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Supporting Information

Precise redox-sensitive cleavage sites for improved bioactivity of siRNA lipopolyplexes

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‡ These authors contributed equally

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Experimental section

Loading of a 2-Chlorotrityl Chloride Resin with an Fmoc protected amino acid

After swelling of 750 mg of a 2-chlorotrityl chloride resin (1.2 mmol chloride) in water-free DCM for 10 min, the first Fmoc protected amino acid (T-shape: 0.75 eq. fmoc-Tyr(tBu)-OH, i-shape: 0.75 eq. Fmoc-Stp(Boc3)-OH, U-shape: 0.75 eq. Fmoc-Lys(fmoc)-OH and DIPEA (1.5 eq.) were added to the resin for 1 h. The reaction solvent was drained and a mixture of DCM/MeOH/DIPEA (80/15/5) was added twice for 10 min. After the removal of the reaction mixture, the resin was washed 5 times with DCM.

About 30 mg of the resin were removed and dried to determine the loading of the resin. Therefore, an exact amount of resin was treated with 1 mL deprotection solution (20 % piperidine in DMF) for 1 h. Afterwards, the solution was diluted and absorption was measured at 301 nm. The loading was then calculated according to the equation: resin load [mmol/g] = (A*1000)/(m [mg]*7800*df) with df as dilution factor.

The resin was treated twice with 20% piperidine in DMF and twice with 20 % piperidine DMF with 2% DBU to remove the fmoc protection group. Reaction progress was monitored by Kaiser test. Afterwards, the resin was washed with DMF, DCM and n-hexane and dried in vacuo.

Syntheses of oligomers containing oleic acid

The coupling procedure of oligomers containing oleic acid (OleA) was the same used for all oligomers (see description in main manuscript). The cleavage of the structures off the resin was performed by incubation with TFA–TIS–H_2O 95 : 2.5 : 2.5 (10 mL g^{-1} resin cooled to 4 °C prior to addition) for 30 min. followed by immediate precipitatation in 40 mL of pre-cooled MTBE–n-hexane 1 : 1. The oleic acid containing oligomers were then purified by size exclusion chromatography using an Äkta purifier system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), a Sephadex G-10 column and 10 mM hydrochloric acid solution–acetonitrile 7 : 3 as solvent. The oligomers were lyophilized. Oligomer sequences were validated by mass spectrometry and ^{1}H-NMR.

siRNA polyplex stability in 90% serum

Polyplexes were formed using 2.5 µg siRNA in 6.25 µL HBG mixed with the oligomer at N/P 12 resulting in a total volume of 12.5 µL. Afterward the incubation 112.5 µl fetal bovine serum (FBS) was added to the samples. All samples had a final concentration of 90 % FBS. The samples were incubated at 37 °C for 2 h. 20 µL of the samples and 4 µL loading buffer were carefully mixed and a siRNA binding assay (see main manuscript) was performed.

Particle Size and Zeta Potential

For dynamic light scattering (DLS) measurements the polyplex solution was measured in a folded capillary cell (DTS 1070) using a Zetasizer Nano ZS with backscatter detection (Malvern Instruments, Worcestershire, UK). Polyplexes were formed using 1.5 µg siRNA in 30 µL HBG mixed with the oligomer at N/P 12 resulting in a total volume of 60 µL. For size measurements, the equilibration time was 0 min, the temperature was 25 °C and an automatic attenuator was used. The refractive index of the solvent was 1.330 and the viscosity was 0.8872 mPa•s. Each sample was measured 3 times. For zeta potential measurements, the sample was diluted to 800 µL with 20 mM HEPES buffer. Zeta potentials were calculated by the Smoluchowski equation. Ten to fifteen sub runs lasting 10 s each at 25 °C (n = 3) were measured.

Ellman´s Assay

The oligomers containing ssbb elements were diluted to a concentration of 1.67 mg/mL. 30 µL of the solution was mixed with 170 µL working solution (2.44 mL Ellman´s buffer (0.2 M Na_2HPO_4, 1 mM EDTA, pH 8.0) and 60 µL DTNB solution in methanol (c = 4 mg/mL)). After 15 min incubation at 37 °C absorption was measured at 412 nm using a GENESYS™ UV-VIS spectrophotometer (Thermo Scientific). The percentage of free mercapto groups is based on the theoretical amount (100 %) of thiols in case of complete cleavage.

Proton ^{1}H NMR spectroscopy

^{1}H NMR spectra were recorded using an AVANCE III HD 500 (500 MHz) by Bruker with a 5 mm CPPBBO probe. All spectra were recorded without TMS as internal standard and therefore all signals were calibrated to the residual proton signal of the deuterium oxide (D_2O) solvent, or chloroform-d ($CDCl_3$). Chemical shifts are reported in ppm and refer to the solvent as internal standard (D_2O at 4.79; $CDCl_3$ at 4.87). Integration was performed manually. The spectra were analyzed using MestreNova (Ver. 9.0 by MestReLab Research).

MALDI Mass spectrometry

One µL matrix droplet consisting of a saturated solution of Super-DHB (sum of 2,5-dihydroxybenzoic acid and 2-hydroxy-5-methoxybenzoic acid) in acetonitrile / water (1 : 1) containing 0.1 % (v/v) TFA was spotted on a MTP AnchorChip (Bruker Daltonics, Bremen, Germany). After the Super-DHB matrix crystallized, one µL of the sample solution (10 mg/mL in water) was added to the matrix spot. Samples were analyzed using an Autoflex II mass spectrometer (Bruker Daltonics, Bremen, Germany).

Supplement figures and tables

Supplement figures

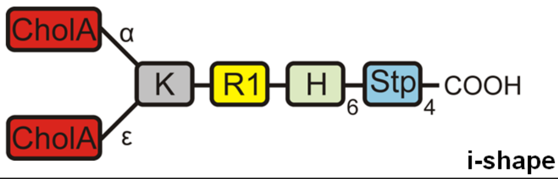
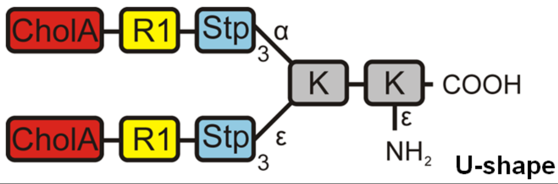
Structures	ID	R1
	*871*	-
	969	ssbb
	*783*	-
	782	ssbb

Fig. S1 Sequence-defined oligomers with i-shape and U-shape topology. Schematic overview of the structures with different modifications (K: lysine, H: histidine, Stp: succinoyl-tetraethylene-pentamine, ssbb: succinoyl-cystamine, CholA: 5β-Cholanic acid). Ids are unique database identification numbers.

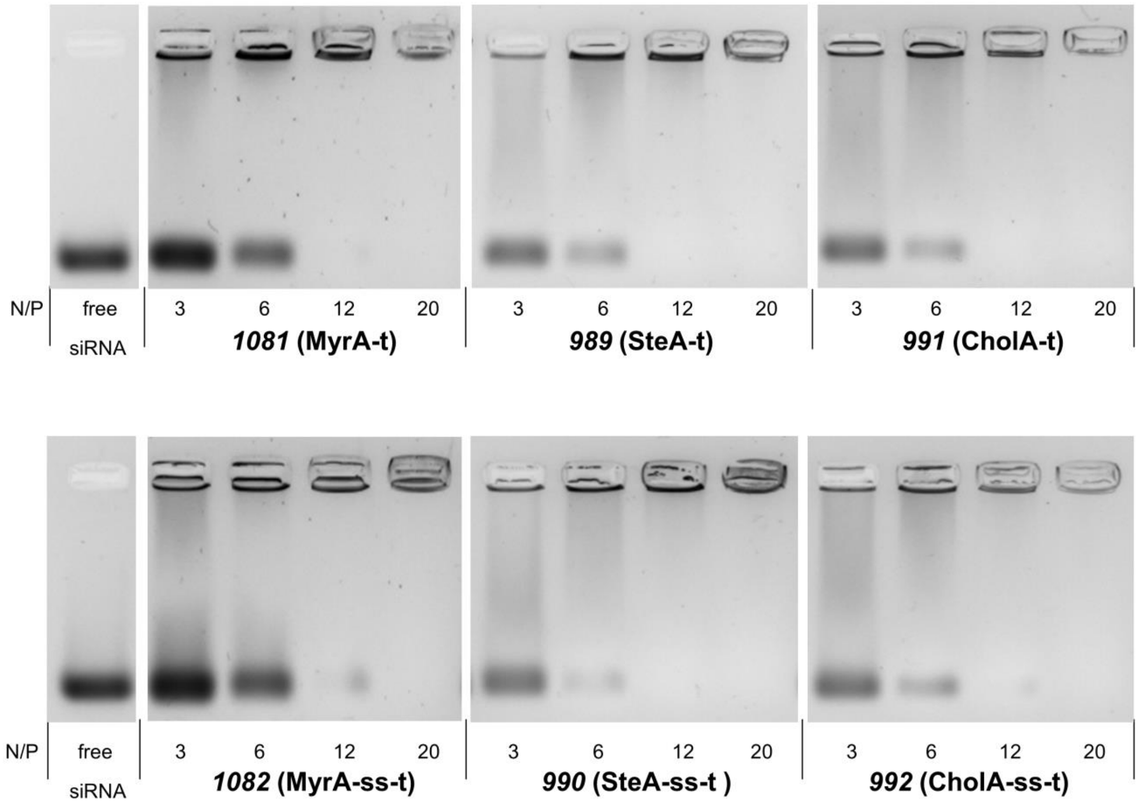


Fig. S2 siRNA binding ability of T-shape structures analyzed with an agarose gel shift assay. The left lane shows the running distance of free siRNA in HBG that is not complexed by lipo-oligomers. Polyplexes were tested for siRNA binding ability at different N/P ratios. Top: stable structures, bottom: reducible structures.

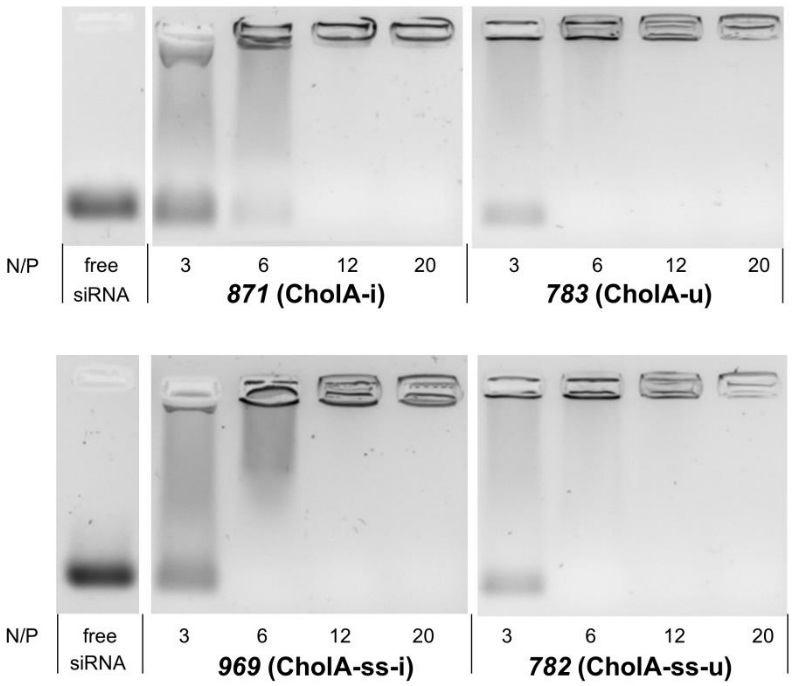


Fig. S3 siRNA binding ability of i-shape and U-shape structures analyzed with an agarose gel shift assay. The left lane shows the running distance of free siRNA in HBG that is not complexed by lipo-oligomers. Polyplexes were tested for siRNA binding ability at different N/P ratios. Top: stable structures, bottom: reducible structures.

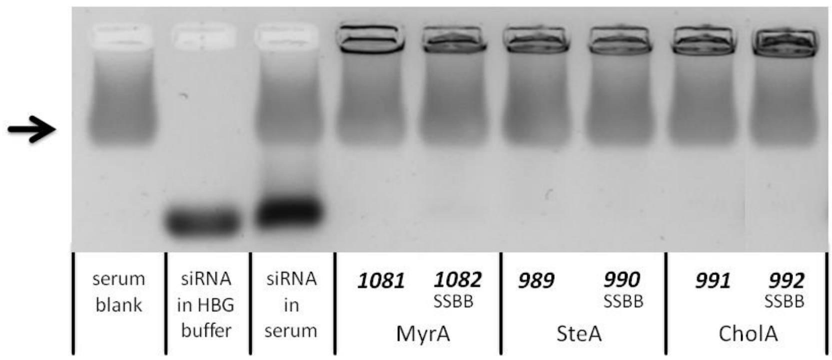


Fig. S4 Gel retardation assay of siRNA polyplexes incubated at N/P 12 for 40 min, followed by treatment with 90 % full serum for two hours at 37 °C. The black arrow points at a band that is caused by serum (see serum blank in band one). Running distance of free siRNA in HBG buffer and in 90 % serum are shown in band two and three.

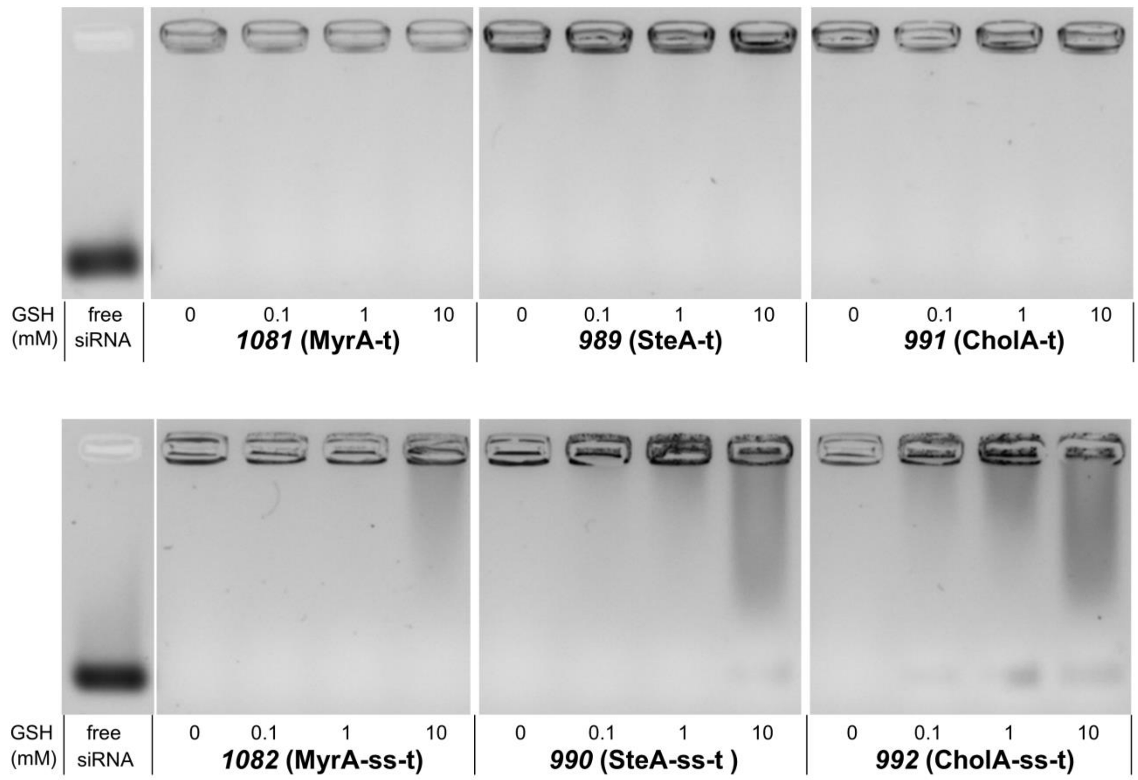


Fig. S5 siRNA binding ability of T-shape structures under reducing conditions analyzed with an agarose gel shift assay. The left lane shows the running distance of free siRNA in HBG that is not complexed by lipo-oligomers. Lipopolyplexes were formed at N/P 20 followed by 90 min treatment at 37 °C with different concentrations of GSH in HEPES buffer pH 7.4. Top: stable structures, bottom: reducible structures.

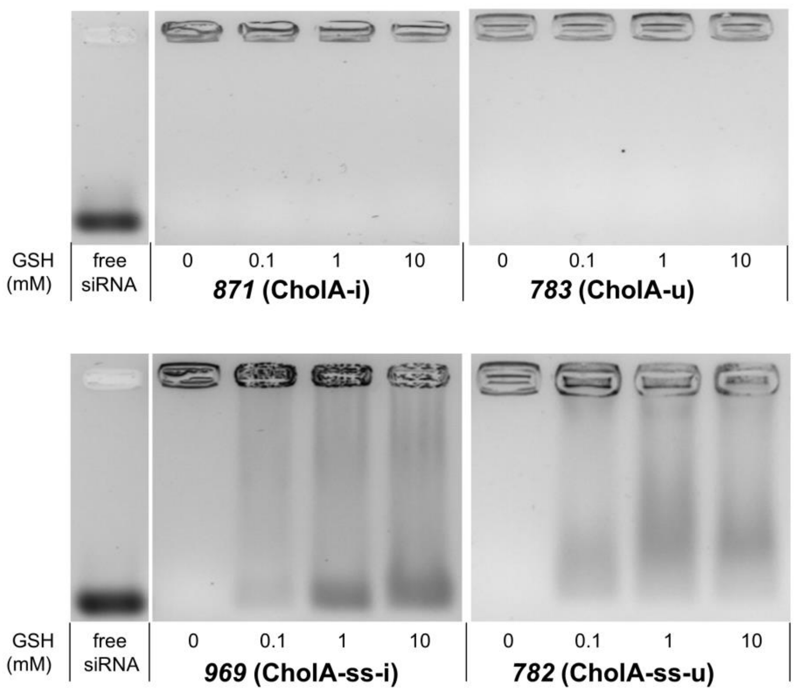


Fig. S6 siRNA binding ability of i-shape and U-shape structures under reducing conditions analyzed with an agarose gel shift assay. The left lane shows the running distance of free siRNA in HBG that is not complexed by lipo-oligomers. Lipopolyplexes were formed at N/P 20 followed by 90 min treatment at 37 °C with different concentrations of GSH in HEPES buffer pH 7.4. Top: stable structures, bottom: reducible structures.

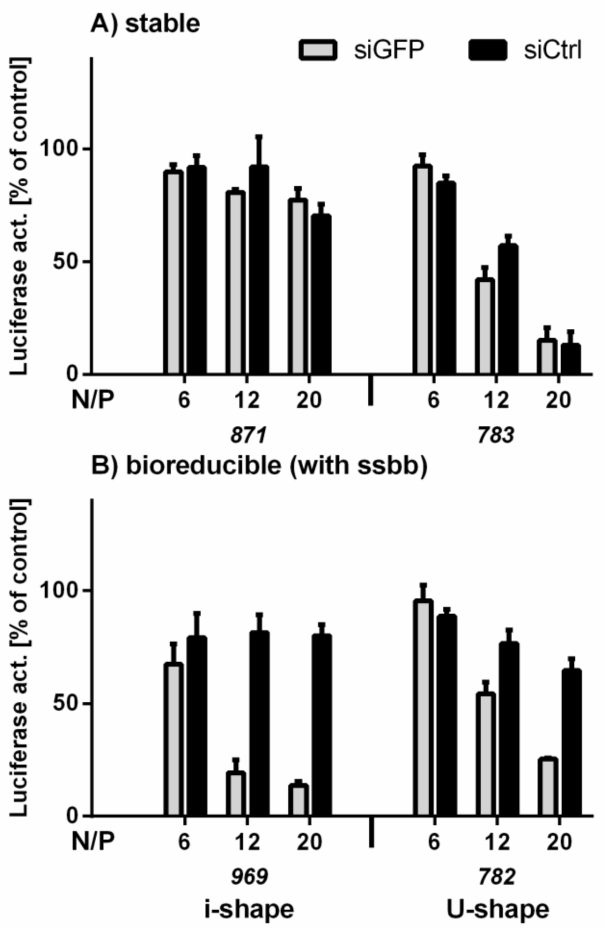


Fig. S7 Gene silencing of i-shape and U-shape oligomers in neuroblastoma cells. Lipopolyplexes with 500 ng / 37 pmol eGFP-targeted siRNA (siGFP) / well respectively control siRNA (siCtrl) at N/P 6, 12 and 20 were tested for eGFPLuc gene silencing in Neuro2A/eGFPLuc cells. A) Lipopolyplexes made of stable structures ***871*** and ***783*** B) Lipopolyplexes made of bioreducible structures ***969*** and ***782***. The luciferase activity of siRNA treated cells is presented related to buffer-treated cells. HBG-treated cells were set to 100 %. Data are presented as mean value (± SD) out of triplicates**.**

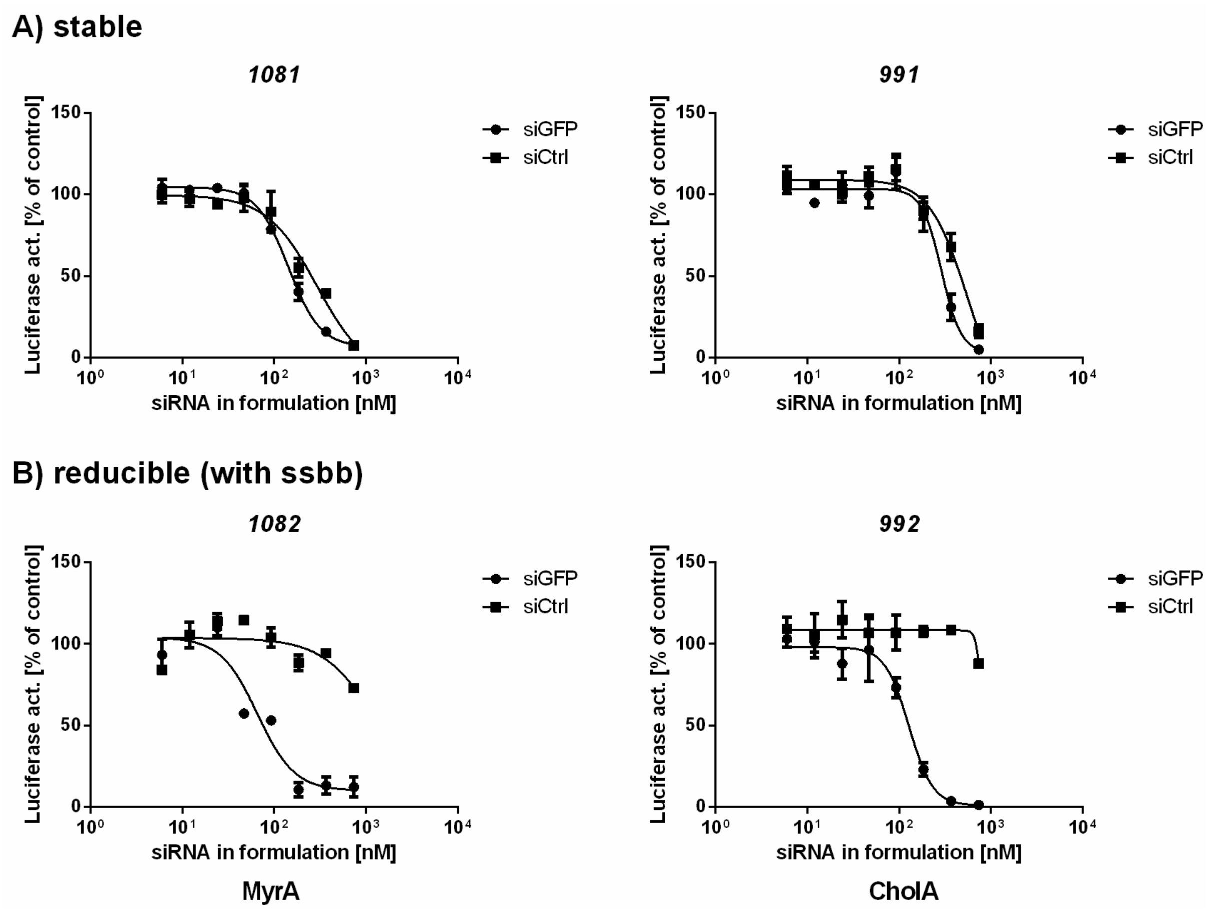


Fig. S8 Dose-dependent gene silencing of T-shape oligomers at N/P 12 in neuroblastoma cells. Lipopolyplexes with eGFP-targeted siRNA (siGFP) respectively control siRNA (siCtrl) were examined for eGFPLuc gene silencing in Neuro2A/eGFPLuc cells. The oligomer amount was adjusted for each formulation to keep it constant at N/P 12. Formulations including siRNA from 6, 12, 27, 47, 93, 185, 370 up to 740 nM were tested. A) Lipopolyplexes made of stable structures ***1081*** and ***991*** B) Lipopolyplexes made of bioreducible structures ***1082*** and ***992***. The luciferase activity of siRNA treated cells is presented related to buffer-treated cells. HBG-treated cells were set to 100 %. Data are presented as mean value (± SD) out of triplicates.

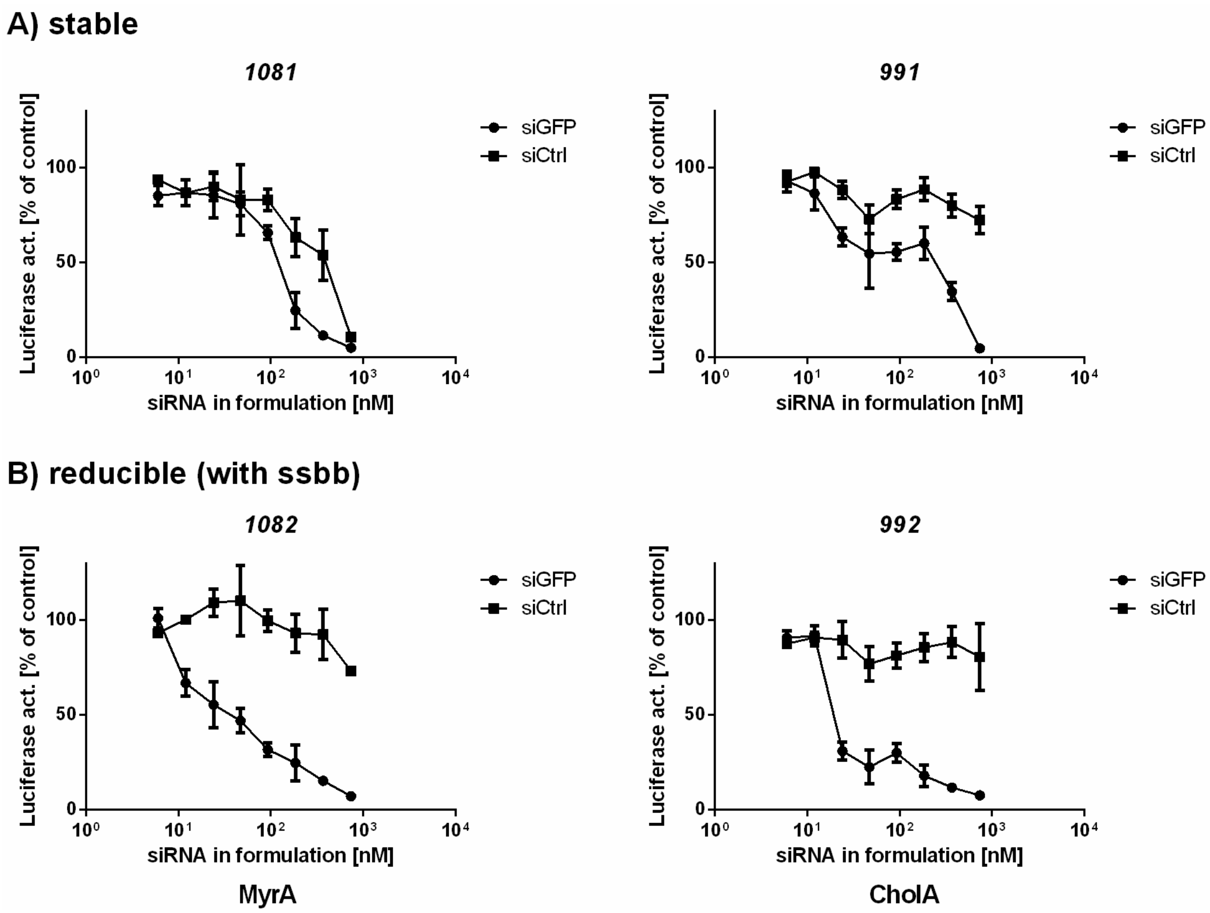


Fig. S9 Dose-dependent gene silencing of T-shape oligomers in neuroblastoma cells. Lipopolyplexes with eGFP-targeted siRNA (siGFP) respectively control siRNA (siCtrl) at constant oligomer amount of 1.44 nmol (N/P 12 at 500 ng siRNA) were examined for eGFPLuc gene silencing in Neuro2A/eGFPLuc cells. Formulations including siRNA from 6, 12, 27, 47, 93, 185, 370 up to 740 nM were tested. A) Lipopolyplexes made of stable structures ***1081*** and ***991*** B) Lipopolyplexes made of bioreducible structures ***1082*** and ***992***. The luciferase activity of siRNA-treated cells is presented related to buffer-treated cells. HBG-treated cells were set to 100 %. Data are presented as mean value (± SD) out of triplicates.

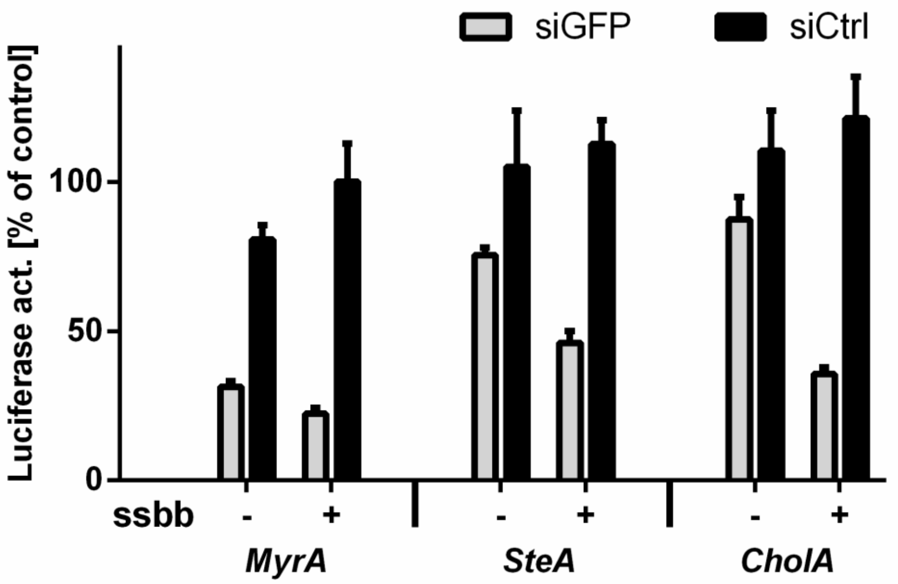


Fig. S10 Gene silencing of T-shape oligomers in prostate cancer cells. Lipopolyplexes with 500 ng eGFP-targeted siRNA (siGFP) respectively control siRNA (siCtrl) at N/P 12 were tested for eGFPLuc gene silencing in DU145/eGFPLuc cells. Lipopolyplexes made of stable structures (***1081***, ***989*** and ***991***) and bioreducible structures (***1082***, ***990*** and ***992***) are shown. The luciferase activity of siRNA treated cells is presented related to buffer-treated cells. HBG-treated cells were set to 100 %. Data are presented as mean value (± SD) out of triplicates.

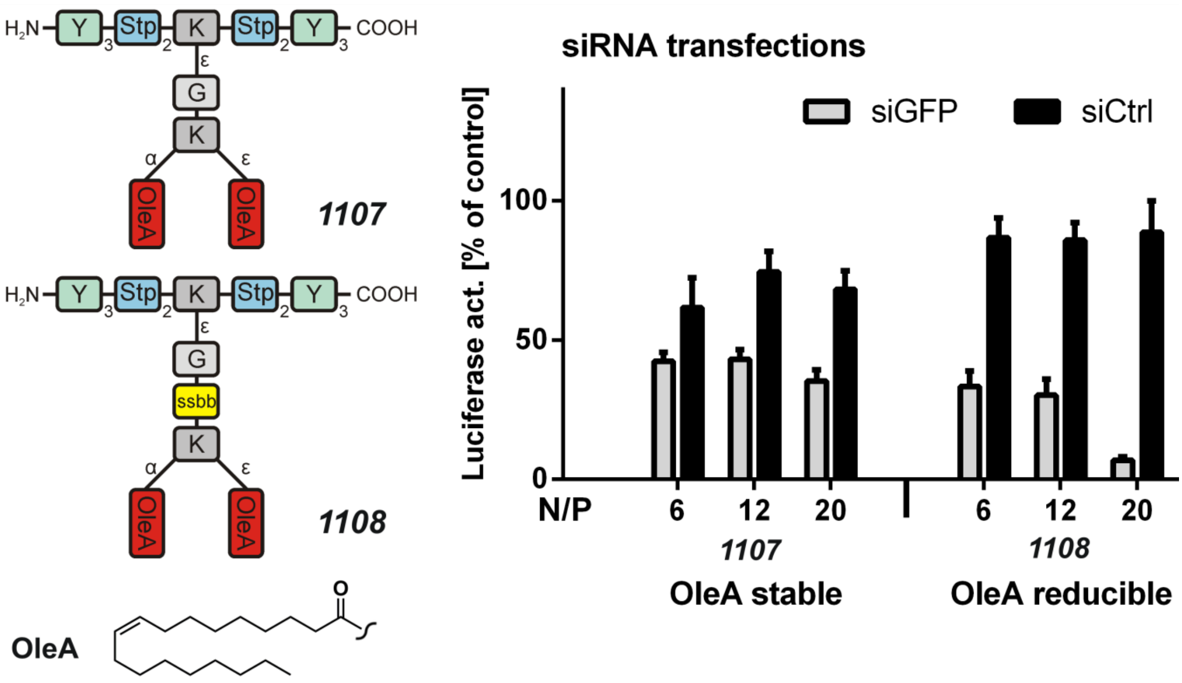


Fig. S11 Gene silencing of oleic acid containing T-shape oligomers in neuroblastoma cells. Lipopolyplexes with 500 ng / 37 pmol eGFP-targeted siRNA (siGFP) / well respectively control siRNA (siCtrl) at N/P 6, 12 and 20 were tested for eGFPLuc gene silencing in Neuro2A/eGFPLuc cells. The luciferase activity of siRNA treated cells is presented related to buffer-treated cells. HBG-treated cells were set to 100 %. Data are presented as mean value (± SD) out of triplicates.

Supplement tables

Table S1 Determination of free thiols in reducible T-shape, i-shape and U-shape structures via Ellman´s assay

Oligomer	Ratio of free thiols (in %)
1082 **(MyrA-ss-t)**	2.0
990 **(SteA-ss-t)**	2.3
992 **(CholA-ss-t)**	2.7
969 **(CholA-ss-i)**	1.2
782 **(CholA-ss-u)**	0.6

Table S2 Particle size (Z-average) and zeta potential of siRNA polyplexes determined with a DLS zetasizer

Oligomer	N/P	z-average [nm]	Mean PDI	Mean Zeta Potentianl [mV]
1081 **(MyrA-t)**	12	105 ± 1,76	0,15 ± 0	27 ± 0,75
1082 **(MyrA-ss-t)**	12	107,7 ± 0,53	0,14 ± 0,02	28,6 ± 0,75
989 **(SteA-t)**	12	125,3 ± 1,03	0,12 ± 0,01	29,3 ± 1,6
990 **(SteA-ss-t)**	12	137,9 ± 1,57	0,13 ± 0,01	26,8 ± 0,85
991 **(CholA-t)**	12	131,7 ± 0,45	0,13 ± 0	29,4 ± 4,16
992 **(CholA-ss-t)**	12	128,3 ± 0,5	0,13 ± 0,01	30,9 ± 0,72
871 **(CholA-i)**	12	275 ± 7,22	0,24 ± 0,01	23,4 ± 0,68
969 **(CholA-ss-i)**	12	237,8 ± 4,21	0,2 ± 0,01	25,2 ± 0,32
783 **(CholA-u)**	12	122,7 ± 2,01	0,26 ± 0,02	31,53 ± 0,67
782 **(CholA-ss-u)**	12	181,23 ± 4,65	0,27 ± 0,01	29,13 ± 3,26

Analytical data

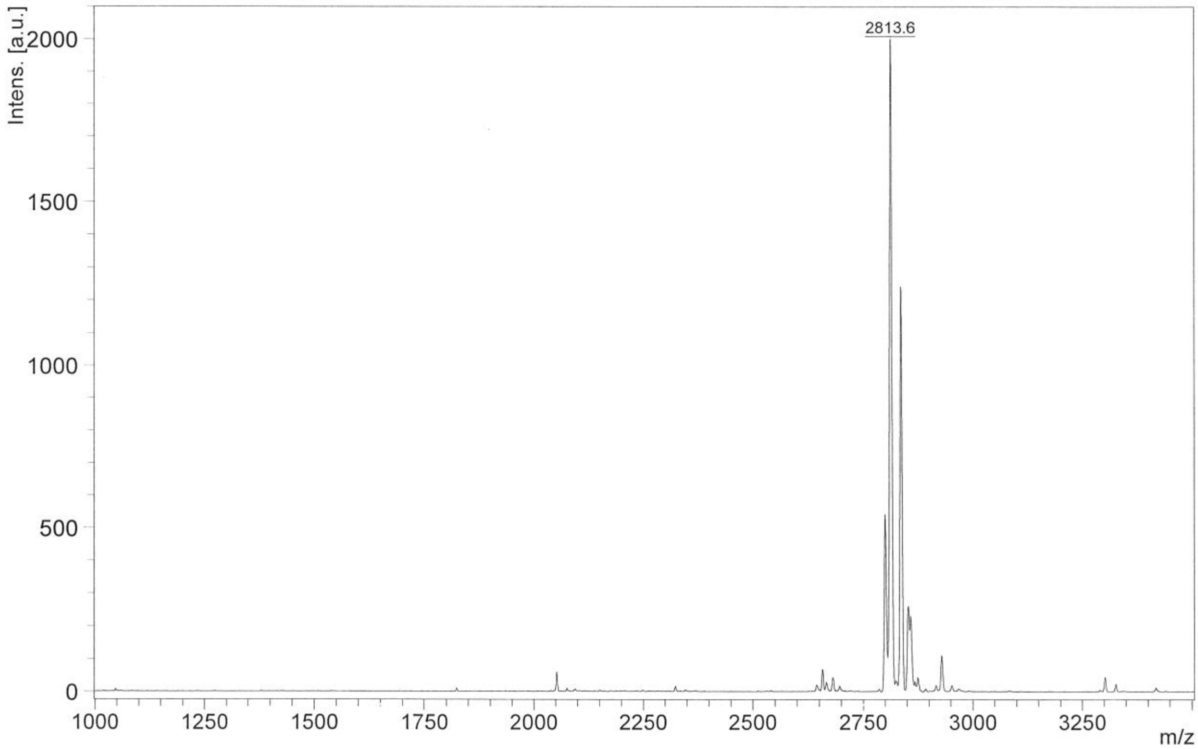
Mass spectra of oligomers

Summarizing table Mass data recorded with a Bruker MALDI-TOF instrument

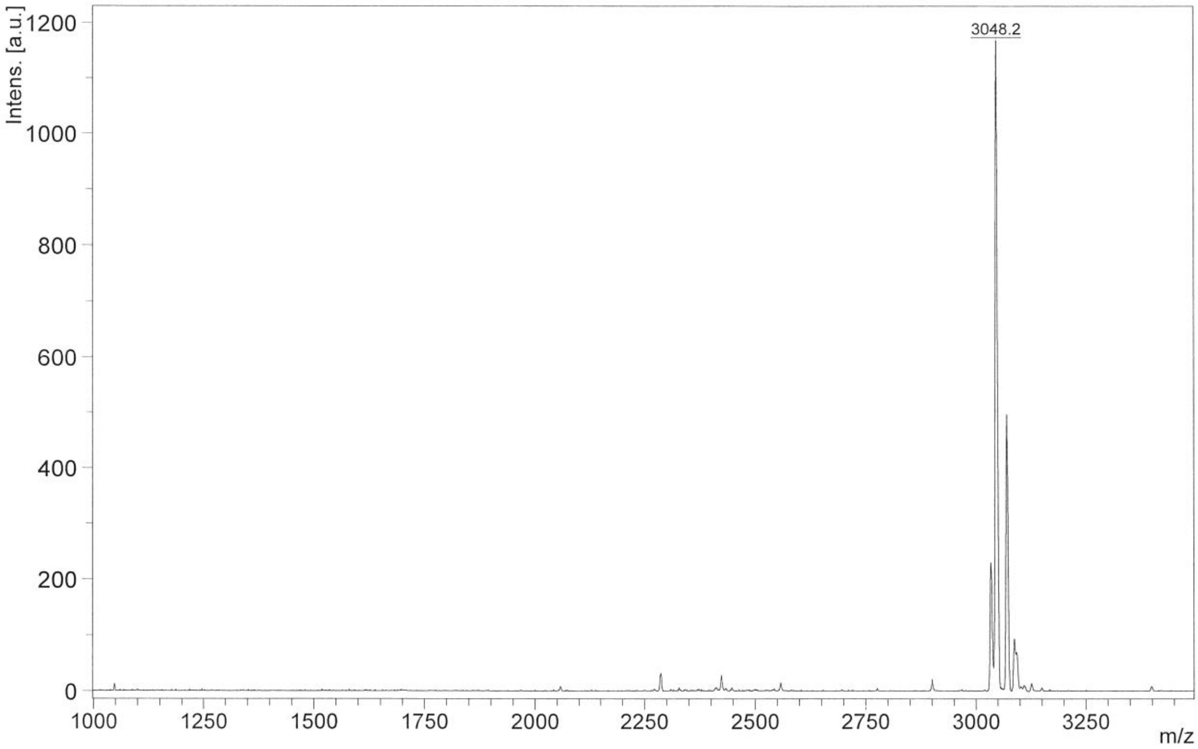
Oligomer	Molecular formula	$[M+H]^+$ calc.	$[M+H]^+$ found
1081 **(MyrA-t)**	$C_{144}H_{235}N_{31}O_{26}$	2815.8	2813.6
1082 **(MyrA-ss-t)**	$C_{152}H_{249}N_{33}O_{28}S_2$	3049.9	3048.2
989 **(SteA-t)**	$C_{152}H_{251}N_{31}O_{26}$	2927.9	2929.3
990 **(SteA-ss-t)**	$C_{160}H_{265}N_{33}O_{28}S_2$	3162.0	3163.6
991 **(CholA-t)**	$C_{164}H_{259}N_{31}O_{26}$	3080.0	3079.0
992 **(CholA-ss-t)**	$C_{172}H_{273}N_{33}O_{28}S_2$	3314.0	3314.2
1107 **(OleA-t)**	$C_{152}H_{247}N_{31}O_{26}$	2923.9	2922.9
1108 **(OleA-ss-t)**	$C_{160}H_{261}N_{33}O_{28}S_2$	3158.0	3156.3
871 **(CholA-i)**	$C_{138}H_{232}N_{40}O_{18}$	2738.9	2739.2
969 **(CholA-ss-i)**	$C_{146}H_{246}N_{42}O_{20}S_2$	2972.9	2973.2
783 **(CholA-u)**	$C_{132}H_{252}N_{34}O_{17}$	2587.0	2587.2
782 **(CholA-ss-u)**	$C_{148}H_{280}N_{38}O_{21}S_4$	3055.0	3056.1
740 **(Test structure)**	$C_{72}H_{119}N_{19}O_{12}S_2$	1506.9	1506.1

Full spectra

1081 **(MyrA-t)**



***1082* (MyrA-ss-t)**



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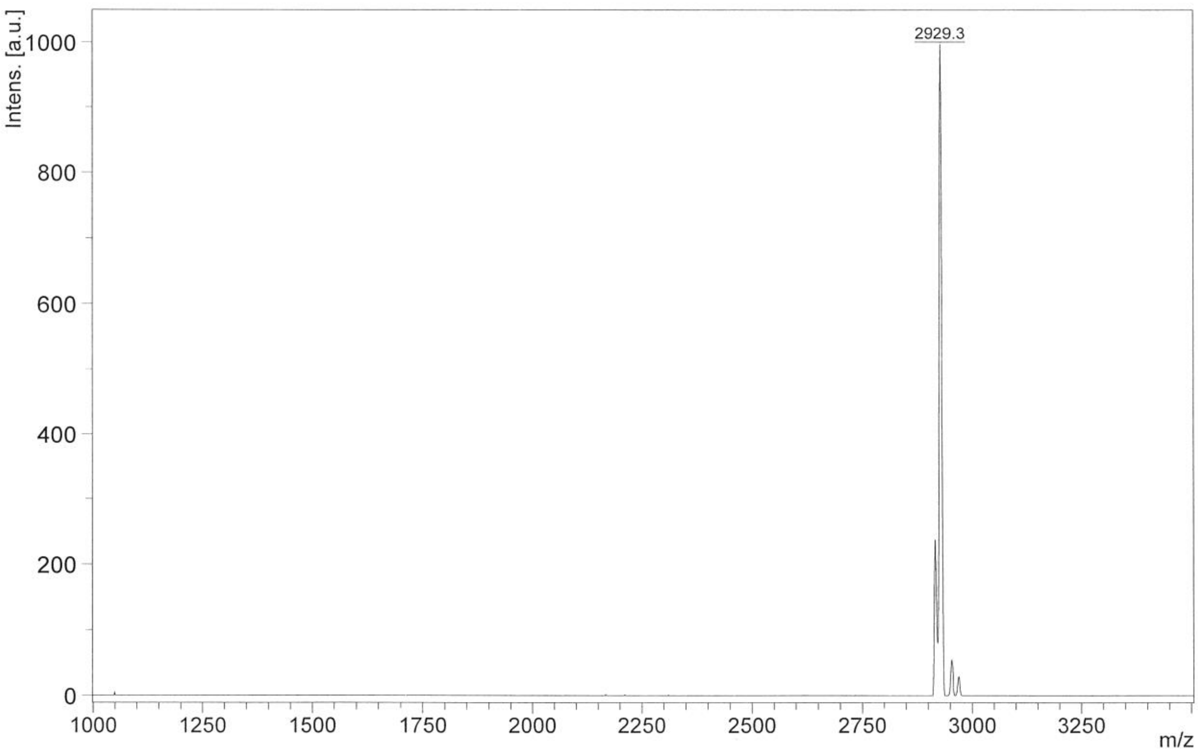

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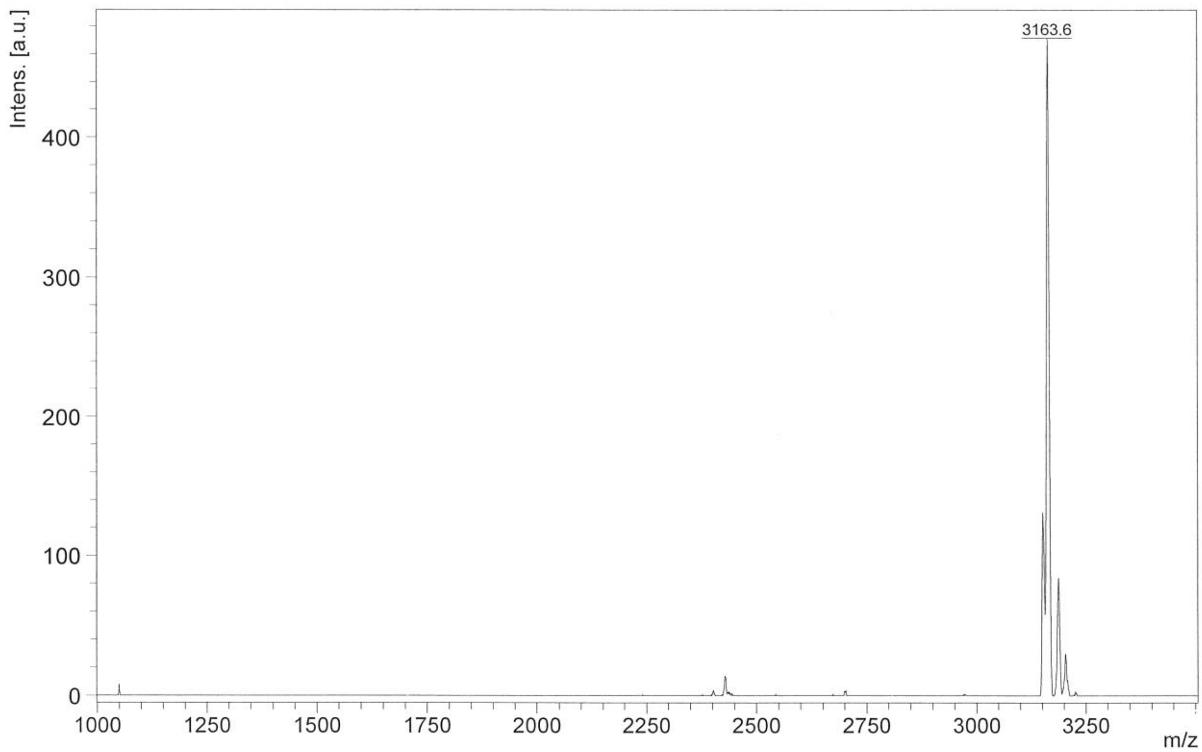

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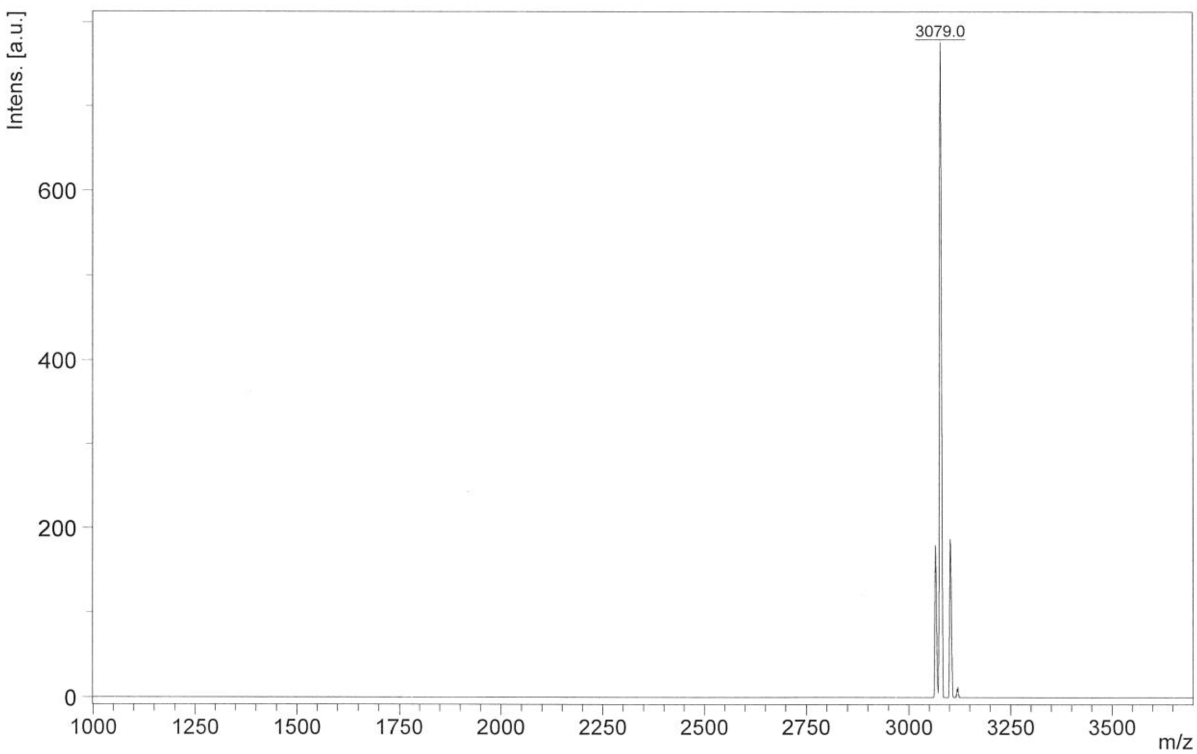

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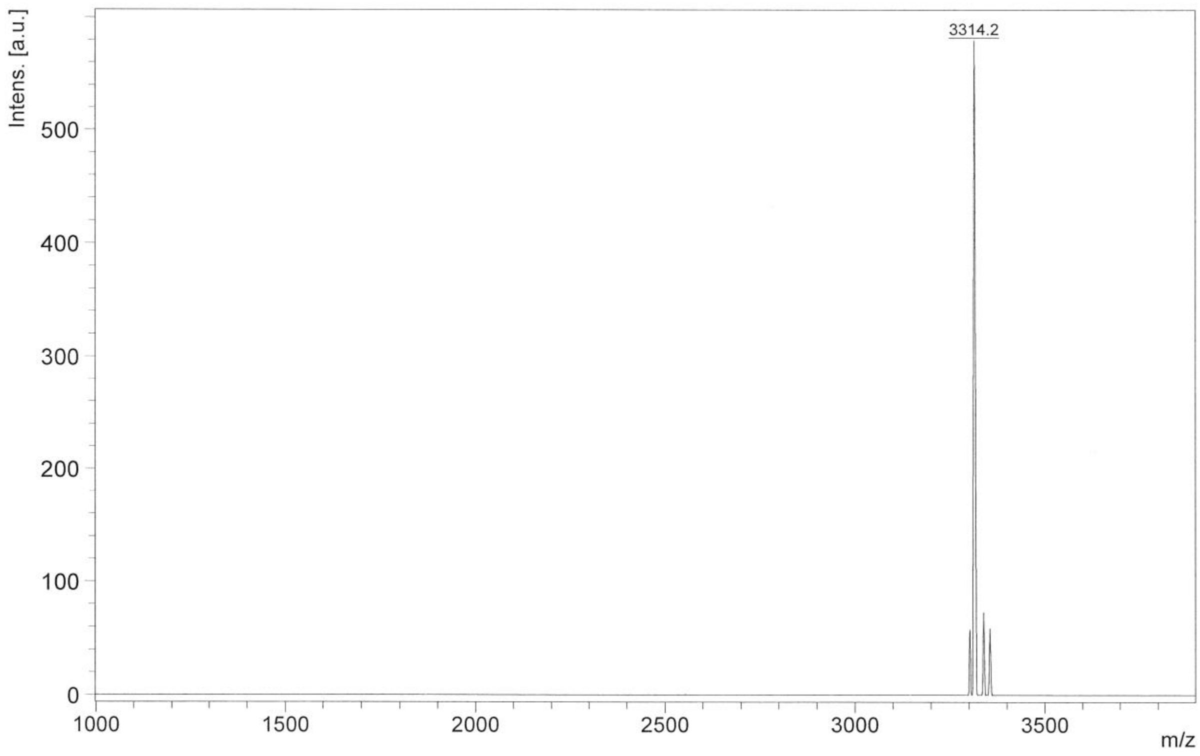
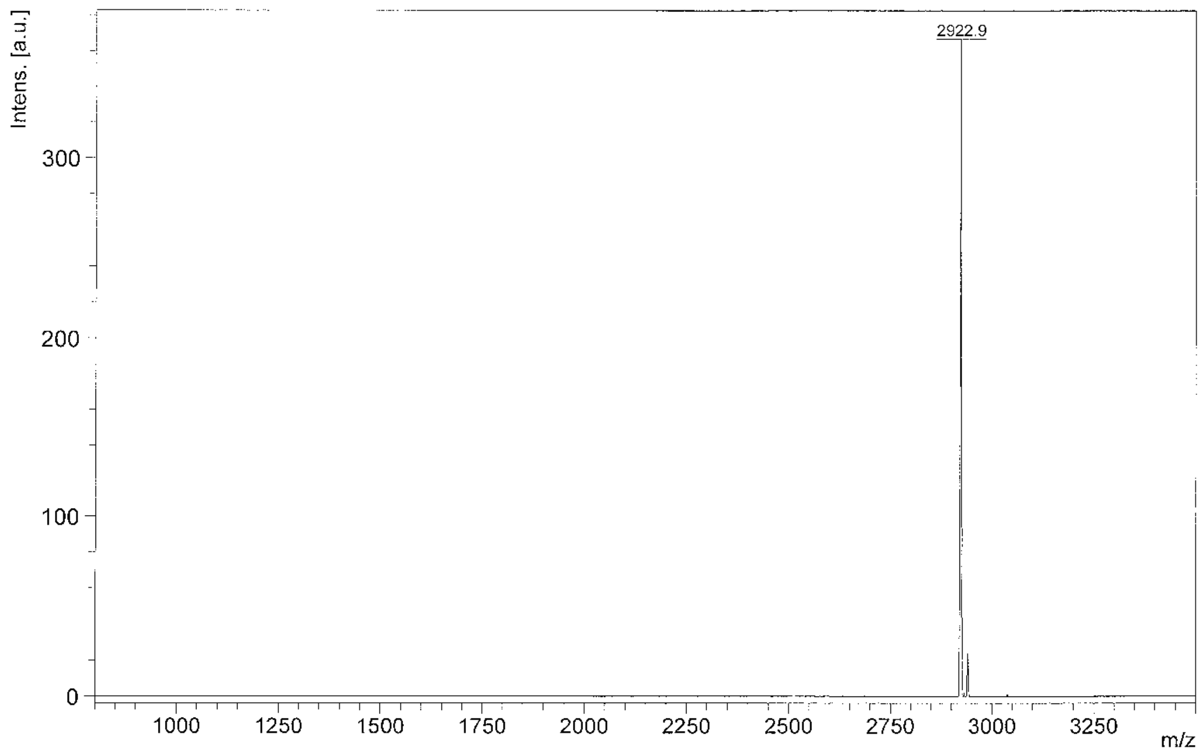
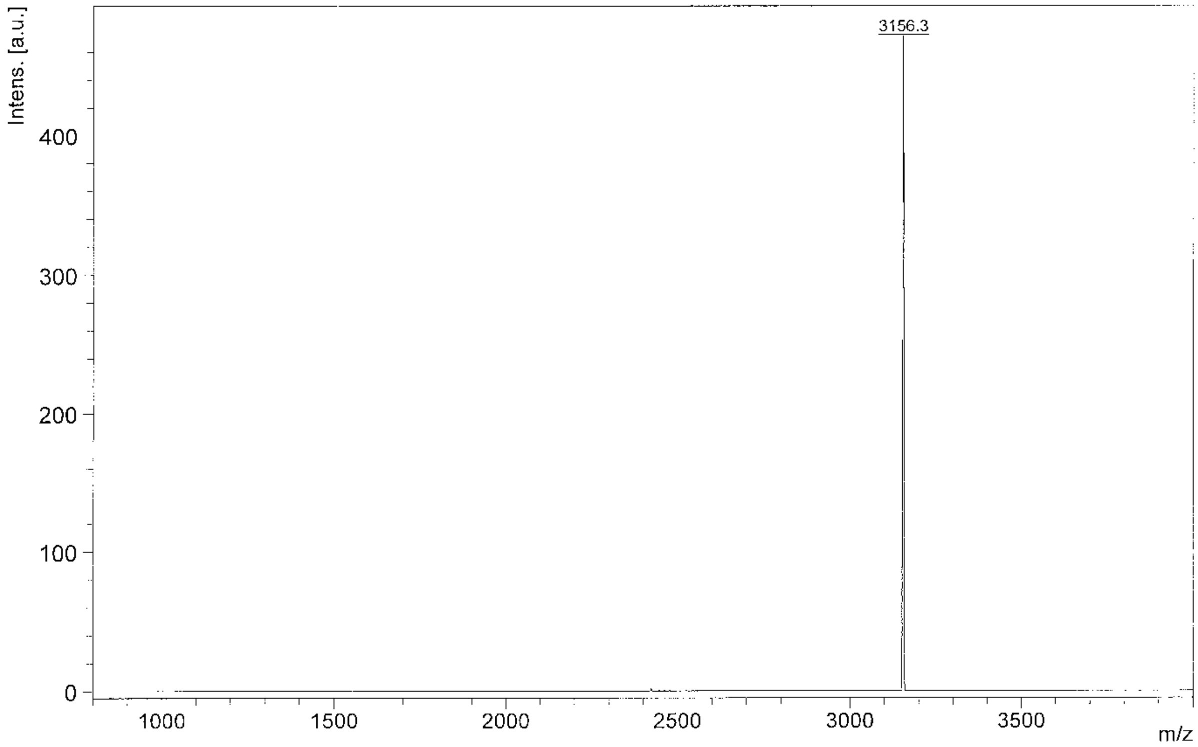


1107 **(OleA-t)**



***1108* (OleA-ss-t)**



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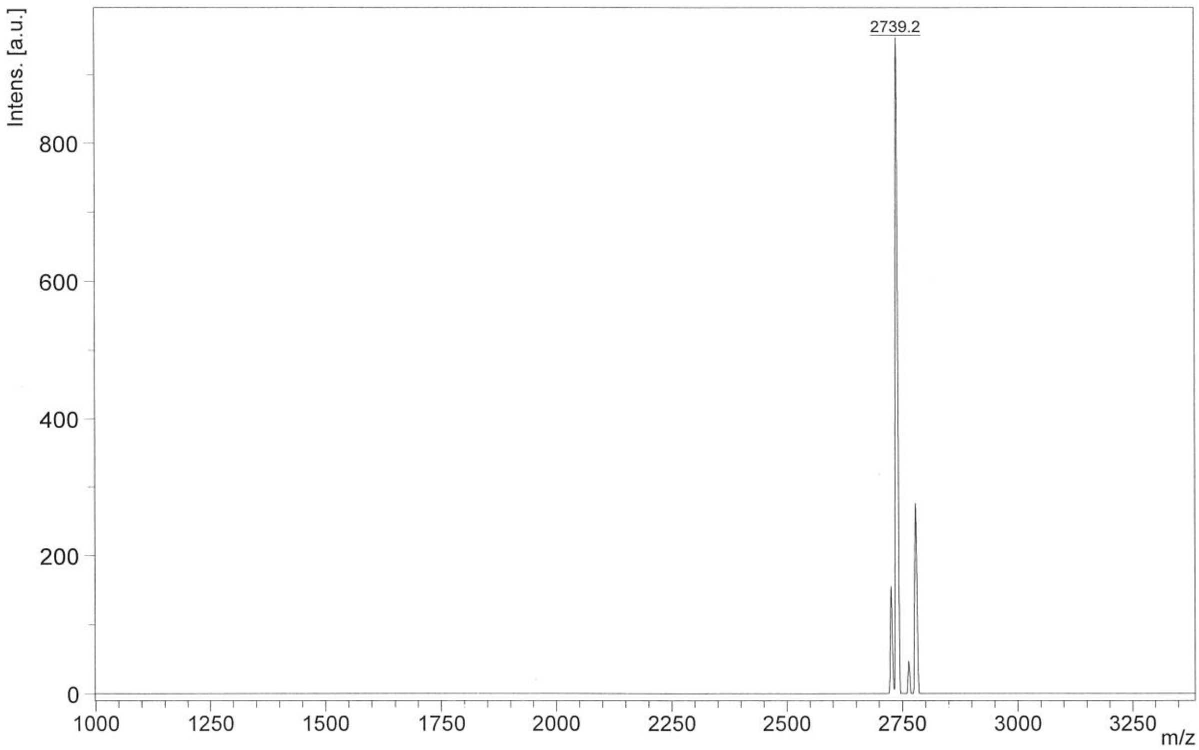

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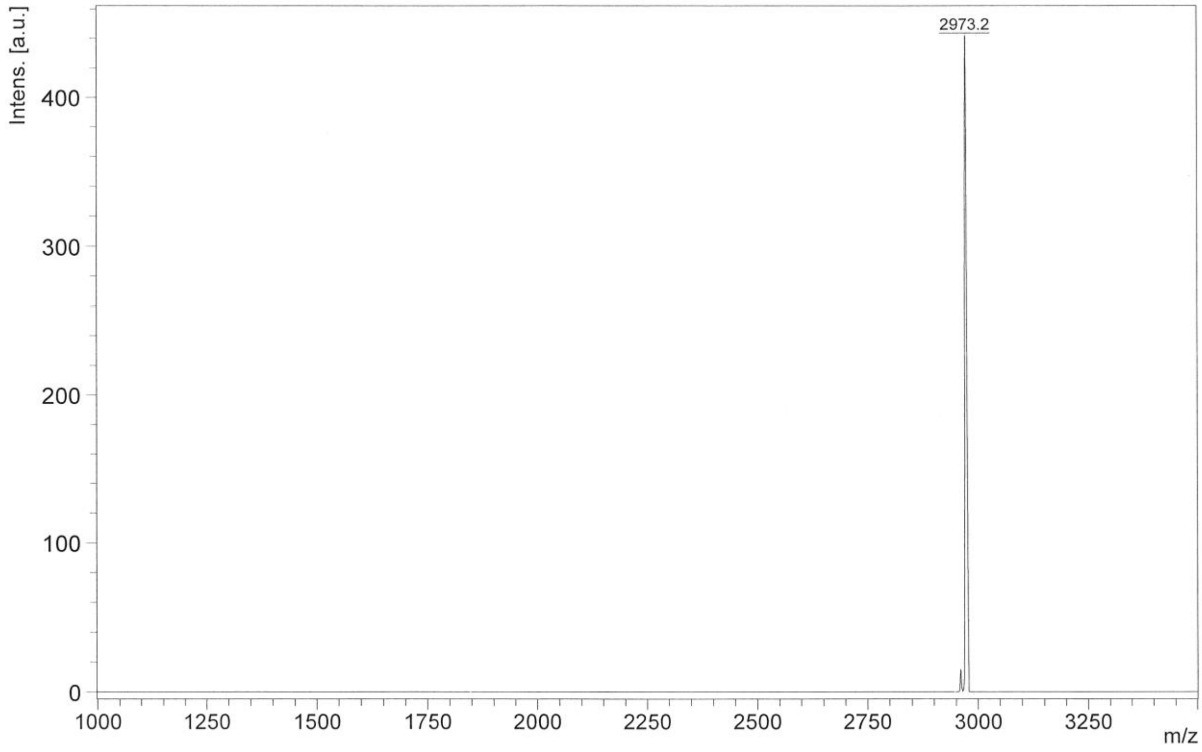

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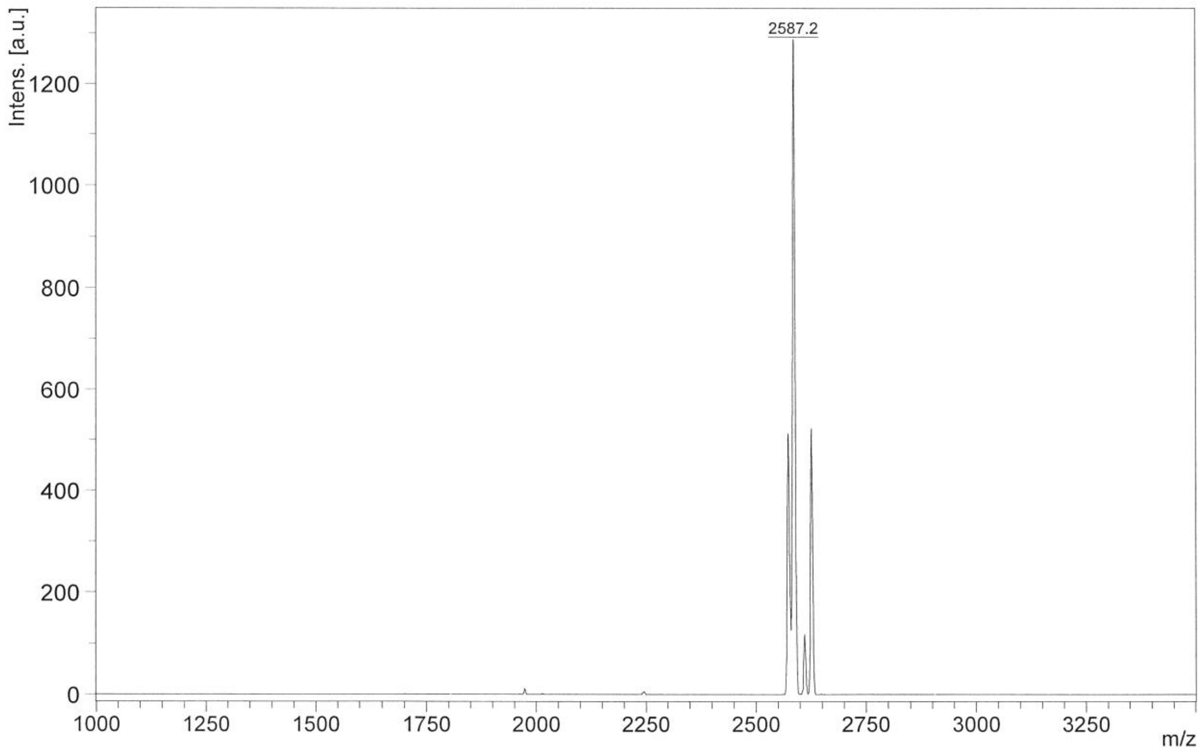
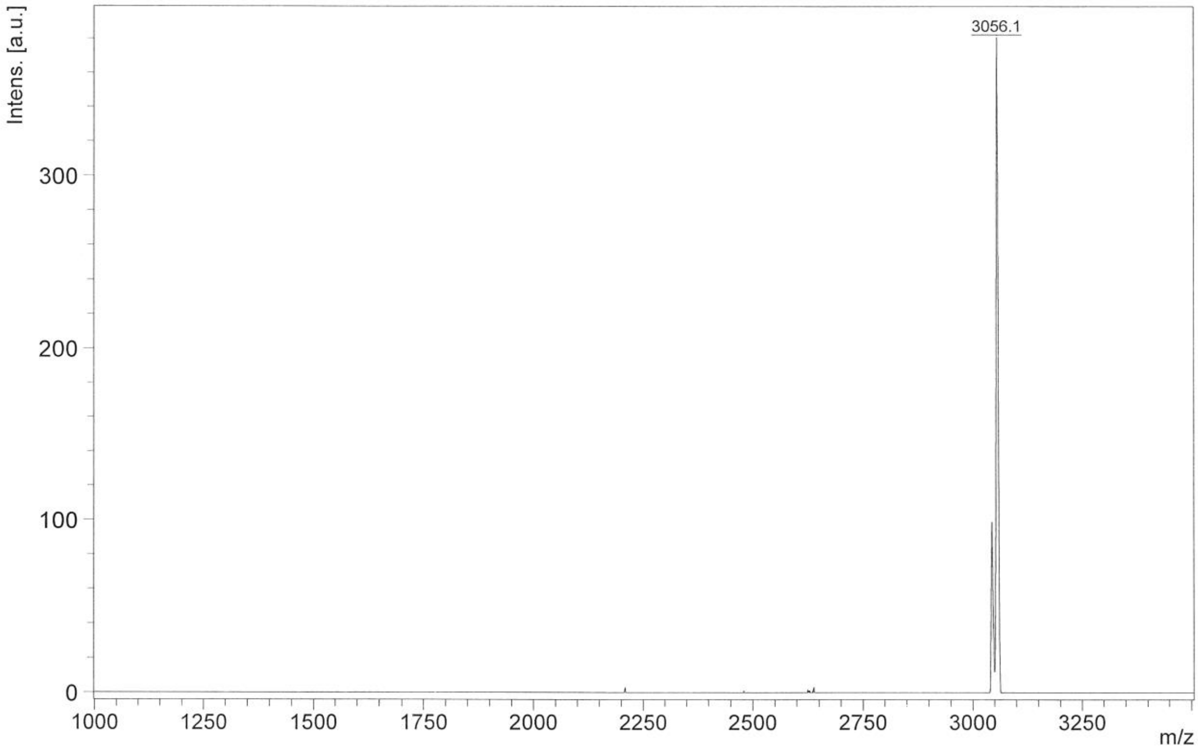


782 **(CholA-ss-u)**



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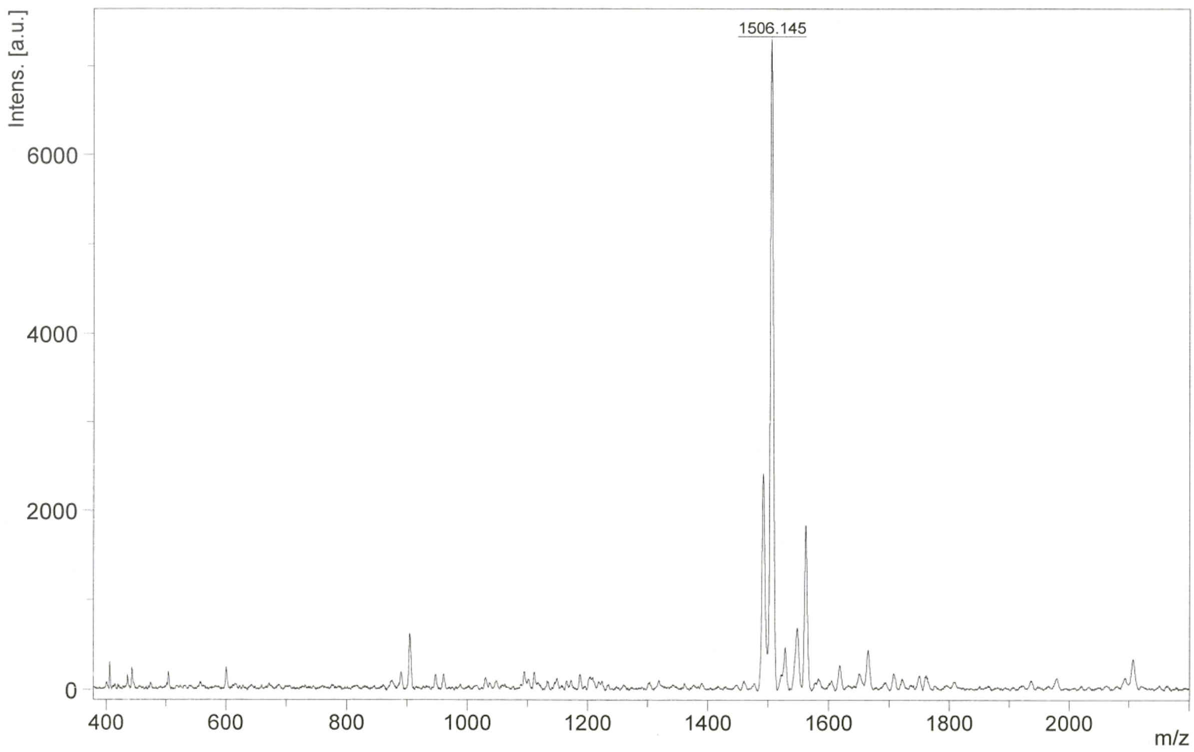
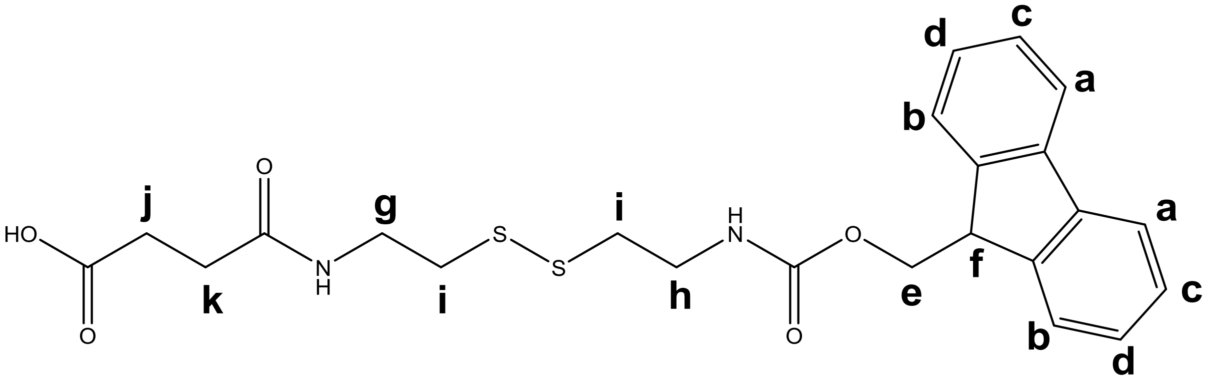
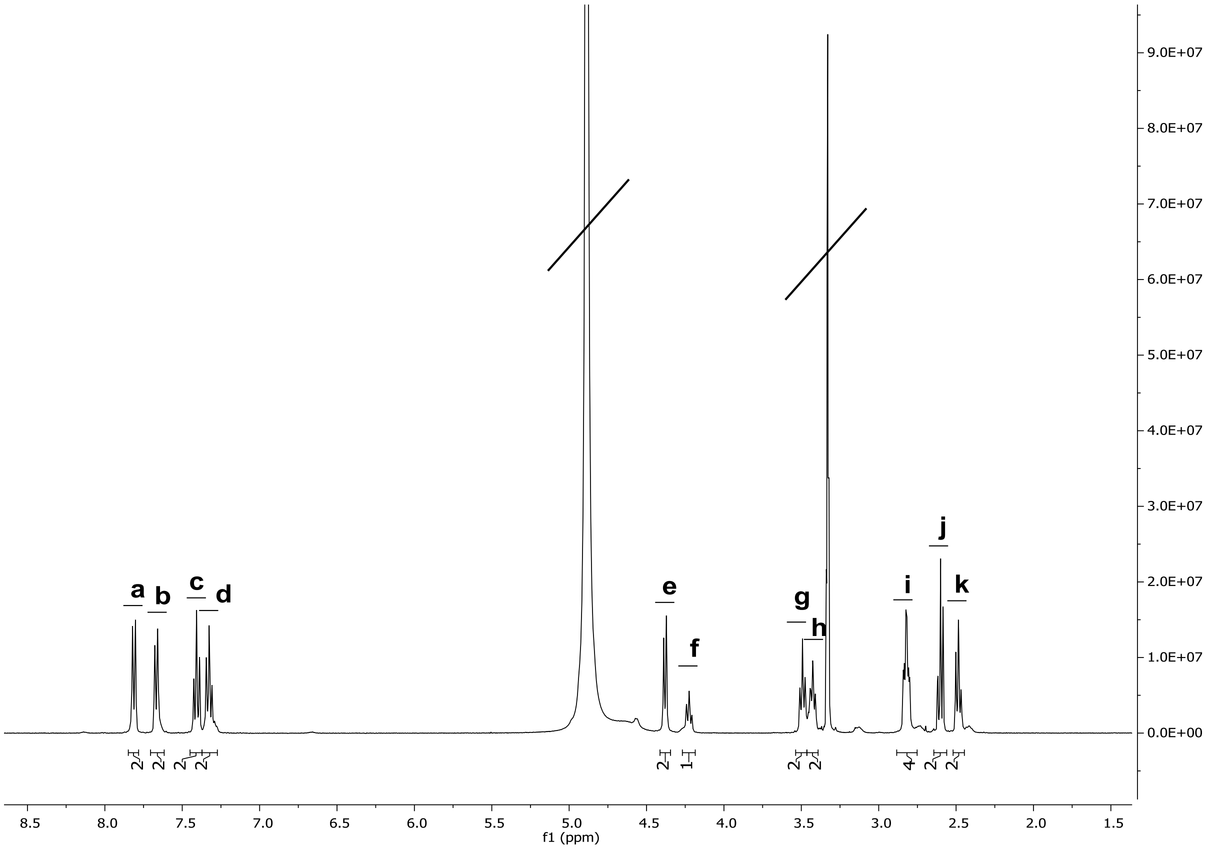


[1]H Proton NMR Spectra:

Disulfide linker (ssbb)

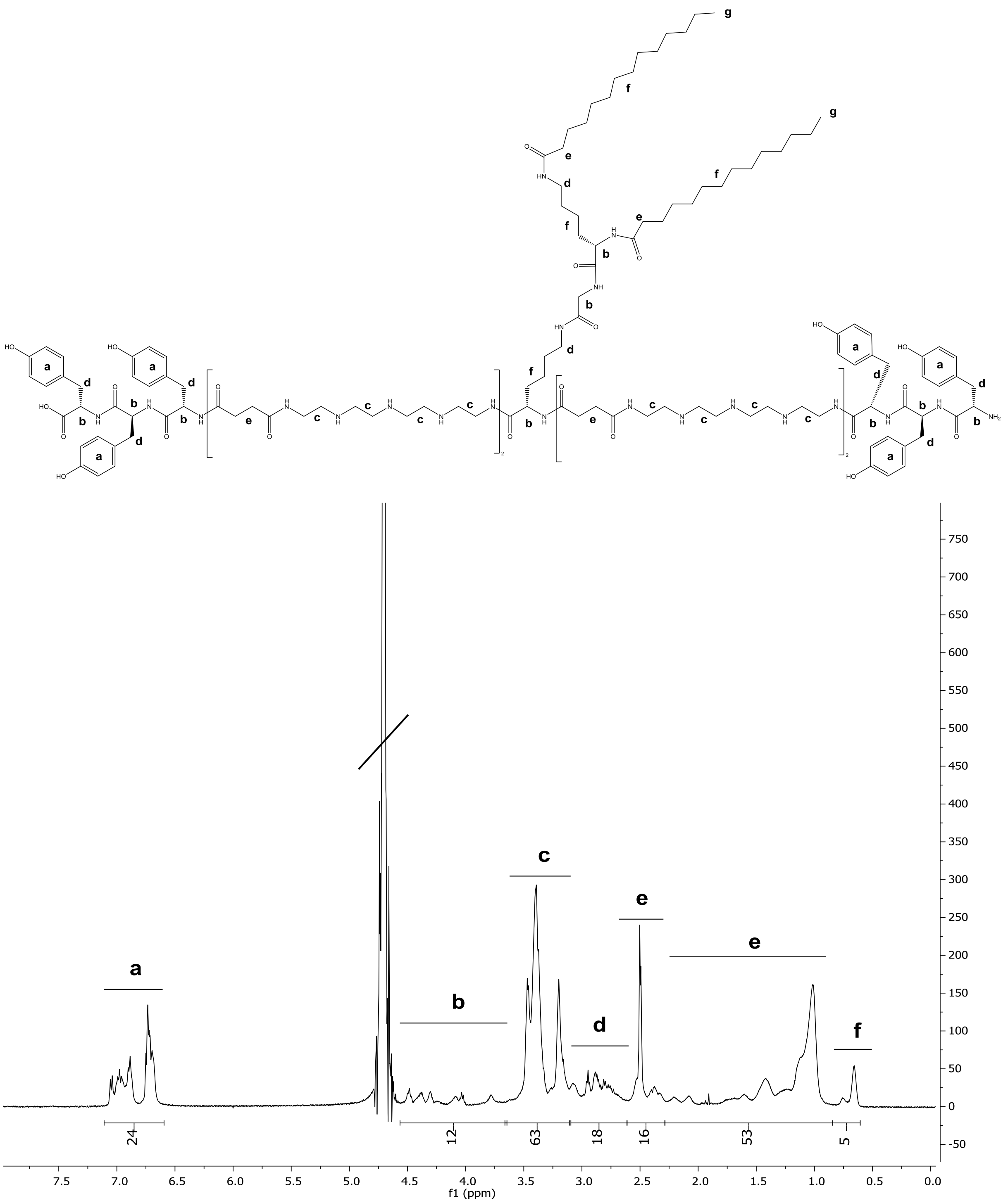




1-(9H-fluoren-9-yl)-3,12-dioxo-2-oxa-7,8-dithia-4,11-diazapentadecan-15-oic acid (ssbb) ^{1}H NMR (500 MHz, Methanol-d4) δ (ppm) 7.81 (d, J = 7.5 Hz, H*a*, 2H), 7.67 (d, J = 7.4 Hz, H*b*, 2H), 7.41 (t, J = 7.4 Hz, H*c*, 2H), 7.33 (t, J = 7.4 Hz, H*d*, 2H), 4.38 (d, J = 6.9 Hz, H*e*, 2H), 4.22 (t, J = 6.8 Hz, H*f*, 1H), 3.49 (t, J = 6.7 Hz, H*g*, 2H), 3.43 (t, J = 6.7 Hz, H*h*, 2H), 2.76-2.87 (m, H*i*, 4H), 2.60 (t, J = 6.6 Hz, H*j*, 2H), 2.48 (t, J = 6.8 Hz, H*k*, 2H).

1081**:**

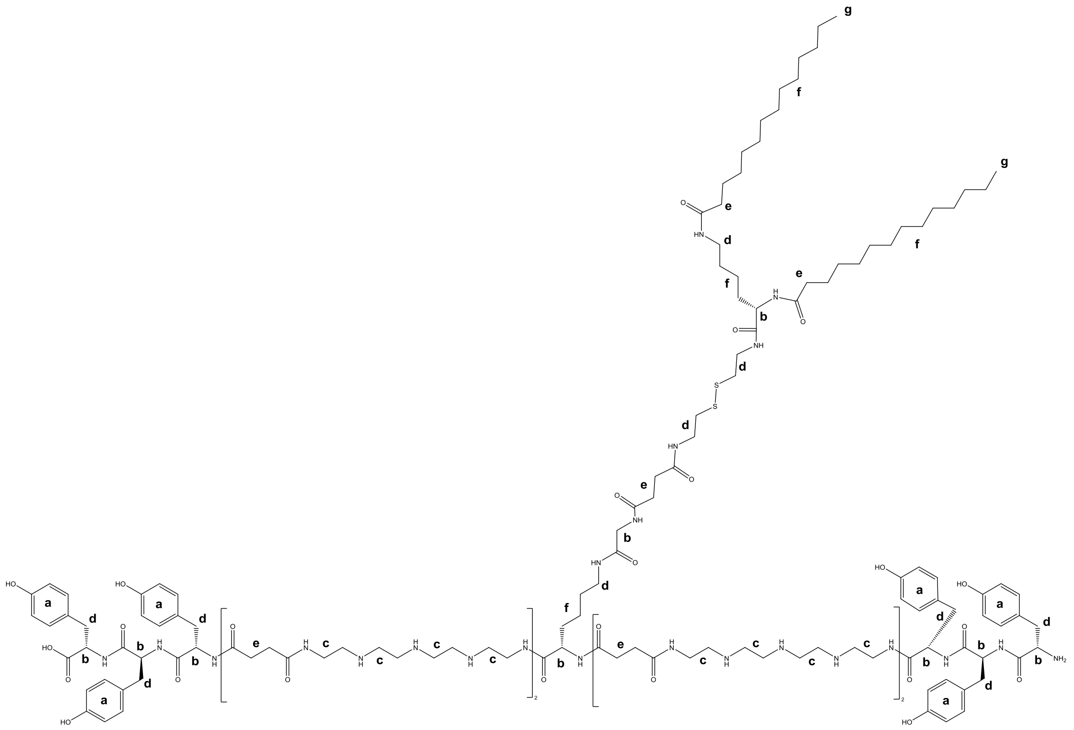
Sequence (C→N): Y_3-Stp_2-K-ε[G-K-α,ε$(MyrA)_2$]αStp_2-Y_3

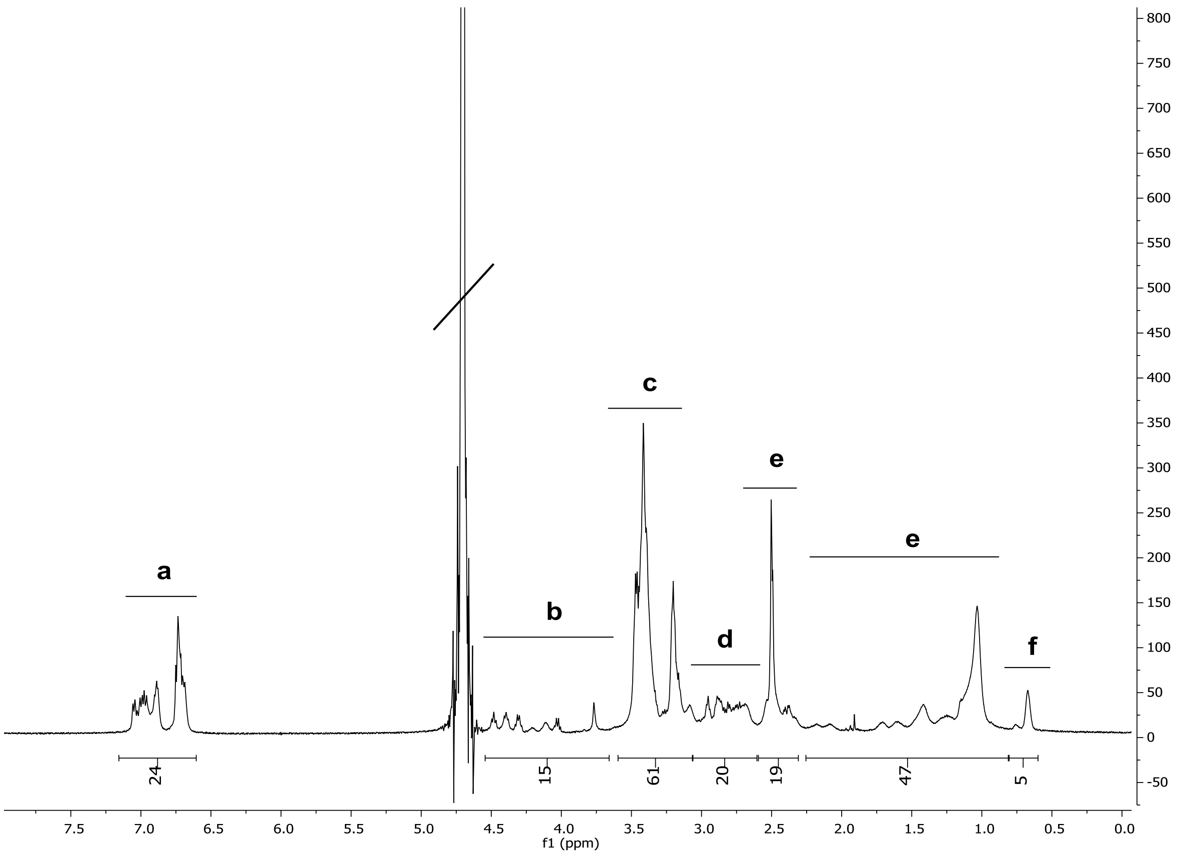


¹H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.60-0.85 (s, 6 H, -CH3 myristic acid), 0.85-2.30 (m, 56 H, βγδH lysine, myristic acid), 2.3-2.6 (m, 20 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- myristic acid), 2.6-3.10 (m, 16 H, εH lysine and tyrosine), 3.10-3.65 (m, 64 H, -CH2- Tp), 3.65-4.55 (m, 10 H, αH amino acids), 6.60-7.10 (m, 24 H, -CH- tyrosine).

***1082*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-ssbb-K-α,ε$(MyrA)_2$]αStp_2-Y_3

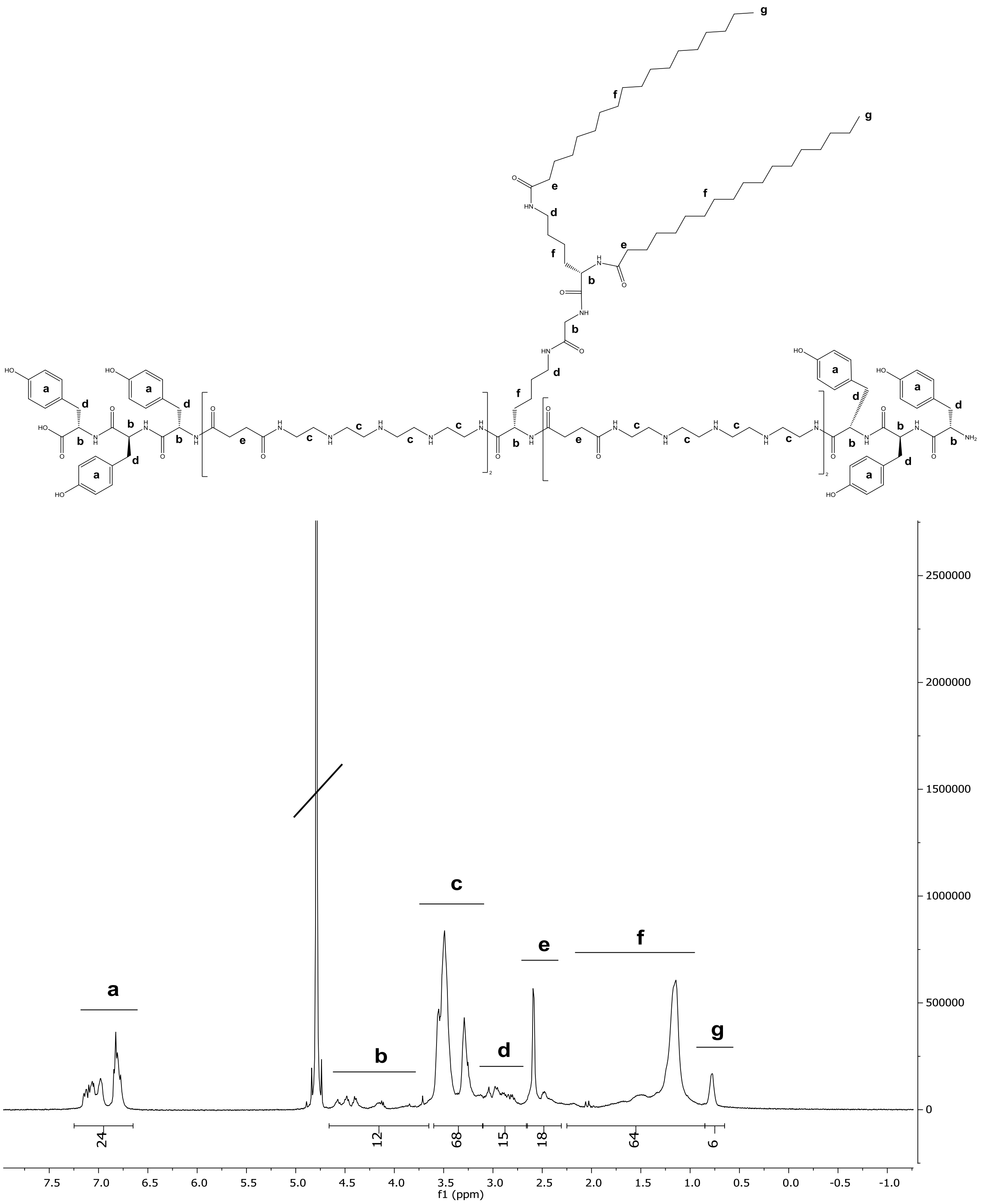




^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.60-0.80 (s, 6 H, -CH3 myristic acid), 0.80-2.25 (m, 56 H, βγδH lysine, myristic acid), 2.3-2.6 (m, 24 H, -CO-CH2-CH2-CO- Stp and ssbb, -CO-CH2- myristic acid), 2.60-3.05 (m, 24 H, εH lysine and tyrosine, -CH2- ssbb), 3.05-3.60 (m, 64 H, -CH2- Tp), 3.65-4.55 (m, 10 H, αH amino acids), 6.60-7.15 (m, 24 H, -CH- tyrosine).

***989*:**

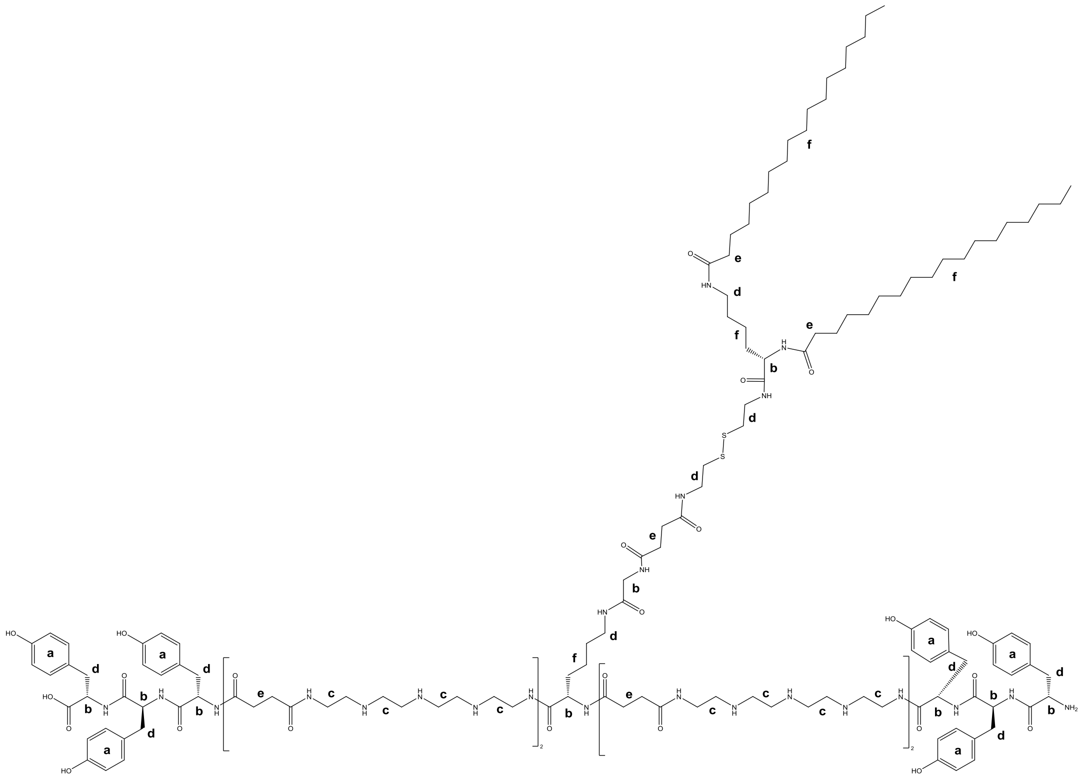
Sequence (C→N): Y_3-Stp_2-K-ε[G-K-α,ε$(SteA)_2$]αStp_2-Y_3

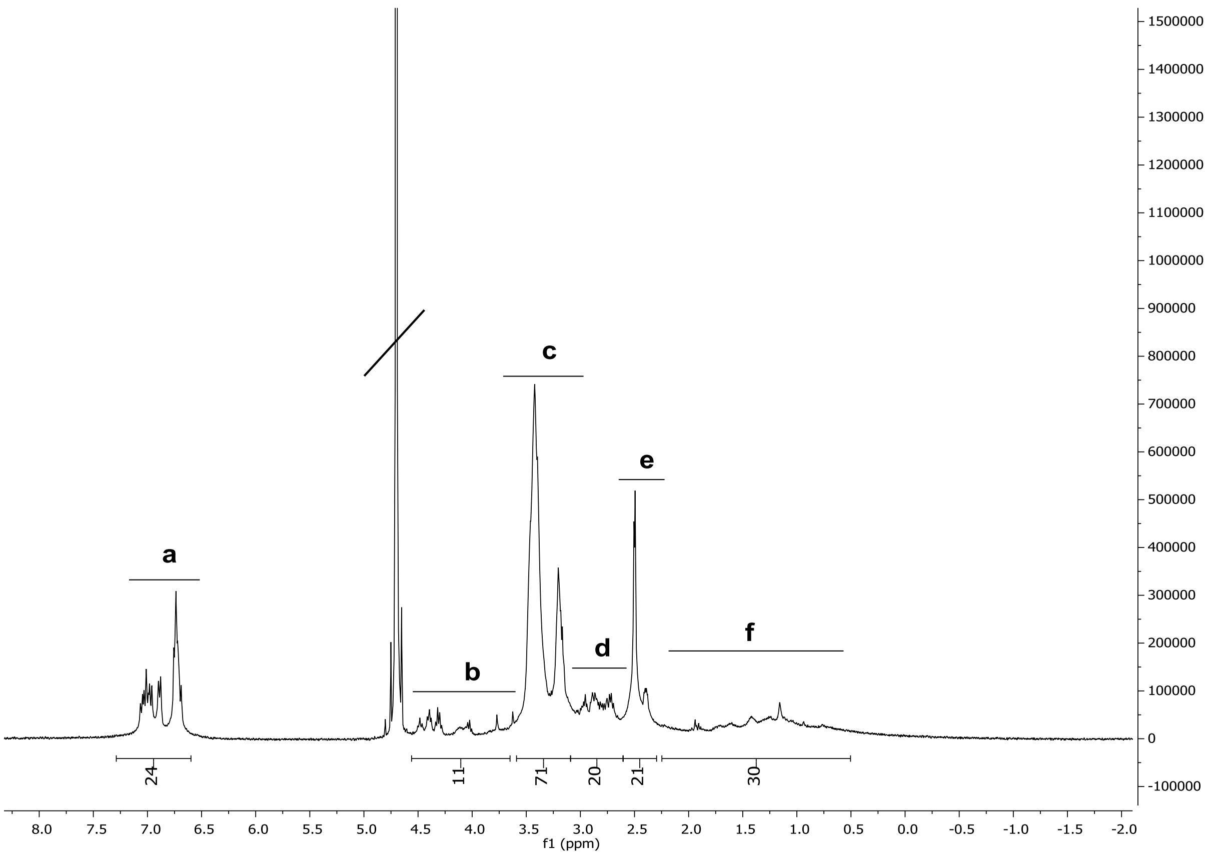


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.65-0.85 (s, 6 H, -CH3 stearic acid), 0.85-2.25 (m, 76 H, βγδH lysine, -CH2- stearic acid), 2.3-2.65 (m, 20 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- stearic acid), 2.65-3.1 (m, 16 H, εH lysine and tyrosine), 3.1-3.6 (m, 64 H, -CH2- Tp), 3.65-4.65 (m, 10 H, αH amino acids), 6.65-7.25 (m, 24 H, -CH- tyrosine).

***990*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-ssbb-K-α,ε$(SteA)_2$]αStp_2-Y_3

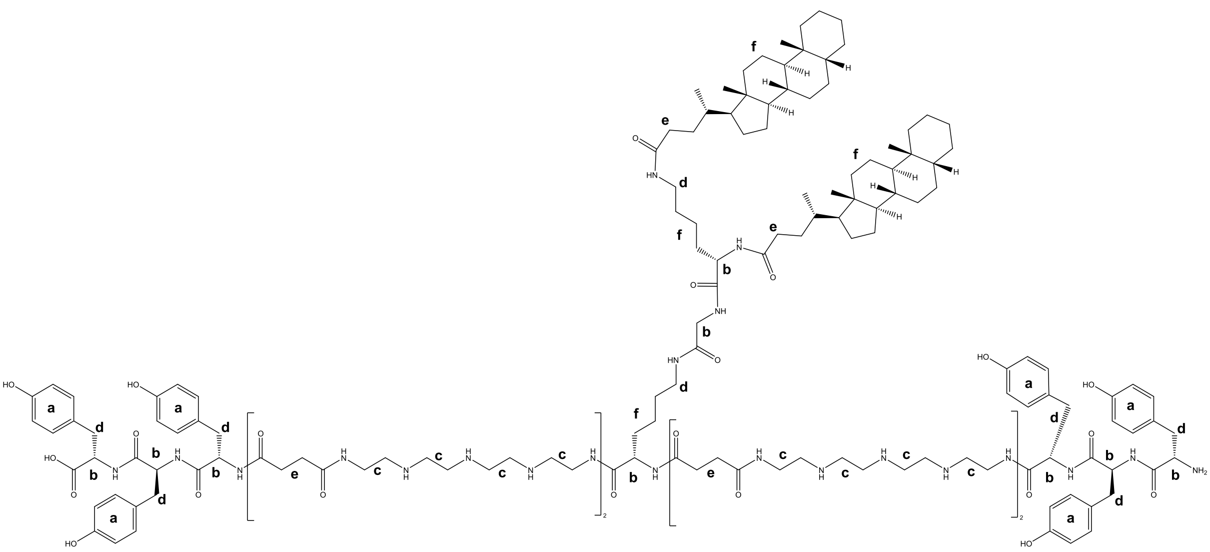


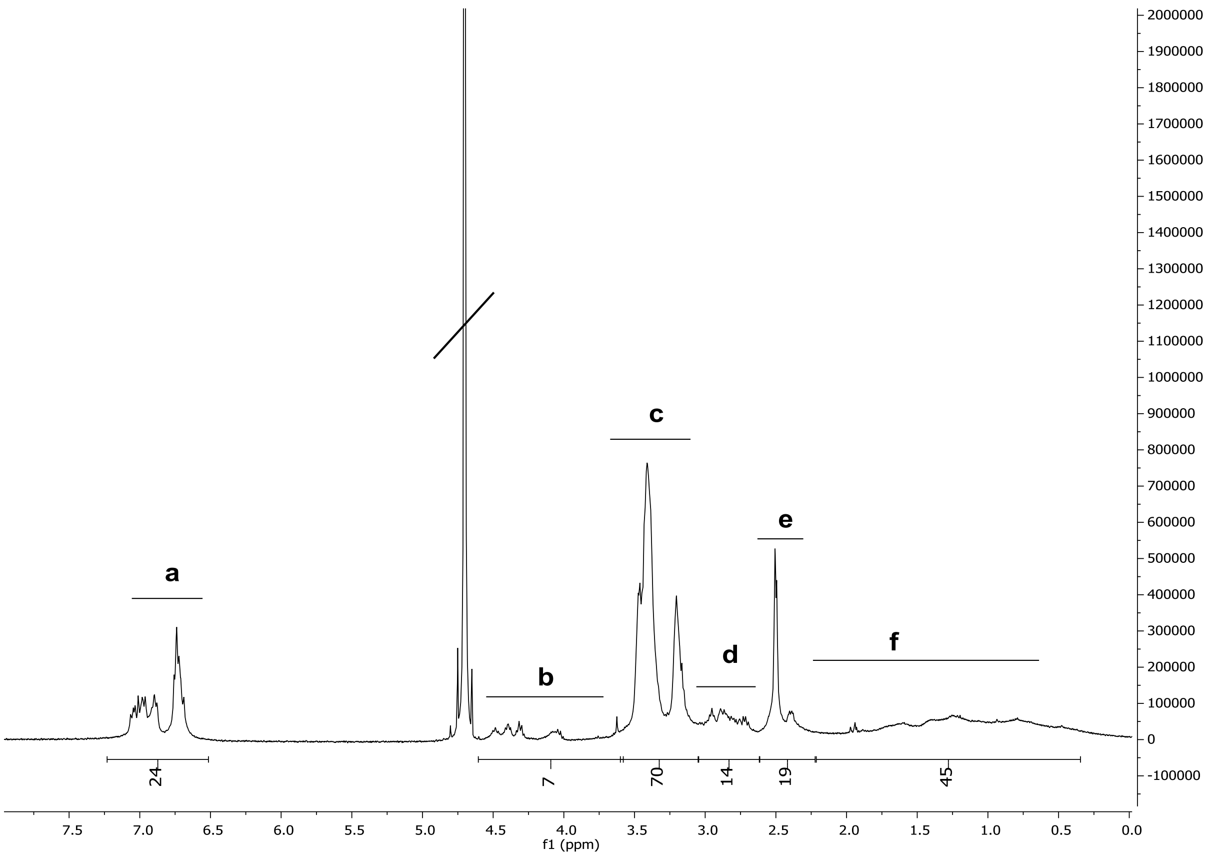


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.50-2.25 (m, 82 H, βγδH lysine, -CH2- and -CH3 stearic acid), 2.3-2.6 (m, 24 H, -CO-CH2-CH2-CO- Stp and ssbb, -CO-CH2- stearic acid), 2.6-3.1 (m, 24 H, εH lysine and tyrosine, -CH2- ssbb), 3.1-3.6 (m, 64 H, -CH2- Tp), 3.65-4.55 (m, 10 H, αH amino acids), 6.6-7.3 (m, 24 H, -CH- tyrosine).

***991*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-K-α,ε$(CholA)_2$]αStp_2-Y_3

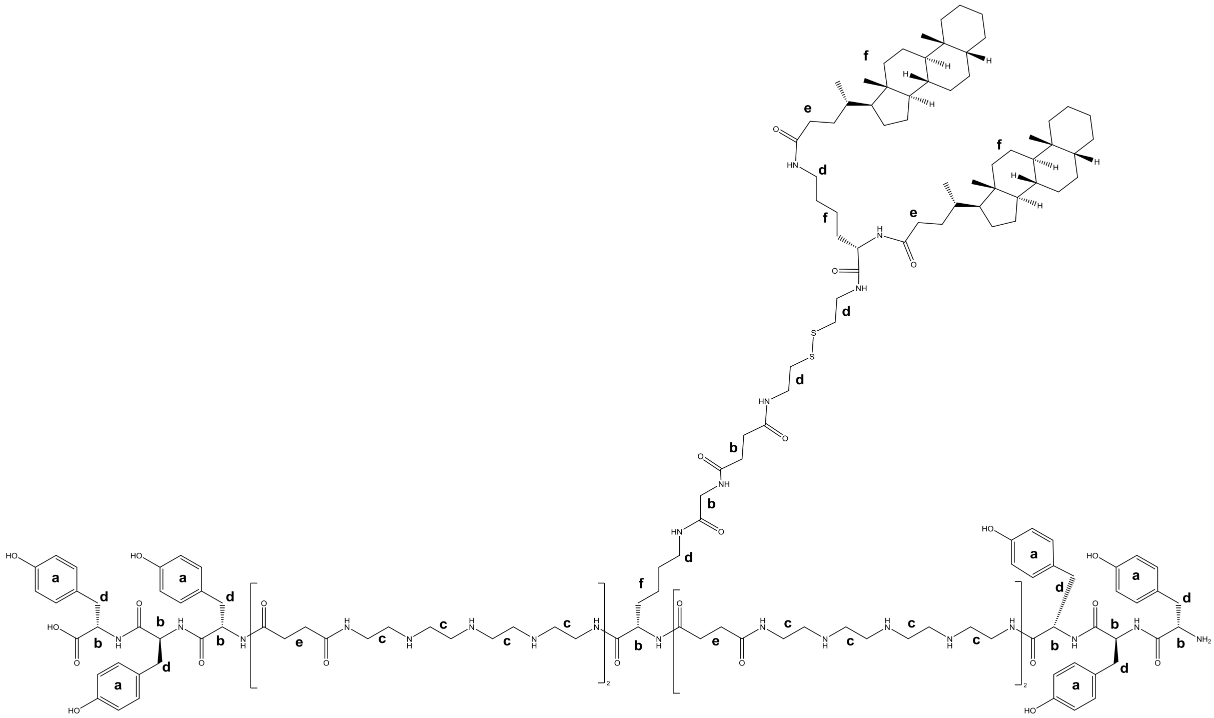


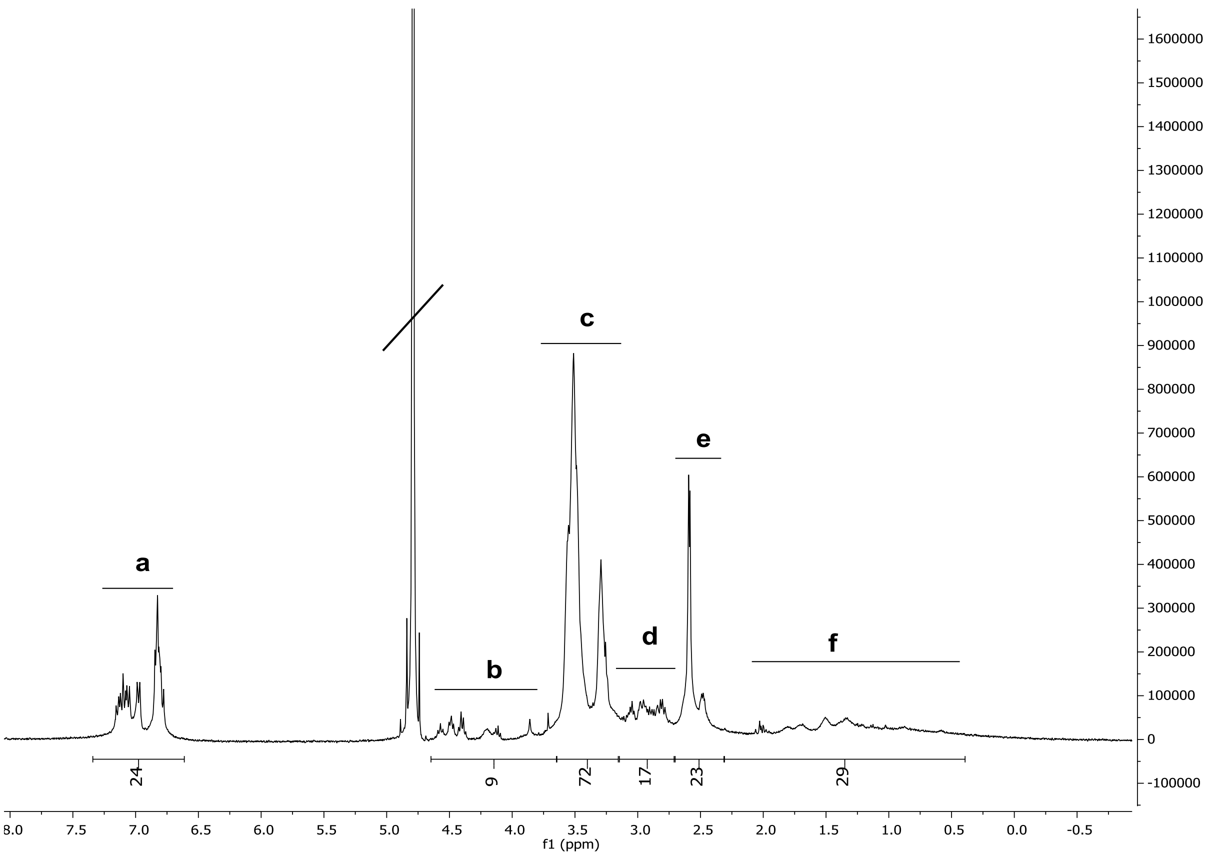


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.35-2.20 (m, 88 H, βγδH lysine, cholanic acid), 2.2-2.6 (m, 20 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- cholanic acid), 2.6-3.05 (m, 16 H, εH lysine and tyrosine), 3.05-3.60 (m, 64 H, -CH2- Tp), 3.60-4.60 (m, 10 H, αH amino acids), 6.50-7.25 (m, 24 H, -CH- tyrosine).

***992*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-ssbb-K-α,ε$(CholA)_2$]αStp_2-Y_3

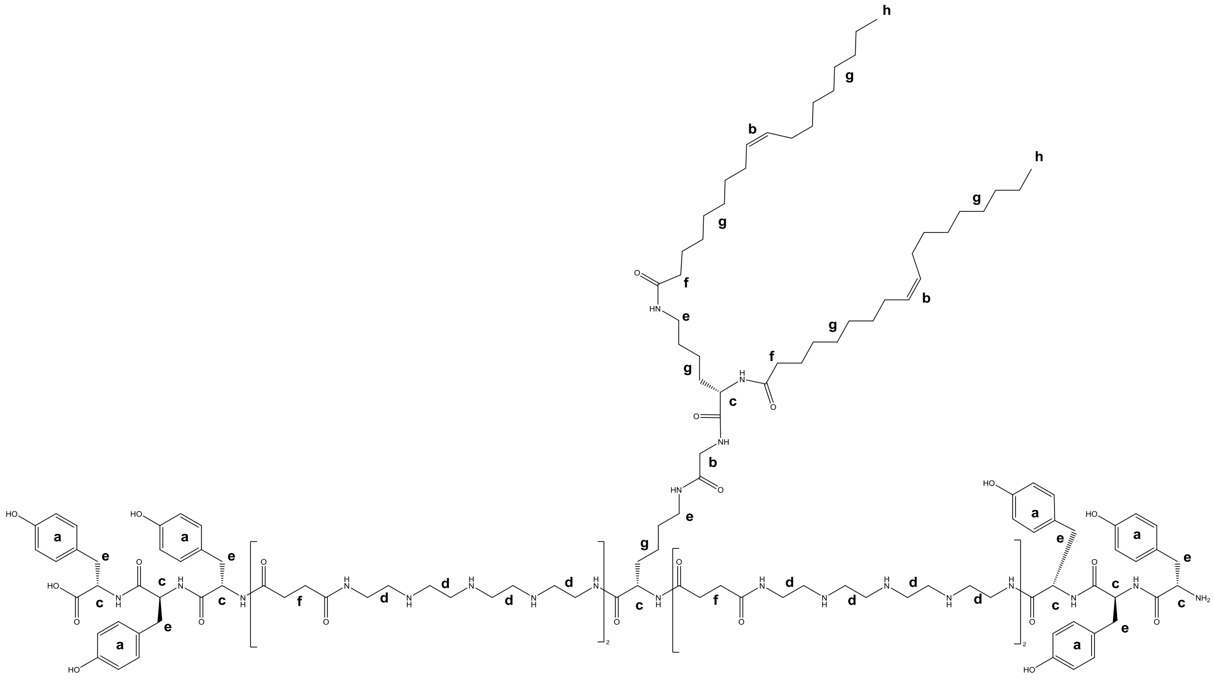


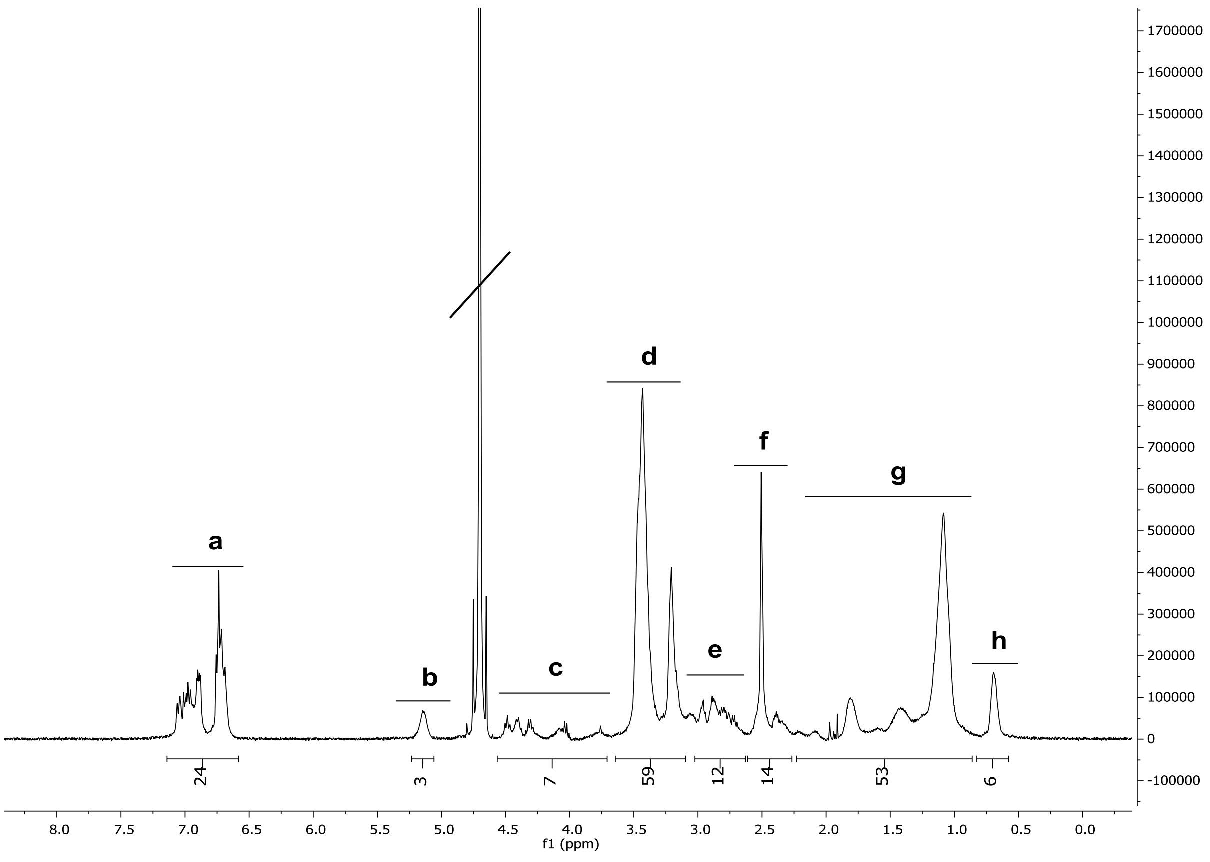


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.40-2.30 (m, 88 H, βγδH lysine, cholanic acid), 2.3-2.7 (m, 24 H, -CO-CH2-CH2-CO- Stp and ssbb, -CO-CH2- cholanic acid), 2.70-3.15 (m, 24 H, εH lysine and tyrosine, -CH2-ssbb), 3.15-3.80 (m, 64 H, -CH2- Tp), 3.65-4.65 (m, 10 H, αH amino acids), 6.60-7.35 (m, 24 H, -CH- tyrosine).

***1107*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-K-α,ε$(OleA)_2$]αStp_2-Y_3

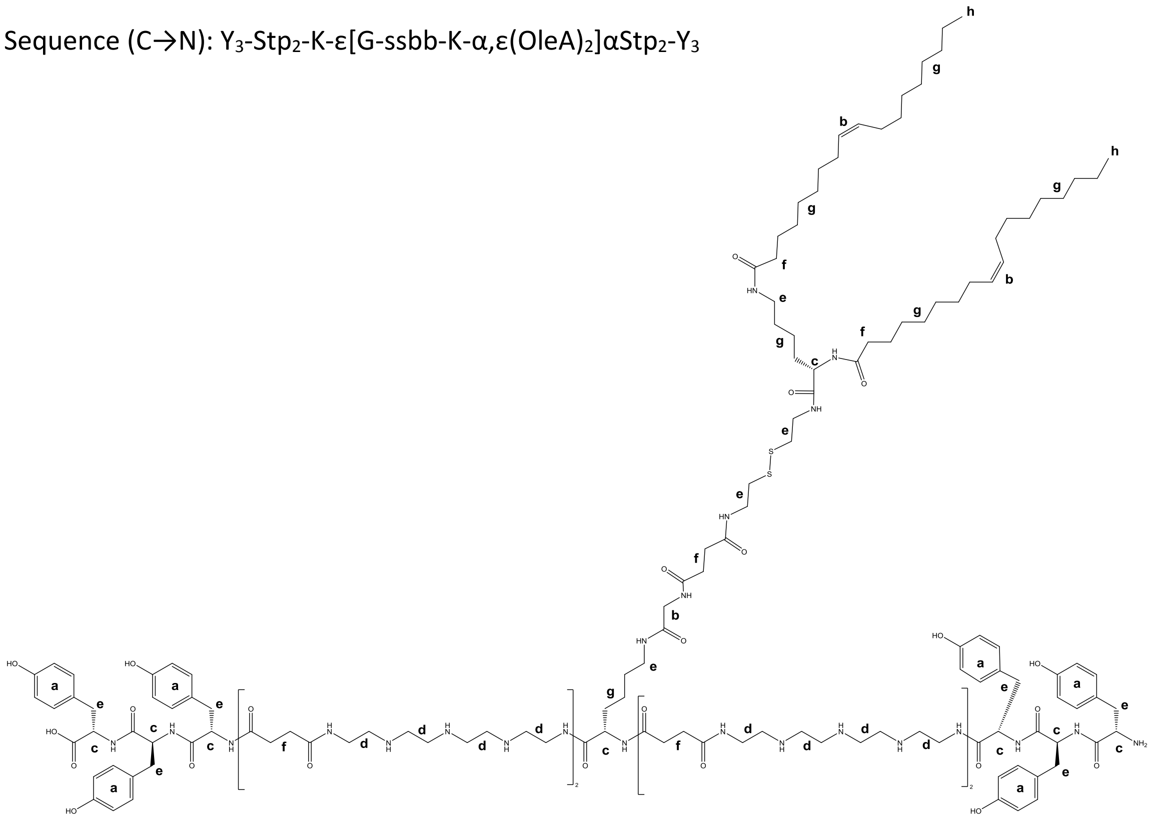


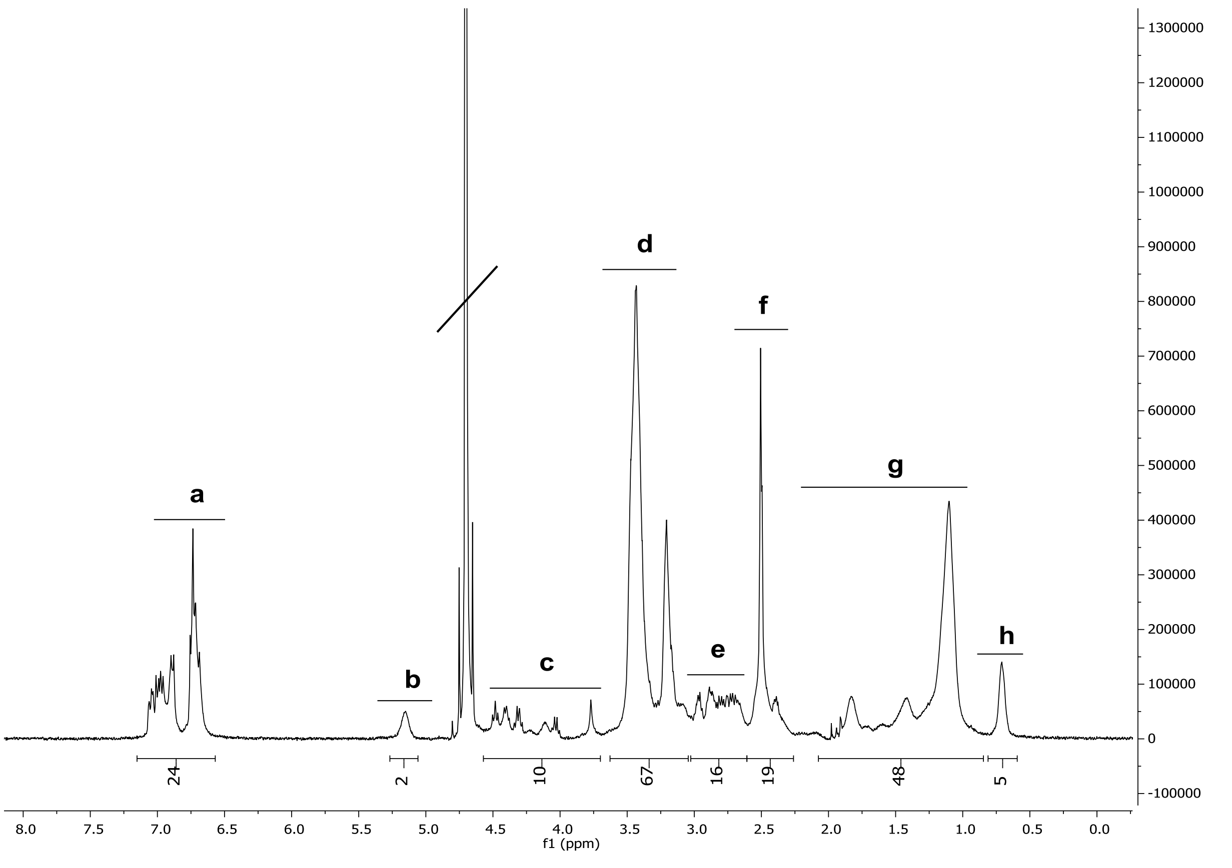


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.60-0.85 (s, 6 H, -CH3 oleic acid), 0.85-2.25 (m, 72 H, βγδH lysine, -CH2- oleic acid), 2.25-2.60 (m, 20 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- oleic acid), 2.65-3.1 (m, 16 H, εH lysine and tyrosine), 3.1-3.65 (m, 64 H, -CH2- Tp), 3.70-4.55 (m, 10 H, αH amino acids), 5.05 – 5.25 (s, 4 H, -CH=CH- oleic acid), 6.60 -7.15 (m, 24 H, -CH- tyrosine).

***1108*:**

Sequence (C→N): Y_3-Stp_2-K-ε[G-ssbb-K-α,ε$(OleA)_2$]αStp_2-Y_3

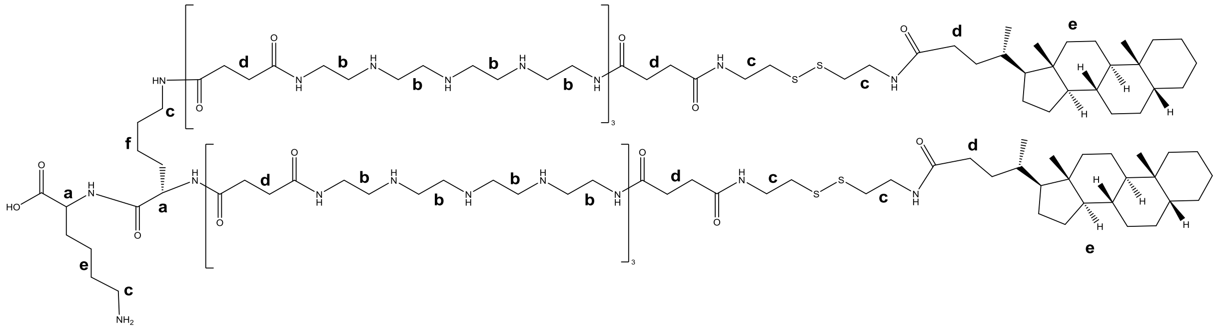


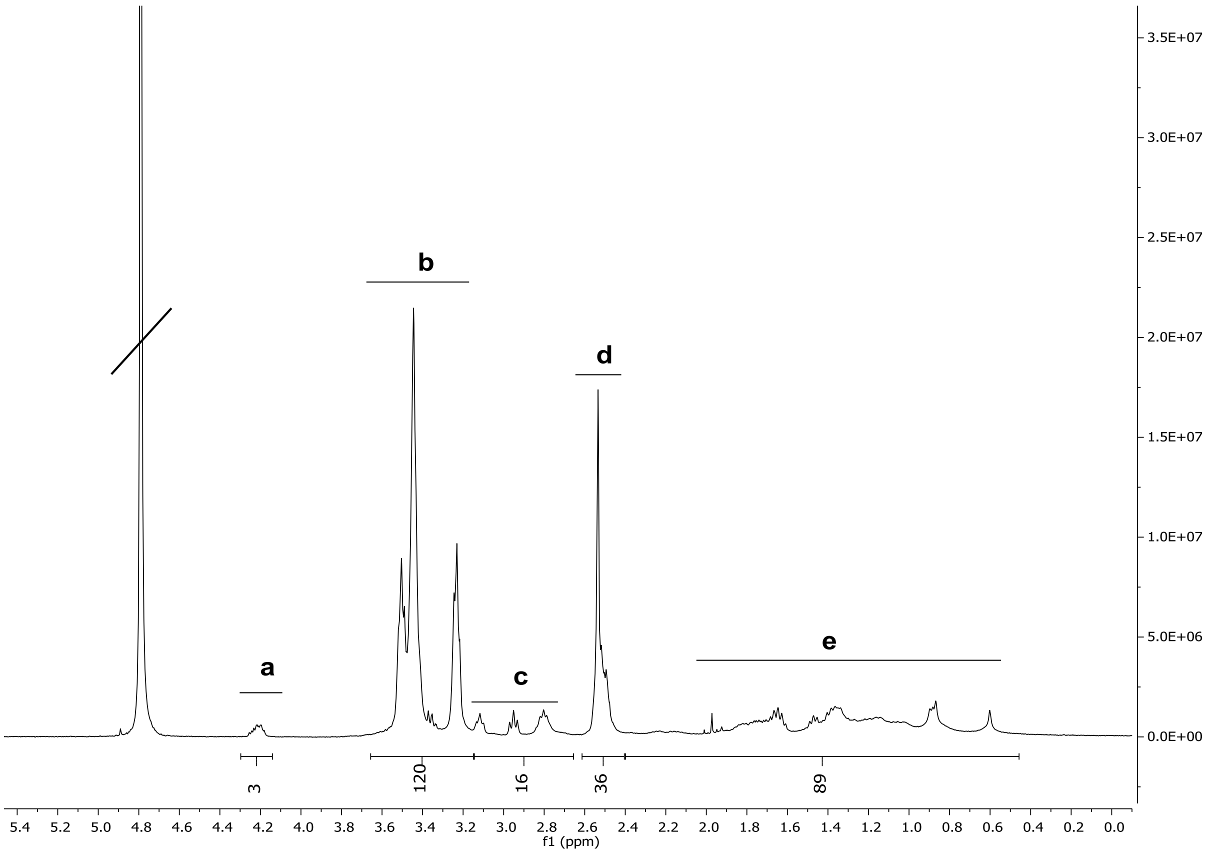


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.60-0.80 (s, 6 H, -CH3 oleic acid), 0.85-2.10 (m, 72 H, βγδH lysine, -CH2- oleic acid), 2.25-2.60 (m, 22 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- oleic acid), 2.60-3.0 (m, 22 H, εH lysine and tyrosine), 3.05-3.65 (m, 64 H, -CH2- Tp), 3.70-4.60 (m, 10 H, αH amino acids), 5.00 – 5.25 (s, 4 H, -CH=CH- oleic acid), 6.55 -7.15 (m, 24 H, -CH- tyrosine).

***782*:**

Sequence (C→N): K-αK-α,ε[Stp3-ssbb-(CholA)$_2$]$_2$

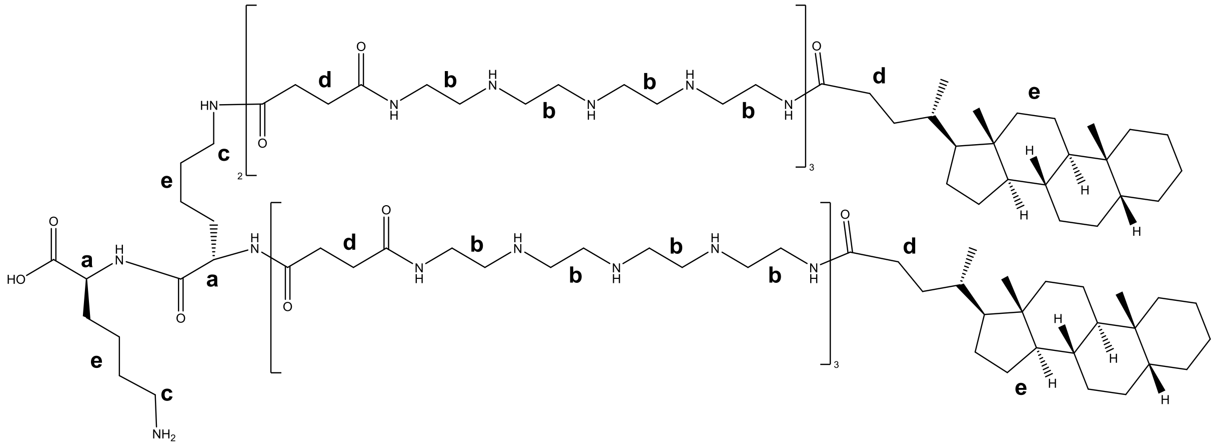


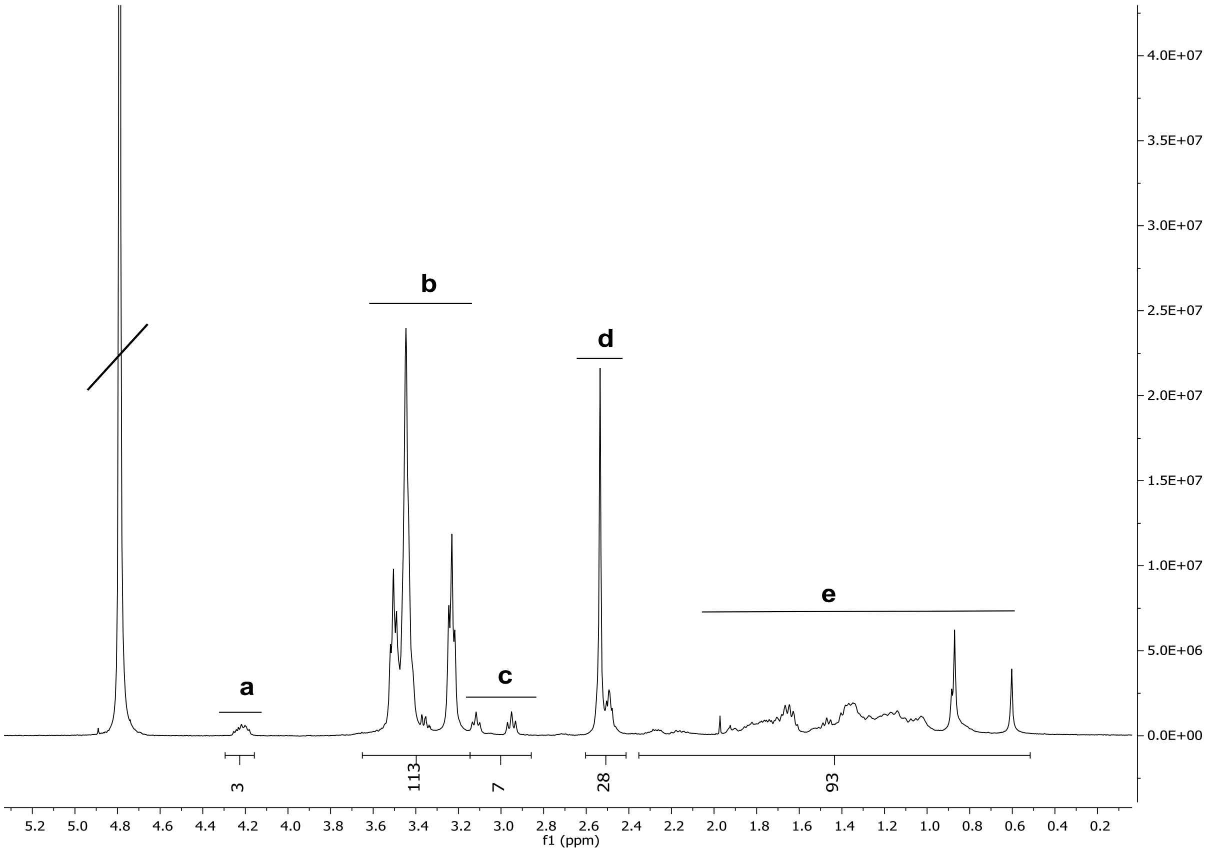


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.45-2.40 m, 88 H, βγδH lysine, cholanic acid), 2.40-2.60 (m, 36 H, -CO-CH2-CH2-CO- Stp and ssbb, -CO-CH2- cholanic acid), 2.65-3.15 (m, 12 H, εH lysine, -CH2- ssbb), 3.15-3.65 (m, 96 H, -CH2- Tp), 4.15-4.30 (m, 2 H, αH lysines).

***783*:**

Sequence (C→N): K-αK-α,ε[Stp3-(CholA)₂]₂

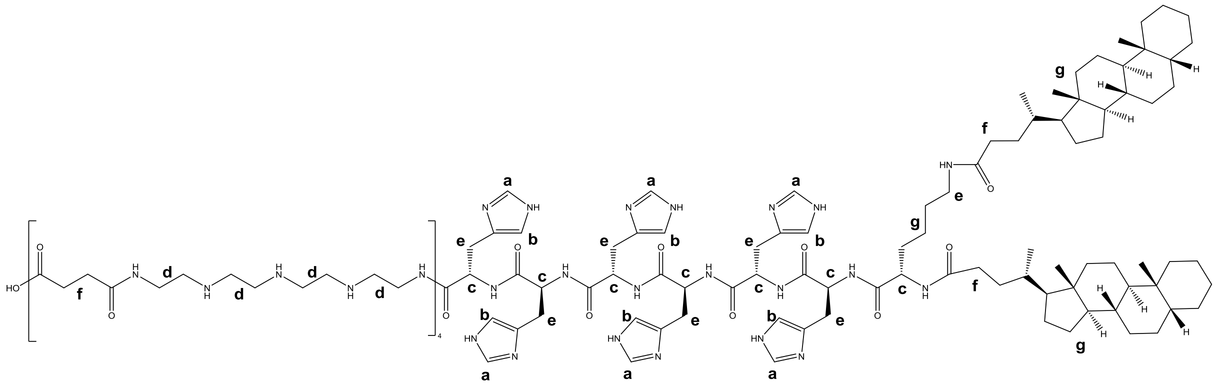


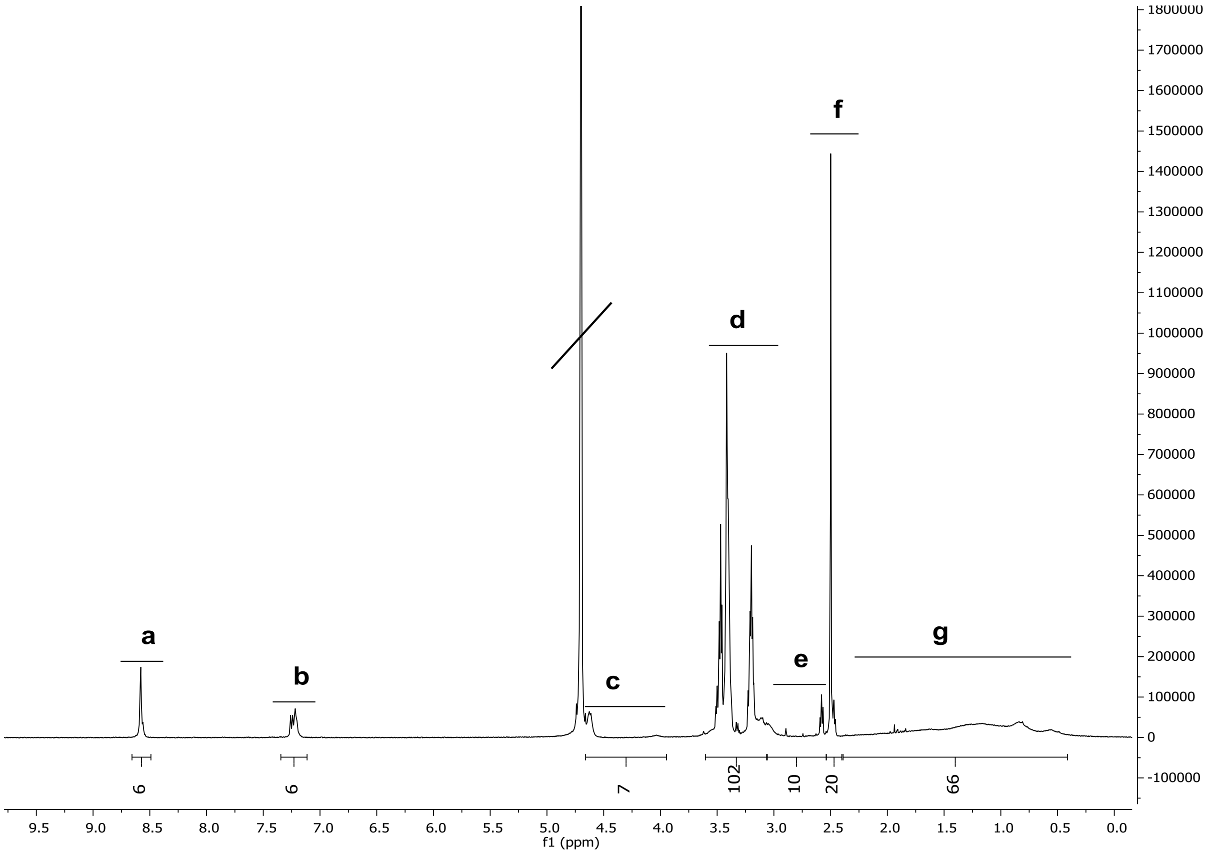


^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.50-2.35 m, 88 H, βγδH lysine, cholanic acid), 2.40-2.60 (m, 28 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- cholanic acid), 2.85-3.15 (m, 4 H, εH lysine), 3.15-3.65 (m, 96 H, -CH2-Tp), 4.15-4.30 (m, 2 H, αH lysines).

***871*:**

Sequence (C→N): Stp_4-H_6-K-α,ε$(CholA)_2$

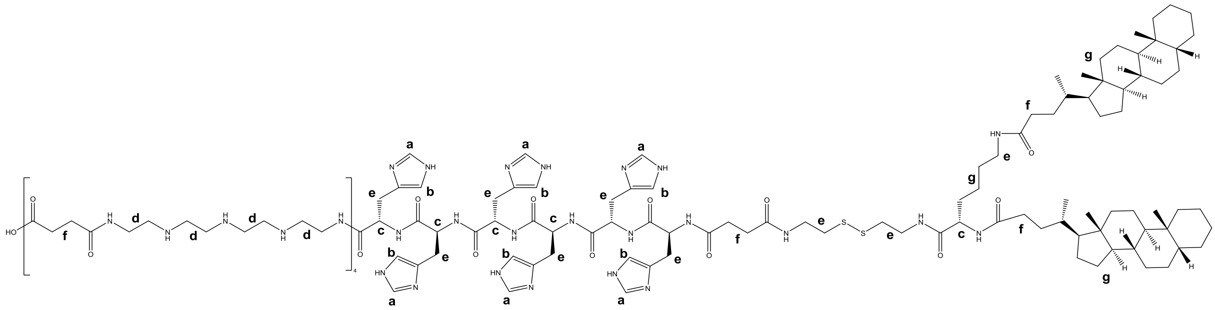


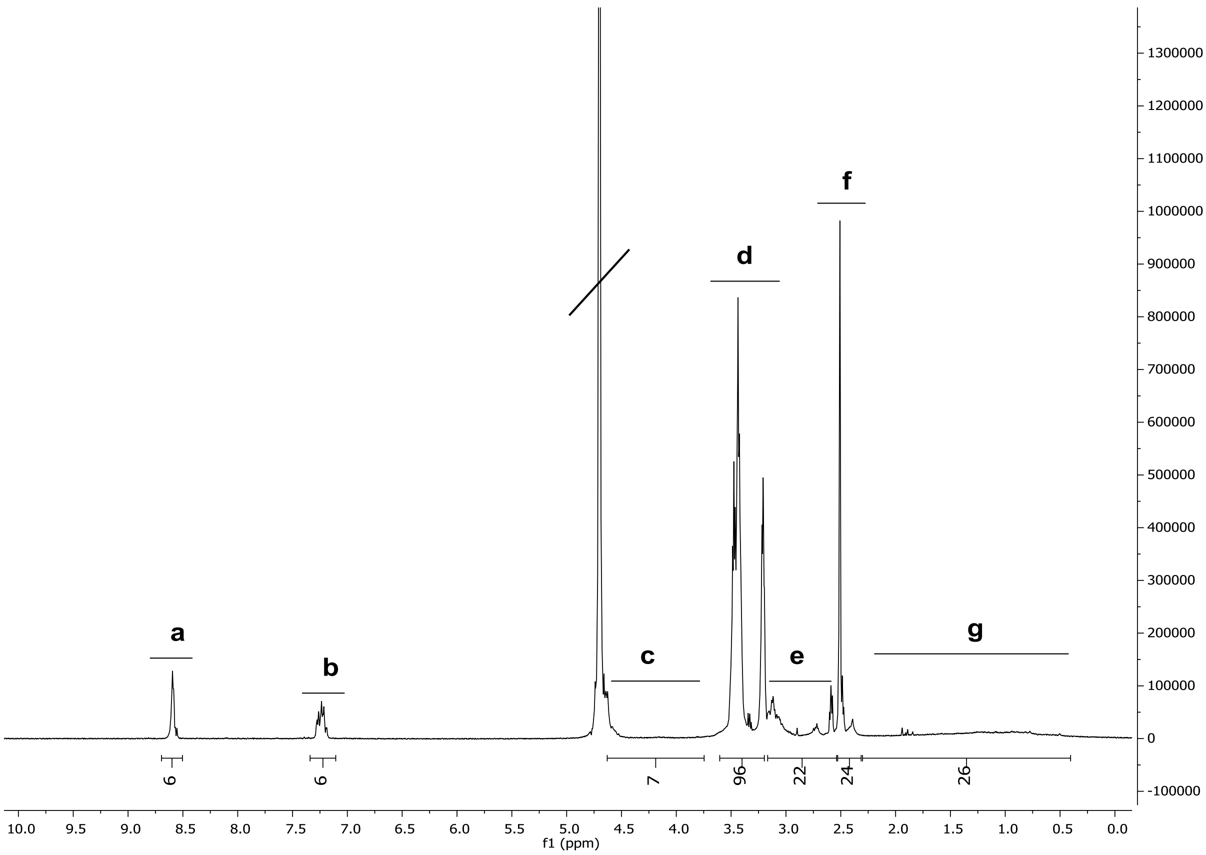


¹H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.40-2.30 m, 82 H, βγδH lysine, cholanic acid), 2.40-2.55 (m, 20 H, -CO-CH2-CH2-CO- Stp, -CO-CH2- cholanic acid), 2.55-3.05 (m, 14 H, εH lysine and histidine), 3.05-3.60 (m, 64 H, -CH2- Tp), 3.95-4.65 (m, 7 H, αH lysines and histidines), 7.10-7.35 (d, 6 H, aromatic H histidine), 8.5-8.65 (m, 6 H, aromatic H histidine).

969:

Sequence (C→N): Stp_4-H_6-ssbb-K-α,ε$(CholA)_2$

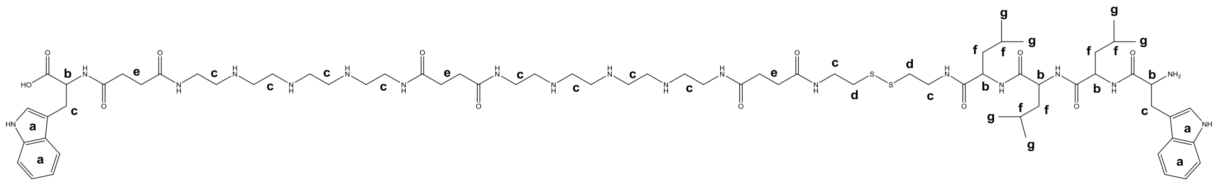


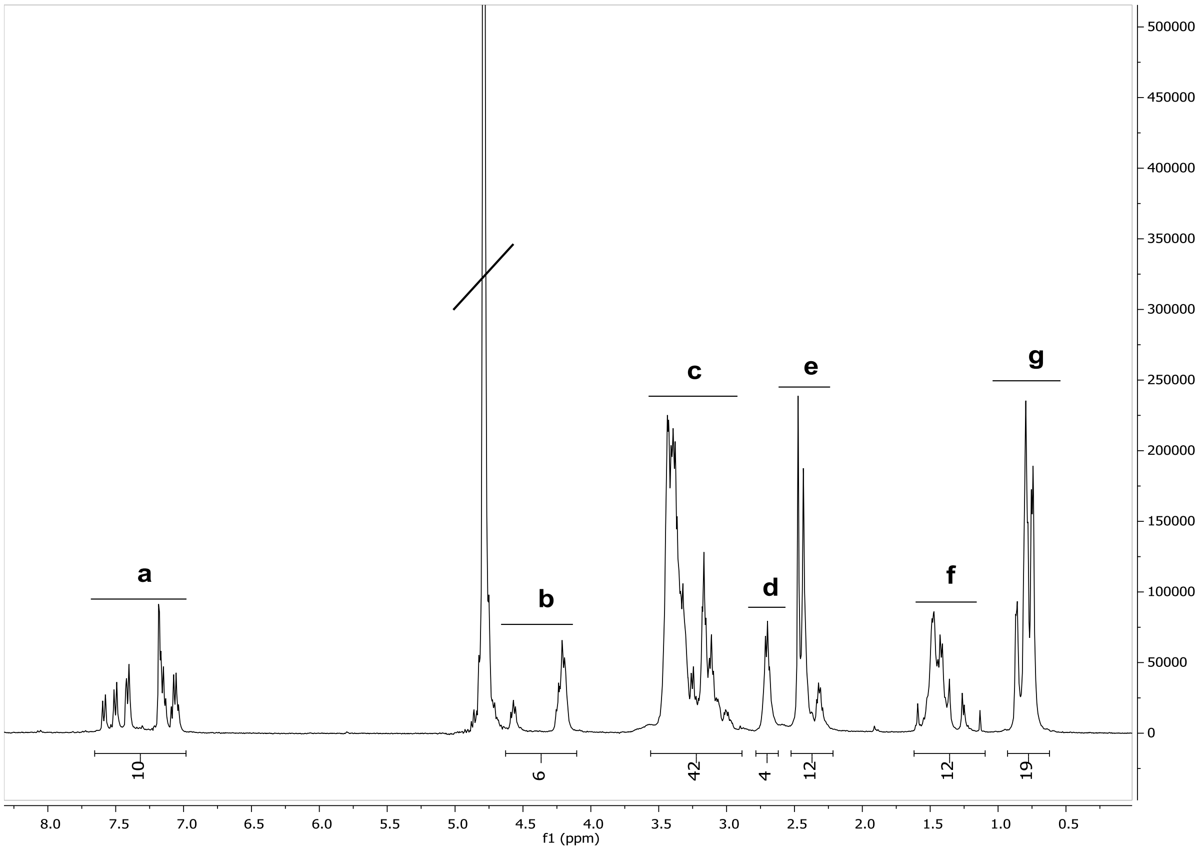


[1]H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.40-2.30 m, 82 H, βγδH lysine, cholanic acid), 2.30-2.55 (m, 24 H, -CO-CH2-CH2-CO- Stp and ssbb, -CO-CH2- cholanic acid), 2.55-3.15 (m, 22 H, εH lysine and histidine, -CH2-ssbb), 3.15-3.65 (m, 64 H, -CH2- Tp), 3.75-4.65 (m, 7 H, αH lysines and histidines), 7.10-7.35 (d, 6 H, aromatic H histidine), 8.50-8.70 (m, 6 H, aromatic H histidine).

740 (test structure):

Sequence (C→N): W-Stp$_2$-ssbb-L$_3$-W





^{1}H NMR (500 MHz, Deuterium oxide) δ (ppm) = 0.60-0.95 (m, 18 H, δH leucine), 1.10-1.60 (m, 12 H, βγH leucine), 2.20-2.50 (m, 12 H, -CO-CH2-CH2-CO- Stp and ssbb), 2.60-2.80 (m, 4 H, -CH2-SS-CH2-), 2.90-3.55 (m, 40 H, -CH2- Tp and ssbb, εH tryptophane), 4.10-4.60 (m, 5 H, αH tryptophanes and leucines), 7.00-7.65 (m, 10 H, aromatic H tryptophane).