Supporting Information for:

Lipidated Peptide Dendrimers Killing Multidrug Resistant Bacteria

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Materials and Reagents

Amino acids and their derivatives were either purchased from Advanced ChemTech, Novabiochem, Iris Biotech GMBH, Sigma Aldrich, Fluka, PolyPeptide, and GL BioChem. Amino acids were used as the following derivatives: : Fmoc-ß-Ala-OH, Fmoc-Arg(Pbf)-OH, 4-(Fmoc)-aminomethylbenzoic acid (Amba), Fmoc-Asn(Trt)-OH, Fmoc-Dab(Boc)-OH, , Fmoc-Glu(OtBu)-OH, Fmoc-His(Boc)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Lys(Alloc)-OH, Fmoc-Met-OH, Fmoc-Orn(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Val-OH, Fmoc-γ-Abu-OH (Gaba). TentaGel S NH_2 (loading: 0.32 mmol/g) and TentaGel S RAM (loading: 0.22-0.26 $mmol \cdot g^{-1}$) resins were purchased from Rapp Polymere. OxymaPure (hydroxyiminocyanoacetic acid ethyl ester), DIC (*N*,*N*'-diisopropyl carbodiimide) and PyBOP were purchased from Iris Biotech GMBH. 5(6)-carboxyfluorescein (CF) was from Sigma. Egg phosphatidylcholine (EPC), egg phosphatidylglycerol (EPG) 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 Tunable 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, amino acid analyses and diffusion-NMR measurements were provided by Mass Spectrometry, Protein Analysis and NMR services respectively of the Department of Chemistry and Biochemistry at the University of Berne.

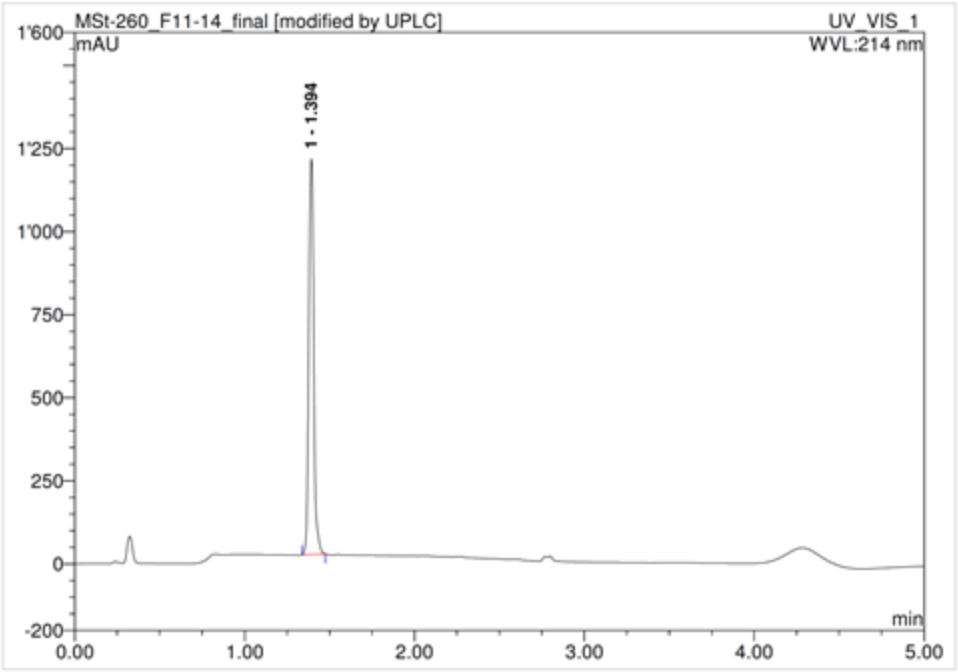
Synthesis

Peptide Dendrimers. Peptide dendrimers were synthesized using standard solid phase peptide synthesis.[1] The resin (TentaGel S RAM) 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.). For further couplings, the resin was acylated with one of the protected amino acids (3 eq./amine) either PyBOP (benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate) (3 eq./amine) and DIPEA (*N*,*N*-Diisopropylethylamine) (5 eq./amine) or OxymaPure (ethyl-2-cyano-2-(hydroxyimino)acetate) (5 eq./amine) and DIC (*N*,*N*′-Diisopropylcarbodiimide) (5 eq./amine) in NMP. Amino acids, derivatives or diamino acids were coupled for 1 h (G0), 2 h (G1), 3 h (G2), 4 h (G3). The completion of the reaction was checked using 2,4,6-trinitrobenzenesulfonic acid solution (TNBS). The coupling was repeated after a positive test. Capping of unreacted peptide chains was carried out with a solution of acetic anhydride and DCM (1:1 v/v) for 15 min. After each coupling, the resin was deprotected with 20% piperidine in DMF (2 × 10 min). After the final deprotection, the resin was washed twice with MeOH and dried under vacuum before the cleavage was carried out using TFA/TIS/H_2O (94:5:1 v/v/v) during 4.5 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.

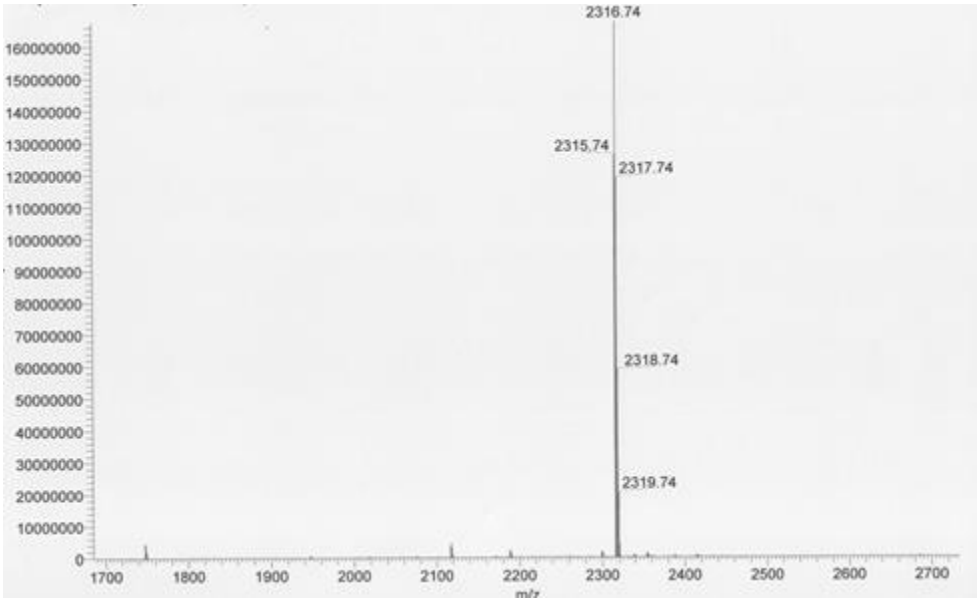
Lipidated Peptide Dendrimers. Synthesis was carried out as above with the following modifications. Fmoc-Lys(Alloc)-OH was attached first to the resin. Before deprotection of the last Fmoc-group the Alloc protecting group was removed under dry conditions with 0.25 eq of $Pd(PPh_3)_4$ and 25 eq. of $PhSiH_3$ in 10 mL dry DCM.[2] This step was repeated twice with washing of the resin with 2x dry DCM in between. After the second cycle, the resin was washed for 1 h two times with DCM and the lipid chain was attached to dendrimer with carboxylic acid (5 eq.), either HOBT (5 eq.), DIC (5 eq.) and DIPEA (3 eq.) in NMP/DCM (1/1 v/v) or OxymaPure (5 eq.) and DIC (5 eq.) in NMP was added to the resin and stirred for one time overnight and one time 2h. Last Fmoc-group was deprotected and cleavage was carried out using TFA/TIS/H_2O (94:5:1 v/v/v) during 4.5 h. Crude was purified by RP-HPLC and obtained as TFA salt after lyophilisation.

TNS01 $(KL)_4(KKL)_2KKLK(C_6)$. From Tenta Gel S RAM® resin (300 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (23.0 mg, 12.7%). Anal. RP-HPLC: t_R = 1.39 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{114}H_{222}N_{30}O_{19}$ calc./found 2315.73/2315.74 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

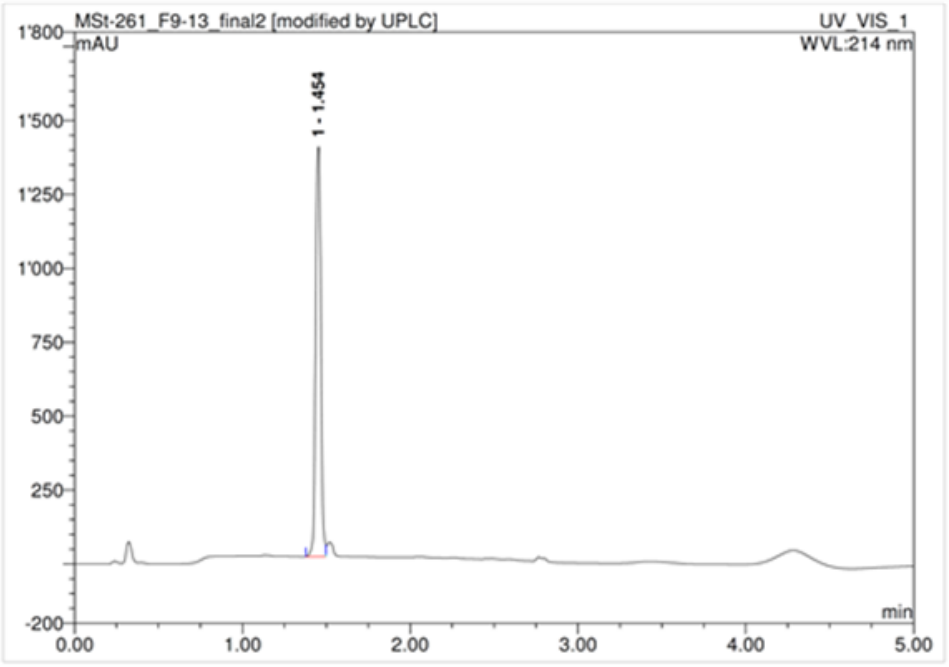


Mass spectrum, HRMS (NSI+):

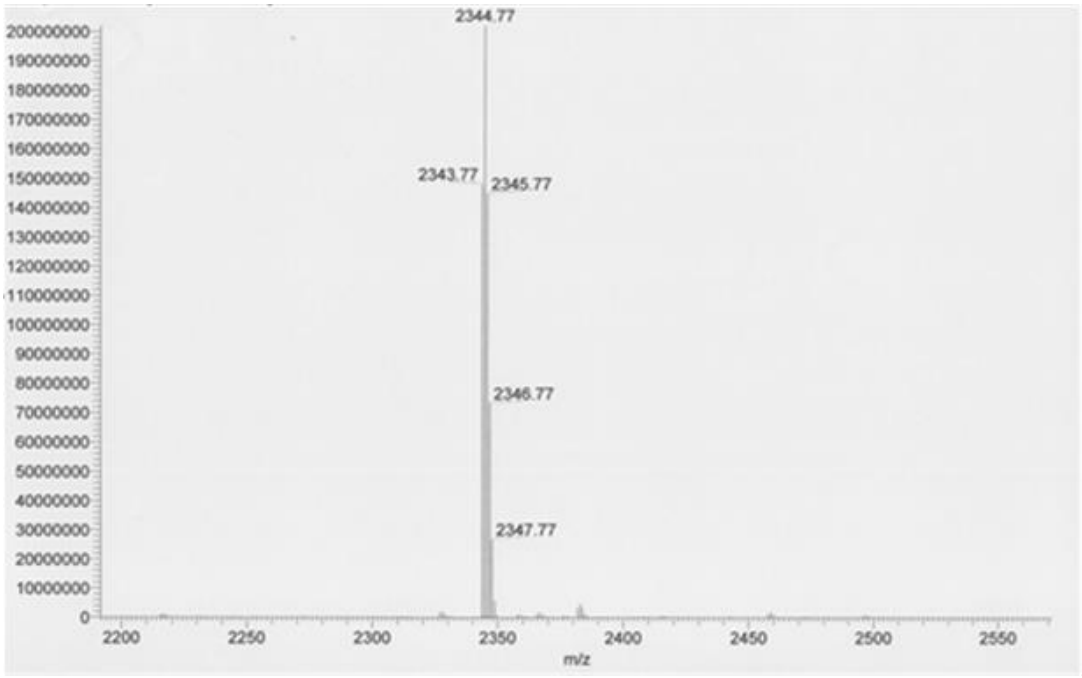


TNS02 $(KL)_4(KKL)_2KKLK(C_8)$. From Tenta Gel S RAM® resin (350 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (27.4 mg, 12.8%). Anal. RP-HPLC: t_R = 1.45 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{116}H_{226}N_{30}O_{19}$ calc./found 2343.76/2343.77 Da $[M]^+$.

Analytical RP-HPLC chromatogram

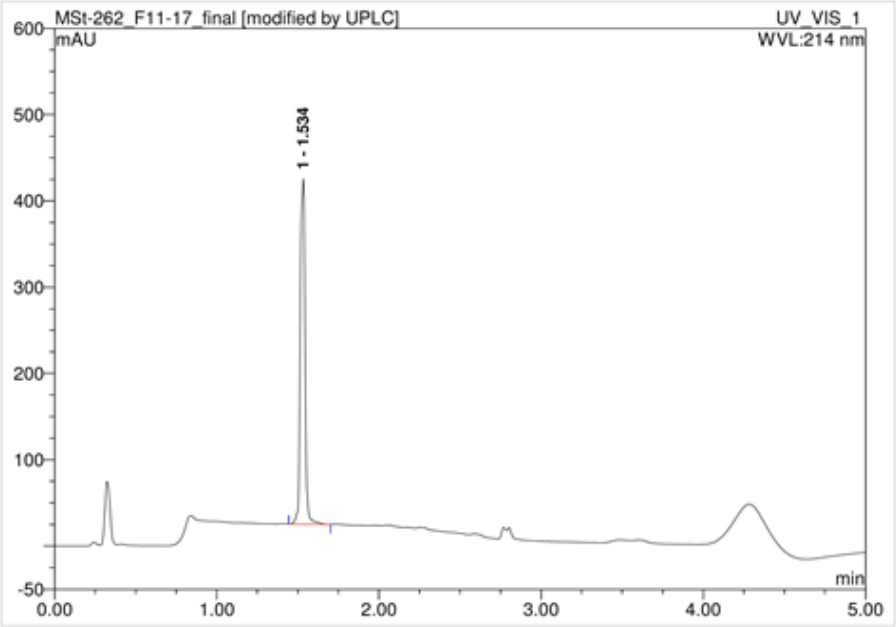


Mass spectrum, HRMS (NSI+):

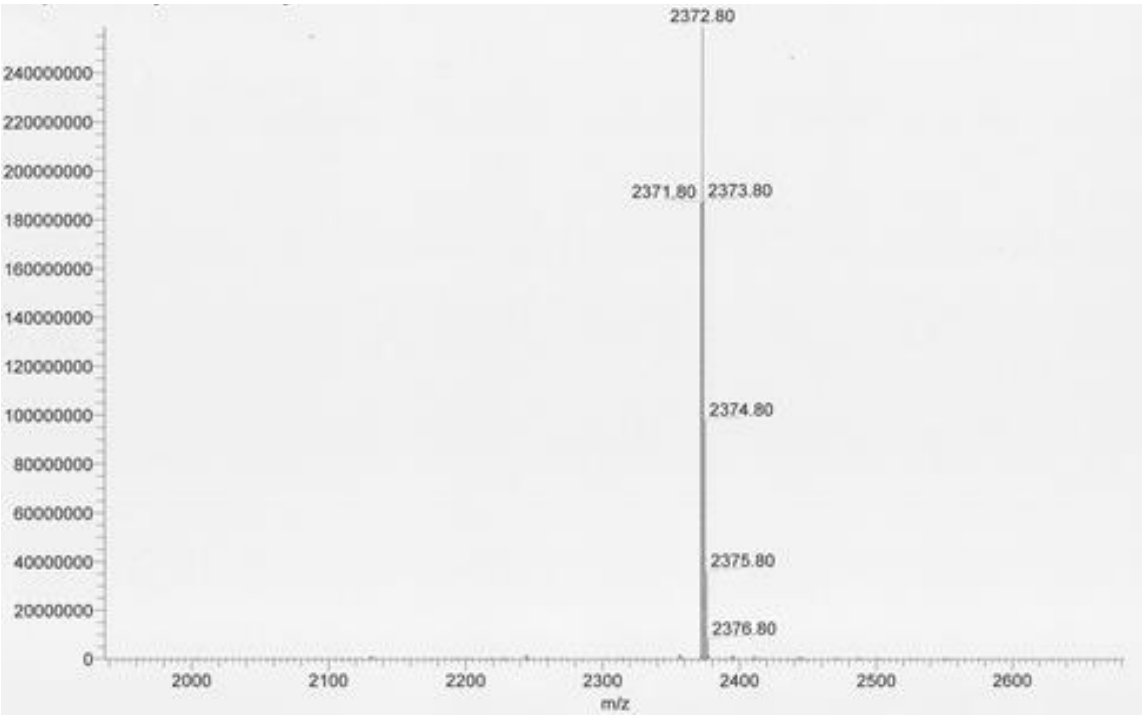


TNS03 $(KL)_4(KKL)_2KKLK(C_{10})$. From Tenta Gel S RAM® resin (500mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (84 mg, 29.5%). Anal. RP-HPLC: t_R = 1.53 min (A/D = 100/0 to 0/100 in 5.0 min, λ = 214 nm) HRMS (NSI+): $C_{118}H_{230}N_{30}O_{19}$ calc./found 2371.80/2371.80 Da [M]

Analytical RP-HPLC chromatogram:

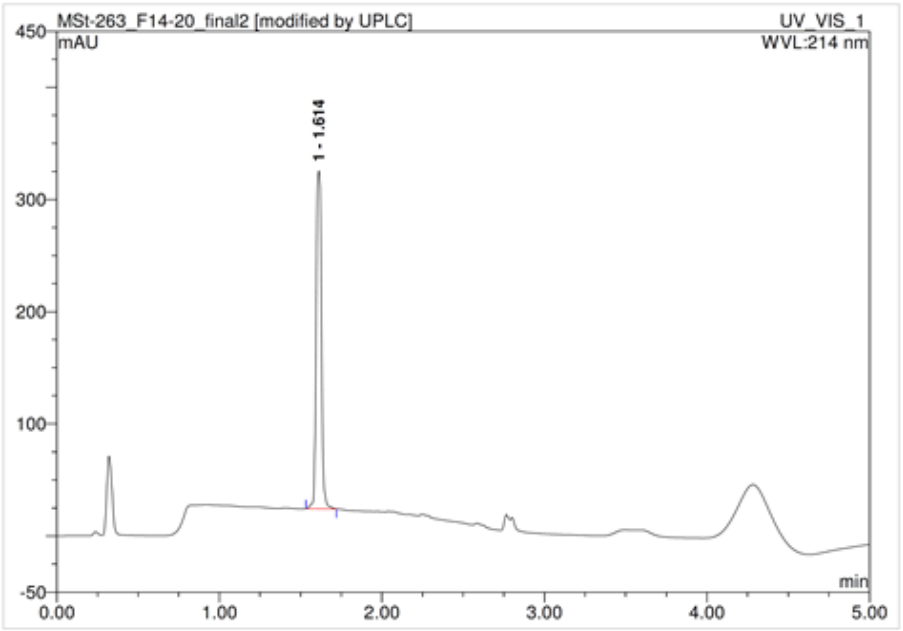


Mass spectrum, HRMS (NSI+):

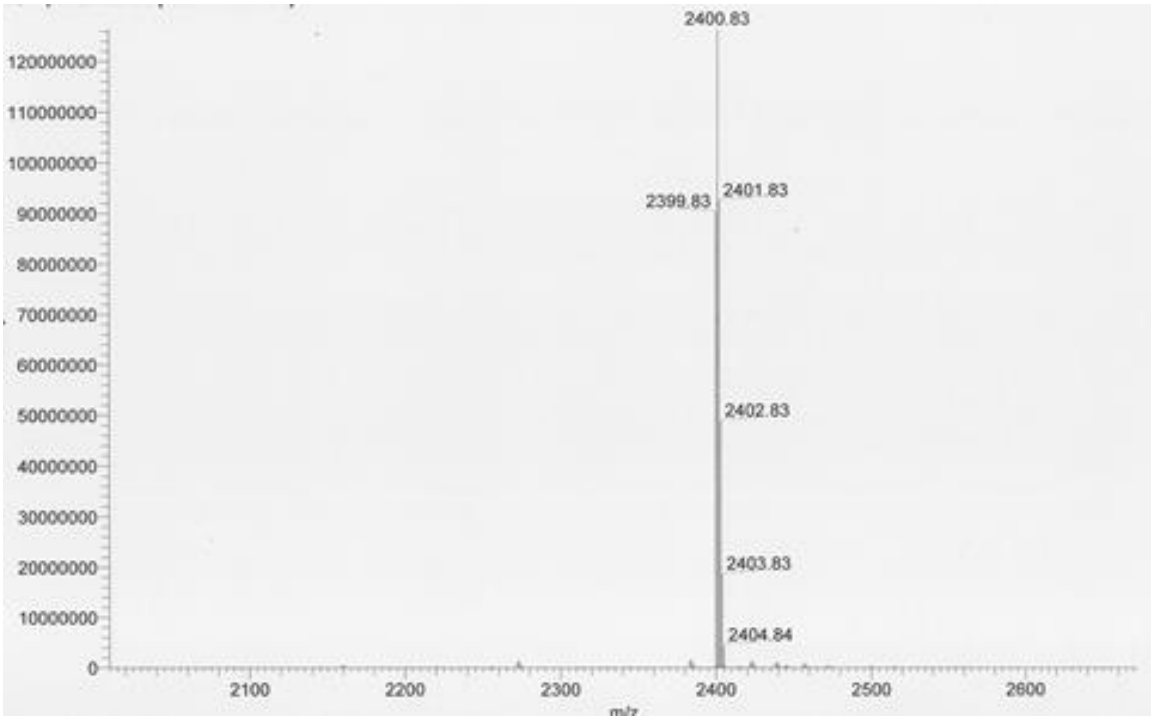


TNS04 $(KL)_4(KKL)_2KKLK(C_{12})$. From Tenta Gel S RAM® resin (350 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (39.8 mg, 18.2%). Anal. RP-HPLC: t_R = 1.61 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{120}H_{234}N_{30}O_{19}$ calc./found 2399.83/2399.83 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

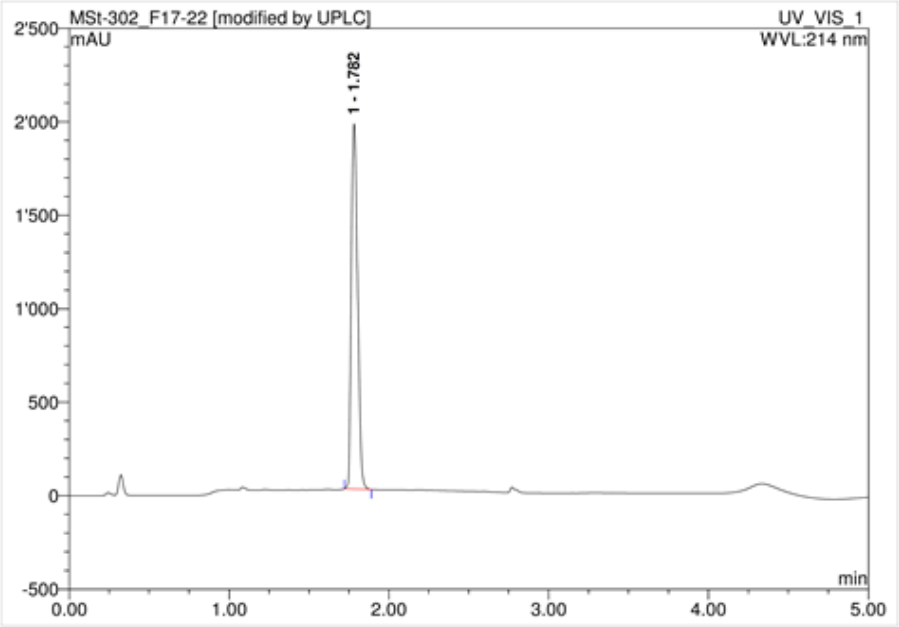


Mass spectrum, HRMS (NSI+):

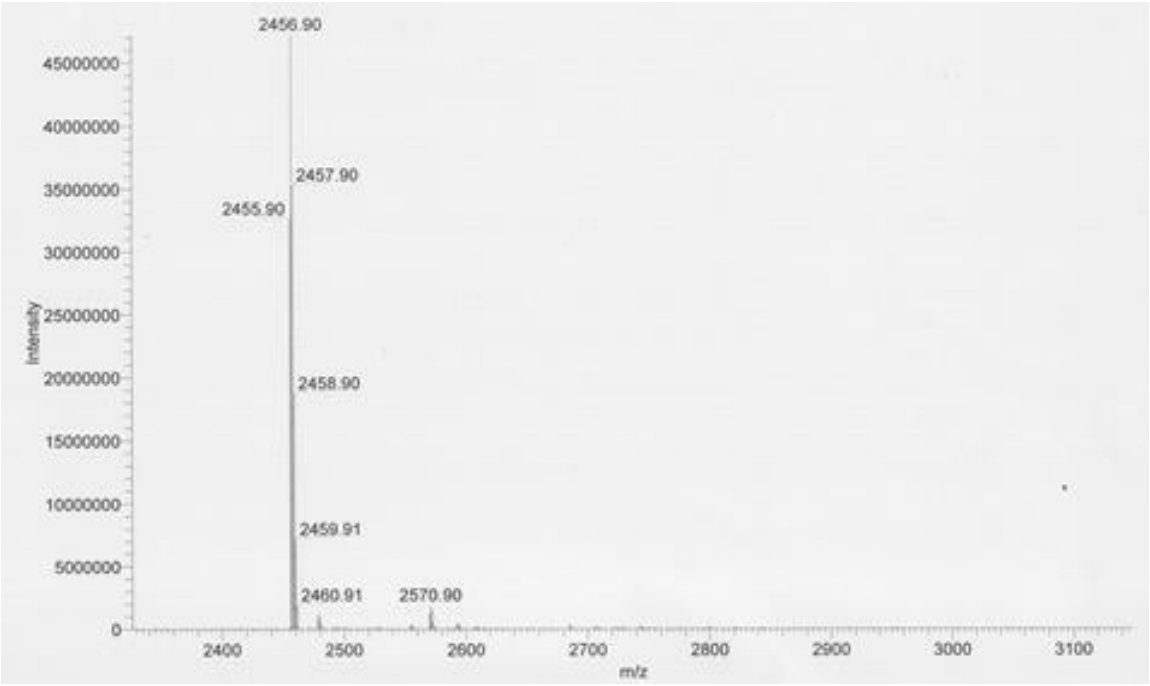


TNS05 $(KL)_4(KKL)_2KKLK(C_{16})$. From Tenta Gel S RAM® resin (350 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (30.24 mg, 13.5%). Anal. RP-HPLC: t_R = 1.78 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{124}H_{242}N_{30}O_{19}$ calc./found 2455.89/2455.90 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

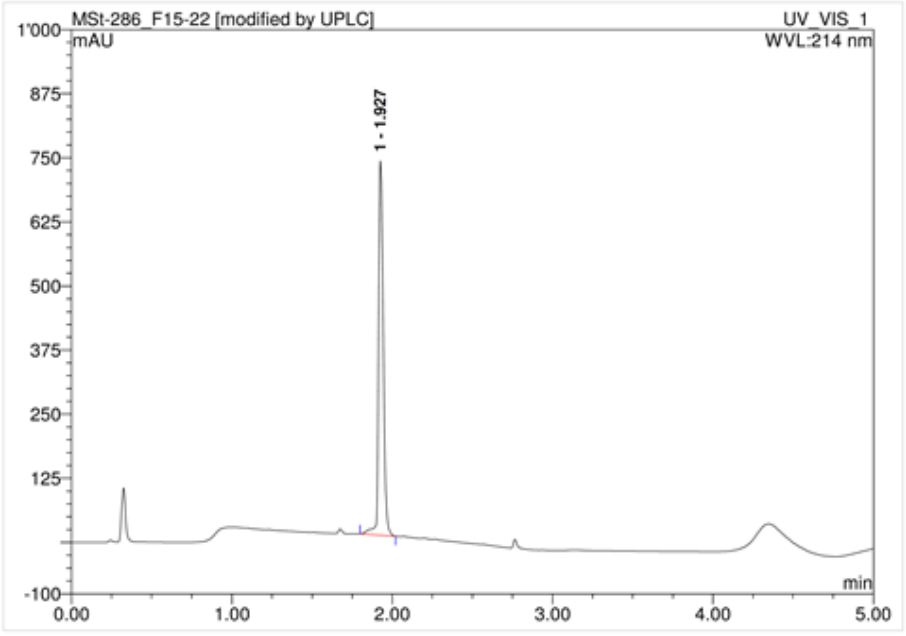


Mass spectrum, HRMS (NSI+)

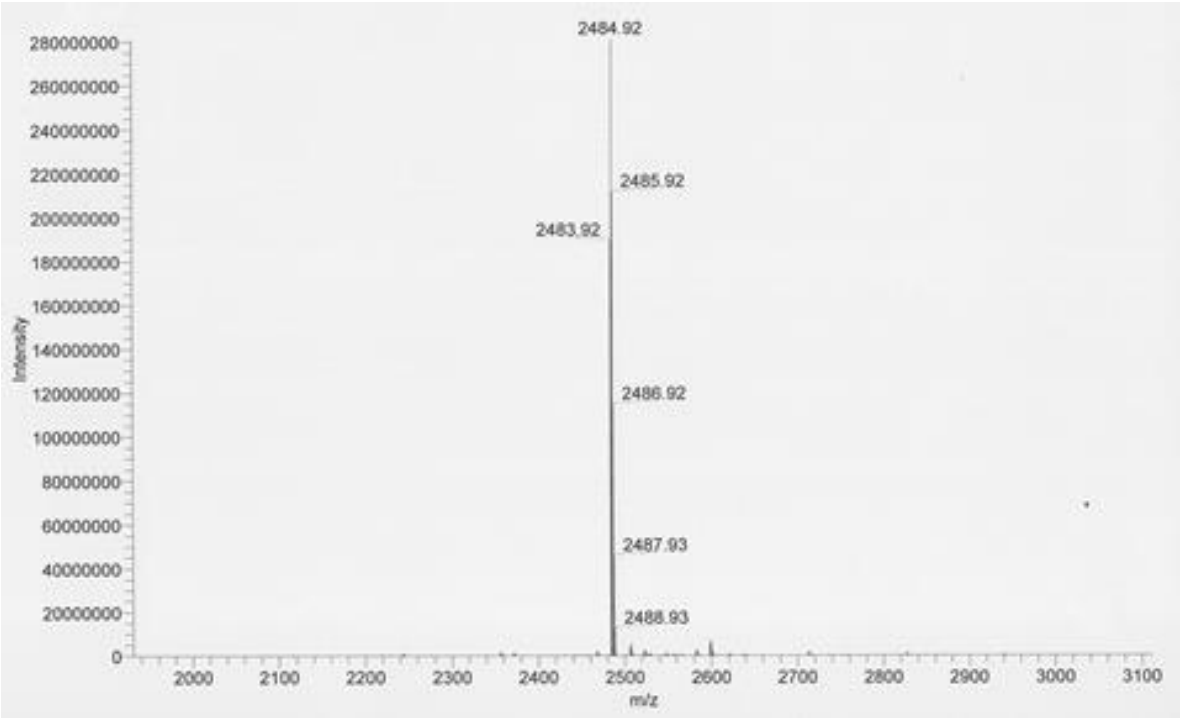


TNS06 $(KL)_4(KKL)_2KKLK(C_{18})$. From Tenta Gel S RAM® resin (500 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (52.6 mg, 16.2%). Anal. RP-HPLC: t_R = 1.93 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{126}H_{246}N_{30}O_{19}$ calc./found 2483.92/2483.92 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

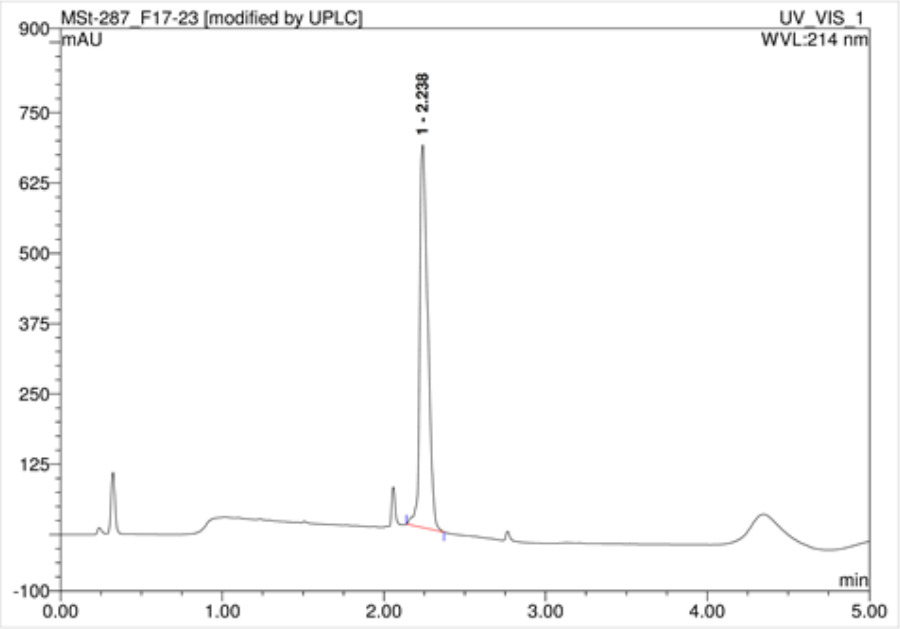


Mass spectrum, HRMS (NSI+)

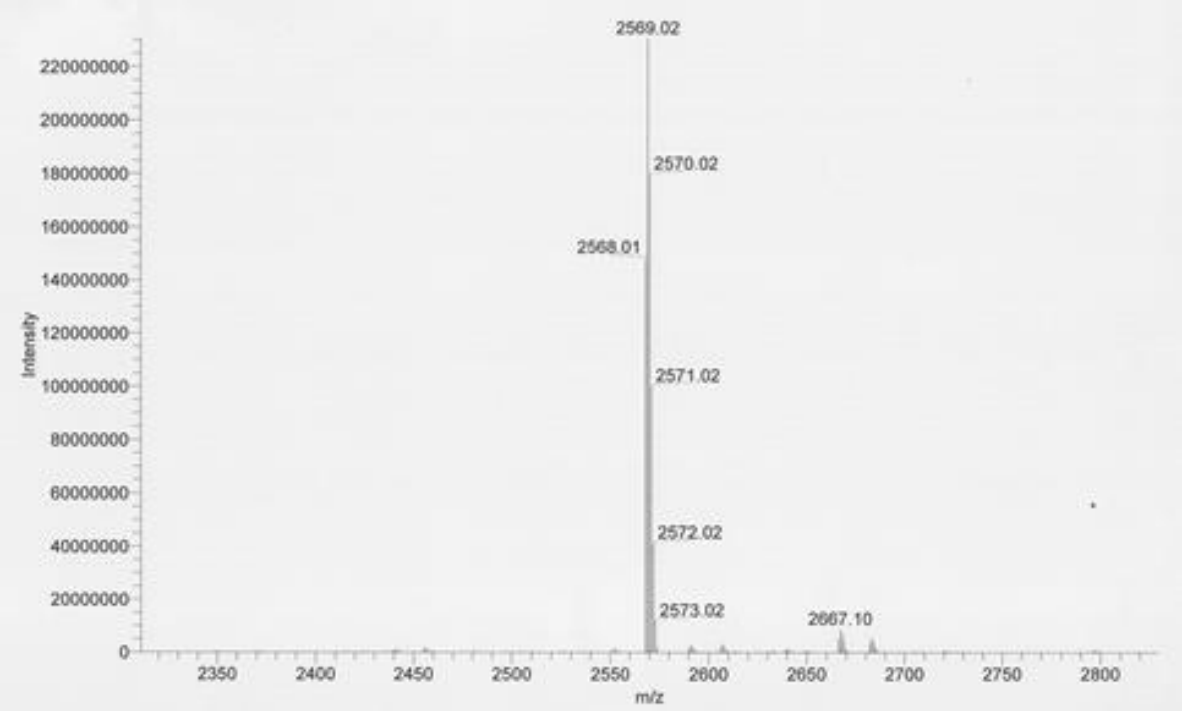


TNS07 $(KL)_4(KKL)_2KKLK(C_{24})$. From Tenta Gel S RAM® resin (500 mg, 0.26 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (56.7 mg, 16.9%). Anal. RP-HPLC: t_R = 2.24 min (A/D = 100/0 to 0/100 in 5.0 min., λ = 214 nm). HRMS (NSI+): $C_{132}H_{258}N_{30}O_{19}$ calc./found 2568.01 /2568.01 Da $[M]^+$.

Analytical RP-HPLC chromatogram:

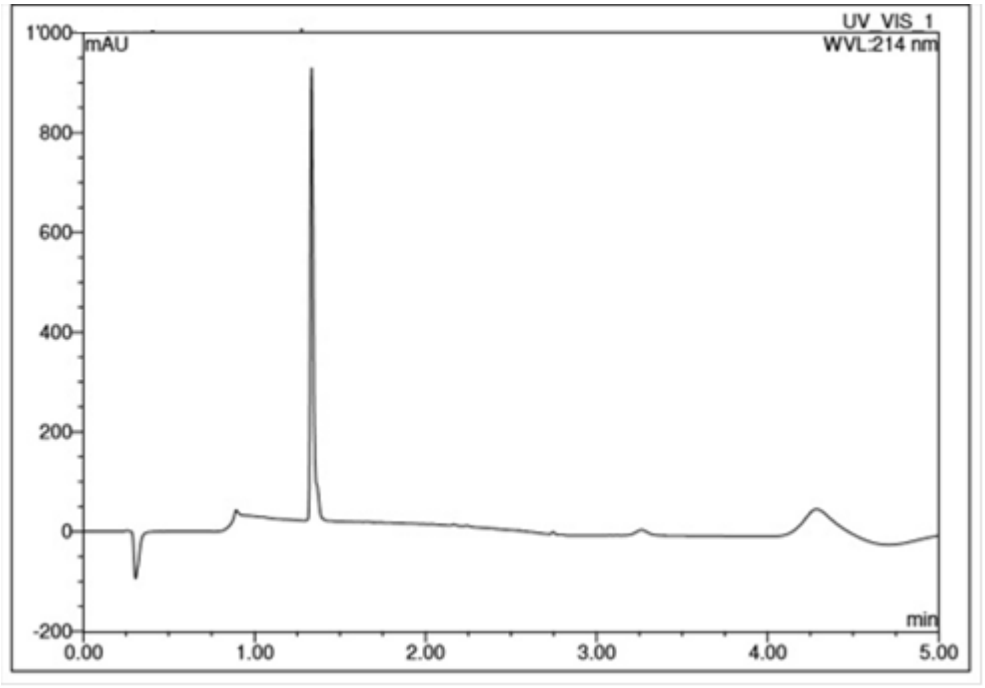


Mass spectrum, HRMS (NSI+)

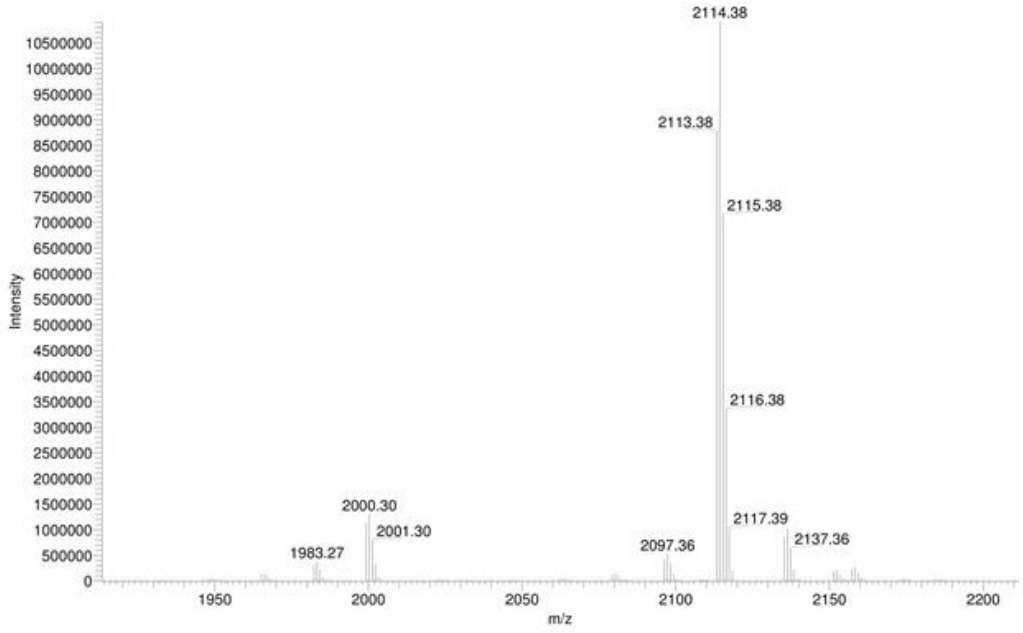


TNS08 $(OF)_4(KBL)_2KKL$. From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (44 mg, 17.3%). Anal. RP-HPLC: t_R = 1.37 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{106}H_{176}N_{28}O_{17}$ calc./found 2113.38/2113.38 Da [M].

Analytical RP-HPLC chromatogram:

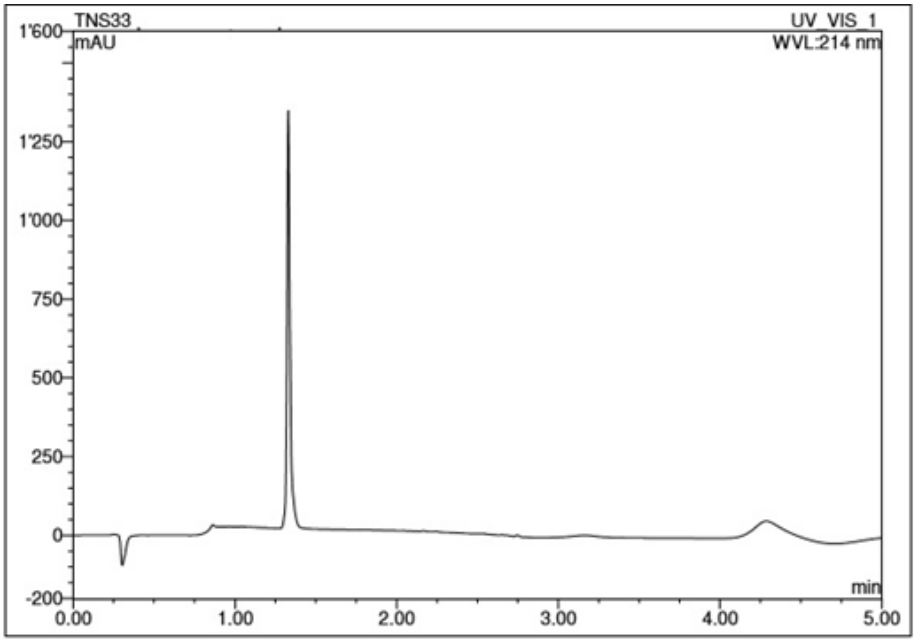


Mass spectrum, HRMS (NSI+)

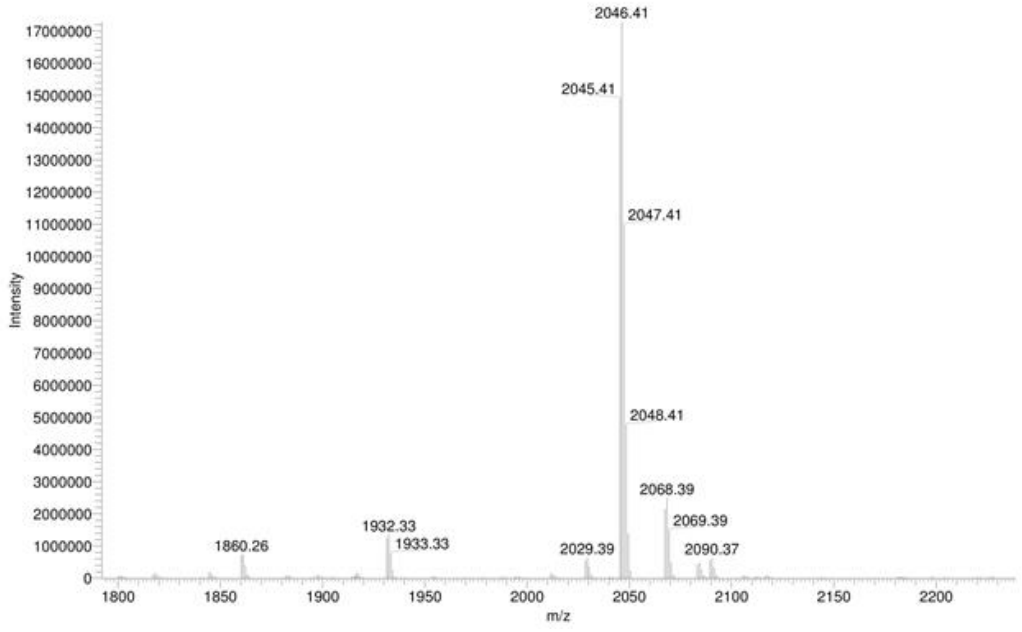


TNS09 $(OL)_4(KBF)_2KKL$. From Tenta Gel S RAM® resin (280 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (30 mg, 21.5%). Anal. RP-HPLC: t_R = 1.36 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{100}H_{180}N_{28}O_{17}$ calc./found 2045.41/2045.41 Da [M].

Analytical RP-HPLC chromatogram:

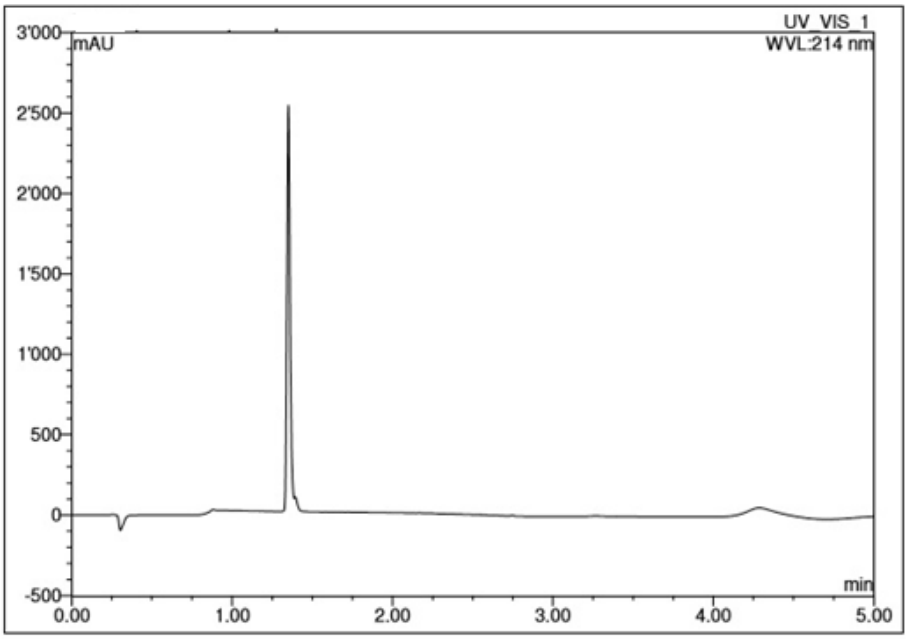


Mass spectrum, HRMS (NSI+)

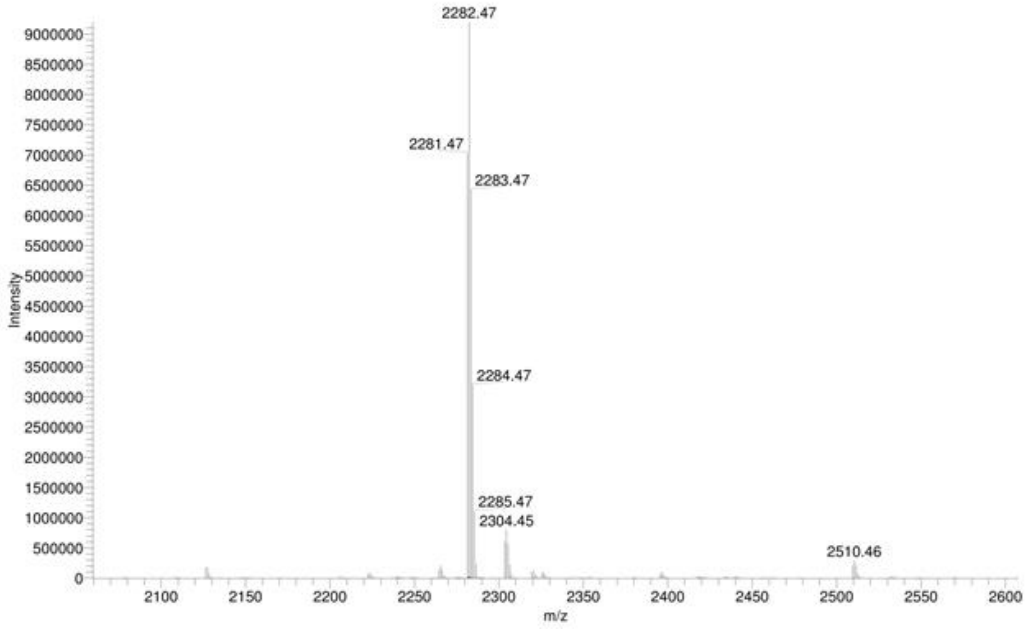


TNS10 $(RF)_4(KBL)KKL$. From Tenta Gel S RAM® resin (630 mg, 0.24mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (44 mg, 12.8%). Anal. RP-HPLC: t_R = 1.38 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{110}H_{184}N_{36}O_{17}$ calc./found 2281.46/2281.47 Da [M].

Analytical RP-HPLC chromatogram:

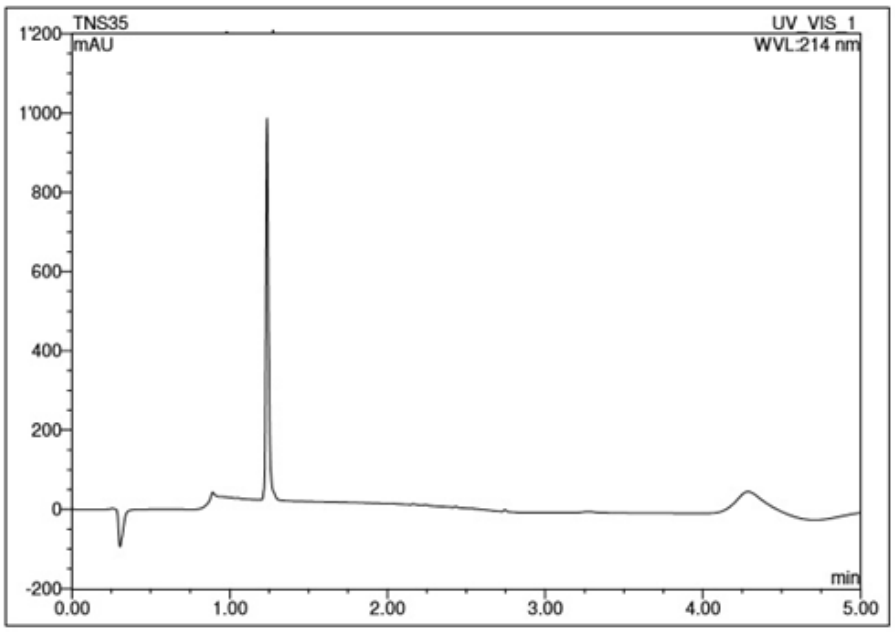


Mass spectrum, HRMS (NSI+)

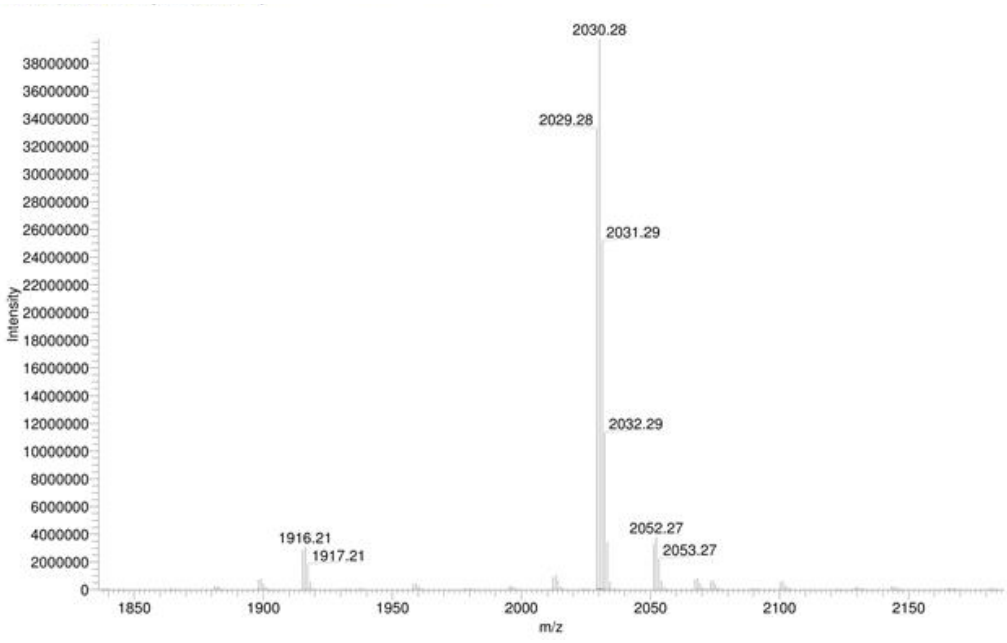


TNS11 $(OF)_4(KB\beta A)_2KKL$. From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (38 mg, 15.6%). Anal. RP-HPLC: t_R = 1.27 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{100}H_{164}N_{28}O_{17}$ calc./found 2029.28/2029.28 Da [M].

Analytical RP-HPLC chromatogram:

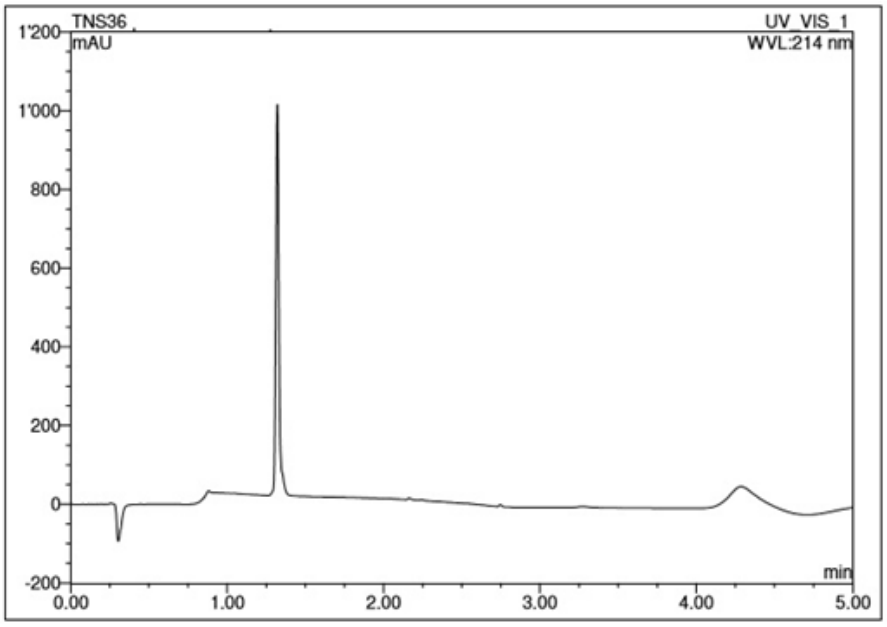


Mass spectrum, HRMS (NSI+)

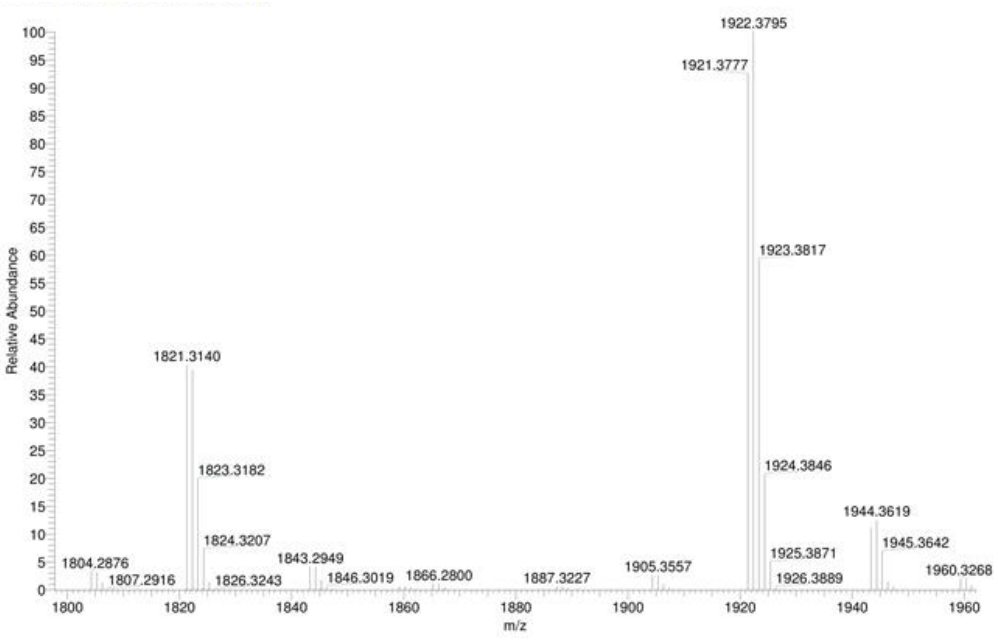


TNS12 $(BL)_4(KBL)_2KKL$. From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (28 mg, 12.1%). Anal. RP-HPLC: t_R = 1.35 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{90}H_{176}N_{28}O_{17}$ calc./found 1921.37/1921.37 Da [M].

Analytical RP-HPLC chromatogram:

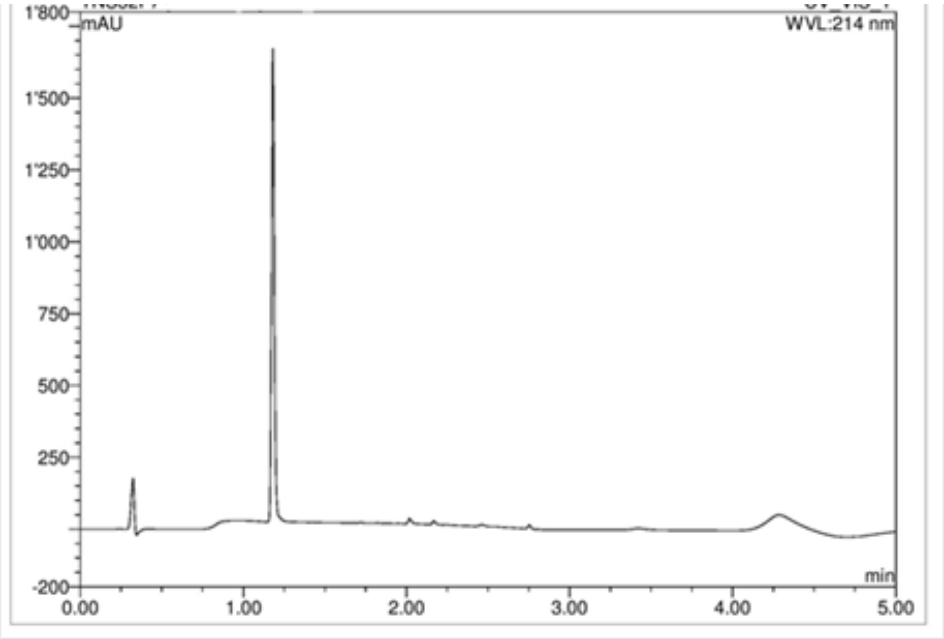


Mass spectrum, HRMS (NSI+)

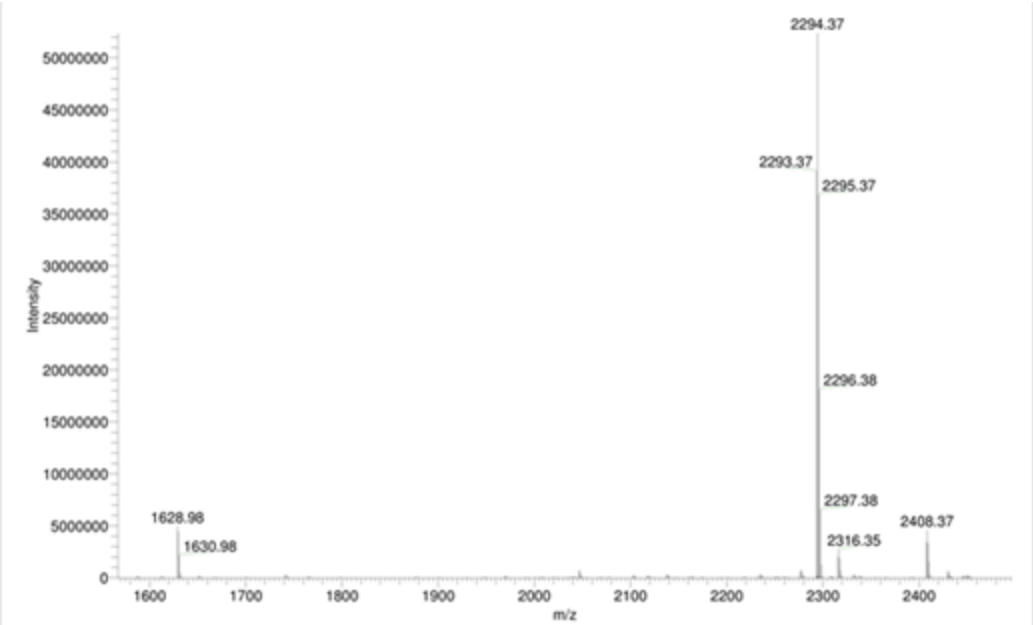


TNS13 $(RX)_4(KBF)_2KKL$. From Tenta Gel S RAM® resin (320 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (28 mg, 15.2%). Anal. RP-HPLC: t_R = 1.27 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{112}H_{172}N_{36}O_{17}$ calc./found 2293.37/2293.37 Da [M].

Analytical RP-HPLC chromatogram:

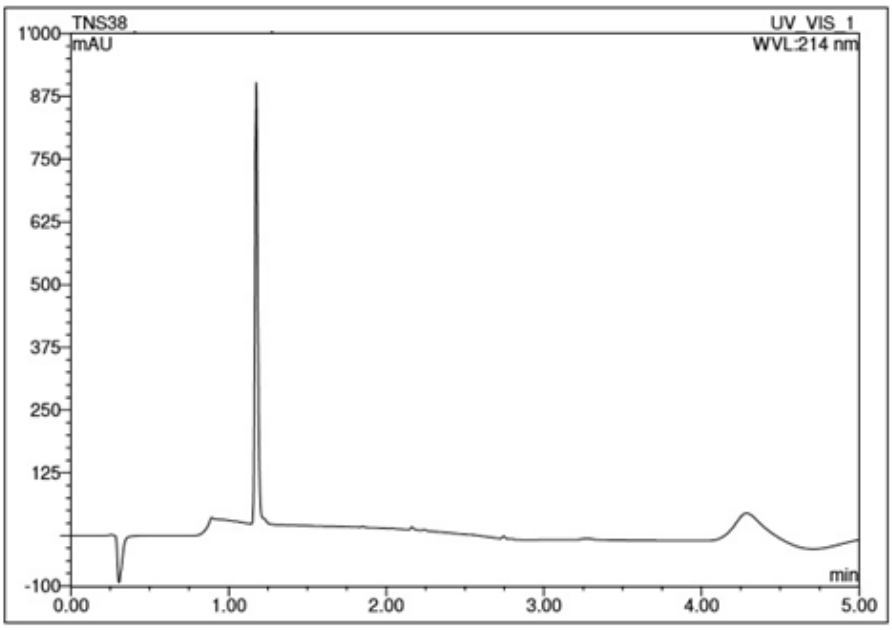


Mass spectrum, HRMS (NSI+)

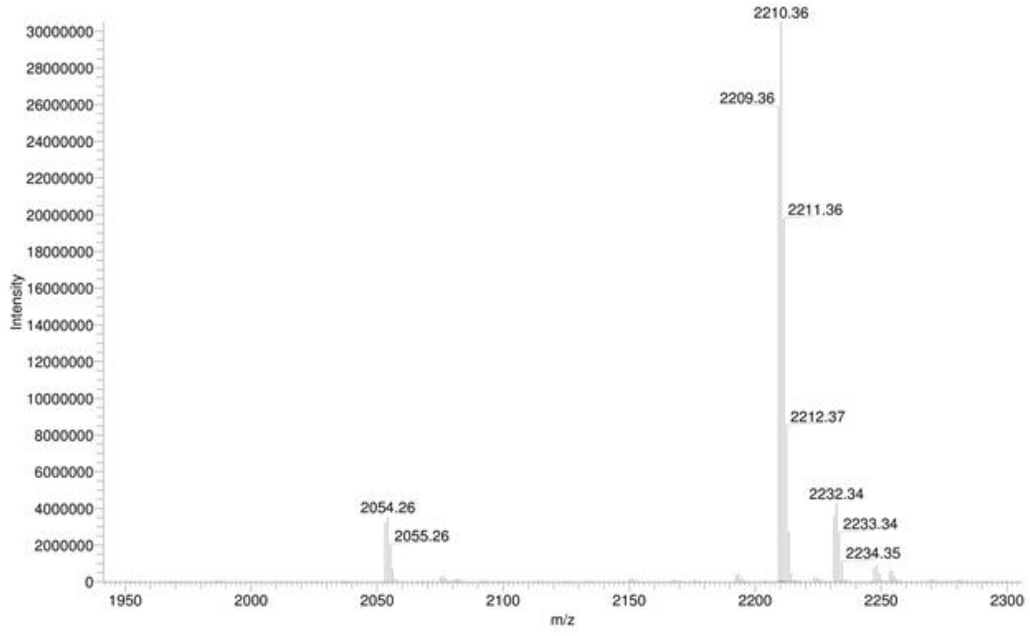


TNS14 $(RE)_4(KBL)_2KKL$. From Tenta Gel S RAM® resin (430 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (32 mg, 14.10%). Anal. RP-HPLC: t_R = 1.21 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{94}H_{176}N_{36}O_{25}$ calc./found 2209.36 /2209.36 Da[M].

Analytical RP-HPLC chromatogram:

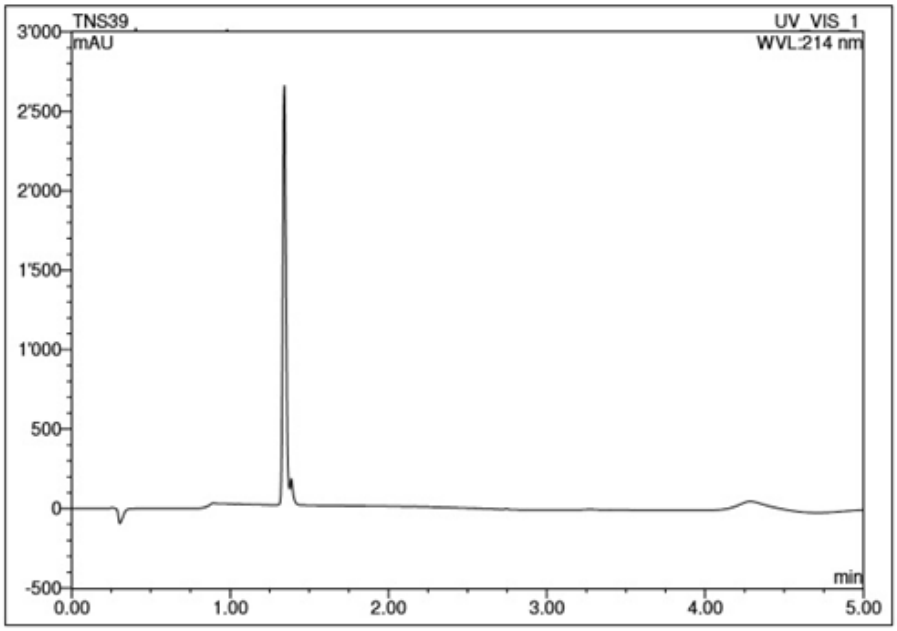


Mass spectrum, HRMS (NSI+)

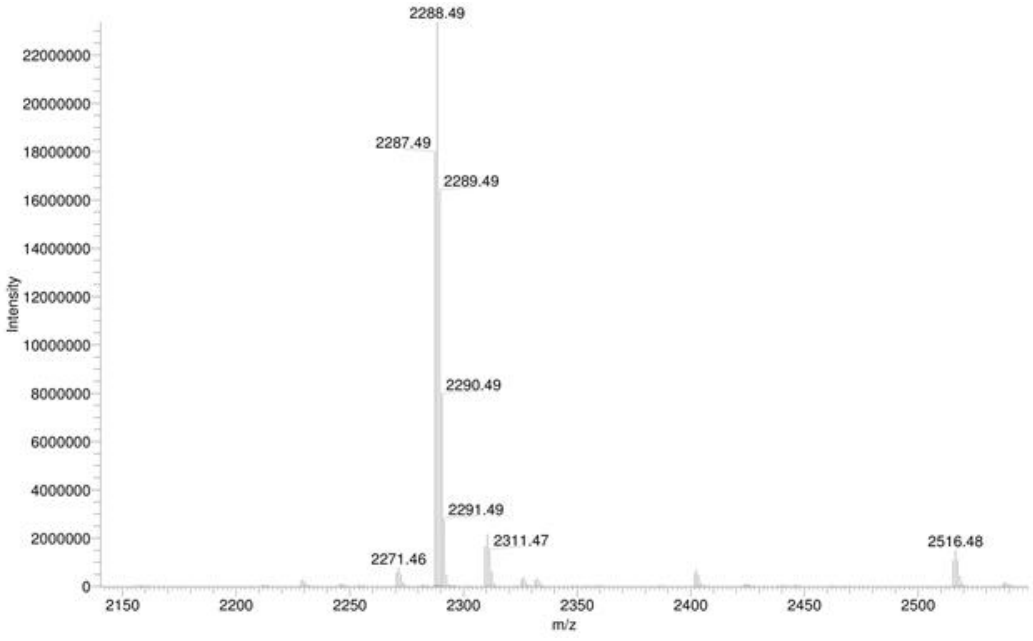


TNS15 $(RL)_4(KHF)_2KKL$. From Tenta Gel S RAM® resin (630 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (42 mg, 12.2%). Anal. RP-HPLC: t_R = 1.38 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{108}H_{186}N_{38}O_{17}$ calc./found 2287.49/2287.49 Da [M].

Analytical RP-HPLC chromatogram:

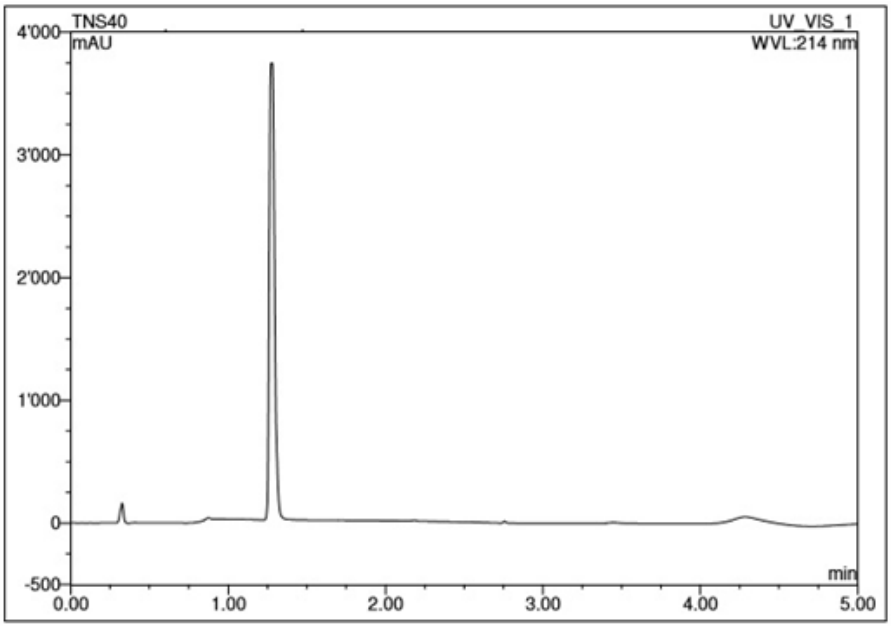


Mass spectrum, HRMS (NSI+)

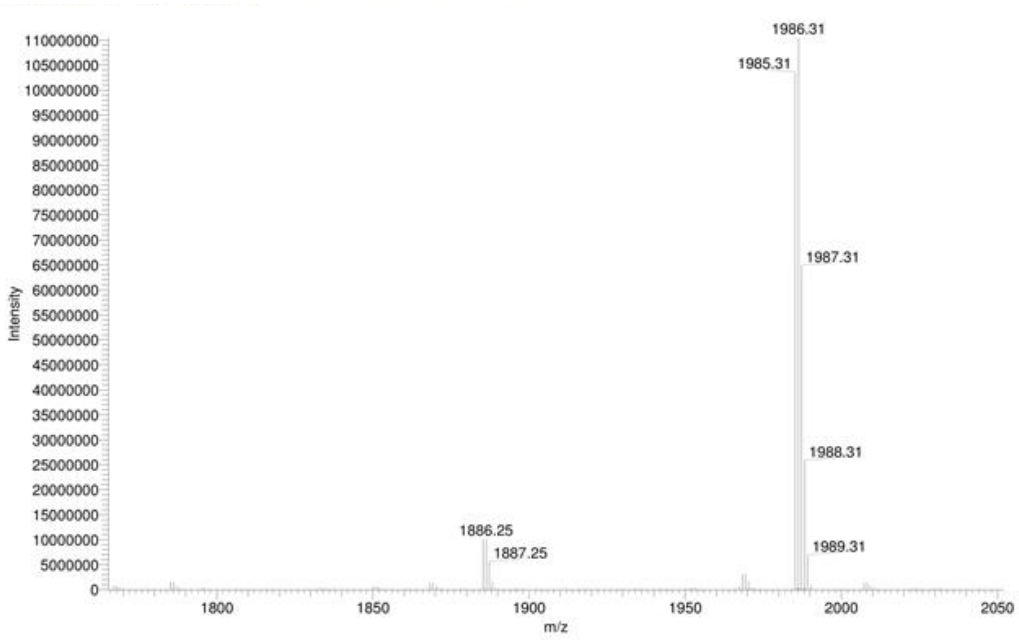


TNS16 $(BT)_4(KRL)_2KKL$. From Tenta Gel S RAM® resin (660 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (36 mg, 11.4%). Anal. RP-HPLC: t_R = 1.18 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{86}H_{168}N_{32}O_{21}$ calc./found 1985.30/1985.31 Da [M].

Analytical RP-HPLC chromatogram:

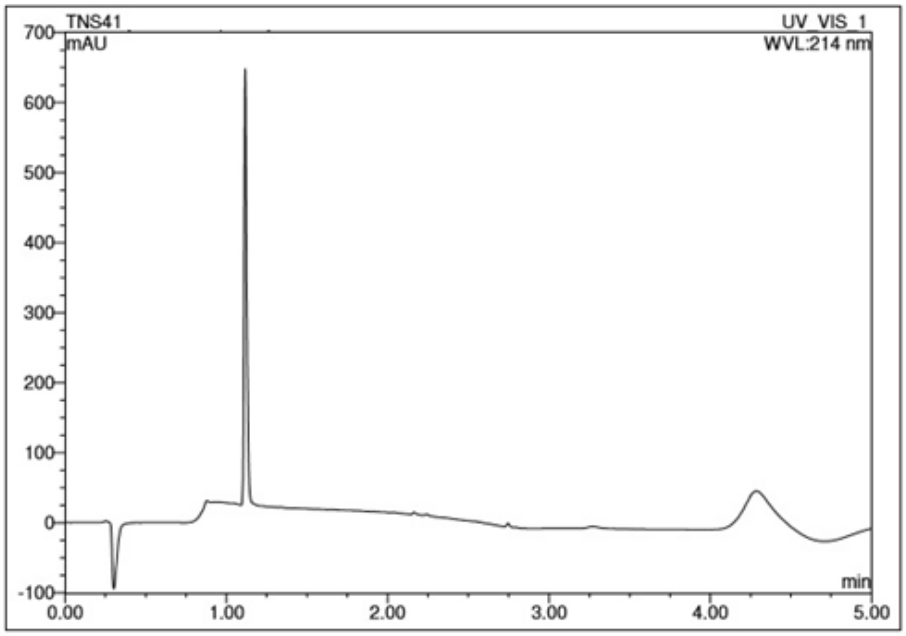


Mass spectrum, HRMS (NSI+)

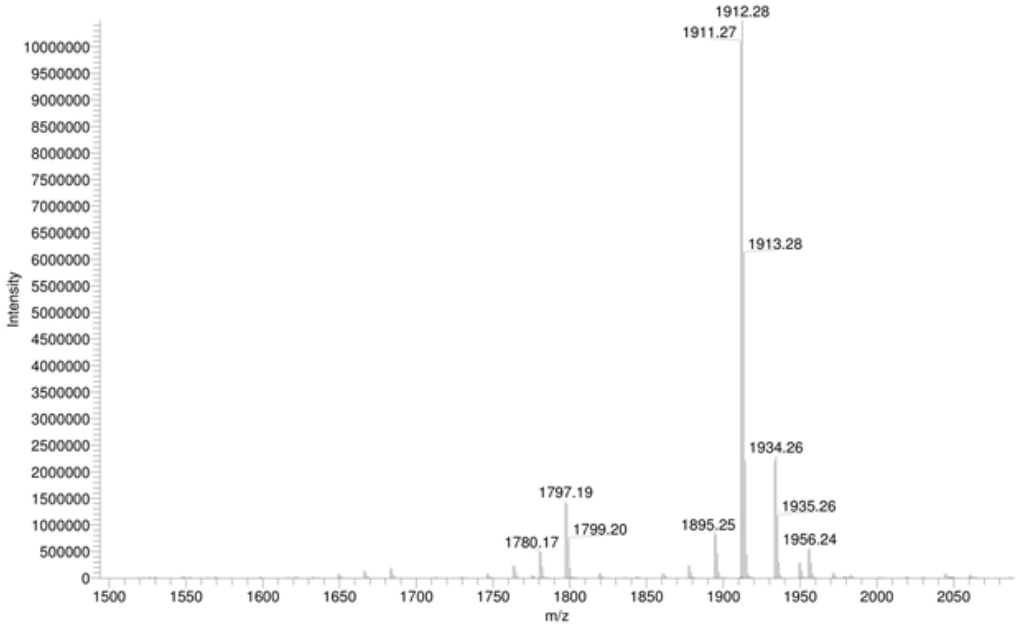


TNS17 $(OZ)_4(KHV)_2KKL$. From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (12 mg, 5.2%). Anal. RP-HPLC: t_R = 1.15 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{88}H_{162}N_{30}O_{17}$ calc./found 1911.27 /1911.27 Da [M].

Analytical RP-HPLC chromatogram:

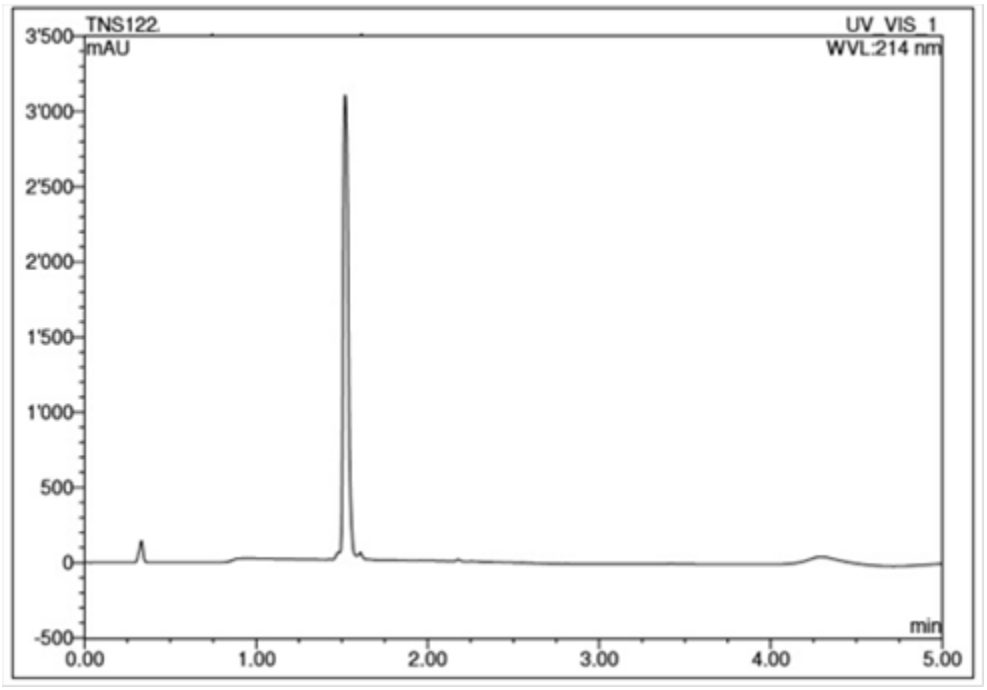


Mass spectrum, HRMS (NSI+)

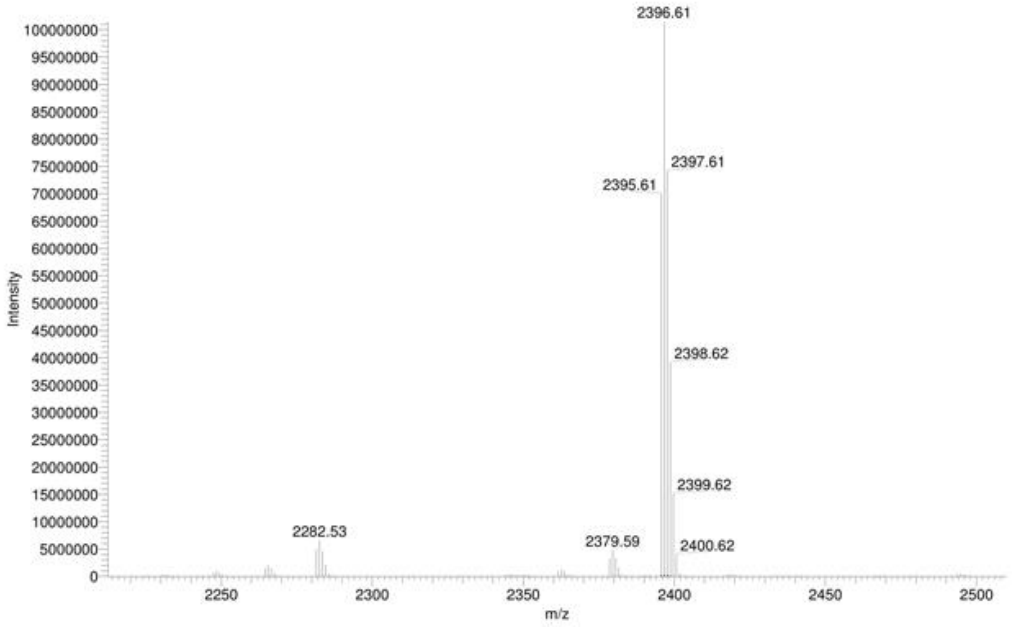


TNS18 $(OF)_4(KBL)_2KKLK(C_{10})$. From Tenta Gel S RAM® resin (500 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (121 mg, 41.7%). Anal. RP-HPLC: t_R = 1.54 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{122}H_{206}N_{30}O_{19}$ calc./found 2395.61/2395.61 Da [M].

Analytical RP-HPLC chromatogram:

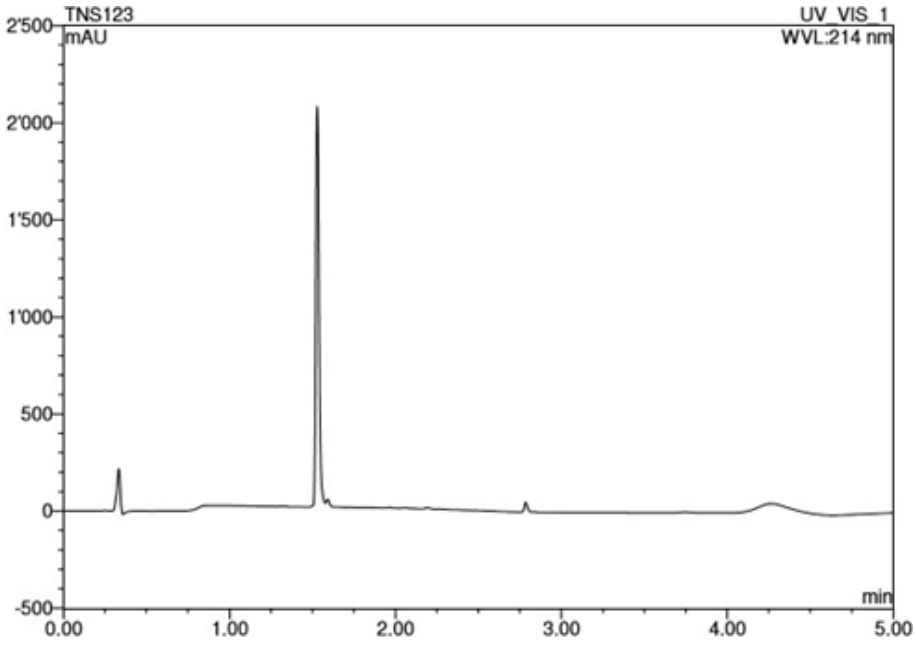


Mass spectrum, HRMS (NSI+)

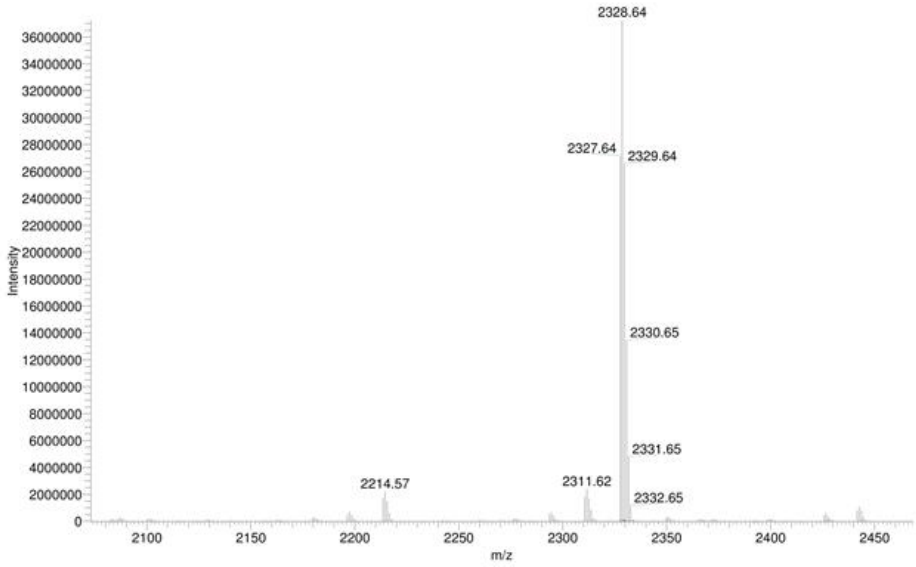


TNS19 $(OL)_4(KBF)_2KKLK(C_{10})$. From Tenta Gel S RAM® resin (530 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (28 mg, 9.4%). Anal. RP-HPLC: t_R = 1.53 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+): $C_{116}H_{210}N_{30}O_{19}$ calc./found 2327.64/2327.64 Da[M].

Analytical RP-HPLC chromatogram:

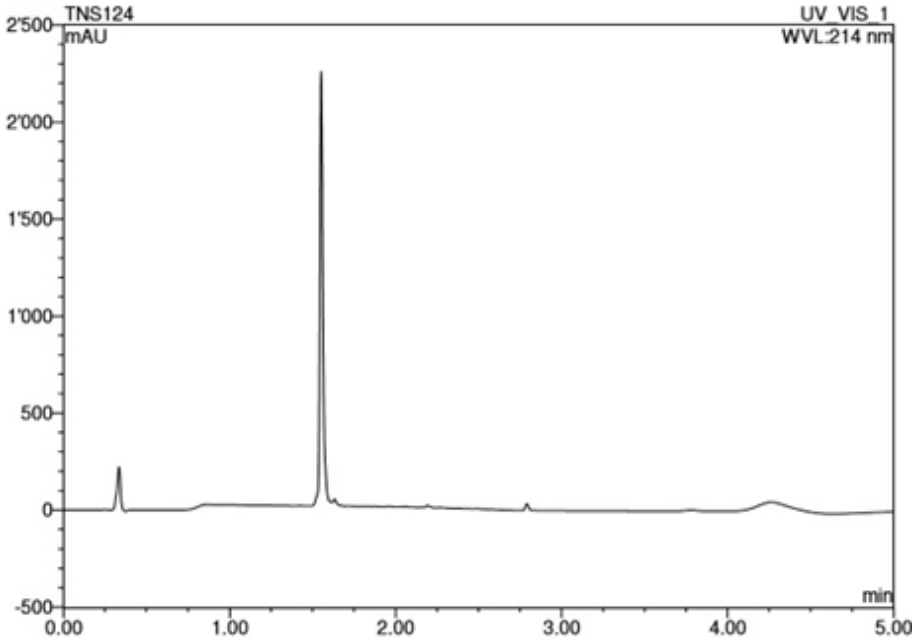


Mass spectrum, HRMS (NSI+)

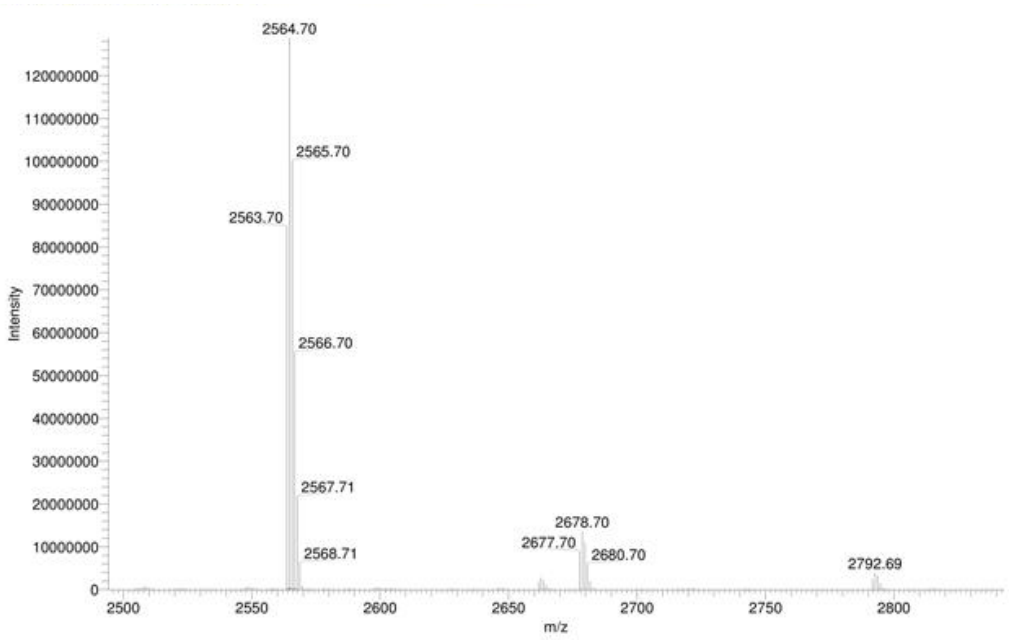


TNS20 $(RF)_4(KBL)_2KKLK$-C10 From Tenta Gel S RAM® resin (350 mg, 0.24 mmol·g^{-1}), the dendrimer was obtained as a white foamy solid after preparative RP-HPLC purification (24 mg, 11.2%). Anal. RP-HPLC: t_R = 1.55 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) HRMS (NSI+):$C_{126}H_{214}N_{38}O_{19}$ calc./found 2563.69/2563.70 Da[M].

Analytical RP-HPLC chromatogram:



Mass spectrum, HRMS (NSI+)



Broth Microdilution Assay

Broth Microdilution Method I (Data in Table 1). Antimicrobial activity was assayed against *Bacillus subtilis* (strain BR151), *Escherichia coli* (strain DH5α) and *Pseudomonas aeruginosa* (strain PA01). To determine the minimal inhibitory concentration (MIC), broth microdilution method was used.[3] A colony of bacteria was grown in LB-medium overnight at 37°C. The concentration was quantified by measuring absorbance at 600 nm and diluted to OD_{600} = 0.1 ($1x10^8$ CFU·mL^{-1}). The samples were prepared as stock solutions of 1 mg·mL^{-1} in H_2O and diluted serially by 2/3 in nutrient LB in a 96-well microtiter plate (Corning-Costar, polypropylene, untreated). The sample solutions (50 µL) were mixed with the diluted bacterial suspension with an OD_{600nm} of 0.001 (50 µL), resulting in the final desired concentration of $5x10^5$ CFU·mL^{-1}. The plates were incubated at 37°C until satisfactory growth (18 - 24h). For each test, two columns of the plate were kept for sterility control (broth only) and growth control (broth with bacterial inoculums, no antibiotics). 10 µL of a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (0.1% in H_2O) was added to each well. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the peptide dendrimer that inhibited visible growth of the tested bacteria (yellow) with the unaided eye.

Broth Microdilution Method II (Data in Table 2-4). Antimicrobial activity was assayed against *P. aeruginosa* PA01 (WT), *P. aeruginosa* (PT1482 (A-), PT1485 (A-B-), (LPS mutants)), *P. aeruginosa* (PEJ2.6, PEJ9.1, ZEM-1A, ZEM-9A (multiple drug resistant)), *Acinetobacter baumannii* (*A. baumannii* 19606), *E. coli* (*E. coli* (CP46), *E. coli* W3110 (TE823), *E. coli* MG1665 (TE824)) and *Stenotrophomonas maltophilia* (*St. maltophilia* (CP127)) (clinical isolates from Université de Genève / Centre Médical Universitaire). *Staphylococcus aureus* (*S.aureus* COL, *S.aureus* Newman) and *Staphylococcus epidermidis* were used as gram positive bacteria. To determine the minimal inhibitory concentration (MIC), broth microdilution method was used.[3] A colony of bacteria was grown in MH-medium overnight at 37°C. The samples were prepared as stock solutions of 8 mg·mL^{-1} in H_2O, diluted to the beginning concentration of 32, 64, 128 or 256 µg·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.[4]

Broth Microdilution Method III (Data in Table 2). Microdilution broth method was used to determine the minimum inhibitory concentration of peptide dendrimers against *P.aeruginosa* (PA01) in MH-medium with 30% human serum.[3] A colony of bacteria was grown in MH-medium overnight at 37°C. Microtiter plate, column 2-12 was filled with 150 µL MH-medium with 30% human serum. The samples were prepared as stock solutions of 8 mg·mL^{-1} in H_2O and diluted to the beginning concentration of 32, 64, 128 or 256 µg·mL^{-1} in 300 µL MH-medium with 30% human serum. This was transferred to the first well of 96-well microtiter

plate and diluted serially by ½. The bacterial suspension was prepared same as Broth microdilution Method II and 4 µL of diluted bacterial suspension added to the each sample solution in microtiter plate. 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.[4]

Hemolysis Assay

To determine the minimal hemolytic concentration (MHC) stock solutions of 8 $mg \cdot mL^{-1}$ of the peptide dendrimers in H_2O were prepared and 50 µL were diluted serially by 1/2 in 50 µL PBS (pH 7.4) in 96-well plate (Corning-Costar or Nunc, polystyrene, untreated). 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 a 15 mL Falcon tube up to 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]

Library Synthesis

The peptide dendrimer library was prepared from 1.5 g of TentaGel S NH_2 resin (loading 0.32 $mmol \cdot g^{-1}$, 0.48 mmol), which was suspended in 10 mL DCM in a 20 mL syringe for 10 minutes and deprotected with 20% piperidine in DMF (20 min). After washing 3 times with about 8 mL of NMP, MeOH, DCM the hydroxyethyl photolinker (4-[4(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]butanoic acid) (1.5 eq., 0.72 mmol, 215 mg) was attached to the resin in the presence of PyBOP (1.5 eq., 0.72 mmol, 374 mg) and DIPEA (3 eq., 1.44 mmol, 185 mg) was added to reaction vessel and stirred in the dark for 2 hours. The TNBS test was used to detect the presence of unreacted amine groups on solid support after washing 3 times with about 8 mL of NMP, MeOH, DCM. The attachment of the first amino acid to photolinker was done using MSNT (1-mesitylene-2-sulfony)-3-nitro-1*H*-1,2,4-triazole) with 1-MeIm (1-methylimidazole). The resin was placed in a syringe, dried overnight at high vacuum as well as MSNT (6 eq., 2.88 mmol, 852 mg) and Fmoc-Leu-OH (6eq, 2.88 mmol, 1016.6 mg). The beads were swelled with 6 mL dry DCM in argon. In a round bottom flask Fmoc-Leu-OH and MSNT were suspended in 4 mL dry DCM under argon separately. After the resin was dried under vacuum 1-MeIm (6eq, 2.88 mmol, 229 µL) was added to Fmoc-Leu-OH suspension. This mixture was then added to the syringe and shaken at room temperature for 2 h. After washing 3 times with about 8 mL of NMP, MeOH, DCM the coupling was repeated two times and the last coupling overnight.[6] The Fmoc-protecting groups of the resin were removed as described previously.

For further couplings, the resin was acylated with one of the amino acids (3 eq/amine,) in the presence of PyBOP (3 eq/amine) and DIPEA (5 eq/amine) in about 6 mL NMP. Amino acids, derivatives or diamino acids were coupled for 1 h (G0), 2 h (G1), 3 h (G2). The completion of the reaction was tested with TNBS test or chloranil test. The coupling was repeated after a positive test. After each coupling, the resin in each syringe was deprotected with 20% piperidine in DMF (20 min) followed by TNBS or chloranil test. Split and mix synthesis was started from first generation and resin batch divided equally according to the number of variable amino acids in each position. After attaching each corresponding amino acid, resin batches were mixed together, agitated well and split equally. After each coupling or deprotection resin was successively washed 3 times with about 8 mL of NMP, MeOH, DCM. At the end of the synthesis, the Fmoc protected library was dried and stored at -18 °C. For all the steps, the syringe was covered with aluminum foil to prevent exposure to light.

Bead Diffusion Assay

Before screening of the library, the Fmoc protecting groups were removed with 20% piperidine in DMF (20 min) followed by removal of side chain protecting groups with TFA/TIS/H_2O (94:5:1) resulting in a dendrimer library on-beads. Antimicrobial screening was performed by washing the beads (10 mg, 3×5 mL) and (swelled 2×1h) in mQ-H_2O. Resin was dried under vacuum and poured on a Petri dish (diameter: 9 cm), then resin beads were partially irradiated under an Hg lamp (100W) with a 366 nm filter from 30 min up to 4 hours. *P.aeruginosa* PA01 bacteria were grown in LB overnight at 37°C. The concentration was quantified by measuring absorbance at 600 nm and diluted to an OD_{600} of 0.1. Bacteria were spread with a cotton swab on an agar plate; the irradiated beads were poured on it and incubated during 18 h until satisfactory growth. A solution of MTT (0.1% in H_2O) was sprayed on the plate. Under a microscope a clear zone (no bacteria) appeared on the plate around some of the beads. These active beads were picked, washed with EtOH and subjected to sequence determination by amino acid analysis.[7] Bead analysis results are given in Tables S1 and S2 below.

Table S1. Sequences determined from beads displaying a clearing zone.

Bead	Clearing[a)]	Sequence[b)]	Cpd nr.[c)]	Bead	Clearing[a)]	Sequence[b)]	Cpd nr.[c)]
BD1	Large	$(OF)_4(KBL)_2KKL$	TNS08	BD34	Medium	$(OF)_4(KHL)_2KKL$	
BD2	Large	$(OF)_4(KBL)_2KKL$	TNS08	BD35	Medium	$(OL)_4(KBX)_2KKL$	
BD3	Large	$(OF)_4(KBL)_2KKL$	TNS08	BD36	Medium	$(RL)_4(KBX)_2KKL$	
BD4	Large	$(OF)_4(KBL)_2KKL$	TNS08	BD37	Medium	$(RL)_4(KBF)_2KKL$	
BD5	Large	$(OF)_4(KBL)_2KKL$	TNS08	BD38	Medium	$(BP)_4(KBL)_2KKL$	
BD6	Large	$(RF)_4(KBL)_2KKL$	TNS10	BD39	Medium	$(BL)_4(KBL)_2KKL$	TNS12
BD7	Large	$(RL)_4(KHF)_2KKL$	TNS15	BD40	Small	$(OL)_4(KBF)_2KKL$	TNS09
BD8	Large	$(RX)_4(KBF)_2KKL$	TNS13	BD41	Small	$(OL)_4(KBF)_2KKL$	TNS09
BD9	Large	$(RE)_4(KBL)_2KKL$	TNS14	BD42	Small	$(OL)_4(KBF)_2KKL$	TNS09
BD10	Large	$(OL)_4(KBF)_2KKL$	TNS09	BD43	Small	$(OL)_4(KBF)_2KKL$	TNS09
BD11	Large	$(OL)_4(KBF)_2KKL$	TNS09	BD44	Small	$(OL)_4(KBF)_2KKL$	TNS09
BD12	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD45	Small	$(OF)_4(KBL)_2KKL$	TNS08
BD13	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD46	Small	$(OF)_4(KBL)_2KKL$	TNS08
BD14	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD47	Small	$(OF)_4(KBL)_2KKL$	TNS08
BD15	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD48	Small	$(OF)_4(KBL)_2KKL$	TNS08
BD16	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD49	Small	$(RF)_4(KBL)_2KKL$	TNS10
BD17	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD50	Small	$(RF)_4(KBL)_2KKL$	TNS10
BD18	Medium	$(OL)_4(KBF)_2KKL$	TNS09	BD51	Small	$(RF)_4(KBL)_2KKL$	TNS10
BD19	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD52	Small	$(RF)_4(KBL)_2KKL$	TNS10
BD20	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD53	Small	$(OX)_4(KBL)_2KKL$	
BD21	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD54	Small	$(RF)_4(KBF)_2KKL$	TNS10
BD22	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD55	Small	$(RL)_4(KBZ)_2KKL$	
BD23	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD56	Small	$(OL)_4(KOF)_2KKL$	
BD24	Medium	$(OF)_4(KBL)_2KKL$	TNS08	BD57	Small	$(OF)_4(KHZ)_2KKL$	
BD25	Medium	$(RL)_4(KBT)_2KKL$		BD58	Small	$(R\beta A)_4(KBT)_2KKL$	
BD26	Medium	$(RX)_4(KHL)_2KKL$		BD59	Small	$(RT)_4(KBL)_2KKL$	
BD27	Medium	$(BF)_4(KHX)_2KKL$		BD60	Small	$(O\beta A)_4(KOF)_2KKL$	
BD28	Medium	$(RF)_4(KBL)_2KKL$	TNS10	BD61	Small	$(HF)_4(KBL)_2KKL$	
BD29	Medium	$(R\beta A)_4(KHL)_2KKL$		BD62	Small	$(OF)_4(KBX)_2KKL$	
BD30	Medium	$(HF)_4(KOL)_2KKL$		BD63	Small	$(OF)_4(KB\beta A)_2KKL$	TNS11
BD31	Medium	$(OX)_4(KBF)_2KKL$		BD64	Small	$(RL)_4(KBF)_2KKL$	
BD32	Medium	$(R\beta A)_4(KHX)_2KKL$		BD65	None	$(BT)_4(KRL)_2KKL$	TNS16
BD33	Medium	$(OF)_4(KHL)_2KKL$		BD66	None	$(OZ)_4(KHV)_2KKL$	TNS17

[a)] Size of the clearing zone surrounding the bead after staining with MTT. [b)] Sequence of the dendrimer as determined from amino acid analysis of the picked bead. One-letter codes for amino acids, O = ornithine, X = 4-aminomethyl-benzoic acid, Z = γ-aminobutyric acid, B = diaminobutyric acid, *K* = branching lysine, βA = β-alanine [c)] Numbers were assigned only to sequences that were resynthesized.

Table S2. Sequences determined from beads picked randomly before photolysis.

Bead	Sequence[a)]	Bead	Sequence[a)]
BDC1	$(BF)_4(KOT)_2KKL$	BDC14	$(OL)_4(KHZ)_2KKL$
BDC2	$(RE)_4(KBE)_2KKL$	BDC15	$(RN)_4(KOP)_2KKL$
BDC3	$(RN)_4(KRV)_2KKL$	BDC16	$(HN)_4(KBF)_2KKL$
BDC4	$(RF)_4(KBL)_2KKL$	BDC17	$(ON)_4(KRZ)_2KKL$
BDC5	$(BE)_4(KRN)_2KKL$	BDC18	$(HZ)_4(KOL)_2KKL$
BDC6	$(HZ)_4(KRN)_2KKL$	BDC19	$(OP)_4(KHL)_2KKL$
BDC7	$(OT)_4(KBE)_2KKL$	BDC20	$(RF)_4(KOV)_2KKL$
BDC8	$(BX)_4(KOX)_2KKL$	BDC21	$(OP)_4(KRL)_2KKL$
BDC9	$(HT)_4(KBE)_2KKL$	BDC22	$(OL)_4(KBX)_2KKL$
BDC10	$(HT)_4(KBV)_2KKL$	BDC23	$(HX)_4(KBE)_2KKL$
BDC11	$(RF)_4(KBV)_2KKL$	BDC24	$(HF)_4(KBX)_2KKL$
BDC12	$(BX)_4(KHL)_2KKL$	BDC25	$(OZ)_4(KBL)_2KKL$
BDC13	$(RE)_4(KBE)_2KKL$		

[a)] Sequence of the dendrimer as determined from amino acid analysis of the picked bead. One-letter codes for amino acids, O = ornithine, X = 4-aminomethyl-benzoic acid, Z = γ-aminobutyric acid, B = diaminobutyric acid, *K* = branching lysine, βA = β-alanine.

Time-kill Experiments

A small quantity of the *Pseudomonas aeruginosa* PAO1 glycerol stock (stored at -80°C) was streaked on to Luria-Bertani (LB) agar plate and incubated at 37°C overnight. A single colony of *Pseudomonas aeruginosa* was grown with shaking at 180 rpm in LB-broth (5 mL) and overnight at 37°C. The resulting bacterial culture was diluted to OD_{600} = 0.004 in fresh MH-medium and mixed 1:1 with antibiotics (dissolved in MH-medium) of interest in a 96 well plate. The plate was then incubated at 37°C shaking at 180 rpm. OD_{600} were measured every hour. The assay was performed in triplicate using the concentrations of 2×MIC for Tobramycin (0.5 $\mu g \cdot mL^{-1}$), Polymyxin B (0.5 $\mu g \cdot mL^{-1}$), G3KL (8 $\mu g \cdot mL^{-1}$), TNS03 (8 $\mu g \cdot mL^{-1}$), and TNS18 (8 $\mu g \cdot mL^{-1}$) or no compound (growth control). Bacterial growth was quantified after 0, 0.5, 1, 2, 3, 4, 5, 6 and 7 hours incubation at 37 °C by plating 10-fold dilutions on LB agar. Plates were incubated overnight at 37°C and the number of individual colonies counted at each time-point.

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 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 by addition of 100 µL of 0.1 M $ZnSO_4$/acetonitrile (1:1) solution, 10 min in ice and 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.[8] Experiments were done in triplicates.

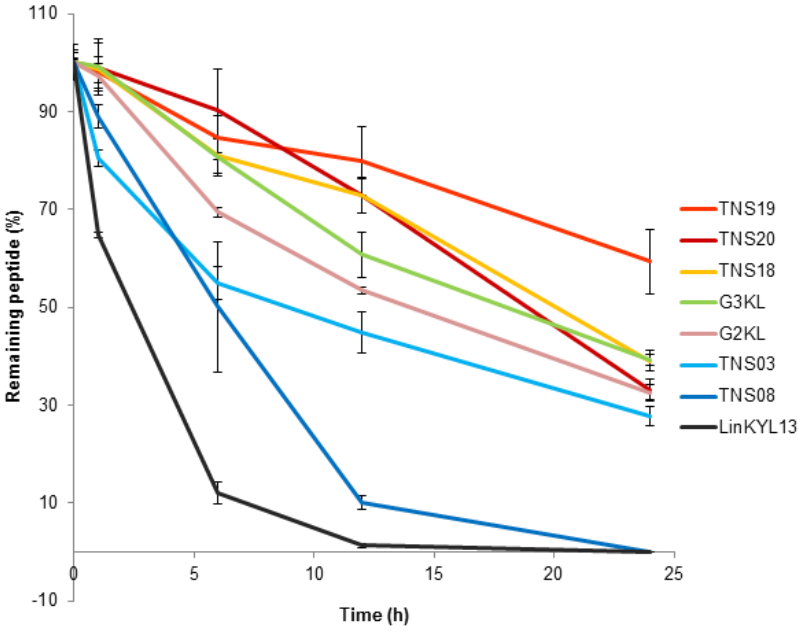


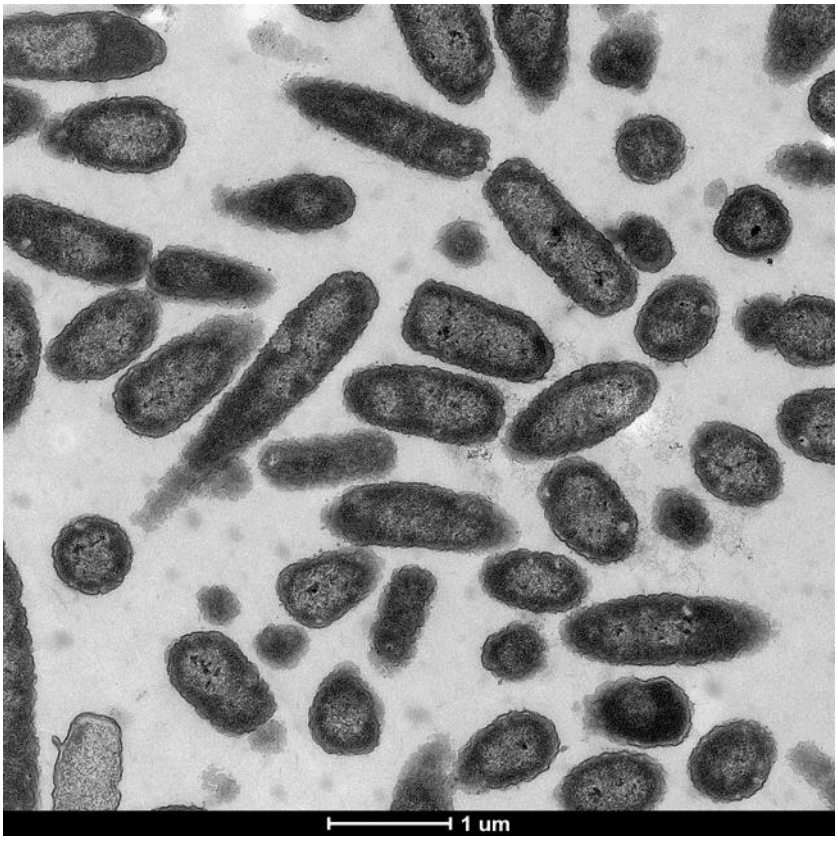
Figure S1. Stability of peptide dendrimers measured over 24 hours in 25% human serum with 0.1 M Tris-HCl pH 7.5 buffer. Average ± standard deviation from triplicate measured is given. LinKYL13 is linear peptide (KYKKALKKLAKLL) which was used as positive control.

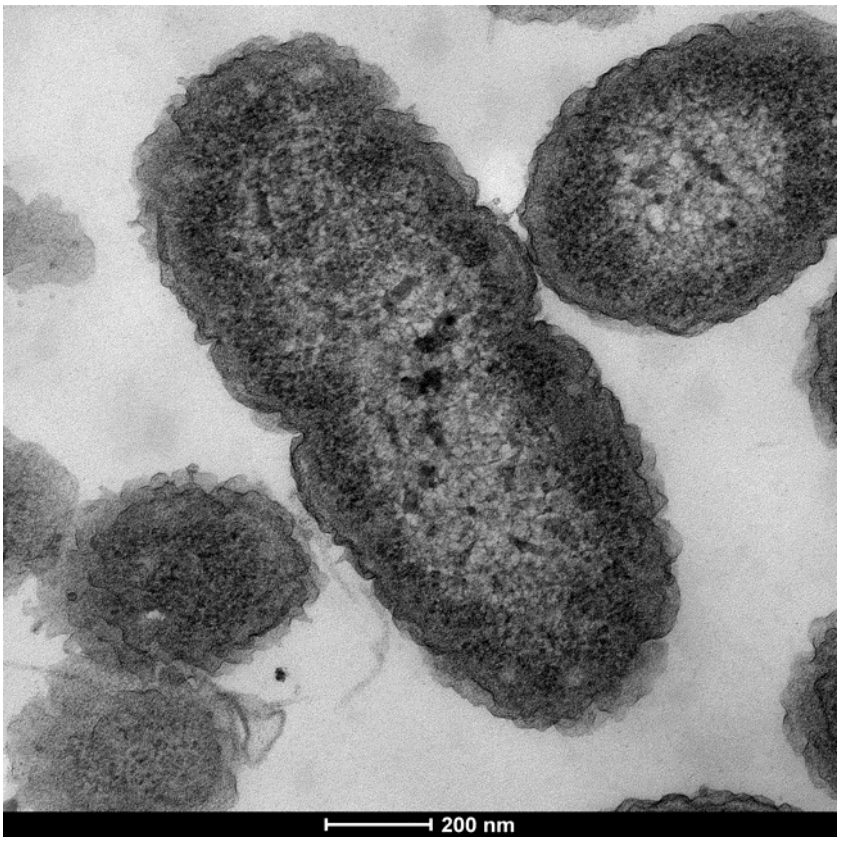
Transmission Electron Microscopy

The culture of *Pseudomonas aeruginosa* PAO1 in the exponential phase was washed with PBS and treated 10 x MIC of the corresponding compound in M63 minimal medium. 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, PAO1 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 0.1 M Na-cacodylate-buffer 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 witch 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 uranyl acetate and lead citrate with an ultrostainer (Leica Microsystems, Vienna, Austria). Sections were then examined with a Tecnai Spirit transmission electron microscope equipped with two digital cameras (Olympus-SIS Veleta CCD Camera, FEI Eagle CCD Camera).

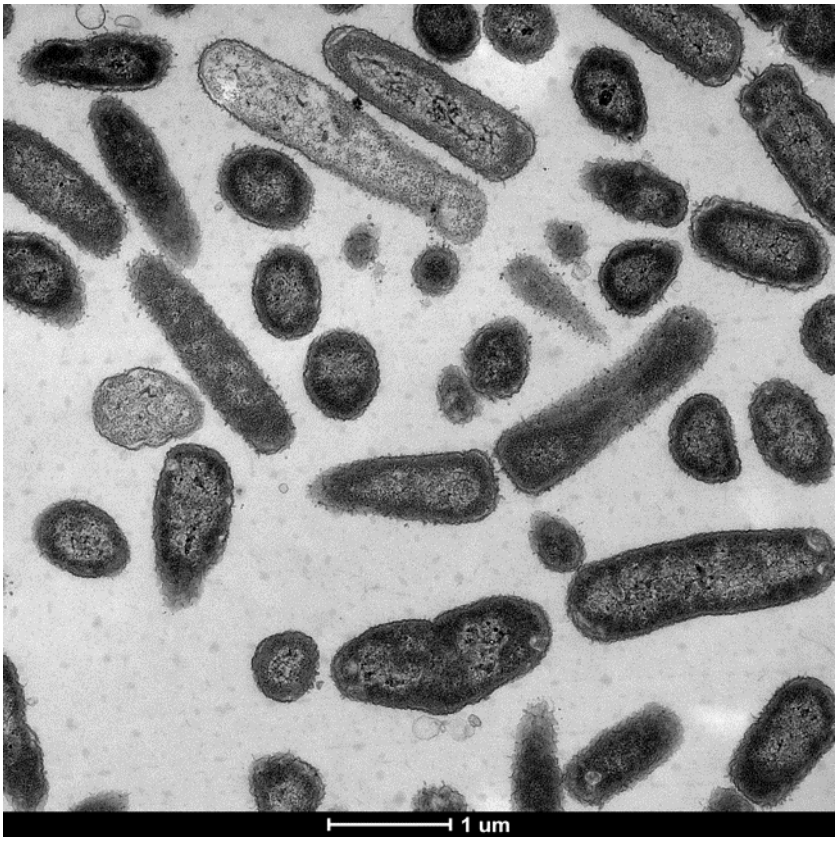
Figure S2. Next 6 pages: Enlarged TEM images from Figure 4, showing *P. aeruginosa* cells treated for 60 min. at 10×MIC with Polymyxin B (5 µg/mL), **TNS18** (40 µg/mL), **TNS03** (40 µg/mL), and **G3KL** (40 µg/mL) and **G2KL** (128 µg/mL).

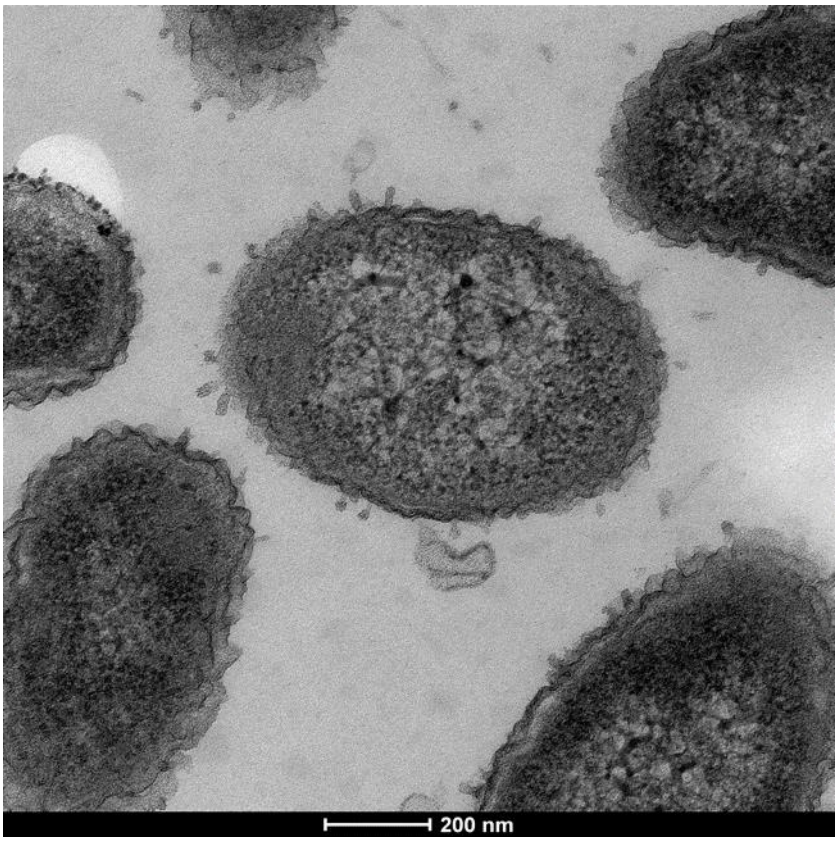
Control no cpd. added



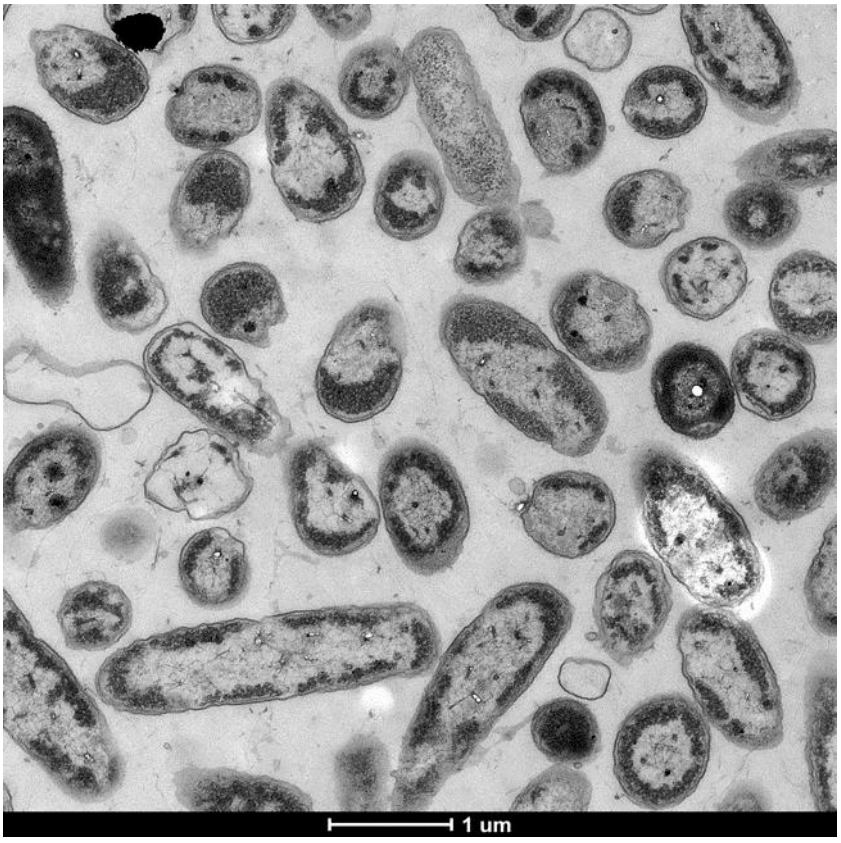


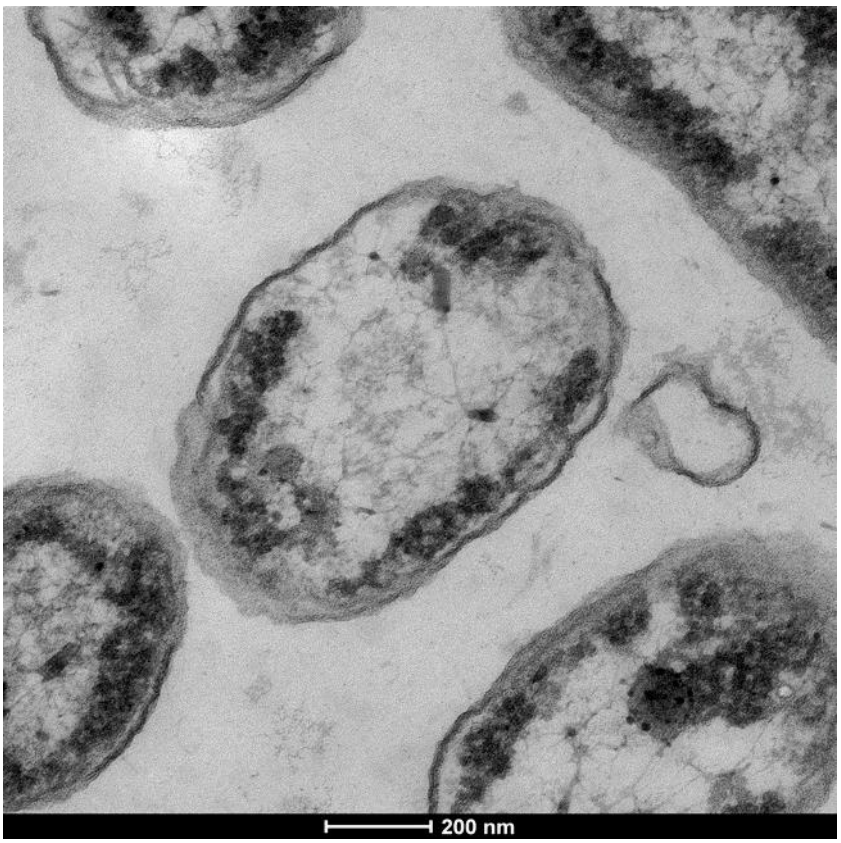
Polymyxin B 5 μg/mL



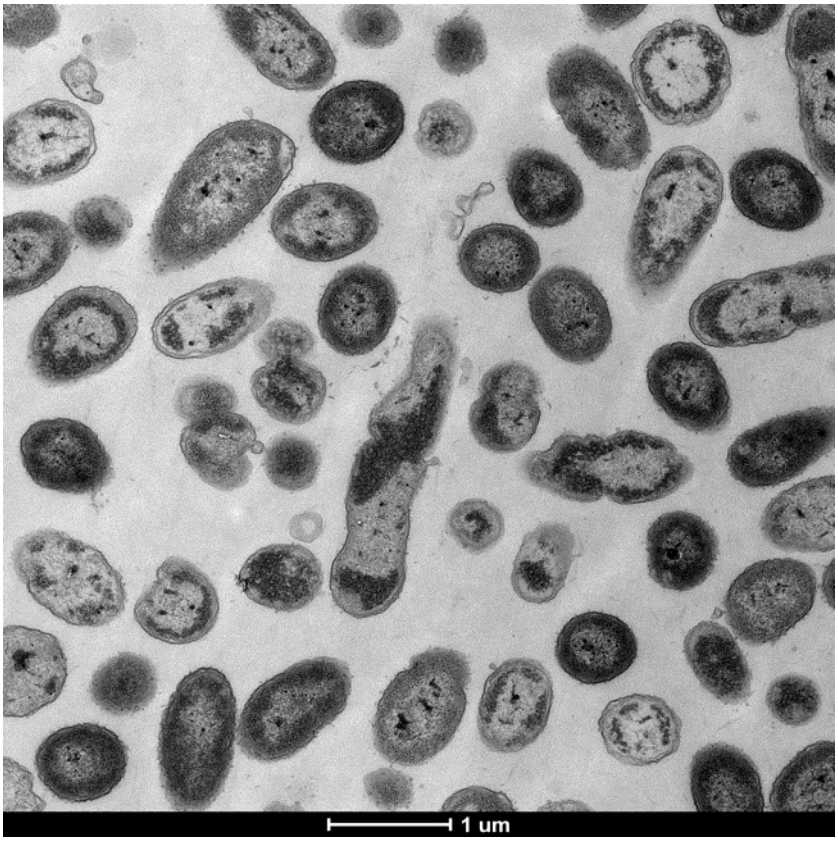


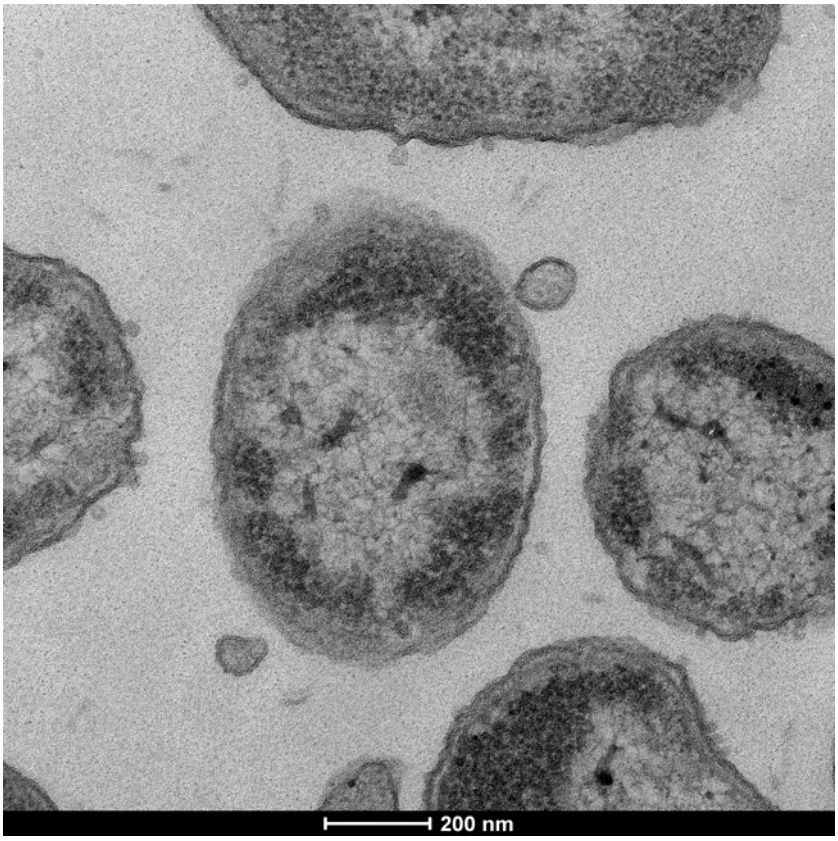
TNS18 40 μg/mL



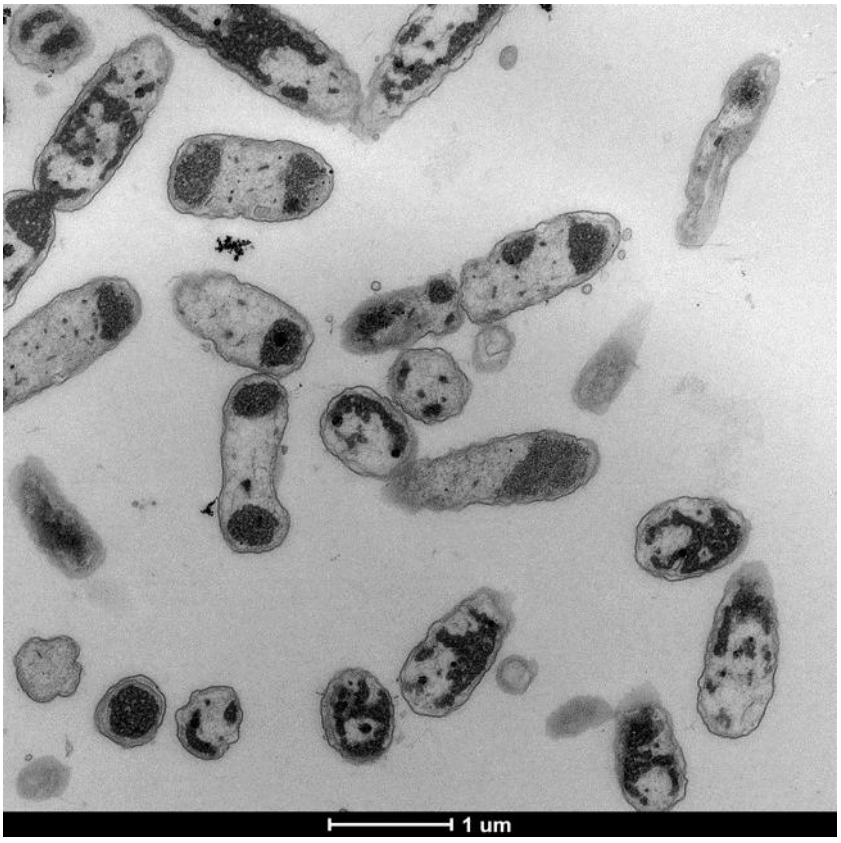


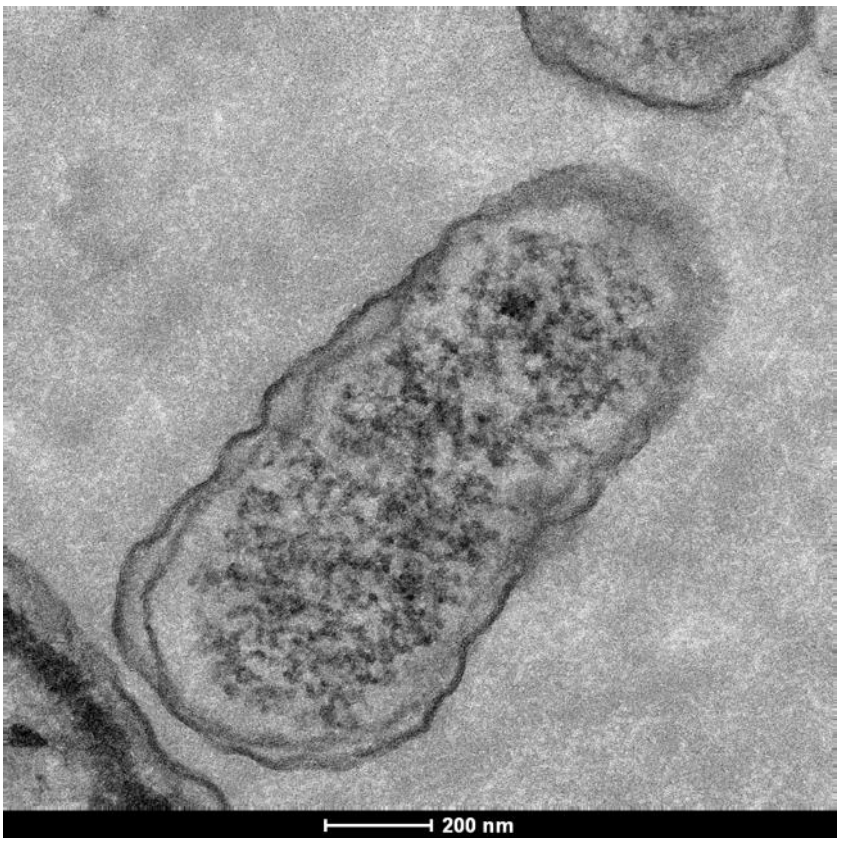
TNS03 40 μg/mL



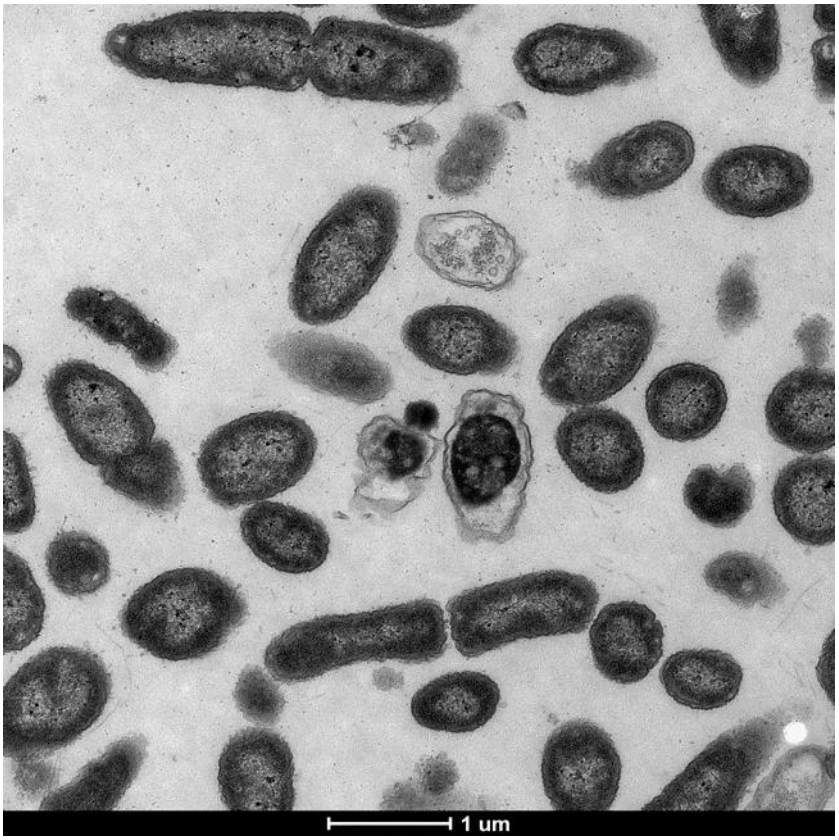


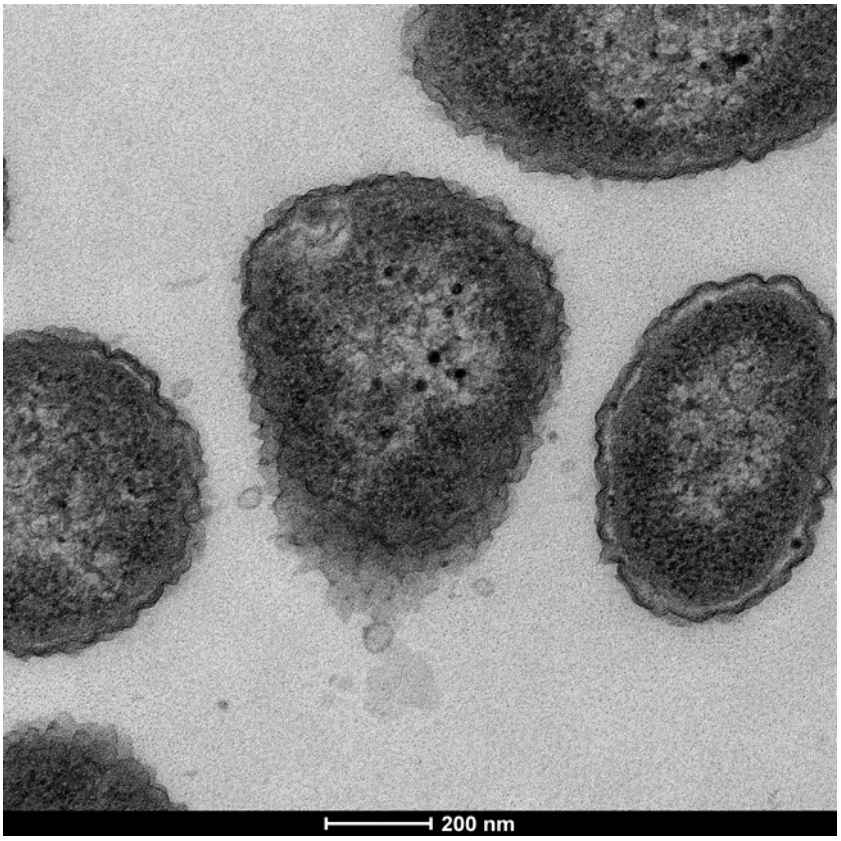
G3KL 40 μg/mL





G2KL 128 μg/mL





Vesicle Leakage Experiments

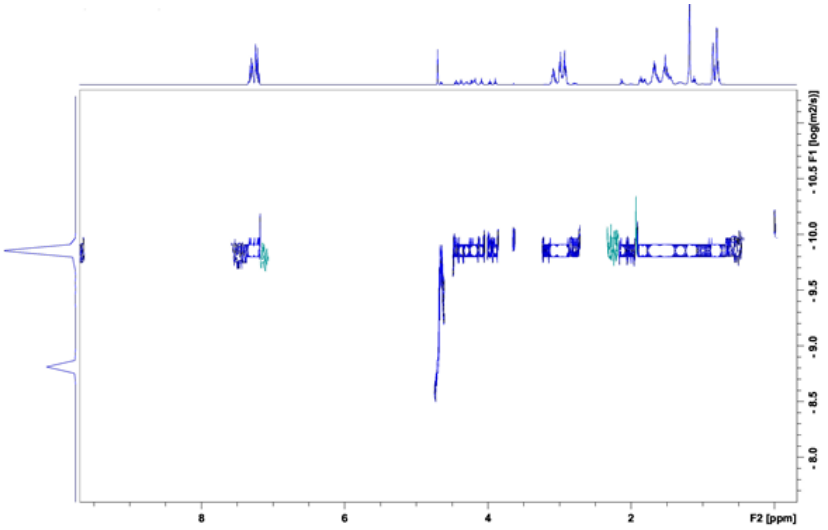
Egg PC or Egg PG thin lipid layer was prepared by evaporating a solution of 100 mg Egg PC or Egg PG in 4 mL MeOH/$CHCl_3$ (1:1) on a rotary evaporator at room temperature and then *in vacuo* overnight. The resulting film was hydrated with 4 mL buffer (50 mM CF, 10 mM TRIS, 107 mM NaCl, pH 7.4) for 30 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 stock solutions (37.5 µL) were diluted to ~3000 µL with a buffer (10 mM TRIS, 107 mM NaCl, pH 7.4) and compound which subjected to analysis, placed in a thermostated fluorescence cuvette (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 after addition of correct volume of peptide dendrimer which dissolved in mQ water, which make final concentrations of peptide dendrimer 1, 5, 10, 15, 20, 200 µg·mL^{-1} at t = 50 s. 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 *I(t)* using $I(t) = (I_t - I_0) / (I_\infty - I_0)$ where I_0 I_t at peptide dendrimer addition, $I_\infty = I_t$ at saturation of lysis.[9]

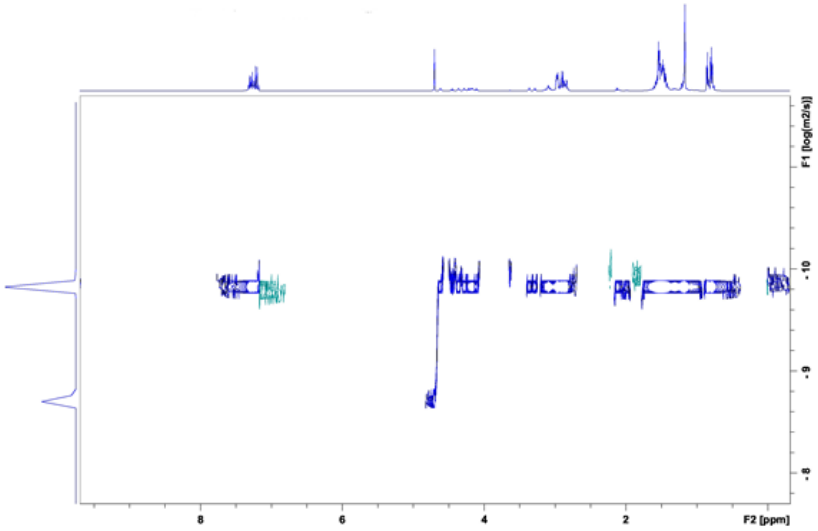
DOSY NMR

Standard diffusion NMR experiments were performed using a Bruker DRX500 with diluted solutions of dendrimer (5-10 mg·mL^{-1}) in D_2O (pH~3, at 303 K), D_2O (pH~7, at 303 K) and D_2O/20%TFE (pH~7, at 303 K). The gradient with a maximum strength of $50 \cdot 10^{-4}$ T·cm^{-1} was calibrated using the HOD proton signal in D_2O (99.997%). The diffusion time Δ was either 80 ms or 100 ms, 120 ms, 150 ms and the gradient duration δ was 7 ms. Data analysis was performed by using the Bruker Simfit software and the diffusion coefficient D [$m^2 \cdot s^{-1}$] was derived from peak integrals or intensities.

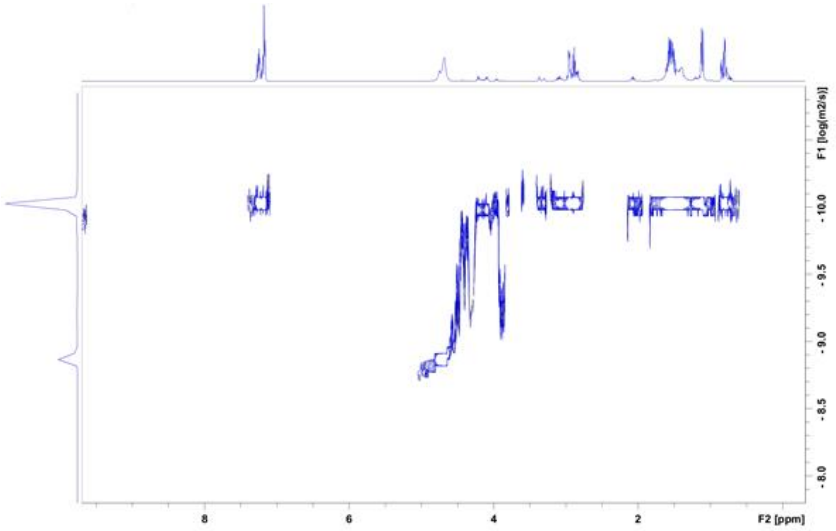
TNS18 DOSY NMR (400 MHz, D_2O, pH~3)



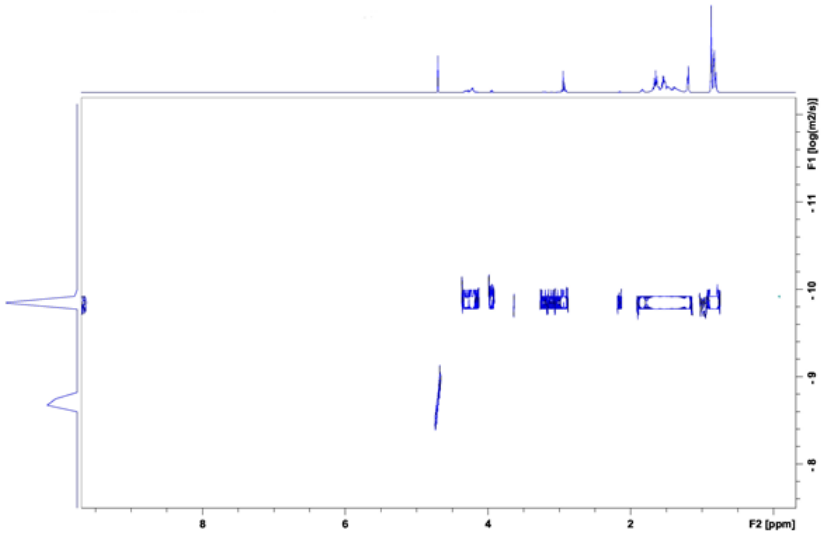
TNS18 DOSY NMR (400 MHz, D_2O, pH~7)



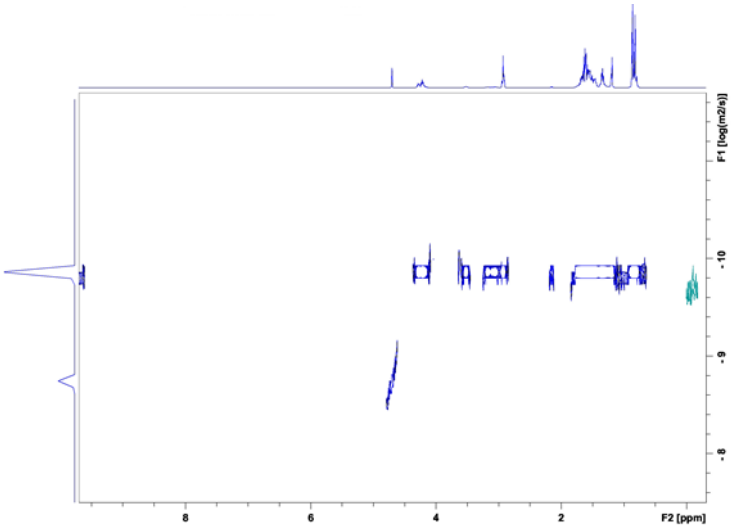
TNS18 DOSY NMR (400 MHz, D_2O/20%TFE, pH~7)



TNS03 DOSY NMR (400 MHz, D_2O, pH~3)



TNS03 DOSY NMR (400 MHz, D_2O, pH~7)



TNS03 DOSY NMR (400 MHz, D_2O/20%TFE, pH~7)

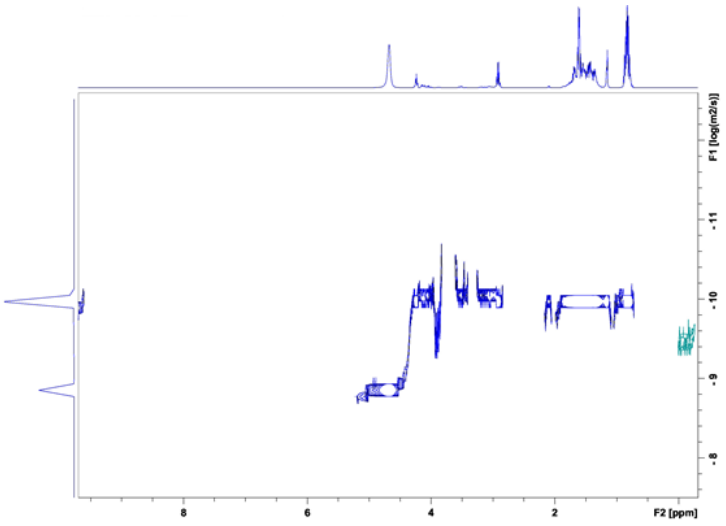


Figure S3. Diffusion NMR spectra of TNS18 and TNS 03. Measurements were done in different condition D_2O (pH~3, at 303 K), D_2O (pH~7, at 303 K) and D_2O/20%TFE (pH~7, at 303 K).

Table S3. DOESY NMR data.

Cpd.	Solvent pH 3	D x10^{-10} ($m^2 \cdot s^{-1}$)	R_h [a] (nm)	Solvent pH 7.4	D x10^{-10} ($m^2 \cdot s^{-1}$)	R_h (nm)	Solvent pH 7.4	D x10^{-10} ($m^2 \cdot s^{-1}$)	R_h (nm)
G2KL	D_2O	1.23±0.01	1.65	D_2O	1.30±0.01	1.55	20% TFE	0.89±0.02	2.27
G2KLC$_{10}$	D_2O	1.12±0.01	1.72	D_2O	1.24±0.01	1.64	20% TFE	0.83±0.04	2.39
TNS34	D_2O	1.23±0.01	1.65	D_2O	1.41±0.04	1.44	20% TFE	0.88±0.04	2.24
TNS122	D_2O	1.20±0.01	1.69	D_2O	1.28±0.01	1.58	20% TFE	0.86±0.02	2.30

[a] R_h is the hydrodynamic radius, calculated from diffusion coefficient D which is the median values from intensity fit analysis of different ^1H signals. $R_h = kT/6\pi\eta D$ Stokes-Einstein equation with Boltzmann-constant $k = 1.380*10^{-23}$ JK^{-1}, temperature T = 303 K and viscosity η = 1.089 mPas for D_2O, 1.118 mPas for D_2O/20%TFE and 1.780 mPas for TFE.

Critical Micellar Concentration

The critical micellar concentration (CMC) was measured using the Nile red method[10] modified for the 96-well plate format. Nile red (Sigma aldrich, Buchs, CH) was diluted in methanol at a concentration of 2 µM and 5 µL was added to each well of a TPP 96-well plate (Faust Laborbedarf AG, Schaffhausen) and dried under the fume hood air flow at room temperature for 1h. Serial dilution of **TNS18**, **G3KL**, Polymyxin B and dodecylphosphocholine (DPC, Avanti polar lipids, Alablaster, USA) were performed in mQ water starting from 10 $mg \cdot mL^{-1}$ to 5 $\mu g \cdot mL^{-1}$ and 50 µL was added to the plate containing the dried Nile red fluorophore. The plate was incubated for 3h before measurement of fluorescence at λ_{ex}= 540 nm and λ_{em}= 645 nm on a Tecan Infinite M1000 Pro plate reader. CMC value was determined at the inflection point at 0.3125 $mg \cdot mL^{-1}$corresponding to 0.9 mM for DPC, in agreement with values between 0.9 and 1.1 mM from literature.[11]

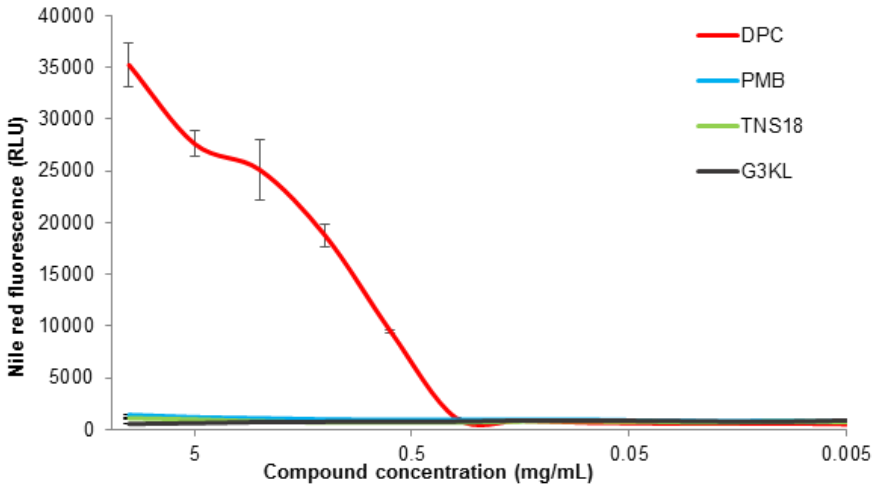


Figure S4. Critical micelle concentration determination of TNS18, G3KL, Polymyxin B (PMB) and Dodecylphosphocholine (DPC) in water in presence of Nile red. Experiments were performed in duplicate.

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 R 100-QS 0.1 cm cuvette. Stock solution (1 $mg \cdot mL^{-1}$) of dendrimers 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, 8 mM final and TFE (0, 5, 10, 15, 20 or 50%). 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.[12]

Molecular Dynamics

The dendrimer models were built by processing the GROMACS topologies of the linear peptides of the same sequence using in house software. The initial starting conformation was generated using CORINA software for the simulated annealing molecular dynamics (SA-MD) procedure and PyMol (Molecular Graphics System, version 1.8 (Schrödinger, LLC)) for the simulations of prefolded helical dendrimers.

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

The energy was minimized using a steepest gradient method to remove any close contacts before the system was subjected to a two-phase position-restrained MD equilibration procedure. The system was first allowed to evolve for 1.0 ns 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 50 ns in the NPT (P is the system pressure) ensemble at 1.0 bar.

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

Simulated Annealing Molecular Dynamics

The NPT pre-equilibrated system was used as the starting point for the SA-MD run. The system was heated to 450 K and high energy conformers were sampled at 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 ns. Each of the conformers was then cooled from 450 K to 300 K over 50 ns and allowed to evolve for an additional 200 ns. Out of the 10 trajectories, 7 showed stable compact folding by radius of gyration and H-bond analysis and were used in the subsequent analyses. To obtain a representative conformer for each SA-MD run, the last 100 ns (10001 frames, 150 → 250 ns) of each run were clustered using an RMSD cut-off of 0.07 nm. In all trajectories of **TNS18**, this optimized cut-off produced a large number of clusters (339–5519) combined with a very large percentage of structures (542–9531) in the top cluster indicative of the stability of the one main conformer. Consequently, the center structure of each cluster was used as a representative structure for each trajectory.

Molecular Dynamics of Prefolded Helical Dendrimers

The stability of the helical structure in the peptide dendrimers under different conditions was assessed by determining the unfolding kinetics. In this case, the MD uses a model where all the residues were initially arranged in a helical conformation. After system pre-equilibration (*vide supra*), the structures were subjected to MD at 300 K during 500 ns in water, 0.15 M NaCl with or without 20% v/v TFE. The unfolding the main α-peptide chain helix was followed by computing the RMSD of its backbone. In all cases, the TFE was found to contribute to the stabilization of the central α-helix.

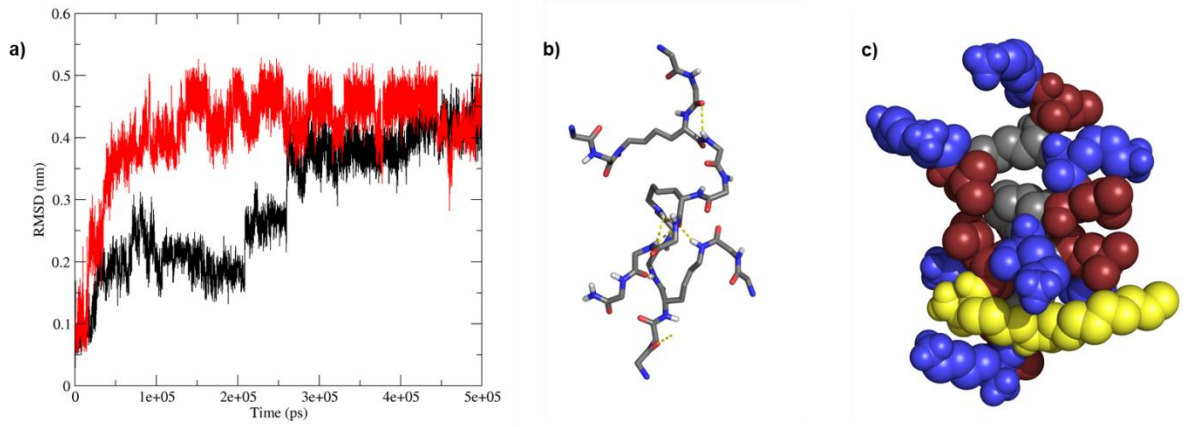


Figure S5. Molecular dynamics of **TNS03**. a) RMSD of the α-peptide chain backbone. b) stick representation of the backbone and H-bonds at 100 ns in presence of 20% TFE at 300 K c) CPK representation of the structure in b), Red: Leu, Blue: Lys, Gray: branching Lys and Yellow: LysC10.

Animal Model Studies

Experiments were performed in accordance with Gansu University of Chinese Medicine animal care ethics approval and guidelines, as per animal care certificate 2016-106. A dose of 0.5 mL of 3.2×10^8 CFU/mL per mouse of *A. baumannii* strain ID abaX1605034, or 0.5 mL of 1.5×10^8 CFU/mL *E. coli* strain ID SP1708, was suspended in sterilised 0.9% saline and injected intraperitoneally into SPF grade BALB/c mice. Peptide dendrimers **TNS18** and **G3KL** as acetate salts were resuspended in sterilised 0.9% saline as 1 $mg\cdot mL^{-1}$ and administered intraperitoneally at 5 $mg\cdot kg^{-1}$, 3 times (8h injection interval). Control antibiotics were similarly administered (see Figure 7). All mice were observed in 72 hours and mice mortality was recorded in each hour. The animals were euthanized 72h post-infection.

Table S4. Resistance profiles for MDR strains.

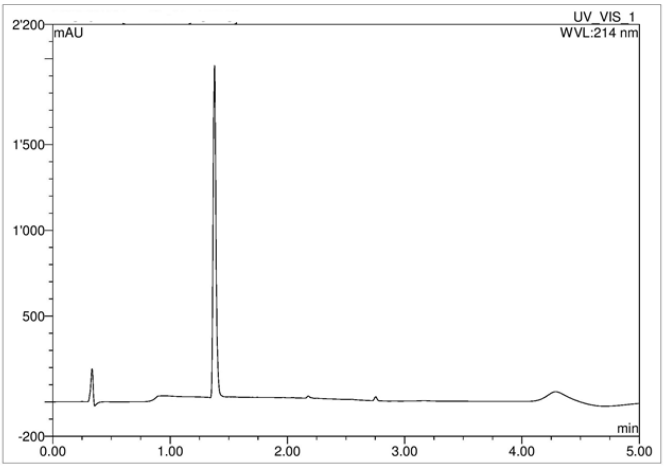
Strain	Resistant Antibiotics
MDR A. baumannii strain X1605034(20-3-5), isolated from Lanzhou General Military Hospital, ICU patient	Aztreonam, Amoxicillin / clavulanic acid, Piperacillin / tazobactam, Ampicillin, Sulfamethoxazole, Ciprofloxacin, Gentamicin, Cefepime, Cefatriaxone、Cefoxitin, Cefazolin, Tobramycin, Imipenem
MDR *Escherichia coli* SP1708, isolated from Lanzhou General Military Hospital, ICU patient.	Aztreonam, Ampicillin, Sulfamethoxazole, Ciprofloxacin, Gentamicin, Cefepime, Ceftriaxone, Cefazolin, Levofloxacin

Ion Exchange

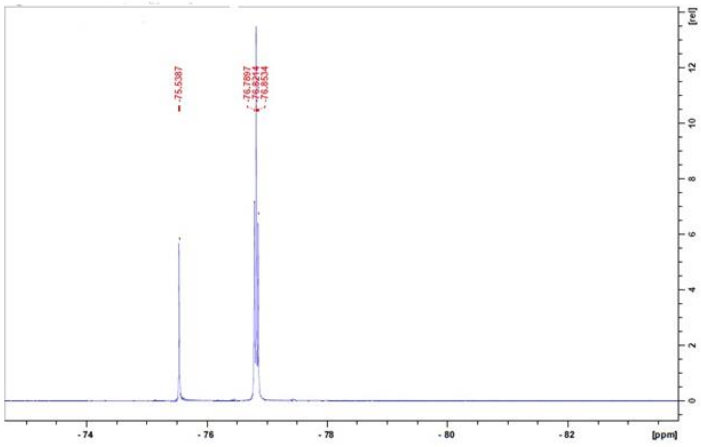
Peptide dendrimers were obtained as trifluoro-acetate salt after HPLC purification. The counter ion was then exchanged to acetate using anion resin (AG1-X2, quaternary ammonium). The resin was swelled 10 minutes in 1.6 M acetic acid (10 mL) and then washed. This step was repeated two more times and the resin was washed three times with 0.16 M acetic acid (10 mL). The peptide dendrimer was dissolved in mQ-H_2O (approximately 40 mg in 4 mL), then poured on the resin and kept for 1 hour with rotatory stirring.[13] Peptide solution was filtered from resin and this exchange step was repeated one more time. Exchanged fractions were subjected to ^{19}F NMR using a Bruker 300 MHz with diluted solution of peptide dendrimer (4-8 mg/mL) in D_2O. Trifluoro ethanol was used as the internal standard.

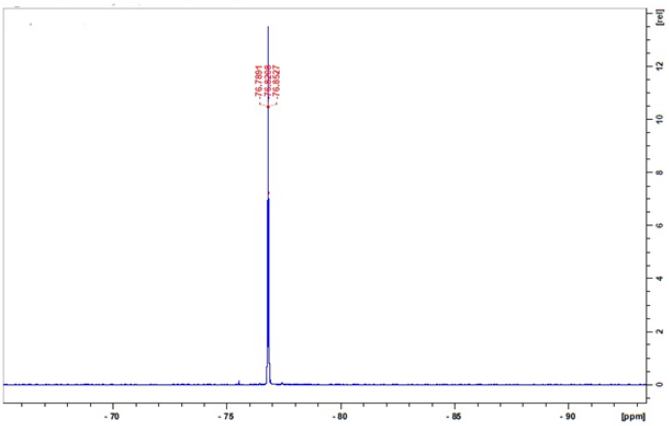
G3KL acetate. Dendrimer **G3KL** (94 mg of HPLC purified trifluoroacetate salt) was exchanged from trifluoroacetate salt to acetate salt using AG1-X2, quaternary ammonium resin (1 g), the dendrimer was obtained as a white foamy solid after exchange (80 mg, 99%). Anal. RP-HPLC: t_R = 1.38 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) ^{19}F NMR (300 MHz, D_2O) before exchange, δ = -76.78 to -76.85 (internal standard trifluoro ethanol), δ = -75.53 (trifluoro acetate): after exchange δ = -76.78 to -76.85 (internal standard trifluoro ethanol).

Analytical RP-HPLC chromatogram:



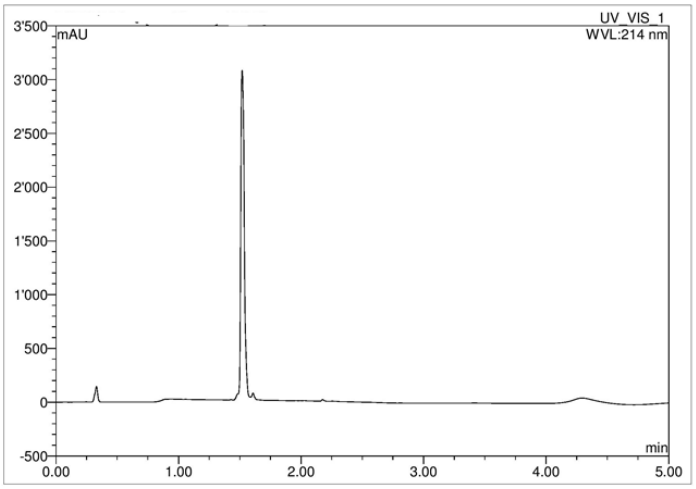
^{19}F NMR:



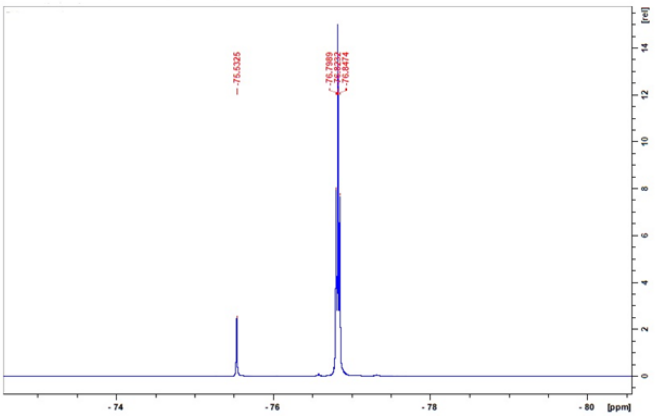


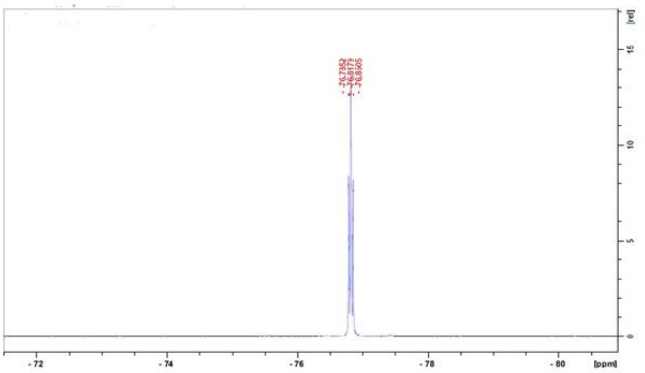
TNS18 acetate. Dendrimer **TNS18** (70 mg of HPLC purified trifluoroacetate salt) was exchanged from trifluoroacetate salt to acetate salt using AG1-X2, quaternary ammonium resin (800 mg), the dendrimer was obtained as a white foamy solid after exchanged (54 mg, 93%). Anal. RP-HPLC: t_R = 1.52 min (A/D = 100/0 to 0/100 in 5.00 min, λ = 214 nm) ^{19}F NMR (300 MHz, D_2O) before exchange, δ = -76.79 to -76.85 (internal standard trifluoro ethanol), δ = -75.53 (trifluoro acetate): after exchange δ = -76.79 to -76.85 (internal standard trifluoro ethanol).

Analytical RP-HPLC chromatogram:



^{19}F NMR:





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