Supporting Information for:

Stereorandomization as a Method to Probe Peptide Bioactivity

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Materials and Reagents

Amino acids and their derivatives were either purchased from Advanced ChemTech, Novabiochem, Iris Biotech GMBH, Sigma Aldrich, PolyPeptide, Space peptides and GL BioChem. Amino acids were used as the following derivatives Fmoc-Arg(Pbf)-OH, Fmoc-D-Arg(Pbf)-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-Pro-OH, Fmoc-D-Pro-OH, Fmoc-Fmoc-Val-OH, Fmoc-D-Val-OH, Fmoc-Trp(Boc)-OH, Fmoc-D-Trp(Boc)-OH, Fmoc-Ile-OH, Fmoc-D-Ile-OH, Fmoc-Gln(Trt)-OH, Fmoc-D-Gln(Trt)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-D-Tyr(tBu)-OH, Fmoc-Ala-OH and Fmoc-D-Ala-OH. Rink Amide AM resin (loading: 0.38 $mmol \cdot g^{-1}$) and 2-Chlorotrityl chloride resin (loading: 1.2 $mmol \cdot g^{-1}$) were purchased from Sigma Aldrich. TentaGel S RAM (loading: 0.24 $mmol \cdot g^{-1}$) resin was purchased from Rapp Polymere. OxymaPure (hydroxyiminocyanoacetic acid ethyl ester) and DIC (*N*,*N*'-diisopropyl carbodiimide) were purchased from Iris Biotech GMBH. 5(6)-carboxyfluorescein (CF) was from Sigma. Egg phosphatidylcholine (EYPC), egg phosphatidylglycerol (EYPG) and a Mini-Extruder used for vesicle preparation were from Avanti Polar Lipids. Peptide dendrimer synthesis was performed manually in polypropylene syringes fitted with a polyethylene frit, a Teflon stopcock and stopper. 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). Analytical RP-HPLC was using HPLC-grade acetonitrile and mQ (Milli-Q) deionized water. The elution solutions were: A mQ deionized water containing 0.05% TFA; D mQ 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 Tuneable Absorbance Detector. Preparative RP-HPLC was using HPLC-grade acetonitrile and mQ deionized water. The elution solutions were: A mQ deionized water containing 0.1% TFA; D mQ deionized water/acetonitrile (10:90, v/v) containing 0.1% TFA. MS spectra were provided by Mass Spectrometry, Protein Analysis and NMR services respectively of the Department of Chemistry and Biochemistry at the University of Berne.

Peptide Synthesis

Linear peptides and peptide dendrimers were synthesized by using both automated and manual synthesis. Automated microwave synthesis was performed with Biotage peptide synthesizer under SPPS conditions at 0.25 mmol scale using TentaGel resin (loading: 0.24 mmol·g^{-1}) and Rink Amide AM (loading: 0.38 mmol·g^{-1}) resin.[1] Deprotection was performed with 20% (v/v) piperidine in DMF, 2 minutes at room temperature and 5 minutes at 50 °C. Unprotected amino groups of the resin or amino acids acylated with 3 mL of 0.2 M amino acid solution (in DMF), 3 mL of 1.0 M DIC (in DMF) and 2 mL of 1.0 M Oxyma Pure (in DMF), for 10 minutes at 50 °C. Synthesis was programmed, one deprotection and two coupling (G0), one deprotection and two coupling (G1), one deprotection and four coupling (G2), two deprotection and eight coupling (G3). For Stereo randomized peptides during the amino acid preparation each L and D amino acids were mixed according to corresponding chirality ratio and dissolved in DMF.

Some Peptide dendrimers were synthesized manually with resin (0.24 mmol/g), first it was swelled in DCM and the Fmoc-protecting groups of the resin were removed with a solution of 20% piperidine in DMF (2 × 10 min.). Unprotected amino groups of the resin or amino acids acylated with amino acids (5 eq./amine), OxymaPure (7.5 eq./amine) and DIC (10 eq./amine) in DMF. Amino acids, derivatives or diamino acids were coupled for 1 h (G0), two times 1 h (G1), two times 2 h (G2) and three times 4 h (G3). After each coupling, the resin was deprotected with 20% piperidine in DMF (2 × 10 min).

Final deprotection was done in (20% piperidine in DMF, 2 × 10 min) by manually after the synthesis. The resin was washed twice with MeOH and dried under vacuum before the cleavage was carried out using TFA/TIS/H2O (94:5:1 v/v/v) during 4 h. After filtration, the peptide was precipitated with 50 mL ice cold tert-butylmethylether (TBME), centrifuged at 4400 rpm for 15 min, and washed twice with TBME. For purification of the crude peptide, it was dissolved in A (100% mQ-H_2O, 0.1% TFA), subjected to preparative RP-HPLC and obtained as TFA salt after lyophilisation.

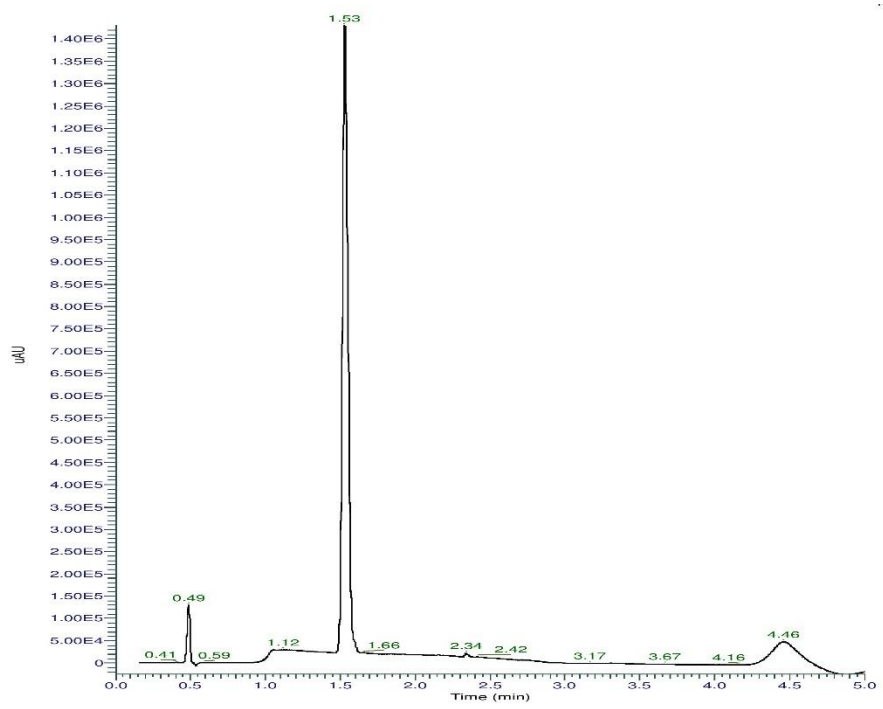
Synthesis of polymyxin B

Fmoc-Dab(Boc)-OH was dissolved in mQ deionized water (36 mL) containing $NaHCO_3$ (1 eq). Allyl bromide (6 eq, 4 mL) and phase transfer catalyst Aliquate 336 (0.85 eq) were dissolved in 32 mL DCM and then added dropwise into the aqueous solution. The reaction was continued at room temperature in overnight with stirring. Then product was extracted to DCM and washed with 200 mL brine and dried over anhydrous Na_2SO_4. Purified compound was subjected to Boc deprotection with 20% TFA in DCM. The reaction was stirred for 3 hours under room temperature and then solvents were removed using rotary evaporator and product was further dried under high vacuum.

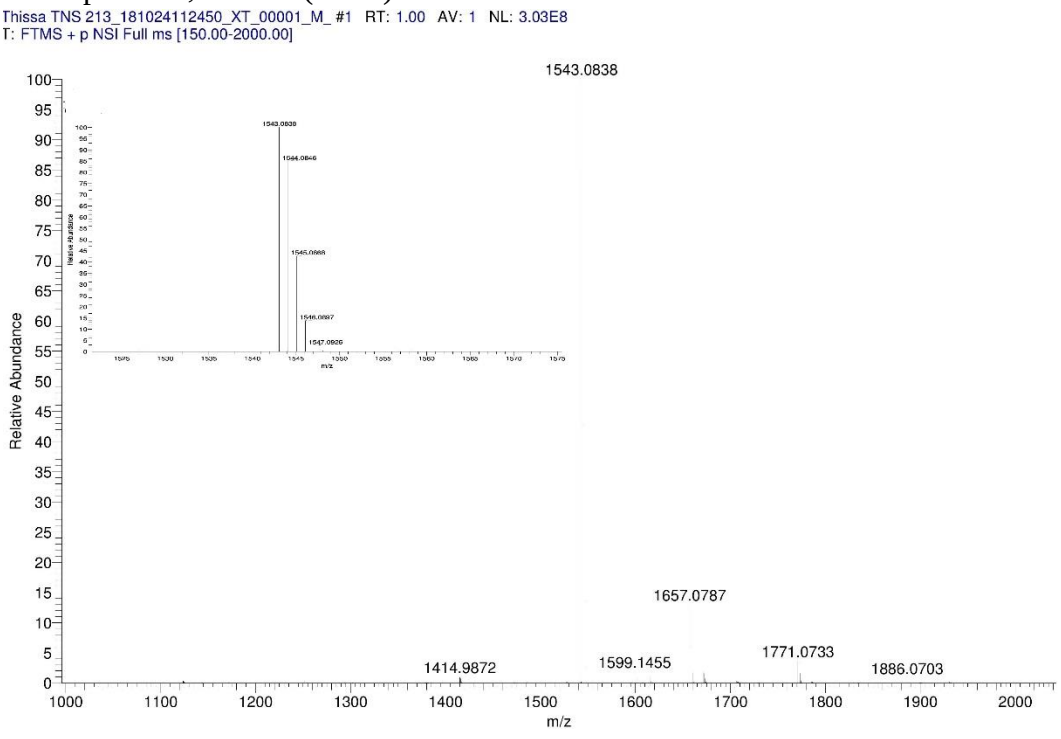
The Fmoc-Dab-OAll (5 eq) attached to 2-Chlorotrityl chloride resin with DIPEA (8 eq) in 4 mL DMF and reaction was continued overnight under inert atmosphere. The Fmoc-protecting group of Fmoc-Dab-OAll of the resin was removed with a solution of 20% piperidine in DMF, 2 minutes at room temperature and 5 minutes at 50 °C. Then peptide chain was continue under SPPS conditions and amino acid bound to resin acylated with amino acids (5 eq./amine), OxymaPure (7.5 eq./amine) and DIC (10 eq./amine) in DMF for 10 minutes at 50 °C. Ivdde protecting group was removed with 3% H_2NNH_2 in DMF for 10min, five times. Then Fmoc-Thr-OH was attached to peptide chain and allyl group was deprotected with $Pd(PPh_3)_4$ (0.25 eq) and $PhSiH_3$ (25 eq) in dry DCM for three times 20 minutes under inert conditions. After Fmoc deprotection, on-resin cyclization was performed with OxymaPure (10 eq./amine) and DIC (15 eq./amine) in DMF.[2] The cleavage was carried out using TFA/TIS/H_2O (94:5:1 v/v/v) during 4 h. After filtration, the peptide was precipitated with 50 mL ice cold *tert*-butylmethylether (TBME), centrifuged at 4400 rpm for 15 min, and washed twice with TBME. For purification of the crude peptide, it was dissolved in A (100% mQ-H_2O, 0.1% TFA), subjected to preparative RP-HPLC and obtained as TFA salt after lyophilisation.

L-SB1 KYKKALKKLAKLL. From TentaGel resin (300 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (56.0 mg, 30.1%). Anal. RP-HPLC: t_R = 1.53 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{75}H_{138}N_{20}O_{14}$ calc./found 1543.07/1543.08 Da [M]+.

Analytical RP-HPLC chromatogram:

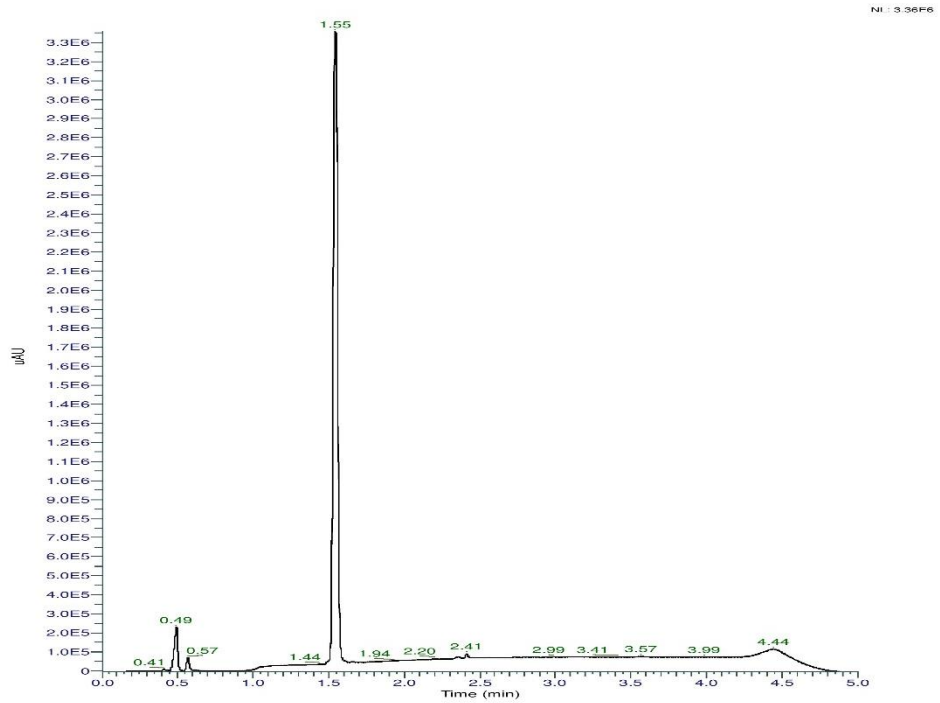


Mass spectrum, HRMS (NSI+)

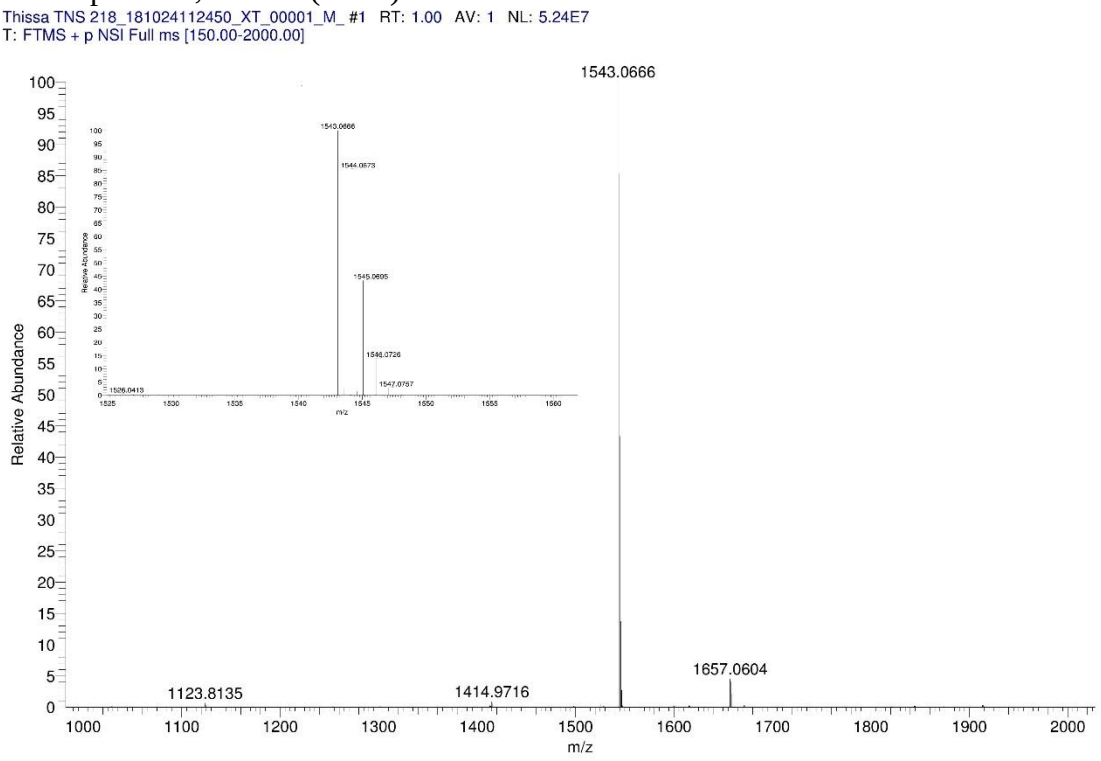


D-SB1 kykkalkklakll. From TentaGel resin (530 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (74.0 mg, 26.1%). Anal. RP-HPLC: t_R = 1.55 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{75}H_{138}N_{20}O_{14}$ calc./found 1543.07/1543.07 Da [M]+.

Analytical RP-HPLC chromatogram:

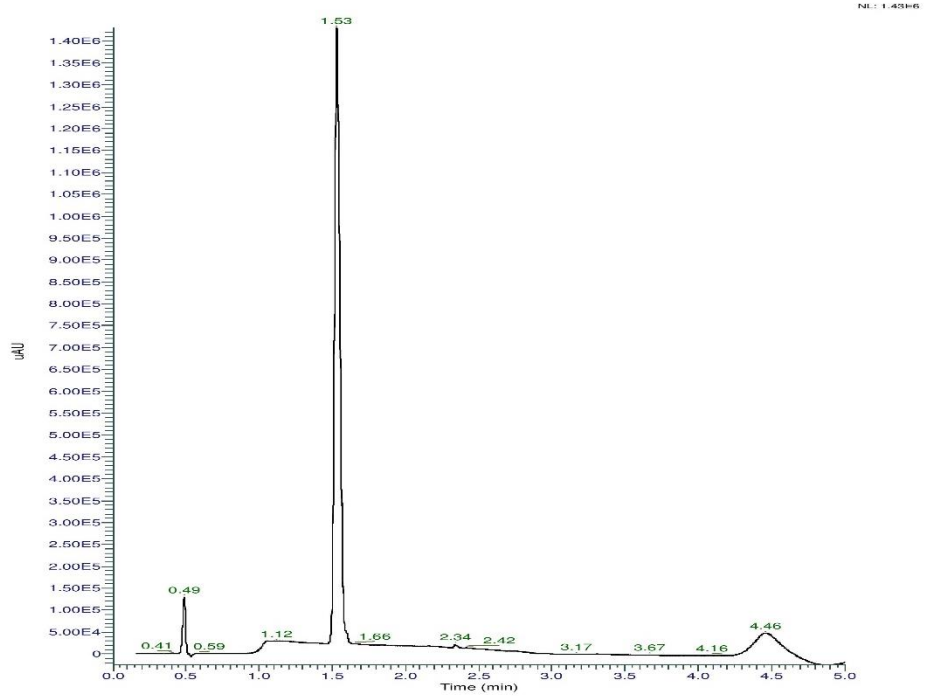


Mass spectrum, HRMS (NSI+)

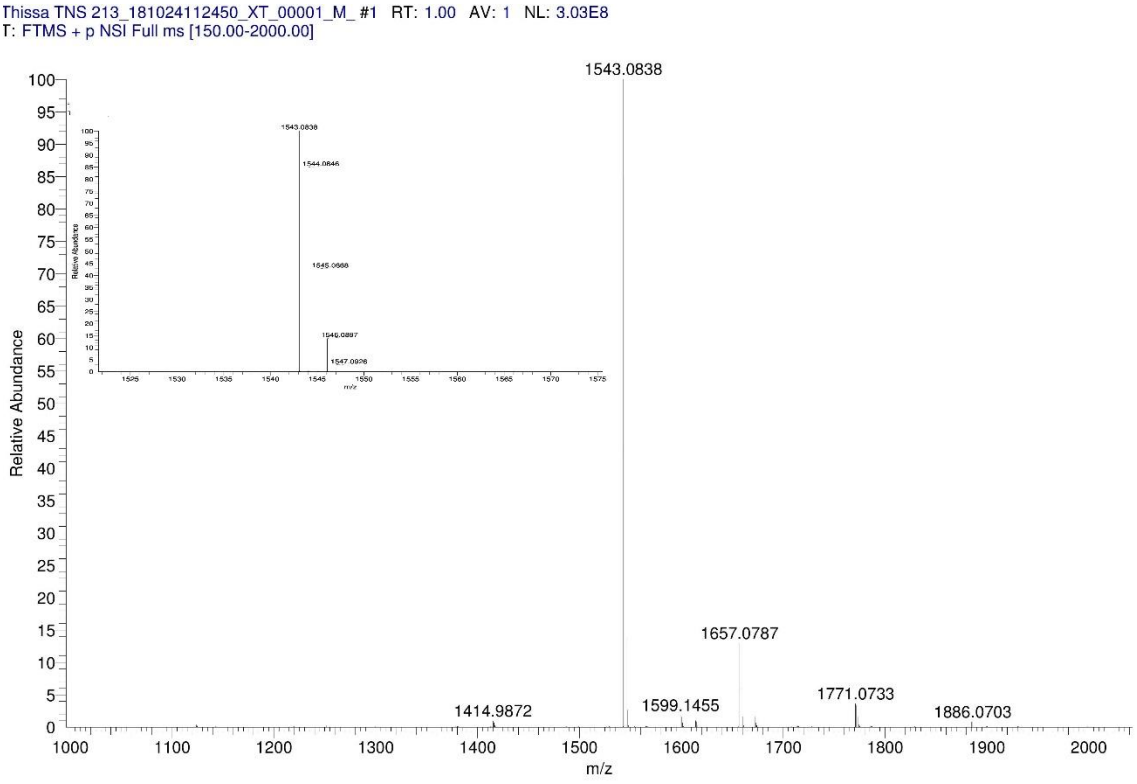


***sr*-SB1** KYKKALKKLAKLL (L:D 1:1). From TentaGel resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (62.0 mg, 22.0%). Anal. RP-HPLC: t_R = 1.53 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{75}H_{138}N_{20}O_{14}$ calc./found 1543.07/1543.08 Da [M]+.

Analytical RP-HPLC chromatogram:

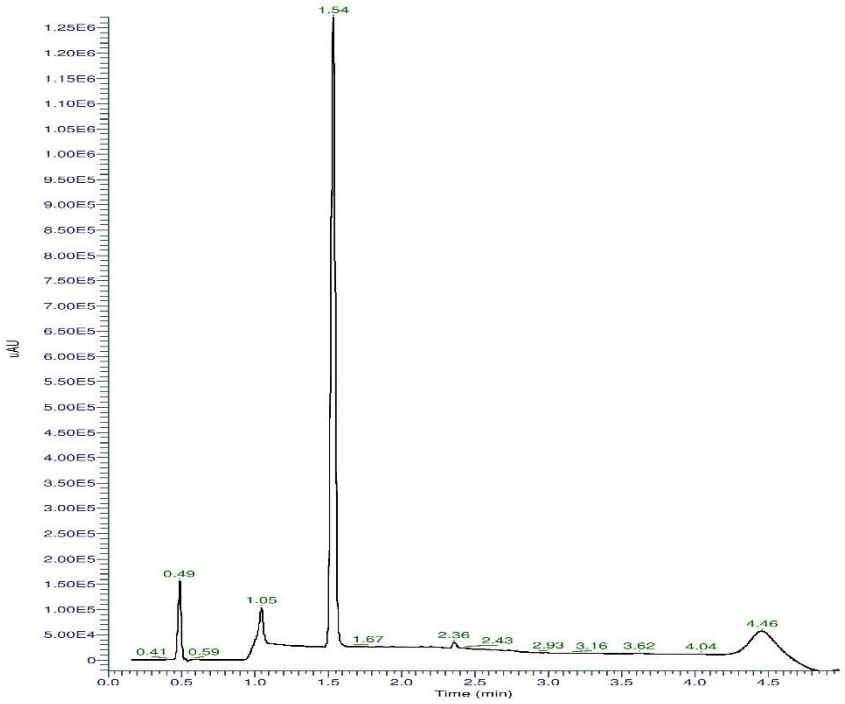


Mass spectrum, HRMS (NSI+)

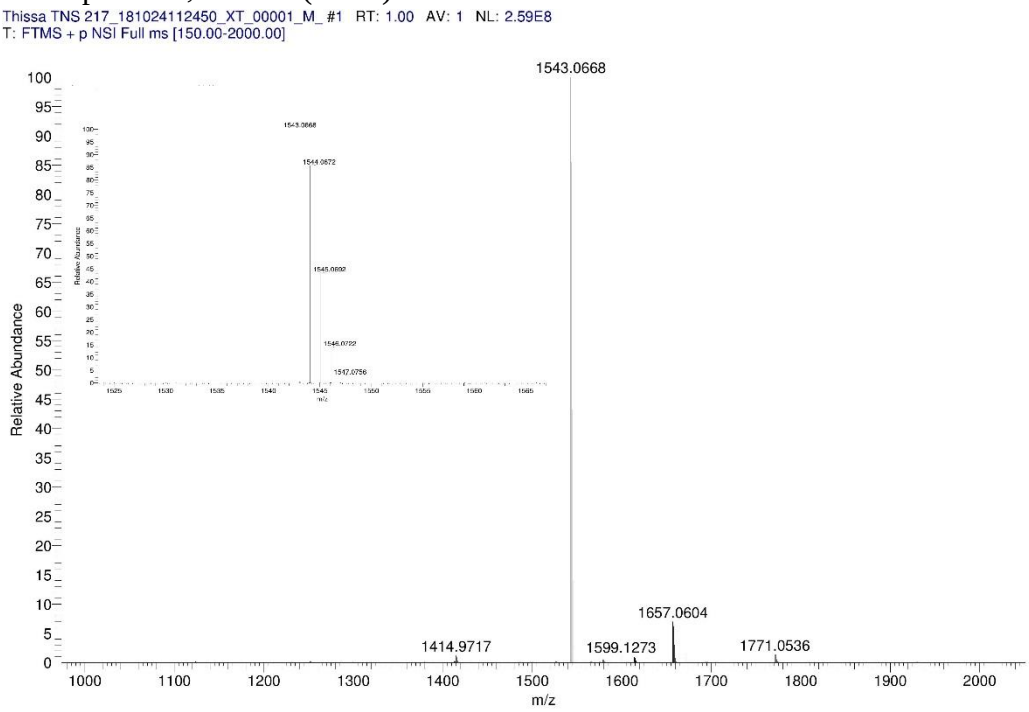


***sr*$^{3/1}$-SB1** KYKKALKKLAKLL (L:D 3:1). From TentaGel resin (520 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (48.0 mg, 17.0%). Anal. RP-HPLC: t_R = 1.54 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{75}H_{138}N_{20}O_{14}$ calc./found 1543.07/1543.07 Da [M]+.

Analytical RP-HPLC chromatogram:

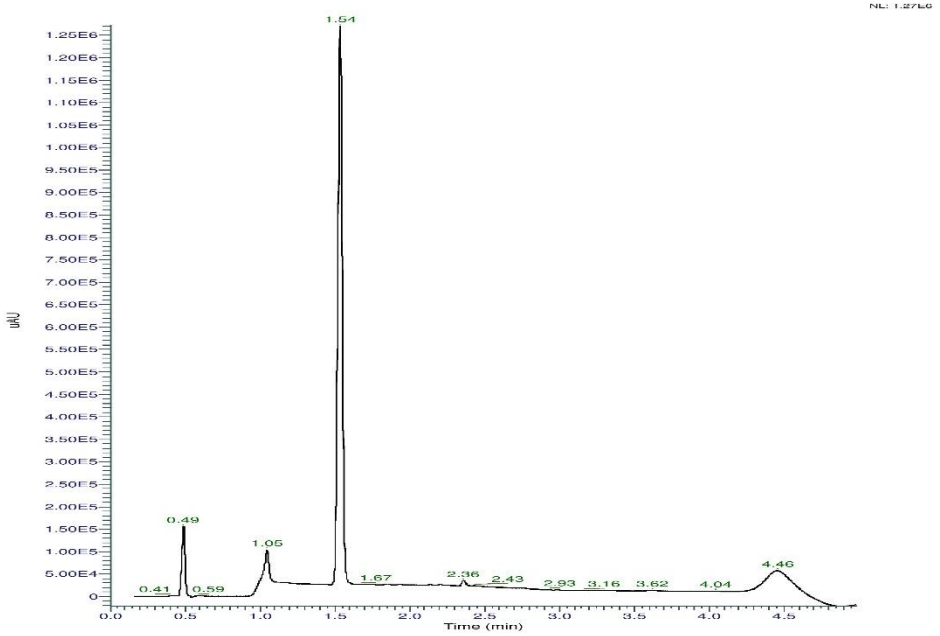


Mass spectrum, HRMS (NSI+)

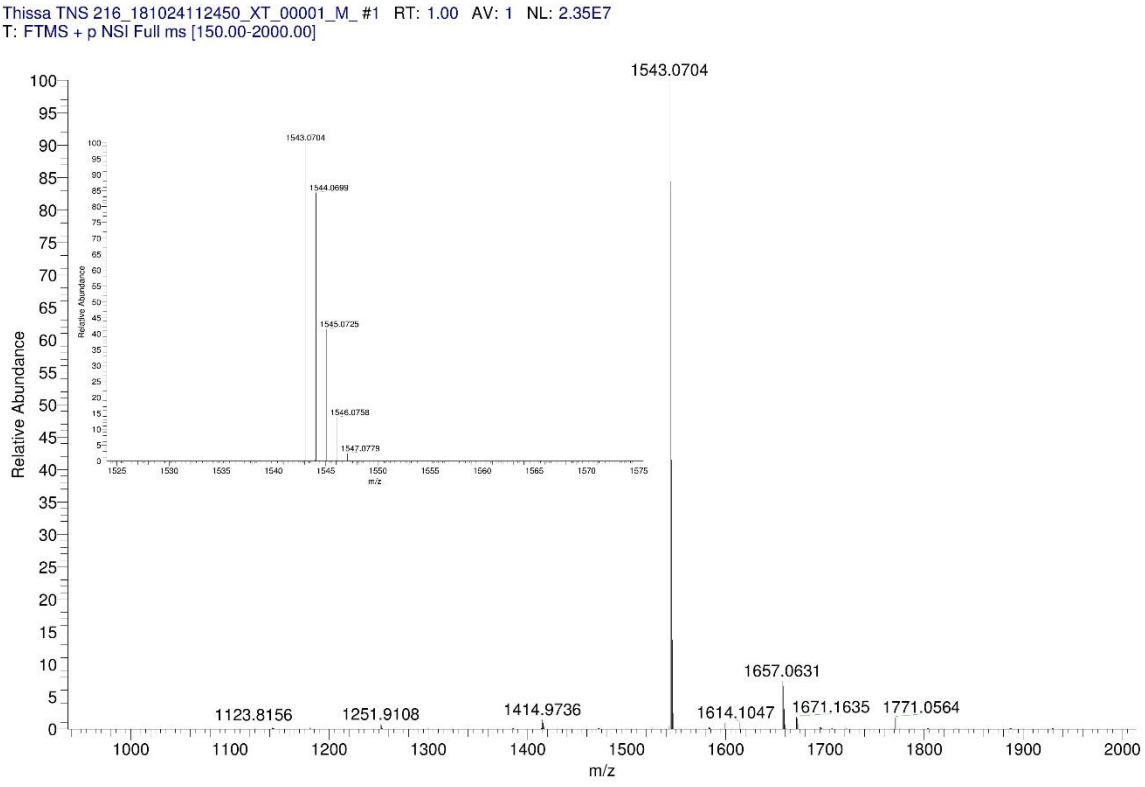


***sr*$^{1/3}$-SB1** KYKKALKKLAKLL (L:D 1:3). From TentaGel resin (510 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (57.0 mg, 20.2%). Anal. RP-HPLC: t_R = 1.54 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{75}H_{138}N_{20}O_{14}$ calc./found 1543.07/1543.07 Da [M]+.

Analytical RP-HPLC chromatogram:

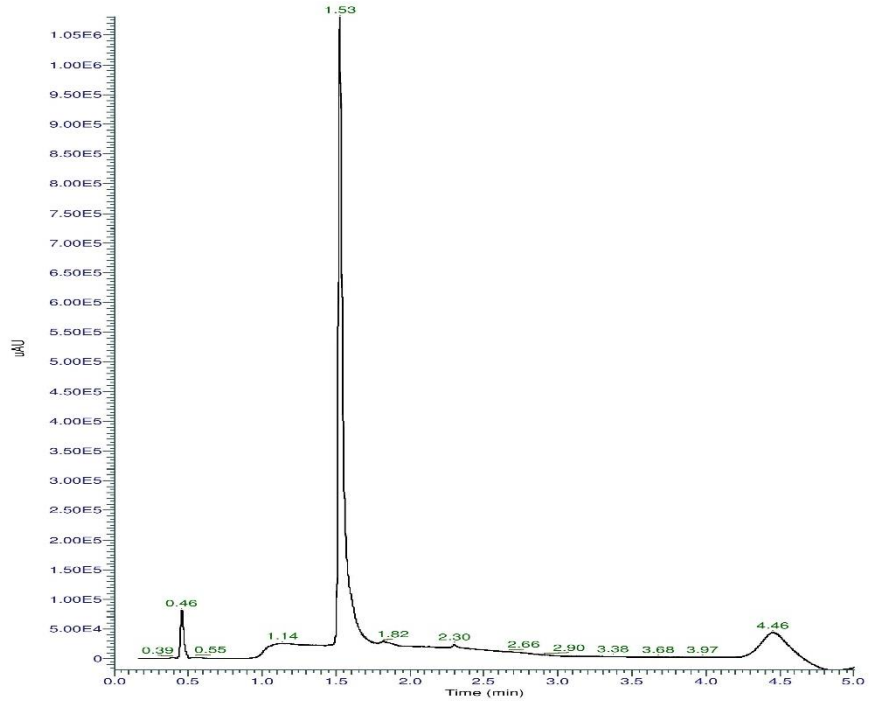


Mass spectrum, HRMS (NSI+)

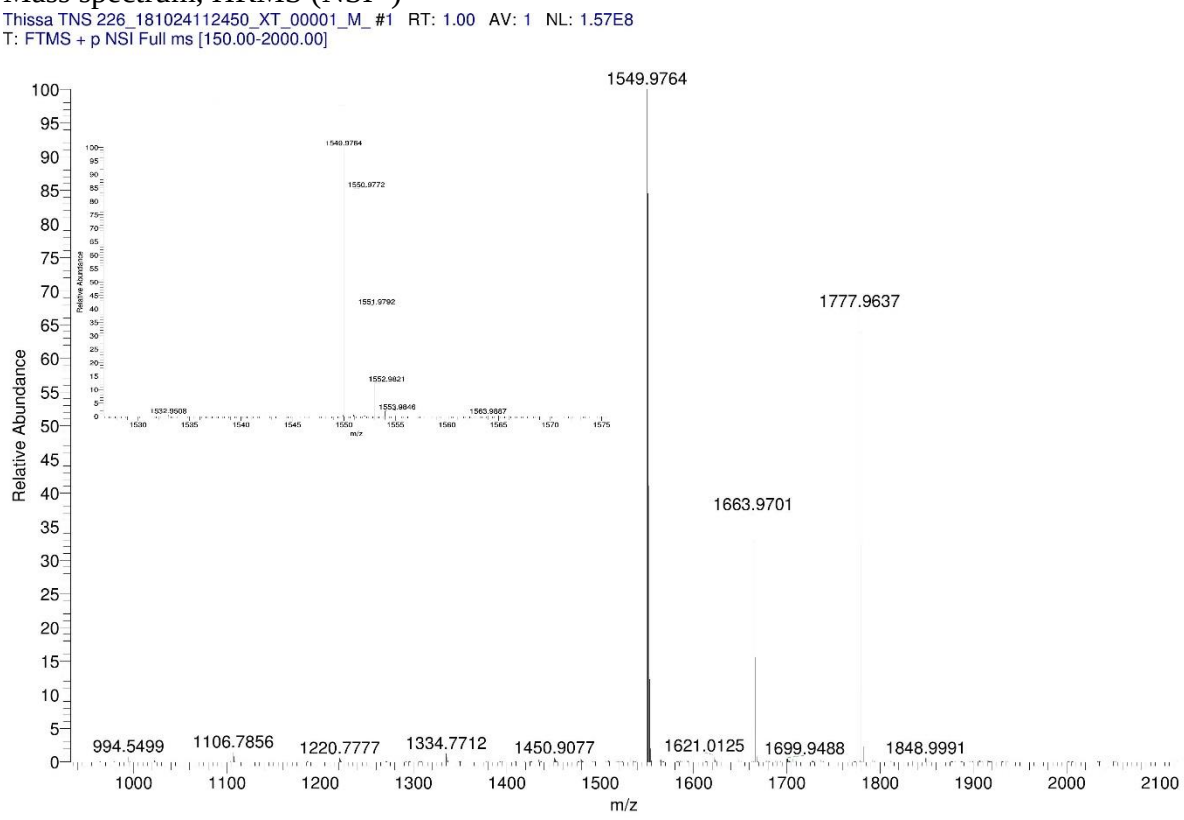


L-DJK5 VQWRAIRVRVIR. From TentaGel resin (490 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (22.0 mg, 8.5%). Anal. RP-HPLC: t_R = 1.53 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{70}H_{123}N_{27}O_{13}$ calc./found 1549.98/1549.98 Da [M]+.

Analytical RP-HPLC chromatogram:

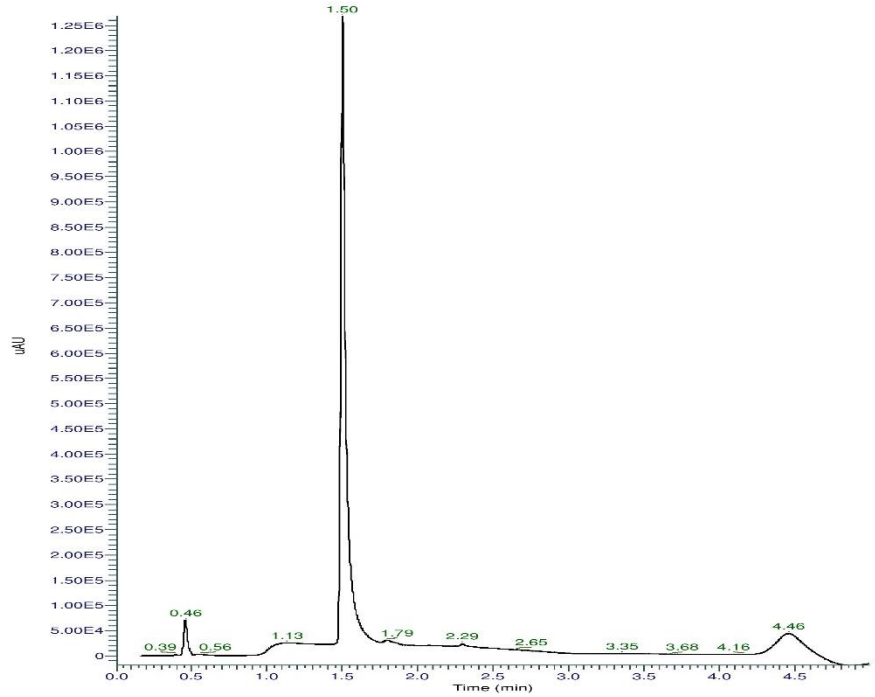


Mass spectrum, HRMS (NSI+)

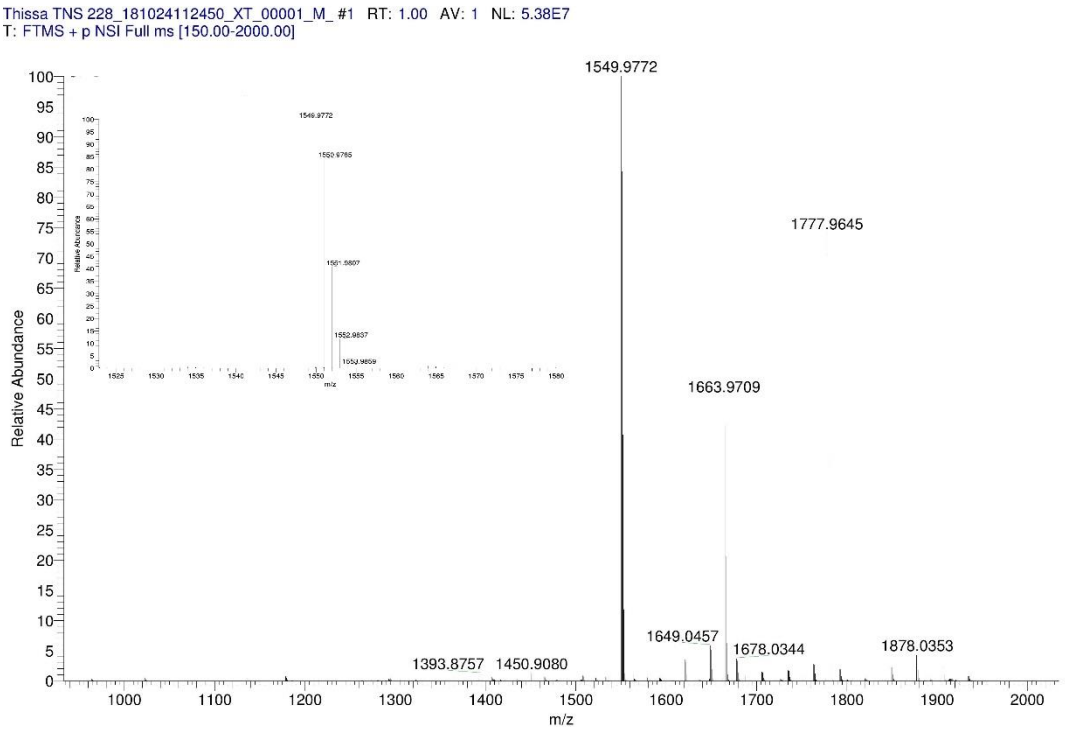


D-DJK5 vqwrairvrvir. From TentaGel resin (310 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (19.0 mg, 7.4%). Anal. RP-HPLC: t_R = 1.50 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{70}H_{123}N_{27}O_{13}$ calc./found 1549.98/1549.98 Da [M]+.

Analytical RP-HPLC chromatogram:

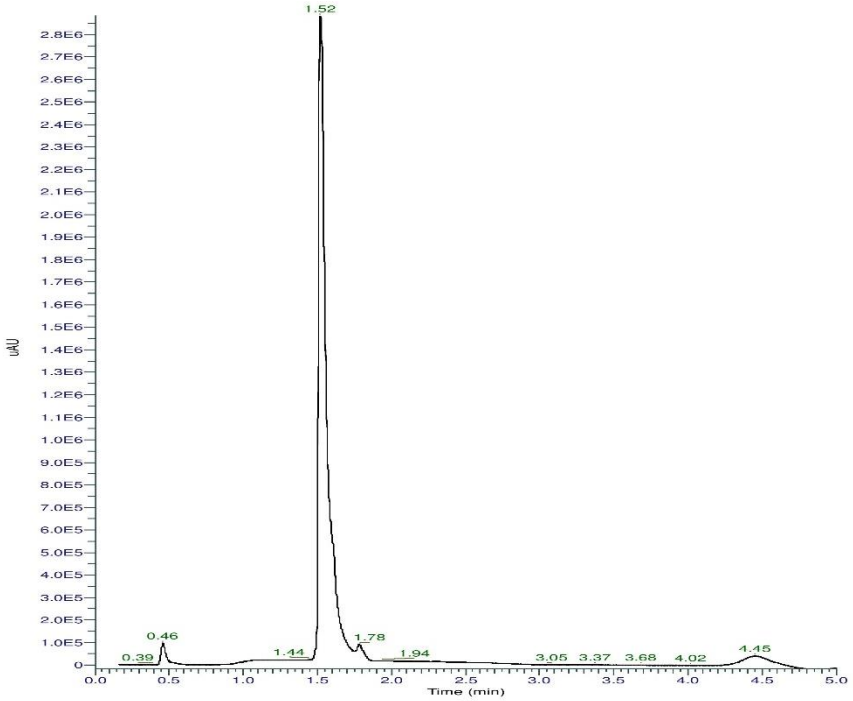


Mass spectrum, HRMS (NSI+)

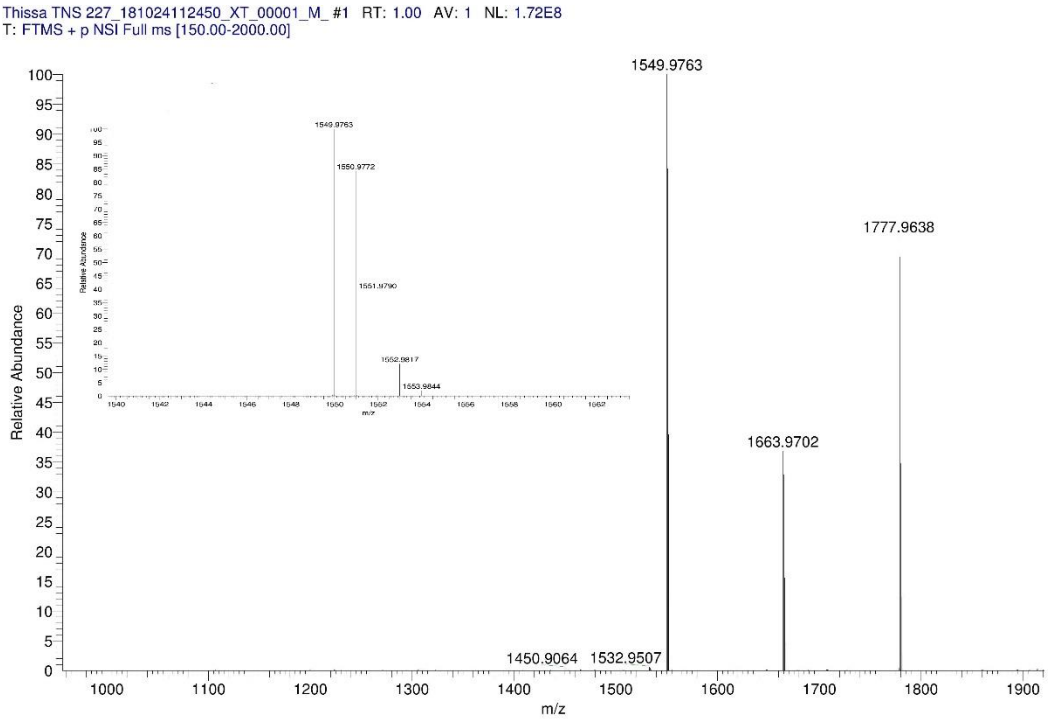


***sr*-DJK5** VQWRAIRVRVIR (L:D 1:1). From TentaGel resin (480 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (13.0 mg, 8.2%). Anal. RP-HPLC: t_R = 1.52 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{70}H_{123}N_{27}O_{13}$ calc./found 1549.98/1549.98 Da [M]+.

Analytical RP-HPLC chromatogram:

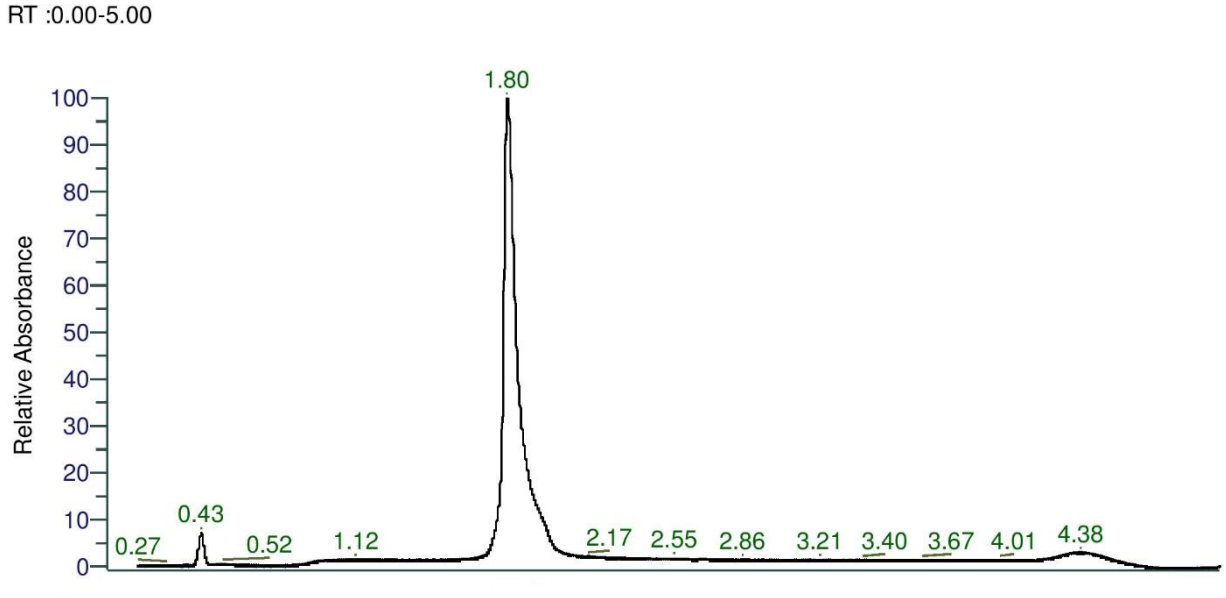


Mass spectrum, HRMS (NSI+)

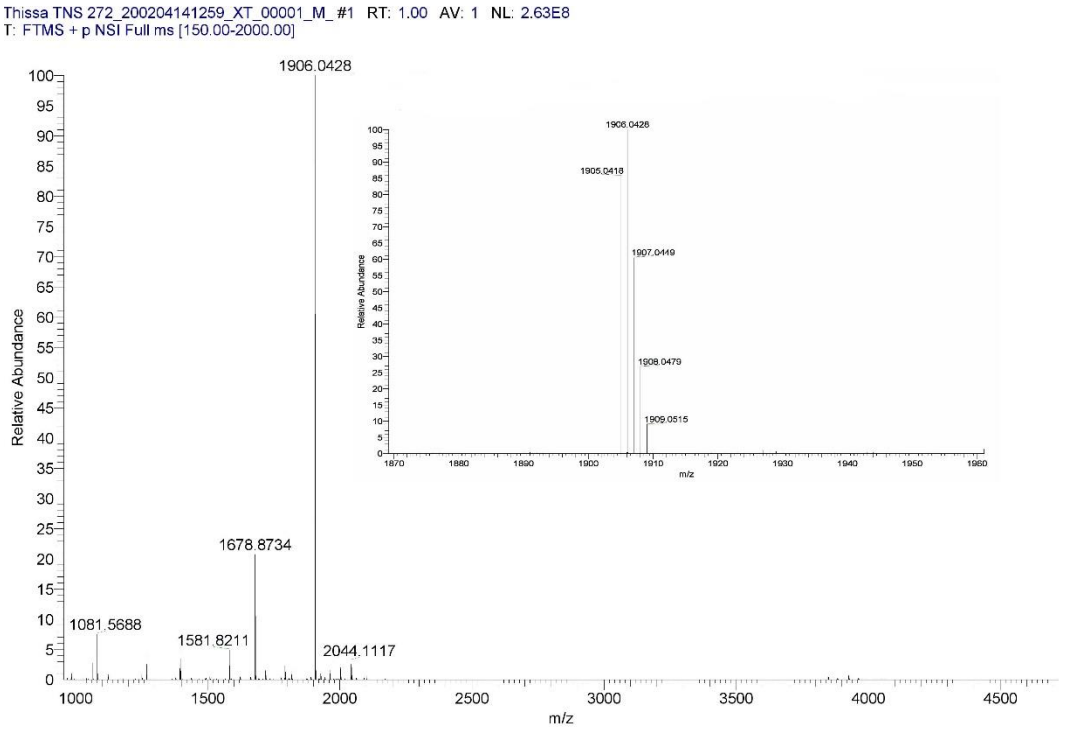


L-Indo ILPWKWPWWPWRR. From Rink Amide AM resin (370 mg, 0.38mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (28.0 mg, 10.4 %). Anal. RP-HPLC: t_R = 1.80 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{100}H_{132}N_{26}O_{13}$ calc./found 1905.05/1905.04 Da [M]+.

Analytical RP-HPLC chromatogram:

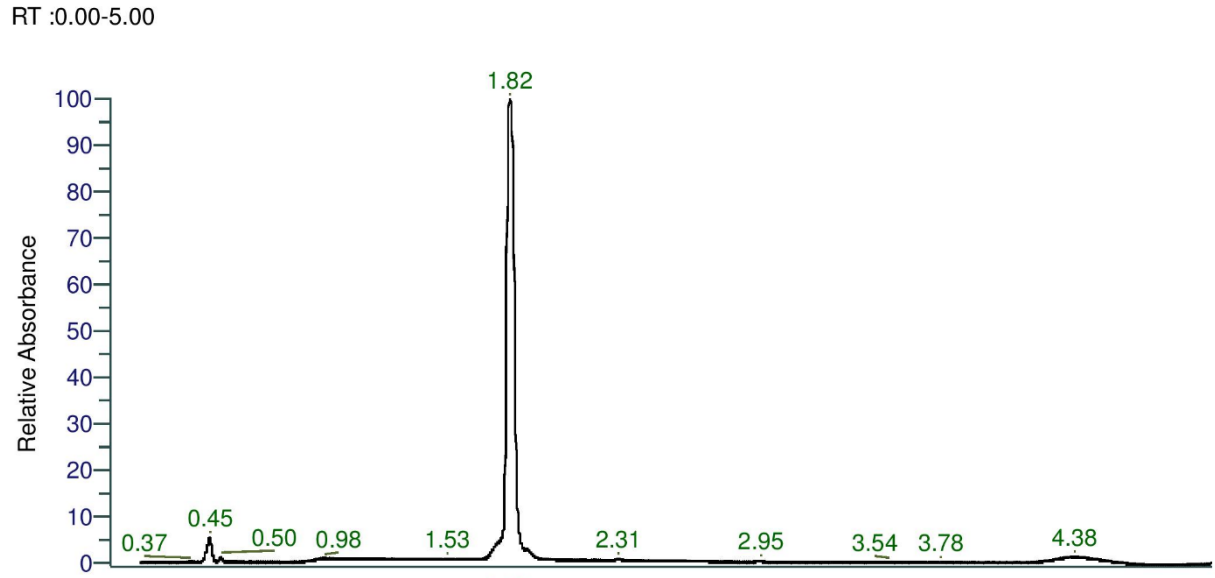


Mass spectrum, HRMS (NSI+)

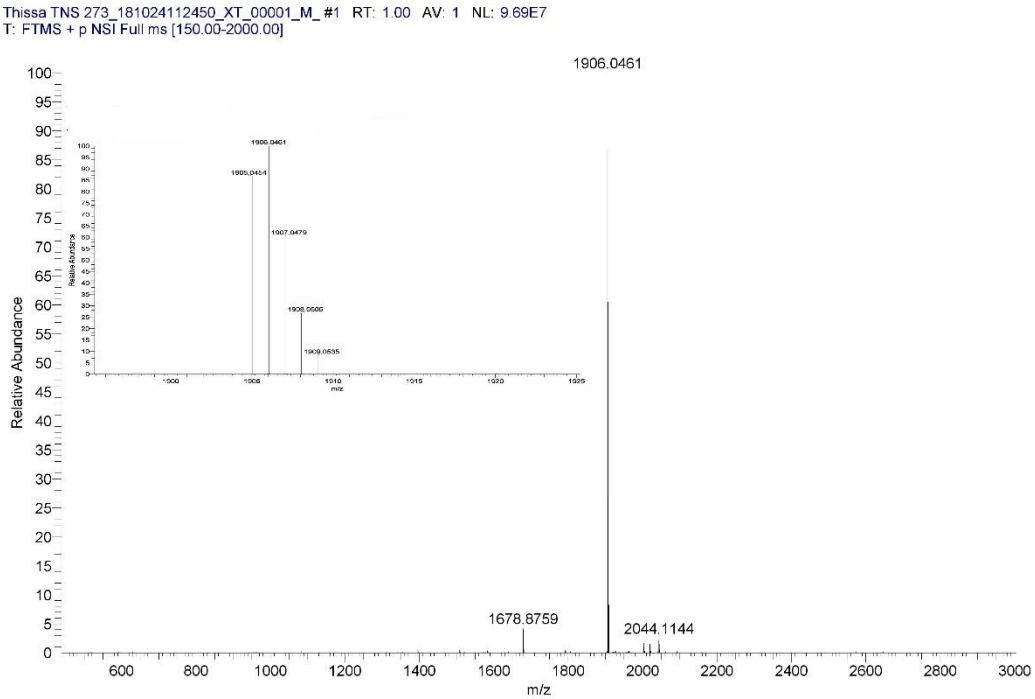


D-Indo ilpwkwpwwpwrr. From Rink Amide AM resin (370 mg, 0.38mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (22.0 mg, 8.2 %). Anal. RP-HPLC: t_R = 1.82 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{100}H_{132}N_{26}O_{13}$ calc./found 1905.05/1905.05 Da [M]+.

Analytical RP-HPLC chromatogram:

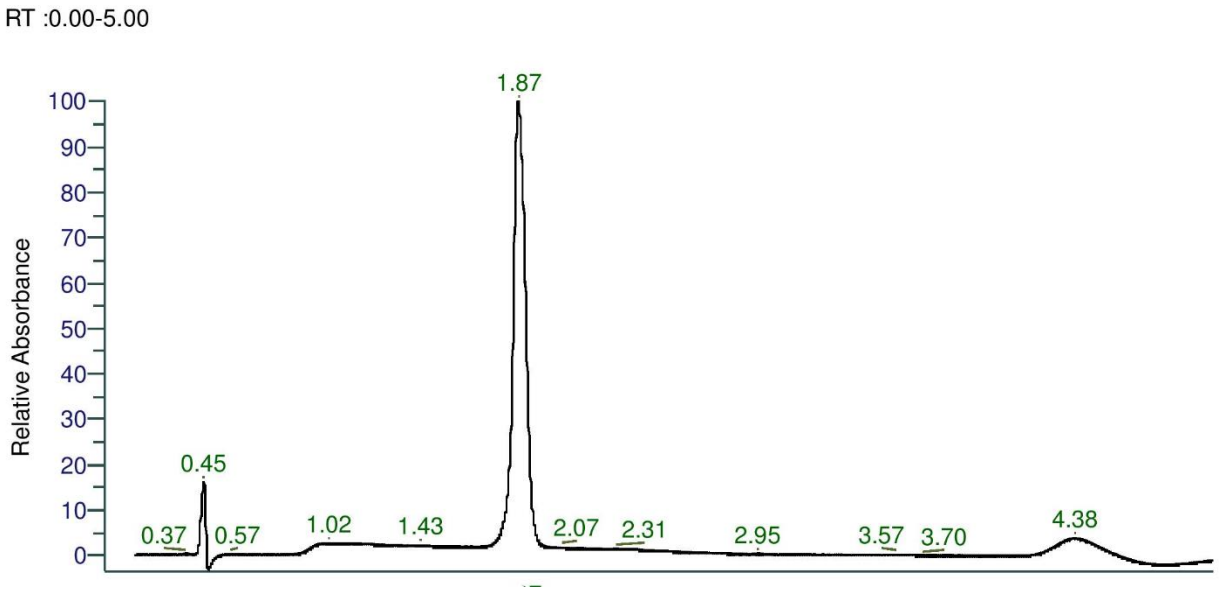


Mass spectrum, HRMS (NSI+)

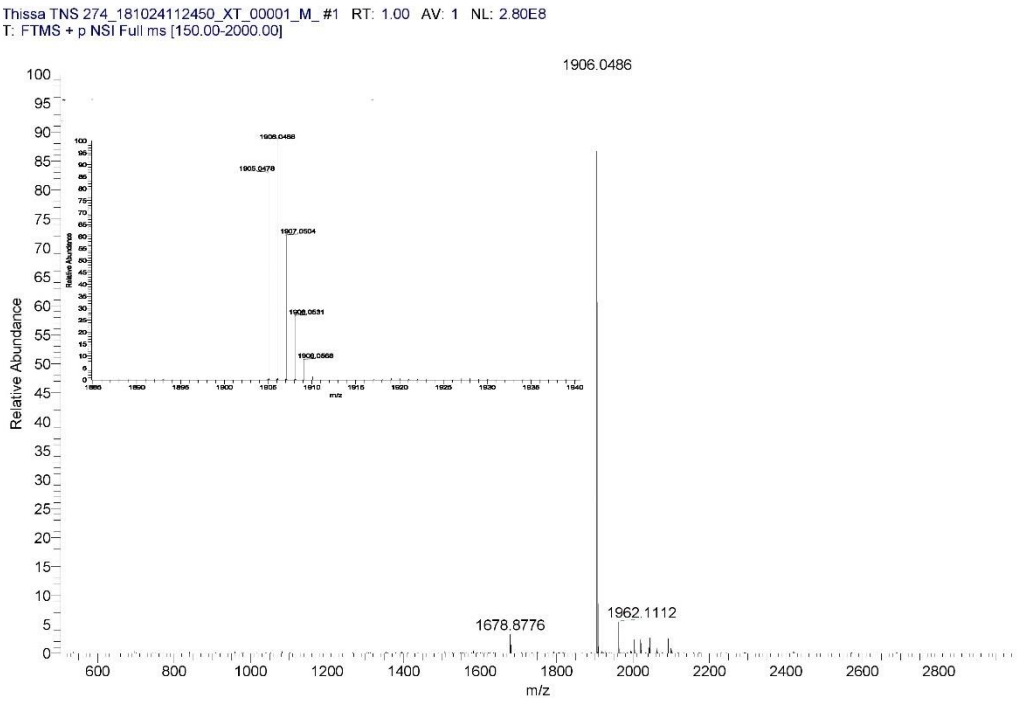


***sr*-Indo** ILPWKWPWWPWRR (L:D 1:1). From Rink Amide AM resin (350 mg, 0.38mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (18.0 mg, 7.1 %). Anal. RP-HPLC: t_R = 1.87 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{100}H_{132}N_{26}O_{13}$ calc./found 1905.05/1905.05 Da [M]+.

Analytical RP-HPLC chromatogram:

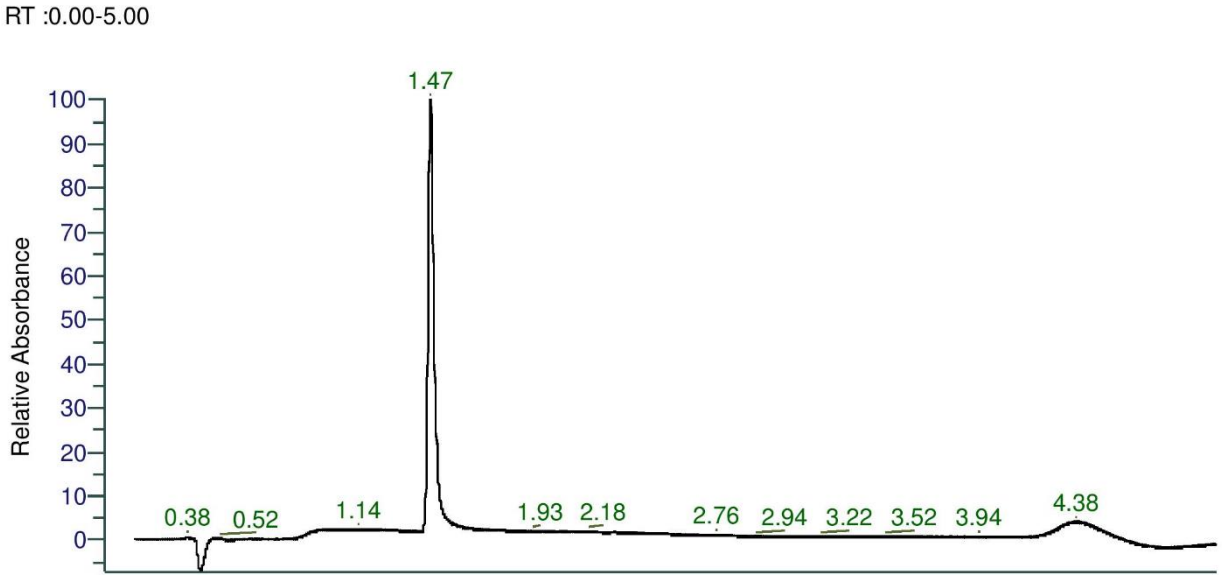


Mass spectrum, HRMS (NSI+)

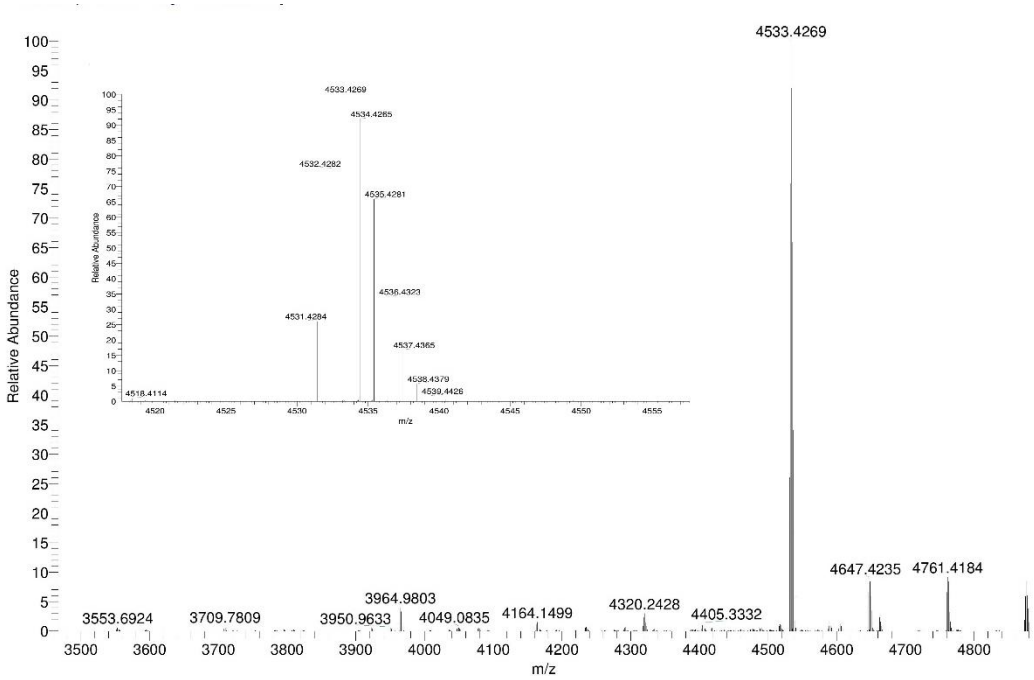


L-G3KL (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*KL. From TentaGel resin (490 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (124.0 mg, 23.2%). Anal. RP-HPLC: t_R = 1.47 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{222}H_{432}N_{60}O_{37}$ calc./found 4531.38/4531.43 Da [M]+.

Analytical RP-HPLC chromatogram:

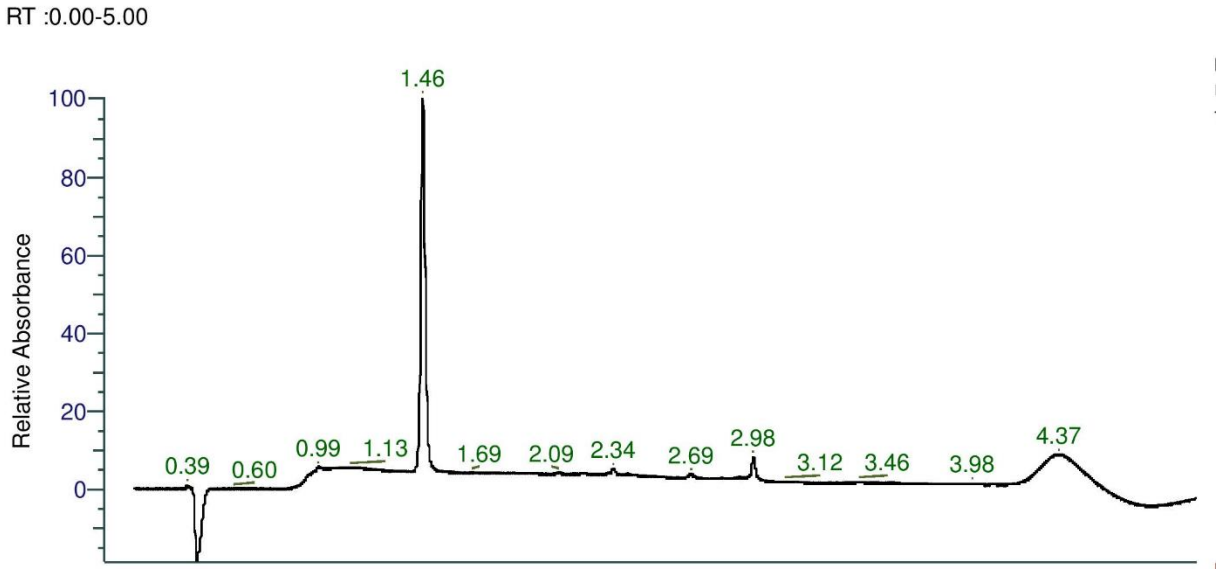


Mass spectrum, HRMS (NSI+)

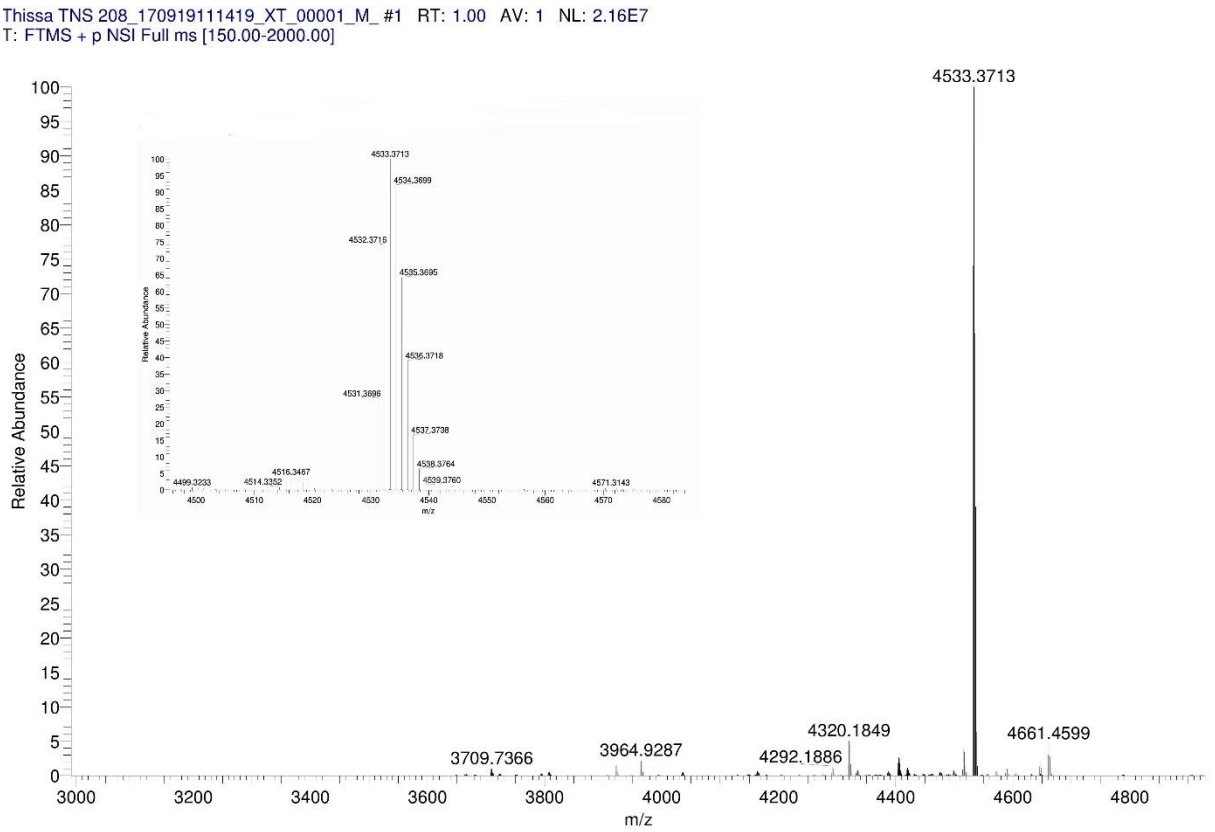


D-G3KL (kl)$_8$(*k*kl)$_4$(*k*kl)$_2$*k*kl. From TentaGel resin (700 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (142.0 mg, 17.1%). Anal. RP-HPLC: t_R = 1.46 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{222}H_{432}N_{60}O_{37}$ calc./found 4531.38/4531.37 Da [M]+.

Analytical RP-HPLC chromatogram:

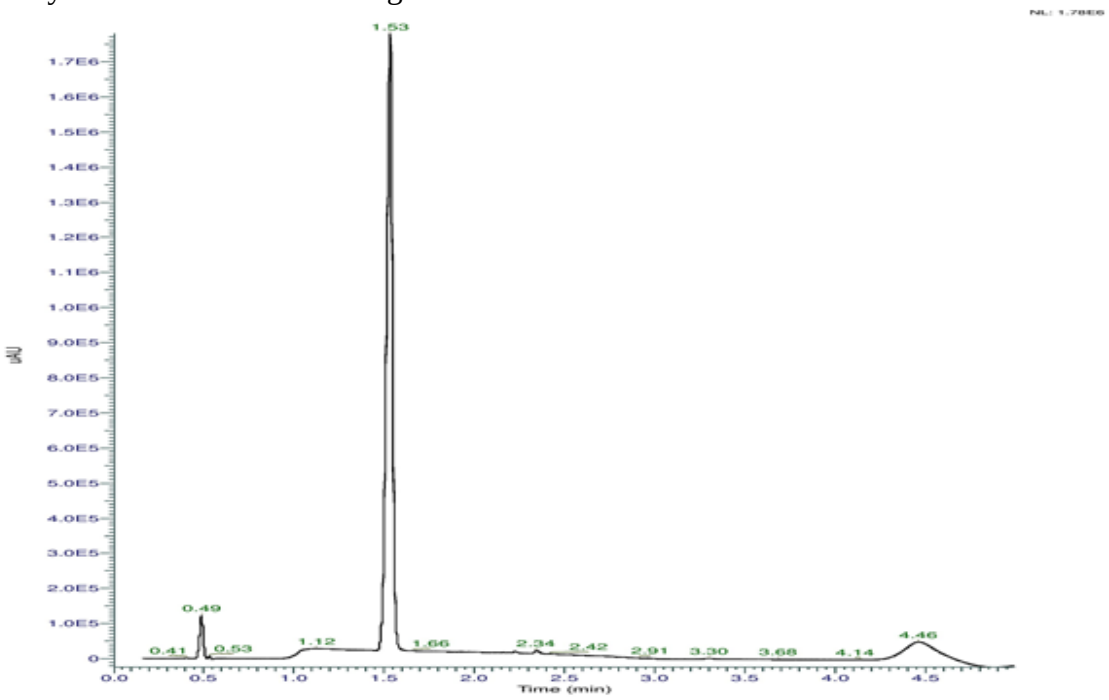


Mass spectrum, HRMS (NSI+)

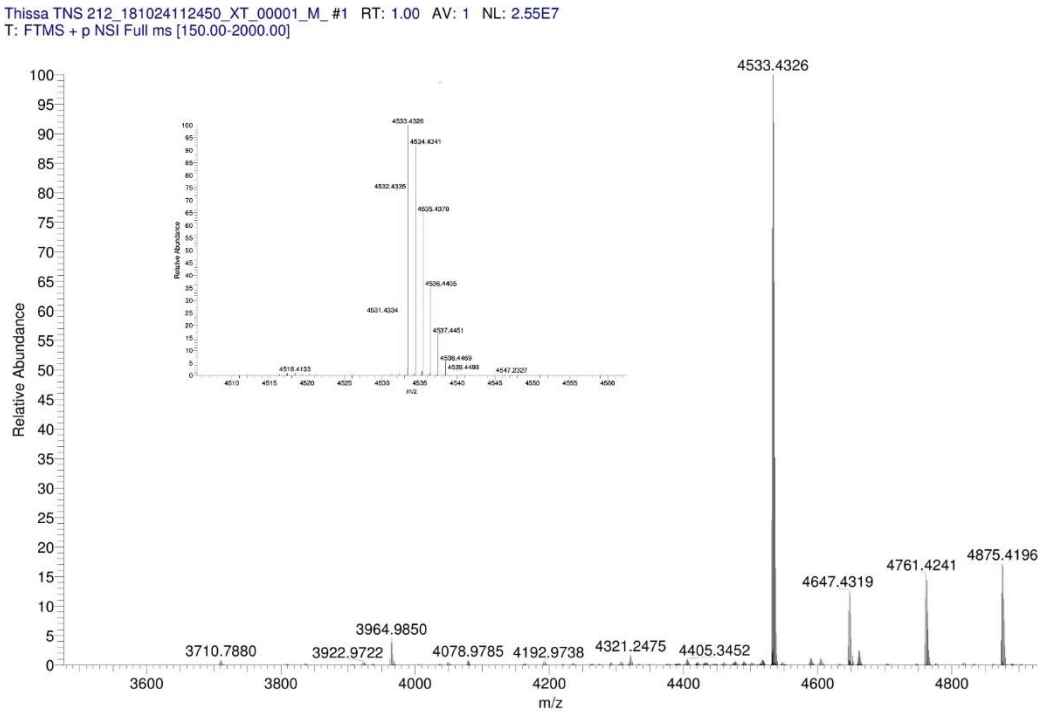


***sr*-G3KL** (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*KL (L:D 1:1). From TentaGel resin (510 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (138.0 mg, 24.3%). Anal. RP-HPLC: t_R = 1.53 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{222}H_{432}N_{60}O_{37}$ calc./found 4531.38/4531.43 Da [M]+.

Analytical RP-HPLC chromatogram:

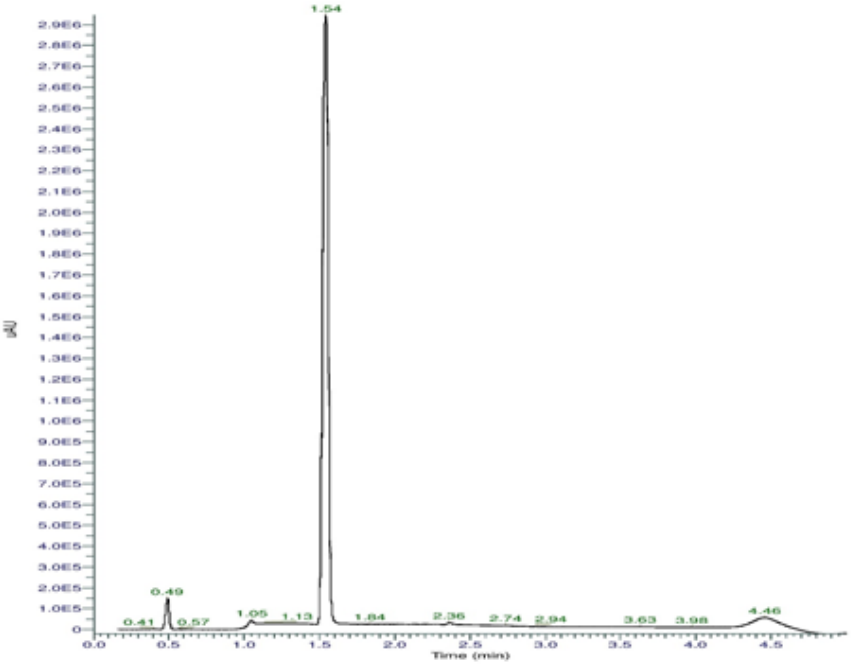


Mass spectrum, HRMS (NSI+)

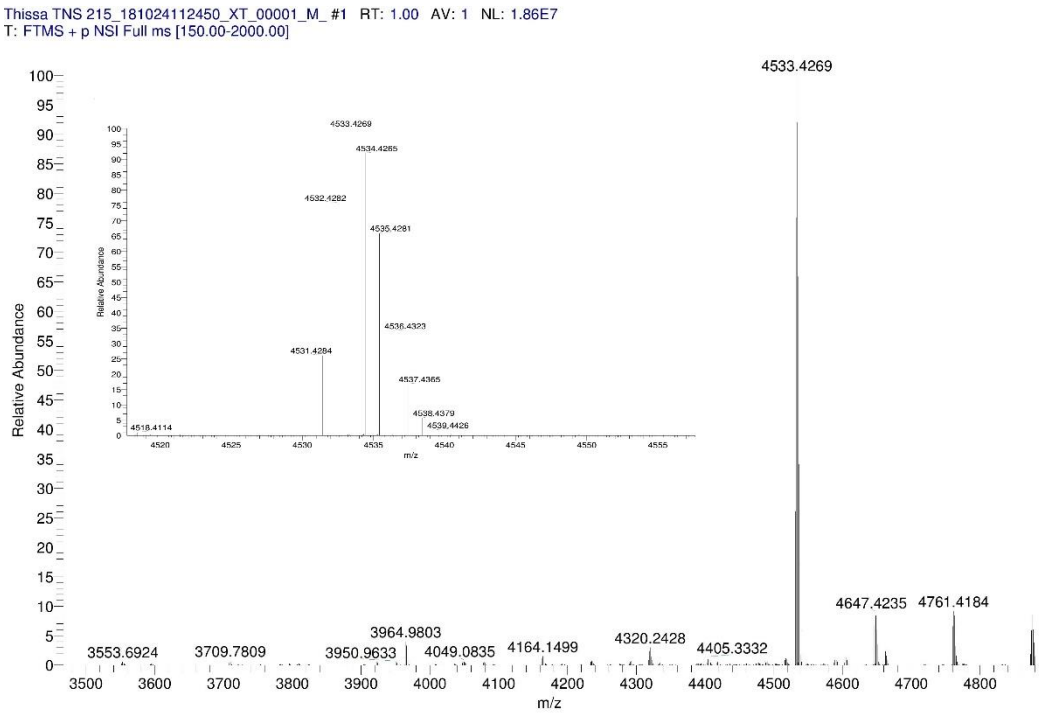


***sr*$^{3/1}$-G3KL** (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*KL (L:D 3:1). From TentaGel resin (520 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (94.0 mg, 11.0%). Anal. RP-HPLC: t_R = 1.54 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{222}H_{432}N_{60}O_{37}$ calc./found 4531.38/4531.43 Da [M]+.

Analytical RP-HPLC chromatogram:

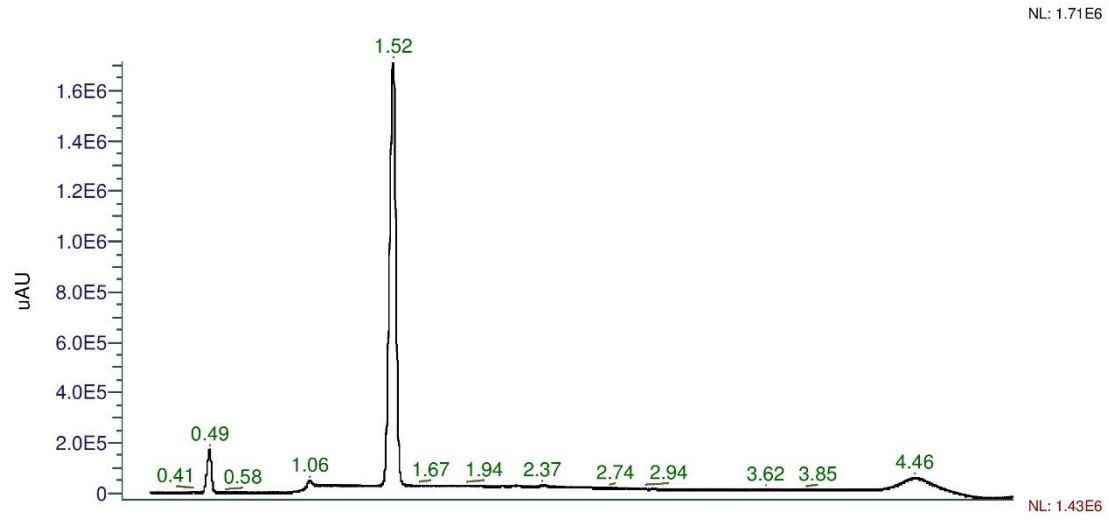


Mass spectrum, HRMS (NSI+)

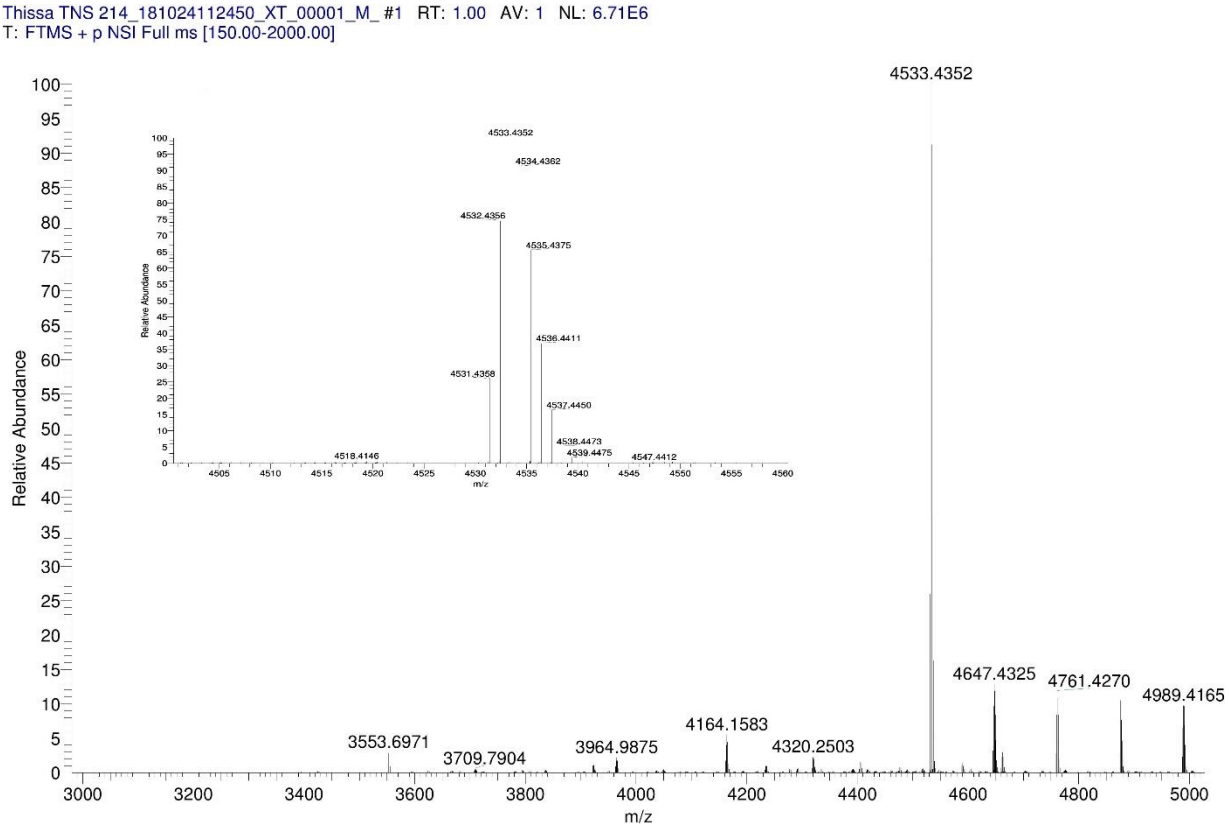


***sr*$^{1/3}$-G3KL** (KL)$_8$(*K*KL)$_4$(*K*KL)$_2$*K*KL (L:D 1:3). From TentaGel resin (510 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (86.0 mg, 10.1%). Anal. RP-HPLC: t_R = 1.52 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{222}H_{432}N_{60}O_{37}$ calc./found 4531.38/4531.44 Da [M]+.

Analytical RP-HPLC chromatogram:

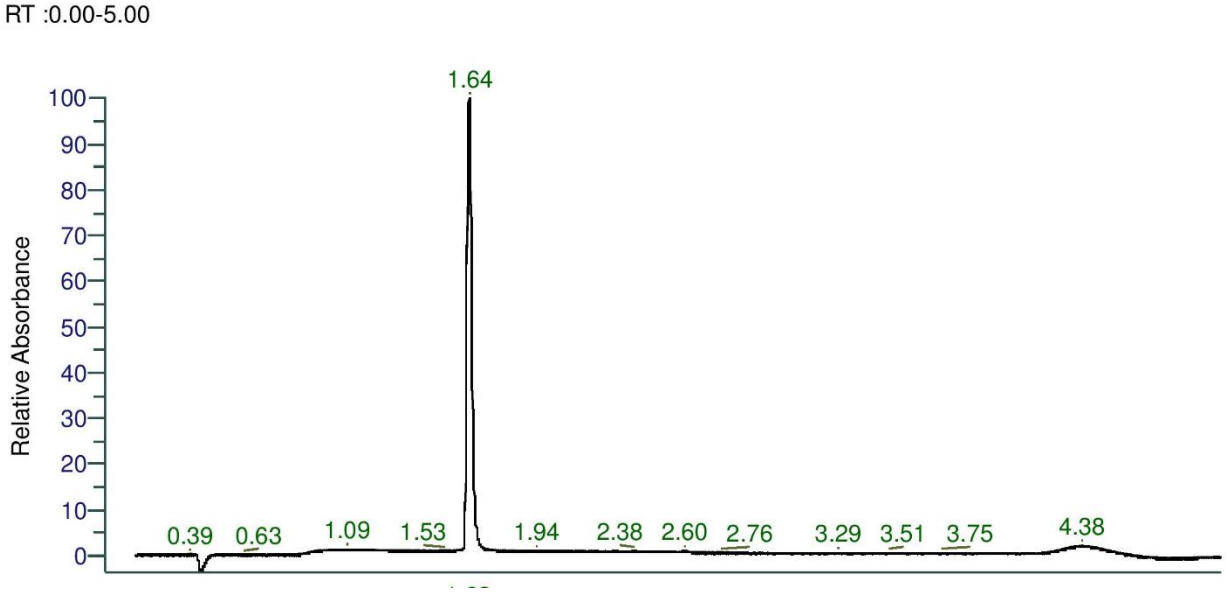


Mass spectrum, HRMS (NSI+)

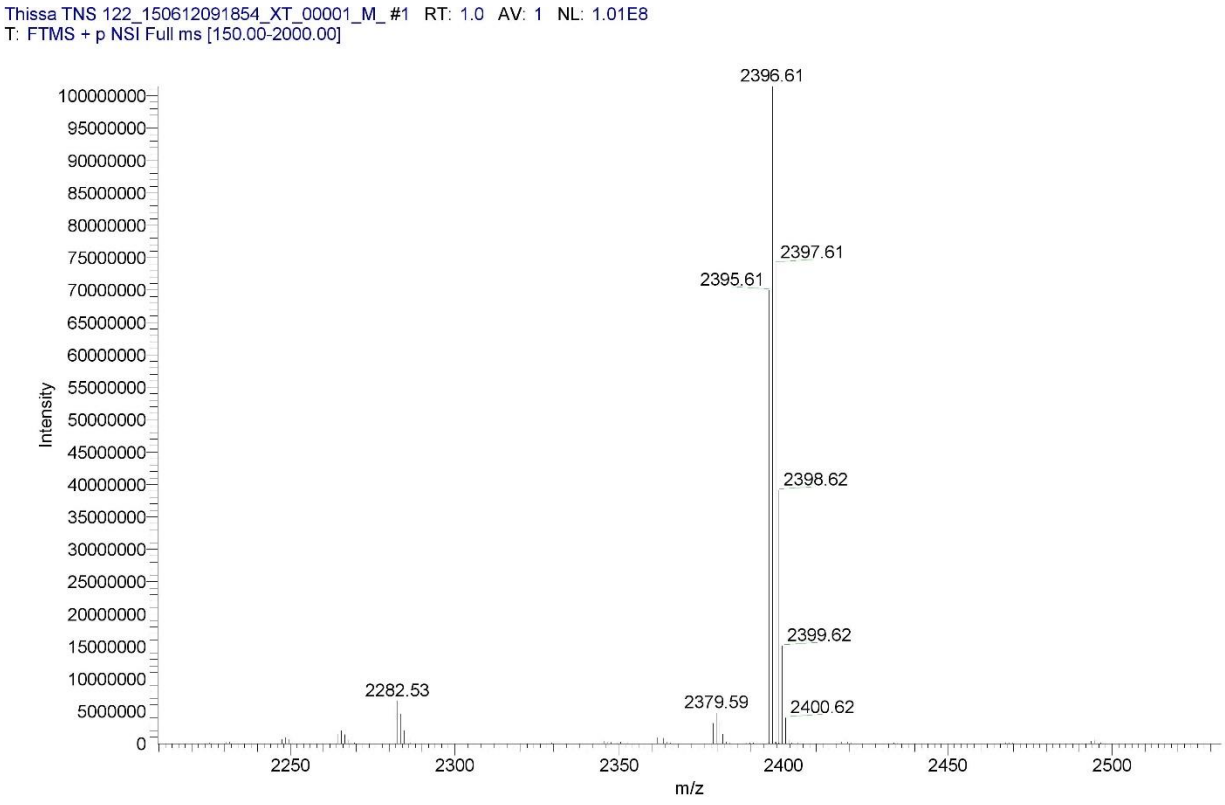


L-TNS18 $(OF)_4(KBL)_2KKLK(C_{10})$. From TentaGel resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (121.0 mg, 41.7 %). Anal. RP-HPLC: t_R = 1.64 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{122}H_{206}N_{30}O_{19}$ calc./found 2395.61/2395.61 Da [M]+.

Analytical RP-HPLC chromatogram:

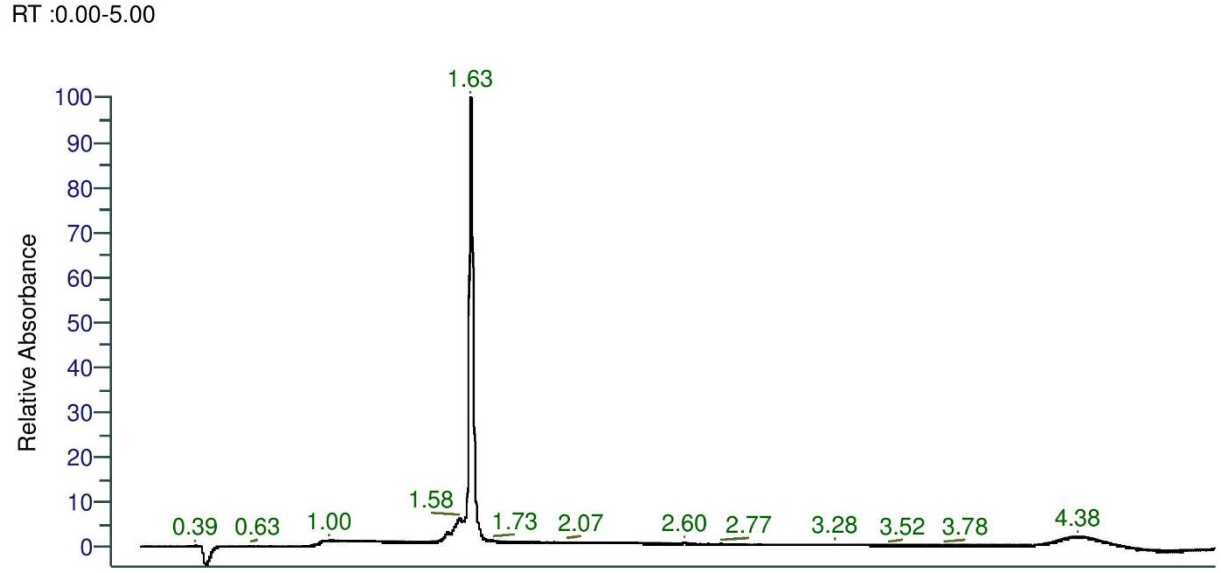


Mass spectrum, HRMS (NSI+)

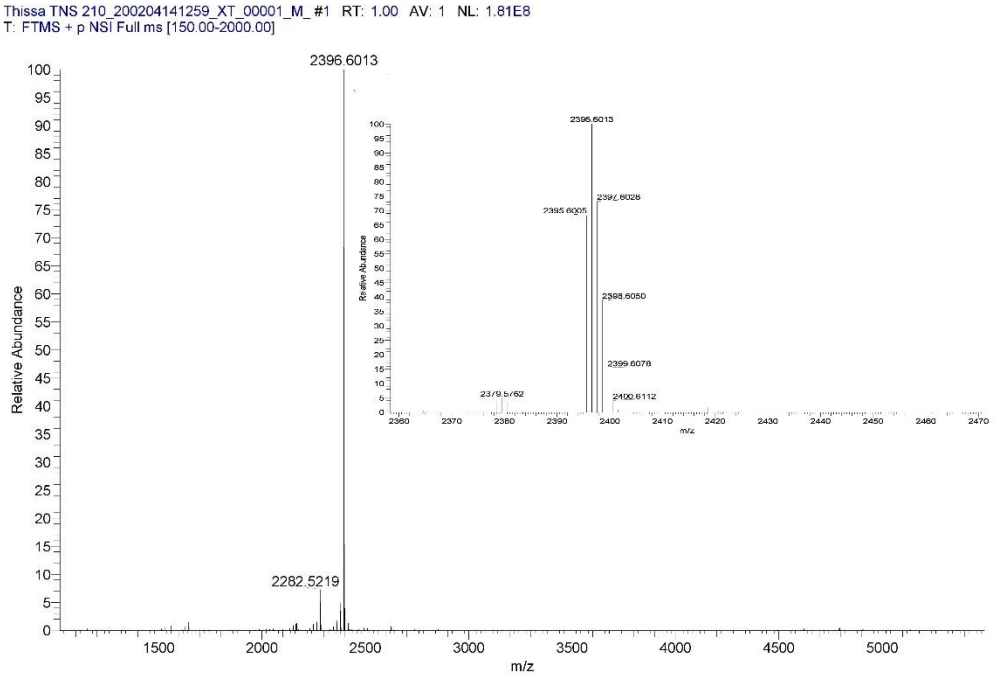


D-TNS18 (of)$_4$(*k*bl)$_2$*k*klk(C$_{10}$). From TentaGel resin (1911 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (156.0 mg, 14.2 %). Anal. RP-HPLC: t_R = 1.63 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{122}H_{206}N_{30}O_{19}$ calc./found 2395.61/2395.60 Da [M]+.

Analytical RP-HPLC chromatogram:

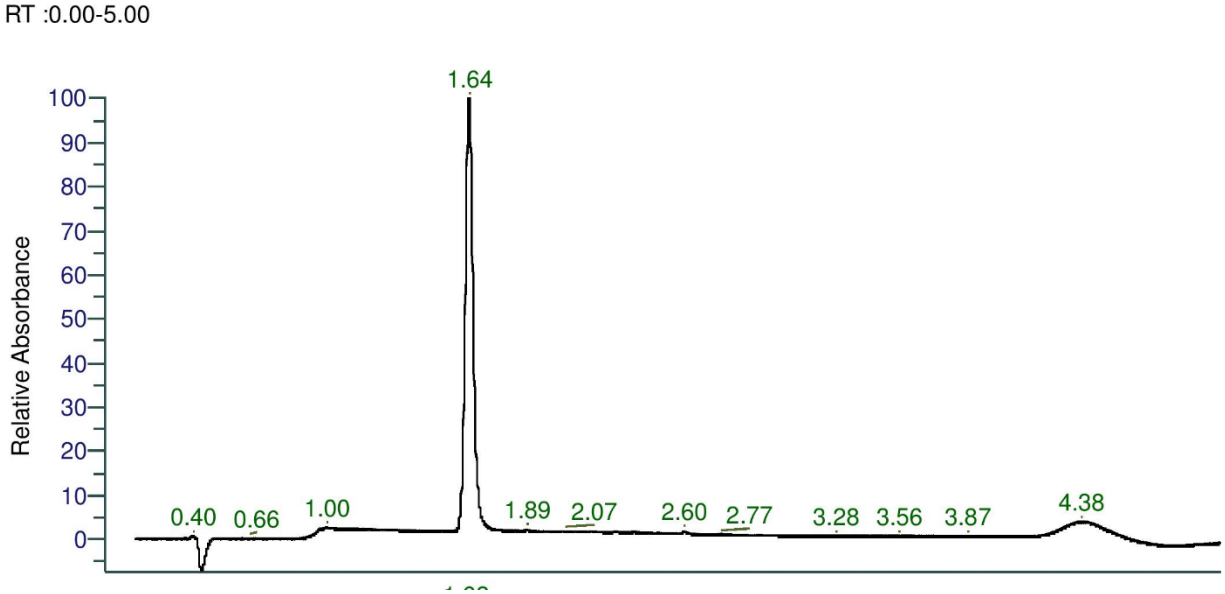


Mass spectrum, HRMS (NSI+)

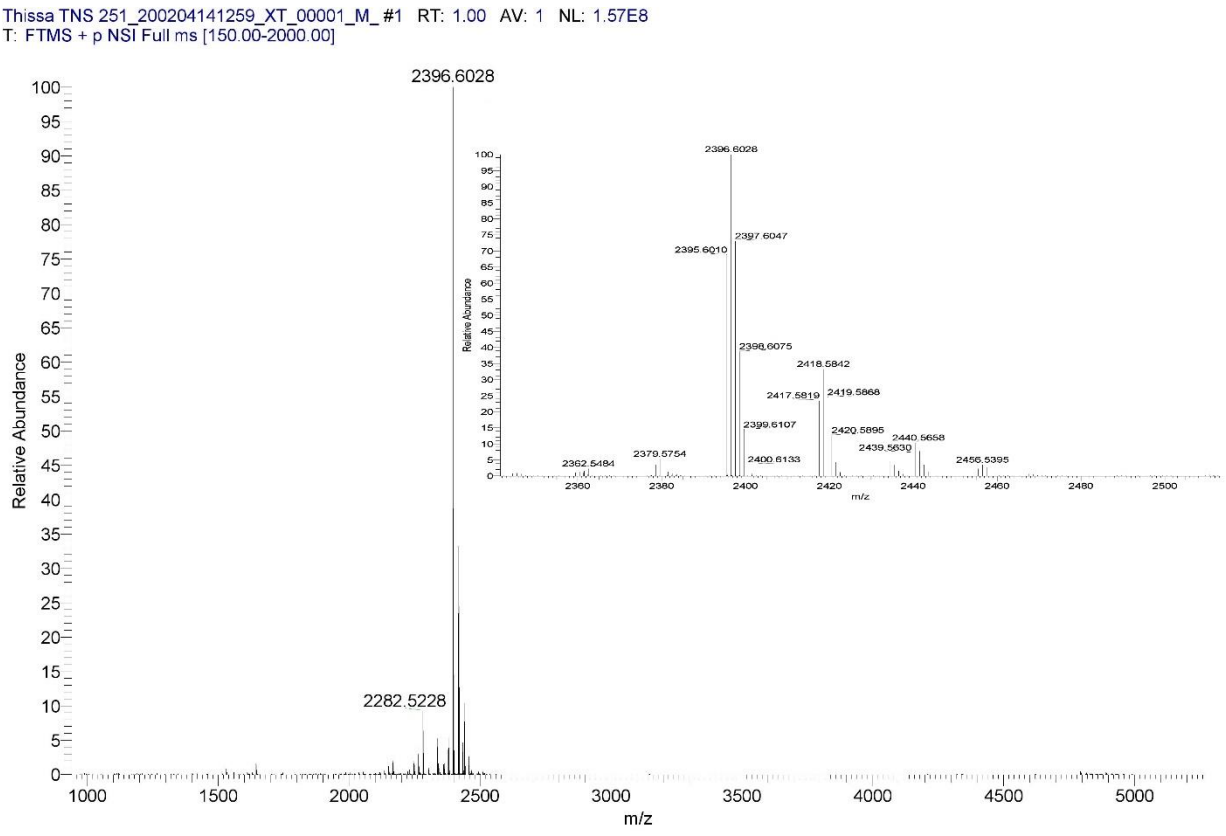


***sr*-TNS18** $(OF)_4(KBL)_2KKLK(C_{10})$ (L:D 1:1). From TentaGel resin (170 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (8.0 mg, 3.8 %). Anal. RP-HPLC: t_R = 1.64 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{122}H_{206}N_{30}O_{19}$ calc./found 2395.61/2395.60 Da [M]+.

Analytical RP-HPLC chromatogram:

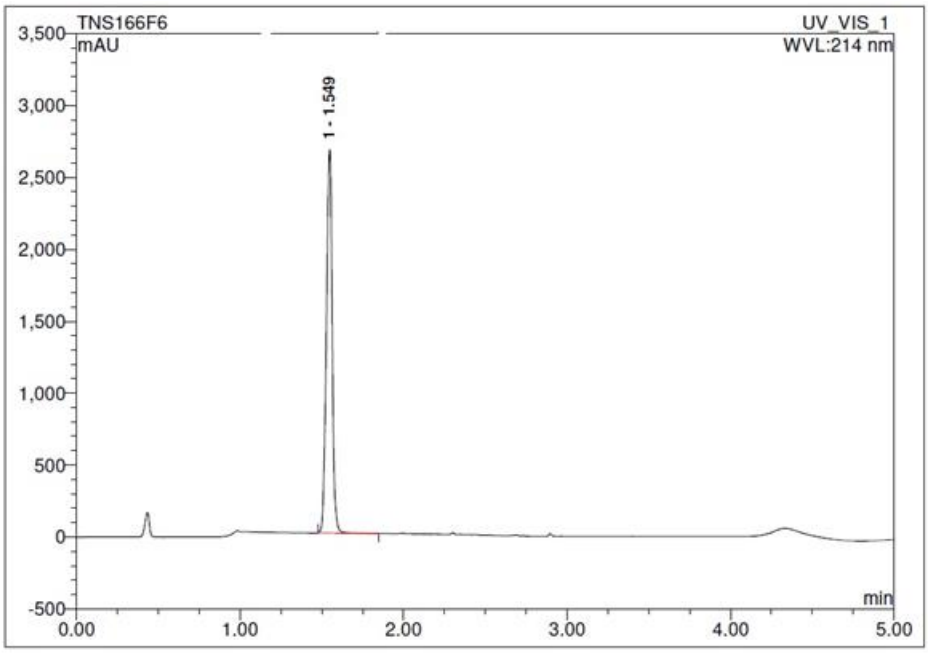


Mass spectrum, HRMS (NSI+)

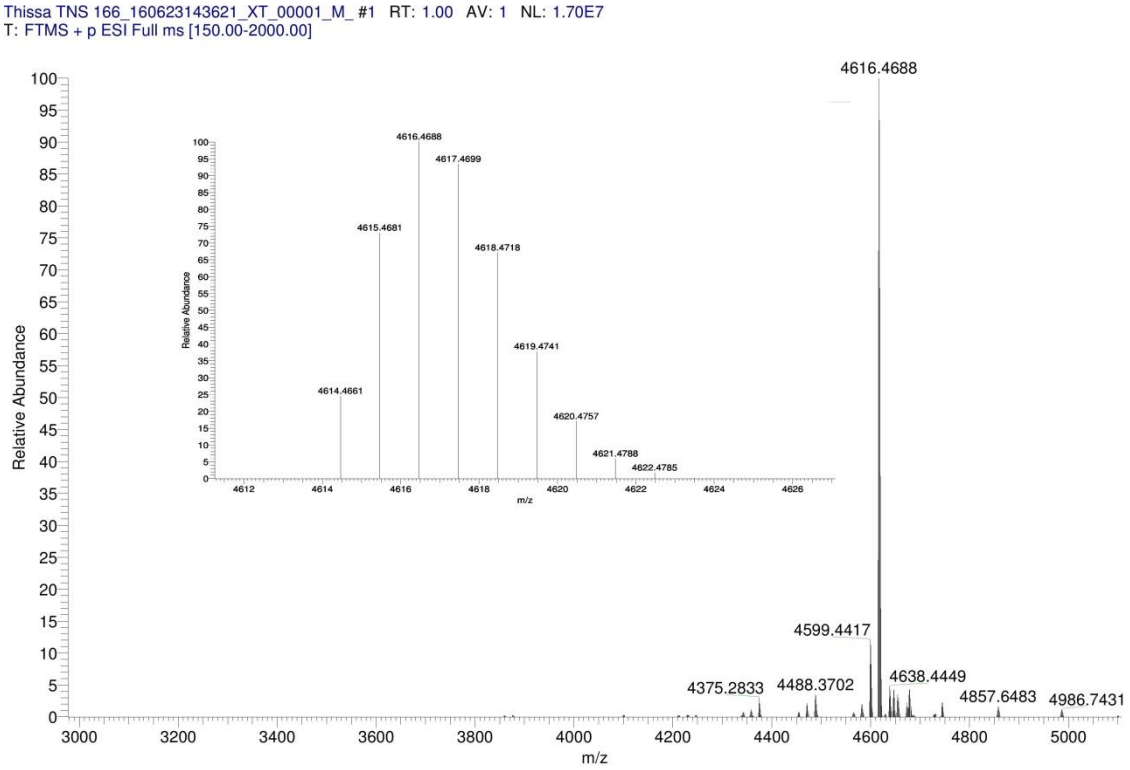


L-T25 (KL)$_8$(*K*KL)$_4$(*K*LL)$_2$*K*KLL. From Spheritide resin (455 mg, 0.19 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (26 mg, 6.5%). Anal. RP-HPLC: t_R = 1.55 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{228}H_{441}N_{59}O_{38}$ calc./found 4614.44/4614.47 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

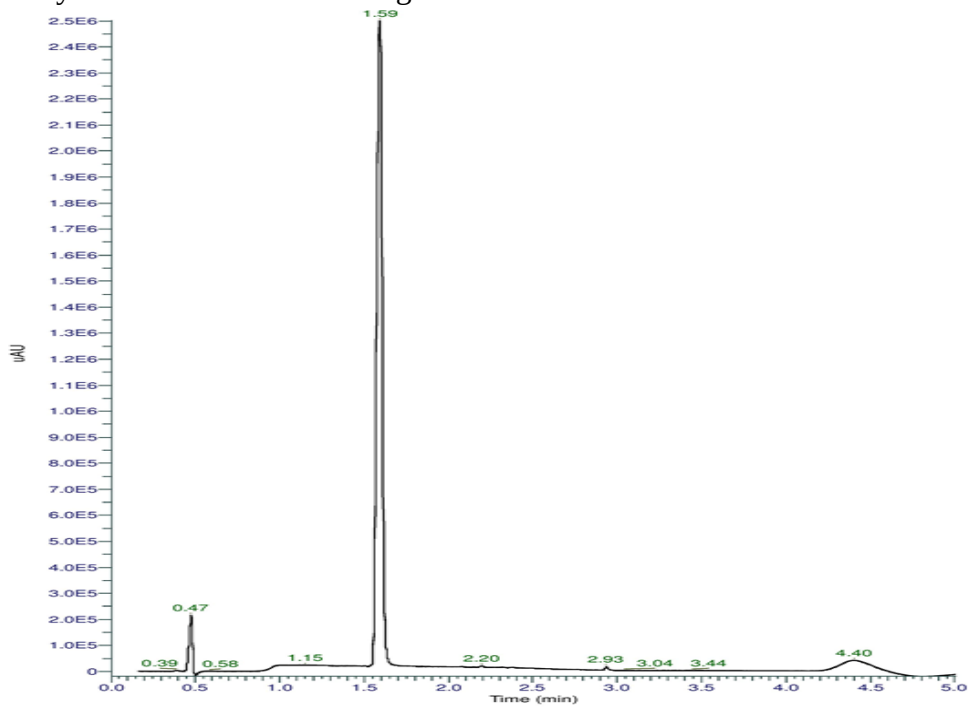


Mass spectrum, HRMS (NSI+)

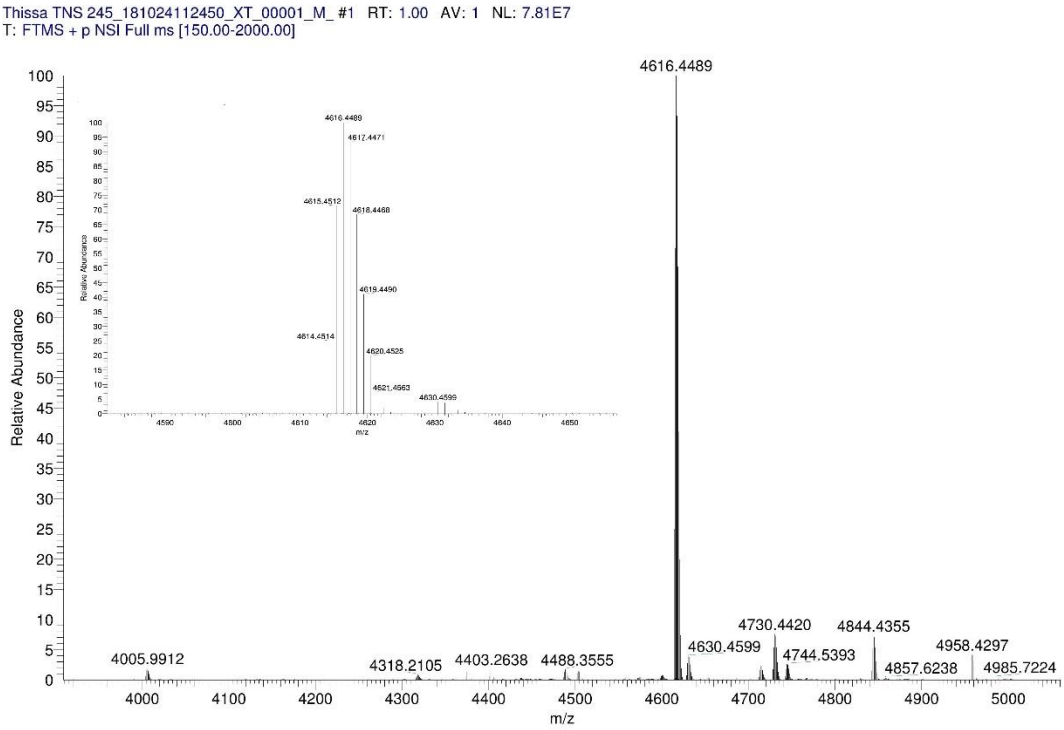


D-T25 (kl)$_8$(*k*kl)$_4$(*k*ll)$_2$*k*kll. From TentaGel resin (520 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (57.0 mg, 6.7%). Anal. RP-HPLC: t_R = 1.59 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{228}H_{441}N_{59}O_{38}$ calc./found 4614.44/4614.45 Da [M]+.

Analytical RP-HPLC chromatogram:

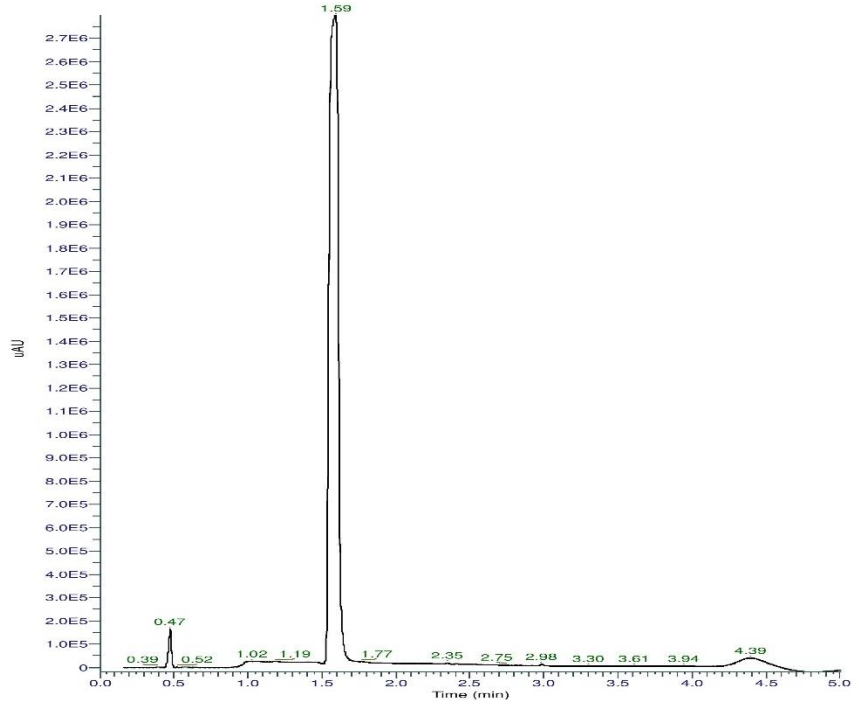


Mass spectrum, HRMS (NSI+)

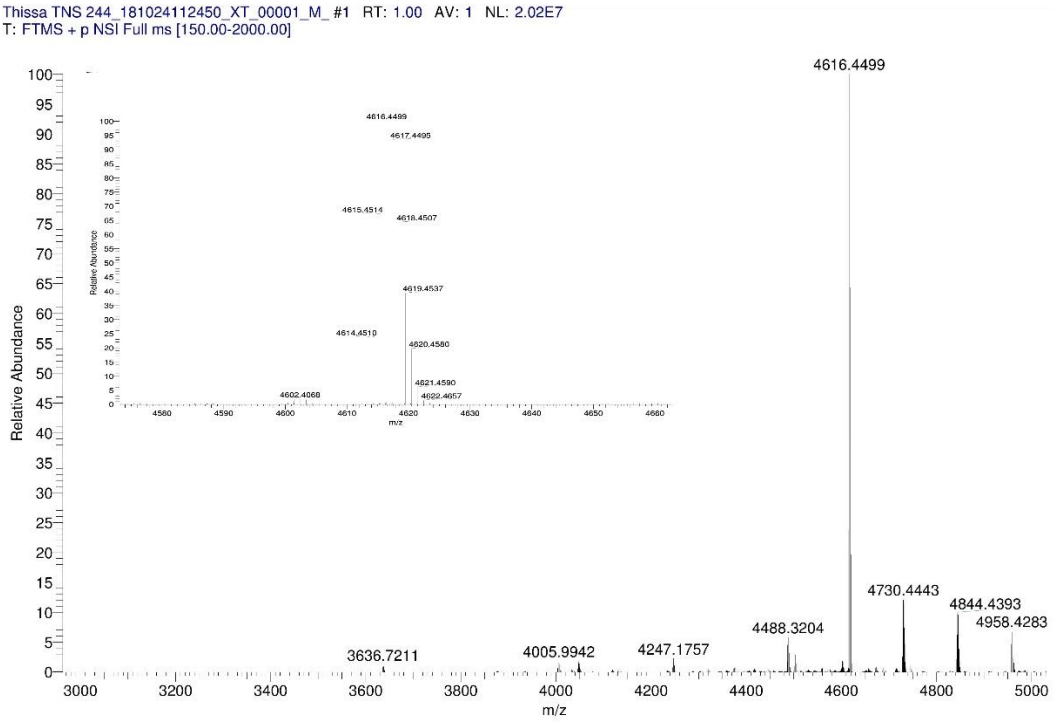


***sr*-T25** $(KL)_8(KKL)_4(KLL)_2KKLL$ (L:D 1:1). From TentaGel resin (10 g, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (3854.0 mg, 22.9%). Anal. RP-HPLC: t_R = 1.59 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{228}H_{441}N_{59}O_{38}$ calc./found 4614.44/4614.44 Da [M]+.

Analytical RP-HPLC chromatogram:

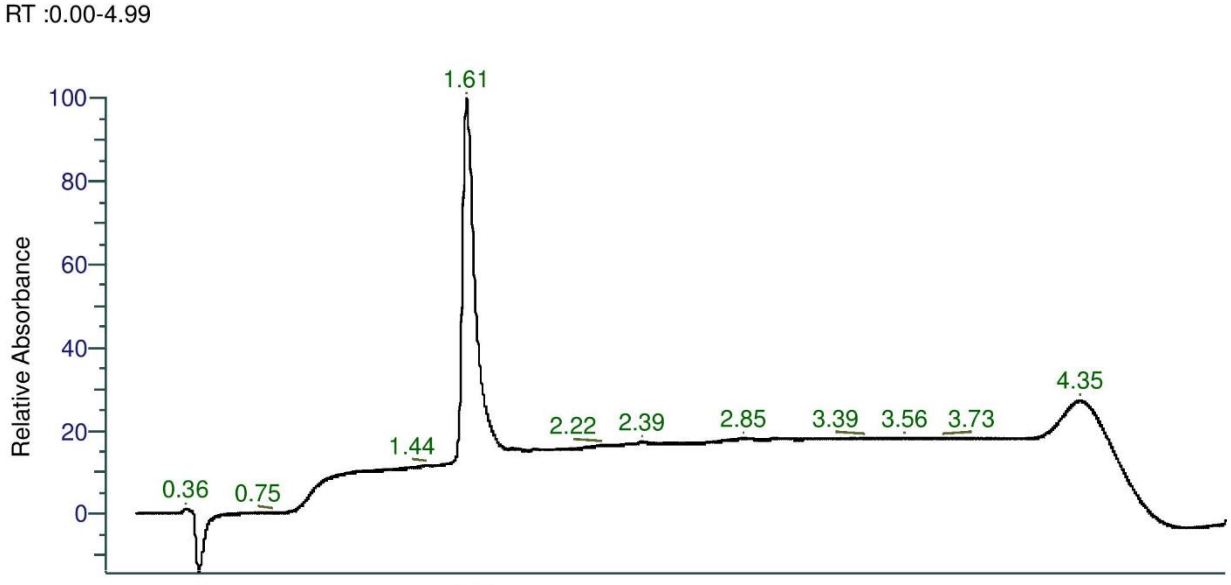


Mass spectrum, HRMS (NSI+)

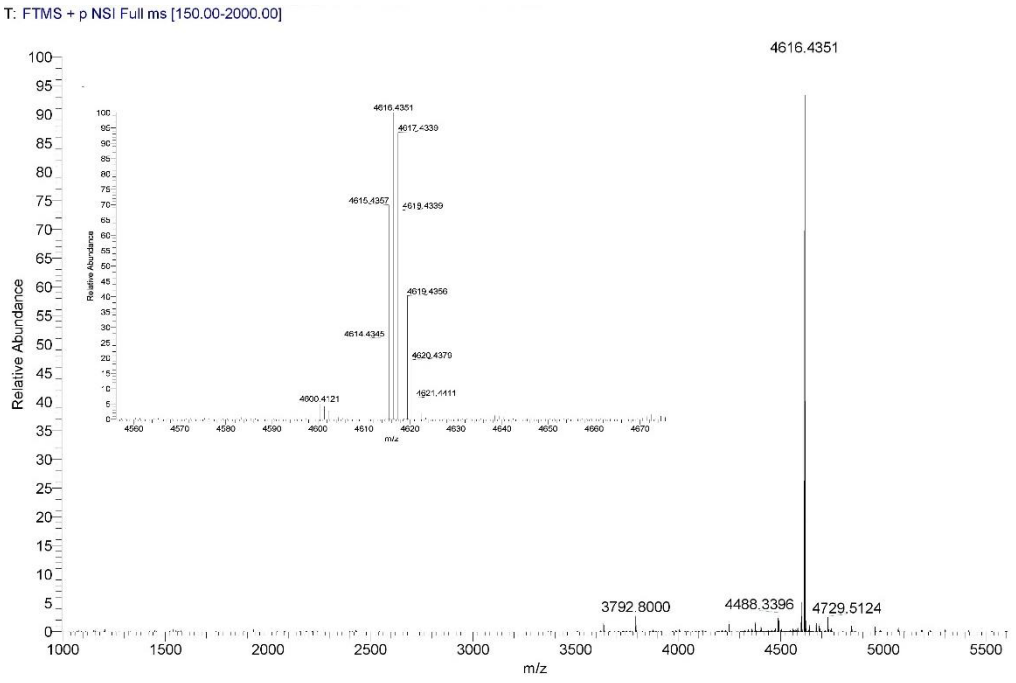


***sr*-T25a** (<u>KL</u>)$_8$(<u>*K*KL</u>)$_4$(*K*LL)$_2$*K*KLL (G2 and G3 stereorandomized). From Rink Amide AM resin (310 mg, 0.38 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (34.0 mg, 6.2%). Anal. RP-HPLC: t_R = 1.61 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{228}H_{441}N_{59}O_{38}$ calc./found 4614.44/4614.43 Da [M]+.

Analytical RP-HPLC chromatogram:

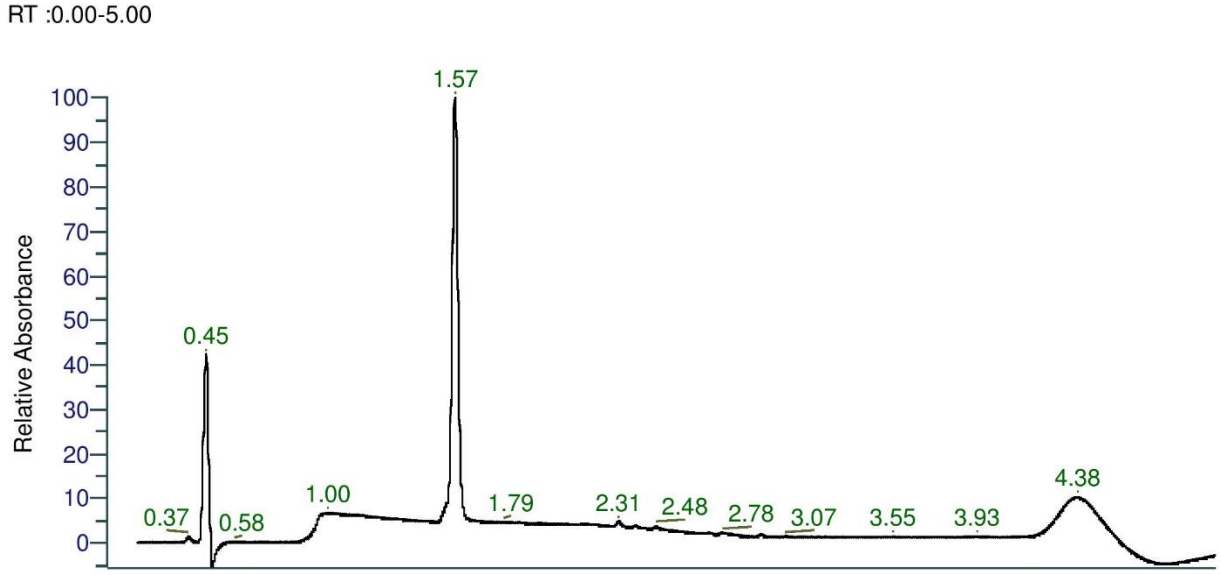


Mass spectrum, HRMS (NSI+)

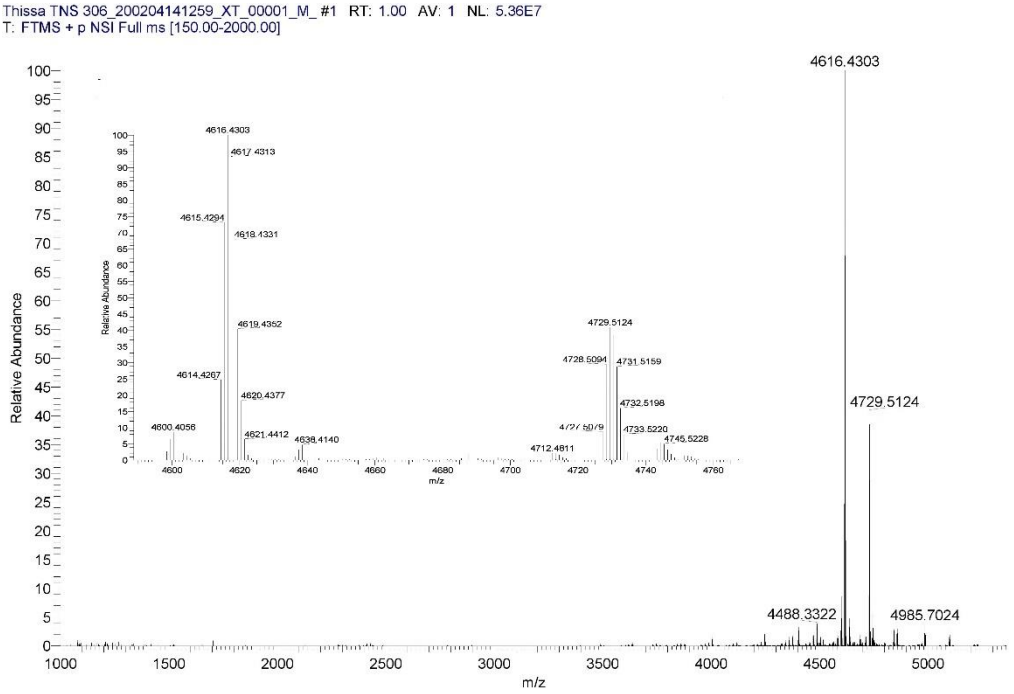


***sr*-T25b** (KL)$_8$(*K*KL)$_4$(<u>*K*LL</u>)$_2$<u>*K*KLL</u> (G0 and G1 stereorandomized) From Rink Amide AM resin (280 mg, 0.38 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (26.0 mg, 5.3%). Anal. RP-HPLC: t_R = 1.57 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{228}H_{441}N_{59}O_{38}$ calc./found 4614.44/4614.43 Da [M]+.

Analytical RP-HPLC chromatogram:

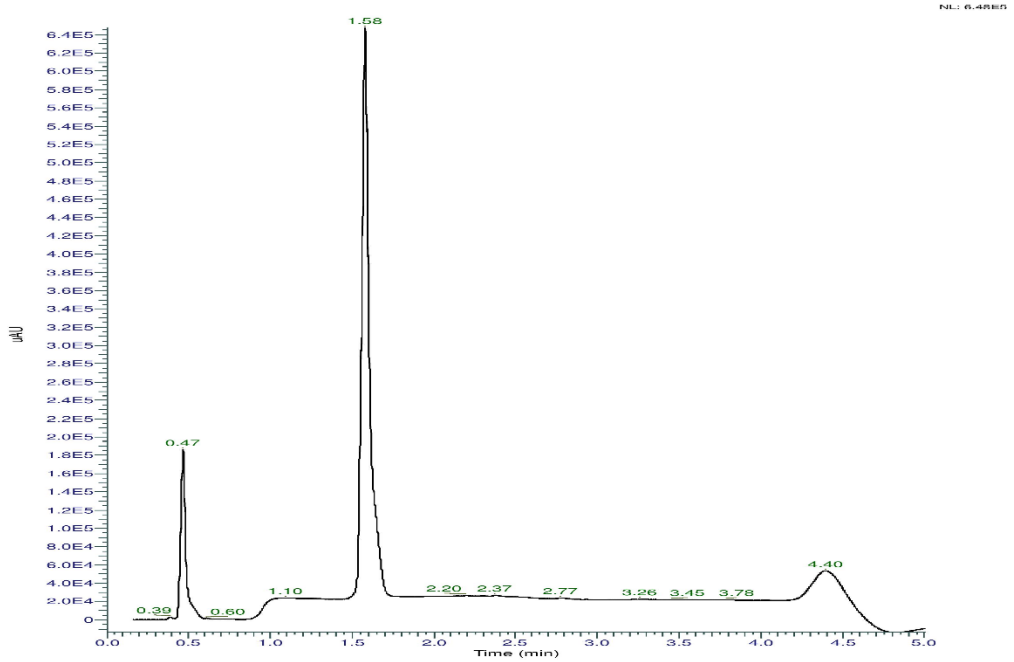


Mass spectrum, HRMS (NSI+)

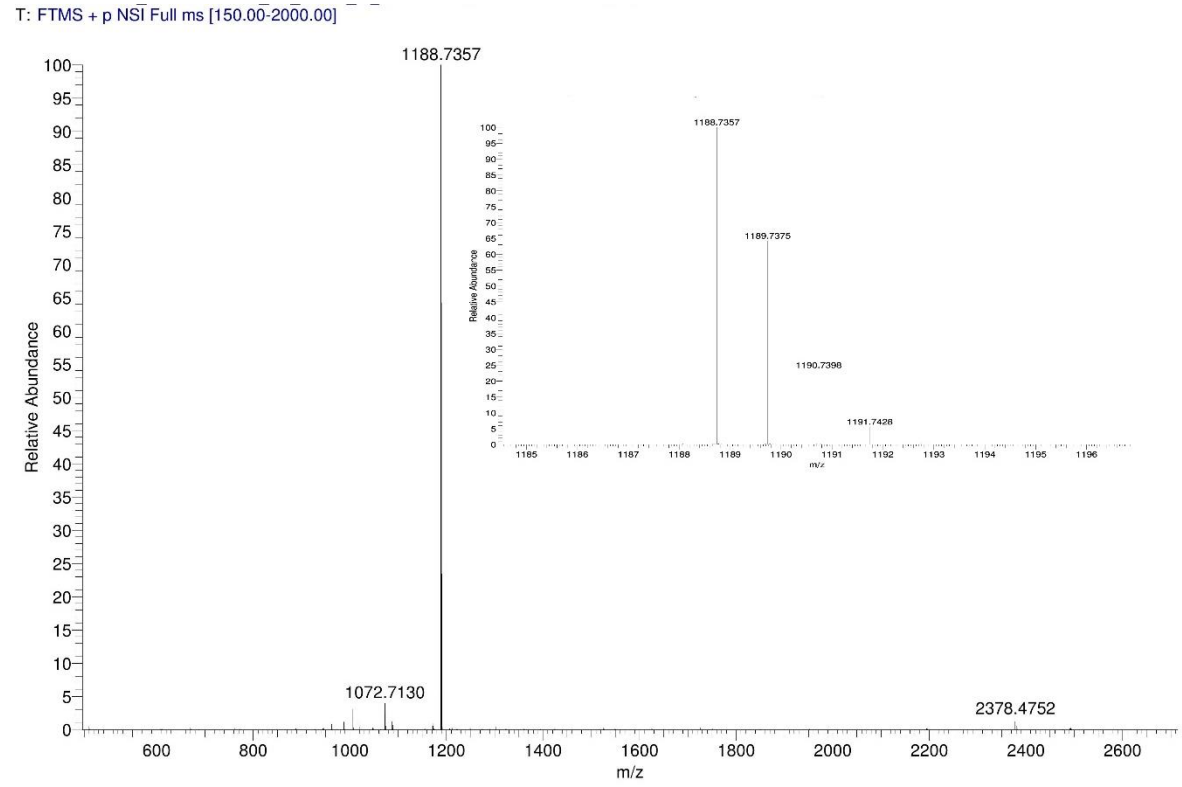


PMB2 6-methylheptanoic acid-Dab^{L}-Thr^{L}-Dab^{L}-Dab^{L}-Dab^{L}-Phe^{D}-Leu^{L}-Dab^{L}-Dab^{L}-Thr^{L}. From CTC resin (400 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (26.0 mg, 3 %). Anal. RP-HPLC: t_R = 1.58 min. (A/D = 100/0 to 0/100 in 2.2 min., λ= 214 nm). HRMS (NSI+): $C_{55}H_{96}N_{16}O_{13}$ calc./found 1188,73/1188,74 Da [M]+

Analytical RP-HPLC chromatogram:

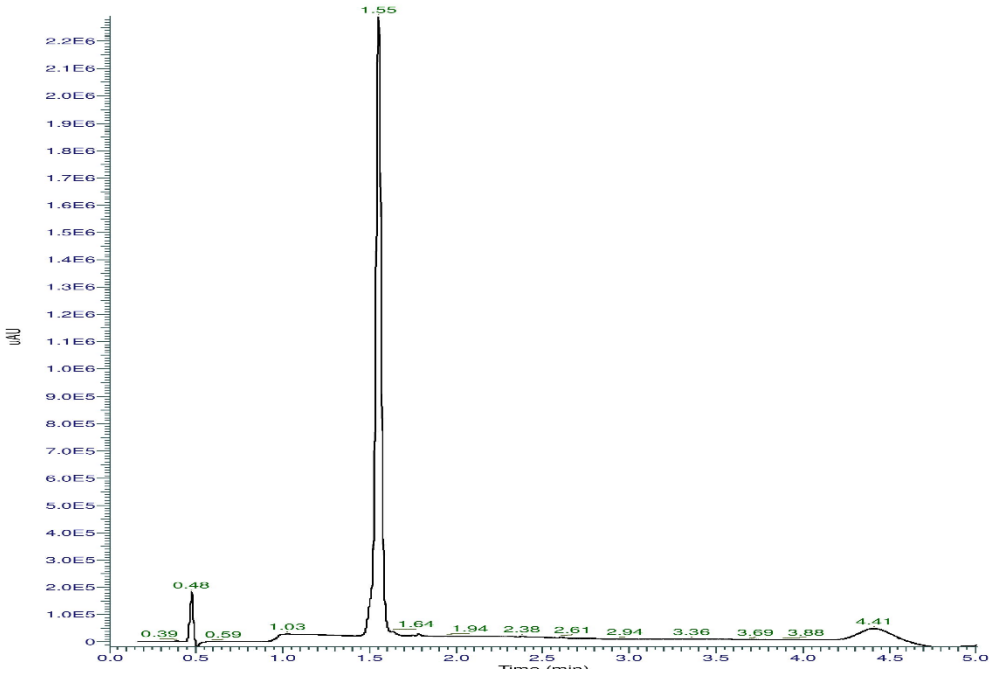


Mass spectrum, HRMS (NSI+)

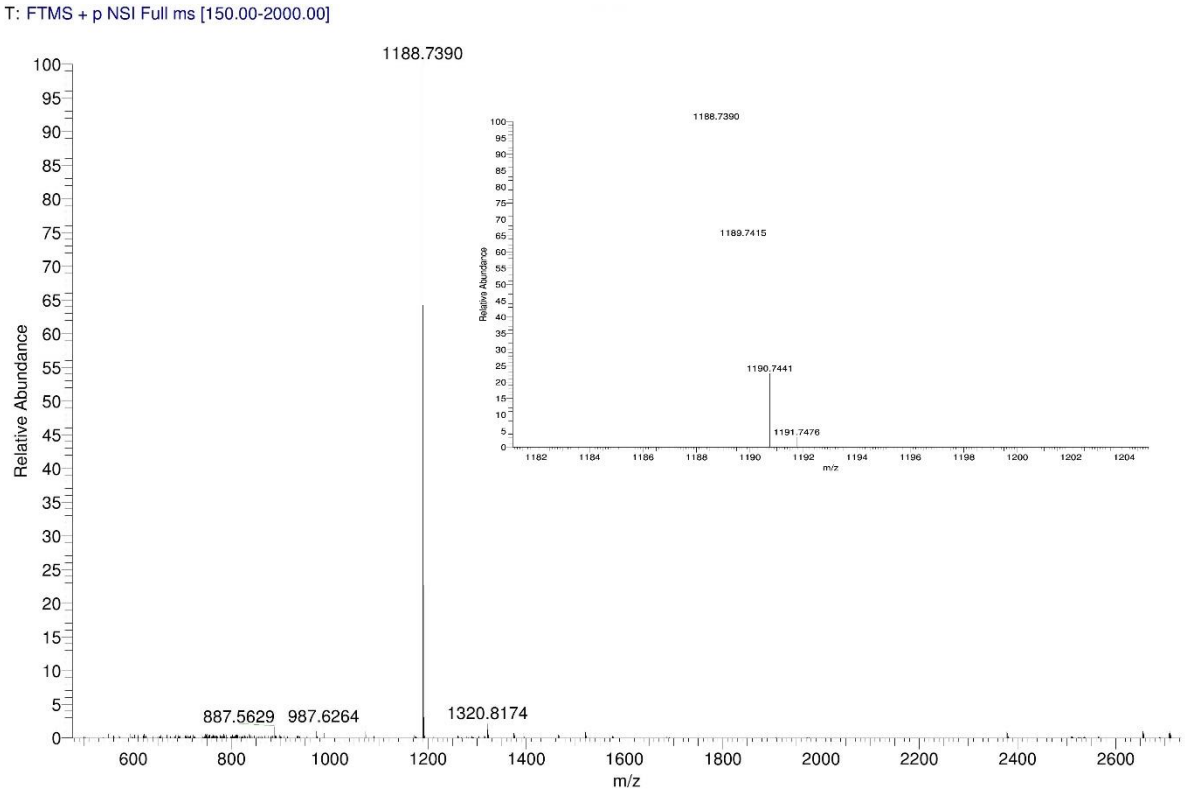


***sr*-PMB2** 6-methylheptanoic acid-Dab*-Thr*-Dab*-Dab*-Dab*-Phe*-Leu*-Dab*-Dab*-Thr* (*racemized positions). From CTC resin (500 mg, 1.2 mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (148.0 mg, 14%). Anal. RP-HPLC: t_R = 1.55 min. (A/D = 100/0 to 0/100 in 2.2 min., λ = 214 nm). HRMS (NSI+): $C_{55}H_{96}N_{16}O_{13}$ calc./found 1188,73/1188,74 Da [M]+

Analytical RP-HPLC chromatogram:

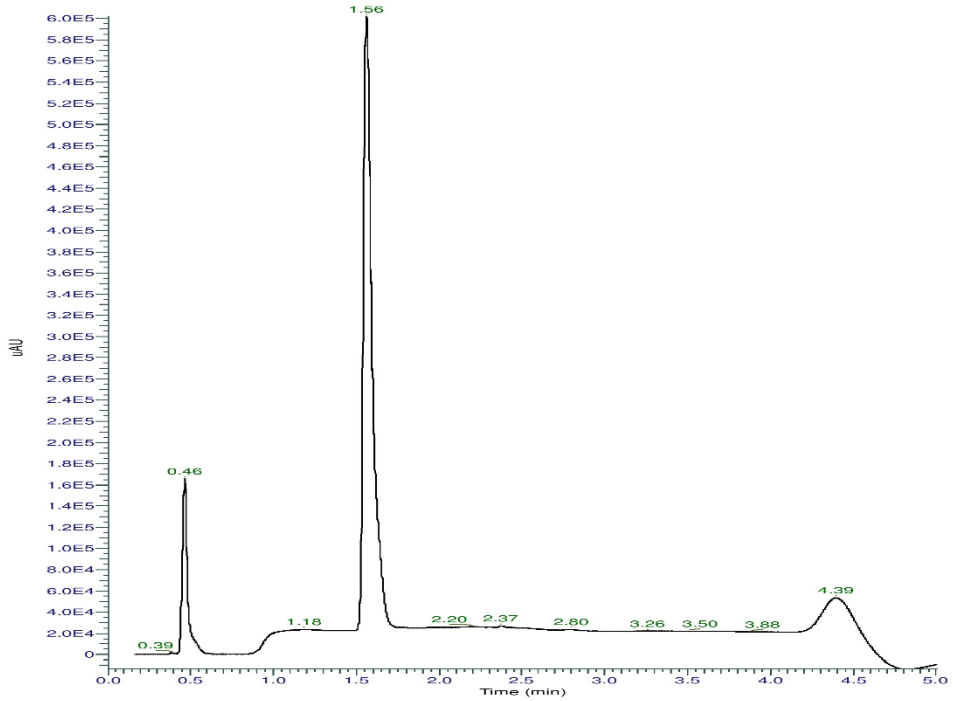


Mass spectrum, HRMS (NSI+)

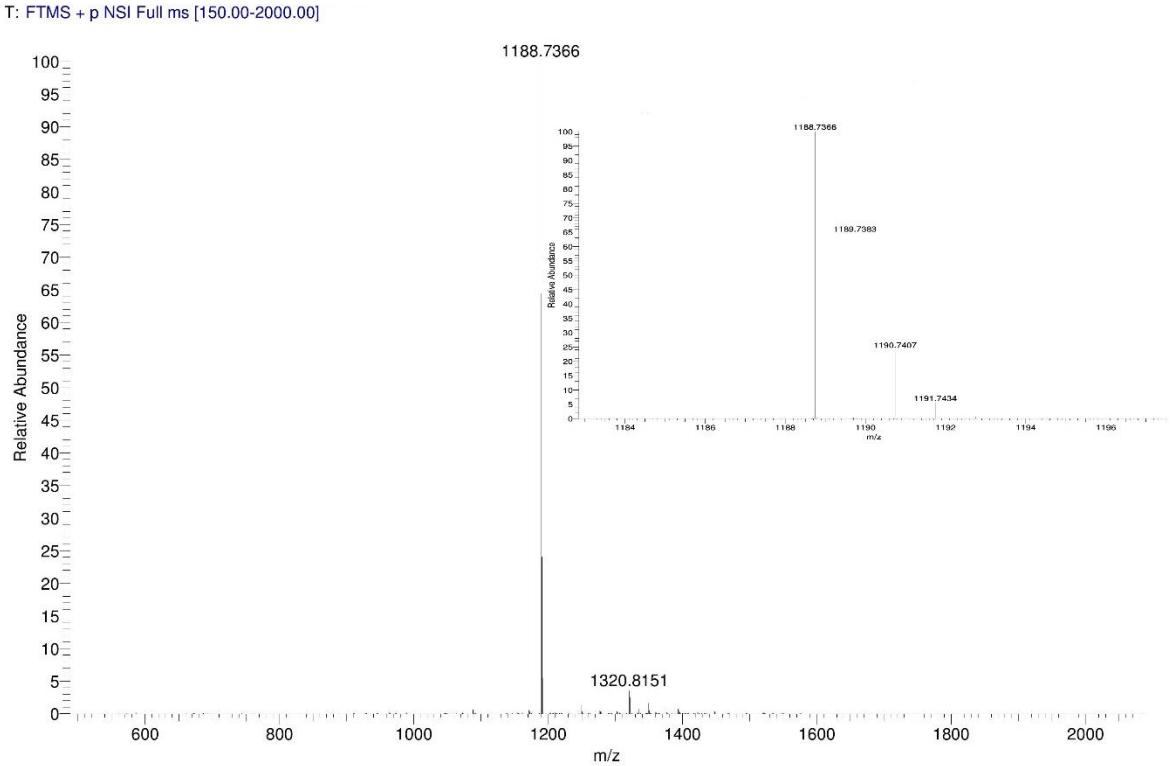


***sr*-PMB2a** 6-methylheptanoic acid-Dab*-Thr^{L}-Dab*-Dab*-Dab^{L}-Phe^{D}-Leu*-Dab^{L}-Dab*-Thr* (*racemized positions). From CTC resin (250 mg, 1.2 mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (24.0 mg, 5 %). Anal. RP-HPLC: t_R = 1.56 min. (A/D = 100/0 to 0/100 in 2.2 min., λ= 214 nm). HRMS (NSI+): $C_{55}H_{96}N_{16}O_{13}$ calc./found 1188,73/1188,74 Da [M]+

Analytical RP-HPLC chromatogram:

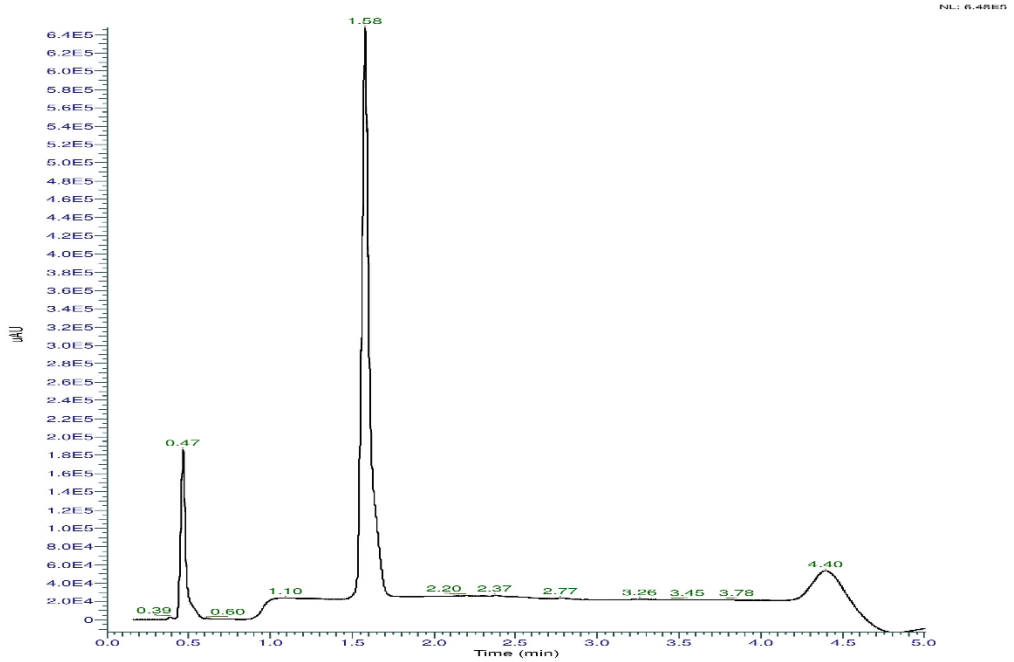


Mass spectrum, HRMS (NSI+)

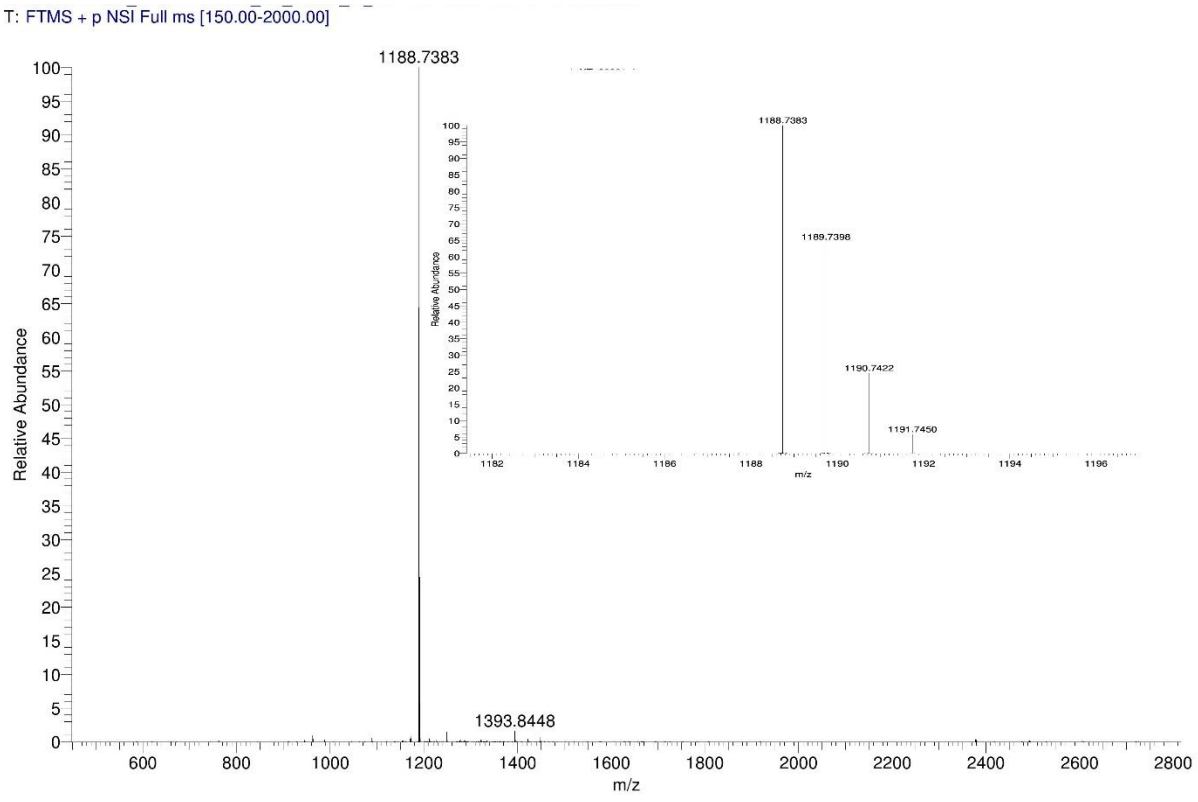


***sr*-PMB2b** 6-methylheptanoic acid-Dab^{L}-Thr*-Dab*-Dab*-Dab^{L}-Phe^{D}-Leu^{L}-Dab^{L}-Dab*-Thr*(*racemized positions). From CTC resin (350 mg, 1.2 mmol·g^{-1}), the peptide was obtained as a white foamy solid after preparative RP-HPLC purification (18.0 mg, 3 %). Anal. RP-HPLC: t_R = 1.58 min. (A/D = 100/0 to 0/100 in 2.2 min., λ= 214 nm). HRMS (NSI+): $C_{55}H_{96}N_{16}O_{13}$ calc./found 1188,73/1188,74 Da [M]+

Analytical RP-HPLC chromatogram:



Mass spectrum, HRMS (NSI+)



Broth Microdilution Assay

Broth Microdilution Method I

Antimicrobial activity was assayed against *Pseudomonas aeruginosa* (*P. aeruginosa* PAO1), *Acinetobacter baumannii* (*A. baumannii* ATCC19606), *Escherichia coli* (*E. coli* W3110), *Klebsiella pneumoniae* (*K. pneumoniae* NCTC418). To determine the minimal inhibitory concentration (MIC), broth microdilution method was used.[3] A colony of bacteria was grown in MH (Mueller Hinton) medium overnight at 37°C. The samples were prepared as stock solutions of 8 $mg \cdot mL^{-1}$ in H_2O, diluted to the initial concentration of 64 or 128 $\mu g \cdot mL^{-1}$ in 300 µL MH-medium, added to the first well of 96-well microtiter plate (TPP, untreated) and diluted serially by ½. The concentration of the bacteria was quantified by measuring absorbance at 600 nm and diluted to OD_{600} = 0.022 in MH-medium. The sample solutions (150 µL) were mixed with 4 µL diluted bacterial suspension with a final inoculation of about of $5x10^5$ CFU. The plates were incubated at 37°C until satisfactory growth (~18 h). For each test, two columns of the plate were kept for sterility control (broth only) and growth control (broth with bacterial inoculums, no antibiotics). The MIC was defined as the lowest concentration of the peptide dendrimer that inhibited visible growth of the tested bacteria, as detected after treatment with MTT.

Broth Microdilution Method II

Antimicrobial activity was assayed against *Pseudomonas aeruginosa* (PEJ2.6, PEJ9.1, ZEM-1A, ZEM9A (multiple drug resistant)), *Acinetobacter baumannii* (*A. baumannii* ATCC19606), *Escherichia coli* (*E. coli* bla OXA-48), *Citrobacter freundii* (*C. freundii* bla OXA-48), *Enterobacter cloacae* (*E. cloacae* bla NDM), *Klebsiella pneumoniae* (*K. pneumoniae* bla OXA-48) (clinical isolates from Université de Genève / Centre Médical Universitaire). To determine the minimal inhibitory concentration (MIC), broth microdilution method was used.[3] A colony of bacteria was grown in MH (Mueller Hinton) medium overnight at 37°C. The samples were prepared as stock solutions of 8 $mg \cdot mL^{-1}$ in H_2O, diluted to the initial concentration of 64 or 128 $\mu g \cdot mL^{-1}$ in 300 µL MH-medium, added to the first well of 96-well microtiter plate (TPP, untreated) and diluted serially by ½. The concentration of the bacteria was quantified by measuring absorbance at 600 nm and diluted to OD_{600} = 0.0022 in MH-medium. The sample solutions (150 µL) were mixed with 4 µL diluted bacterial suspension with a final inoculation of about of $5x10^5$ CFU. The plates were incubated at 37°C until satisfactory growth (~18 h). For each test, two columns of the plate were kept for sterility control (broth only) and growth control (broth with bacterial inoculums, no antibiotics). The MIC was defined as the lowest concentration of the peptide dendrimer that inhibited visible growth of the tested bacteria.

Table S1. Activity of AMPD **T25** against multidrug resistant bacteria.[a)]

	ZEM9A	ZEM-1A	PEJ9.1	PEJ2.6	*A.baumannii* ATCC19606	*E. coli* bla OXA-48	*C. freundii* bla OXA-48	*E. cloacae* bla NDM	*K. pneumoniae* bla OXA-48
L-**T25**	8	8	16	8	8	8	8	16	16
D-**T25**	8	8	16	8	8	16	16	16	16
sr-**T25**	>64	8	32	8	8	8	8	16	16

a) The MIC in µg/mL, was determined in Muller-Hinton medium after incubation for 16-20 hours at 37 °C. The results represent two independent experiments performed in duplicate.

Biofilm Inhibition Assay

A colony of bacteria was grown in LB medium (Lysogeny broth) medium overnight at 37°C. 96-well sterile, U-bottomed polystyrene microtiter plates (TPP Switzerland) were prepared by adding 200 µL of sterile deionized water to the peripheral wells to decrease evaporation from test wells. Aliquots of 180 µL of culture medium (0.25% (w/v) nutrient broth no. 2, Oxoid) containing desired concentration of the test compound were added to the internal wells as triplicate of each compound. The concentration of the bacteria was quantified by measuring absorbance at 600 nm and diluted to OD_{600} = 1.0 in LB medium. Then bacteria were resuspended in culture medium (0.25% (w/v) nutrient broth no. 2, Oxoid). The sample solutions (180 µL) were mixed with 20 µL of bacterial suspension and plates were incubated in a humid environment for 24 hours at 37 °C. After that wells were washed twice with 200 µL sterile deionized water before incubated with 200 µL 0.25% (w/v) nutrient broth containing 0.5 mM WST-8 and 20 mM phenazine ethosulfate for 3 hours at 37 °C. Then absorbance was measured at 450 nm with a plate reader (SpectraMax250 from Molecular Devices).[4]

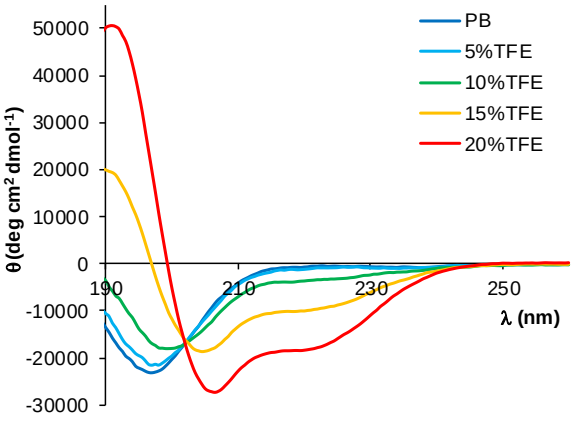
Hemolysis Assay

Minimum hemolytic concentration (MHC) was determined by serial dilution of a stock solution of 8 $mg \cdot mL^{-1}$ of the peptide dendrimers in H_2O. 50 µL of the solution was diluted serially by ½ with 50 µL of PBS (pH 7.4) in 96-well plate (Corning-Costar or Nunc, polystyrene, untreated) and 50 µL of blood sample, prepared as described below, was added resulting in final concentration range 2000 – 0.98 µg/mL). Human red blood cells (hRBC) were obtained from Interregionale Blutspende SRK AG, Bern and then centrifuged 1.5 mL of whole blood at 3000 rpm for 15 minutes. Plasma was discarded, and the pellet was re-suspended in 5 mL of PBS. The washing was repeated three times and the remaining pellet was re-suspended in 10 mL of PBS at a final hRBC concentration of 5%. The hRBC suspension (50 µL) was added to each well and the plate was incubated at room temperature for 4 hours. Minimal hemolytic concentration (MHC) end points were determined by visual inspection of the wells after the incubation period. Controls on each plate included a blank medium control (50 µL PBS + 50 µL of hRBC suspension) and a hemolytic activity control (mQ-deionized water 50 µL + 50 µL hRBC suspension).[5, 6]

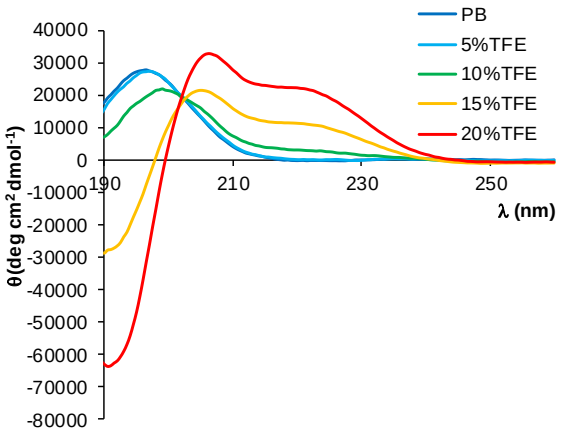
CD Spectroscopy

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 ® 100-QS 0.1 cm cuvette. Stock solutions (1.00 $mg \cdot mL^{-1}$) of dendrimer TFA salts were freshly prepared in mQ-deionized water. For the measurement, the peptides were diluted to 200 $\mu g \cdot mL^{-1}$ with phosphate buffer, pH = 7.4, 6 mM final and TFE (0, 5, 10, 15, 20%). The range of measurement was 190-260 nm, scan rate was 10 $nm \cdot min^{-1}$, pitch 0.5 nm, response 16 sec and band 1.0 nm. The nitrogen flow was kept above 8 $L \cdot min^{-1}$. The blank was recorded under the same conditions and subtracted manually. Each sample was subjected to two accumulations. The cuvettes were washed with 1M HCl, mQ-H_2O and PB buffer before each measurement.[7, 8]

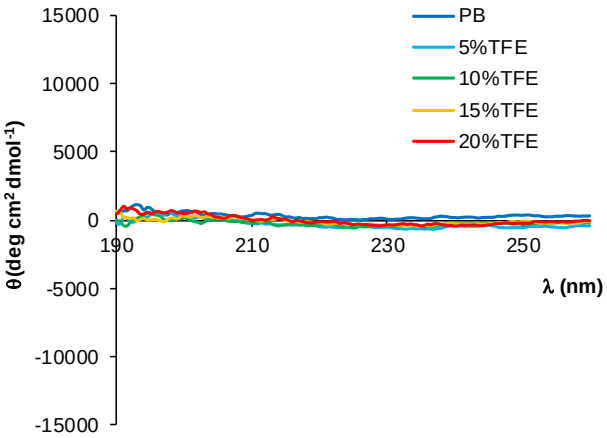
L-**SB1**



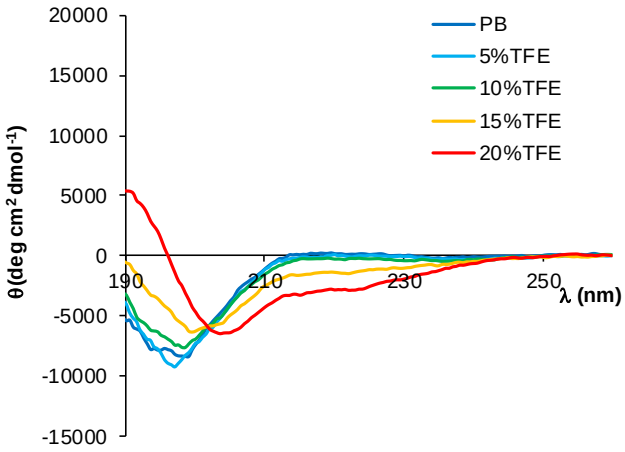
D-**SB1**



Sr-**SB1**



sr$^{3/1}$-**SB1**



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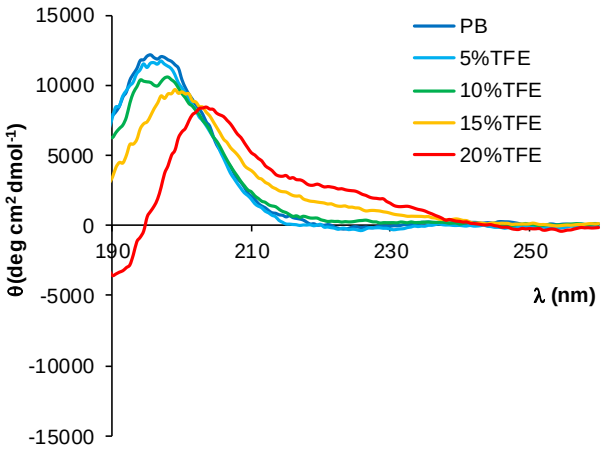
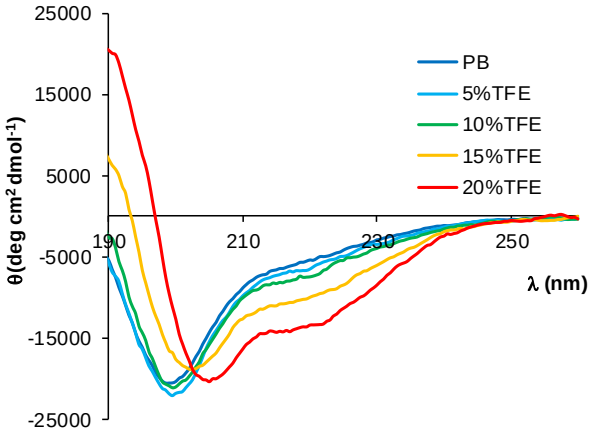


Figure S1: CD spectra of **SB1** analogs (200 μg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

L-**DJK5**



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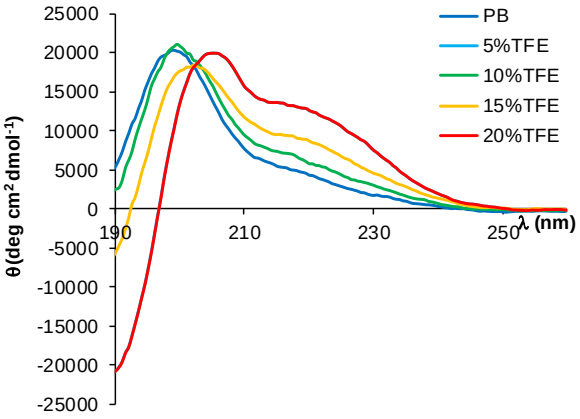


sr-**DJK5**

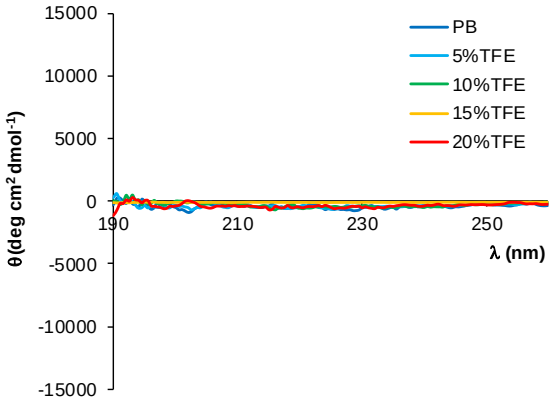
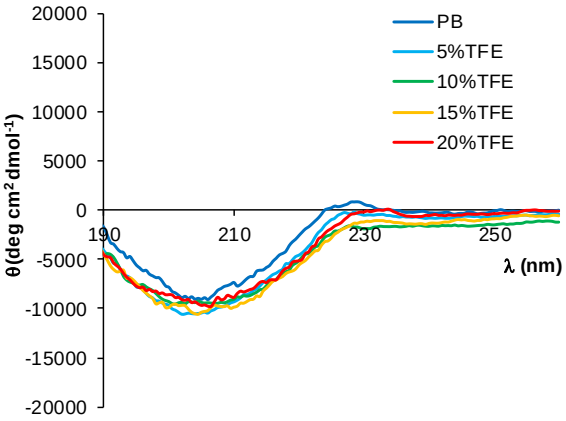
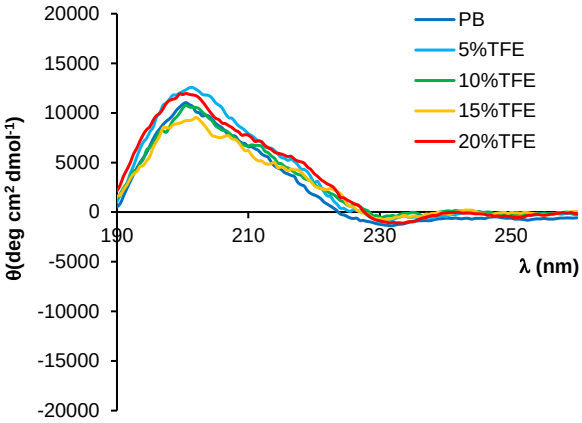


Figure S2: CD spectra of **DJK5** analogs (200 µg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

L-**Indo**



D-Indo



sr-**Indo**

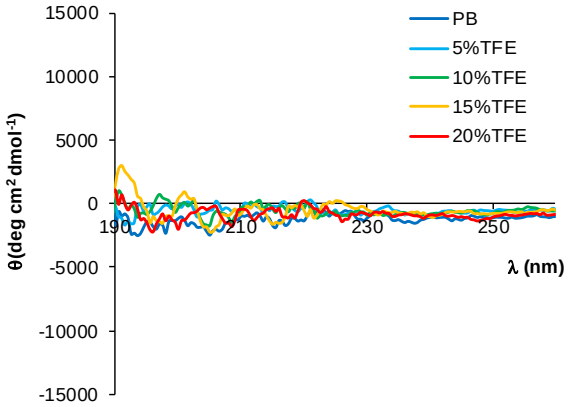
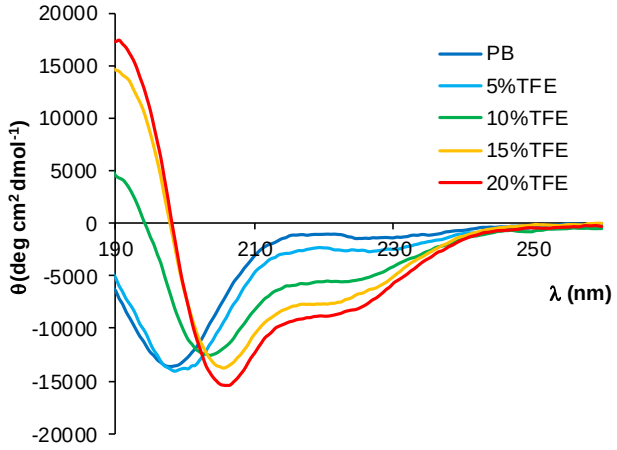
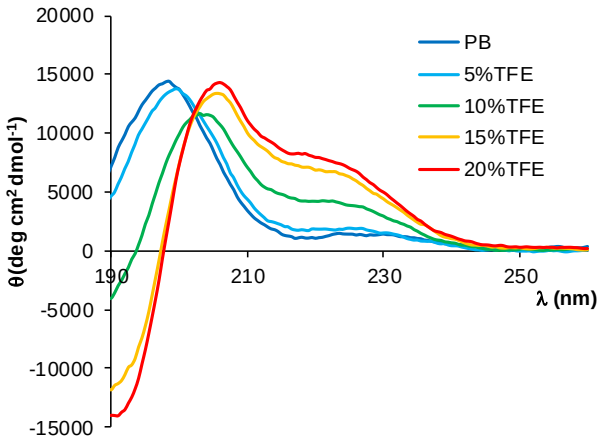


Figure S3: CD spectra of Indolicidin analogs (200 μg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

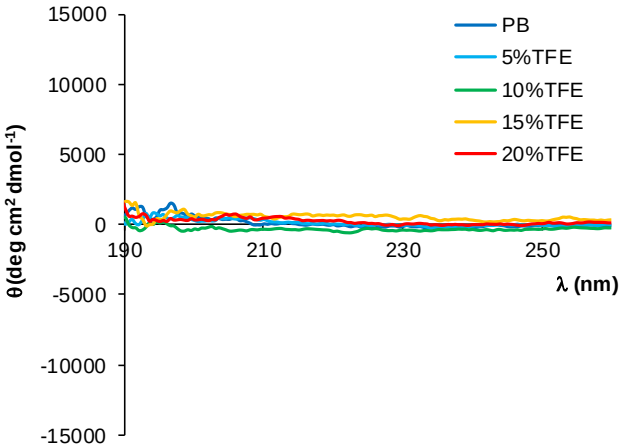
L-G3KL



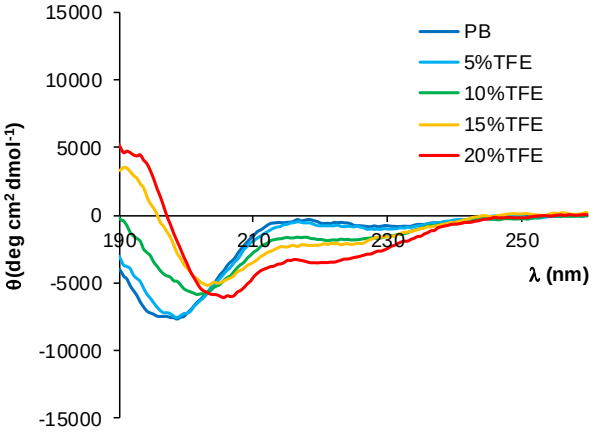
D-G3KL



***sr*-G3KL**



***sr*$^{3/1}$-G3KL**



***sr*$^{1/3}$-G3KL**

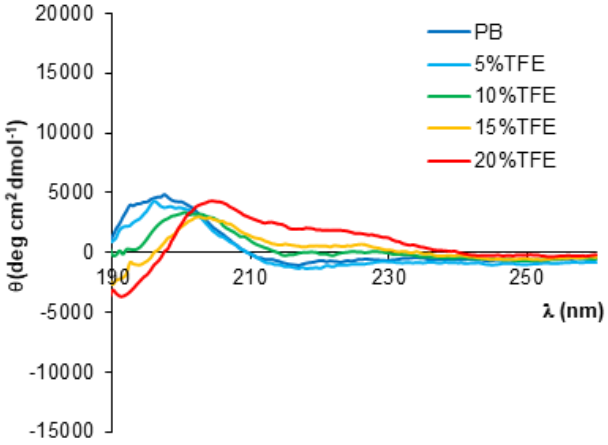
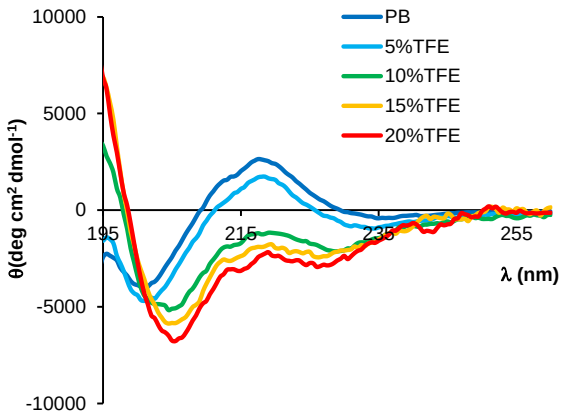
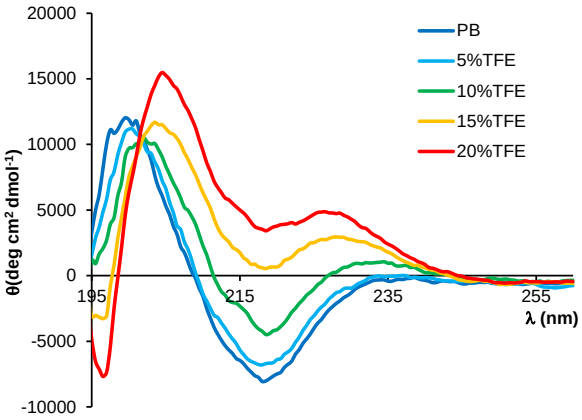


Figure S4: CD spectra of **G3KL** analogs (200 μg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

L-**TNS18**



D-TNS18



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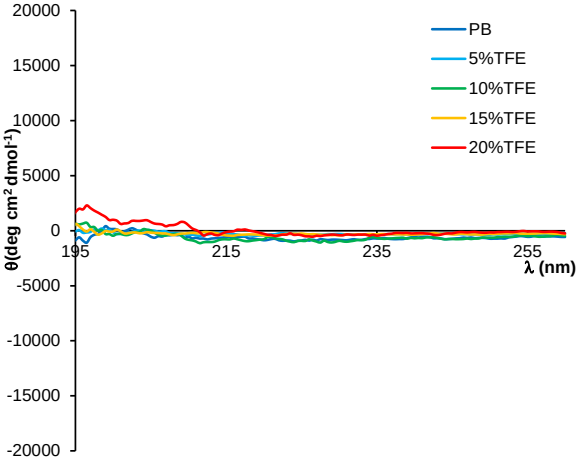
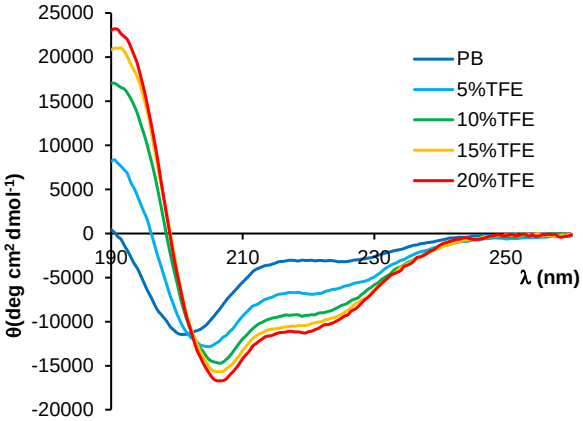


Figure S5: CD spectra of **TNS18** analogs (200 μg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

L-**T25**



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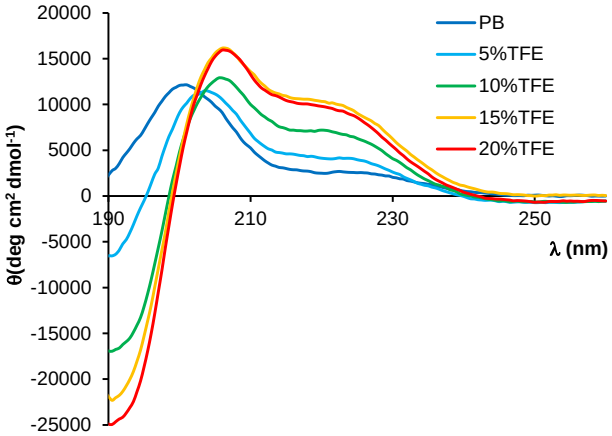

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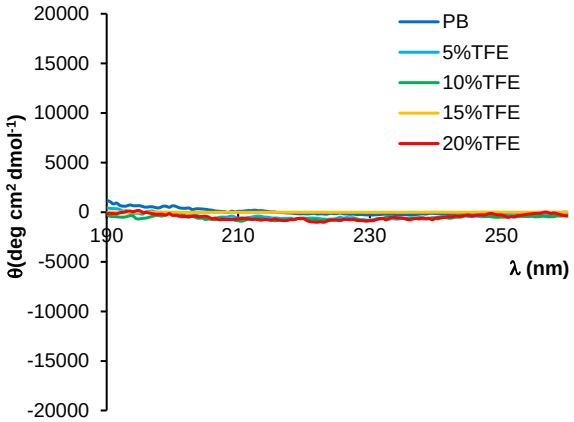
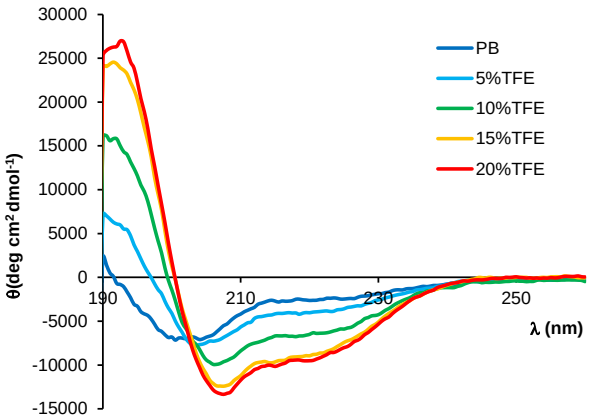


sr-**T25a**



sr-**T25b**

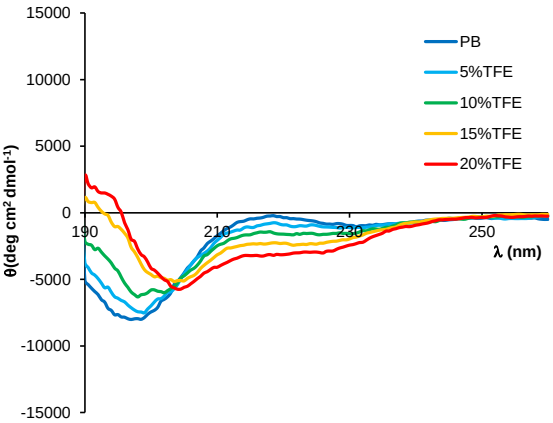


Figure S6: CD spectra of **TNS18** analogs (200 µg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

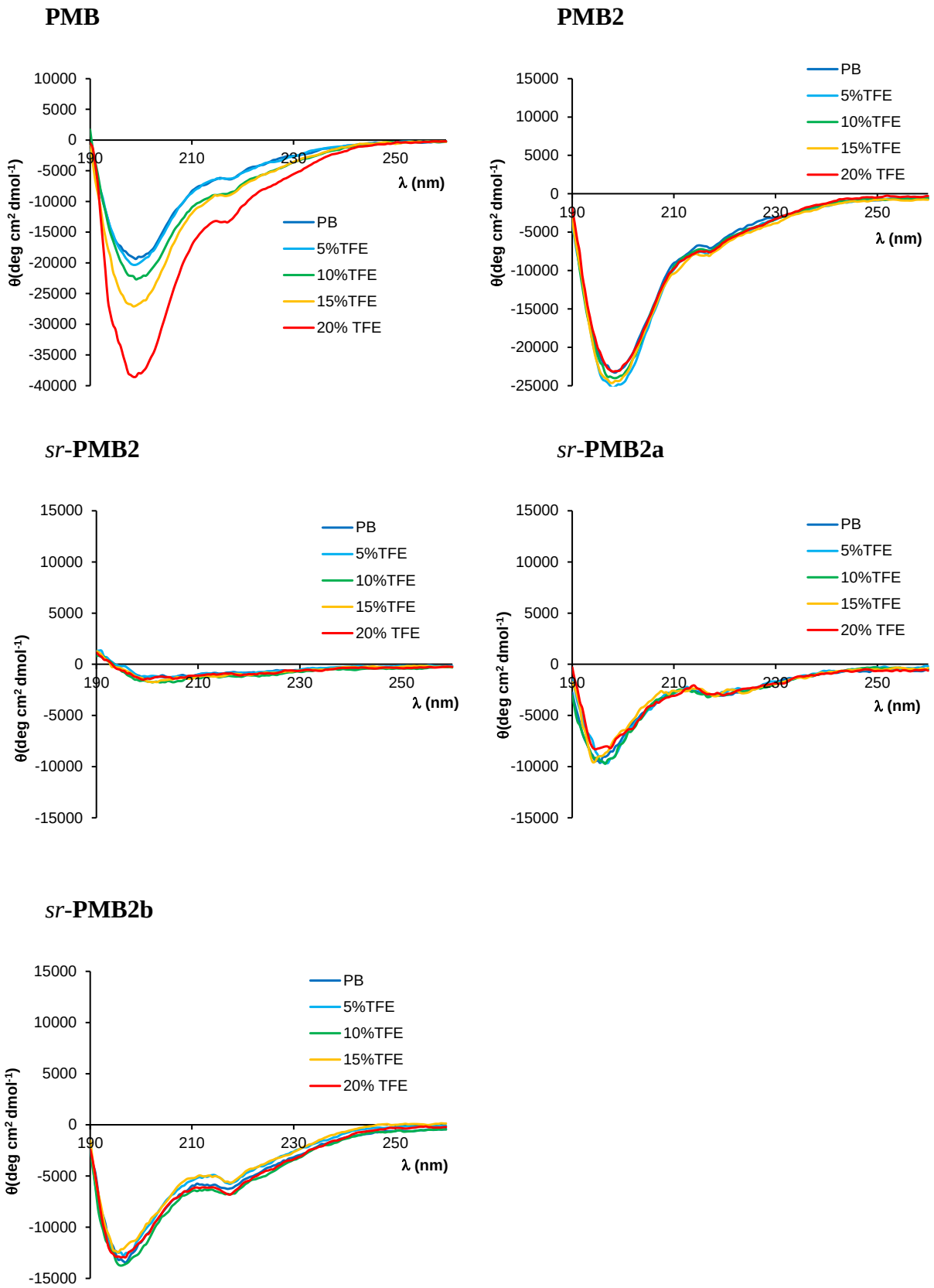


Figure S7: CD spectra of polymyxin B analogs (200 μg/mL) in aqueous phosphate buffer (pH 7.4) with different percentage of trifluoroethanol (TFE).

Serum Stability Assays

Peptides and peptide dendrimers were prepared as 400 µM stock solutions in 0.1 M Tris-HCl pH 7.5 buffer with 4-hydroxybenzoic acid as internal standard (100 $\mu g \cdot mL^{-1}$). 25% Human serum was prepared in 0.1 M Tris-HCl pH 7.5 buffer. Proteolysis was initiated upon addition of 50 µL of the test peptide or peptide dendrimer to 50 µL to human serum (25%) and shaking at 350 rpm and 37 °C. The final peptide concentration was 200 µM. The reactions were analyzed after 0, 1, 6, 12 and 24 hours after addition of 100 µL of 0.1 M $ZnSO_4$/acetonitrile (1:1) solution. The samples were cooled 10 min in ice and the supernatant was collected for each sample after centrifugation at 11,000 rpm for 10 minutes. Supernatants were then dried using speed vacuum. After dissolving the solid in 120 µL mQ-H_2O samples were centrifuged at 11,000 rpm for 10 minutes. Each sample was analyzed by RP-UPLC (flow rate: 1.2 $mL \cdot min^{-1}$. gradient: A/D=100/0 to 0/100 in 10 min). Conversions were calculated by quantification of the remaining peptide and peptide dendrimers determined by integration of the area of the chromatogram peak in analytical RP-HPLC.[9] Experiments were done in triplicates.

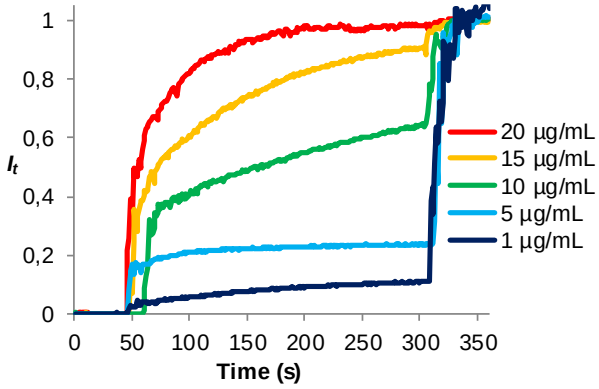
Vesicle Leakage Experiments

Egg PC (phosphatidyl choline/EYPC) or Egg PG (phosphatidyl glycerol/EYPG) thin lipid layer was prepared by evaporating a solution of 100 mg Egg PC or Egg PG 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 (50 mM CF, 10 mM TRIS, 107 mM NaCl, pH 7.4) for 30 min, subjected to freeze-thaw cycles (7x) and extrusion (15x) through a polycarbonate membrane (pore size 100 nm). Extra vesicular components were removed by gel filtration (Sephadex G-50) with 10 mM TRIS, 107 mM NaCl, pH 7.4 buffer. Final conditions: ~ 2.5 mM Egg PC or Egg PG; 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.

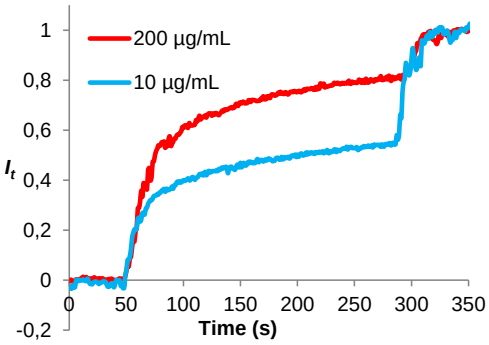
Egg PC or Egg PG vesicle stock solutions (37.5 µL) were diluted to ~3 mL with a buffer (10 mM TRIS, 107 mM NaCl, pH 7.4), placed in a thermostated fluorescence cuvette at 25 °C and gently stirred (final lipid concentration ~31 µM). CF efflux was monitored at λ_{em} 517 nm (λ_{ex} 492 nm) as a function of time. At t = 50 s of suitable volume of peptide dendrimer dissolved in mQ water to produce final concentrations of peptide dendrimer of 1, 5, 10, 15, 20, 200 $\mu g \cdot mL^{-1}$ was added. Finally, 1.2% Triton X-100 30 µL was added to cuvette (0.012% final concentration) at t = 300 s. Fluorescence intensities were normalized to fractional emission intensity It using $I_t = (I - I_0) / (I_\infty - I_0)$ where I is the measured fluorescence, I_0 = I at peptide dendrimer addition, I_∞ = I after complete lysis.[10]

L-**SB1**

EYPG

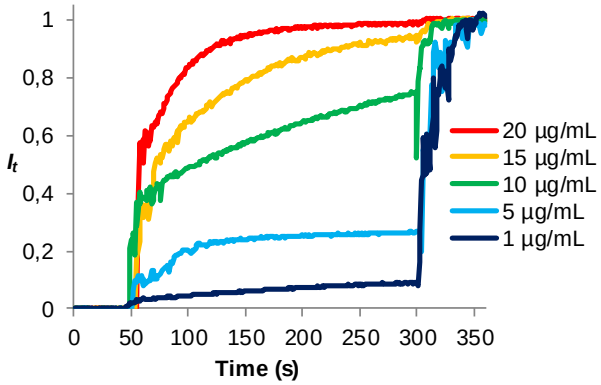


EYPC



D-**SB1**

EYPG



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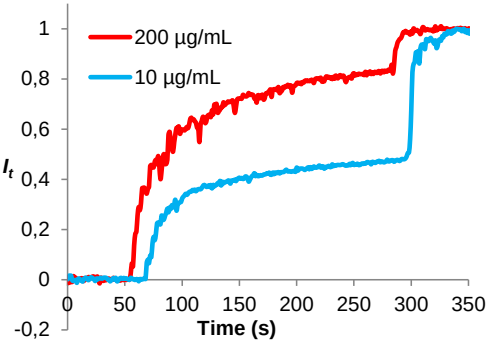
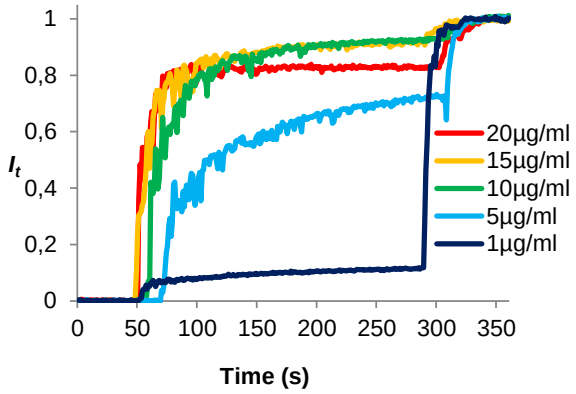
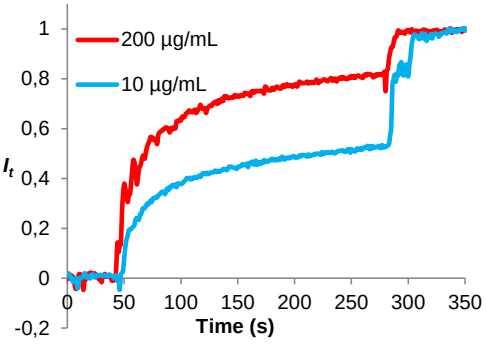


rac-**SB1**

EYPG

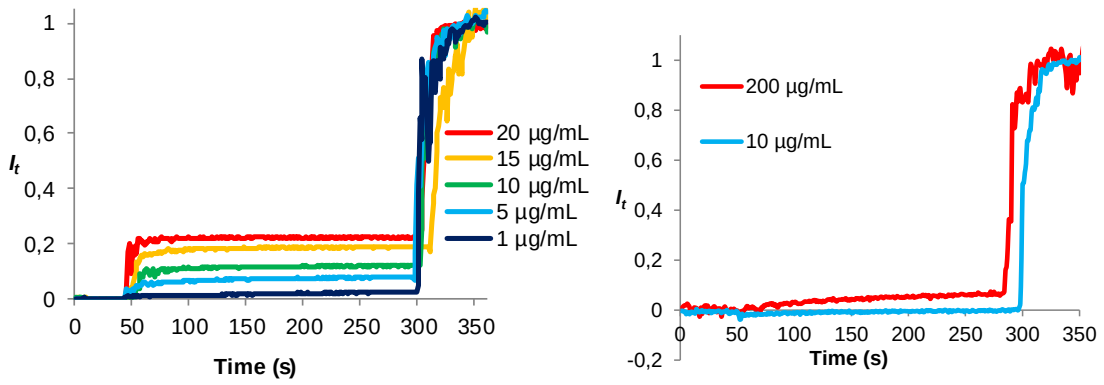


EYPC



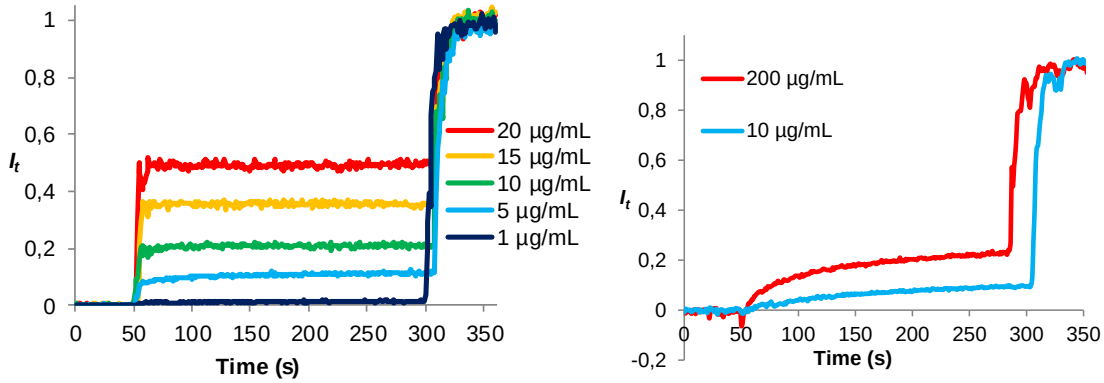
sr-**SB1**

EYPG **EYPC**



sr$^{3/1}$-**SB1**

EYPG **EYPC**



sr$^{1/3}$-**SB1**

EYPG **EYPC**

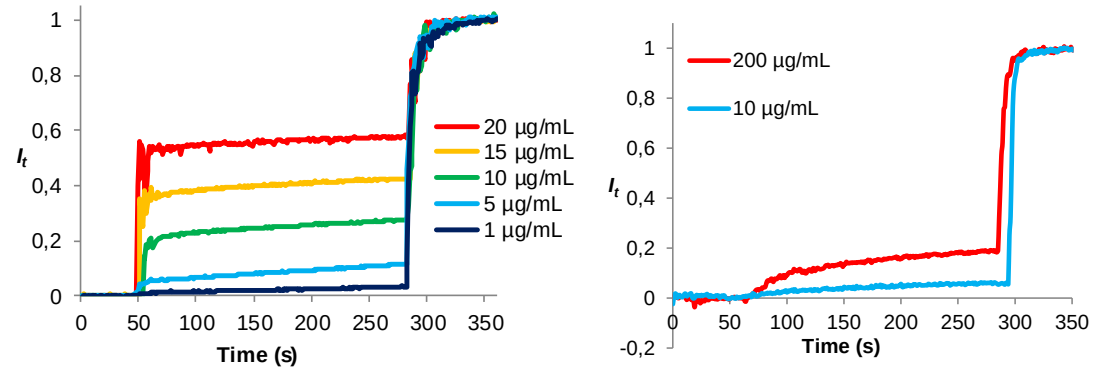
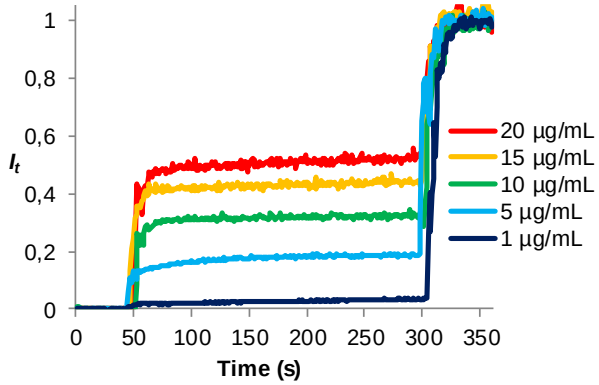


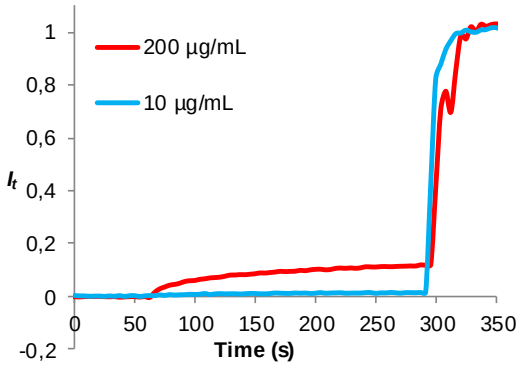
Figure S8: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by **SB1** analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

L-**DJK5**

EYPG

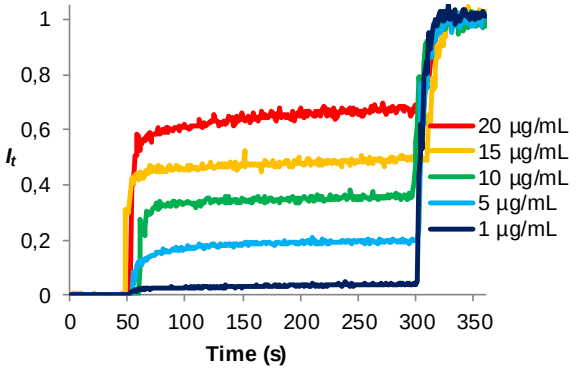


EYPC

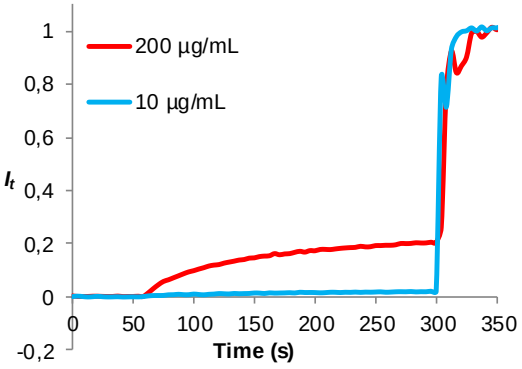


D-**DJK5**

EYPG

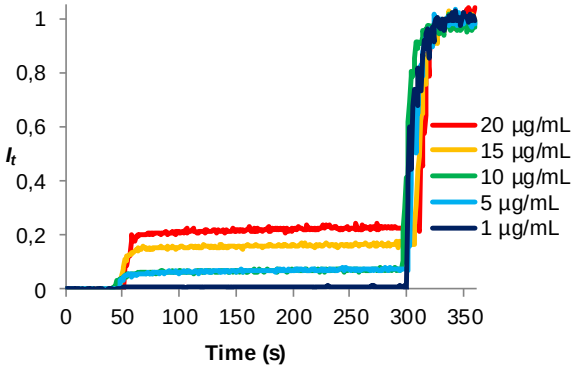


EYPC



sr-**DJK5**

EYPG



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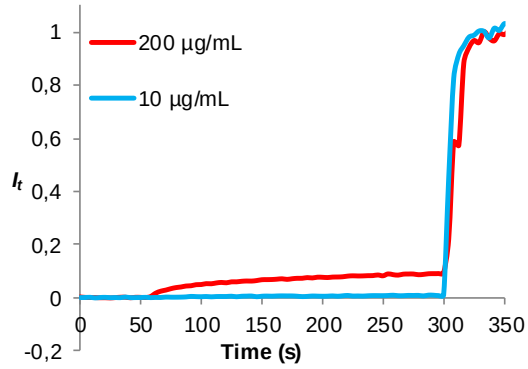
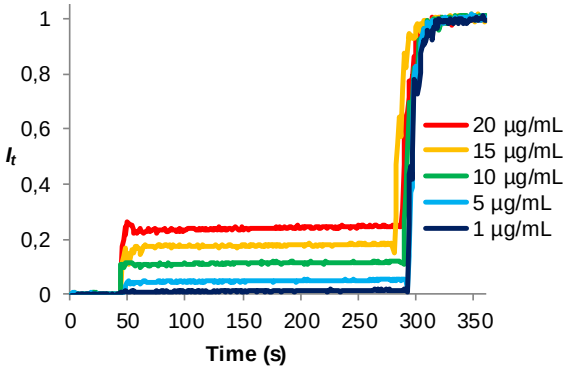


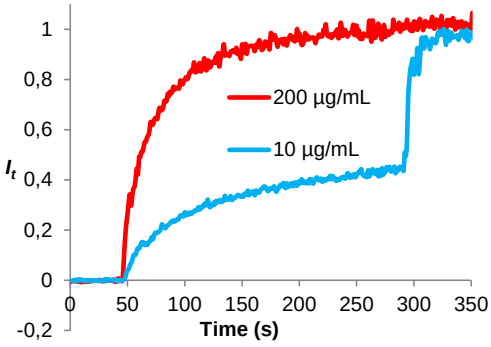
Figure S9: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by **DJK5** analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

L-**Indo**

EYPG

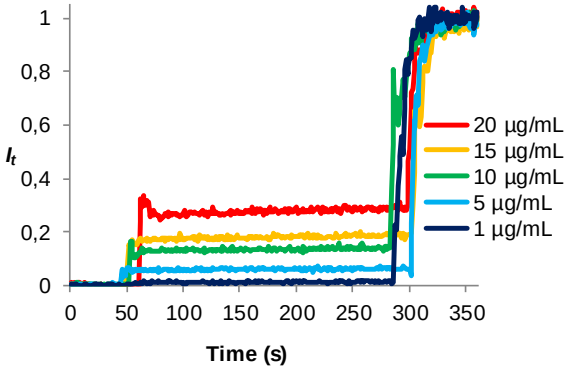


EYPC

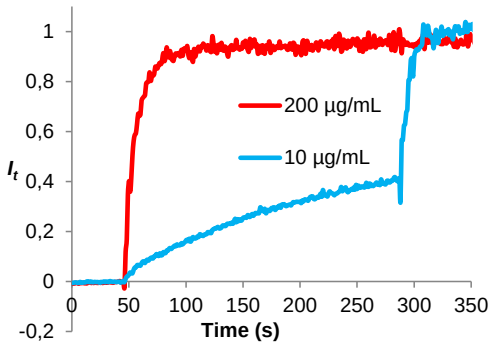


D-**Indo**

EYPG

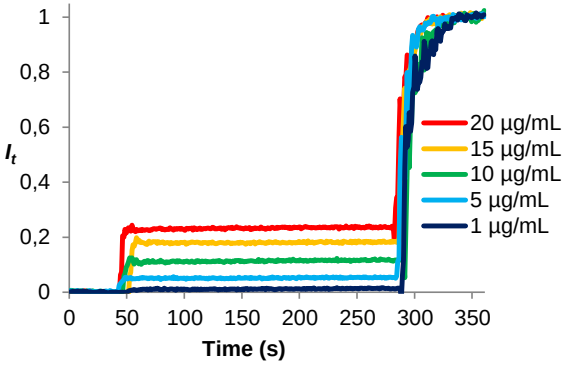


EYPC



sr-**Indo**

EYPG



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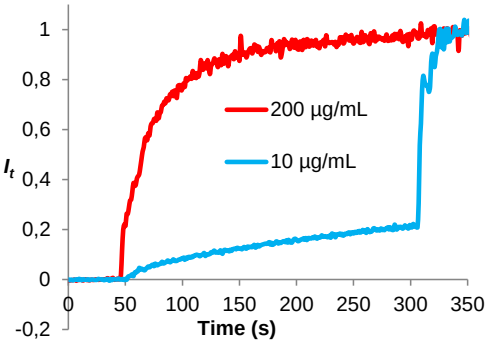
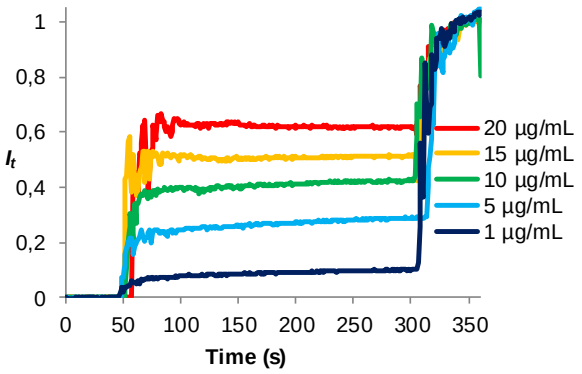


Figure S10: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by indolicidin analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

L-G3KL

EYPG



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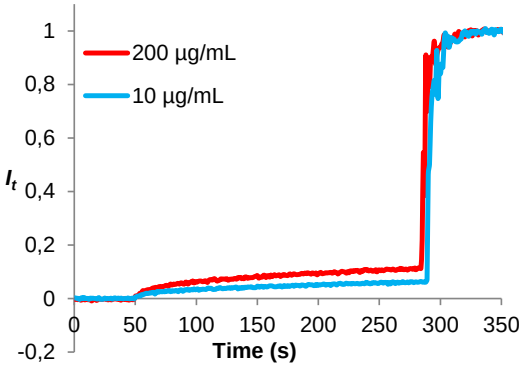
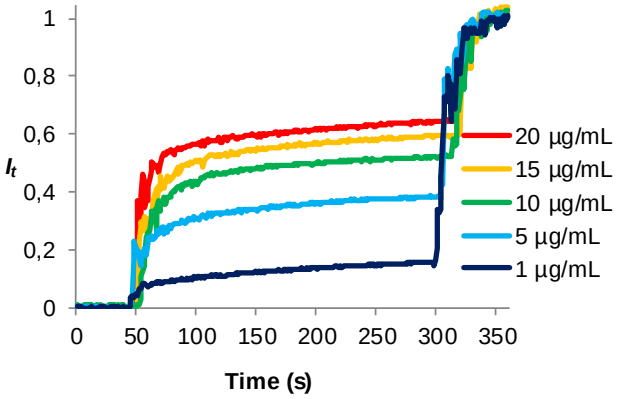


D-**G3KL**

EYPG



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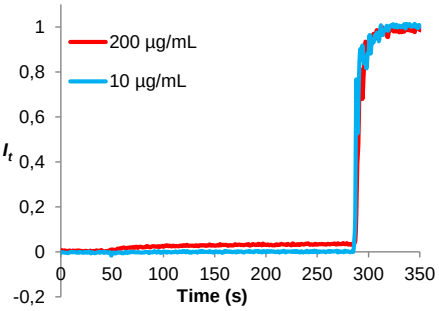
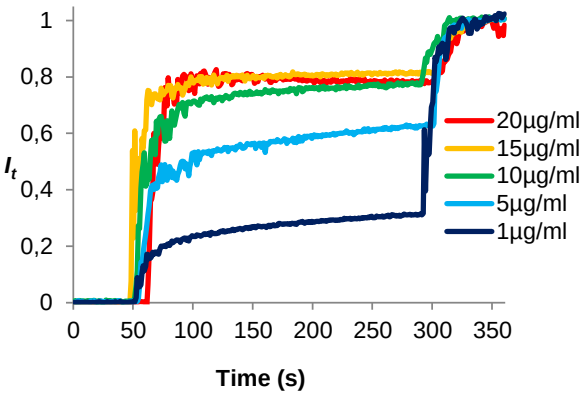


***rac*-G3KL**

EYPG



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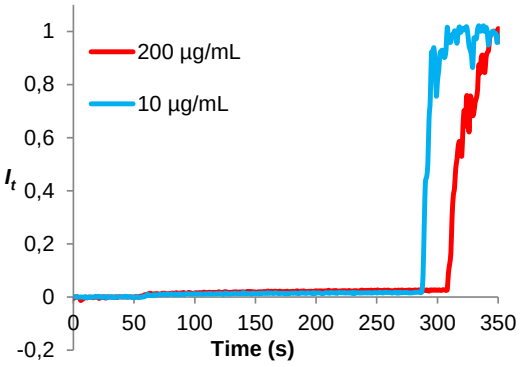

***sr*-G3KL**

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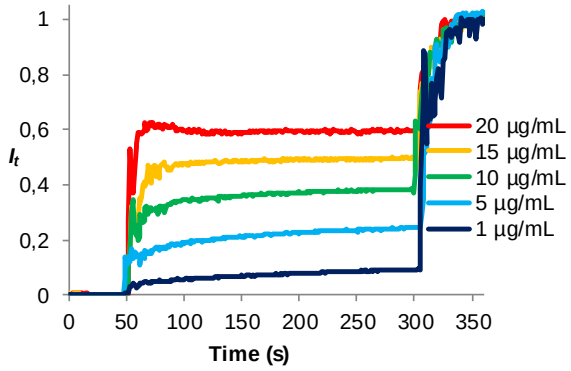

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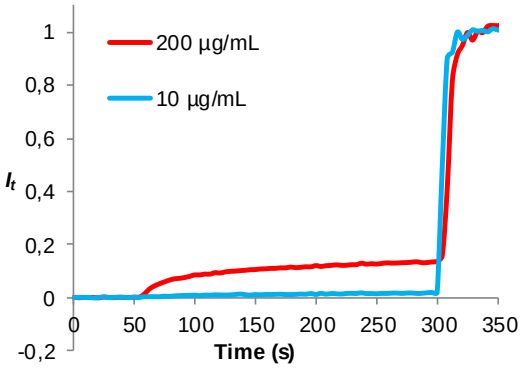
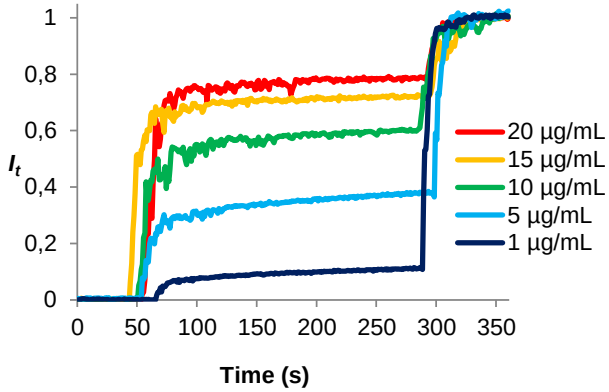


***sr*$^{3/1}$-G3KL**

EYPG



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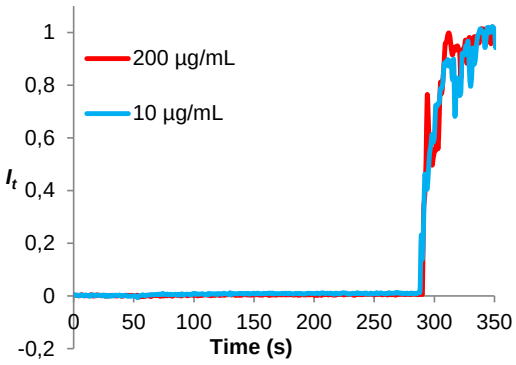

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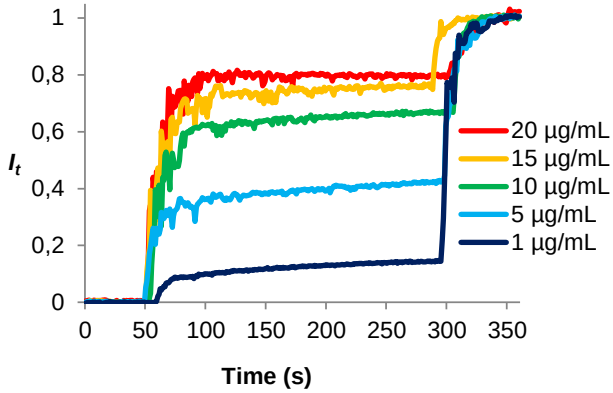

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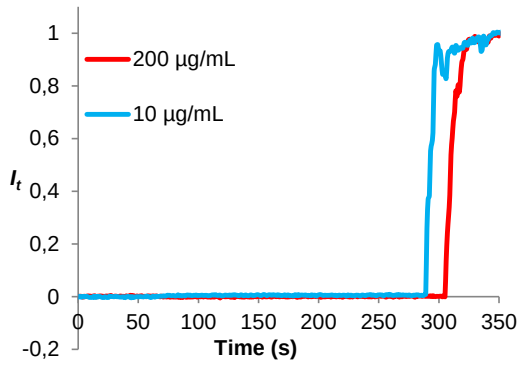
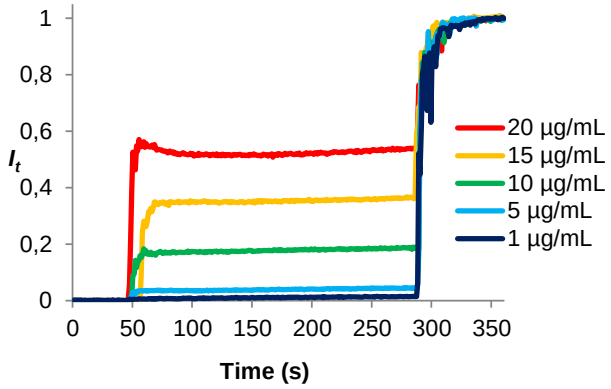


Figure S11: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by **G3KL** analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

L-**TNS18**

EYPG



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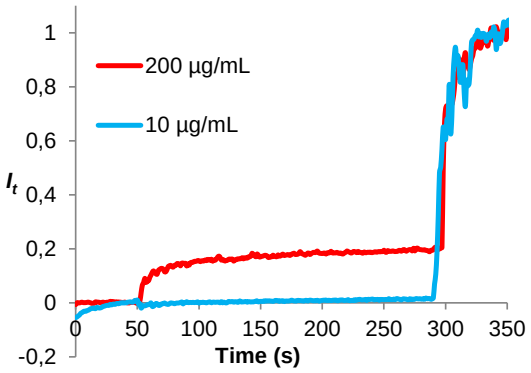
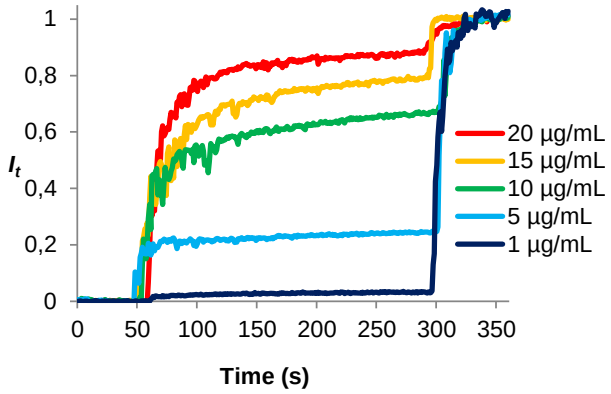


D-**TNS18**

EYPG



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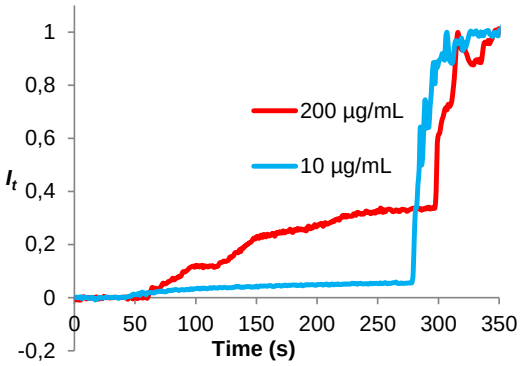
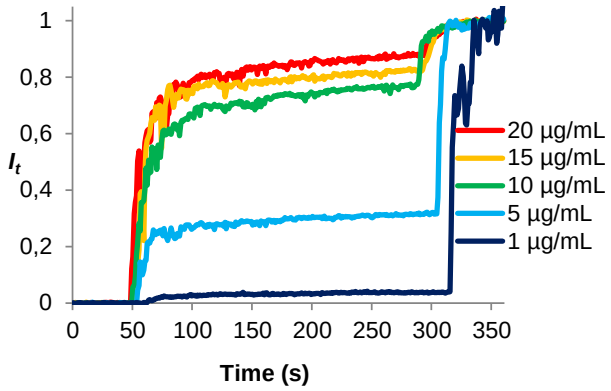


rac-**TNS18**

EYPG



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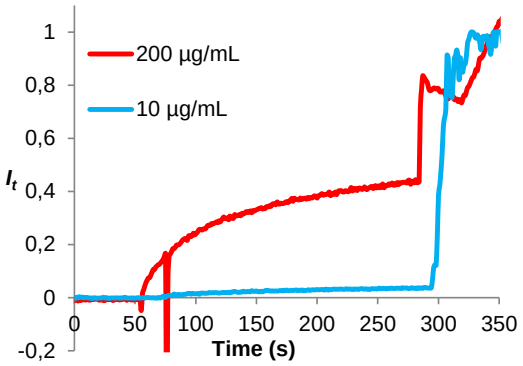


sr-**TNS18**

EYPG **EYPC**

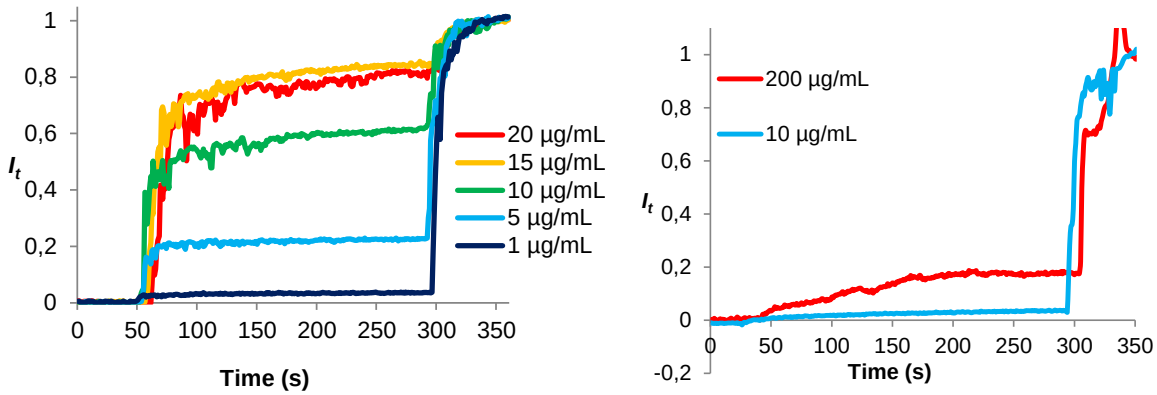
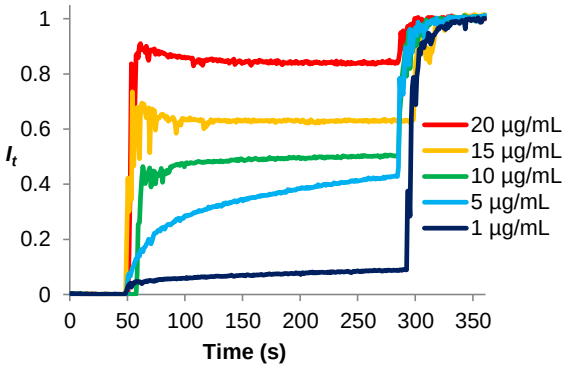


Figure S12: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by **TNS18** analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

L-**T25**

EYPG



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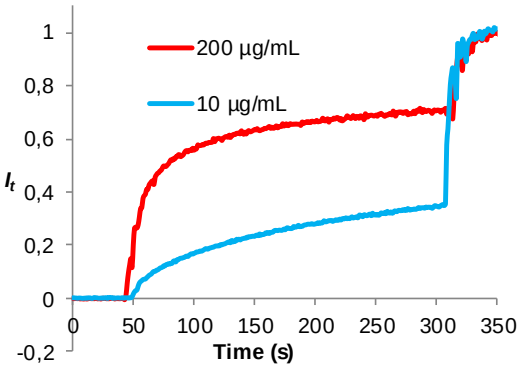
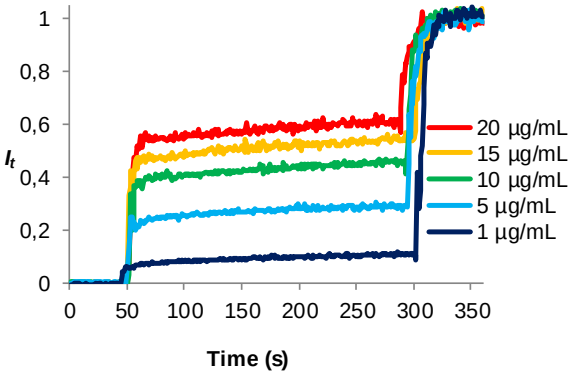


D-**T25**

EYPG



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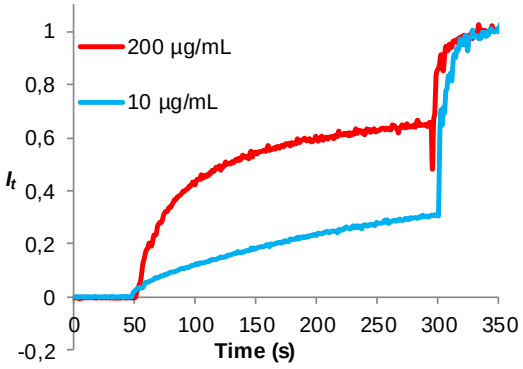
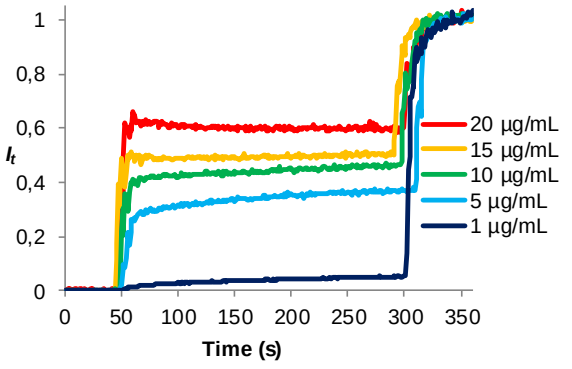


***rac*-T25**

EYPG



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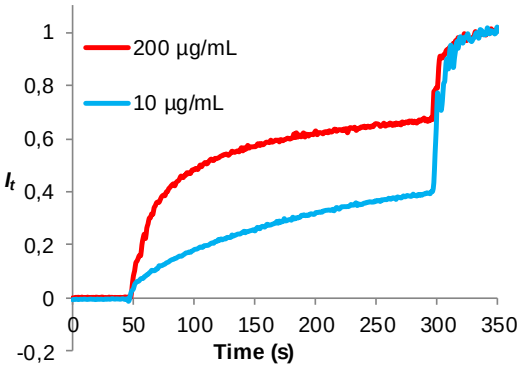
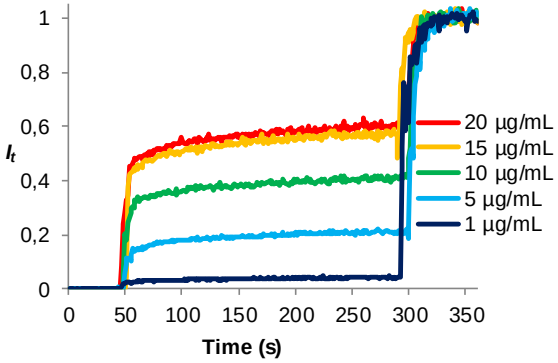


***sr*-T25**

EYPG



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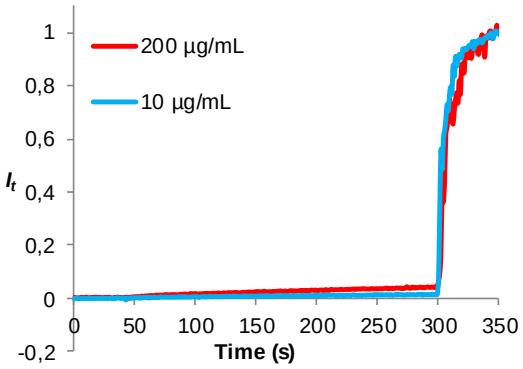
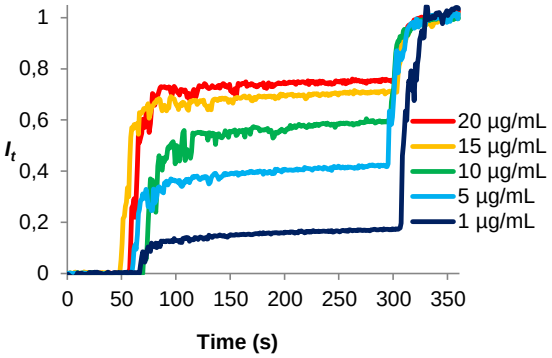


***sr*-T25a**

EYPG



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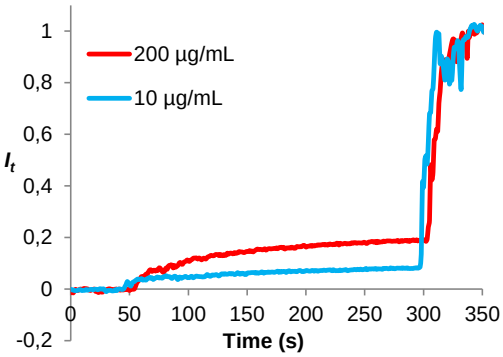
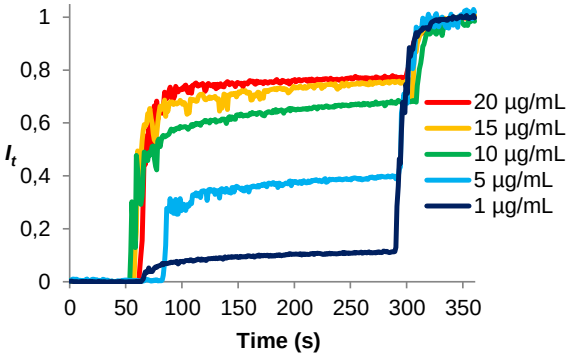


***sr*-T25b**

EYPG



EYPC

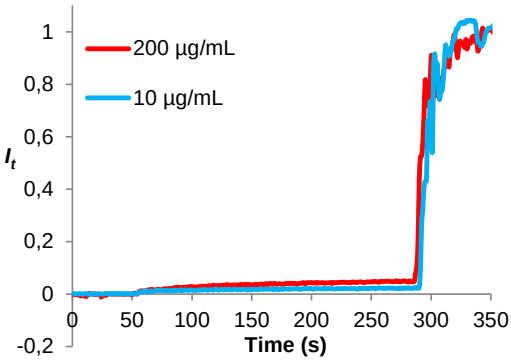
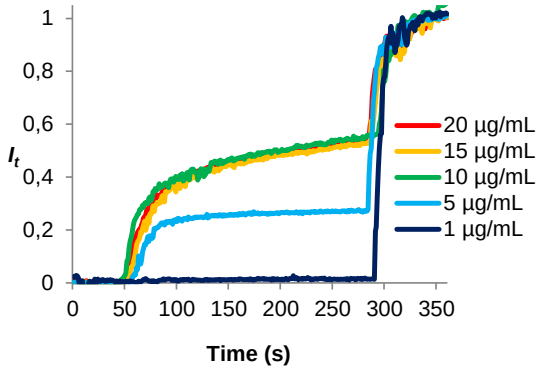


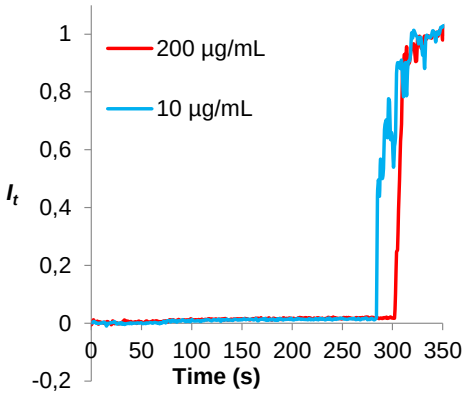
Figure S13: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by **T25** analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

PMB

EYPG

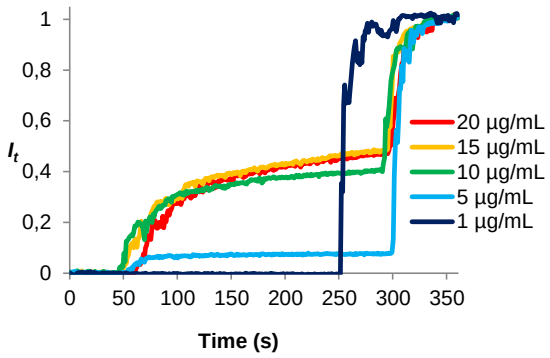


EYPC



PMB2

EYPG



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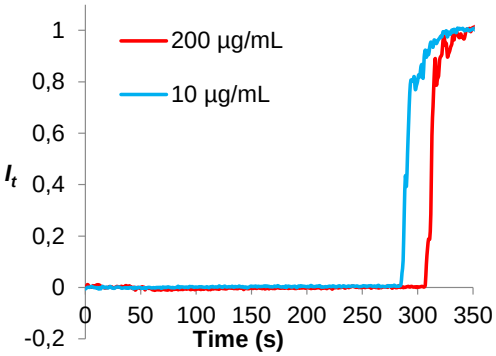
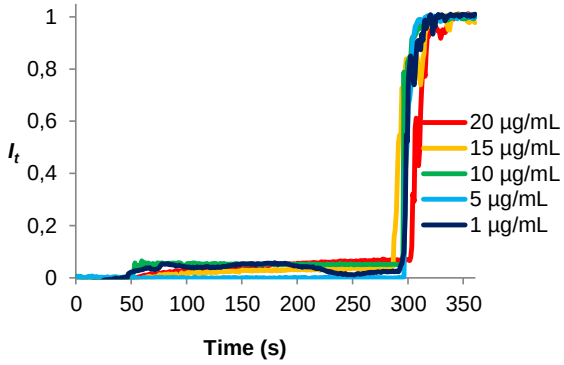


***sr*-PMB2**

EYPG



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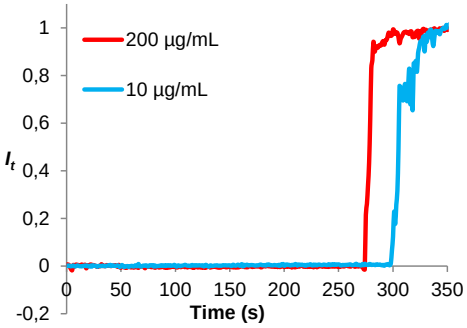
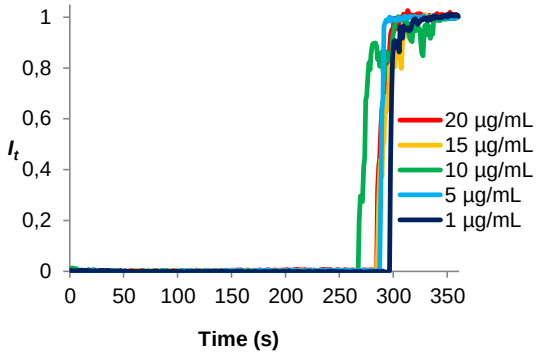


***sr*-PMB2a**

EYPG



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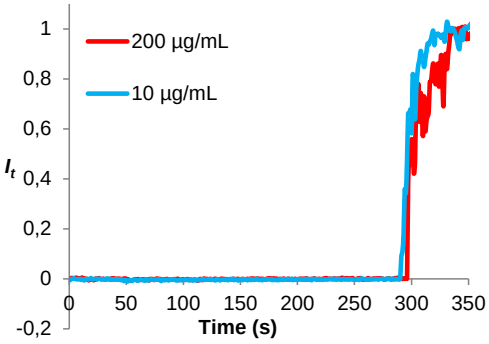
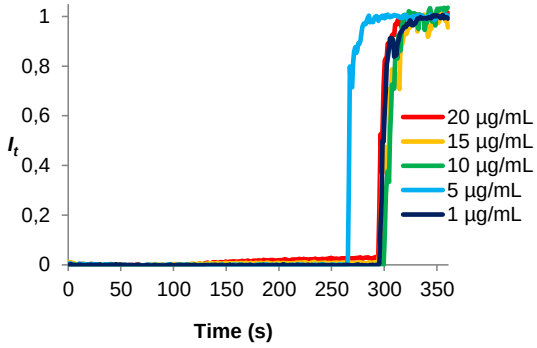


***sr*-PMB2b**

EYPG



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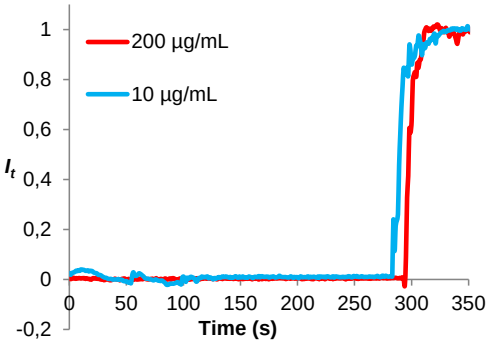


Figure S14: Vesicle leakage experiments using 5(6)-carboxyfluorescein induced by Polymyxin B analogs. LUVs were suspended in buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and the indicated concentration of the compound was added after 50 sec. After 300 seconds 1.2% Triton X-100 was added for full release of fluorescein.

Cell Culture

HeLa, RAW264.7 murine macrophages (Sigma Aldrich), MRC-5, HepG2 and A549 cells were cultured and maintained in DMEM (Dulbeccos modified Eagle medium, Sigma Aldrich) supplemented with 10% FBS (Sigma Aldrich) and 1% penicillin/streptomycin. Cells were incubated in a humidified incubator at 37 °C in the presence of 5% CO_2.

Cytotoxicity

The 96-well plates were coated with poly-L-Lysine (Sigma Aldrich, Buchs, CH) for 30 min. The excess of poly-L-Lysine was removed and the plate was dried in the laminar flow hood at RT. HeLa, MRC-5, HepG2 and A549 cells were seeded into 96 well plates, 1×10^4 cells/well, the day before the experiment. The medium was removed and the compounds at increasing concentration (1 to 300 µM) were added into the wells. The cells were incubated for 24 hours in DMEM containing 10% FBS at 37 °C in the presence of 5% CO_2. The next day, the medium was removed and replaced by the medium containing 10% FBS and 10% AlamarBlue® (Thermo Fisher Scientific, Reinach, CH). The cells were incubated for 3-5 hours at 37 °C with 5% CO_2 in a humidified atmosphere. The fluorescence was then measured on a Tecan Infinite M1000 Pro plate reader at λ_{ex} 560 nm and λ_{em} 590 nm. The value was normalized according to the untreated cells.

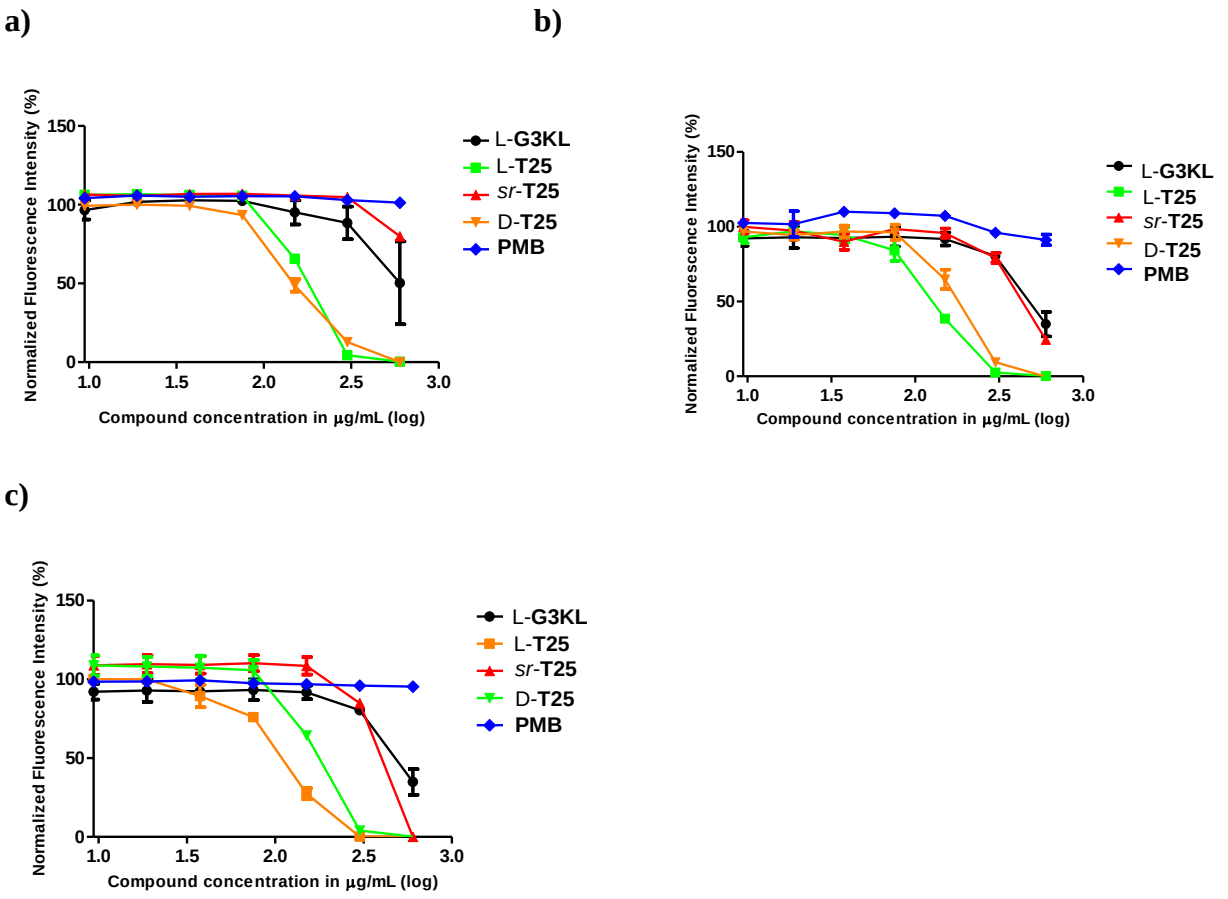


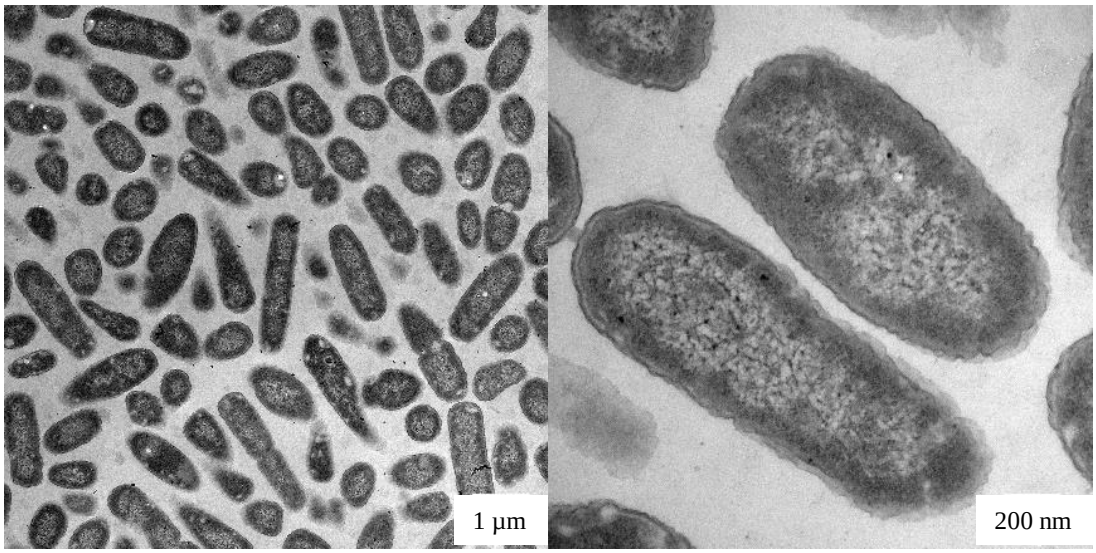
Figure S15: Cytotoxicity of **G3KL**, L-**T25**, *sr*-**T25**, D-**T25** and polymyxin B. (a) MRC5 cells, (b) A549 cells, (c) HeLa cells. The cells were incubated for 24 hours in DMEM containing 10% FBS at 37 °C in the presence of 5% CO_2.

Transmission Electron Microscope (TEM)

Exponential phase of *Pseudomonas aeruginosa* PAO1 or *E. coli* W3110 was adjusted to OD 1.0 and washed with PBS and treated with the peptide dendrimers L-**T25** (40 µg/mL), *sr*-**T25** (40 µg/mL), **d-T25** (40 µg/mL), or the polymyxin derivatives *sr*-**PMB2** (20 µg/mL), *sr*-**PMB2a** (20 µg/mL), *sr*-**PMB2b** (5 µg/mL) and **PMB2** (5 µg/mL) and Polymyxin B (5 µg/mL) in M63 minimal medium supplemented with 1mM $MgSO_4$ and 10% glycerol. Each time, 1 mL of the bacteria were centrifuged after 15, 30 and 60 min at 12'000 rpm for 3 min and fixed overnight with 2.5% glutaraldehyde (Agar Scientific, Stansted, Essex, UK) in 0.15 M HEPES (Fluka, Buchs, Switzerland) with an osmolarity of 670 mOsm and adjusted to a pH of 7.35. The next day, bacteria cells were washed with 0.15 M HEPES three times for 5 min, postfixed with 1% OsO_4 (SPI Supplies, West Chester, USA) in 0.1 M Na-cacodylate-buffer (Merck, Darmstadt, Germany) at 4 °C for 1 h. Thereafter, bacteria cells were washed in 0.05 M maleic acid (Merck, Darmstadt, Germany), pH 5.0, three times for 5 min and dehydrated in 70, 80, and 96% ethanol (Alcosuisse, Switzerland) for 15 min each at room temperature. Subsequently, they were immersed in 100% ethanol (Merck, Darmstadt, Germany) three times for 10 min, in acetone (Merck, Darmstadt, Germany) two times for 10 min, and finally in acetone-Epon (1:1) overnight at room temperature. The next day, bacteria cells were embedded in Epon (Fluka, Buchs, Switzerland) and hardened at 60°C for 5 days.

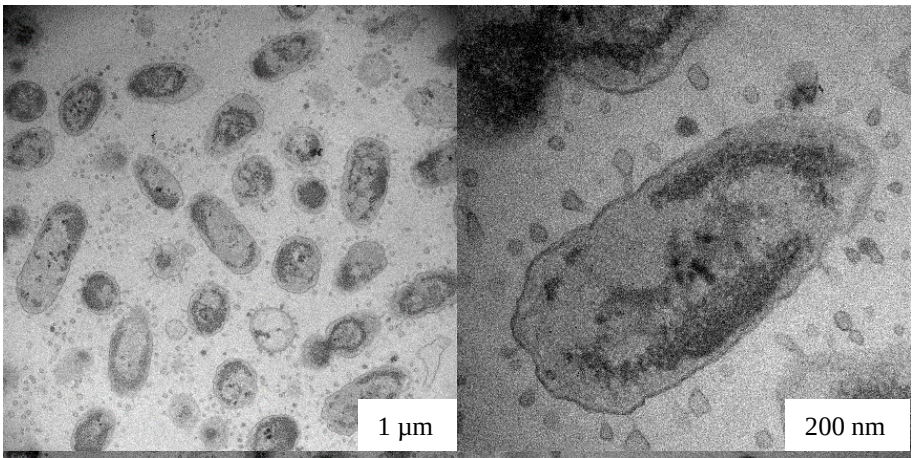
Sections were produced with an ultramicrotome UC6 (Leica Microsystems, Vienna, Austria), first semithin sections (1 µm) for light microscopy which were stained with a solution of 0.5% toluidine blue O (Merck, Darmstadt, Germany) and then ultrathin sections (70-80 nm) for electron microscopy. The sections, mounted on single slot copper grids, were stained with UranyLess (Electron Microscopy Sciences; Hatfield, USA) and lead citrate with an ultrostainer (Leica Microsystems, Vienna, Austria). Sections were then examined with a Tecnai Spirit transmission electron microscope (Tecnai Spirit, FEI, Brno, Czech Republic) equipped with a digital camera (Veleta, Olympus, Soft Imaging System, Münster, Germany).

Control no cpd. added

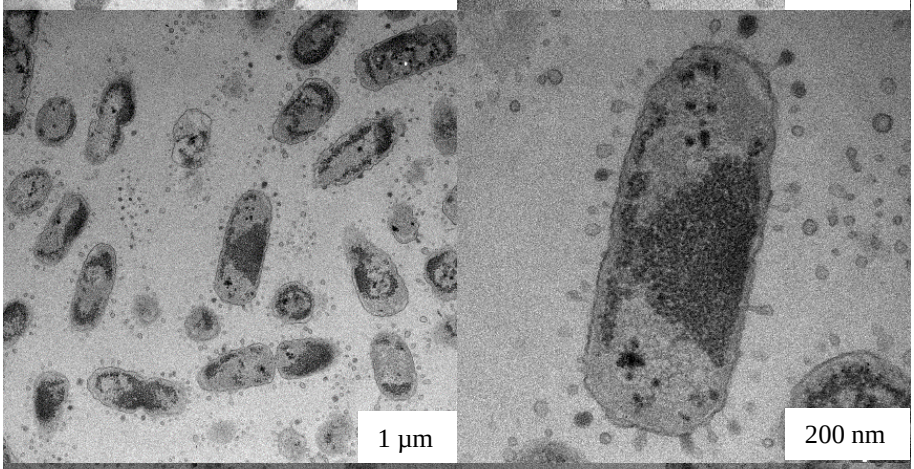


L-**T25** 40 µg/mL

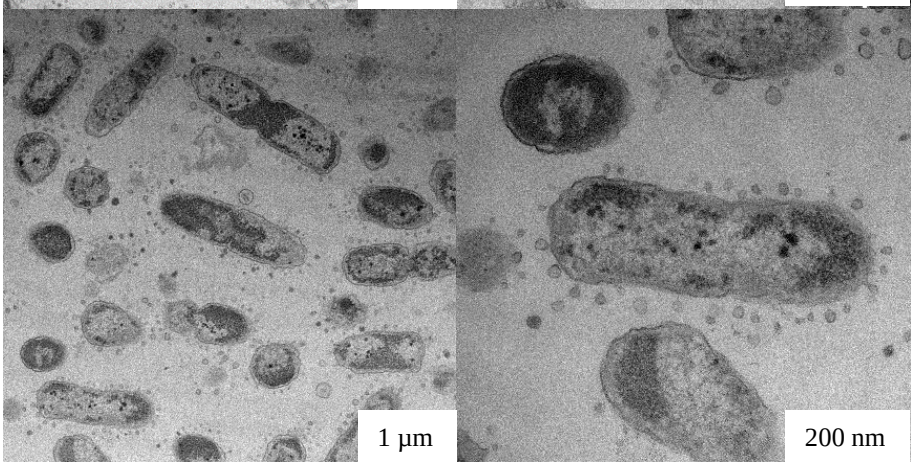
15 min



30 min



60 min



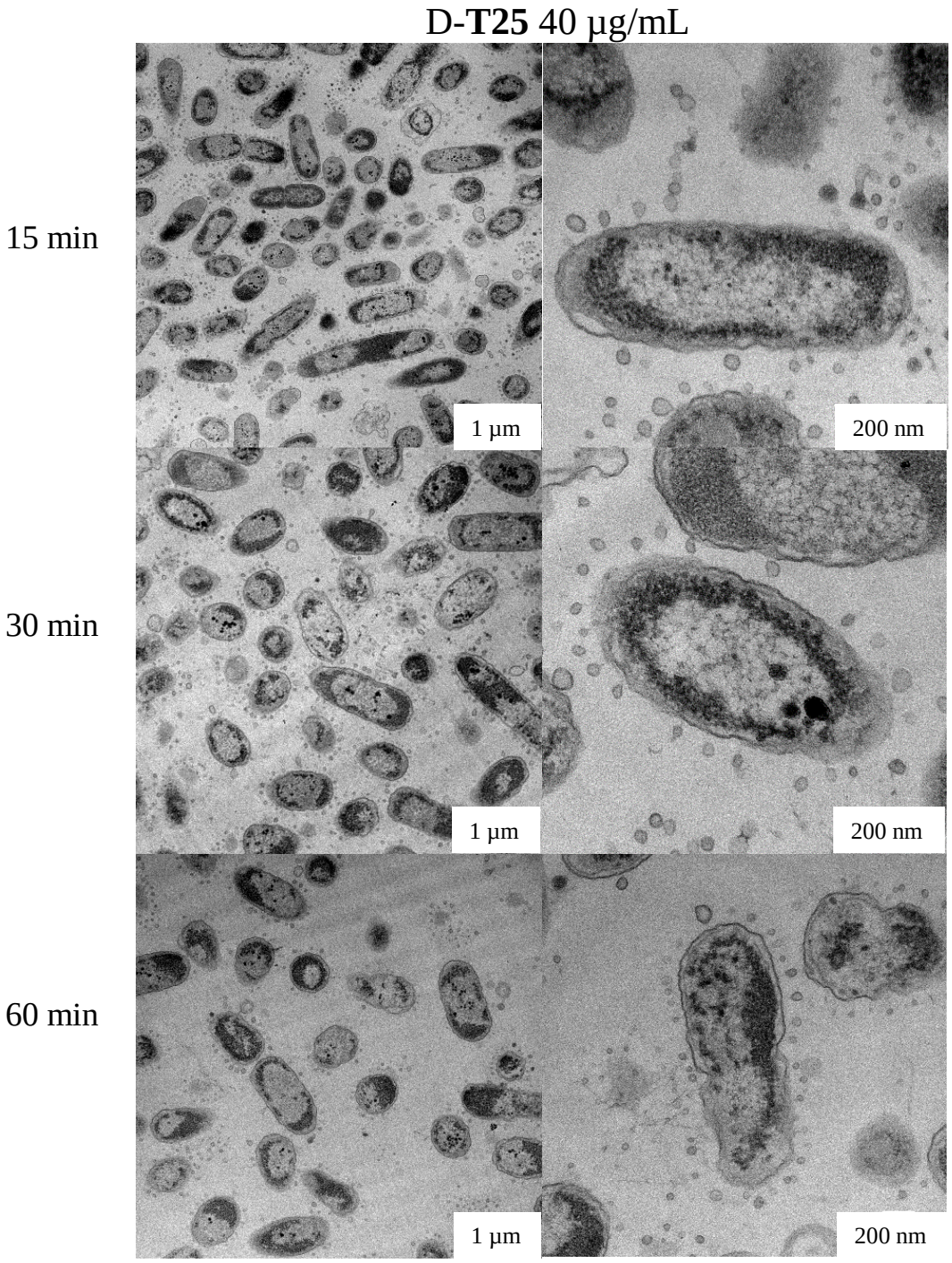
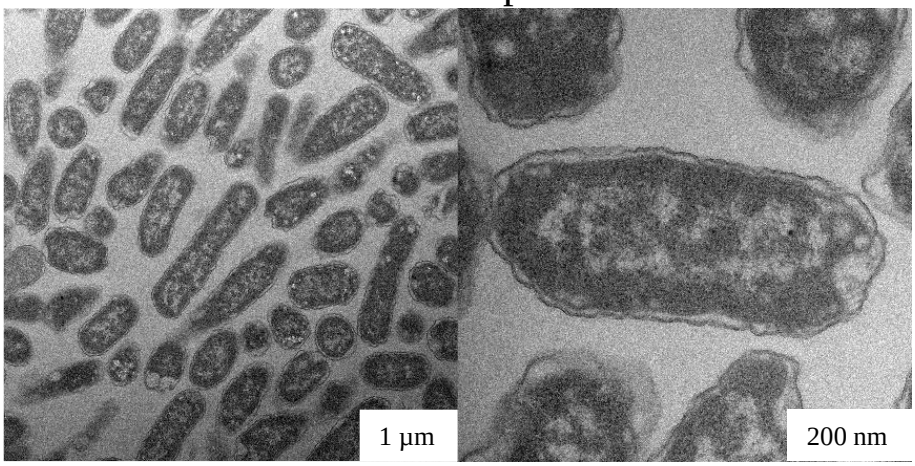


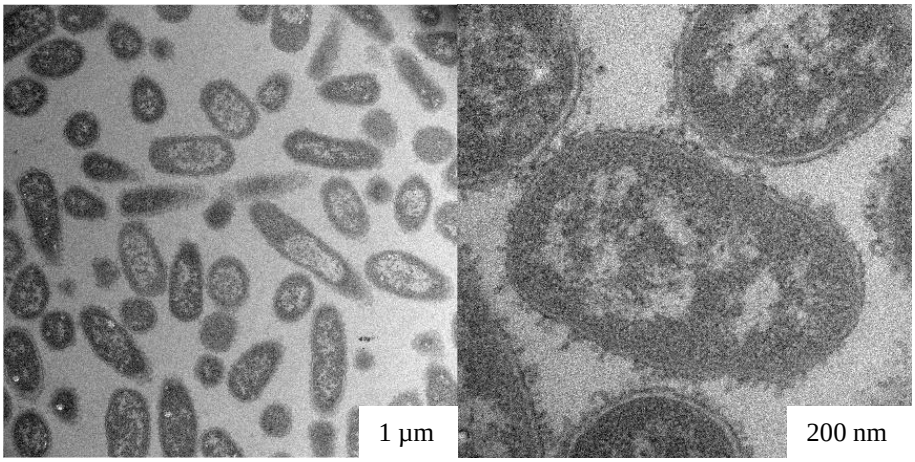
Figure S16: Enlarged TEM images of *P. aeruginosa* cells treated for 15, 30 and 60 min at 10 × MIC with AMPD L-**T25** (40 µg/mL) and D-**T25** (40 µg/mL). Control showed the untreated *P. aeruginosa* cells.

Control no cpd. added



Polymyxin B 5 µg/mL

60 min



sr-**PMB2** 20 µg/mL

60 min

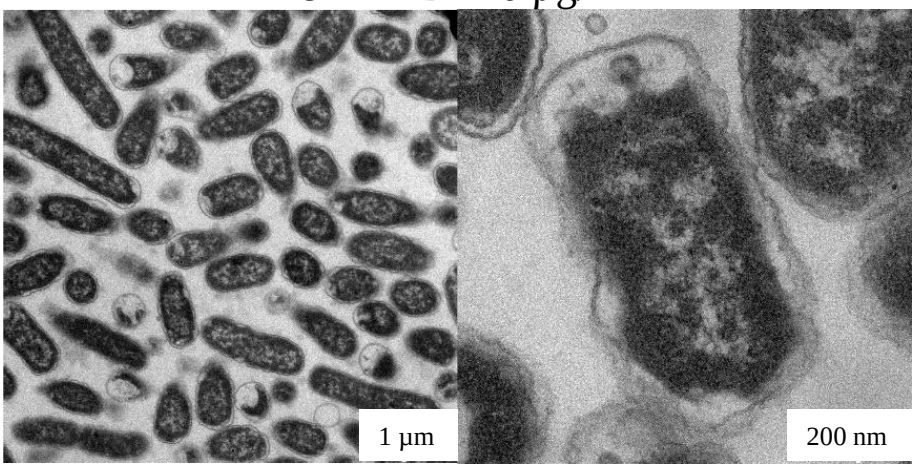


Figure S17: Enlarged TEM images of *E. coli* cells treated for 60 min at 20 × MIC with Polymyxin B (5 µg/mL) and *sr*-**PMB2** (20 µg/mL). Control showed the untreated *E. coli* cells.

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 coordinates of the initial starting conformation were constructed with all the residues in a helical conformation using PyMol software (Version 1.8.x, *Schrödinger, LLC*.) The amine side chains of Lys were protonated, the *N*-termini were free to simulate pH ~ 7.

MD Simulations were performed using GROMACS software version 2019.4 and the Gromos53a6 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. 20 % of the volume of the box was filled with the appropriate number of randomly placed TFE molecules before addition of water. 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 followed by a 500 ns production run.

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.

The final equilibrated structures used in the analysis and were obtained by clustering the last 100 ns of the MD run using the gromacs method on the backbone with a cutoff of 0.3 nm leading to 14 clusters with 71 % of the structures in the main cluster. The central structure of the main cluster was used for generating the models as the most representative equilibrated conformation. The Ramachandran number during the whole trajectory was calculated using backmap Python package (https://github.com/ranjanmannige/backmap) with a timestep of 10 ns between analyzed frames.

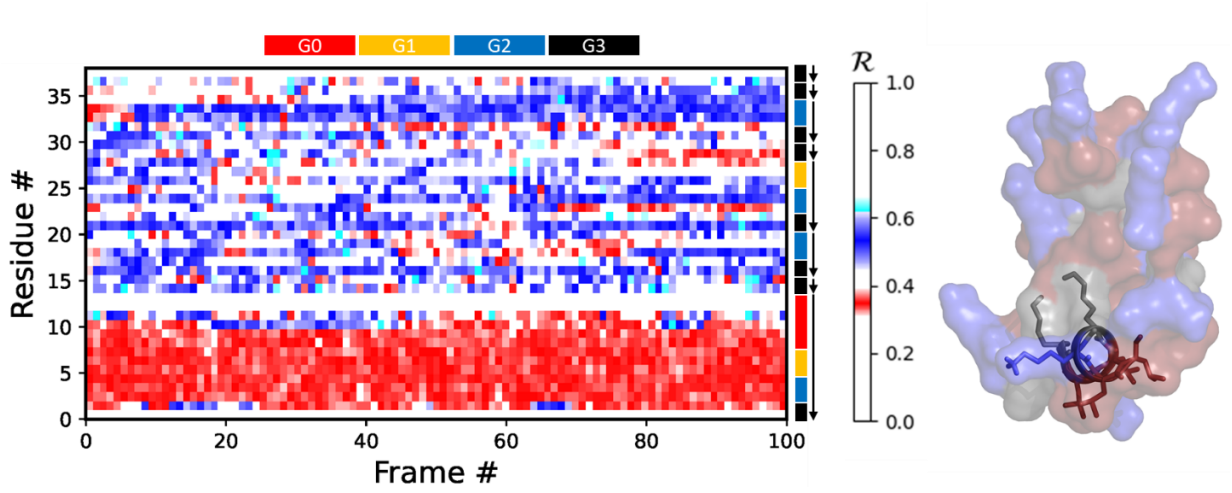


Figure S18: MD analysis: Left, Ramachandran numbers R during the course of the trajectory showing the presence of both helices (at R ~ 0.34, red) and sheets (at R ~ 0.52, blue). The timestep between frames is 10 ns. Residues 1-13 which compose the central a-peptide are in a stable a-helical conformation throughout the simulation.. Right, surface representation on the dendrimer (Lys in blue, Leu in Red, Branching Lys in gray) showing the positioning on the central a-helix (cartoon representation) involving the residues 1-13.

Determination of TNF-α expression level in Murine Macrophages by ELISA

Raw 264.7 mouse macrophages (Sigma Aldrich) were seeded into 96-well plates at 2.5 x 10^4 cells/well the day before the experiment. Cells were treated with 10 µM of Polymyxin B and the polymyxin derivatives (**PMB2,** *sr*-**PMB2**, *sr*-**PMB2a** and *sr*-**PMB2b**) with or without 0.1 µg/mL of LPS in 200 µL of medium containing 1 or 10% FBS for 4 h at 37 °C and 5% CO2. After treatment, the cells were centrifuged at maximal speed for 10 min to obtain cell-free samples and the supernatant was collected and stored at -20°C. The level of TNF-α was measured by ELISA using anti-mouse TNF-α (Peprotech, USA) as recommended by the manufacturer's instructions.

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