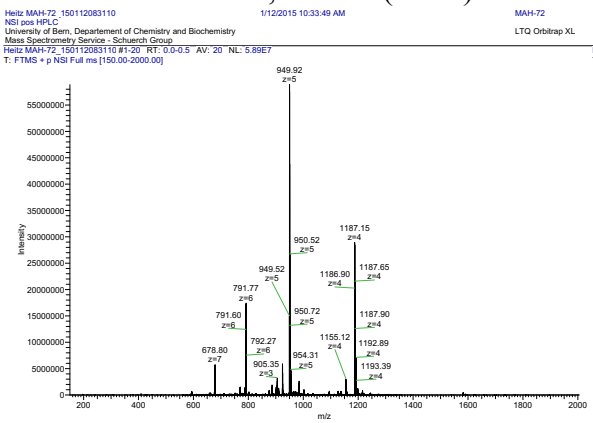
MH46 ((KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*LLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (8.5 mg, 1.17 µmol, 2%). Analytical RP-HPLC: t_R = 3.11 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{453}N_{61}O_{39}$ calc./obs. 4742.53/4742.55 [M+H$^+$].

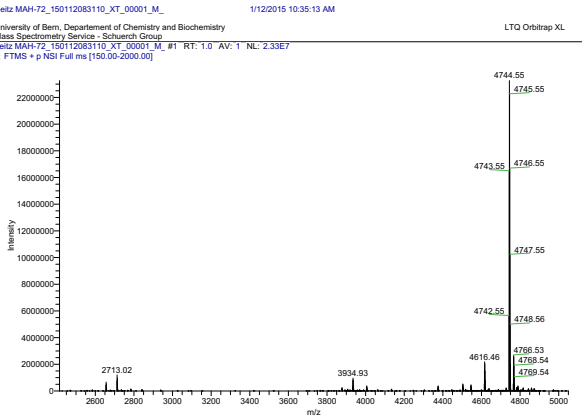


LCMS

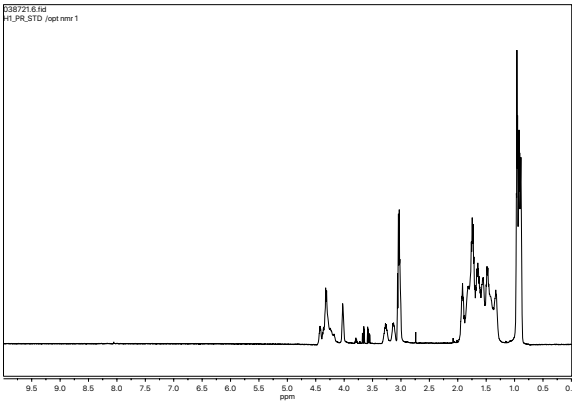


MASS SPECTRUM, HRMS (NSI+):

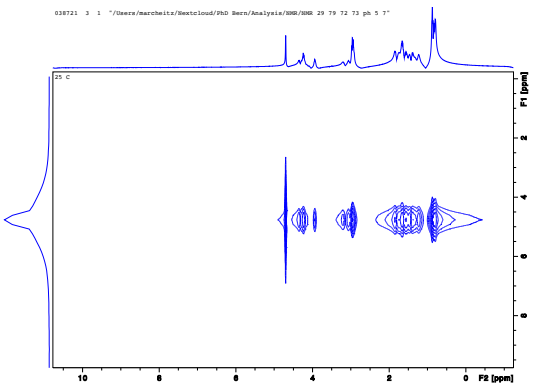


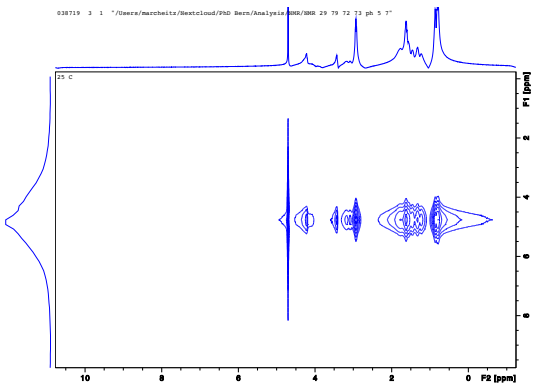


^{1}H NMR

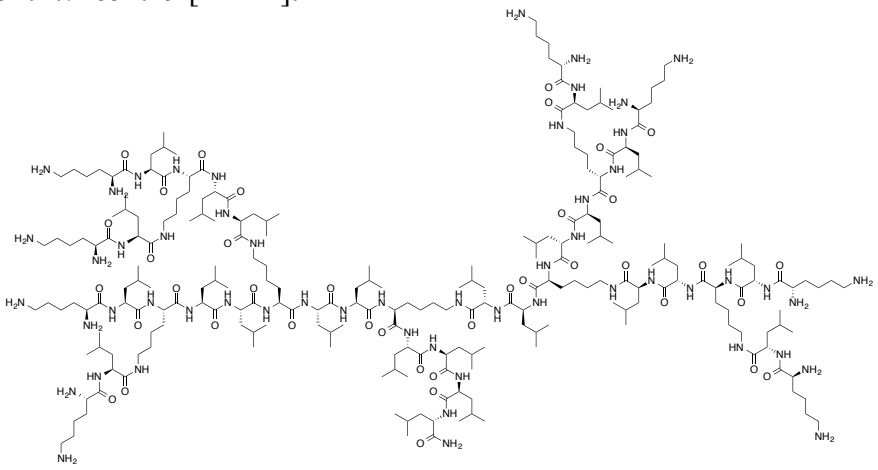


DOSY NMR pH 5 and pH 7.4

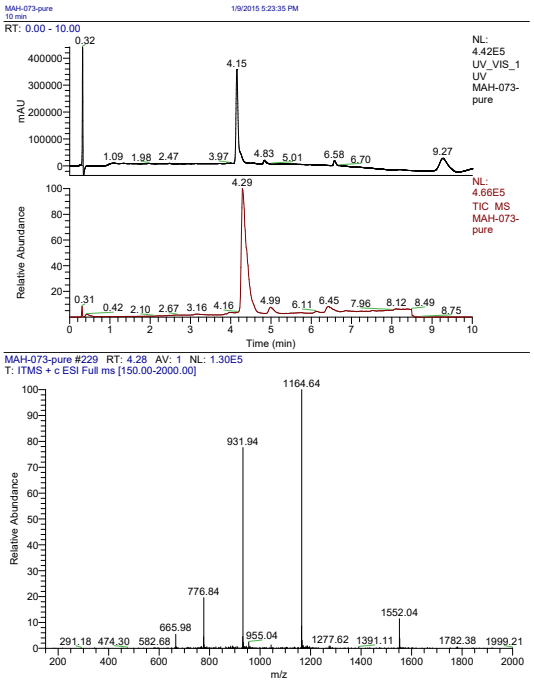




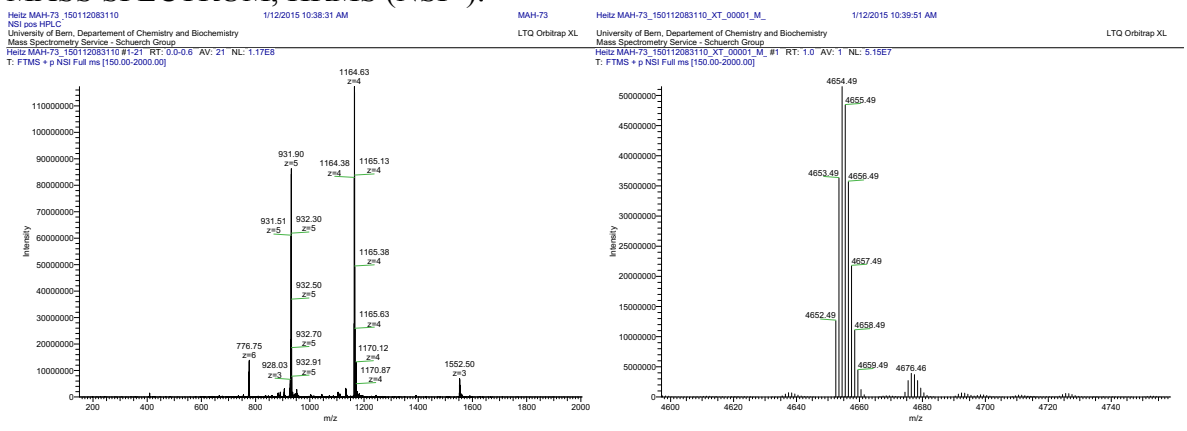
MH47 ((KL)$_8$(*K*LL)$_4$(*K*LL)$_2$*K*LLLL) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (10.1 mg, 1.56 µmol, 2%). Analytical RP-HPLC: t_R = 4.15 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{447}N_{55}O_{39}$ calc./obs. 4652.47/4652.49 [M+H$^+$].



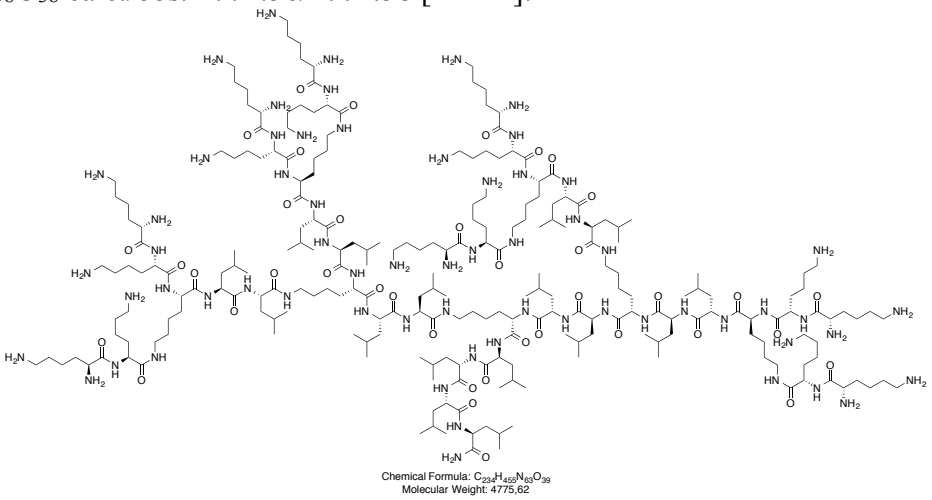
LCMS



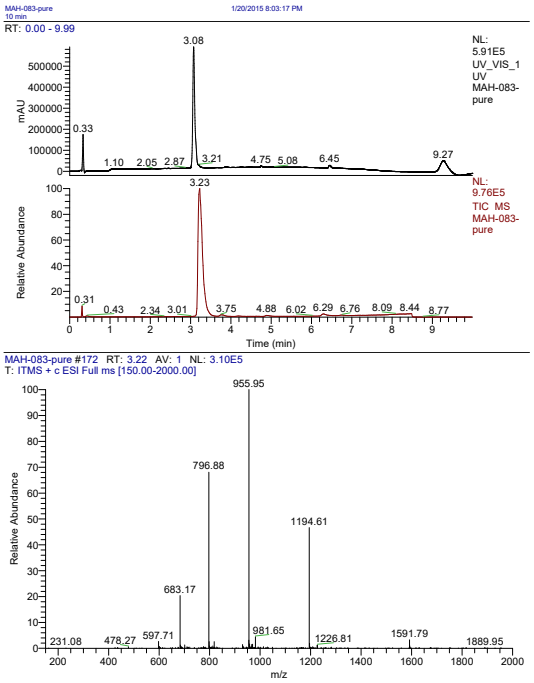
MASS SPECTRUM, HRMS (NSI+):



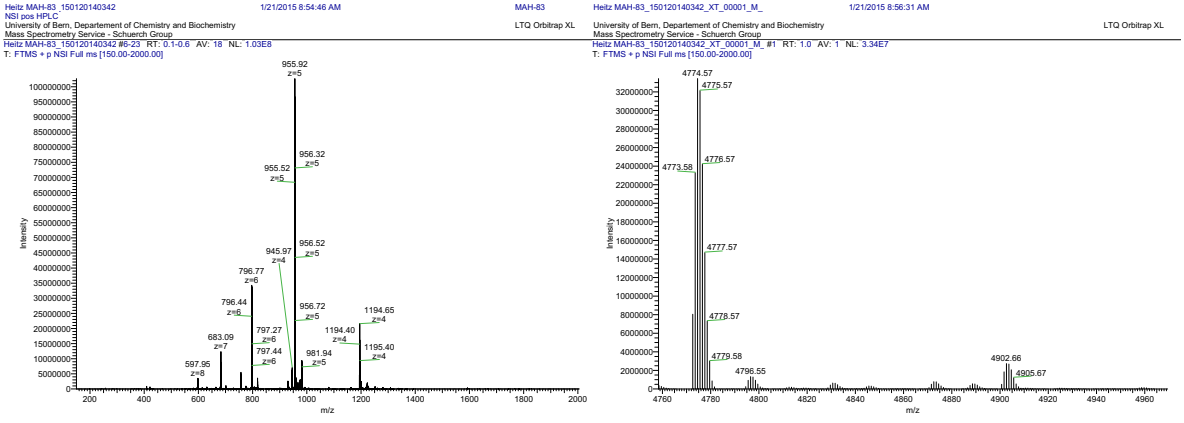
MH48 ((KK)$_8$(*K*LL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the Biotage Initiator+ Alstra synthesizer as foamy colourless solid after preparative RP-HPLC (25.9 mg, 3.45 µmol, 5%). Analytical RP-HPLC: t_R = 3.08 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{228}H_{440}N_{58}O_{38}$ calc./obs. 4772.56/4772.58 [M+H$^+$].



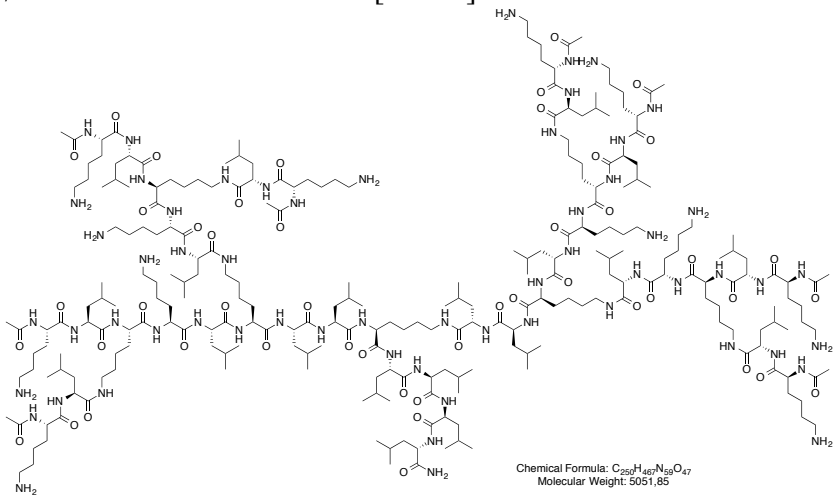
LCMS



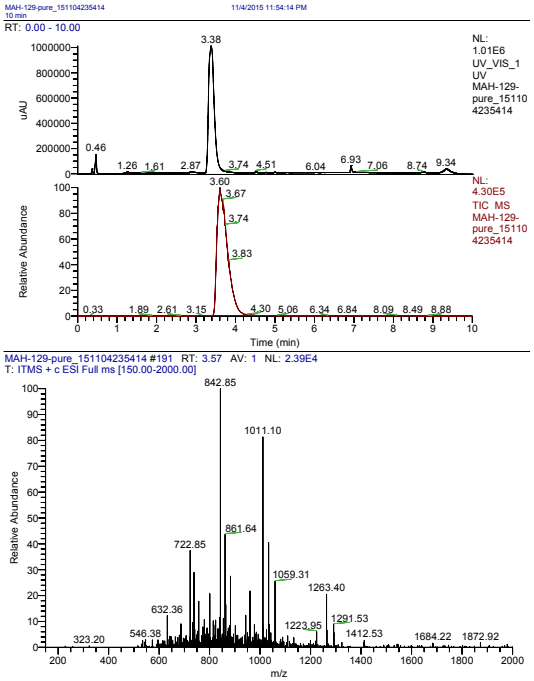
MASS SPECTRUM, HRMS (NSI+):



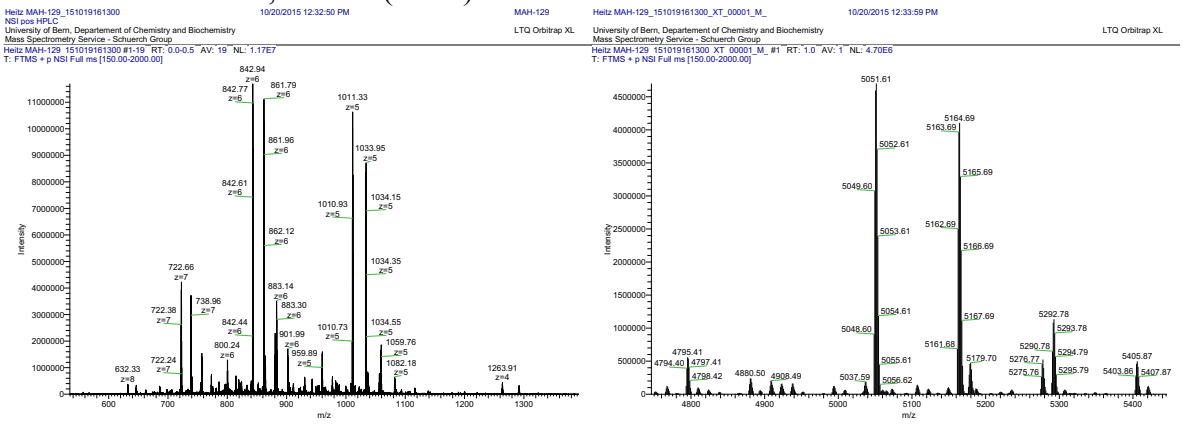
MH49 ((AcKL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (26.5 mg, 4.13 µmol, 5%). Analytical RP-HPLC: t_R = 3.38 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{250}H_{467}N_{59}O_{47}$ calc./obs. 5048.60/5048.60 [M+H$^+$].



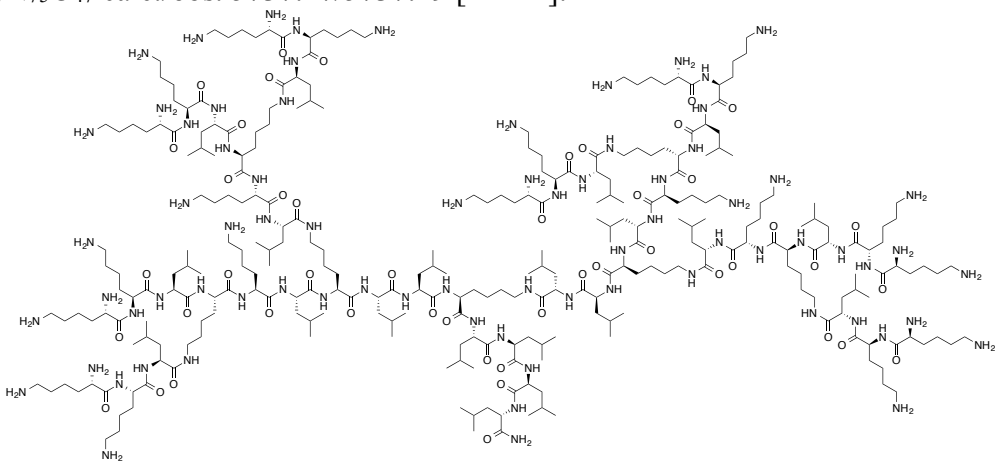
LCMS



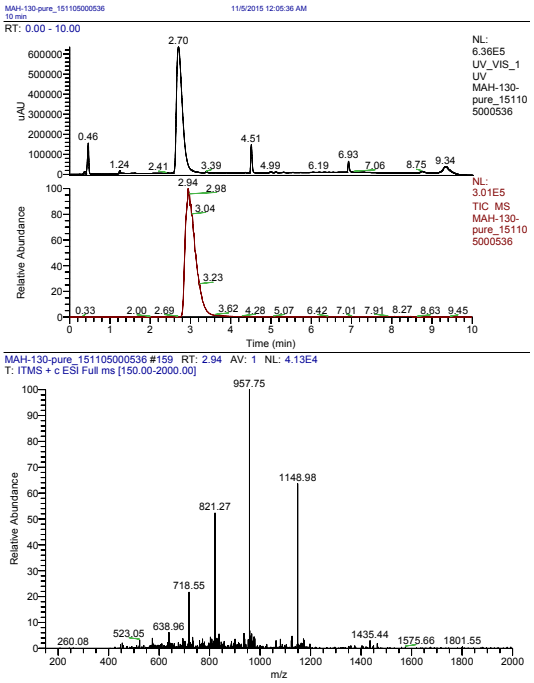
MASS SPECTRUM, HRMS (NSI+):



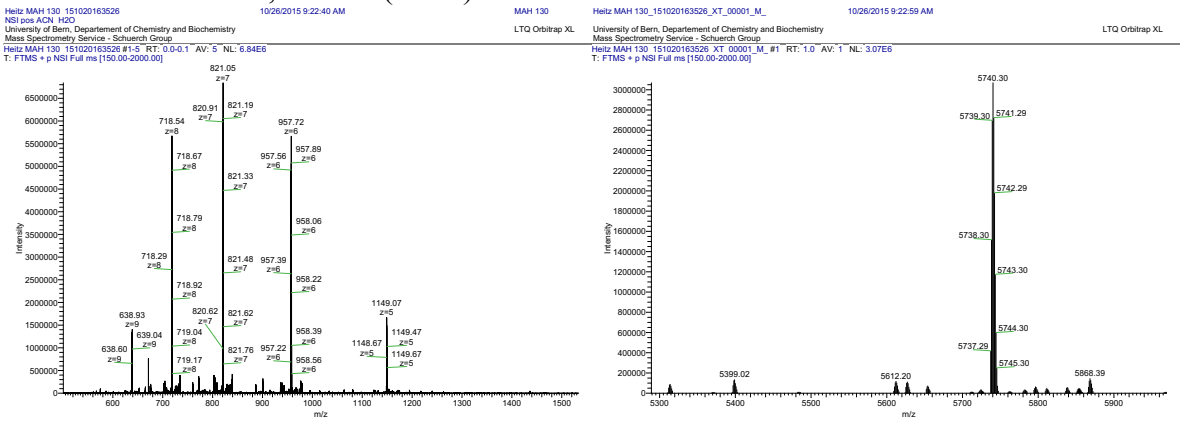
MH50 ((KKL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (31.0 mg, 3.47 µmol, 4%). Analytical RP-HPLC: t_R = 2.70 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{282}H_{547}N_{75}O_{47}$ calc./obs. 5737.27/5737.29 [M+H$^+$].



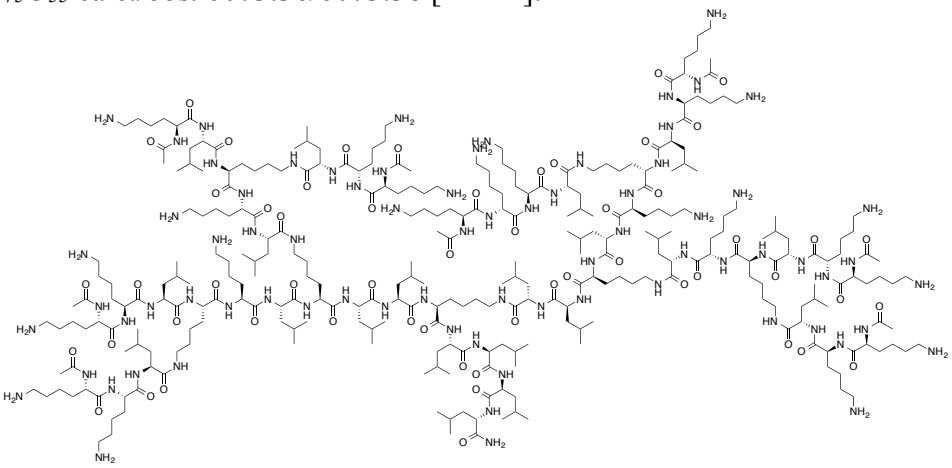
LCMS



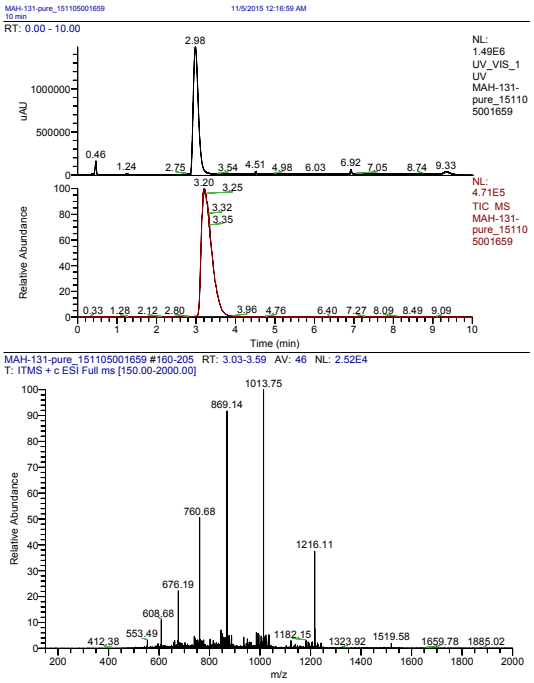
MASS SPECTRUM, HRMS (NSI+):



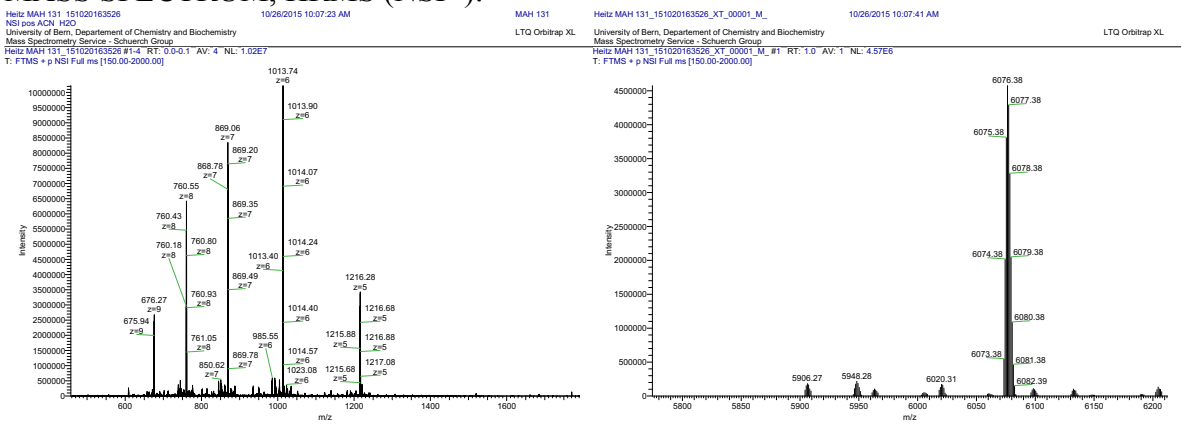
MH51 ((AcKKL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (46.0 mg, 5.5 µmol, 6%). Analytical RP-HPLC: t_R = 2.98 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{298}H_{563}N_{75}O_{55}$ calc./obs. 6073.36/6073.38 [M+H$^+$].



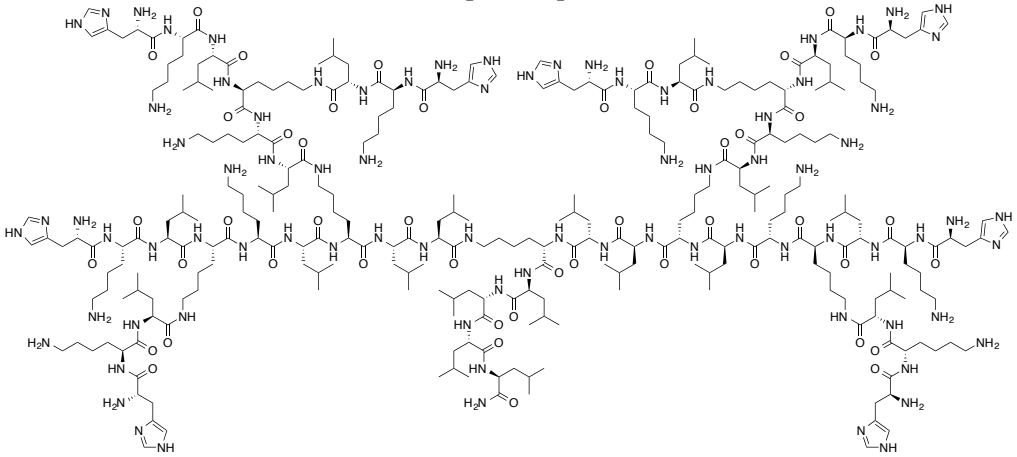
LCMS



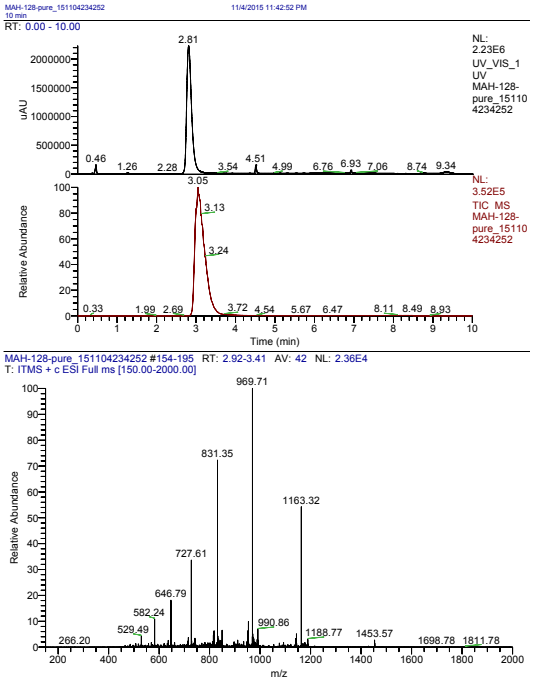
MASS SPECTRUM, HRMS (NSI+):



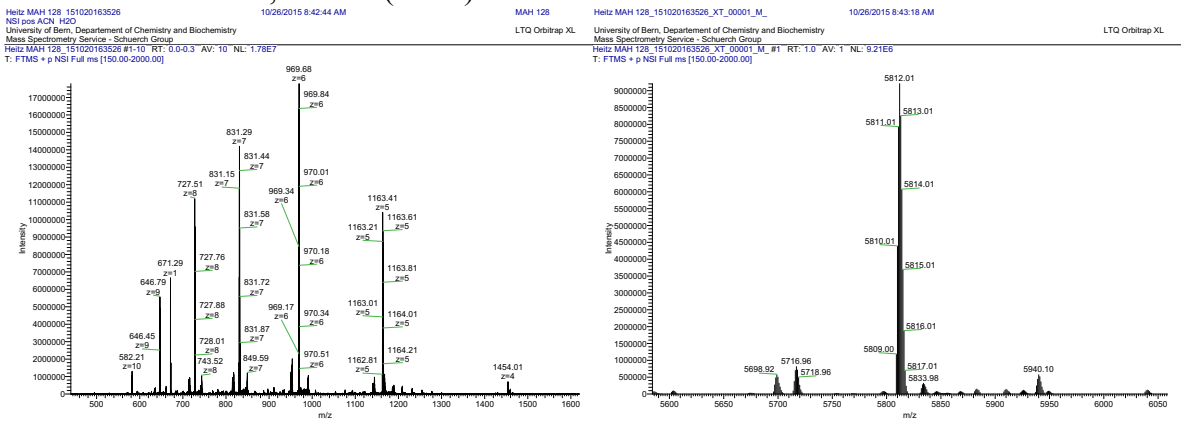
MH52 ((HKL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*LLLL) was obtained from the CEM Liberty Blue synthesizer as foamy colourless solid after preparative RP-HPLC (40.1 mg, 4.93 µmol, 6%). Analytical RP-HPLC: t_R = 2.81 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{282}H_{507}N_{83}O_{47}$ calc./obs. 5808.98/5809.00 [M+H$^+$].



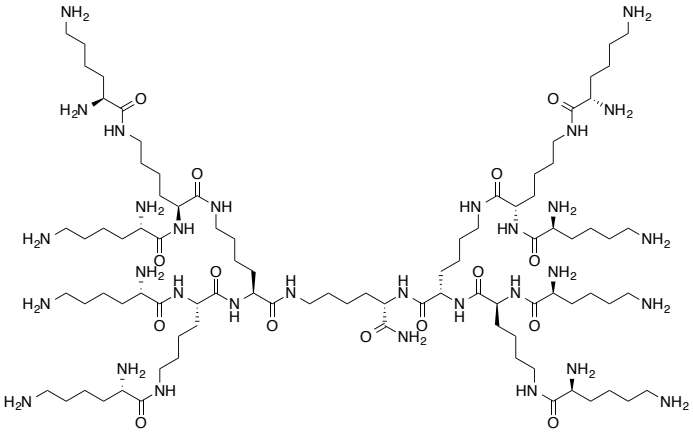
LCMS



MASS SPECTRUM, HRMS (NSI+):

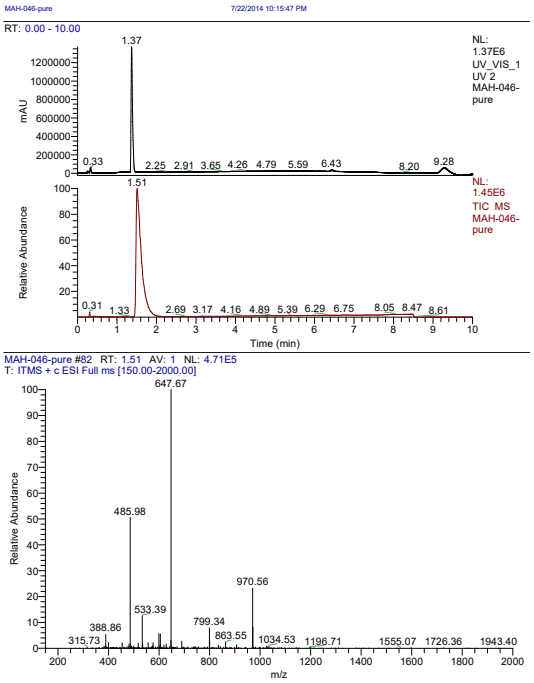


MH53 ((K)$_8$(*K*)$_4$(*K*)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (8.3 mg, 2.2 µmol, 6%). Analytical RP-HPLC: t_R = 1.37 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{90}H_{183}N_{31}O_{15}$ calc./obs. 1938.45/1938.46 [M+H$^+$].

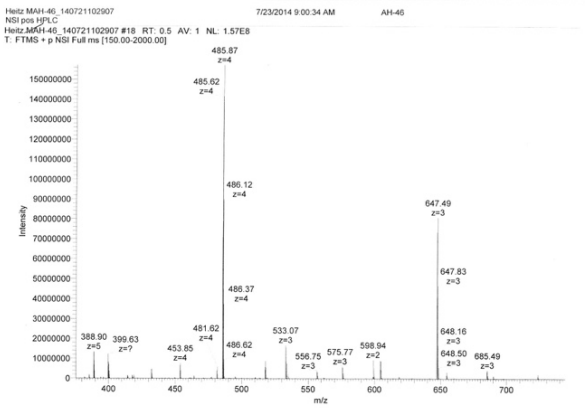


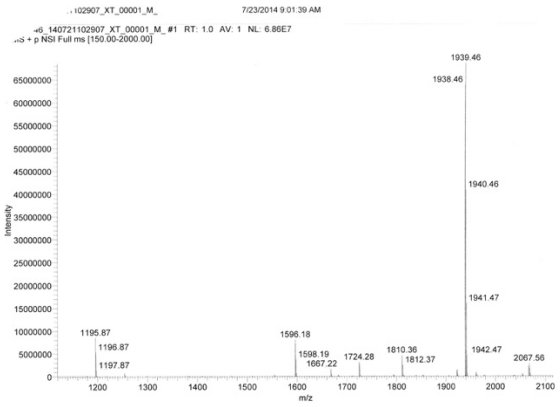
Chemical Formula: $C_{90}H_{183}N_{31}O_{15}$
Exact Mass: 1938,45
Molecular Weight: 1939,66

LCMS

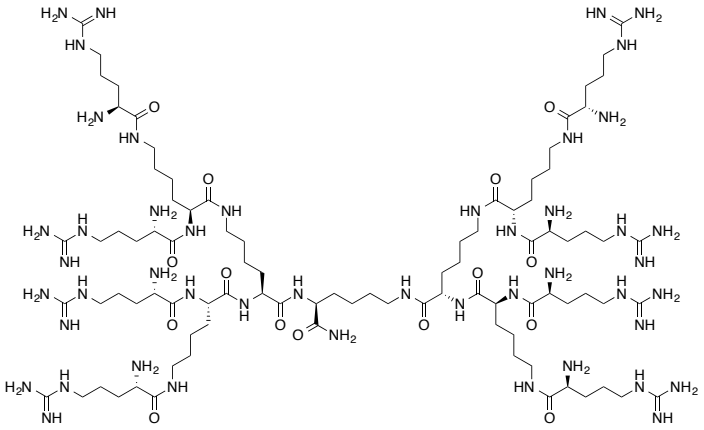


MASS SPECTRUM, HRMS (NSI+):



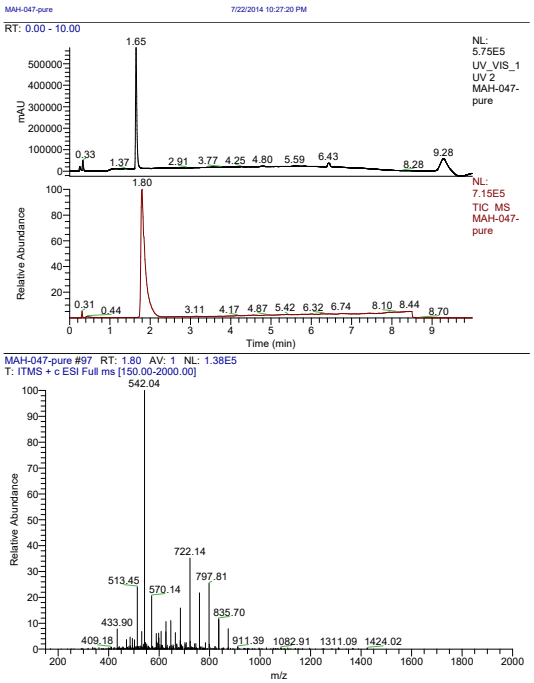


MH54 ((R)$_8$(*K*)$_4$(*K*)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (3.5 mg, 0.9 µmol, 3%). Analytical RP-HPLC: t_R = 1.65 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{90}H_{183}N_{47}O_{15}$ calc./obs. 2162.50/2162.51 [M+H$^+$].

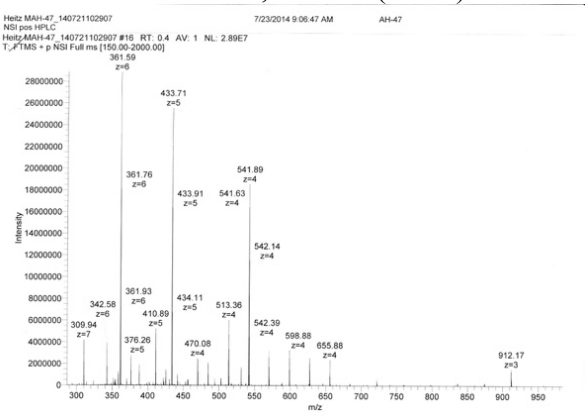


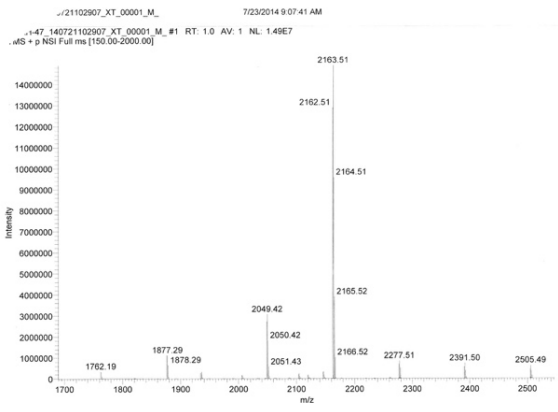
Chemical Formula: $C_{90}H_{183}N_{47}O_{15}$
Exact Mass: 2162,50
Molecular Weight: 2163,77

LCMS

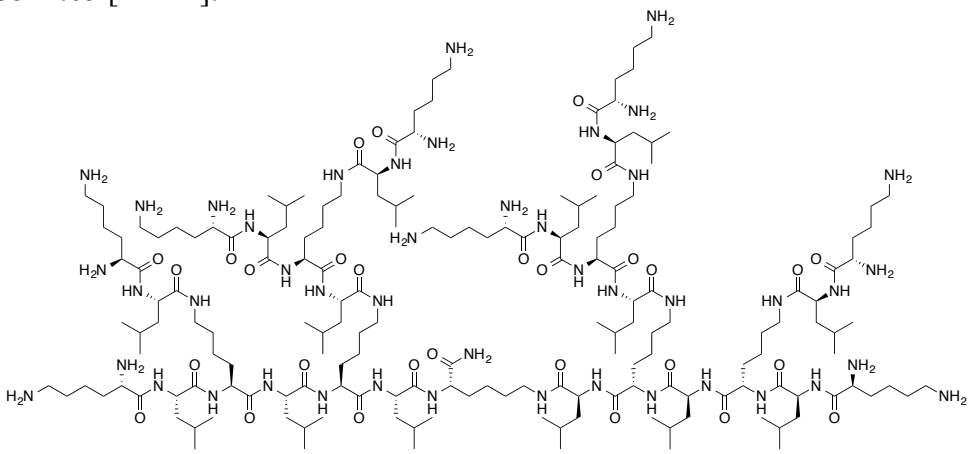


MASS SPECTRUM, HRMS (NSI+):



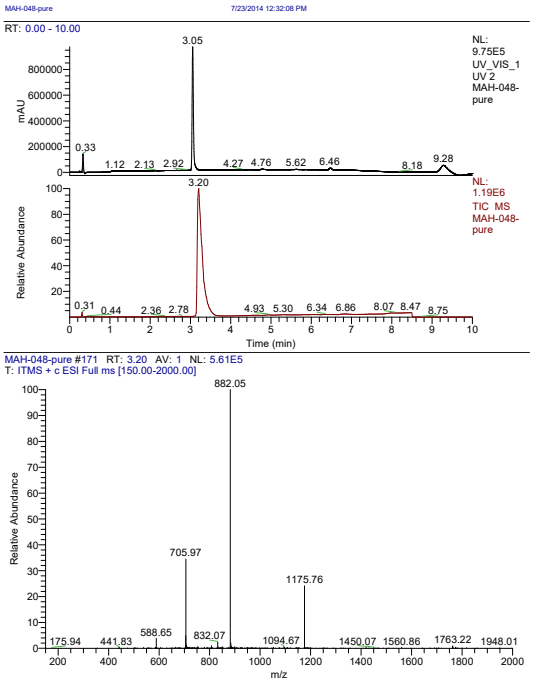


MH55 ((KL)$_8$(*K*L)$_4$(*K*L)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (17.0 mg, 3.2 μmol, 5%). Analytical RP-HPLC: t_R = 3.05 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{174}H_{337}N_{45}O_{29}$ calc./obs. 3521.63/3521.65 [M+H$^+$].

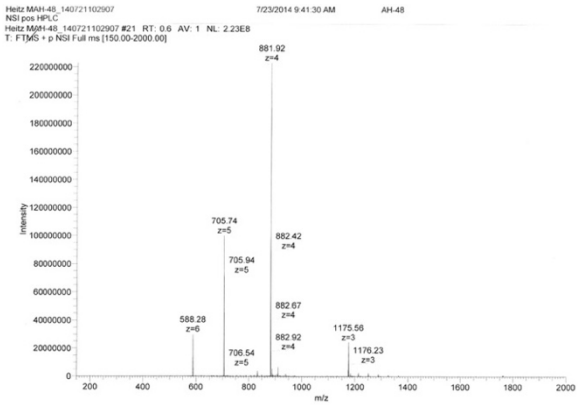


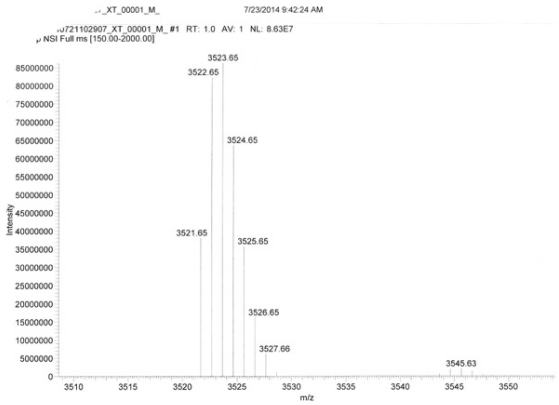
Chemical Formula: $C_{174}H_{337}N_{45}O_{29}$
Molecular Weight: 3523,90

LCMS

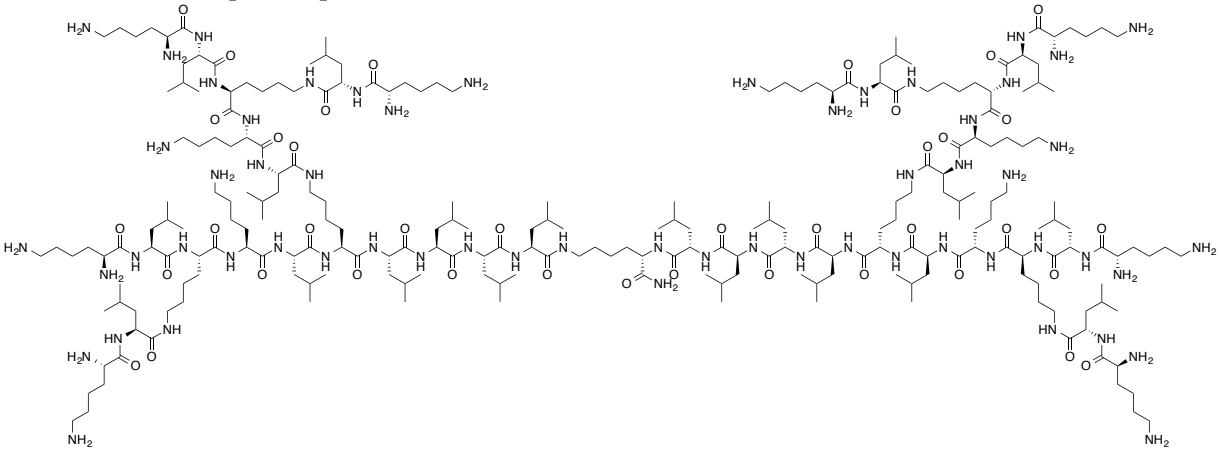


MASS SPECTRUM, HRMS (NSI+):



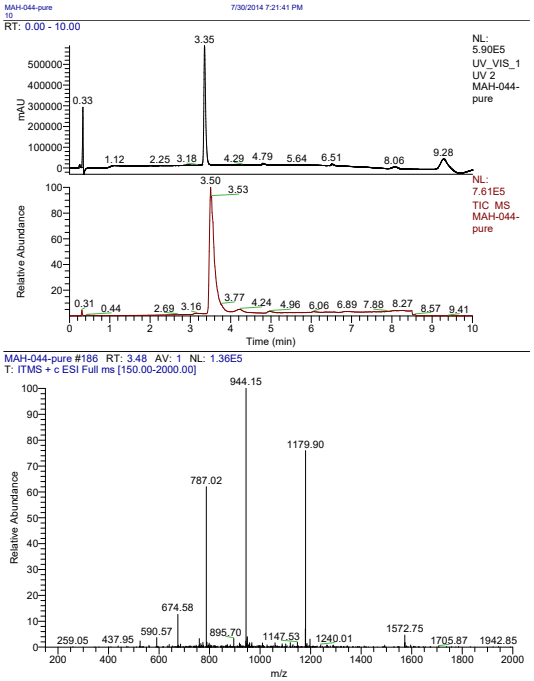


MH56 ((KL)$_8$(*K*KL)$_4$(*K*LLLL)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (5.5 mg, 0.8 µmol, 1%). Analytical RP-HPLC: t_R = 3.35 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{234}H_{451}N_{59}O_{39}$ calc./obs. 4712.51/4712.52 [M+H$^+$].

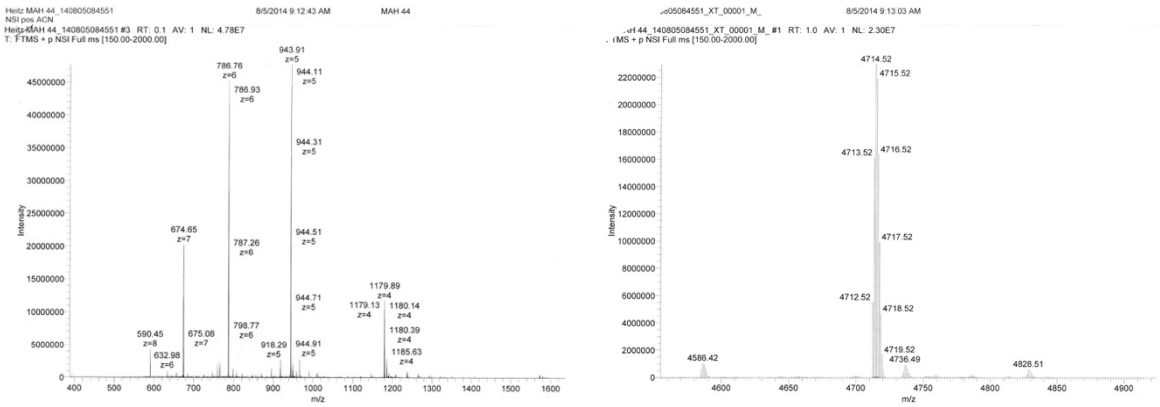


Chemical Formula: $C_{234}H_{451}N_{59}O_{39}$
Molecular Weight: 4715,46

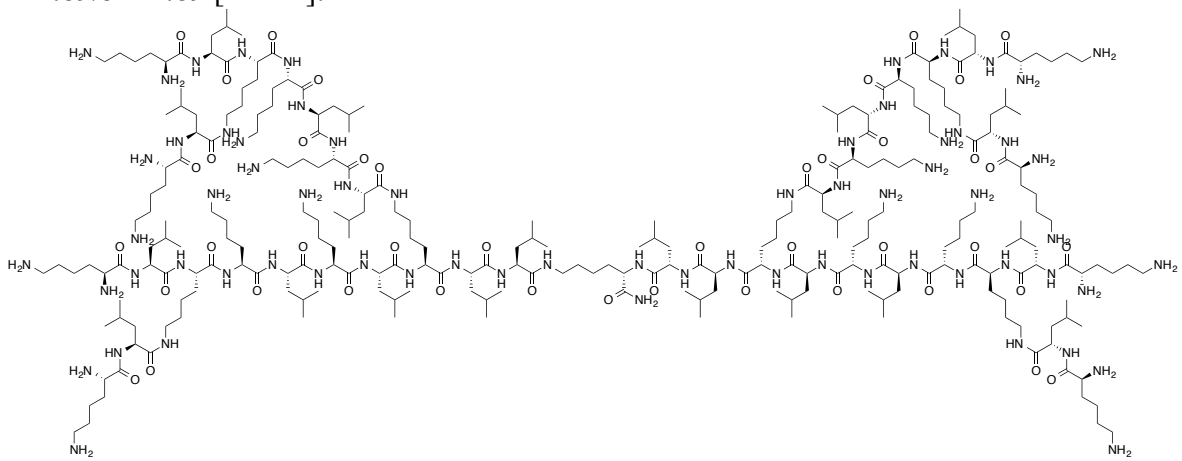
LCMS



MASS SPECTRUM, HRMS (NSI+):

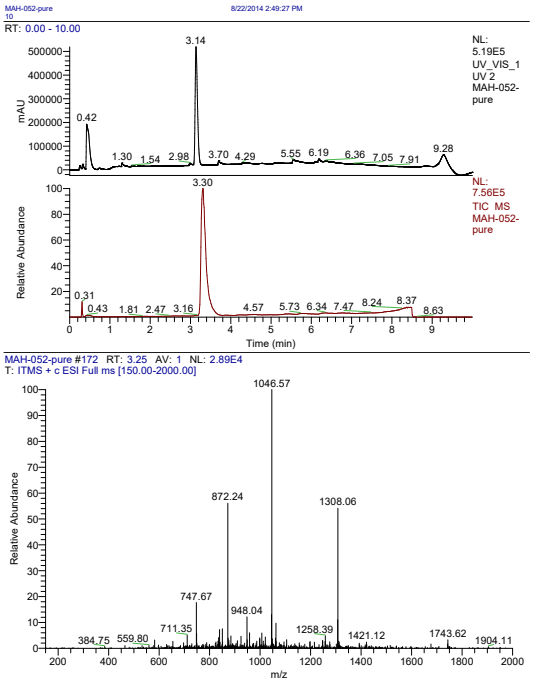


MH57 ((KL)$_8$(*K*KLKL)$_4$(*K*LL)$_2$*K*) was obtained after manual synthesis as foamy colourless solid after preparative RP-HPLC (6.6 mg, 0.83 μmol, 1%). Analytical RP-HPLC: t_R = 3.14 min (100% A to 100% D in 7.5 min, λ= 214 nm). MS (ESI+): $C_{258}H_{499}N_{67}O_{43}$ calc./obs. 5224.89/5224.89 [M+H$^+$].



Chemical Formula: $C_{258}H_{499}N_{67}O_{43}$
Molecular Weight: 5228,26

LCMS



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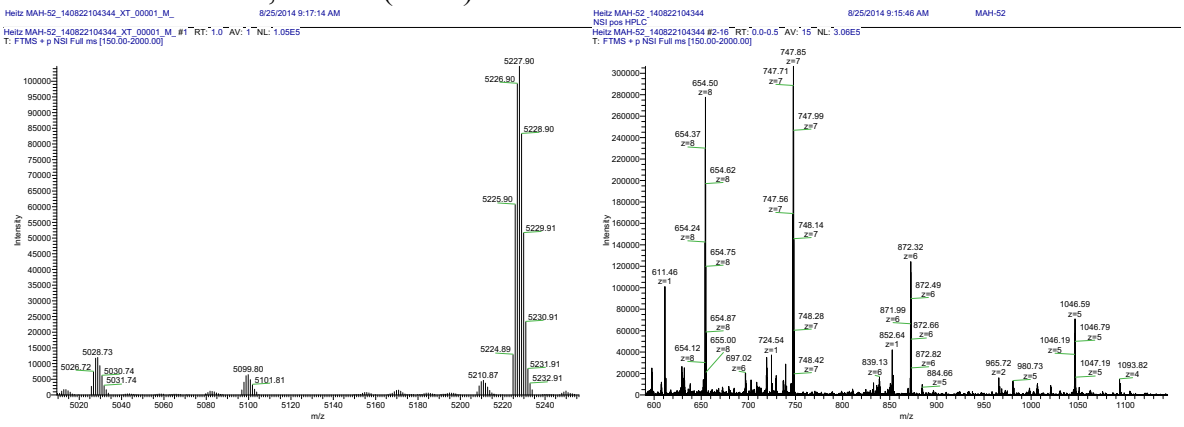

1.3 Peptide Dendrimers and siRNA

Table S1. Analogs of hits **MH06**, **MH13** and **MH18**.

No.	Sequence[a]	Yield[b] mg (%)	MS[c] calc./obs.	Free siRNA [%][d]	Cellular uptake [GMFI][e]	GAPDH activity [%][f]
Additional G0 with lysines and acylated lysines:						
MH27	$(KL)_8(KKL)_4(KLL)_2KGSK(C_6)$	24.5 (5)	4630.40/4630.40	15.2±1.1	n.d.	112±14
MH28	$(KL)_8(KKL)_4(KLL)_2KGSK(C_{12})$	11.9 (2)	4714.49/4714.50	8.8±0.5	n.d.	109±12
MH29	$(KL)_8(KKL)_4(KLL)_2KGSK(C_{18})$	7.1 (1)	4798.59/4798.59	7.2±0.8	n.d.	86±2
MH30	$(KL)_8(KKL)_4(KLL)_2K$	37.7 (8)	4260.18/4260.19	8.5±1.1	4±3	95±16
MH31	$(KL)_8(KKL)_4(KLL)_2KK$	12.0 (3)	4388.27/4388.28	9.1±1.3	n.d.	98±11
MH32	$(KL)_8(KKL)_4(KLL)_2KKK$	6.6 (2)	4516.37/4516.38	9.5±1.5	n.d.	97±12
MH33	$(KL)_8(KKL)_4(KLL)_2KK$(Lit)	14.5 (3)	4746.56/4746.56	9.8±0.8	21±10	109±6
MH34	$(KL)_8(KKL)_4(KLL)_2KK$(Por)	24.5 (6)	5028.50/5028.51	0.8±0.5	n.d.	86±15
MH35	$(KL)_8(KKL)_4(KLL)_2KK(C_8)K(C_8)K(C_8)$	10.9 (2)	5022.77/5022.79	5.8±0.9	85±2	46±8
MH36	$(KL)_8(KKL)_4(KLL)_2KK$(AcLL)K(AcLL)	5.0 (1)	5052.72/5052.74	11.1±1.1	28±8	78±4
MH37	$(KL)_8(KKL)_4(KLL)_2KK$(LLL)K(LLL)	2.1 (1)	5194.87/5194.89	9.3±0.6	40±21	79±2
MH38	$(KL)_8(KKL)_4(KLL)_2KK$(LLLL)LLLL	13.3 (3)	5292.94/5292.95	3.3±0.8	274±45	43±15
Additional G0 with oligopeptide:						
MH39	$(KL)_8(KKL)_4(KLL)_2K$PPPP	7.2 (1)	4648.39/4648.39	9.2±0.9	n.d.	57±8
MH40	$(KL)_8(KKL)_4(KLL)_2K$NleNleNleNle	5.1 (1)	4712.51/4712.53	3.9±0.1	127±24	23±1
MH41	$(KL)_8(KKL)_4(KLL)_2K$Ado	47.0 (13)	4457.35/4457.36	9.6±0.7	16±4	94±9
MH42	$(KL)_8(KKL)_4(KLL)_2K$AdoAdo	54.5 (15)	4564.53/4654.54	9.8±0.7	15±8	82±6
Additional designs:						
MH53	$(K)_8(K)_4(K)_2K$	8.3 (6)	1938.45/1938.46	24.1±1.8	n.d.	95±8
MH54	$(R)_8(K)_4(K)_2K$	3.5 (3)	2162.5/2162.51	9±0.3	n.d.	92±23
MH55	$(KL)_8(KL)_4(KL)_2K$	17.0 (5)	3521.63/3521.65	11.2±1.6	n.d.	96±6
MH56	$(KL)_8(KKL)_4(KLLL)_2K$	5.5 (1)	4712.51/4712.52	7±0.6	58±10	87±11
MH57	$(KL)_8(KKLKL)_4(KLL)_2K$	6.6 (1)	5224.89/5224.89	11.9±0.6	n.d.	106±1

[a] One-letter code amino acids are used, for the core three letter code may be used for some moieties with Lit= lithocholic acid, Por= 5-(4-Carboxyphenyl)-10,15,20-triphenylporphyrin and Ado= 12-amino-dodecanoic acid (Ado), *K* is the branching lysine residue, the C-terminus are carboxamide $CONH_2$, all N-termini are free. [b] Isolated yields as trifluoroacetate salt after preparative HPLC purification. [c] ESI MS, see also supporting information. [d] Fluorescence from intercalation in siRNA (20 nM) in complex with peptide dendrimer (N/P 10, 420 nM, 3 µg/mL) by Quant-it™ microRNA performed in triplicate normalized to the value of siRNA alone. [e] GMFI = Geo Mean Fluorescence Intensity of internalized FAM-siRNA in HeLa cells after 4 h transfection by FAM-siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) from two independent experiments normalized to L2000. [f] GAPDH activity in HeLa cells after 4 h transfection by siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS and normalized to siNC (negative control). Experiments in three independent triplicates. Bar graphs representation and parallel transfection with siNC are present in Figure S1. n.d.= not determined.

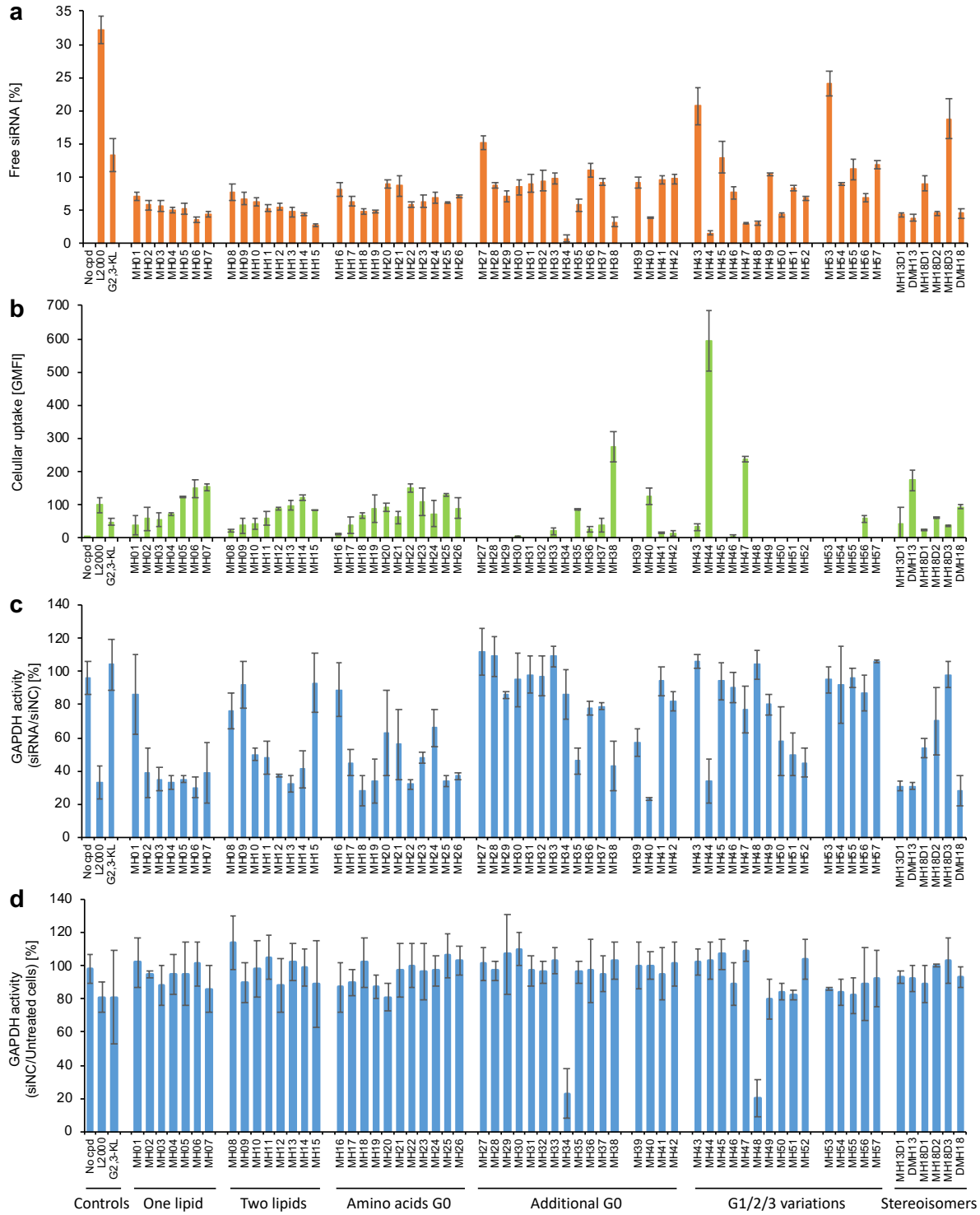

Figure S1. (a) Fluorescence from intercalation in siRNA (20 nM) in complex with peptide dendrimer (N/P 10, 420 nM, 3 µg/mL) or L2000 (2:1, 532 ng/mL) by Quant-it™ microRNA performed in triplicate and normalized to the value of siRNA alone. **(b)** GMFI = Geo Mean Fluorescence Intensity of internalized FAM-siRNA in HeLa cells after 4 h transfection by FAM-siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) from two independent experiments normalized to L2000. **(c)** GAPDH activity in HeLa cells after 4 h transfection by siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS and normalized to siNC. **(d)** GAPDH activity in HeLa cells after 4 h transfection by siNC (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS and normalized to untreated cells.

1.3.1 Cell Culture, Transfection Reagents and siRNA

HeLa, HEK-293, CHO, PC-3, HT-1080, SH-SY5Y, Caco-2 and MCF-7 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. The sequence of the siRNA targeting GAPDH (AM 4631) for the sense strand is GGUCAUCCAUGACAACUUUdTdT and for the antisense strand AAAGUUGUCAUGGAUGACCdTdT. In the case of FAM-siRNA (AM 4650) or Cy3-siRNA (AM4649) targeting GAPDH, the sequences are identical with a 5-carboxyfluorescein or a Cyanine 3 attached to the 5' end of the sense strand. The sequence of the validated negative control siRNA (siNC, AM 4635) for the sense strand is AGUACUGCUUACGAUACGGdTdT and for the antisense strand CCGUAUCGUAAGCAGUACUdTdT. The siRNAs are consisting of phosphodiester bonds and containing two nucleotide overhangs (dTdT) at the 3'-end (Thermo Fisher Scientific). Lipofectamine® 2000 (L2000) was obtained from Thermo Fisher Scientific and used as positive control with the transfection protocol in accordance with the manufacturer's instructions.

1.3.2 siRNA Transfection and Protein Quantitation

HeLa, CHO, PC3, SH-SY5Y, Caco-2 and MCF-7 cells were seeded in TPP 96-well plates (Faust Laborbedarf AG, Schaffhausen) at 5×10^3 cells per well the day before transfection. The 96-well plates were coated with poly-L-Lysine (Sigma aldrich, Buchs, CH) for 1 h and dried under a flow of air in the fumehood at RT before plating HEK-293 and HT-1080 at $5x10^3$ cells per well the day before transfection. Cells were always used in their exponential phase for transfection and with a number of passages between 3 to 20. The siRNA transfection complexes were formed in OptiMEM by mixing siRNA (1–20 pmol, 0.02–0.4 µL from a 50 µM Milli-Q water solution in 6.25 µL OptiMEM) with peptide dendrimers (N/P ratio of 1–100, 21–420 pmol, 0.1–3.5 µl from a 1 mg/mL Milli-Q water solution in 6.25 µL OptiMEM) or L2000 (w/w ratio of 0.5:1–2:1, 0.0665–0.266 µg, 0.0665–0.266 µL from the 1 mg/mL commercial solution in 6.25 µL OptiMEM) at room temperature for 30 minutes (12.5 µL, concentration of 80–1600 nM siRNA and 1.68–33.6 µM i.e. 12–240 µg/mL peptide dendrimers or 5.32–21.28 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 10–200 nM siRNA and 0.210–4.2 µM i.e. 1.5–30 µg/mL peptide dendrimers or 0.665–2.66

µg/mL L2000). Following removal of the complete medium from the cells, 100 µL of the transfection were added to each well. The plate was then incubated for 4–72 h at 37 °C in a humidified atmosphere in 5% carbon dioxide. The transfection complexes were replaced by full growth medium following the transfection. The siGAPDH gene knockdown assays were conducted 48 h or 72 h hours following transfection by measuring the level of GAPDH protein according to the manufacturer's instructions of the KDalert GAPDH assay kit (Thermo Fisher Scientific, Reinach, CH). Briefly, medium was removed, cells were lysed with 200 µL cold lysis buffer, 2–10 µL of lysis buffer from each well was taken to a new 96 well plate containing 90 µL Master Mix and fluorescence measured at λ_{ex}= 560 nm and λ_{em}= 590 nm on a Tecan Infinite M1000 Pro plate reader.

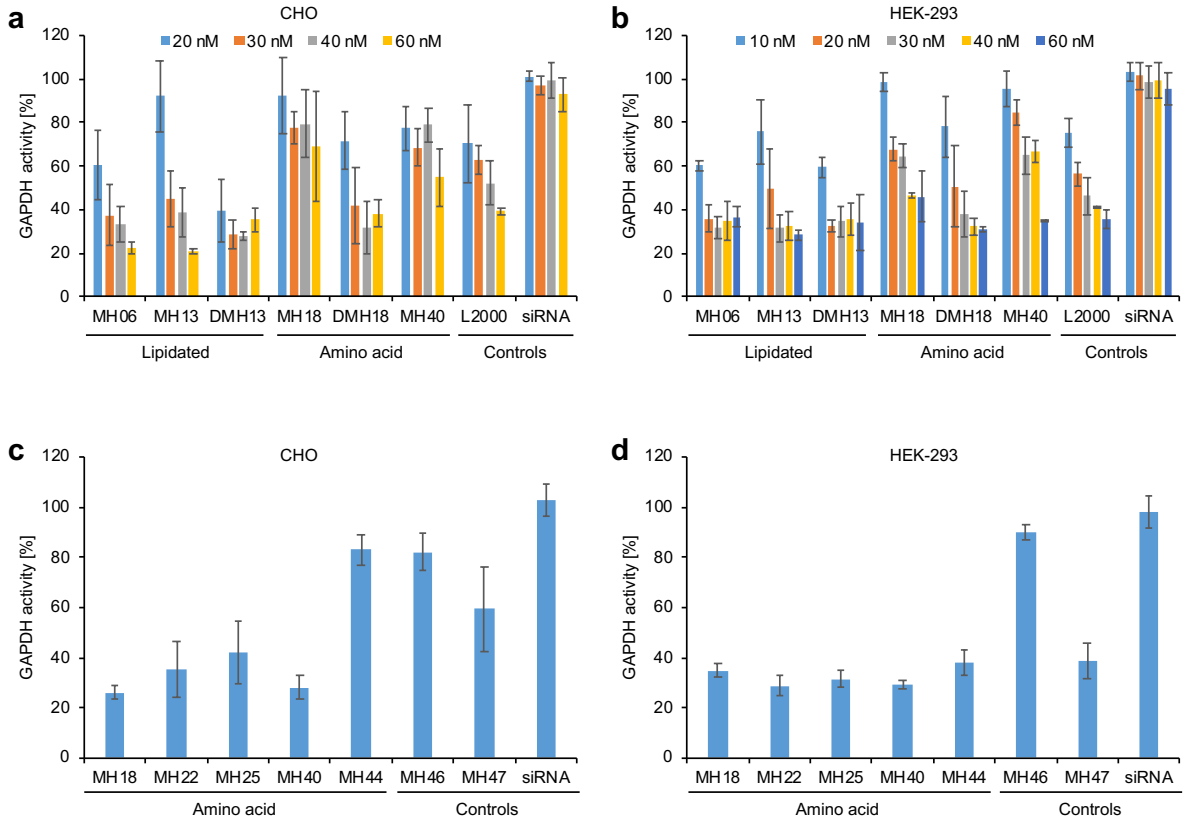

Figure S2. GADPH activity in **(a)** CHO and **(b)** HEK-293 cell lines after 4 h transfection by siRNA (10–60 nM) and peptide dendrimers (N/P 10, 0.21–1.26 µM, 1.5–9 µg/mL) or L2000 (2:1, 0.266–1.596 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS. GAPDH activity in **(c)** CHO and **(d)** HEK-293 cell lines after 4 h transfection peptide dendrimers (N/P 10, 2.1 µM) and siRNA (100 nM) followed by 48 h incubation in DMEM supplemented with 10% FCS. Results were normalized to the value of the parallel transfection with siNC (negative control). All experiments were carried out in triplicate in three independent experiments.

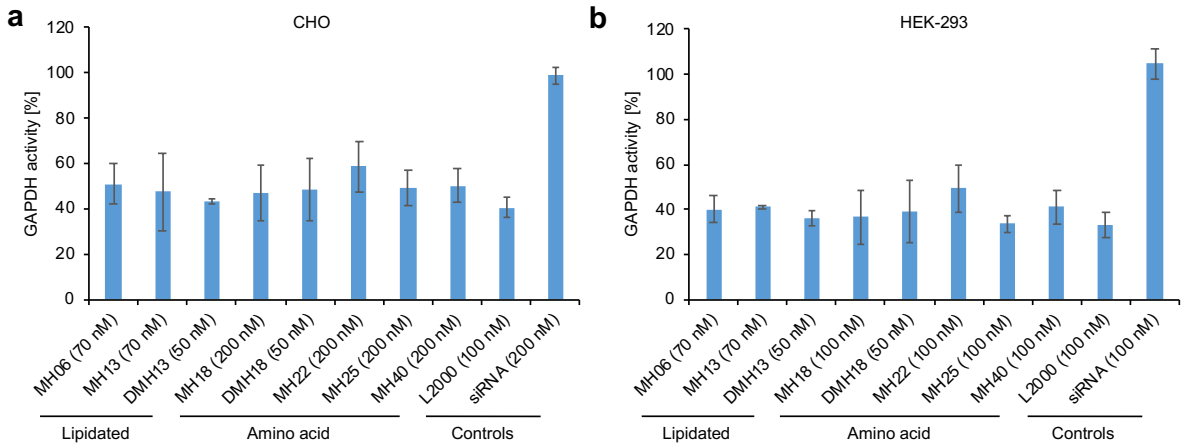

Figure S3. GAPDH activity in **(a)** CHO and **(b)** HEK-293 cells after 8 h transfection in presence of 10% serum by siRNA (50–200 nM) and peptide dendrimers (N/P 10, 1.05–4.2 µM, 7.5–30 µg/mL) or L2000 (2:1, 2.66 µg/mL) followed by 72 h incubation in DMEM supplemented with 10% FCS. Results were normalized to the value of the parallel transfection with siNC (negative control). All experiments were carried out in triplicate.

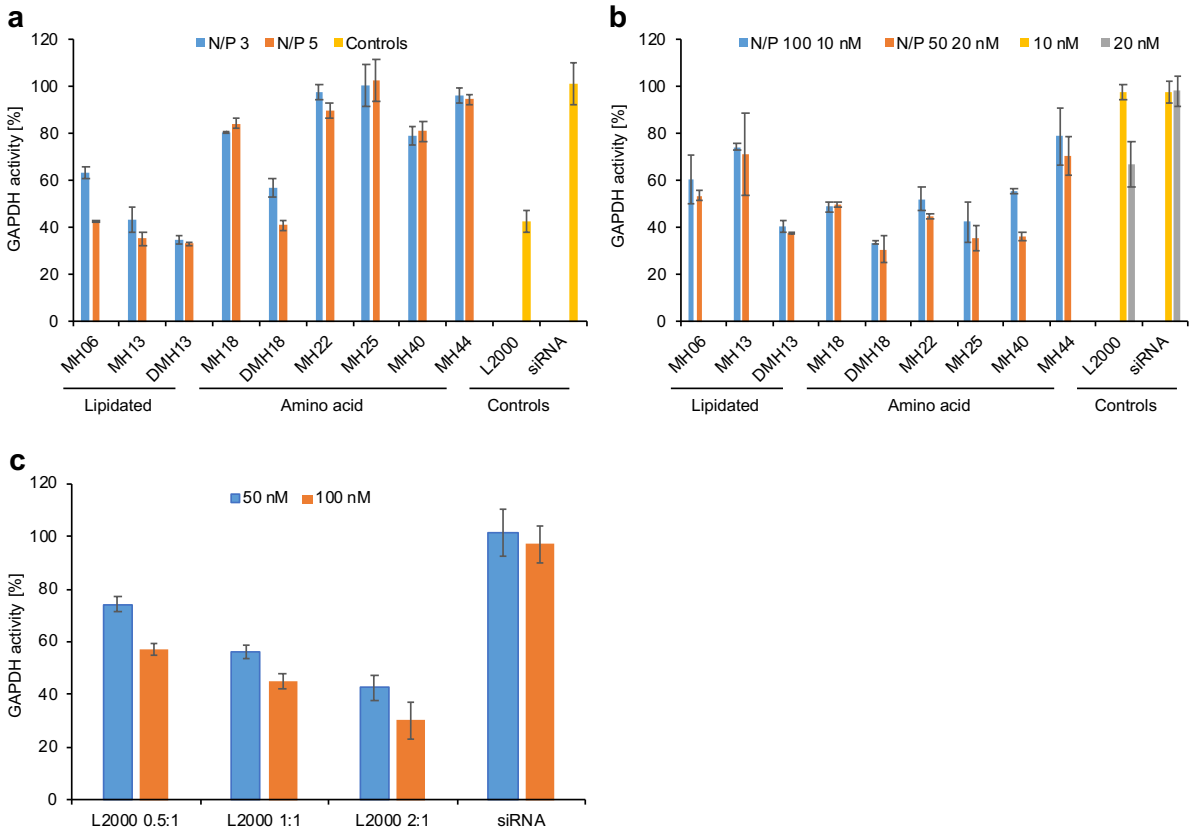

Figure S4. (a) GADPH activity in HeLa cells after 4 h transfection by siRNA (50 nM) and peptide dendrimers (N/P 3 and 5, 0.315 and 0.525 µM, 2.25–3.75 µg/mL) or L2000 (2:1, 1.33 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS. **(b)** GADPH activity in HeLa cells after 4 h transfection by siRNA (10 and 20 nM) and peptide dendrimers (N/P 100 and 50, 1.05 and 2.1 µM, 7.5 and 15 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS. **(c)** GADPH activity in HeLa cells after 4 h transfection by siRNA (50 and 100 nM) and L2000 at different ratios (0.5–2:1, 0.3325 to 2.66 µg/mL) followed by 48 h incubation in DMEM supplemented with 10% FCS. Results were normalized to the value of the parallel transfection with siNC (negative control). All experiments were carried out in triplicate in three independent experiments.

1.3.3 siRNA Transfection in Presence of Bafilomycin

HeLa cells were used untreated or 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. After transfection, complexes were removed and replaced with DMEM supplemented with 10% FCS and incubated for 48 h or DMEM supplemented with 10% FCS containing 200 nM Bafilomycin A1, incubated for 24 h then replaced by DMEM supplemented with 10% FCS for 24 h supplementary incubation. Then, HeLa cells were treated for 1 h before transfection, 24 h following transfection or both with Bafilomycin A1. GAPDH protein level was assessed as described above after a total of 48 h.

1.3.4 Cellular Uptake by Flow Cytometry

HeLa, CHO and HEK-293 cells were seeded in 24-well TPP plates (Faust Laborbedarf AG, Schaffhausen) at 1×10^5 cells per well 24 h prior to transfection. Complexes were formed with labelled FAM-siRNA (Thermo Fisher Scientific, Reinach, CH) (12–40 pmol, 0.24–0.8 µL from a 50 µM Milli-Q water solution in 25 µL OptiMEM) and peptide dendrimers (N/P ratio of 10, 252–840 pmol, 2.1–7 µL from a 1 mg/mL Milli-Q water solution in 25 µL OptiMEM) or L2000 (w/w ratio of 2:1, 0.319–1.064 µg, 0.319–1.064 µL from the 1 mg/mL commercial solution in 25 µL OptiMEM) and incubated at room temperature for 30 minutes (50 µL, concentration of 240–800 nM siRNA and 5.04–16.8 µM i.e. 36–120 µg/mL peptide dendrimers or 6.384–21.28 µg/mL L2000). The complexes were then diluted in OptiMEM to a final volume of 400 µL per well (final concentration of 30–100 nM FAM-siRNA and 0.63–2.1 µM i.e. 4.5–15 µg/mL peptide dendrimers or 0.798–2.66 µg/mL L2000). Following removal of the medium, the complexes were added to each well and incubated for 4 h at 37 °C in a humidified atmosphere in 5% carbon dioxide. Then the medium was removed, cells were washed with heparin in OptiMEM (2 mg/ml, 0.5 ml, 3 times), washed twice with PBS and the cell membrane was labeled with CellMask Deep Red plasma membrane stain (Thermo Fisher Scientific, Reinach, CH) at 0.5X in full DMEM growth medium (0.25 µL in 0.5 mL / well), 10 minutes at 37 °C. The cells were washed with PBS (1.0 mL / well, 3 times) and detached from the wells with 250.0 µL of 0.25% Trypsin / EDTA solution at 37 °C. Cells were harvested with 750.0 µL full DMEM growth medium, collected in 1.5 mL eppendorf tubes and centrifuged 5 minutes at 200 RCF. The trypsin solution was discarded, the pellet shaken and the cells were resuspended in 50.0 µL of PBS + 1% FCS. The fluorescence intensity of each cell sample was analyzed by a flow cytometer-microscope coupled

ImageStream X Mark II (Merck Millipore) and processed with the IDEAS® software.

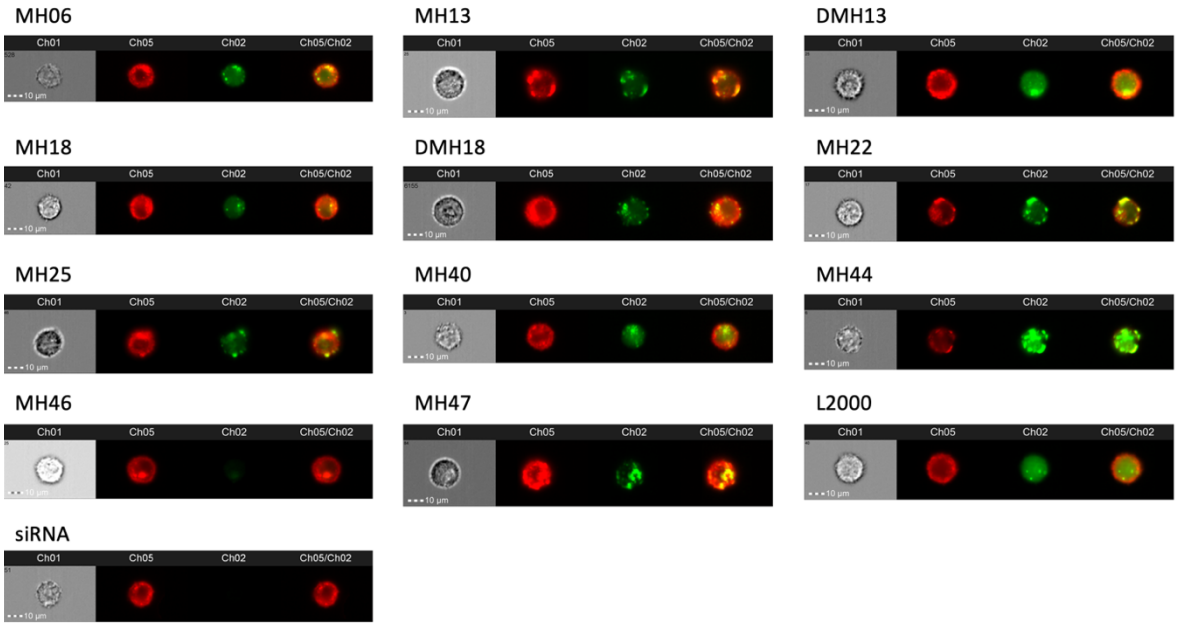

Figure S5. ImageStream X Mark II images of internalized FAM-siRNA in HeLa cells after 4 h transfection by FAM-siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) from flow cytometry experiments.

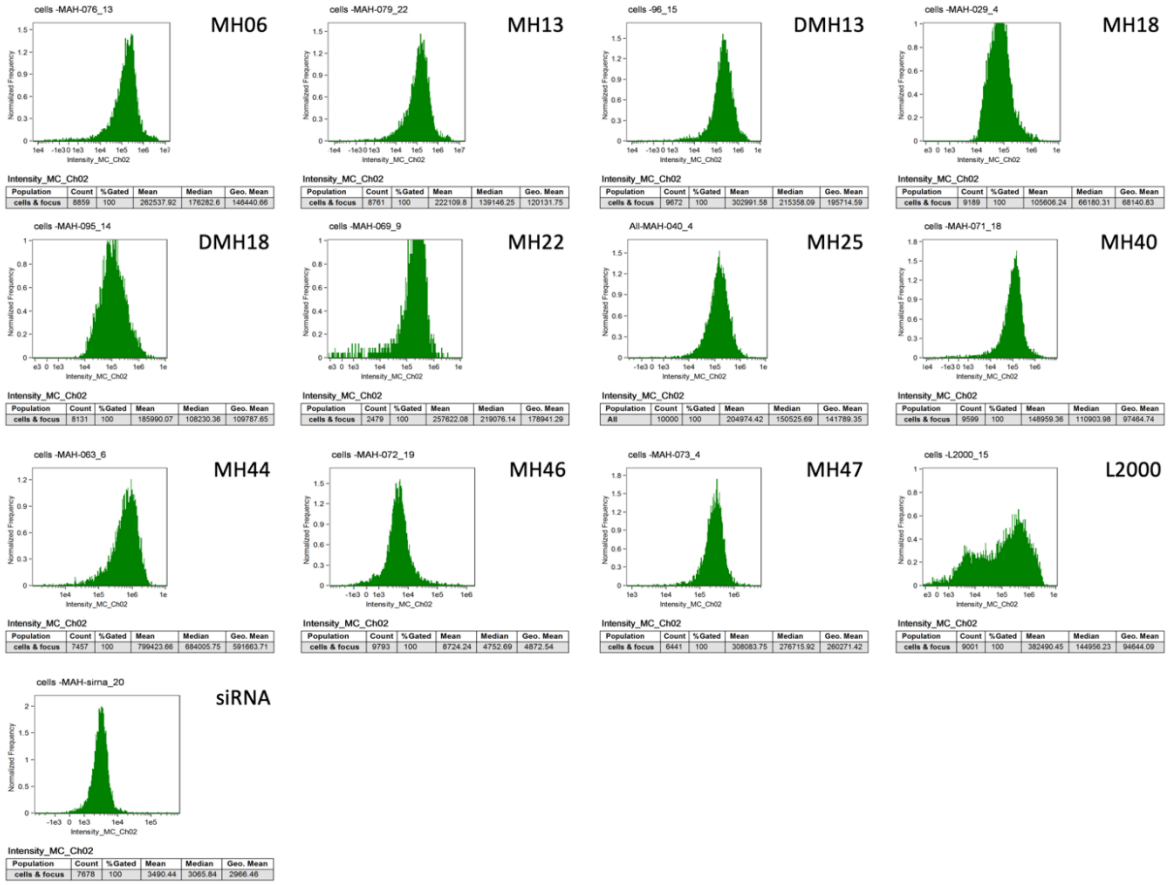

Figure S6. ImageStream X Mark II histograms of internalized FAM-siRNA in HeLa cells after 4 h transfection by FAM-siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) from flow cytometry experiments.

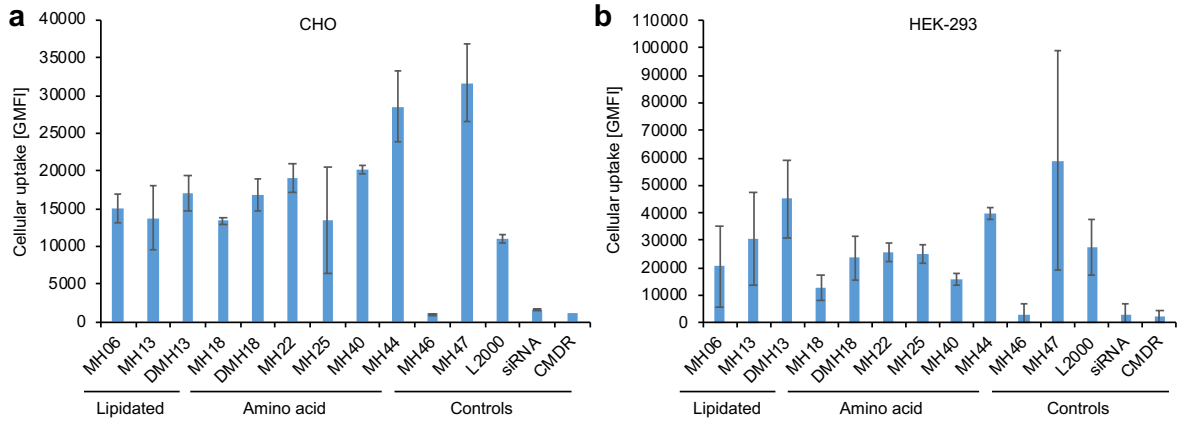

Figure S7. Geo Mean Fluorescence Intensity (GMFI) of internalized FAM-siRNA in **(a)** CHO or **(b)** HEK-293 after 4 h transfection by FAM-siRNA (30 nM) and peptide dendrimers (N/P 10, 0.63 µM, 4.5 µg/mL) or L2000 (2:1, 0.798 µg/mL) from two independent experiments.

1.3.5 Cellular Uptake in Presence of Inhibitors and at Lower Temperature

Cells were treated with Cytochalasin D (25 µg/mL), Nystatin (25 µg/mL) or Chlorpromazine (15 µg/mL) in DMEM supplemented with 10% FCS for 1 h and washed with PBS before transfection in 24-well TPP plates as described above. In the temperature dependent experiment, cells were transfected in the same condition as above for 4 h but at 4 °C. The cells were washed and processed as described above and the fluorescence intensity of each cell sample was analyzed by a flow cytometer-microscope coupled ImageStream X Mark II (Merck Millipore) and processed with the IDEAS® software.

1.3.6 Quantitative RT-PCR

Cells were transfected in TPP 96-well plates as previously described. Following transfections, cells were washed with 50 µl cold PBS and the Cells-to-Ct kit (Thermo Fisher Scientific, Reinach, CH) was used. Briefly, cells were lysed with 49.5 µL lysis buffer + 0.5 µL DNase for 5 minutes, 5 µl of stop solution added and incubated for 2 minutes. Then, 2 µl of cell lysate was mixed with 18 µl of the Master mix containing 1 µL TaqMan GAPDH endogenous control (FAM/MGB, non-primer limited, 433764F, Thermo Fisher Scientific), 1 µL TaqMan 18S Endogenous control (VIC/MGB, primer limited, 4319413E, Thermo Fisher Scientific), 5 µL TaqMan 1-Step qRT-PCR Mix and 11 µL water. RT-PCR was performed in duplex, with GAPDH and 18S primers in the green and yellow channels respectively in a Corbett Rotorgene 6000 (Qiagen). Cycles: RT: 50 °C/5 min, RT inactivation: 95 °C/20 sec, Amplification: 95 °C/15sec and 60 °C/1 min for 40 cycles. mRNA levels were calculated by normalizing the Ct values of GAPDH to the Ct value of 18S and quantified by the $2^{-\Delta\Delta Ct}$ method.

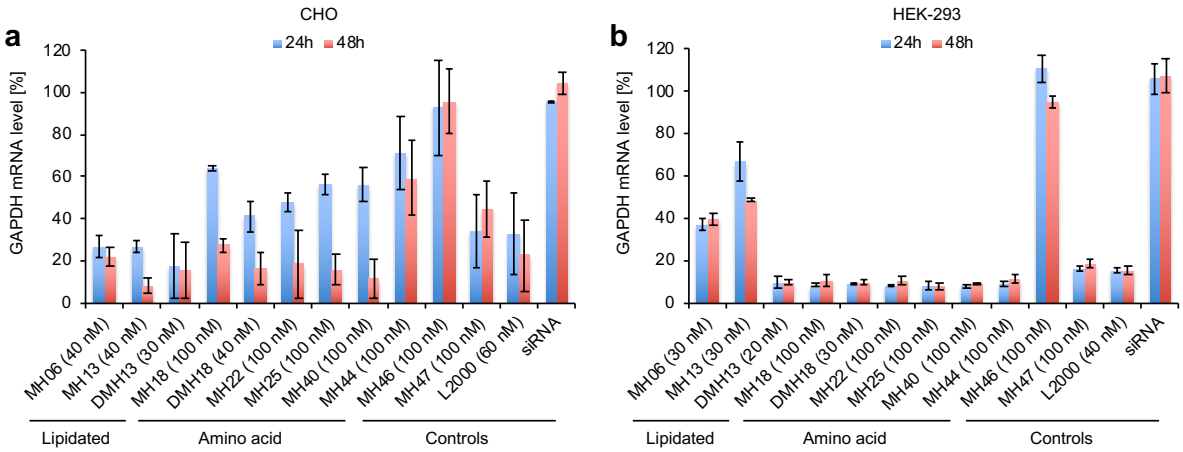

Figure S8. GAPDH mRNA level in **(a)** CHO and **(b)** HEK-293 cells by RT-PCR after 4 h transfection with siRNA (20–100 nM) in complex with dendrimers (N/P 10, 0.42–2.1 µM, 3–15 µg/mL) or L2000 (2:1, 1.064 and 1.596 µg/mL) completed by an incubation in DMEM supplemented with 10% FCS of up to 24 or 48 h. Experiments were carried out in triplicate. Results were normalized to the level of untreated cells and each measurement to the level of 18S. Experiments were carried out in triplicate.

1.3.7 Cell Viability by AlamarBlue® Assay

Cells were transfected in TPP 96-well plates as previously described. Following transfections, the medium was removed and replaced with 10% AlamarBlue® (Thermo Fisher Scientific, Reinach, CH) in DMEM supplemented with 10% FCS. Cells were incubated for 4–24 h 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.

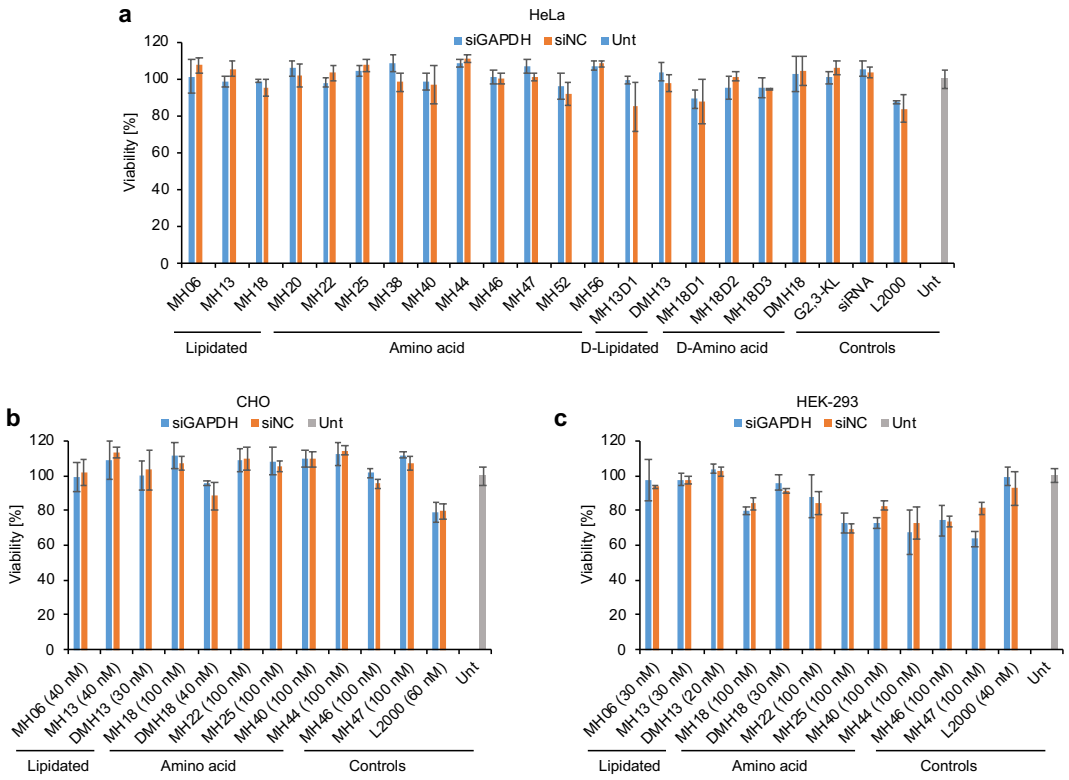

Figure S9. Viability of cells after 4 h transfection by **(a)** siRNA (100 nM) and peptide dendrimers at (N/P 10, 2.1 μM, 15 μg/mL) or L2000 (2:1, 1.33 μg/mL) in HeLa and at different concentration (20–100 nM siRNA and N/P 10, 0.42–2.1 μM i.e. 3–15 μg/mL peptide dendrimers or 2:1, 0.532–2.66 μg/mL L2000) in **(b)** CHO and **(c)** HEK-293 cells. Experiments were carried out in triplicate and results were normalized to the level of untreated cells.

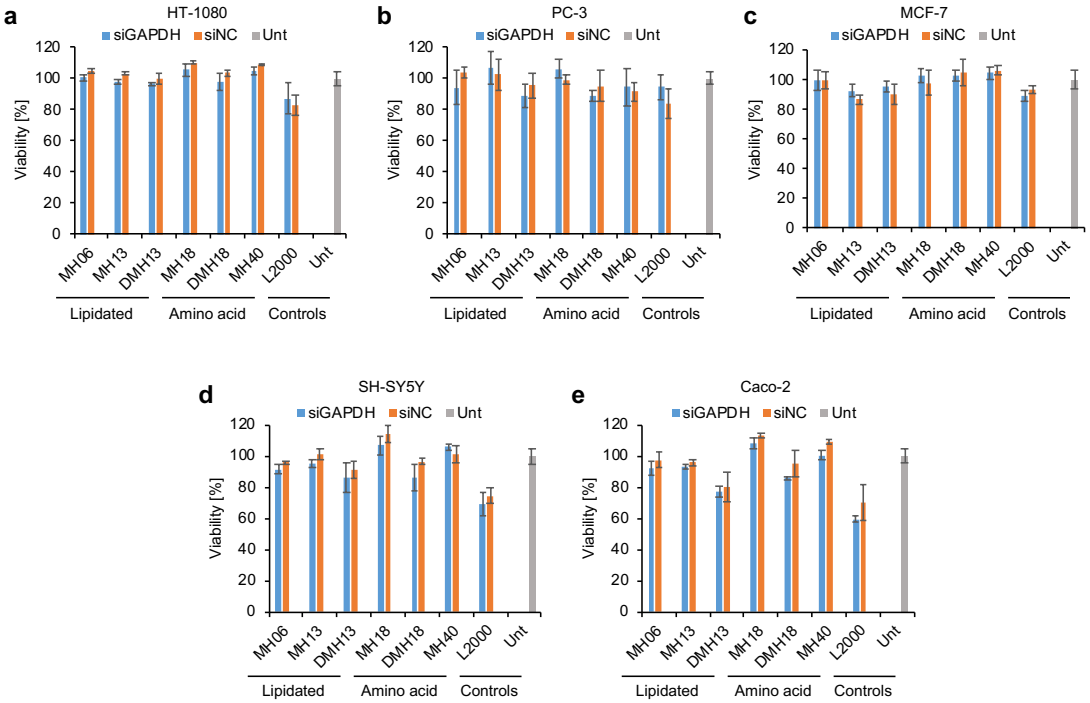

Figure S10. Viability of **(a)** HT-1080, **(b)** PC-3, **(c)** MCF-7, **(d)** SH-SY5Y and **(e)** Caco-2 cells after 4 h transfection by siRNA (50 nM) and peptide dendrimers (N/P 10, 1.05 μM, 7.5 μg/mL) or L2000 (2:1, 1.33 μg/mL). Experiments were carried out in triplicate and results normalized to the level of untreated cells.

1.3.8 Confocal Microscopy

Nunc Lab-Tek II 8-well chambered coverglass plates (Faust Laborbedarf AG, Schaffhausen) were treated with poly-L-Lysine (Sigma Aldrich, Buchs, CH) for 1 h, dried at RT and the day prior transfection the cells were plated at 3×10^4 cells per well. Complexes were formed with labelled FAM-siRNA (12–40 pmol, 0.24–0.8 µL from a 50 µM Milli-Q water solution in 25 µL OptiMEM) and peptide dendrimers (N/P ratio of 10, 252–840 pmol, 2.1–7 µL from a 1 mg/mL Milli-Q water solution in 25 µL OptiMEM) or L2000 (w/w ratio of 2:1, 0.319–1.064 µg, 0.319–1.064 µL from the 1 mg/mL commercial solution in 25 µL OptiMEM) and incubated at room temperature for 30 minutes (50 µL, concentration of 240–800 nM siRNA and 5.04–16.8 µM i.e. 36–15 µg/mL peptide dendrimers or 6.384–21.28 µg/mL L2000). The complexes were then diluted in OptiMEM to a final volume of 400 µL per well (final concentration of 30–100 nM FAM-siRNA and 0.63–2.1 µM i.e. 4.5–15 µg/mL peptide dendrimers or 0.798–2.66 µg/mL L2000). The complexes were added to each well and incubated 4 h at 37 °C in a humidified atmosphere in 5% carbon dioxide following the removal of the full growth medium. Then, the medium was removed, cells were washed with heparin in OptiMEM (2 mg/ml, 0.5 ml, 3 times), washed twice with PBS and the cell membrane was labeled with CellMask Deep Red plasma membrane stain (Thermo Fisher Scientific, Reinach, CH) at 0.5X in full DMEM growth medium (0.25 µL in 0.5 mL / well), 10 minutes at 37 °C. The cells were washed with PBS (1.0 mL / well, 3 times), FluoroBrite DMEM (Thermo Fisher Scientific, Reinach, CH) was added and images were taken on a Zeiss LSM 880 confocal microscope with lense x40/1.3.

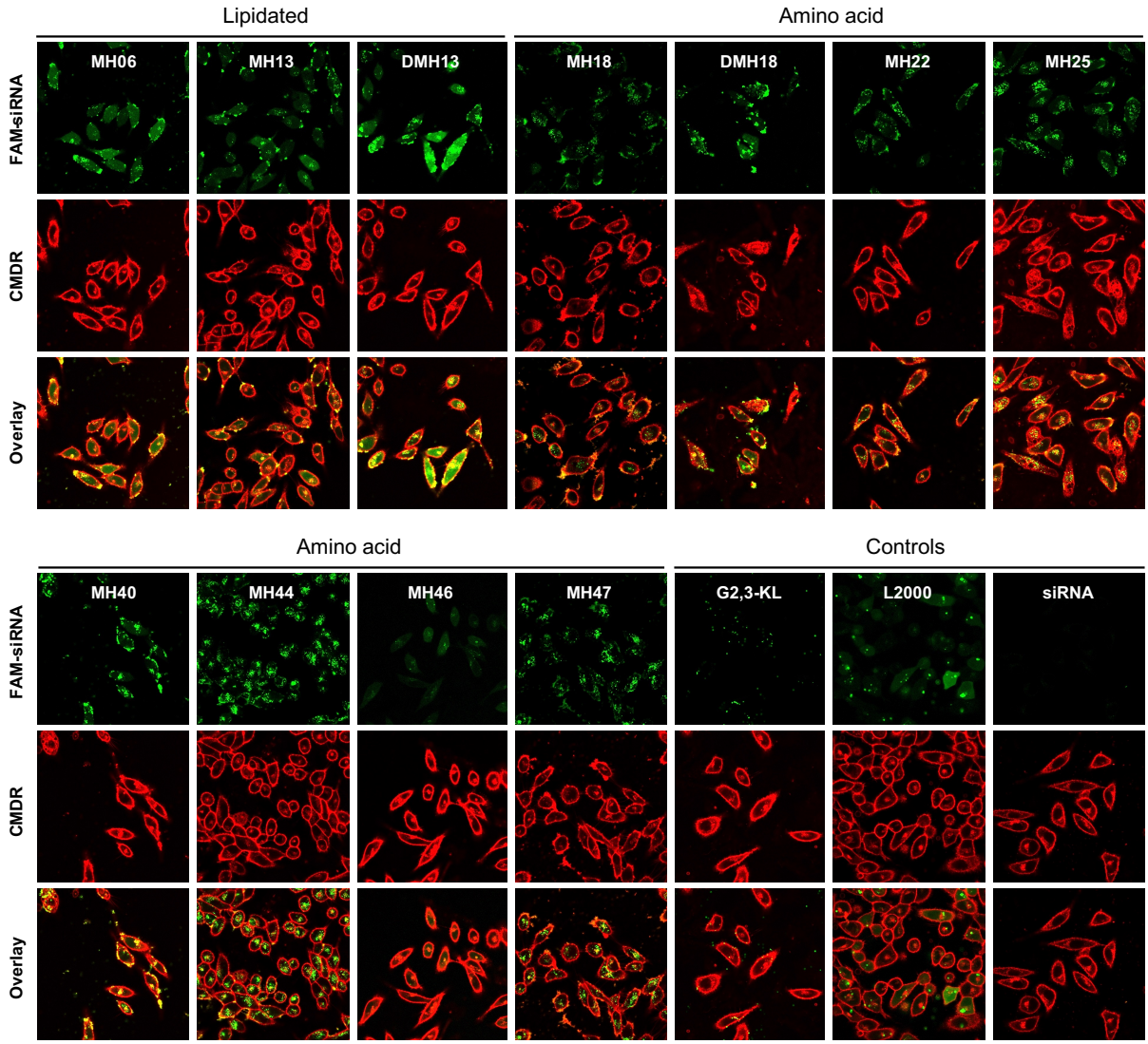

Figure S11. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (100 nM, green) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) in HeLa cells after 4 h transfection. The plasma membrane is stained with Cell mask deep red (red).

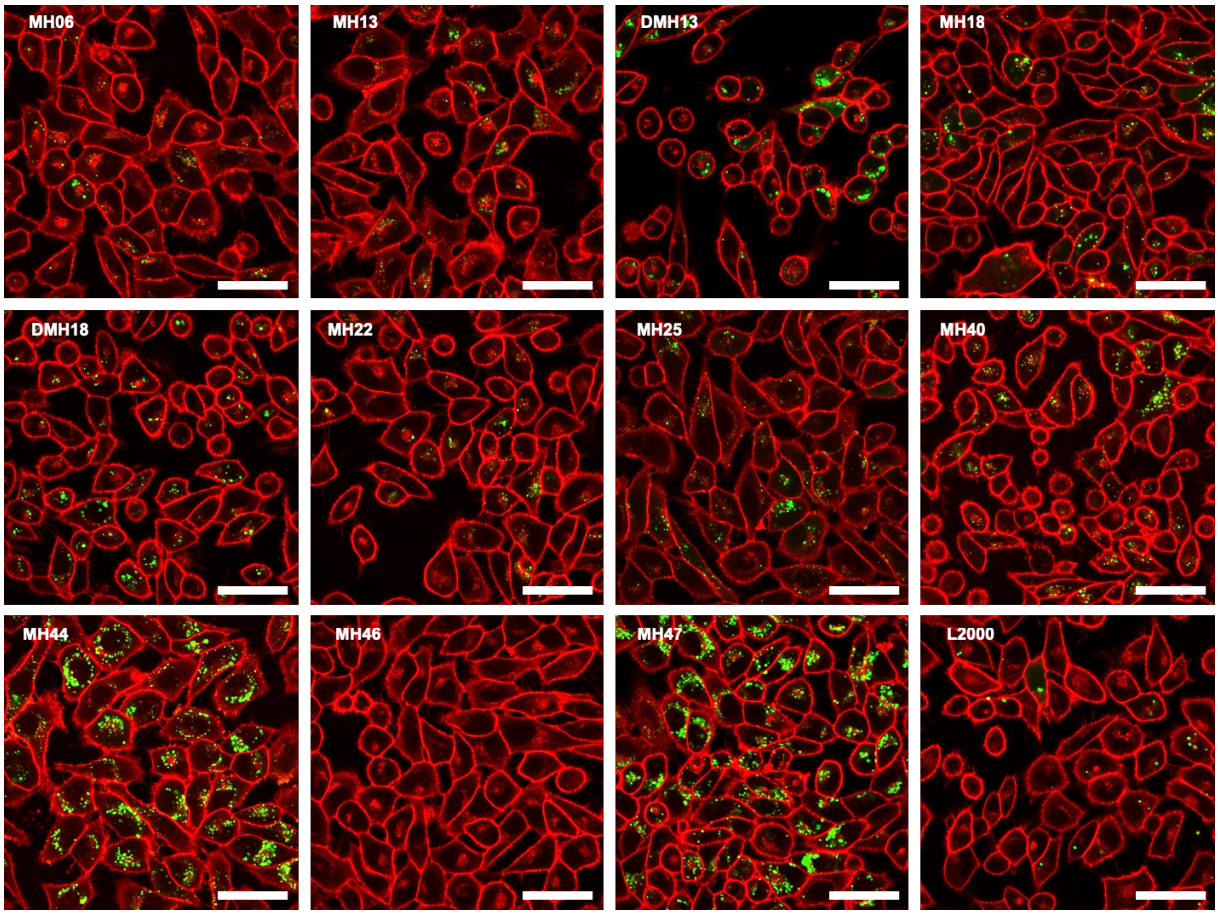

Figure S12. Live cells confocal microscopy of FAM-siRNA (green) in HeLa cells after 24 h. HeLa cells were incubated with FAM-siRNA (100 nM) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) for 4 h in OptiMEM, replaced with DMEM supplemented with 10% FCS and further incubated for 24h. The plasma membrane was stained with Cell mask deep red. White scale bars represent 50 µm (lense x40/1.3). Not overlaid images are shown in Figure S13.

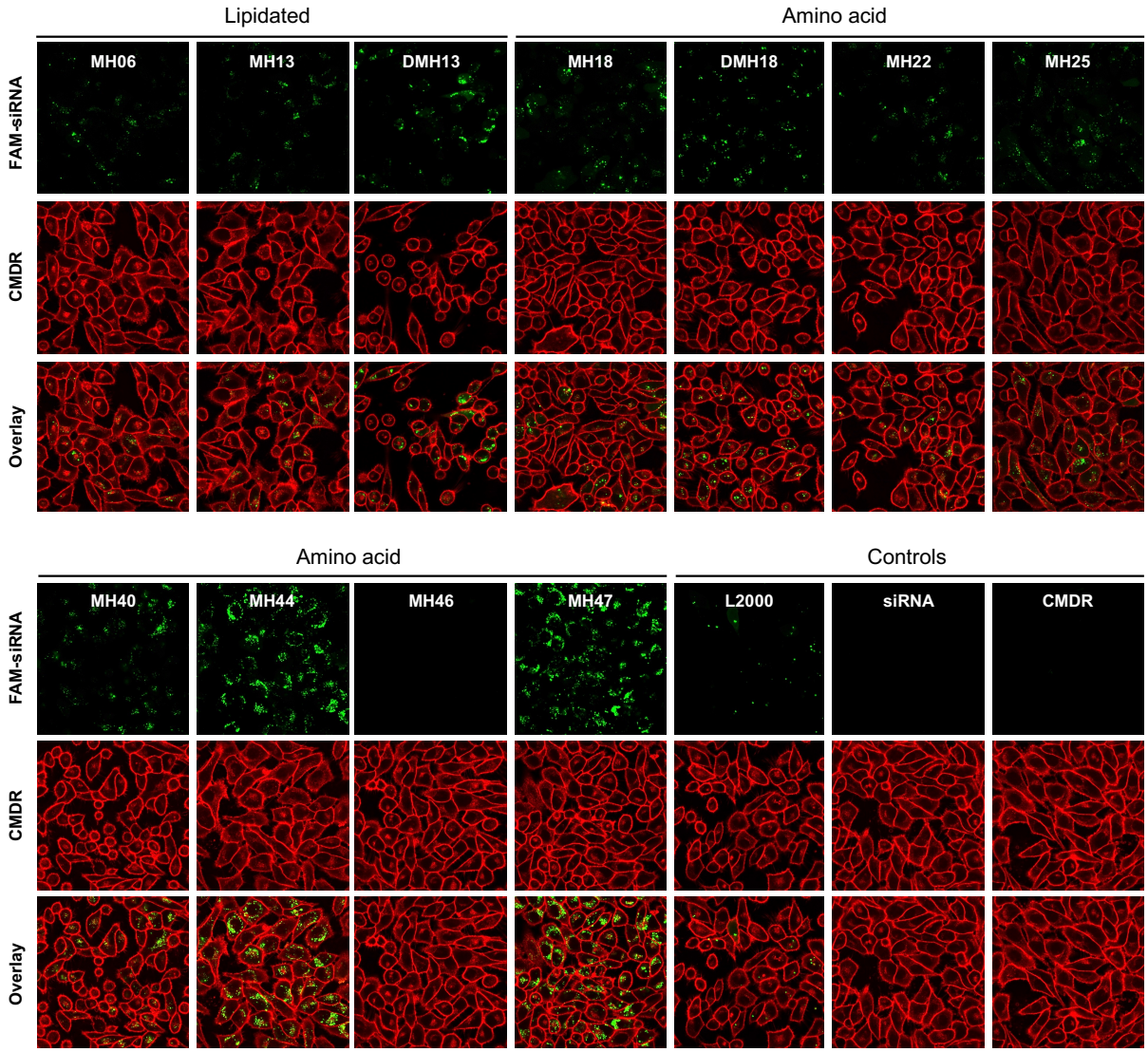

Figure S13. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (100 nM, green) and peptide dendrimers (N/P 10, 2.1 µM, 15 µg/mL) or L2000 (2:1, 2.66 µg/mL) in HeLa cells after 4 h transfection and 24 h incubation in DMEM supplemented with 10% FCS. The plasma membrane is stained with Cell mask deep red (CMDR, red).

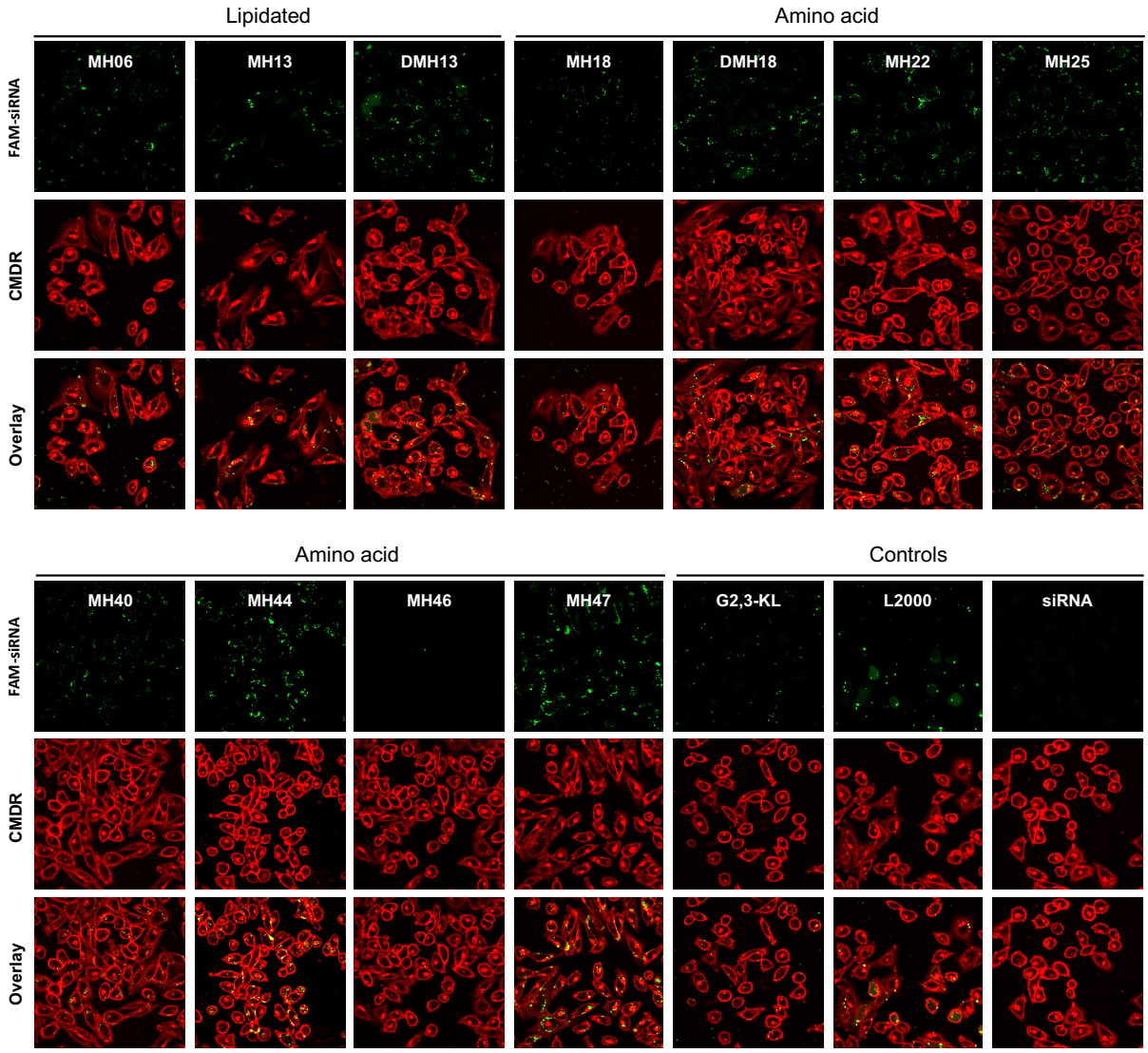

Figure S14. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (30 nM, green) and peptide dendrimers (N/P 10, 0.63 μM, 4.5 μg/mL) or L2000 (2:1, 0.798 μg/mL) in CHO cells after 4 h transfection. The plasma membrane is stained with Cell mask deep red (red).

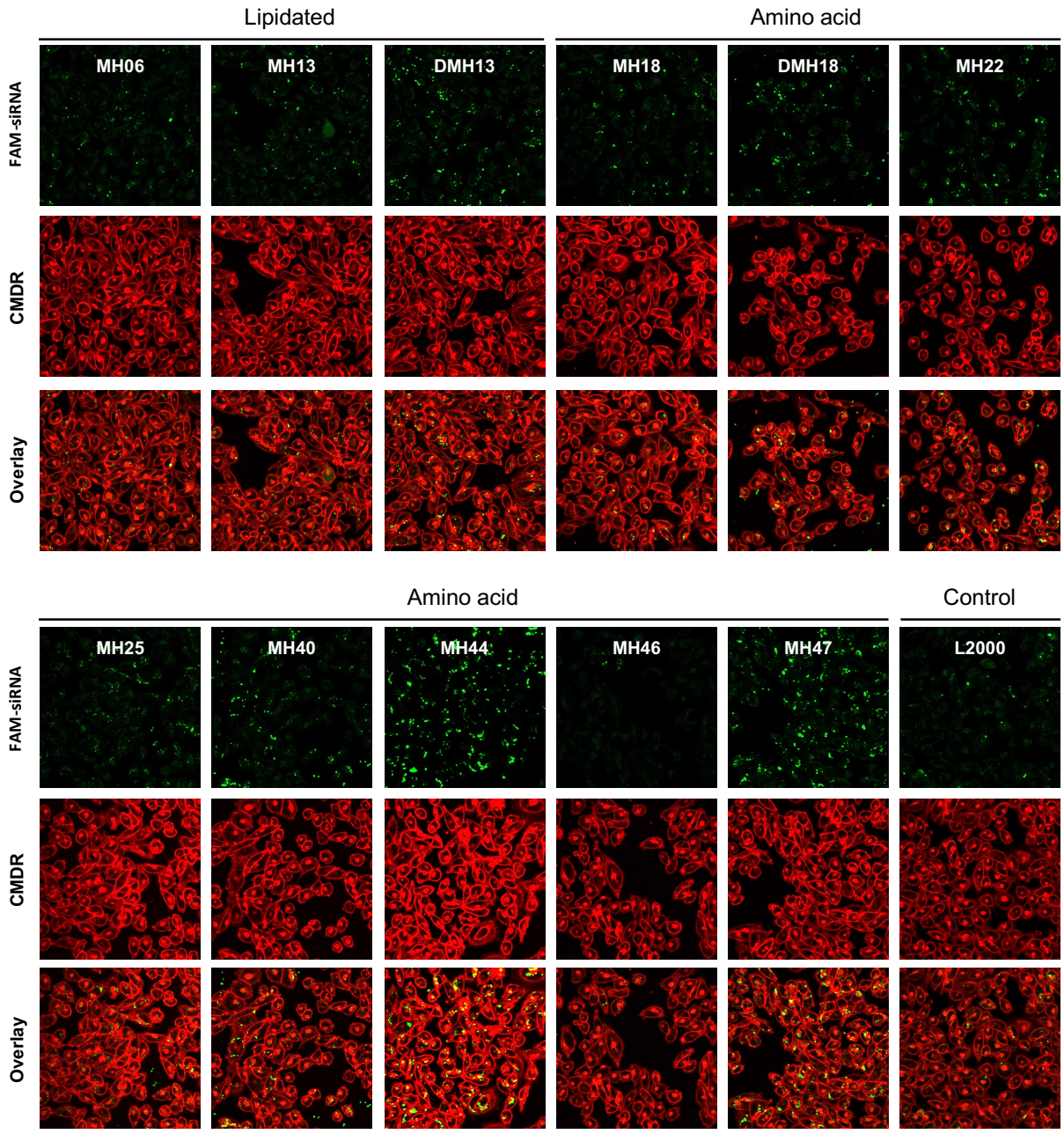

Figure S15. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (30 nM, green) and peptide dendrimers (N/P 10, 0.63 µM, 4.5 µg/mL) or L2000 (2:1, 0.798 µg/mL) in CHO cells after 4 h transfection and 24 h incubation in DMEM supplemented with 10% FCS. The plasma membrane is stained with Cell mask deep red (red).

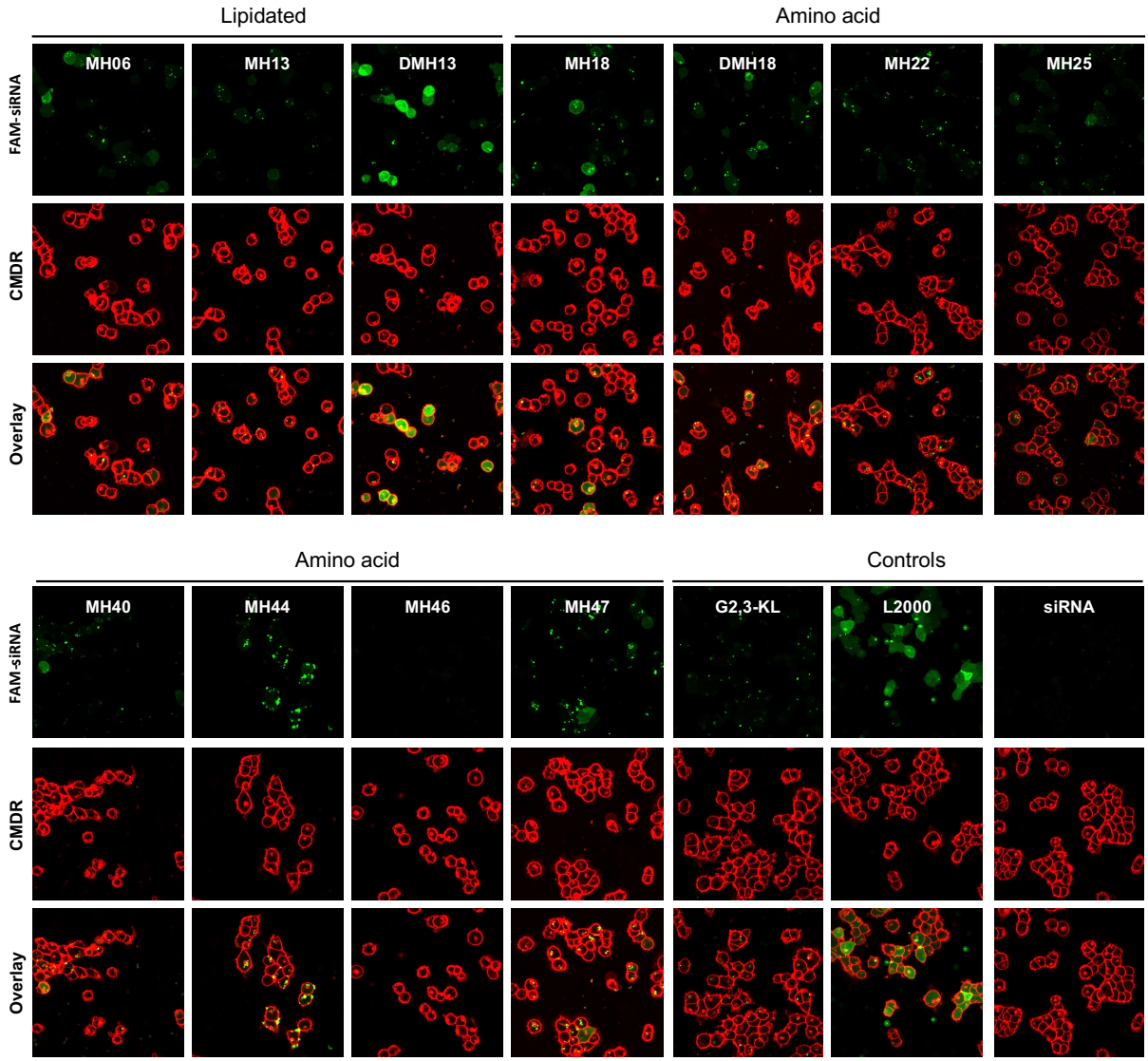

Figure S16. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (30 nM, green) and peptide dendrimers (N/P 10, 0.63 μM, 4.5 μg/mL) or L2000 (2:1, 0.798 μg/mL) in HEK-293 cells after 4 h transfection. The plasma membrane is stained with Cell mask deep red (red).

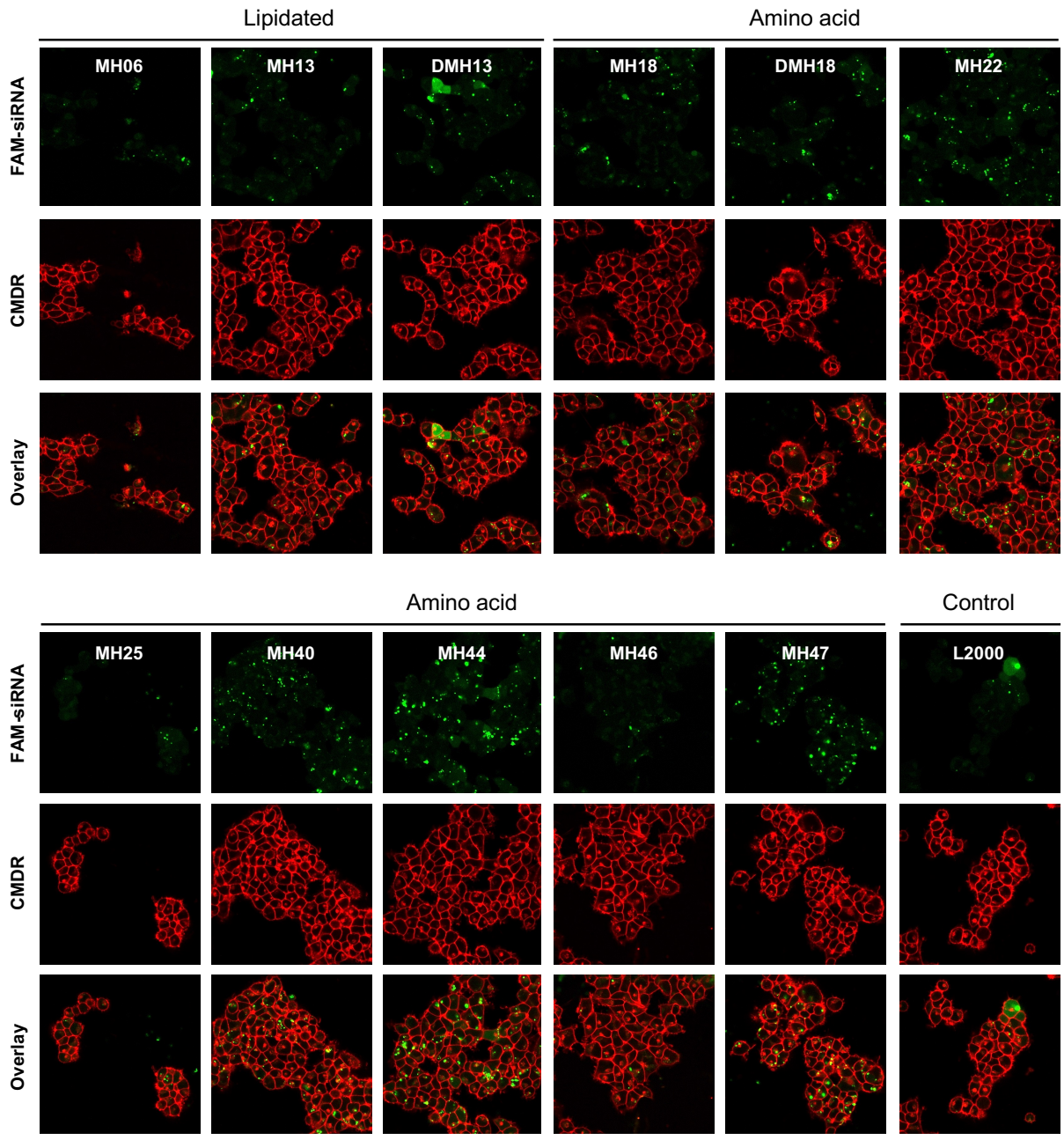

Figure S17. Live cells confocal microscopy with lense ×40/1.3 of FAM-siRNA (30 nM, green) and peptide dendrimers (N/P 10, 0.63 µM, 4.5 µg/mL) or L2000 (2:1, 0.798 µg/mL) in HEK-293 cells after 4 h transfection and 24 h incubation in DMEM supplemented with 10% FCS. The plasma membrane is stained with Cell mask deep red (red).

1.3.9 Free siRNA assay by Quant-iT™ microRNA

The complexes were formed in OptiMEM by mixing siRNA (10 pmol, 0.2 µL from a 50 µM Milli-Q water solution in 6.25 µL OptiMEM pH 5 or pH 7.4) with peptide dendrimers (N/P ratio of 1–10, 42–420 pmol, 0.15–1.5 µL from a 1 mg/mL Milli-Q water solution in 6.25 µL OptiMEM pH 5 or pH 7.4) or L2000 (w/w ratio of 0.2:1–8:1, 0.0266–1.064 µL from the 1 mg/mL commercial solution in 6.25 µL OptiMEM pH 7.4) for 30 min at room temperature (12.5 µL, concentration of 800 nM siRNA and 1.68–16.8 µM i.e. 12–120 µg/mL peptide dendrimers or 0.133–5.32 µg/mL L2000). Then, the Quant-iT™ microRNA Assay Kit (Thermo Fisher Scientific, Reinach, CH) was used following the manufacturer's protocol. Briefly, 1 µl of reagent was diluted in 200 µl of buffer (pH 5 by acidification by 10 mM acetate buffer or standard pH 7.4) 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 20 nM siRNA and 42–420 nM i.e. 0.3–3 µg/mL peptide dendrimers or 0.0532–2.128 µg/mL L2000) and fluorescence measured at λ_{ex}= 500 nm and λ_{em}= 525 nm after 10 min on a Tecan Infinite M1000 Pro plate reader. The Quant-iT™ microRNA signal from the complexes were normalized against a « siRNA only » control to yield the percentage of the signal detected.

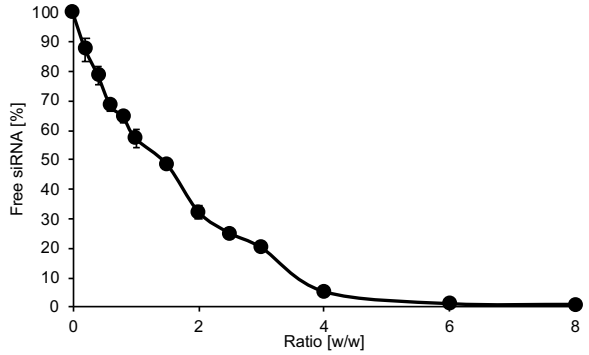

Figure S18. Free siRNA assay by intercalation of Quant-it™ microRNA in complexes of siRNA (20 nM) and L2000 (0.2–8:1, 0.0532–2.128 µg/mL) in function of the N/P ratio. Experiment performed in triplicates.

1.3.10 Competition assay by Quant-iT™ microRNA

The complexes were formed as in the free siRNA 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 0–4 µg/mL heparin, 20 nM siRNA and 420 nM i.e. 3 µg/mL peptide dendrimers or 532 ng/mL L2000). Fluorescence measured at λ_{ex}= 500 nm and λ_{em}= 525 nm was performed on a Tecan Infinite M1000 Pro plate reader. The Quant-iT™ microRNA signal from the complexes were normalized against a « siRNA only » control to yield the percentage of the signal detected.

1.3.11 Free FAM-siRNA Assay by Fluorescence Polarization

The complexes were formed in DPBS (Thermofisher scientific, Reinach, CH) by mixing FAM-siRNA (2 pmol, 0.04 µL from a 50 µM Milli-Q water solution in 6.25 µL DPBS at pH 5 or pH 7.4) with the peptide dendrimers (N/P ratio of 1–10, 4.2–42 pmol, 0.03–0.3 µL from a 1 mg/mL Milli-Q water solution in 6.25 µL DPBS at pH 5 or pH 7.4) or L2000 (w/w ratio of 0.2:1–16:1, 0.00532–0.4256 µg, 0.00532–0.4256 µL from the 1 mg/mL commercial solution in 6.25 µL DPBS at pH 5 or pH 7.4) for 30 min at room temperature (12.5 µL, concentration of 160 nM FAM-siRNA and 0.336–3.36 µM i.e. 2.4–24 µg/mL peptide dendrimers or 0.4256 –34.048 µg/mL L2000). Complexes were then diluted in DPBS at pH 5 or pH 7.4 to 100 µL per well (final concentration of 20 nM FAM-siRNA and 42–420 nM i.e. 0.3–3 µg/mL peptide dendrimers or 0.0532–4.256 µg/mL L2000) and added to a Cellstar black, µClear, Greiner Bio one 96-well plate (Huberlab, Aesh, CH) and fluorescence anisotropy measured at λ_{ex}= 470 nm and λ_{em}= 520 nm on a Tecan Infinite M1000 Pro plate reader. For each measurement, G-factor was calibrated with a solution of fluorescein at 1 nM in 10 mM NaOH set at 20 mP and for testing at pH 5, the polarization of FAM-siRNA alone was set to 55 mP to be comparable with pH 7.4.

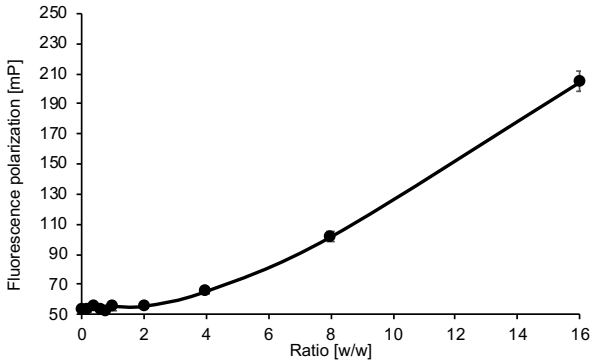

Figure S19. Fluorescence polarization of FAM-siRNA (20 nM) in function of the ratio of L2000 (0.2–16:1, 0.0532–4.256 µg/mL). Experiment performed in triplicates.

1.3.12 Competition Assay by Fluorescence Polarization

The complexes were formed as in the free FAM-siRNA assay above in DPBS and 50 µL added to 50 µL of a serial dilution of 0 to 20 µg/mL heparin in DPBS in a Cellstar black, µClear, Greiner bio one 96-well plate (100 µL, final concentration of 0–4 µg/mL heparin, 20 nM FAM-siRNA and 420 nM i.e. 3 µg/mL peptide dendrimers or 532 ng/mL L2000). Plate was incubated for 2 h at RT and fluorescence anisotropy measured at λ_{ex}= 470 nm and λ_{em}= 520 nm on a Tecan Infinite M1000 Pro plate reader. For each measurement, G-factor was calibrated with a solution of fluorescein at 1 nM in 10 mM NaOH set at 20 mP.

1.3.13 Dynamic Light Scattering (DLS)

Complexes were formed in phosphate buffer (PB) at pH 5 or pH 7.4 with siRNA (80 pmol, 1.6 µL from a 50 µM Milli-Q water solution in 50 µL PB at pH 5 or pH 7.4) and peptide dendrimers (N/P ratio of 10, 1680 pmol, 10–14 µL from a 1 mg/mL Milli-Q water solution in 50 µL PB at pH 5 or pH 7.4) or L2000 (w/w ratio of 2:1, 2.128 µg, 2.128 µL from the 1 mg/mL commercial solution in 50 µL PB at pH 5 or pH 7.4) and incubated at room temperature for 30 minutes (100 µL, final concentration of 800 nM siRNA and 16.8 µM i.e. 120 µg/mL peptide dendrimers or 21.28 µg/mL L2000). 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.

1.3.14 Transmission Electron Microscopy (TEM)

The complexes were formed as previously described in Milli-Q water with siRNA (10 pmol, 0.2 µL from a 50 µM Milli-Q water solution in 6.25 µL Milli-Q water) and peptide dendrimers (N/P ratio of 10, 210 pmol, 1.25–1.75 µL from a 1 mg/mL Milli-Q water solution in 6.25 µL Milli-Q water) or L2000 (w/w ratio of 2:1, 21.28 µg/mL, 0.266 µL from the 1 mg/mL commercial solution in 6.25 µL Milli-Q water) for 30 min at room temperature (12.5 µL, final concentration of 800 nM siRNA and 16.8 µM i.e. 120 µg/mL peptide dendrimers or 21.28 µg/mL L2000). The carbon-coated copper TEM grid (400 mesh, Gloor Instruments AG, Kloten) were glow discharge on a CTA 010 Balzers Union. Then, 5 µL of the complex were dropped on the grid, incubated for 1 min at RT then dried with a filter paper. The grid was washed with water and dried 3 times with filter paper. The grid was then stained with uranyl acetate (2% in 50% alcoholic solution, 6 µl, Gloor Instruments AG, Kloten) for 10 secondes twice. Imaging was performed after stocking/air-dried for around 2 h on a FEI Tecnai spirit transmission electron microscope equipped with two digital cameras (Olympus-SIS Veleta CCD Camera, FEI Eagle CCD Camera).

1.3.15 Dialysis

Complexes were formed with labelled Cy3-siRNA in OptiMEM (80 pmol, 1.6 µL from a 50 µM Milli-Q water solution in 50 µL OptiMEM pH 5 or pH 7.4) and peptide dendrimers (N/P ratio of 1–10, 168–1680 pmol, 1–14 µL from a 1 mg/mL Milli-Q water solution in 50 µL OptiMEM pH 5 or pH 7.4) or L2000 (w/w ratio of 2:1, 0.218–2.128 µg, 0.218–2.128 µL from the 1 mg/mL commercial solution in 50 µL OptiMEM pH 5 or pH 7.4) and incubated at room temperature for 30 minutes (100 µL, concentration of 80–800 nM Cy3-siRNA and 1.68–16.8 µM i.e. 12–120 µg/mL peptide dendrimers or 2.66–21.28 µg/mL L2000). The complexes were then diluted in OptiMEM pH 5 or pH 7.4 to a final volume of 800 µL (final concentration of 100 nM Cy3-siRNA and 0.210–2.1 µM i.e. 1.5–15 µg/mL peptide dendrimers or 0.266–2.66 µg/mL L2000). 500 µL of the complexes were added to Amicon Ultra 0.5 mL dialysis Eppendorfs with a 100 KDa cut-off (Sigma aldrich, Buchs, CH) and centrifugated at 14000 g for 10 minutes. For the second experiment, following centrifugation, lower compartment was replaced, 500 µL of OptiMEM at pH 5 or pH 7.4 was added to the Amicon Ultra 0.5 mL dialysis Eppendorfs top compartment and centrifuged one more time at 14000 g for 10 minutes. Then, 100 µL of the dialysed solutions from before dialysis, after dialysis and supplementary wash were transferred to a TPP 96-well plate (Faust Laborbedarf AG, Schaffhausen) and fluorescence measured at λ_{ex}= 547 nm and λ_{em}= 563 nm on a Tecan Infinite M1000 Pro plate reader.

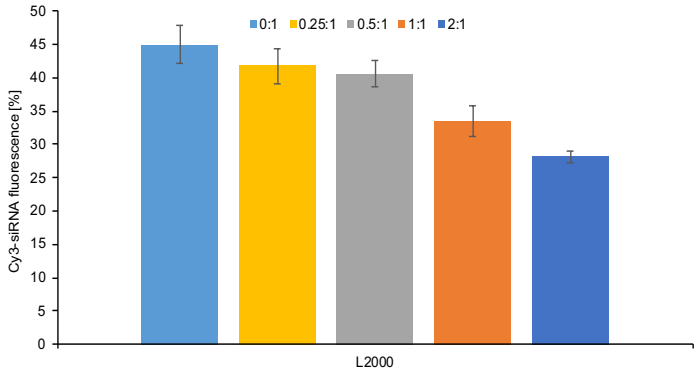

Figure S20. Ratio of fluorescence from before and after dialysis with a 100 KDa cutoff of Cy3-siRNA (100 nM) and L2000 (0.25–2:1, 0.3325–2.66 µg/mL) in OptiMEM. Experiment performed in triplicates.

1.3.16 Vesicle Leakage

Egg yolk phosphatidylcholine (EYPC, Avanti Polar Lipids, Alablaster, USA) thin lipid layer was prepared by evaporating a solution of 100 mg in 4 mL MeOH/$CHCl_3$ (1:1) on a rotary evaporator at room temperature and then *in vacuo* overnight. The resulting film was hydrated with 4 mL buffer A (50 mM 5(6)-carboxyfluorescein (CF, Sigma Aldrich, Buchs, CH), 10 mM TRIS, 10 mM NaCl, pH 7.4) for 30 min, subjected to freeze-thaw cycles (7 times) and extrusion (15 times) through a polycarbonate membrane (pore size 100 nm). Extra vesicular components were removed by gel filtration (Sephadex G-50) with buffer B (10 mM TRIS, 107 mM NaCl, pH 7.4). Final concentrations: ~ 2.5 mM EYPC; inside: 50 mM CF, 10 mM TRIS, 10 mM NaCl, pH 7.4 buffer; outside: 10 mM TRIS, 107 mM NaCl, pH 7.4 buffer.

EYPC stock solutions (10 µL) were diluted to 700-792 µL with the buffer B (10 mM TRIS, 107 mM NaCl, pH 7.4) and placed in a fluorescence cuvette, thermostated (25 °C) and gently stirred (final concentration of EYPC 31 µM, 625 µM CF, 10 mM TRIS and 107 mM NaCl). Complexes were formed with siRNA in buffer B (80 pmol, 1.6 µL from a 50 µM Milli-Q water solution in 50 µL buffer) and peptide dendrimers (N/P ratio of 10, 1680 pmol, 12 µL from a 1 mg/mL Milli-Q water solution in 50 µL buffer B) or L2000 (w/w ratio of 2:1, 2.128 µg, 2.128 µL from the 1 mg/mL commercial solution in 50 µL buffer B) and incubated at room temperature for 30 minutes (100 µL, concentration of 800 nM siRNA and 16.8 µM i.e. 120 µg/mL peptide dendrimers or 21.28 µg/mL L2000).

CF efflux was monitored on an Agilent Cary Eclipse fluorescence spectrophotometer at λ_{ex}= 492 nm and λ_{em}= 517 nm as a function of time for 360 s. At t= 30 s peptide dendrimers (0.8–12 µL from a 1 mg/mL Milli-Q water stock solution), L2000 (0.8–12 µL from the 1 mg/mL commercial solution) or complexes (100 µL from the complexes solutions described above) were added to the cuvette (final concentration of 0–100 nM siRNA and 0.14–2.1 µM / 1–15 µg/mL peptide dendrimers or 1–15 µg/mL L2000). At t= 270 s 1.2% Triton X-100 was added to the cuvette (50 µL, 0.07% final concentration). Fluorescence intensities were normalized to fractional emission intensity I(t) using I(t) = (It - I0)/(I∞ - I0) where I0 is the intensity before addition of the peptide dendrimer, I∞ is intensity at saturation after lysis by Triton X-100.[2]

1.4 Peptide Dendrimers Only

1.4.1 Critical Micellar Concentration (CMC)

Nile red is known to have a higher fluorescence when being surrounded by a hydrophobic environment and this experiment is derived from known procedure but applied to 96-well plates.[3] To see if the CMC determination is working in our hands with our assay, we first performed it with dodecylphosphocholine and found a value of 0.3125 mg/mL, corresponding to 0.9 mM which is in accordance with values between 0.9 and 1.1 mM from literature (data not shown).[4–6] Briefly, Nile red (Sigma aldrich, Buchs, CH) was diluted in methanol at a concentration of 2 µM and 5 µL was 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, L2000 and dodecylphosphocholine (Avanti polar lipids, Alablaster, USA) were performed in 10 mM phosphate buffer (pH 5 or pH 7.4) starting from 1 mg/mL to 0.5 µg/mL and 50 µL was added to the plate containing the dried Nile red fluorophore (final concentration 0.2 µM). For the second experiment, solution of peptide dendrimers at 1 mg/mL in Milli-Q water were diluted to 0.1 mg/mL in 100 µL PB at pH ranging from 7.4 to 4 and added to dried Nile red. 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. CMC values can be determined at the inflection point of the curves.

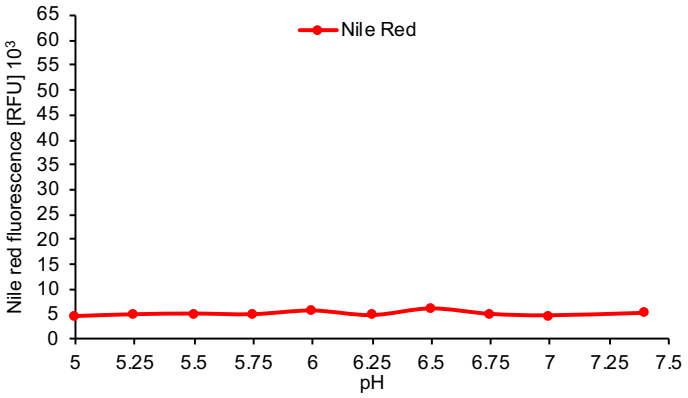

Figure S21. Fluorescence of Nile red alone (final concentration of 0.2 µM) in different pH measured at λ_{ex}= 540 nm and λ_{em}= 615 nm. RFU = Relative fluorescence unit.

1.4.2 Diffusion NMR (DOSY) Measurements

Diffusion NMR experiments were performed using a Bruker DRX500 with solutions of dendrimer (15 mg/mL) in D_2O (pH 5 or 7.4, at 303 K). The gradient with a maximum strength of 50×10^{-4} $T\cdot cm^{-1}$ was calibrated using the HOD proton signal in D_2O (99.997%). The diffusion time was 125 ms and the gradient duration δ was 6 ms. Data analysis was performed by using the Bruker Simfit software and the diffusion coefficient D [m^2s^{-1}] was derived from peak area and intensities. The hydrodynamic radii were calculated from the diffusion coefficient D using the Stokes-Einstein equation $Rh= kT/6\pi\eta D$ with Boltzmann constant $k= 1.380\times10^{-23}$ JK^{-1}, temperature T in K and viscosity $\eta= 1.089$ mPa. s for D_2O at 303 K.

1.4.3 Titration by NaOH

Peptide dendrimers (0.8–1 μmol, 5–7 mg) were diluted in 7–10 mL Milli-Q water (Final concentration of 100 μM) and acidified to pH 3 with 1 M HCl. Then, 0.1 M NaOH was added by step of 2 μL to the solution with a Dosimat plus (Metrohm, Zofingen, Switzerland) and pH measured on a 692 pH/ion meter (Metrohm).

1.4.4 Circular Dichroism (CD) Spectroscopic Measurements.

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 200 μg/mL with PBS buffer (pH = 7.4, 10 mM final concentration). For the solvent dependent studies, the peptides were diluted to 200 μg/mL with phosphate buffer (pH = 5 or 7.4, 8 mM final concentration) and TFE or ACN (0, 5, 10, 15, 20 or 40%). The range of measurement was 185–260 nm, scan rate was 10 nm/min or 20 nm/min, pitch 0.5 nm, response 16 sec. and band 1.0 nm. The nitrogen flow was kept above 8 L/min. The baseline (solvent) was recorded under the same conditions and subtracted manually. Each sample was subjected to two accumulations. The cuvettes were washed with 1M HCl, mQ-deionized H_2O and PBS or PB buffer before each measurement.

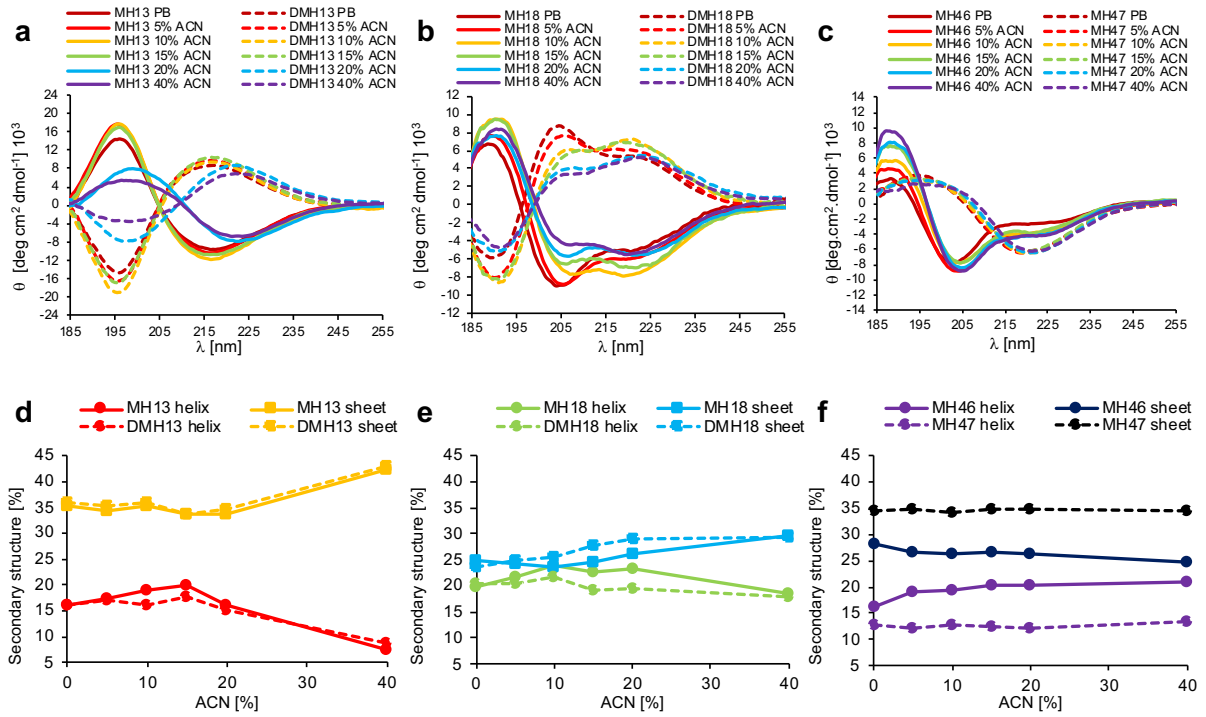

Figure S22. Conformation of transfection peptide dendrimers in solution. **(a,b,c)** Circular dichroism spectra of **MH13**, **DMH13**, **MH18**, **DMH18**, **MH46 and MH47** (200 µg/mL) in 8 mM phosphate buffer (PB) at pH 7.4 with various concentration of acetonitrile. **(d,e,f)** Percentage of α-helix and β-sheet as a function of the concentration of acetonitrile processed by Dichroweb using the CONTIN analysis program and reference set 3.

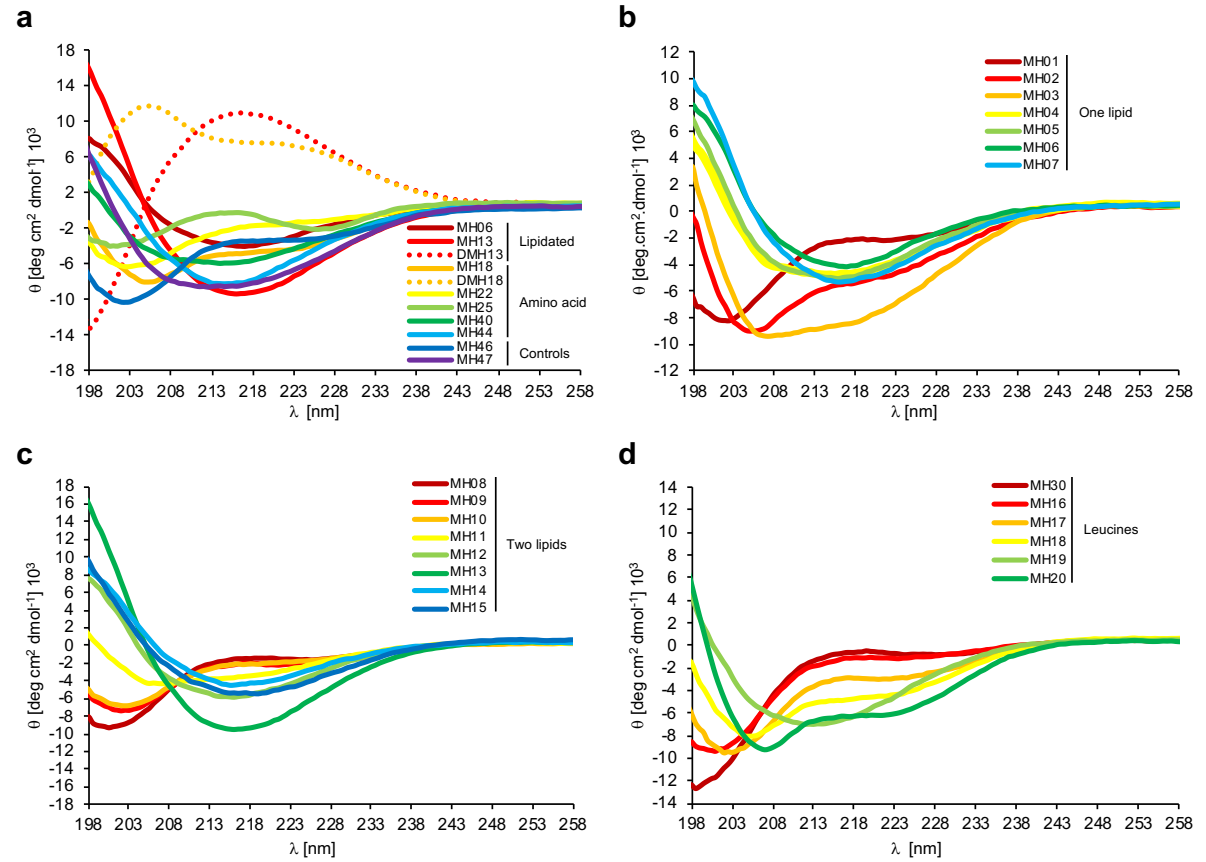

Figure S23. Circular dichroism spectra for the panel of **(a)** selection of compounds, **(b)** mono lipidated, **(c)** doubly lipidated and **(d)** leucines in the G0 of peptide dendrimers in PBS (pH 7.4) at 200 µg/mL in 10 mM aqueous phosphate buffer saline pH 7.4 (PBS).

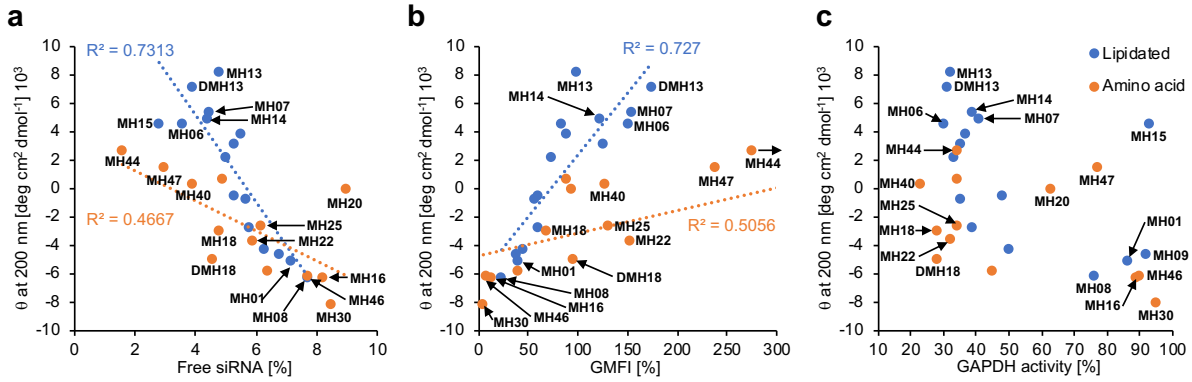

Figure S24. Correlation between θ at 200 nm from circular dichroism and **(a)** free siRNA, **(b)** GMFI and **(c)** GAPDH activity. Data taken from Figure S1, Table 1, Table 2 and Table S1.

1.5 Molecular Dynamics

The dendrimer models were built by processing the GROMACS topologies of the linear peptides of the same sequence using in house software. The initial starting conformation was generated using PyMol (Molecular Graphics System, version 1.8 (Schrödinger, LLC)) by setting the (Φ, Ψ) angle pairs of all the residues in a helical conformation.

Molecular dynamics (MD) simulations were performed using GROMACS software version 2016.1 and the Gomos53a6 force field. A dodecahedral box was created around the dendrimer 1.0 nm from the edge of the dendrimer and filled with extended simple point charge water molecules. Sodium and chloride ions were added to produce an electroneutral solution at a final concentration of 0.15 M NaCl.

The energy was minimized using a steepest gradient method to remove any close contacts before the system was subjected to a two-phase position-restrained MD equilibration procedure. The system was first allowed to evolve for 100 ps in a canonical NVT (N is the number of particles, V the system volume, and T the temperature) ensemble at 300 K before pressure coupling was switched on and the system was equilibrated for an additional 100 ps in the NPT (P is the system pressure) ensemble at 1.0 bar.

All bond lengths were constrained to their equilibrium values by using the LINCS algorithm. The neighbor list for the calculation of nonbonded interactions was updated every five time steps with a cutoff of 1.0 nm with a step size of 2 fs. A twin range cutoff of 1.0 nm was used for both Coulomb and Lennard-Jones interactions. The system was split into two groups, “Protein” and “Non-Protein”, which were coupled separately to a temperature bath using the V-rescale algorithm with a time constant of 0.1 ps while the pressure coupling was conducted using an isotropic Parrinello-Rahman barostat with a time constant of 2.0 ps.

Analysis of Molecular Dynamics – The stability of the helical structure in the peptide dendrimers under different conditions was assessed by determining the unfolding kinetics as evidenced by the radius of gyration and RMSD values. After system pre-equilibration (*vide supra*), the structures were subjected to MD at 300 K during 1000 ns in water, 0.15 M NaCl with or without 20% v/v TFE. The unfolding the main α-peptide chain helix was followed by computing the RMSD of its backbone and the total number of i→i+4 H-bonds. The overall stability of the internal structure was assessed using the total number of backbone H-bonds over time. The spontaneous appearance of β-sheet and random coil secondary structures was evidenced by Ramachandran number analysis.[7] The last 100 ns (10001 structures) of each 1 ms MD run were clustered using the GROMACS method and a cutoff of 0.3 nm and the central structure of the main cluster was used in the analysis. The Ramachandran numbers were computed using the python package as implemented by Mannige R..[8] That same structure was used as representative structure of the equilibrated dendrimer in each of the conditions simulated using the PyMol software for building the 3D models.

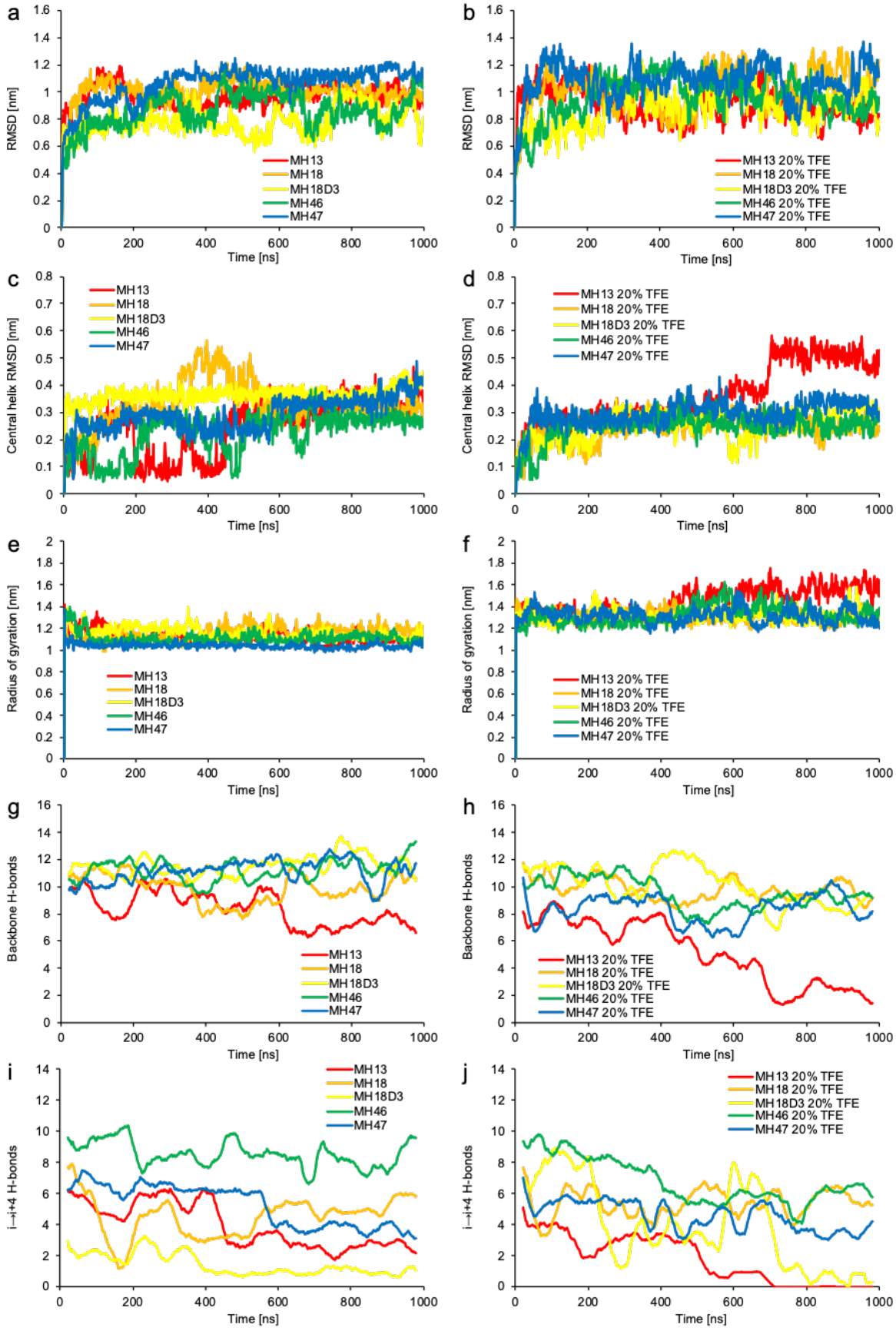

Figure S25. Different properties recorded during the time course of the 1 μs molecular dynamic run. **(a,b)** RMSD, **(c,d)** central helix RMSD, **(e,f)** radius of gyration, **(g,h)** backbone H-bonds and **(i,j)** i-i4 H-bonds in (left) water or (right) 20% trifluoroethanol at pH 5.

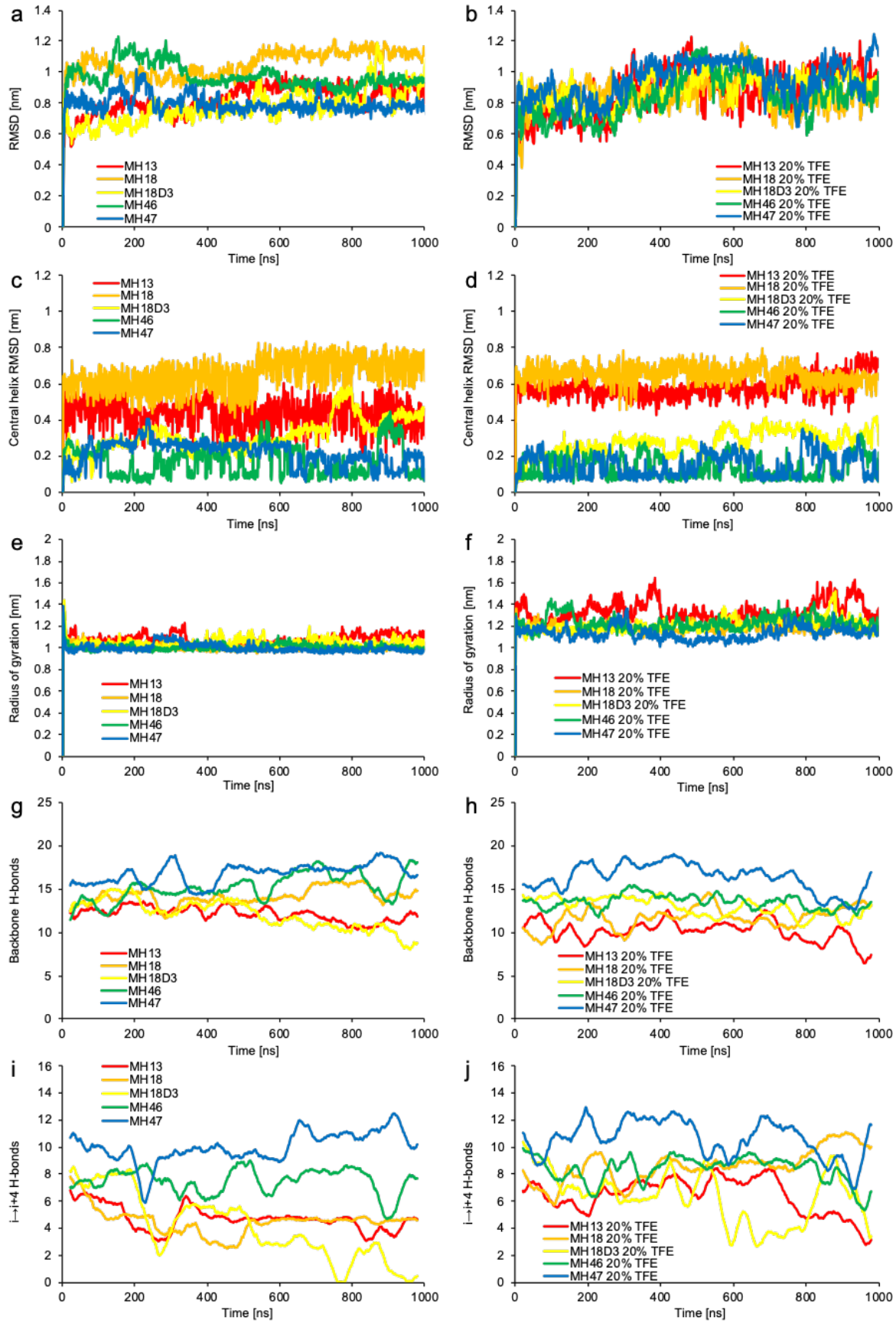

Figure S26. Different properties recorded during the time course of the 1 μs molecular dynamic run. **(a,b)** RMSD, **(c,d)** central helix RMSD, **(e,f)** radius of gyration, **(g,h)** backbone H-bonds and **(i,j)** i-i4 H-bonds in (left) water or (right) 20% trifluoroethanol at pH 7.

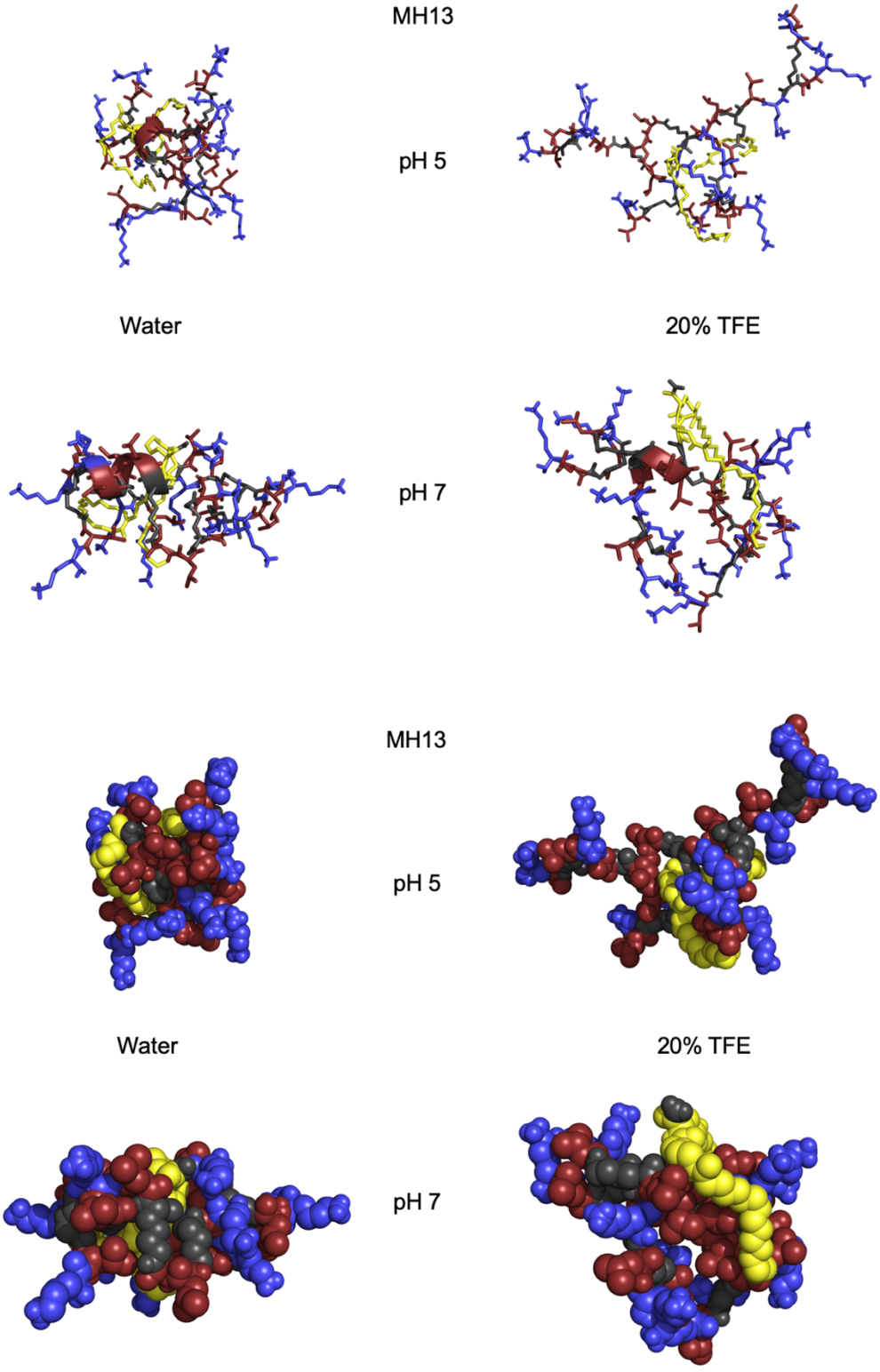

Figure S27. MD structure of the center of the main cluster of the last 100 ns of the simulation represented as stick and cartoon (top panel) and CPK (bottom panel) for **MH13** at pH 5 (top) and 7 (bottom) in absence (left) and presence (right) of 20% trifluoroethanol. Color-coded by residue type (blue = lysine, red = leucine, gray = branching lysine, yellow = lipid chain).

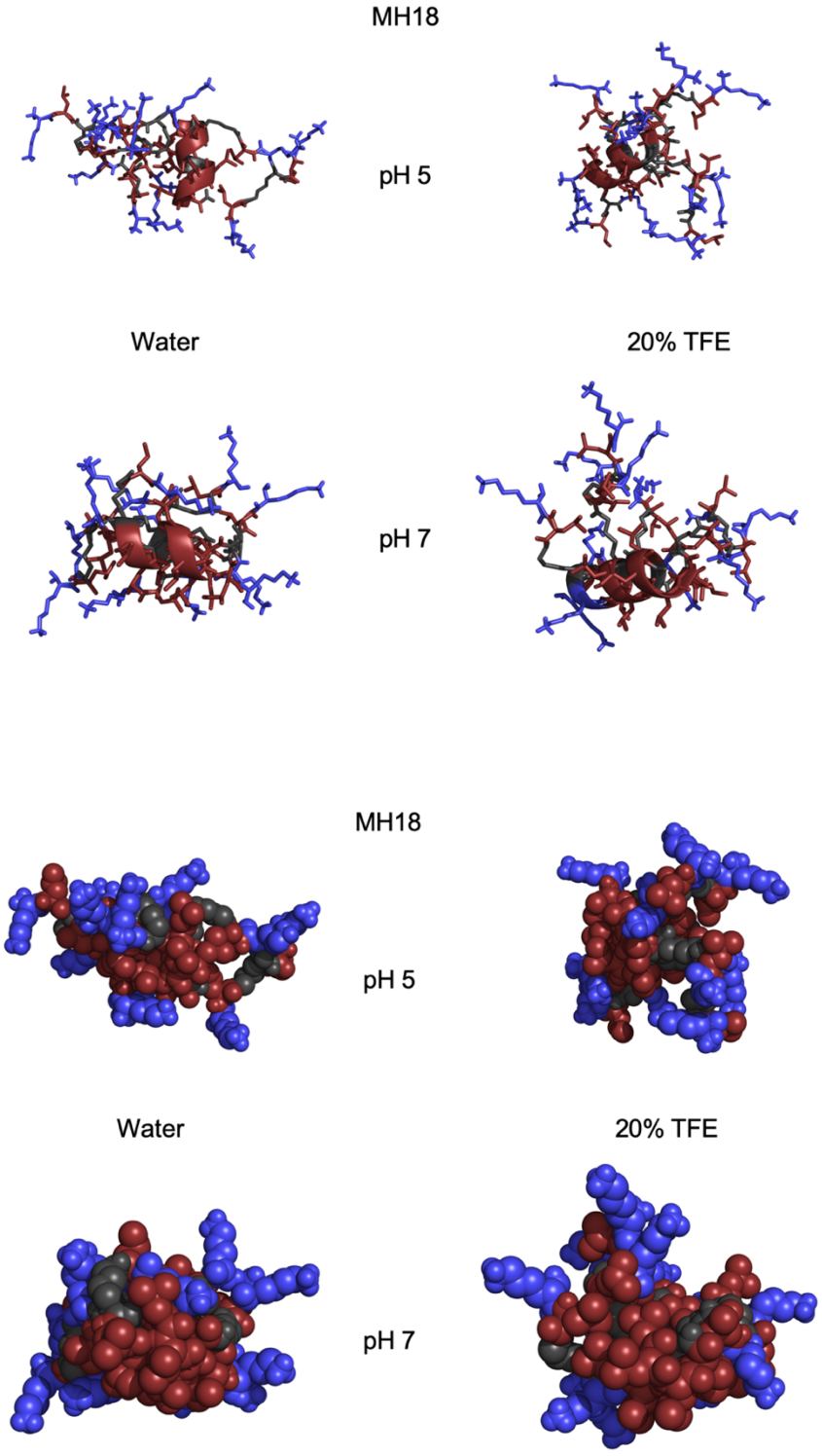

Figure S28. MD structure of the center of the main cluster of the last 100 ns of the simulation represented as stick and cartoon (top panel) and CPK (bottom panel) for **MH18** at pH 5 (top) and 7 (bottom) in absence (left) and presence (right) of 20% trifluoroethanol. Color-coded by residue type (blue = lysine, red = leucine, gray = branching lysine).

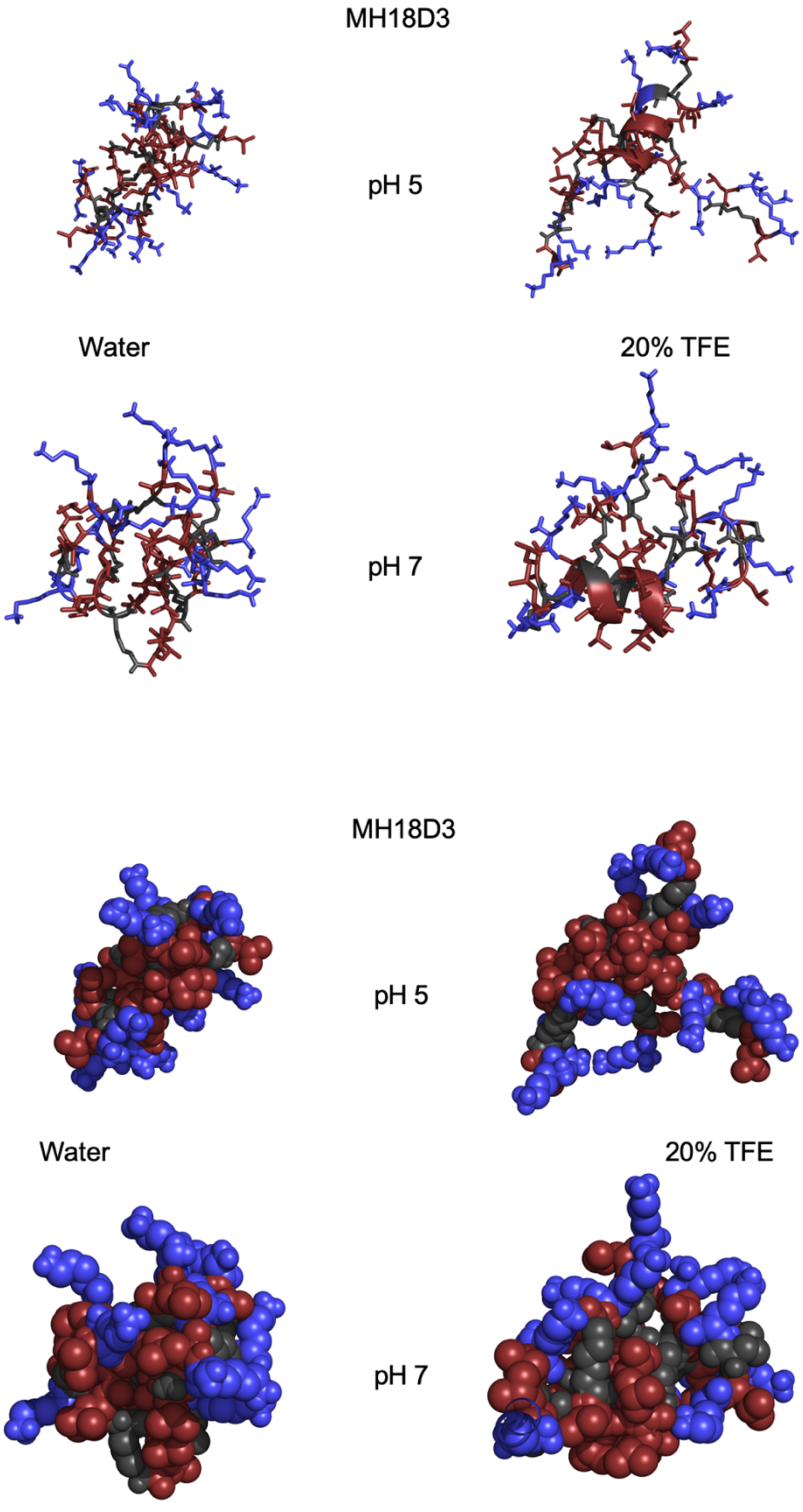

Figure S29. MD structure of the center of the main cluster of the last 100 ns of the simulation represented as stick and cartoon (top panel) and CPK (bottom panel) for **MH18D3** at pH 5 (top) and 7 (bottom) in absence (left) and presence (right) of 20% trifluoroethanol. Color-coded by residue type (blue = lysine, red = leucine, gray = branching lysine).

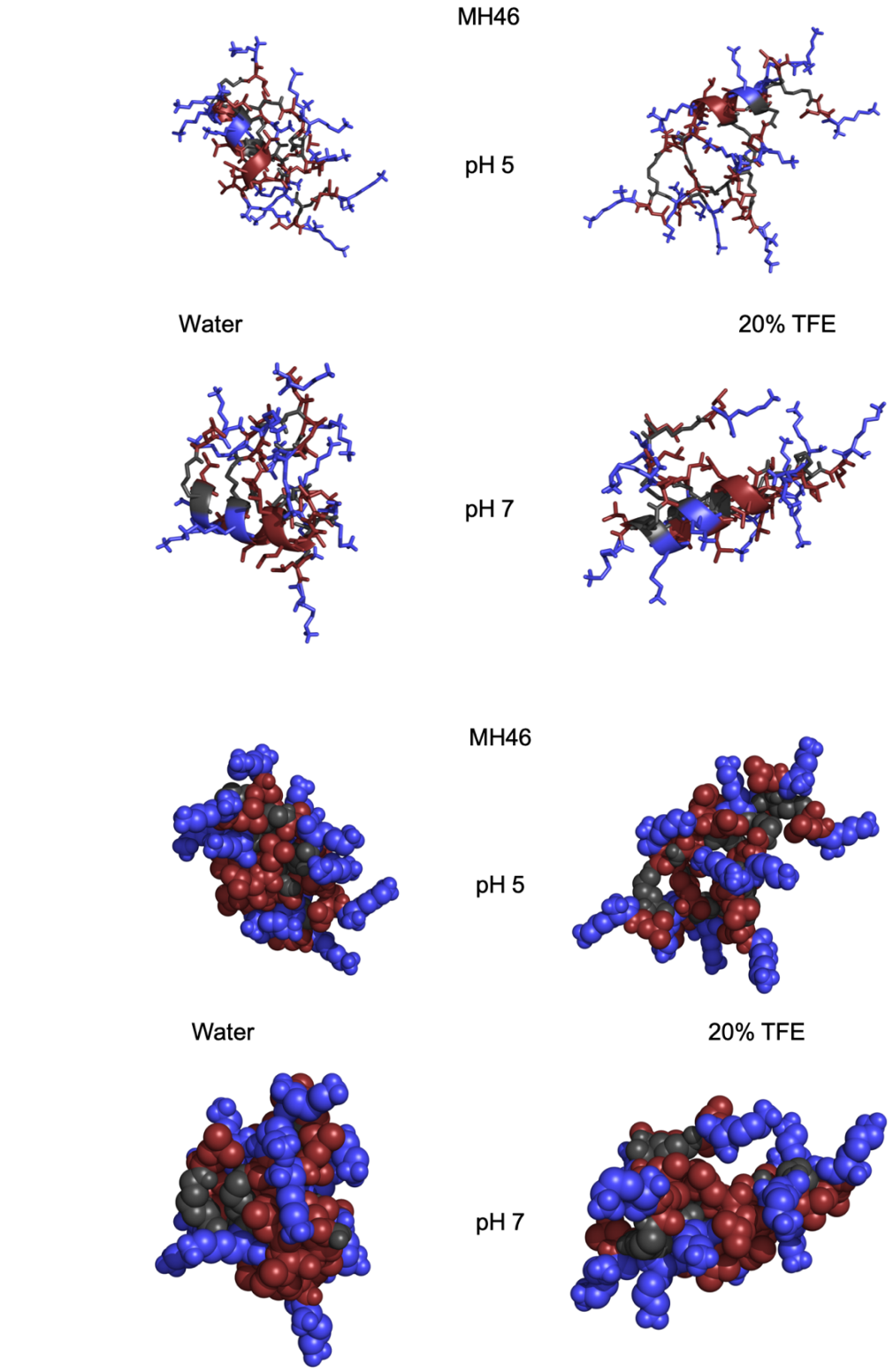

Figure S30. MD structure of the center of the main cluster of the last 100 ns of the simulation represented as stick and cartoon (top panel) and CPK (bottom panel) for **MH46** at pH 5 (top) and 7 (bottom) in absence (left) and presence (right) of 20% trifluoroethanol. Color-coded by residue type (blue = lysine, red = leucine, gray = branching lysine).

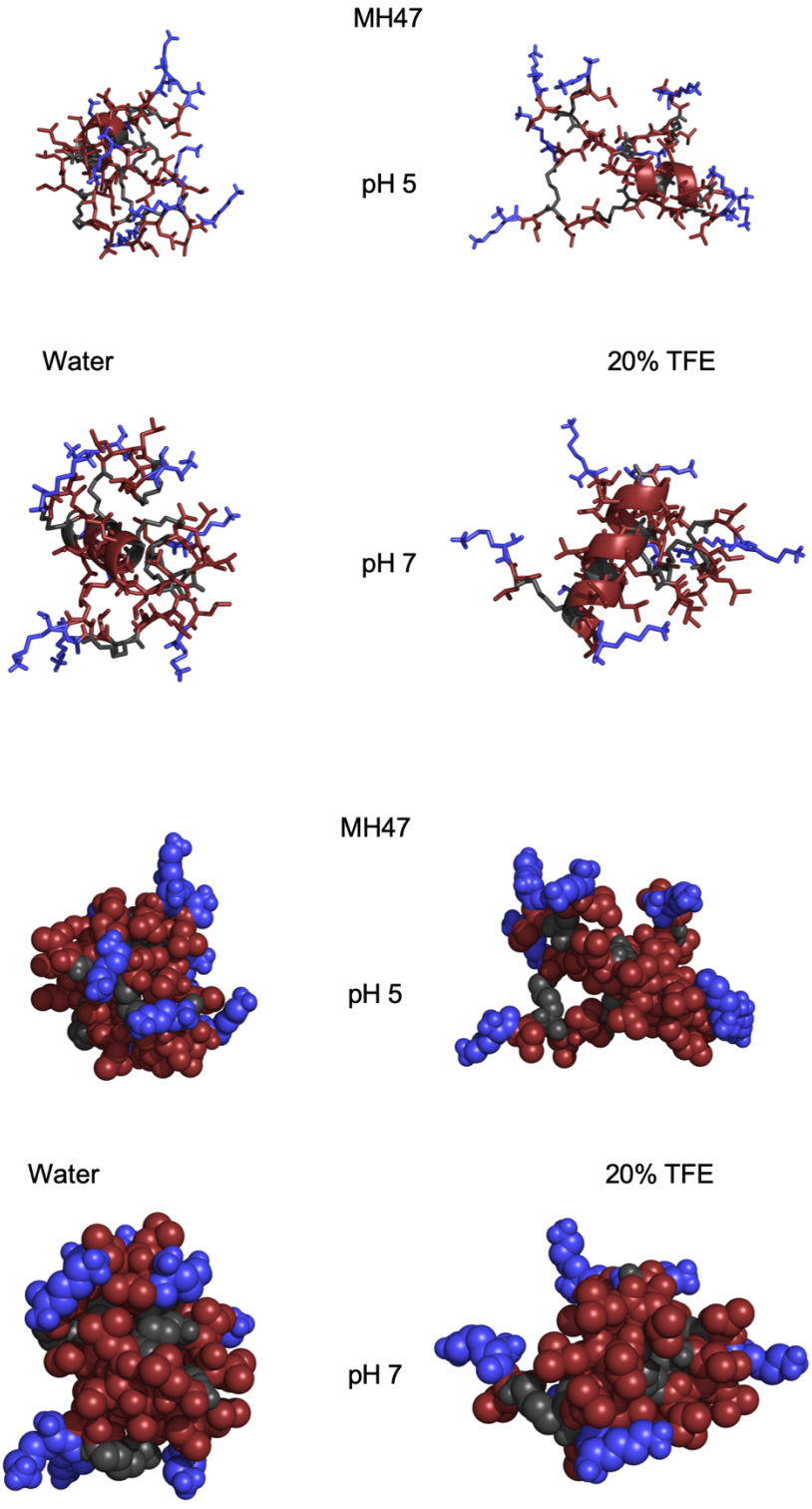

Figure S31. MD structure of the center of the main cluster of the last 100 ns of the simulation represented as stick and cartoon (top panel) and CPK (bottom panel) for **MH47** at pH 5 (top) and 7 (bottom) in absence (left) and presence (right) of 20% trifluoroethanol. Color-coded by residue type (blue = lysine, red = leucine, gray = branching lysine).

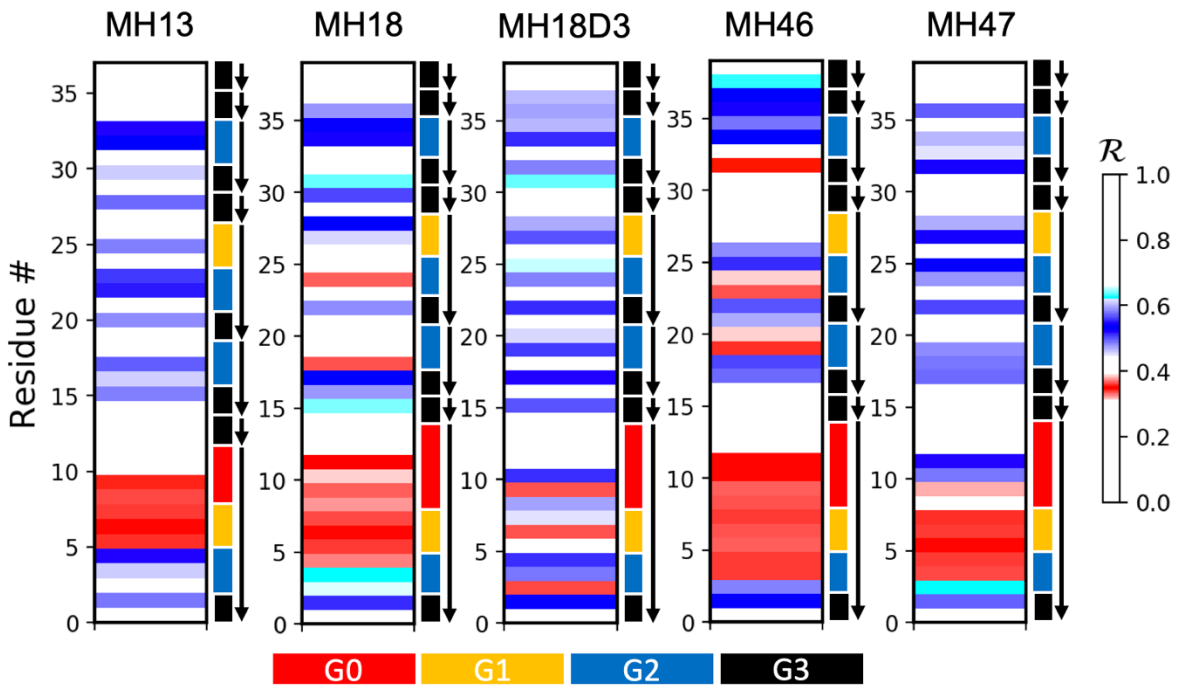

Figure S32. Ramachandran number $\mathcal{R}$ analysis of the internal secondary structures at pH 5 in water. Residues in α-helical conformation ($\mathcal{R} \approx 0.34$) are indicated in red, β-sheet ($\mathcal{R} \approx 0.52$) in blue and loops ($\mathcal{R} \approx 0.62$) in cyan. The position of each residue according to its generation is noted using the color code of Figure 1. The arrows represent continuous α-peptide portions of the dendrimer.

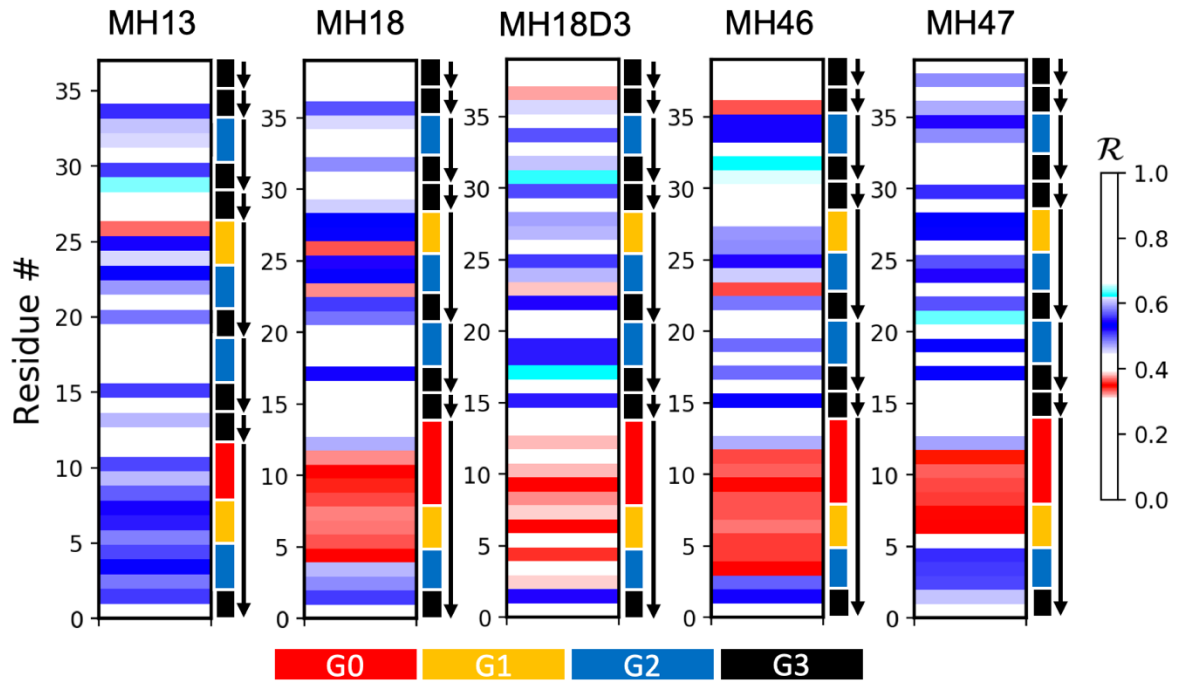

Figure S33. Ramachandran number $\mathcal{R}$ analysis of the internal secondary structures at pH 5 in water in presence of 20% TFE. Residues in α-helical conformation ($\mathcal{R} \approx 0.34$) are indicated in red, β-sheet ($\mathcal{R} \approx 0.52$) in blue and loops ($\mathcal{R} \approx 0.62$) in cyan. The position of each residue according to its generation is noted using the color code of Figure 1. The arrows represent continuous α-peptide portions of the dendrimer.

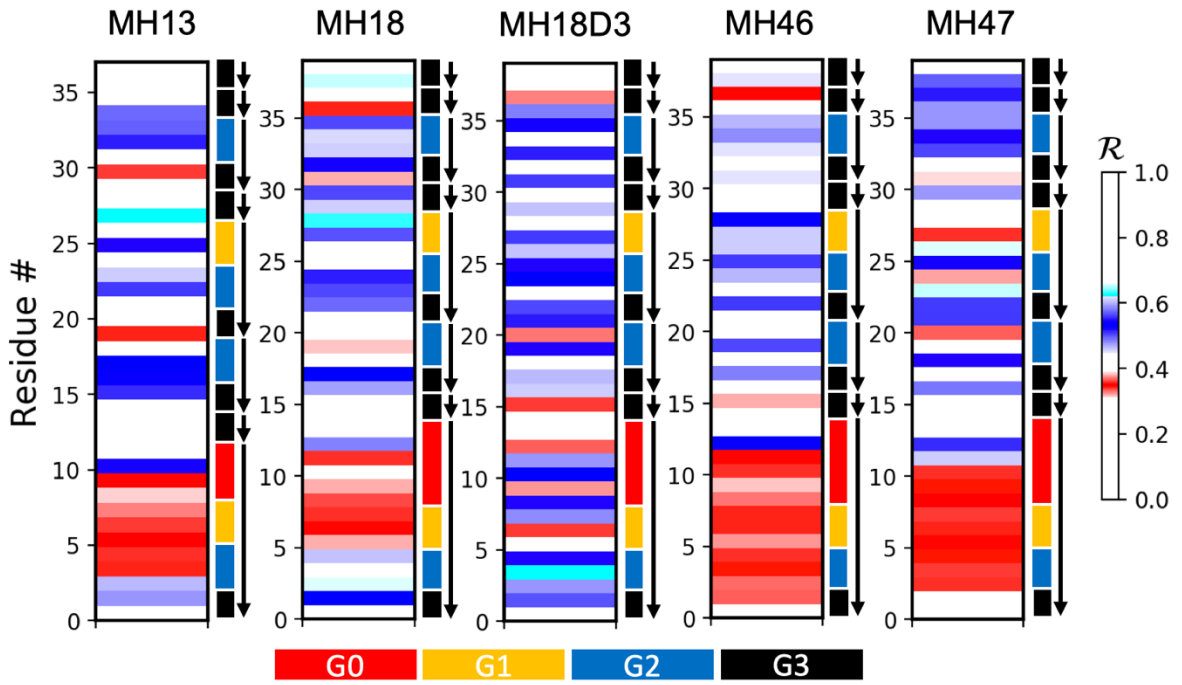

Figure S34. Ramachandran number $\mathcal{R}$ analysis of the internal secondary structures at pH 7 in water. Residues in α-helical conformation ($\mathcal{R} \approx 0.34$) are indicated in red, β-sheet ($\mathcal{R} \approx 0.52$) in blue and loops ($\mathcal{R} \approx 0.62$) in cyan. The position of each residue according to its generation is noted using the color code of Figure 1. The arrows represent continuous α-peptide portions of the dendrimer.

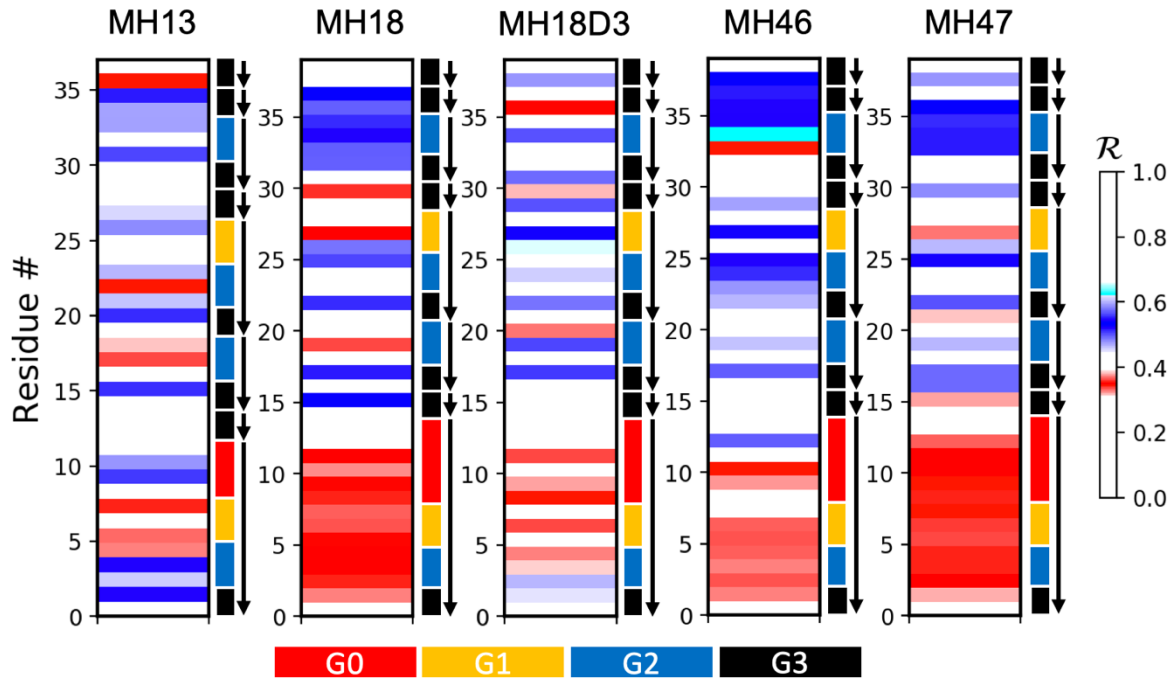

Figure S35. Ramachandran number $\mathcal{R}$ analysis of the internal secondary structures at pH 7 in water presence of 20% TFE. Residues in α-helical conformation ($\mathcal{R} \approx 0.34$) are indicated in red, β-sheet ($\mathcal{R} \approx 0.52$) in blue and loops ($\mathcal{R} \approx 0.62$) in cyan. The position of each residue according to its generation is noted using the color code of Figure 1. The arrows represent continuous α-peptide portions of the dendrimer.

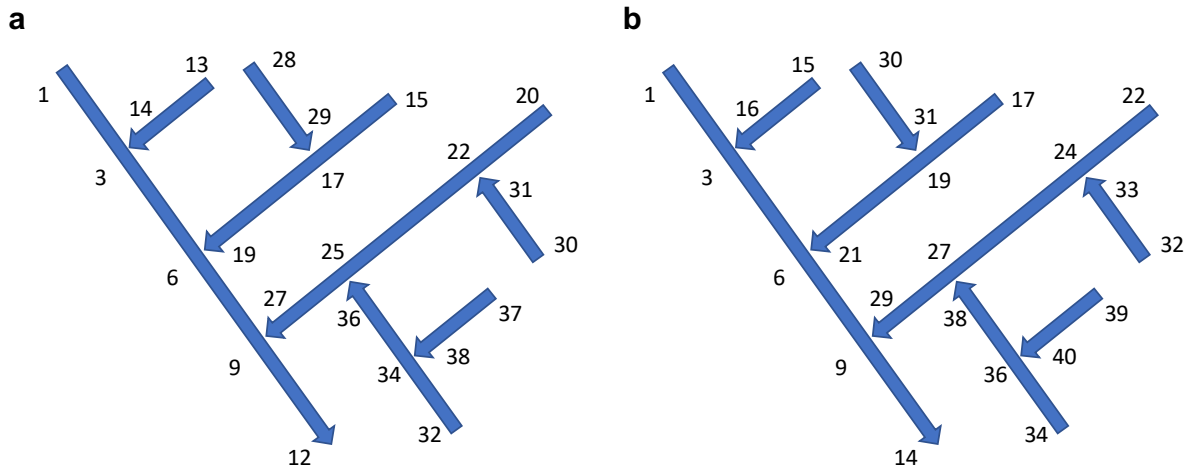

Figure S36. Due to the constraints imposed by the PDB file format (originally designed for linear α-peptides), the residues of the peptide dendrimers were listed as 8 N→C peptides in order to maximize the longest α-peptide fragments for **(a) MH13** and **(b) MH18**, **MH18D3**, **MH46** and **MH47**. The order of the 8 peptides follows the order of the branching lysines to which each of them connect in an iterative way. The numbering depicted reflects the residues in analyses of Figure S32–S35.

2 References

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