Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information for:

Membrane disrupting antimicrobial peptide dendrimers with multiple amino termini

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Material and reagents

All reagents were either purchased from Aldrich, Fluka or Acros Organics. PyBOP, amino acids and their derivatives were purchased from Advanced ChemTech (USA) or Novabiochem (Switzerland). Amino acids were used as the following derivatives: Fmoc-Ala-OH, Fmoc-ß-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Gly-OH, Fmoc-His(Boc)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Lys(Fmoc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Val-OH, Fmoc-Dap(Fmoc)-OH, Fmoc-^{a}Pro(Boc)-OH. TG S RAM (loading: 0.24 mmol·g^{-1} or 0.22 mmol·g^{-1}) and TG RAM HL (loading: 0.39 mmol·g^{-1}) resins were purchased from Rapp Polymere (Germany) while hydroxymethyl-photolinker NovaSyn TG (loading: 0.24 mmol·g^{-1}) was purchased from Novabiochem. Peptide dendrimer syntheses were performed manually in polypropylene syringes fitted with a polyethylene frit, a teflon stopcock and stopper. Solvents were p.a. quality and distilled prior to use. Analytical RP-HPLC was performed in Waters (996 Photo diode array detector) chromatography system using Atlantis column (dC18, 5 µm, 4.6 × 100 mm, flow rate 1.4 mL·min^{-1}). Analytical RP-UHPLC was performed in Dionex ULTIMATE 3000 Rapid Separation LC System (ULTIMATE-3000RS diode array detector) using a Dionex Acclaim® RSLC 120 C18 column (2.2 µm, 120 Å, 3.0 × 50 mm, flow 1.2 ml·min^{-1}). Compounds were detected by UV absorption at 214 nm. Data recording and processing was either done with Waters Empower2 software (analytical RP-HPLC) or with Dionex Chromeleon Management System Version 6.80 (analytical RP-HPLC). Preparative RP-HPLC was performed with a Waters Prep LC4000 chromatography system using a Delta-Pak column (C18, 15 µm, pore size 300 Å, flow rate 80 mL·min^{-1}) or Waters Prep LC2489 chromatography system using a Dr. Maisch Gmbh Reprospher column (C18-DE, 100 x 30 mm, 5 µm, pore size 100 Å, flow rate 60 mL·min^{-1}). Compounds were detected by UV absorption at 214 nm. All RP-HPLC were using HPLC-grade acetonitrile and mQ-deionized water. The elution solutions were: **A** H_2O with 0.1% TFA; **B** H_2O/MeCN (50:50); **C** H_2O/MeCN (10:90) with 0.1% TFA; **D** H_2O/MeCN (40:60) with 0.1% TFA. MS spectra, amino acid analyses and PGSE-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. Yields were determined with quantitative amino acid analysis (AAA) if not noted otherwise.

Combinatorial library

Library synthesis

The peptide dendrimer library was prepared as described previously[1] from 1.5 g -100 mg per split- resin batch of hydroxymethyl-photolinker NovaSyn TG resin (0.24 mmol·g^{-1}) divided equally in 15 reactors. The attachment of the first amino acid to hydroxymethyl resin was done by using MSNT with 1-MeIm.[2] The resin was placed in a syringe and swelled with dry CH_2Cl_2 under nitrogen. In a round bottom flask, the appropriate amino acid (5 eq) was dissolved in dry CH_2Cl_2 (3 mL per mmol) and a few drops of THF. MSNT (5 eq) and 1-methylimidazole (3.75 eq) were added, dissolved, and the mixture was added to the syringe. Then the syringe was agitated at room temperature for 1 h. The resin was washed with CH_2Cl_2 (5 × 5 mL). The coupling was repeated two times. A sensitive color test (alizarin-cyanuric chloride test)[3] was used to detect the presence of hydroxyl groups on solid support, the presence of unreacted hydroxyl groups was indicated by red-colored beads. 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 amino acid (3 eq/G, G = generation) in the presence of PyBOP (3 eq/G) and DIEA (5 eq/G) in NMP.[4] Amino acids, derivatives or diamino acids were coupled for 30 min (G0), 1 h (G1), 2 h (G2), 4 h (G3). The completion of the reaction was checked using 2,4,6-trinitrobenzenesulfonic acid solution (TNBS) or chloranil test.[5] If the beads were red (brown for proline), there were some free amino groups and the resin test was positive. If they were colorless, there were no more free amino groups and the resin test was negative. The coupling was repeated after a positive test. After each coupling, the resin in each syringe was deprotected (20% piperidine in DMF, 2 × 10 min) followed by TNBS or chloranil test (test must be positive). Then the resin batches were mixed together, vortexed during 1 min and split equally. These split-and-mix steps were repeated after each amino acid coupling. After each coupling or deprotection the resin was successively washed with NMP, MeOH and CH_2Cl_2 (3 × 5 mL, with each solvent). At the end of the synthesis, the Fmoc protected library was dried and stored at -18°C. For all the steps, the syringes were covered with aluminum to prevent exposure to light.

Bead diffusion assay

Before the screening of the library, the Fmoc protecting groups were removed and the last amino acid was either acetylated with Ac_2O/CH_2Cl_2 (1:1) for 1 h or not acetylated. The side-chain protecting groups were removed with TFA/TIS/H_2O (94:5:1) resulting in a functional dendrimer library on-beads. Antimicrobial screening was performed by washing the beads (10 mg, 5 × 3

mL) with mQ-H_2O and swelling those (10 mg, 2 × 1 h) in mQ-H_2O. After solution filtration and drying under vacuum, the resin was poured on a petri dish (diameter: 3 cm) and was partially irradiated under an Hg lamp (100 W) with a 366 nm filter for 45 min. *Bacillus subtilis* (strain BR151) bacteria were grown in LB broth during 4 h at 37°C. The concentration was quantified by measuring absorbance at 600 nm and diluted to an OD 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 and subjected to AAA.

Sequence determination

Single dendrimer-containing resin beads were hydrolyzed with 6 M aqueous HCl at 110°C for 22 h. The amino acids were derivatized with phenylisothiocyanate (PITC) and the phenylthiocarbamyl (PTC) derivatives analyzed on a RP-C18 Novapack column.

Table S1. Hits against *B. subtilis*. Beads showing an inhibition disk were picked as hits.

No.		X^4	X^3	X^2	X^1
1	**H1**	Leu	Leu	Phe	Lys
2	**H2**	Leu	Phe	Pro	Lys
3	**H3**	Leu	Gln	Phe	Lys
4		Leu	Phe	Ser	Lys
5		Leu	Leu	Tyr	Lys
6		Arg/Gly	Arg/Gly	Gln	[a]Pro
7		Gly	Phe	Arg	[a]Pro
8		Phe	Val/Pro	Val/Pro	Lys
9		Leu	Gln	Leu	Lys
10		Leu	Phe	Gly	Lys
11		Arg	Ala	Ala	[a]Pro
12		Leu	Leu	Phe	Lys
13		Leu	Arg	Arg	Lys
14		Phe	Ser	Gln/Gly	[a]Pro
15		Arg	ß-Ala	Phe	Lys

Table S2. Non-hits against *B. subtilis*. Beads showing no inhibition disk were picked as hits.

No.	X^4	X^3	X^2	X^1
1	Gly	Gln	βAla	Lys
2	Ala	Gly	Thr	Lys
3	His	Asp	Val	[a]Pro
4	His	Thr	-	Lys
5	Ser	Gln	Tyr	[a]Pro
6	Leu	Gly	Gln	Lys
7	Phe	Gln	Tyr	Lys
8	Arg	Tyr	βAla	[a]Pro
9	Ala	Asp	Gln	[a]Pro
10	Gln	Ala	Val	[a]Pro
11	Gly	Leu	Gln	Lys
12	Arg	Gly	Gln	[a]Pro
13	βAla	Ala	Tyr	Lys
14	Ala	Leu	Arg	Lys
15	βAla	Pro	Pro	Lys
16	Arg	Val	Gln	[a]Pro
17	Ser	Val	Leu	[a]Pro
19	Leu	Tyr	Pro	Lys
20	Gln	Ala	Arg	Lys
21	Gly	His	βAla	[a]Pro
22	Val	Gly	Asp	Lys
23	Asp	Gln	Ala	Lys
24	Tyr	Gln	Ala	Lys
25	His	Arg	Leu	[a]Pro
26	Gln	Ala	Val	[a]Pro
27	Tyr	Gly	Asp	Lys
28	Arg	Gln	Ser	[a]Pro
29	Val	βAla	Gly	[a]Pro
30	Ala	Tyr	Arg	Lys
31	Ala	Gln	Ala	Lys
32	Thr	Ala	Thr	[a]Pro
33	Arg	Gln	Arg	[a]Pro
34	Arg	Gln	Gln	[a]Pro
35	βAla	Val	βAla	Lys
36	Pro	Pro	Pro	Lys
37	Asp	Asp	Asp	Lys
38	Val	Ala	Gln	Lys
39	Ser	Tyr	His	Lys
40	Pro	Asp	His	Lys
41	Gln	Gly	ß-Ala	Lys
42	Ala	Ala	His	Lys
43	ß-Ala	Ala	Gly	Lys
44	Ala	Ala	Ala	[a]Pro
45	Pro	Pro	Pro/Val	[a]Pro
46	Pro	ß-Ala	-	Lys
47	Thr	Pro	Ala	Lys
48	Ser	Asp	Asp	Lys
49	Pro	Thr	Val	Lys
50	Ala	Asp	-	[a]Pro
51	Ala	Asp	Gln	[a]Pro
52	Gly	Ala	Gly	Lys

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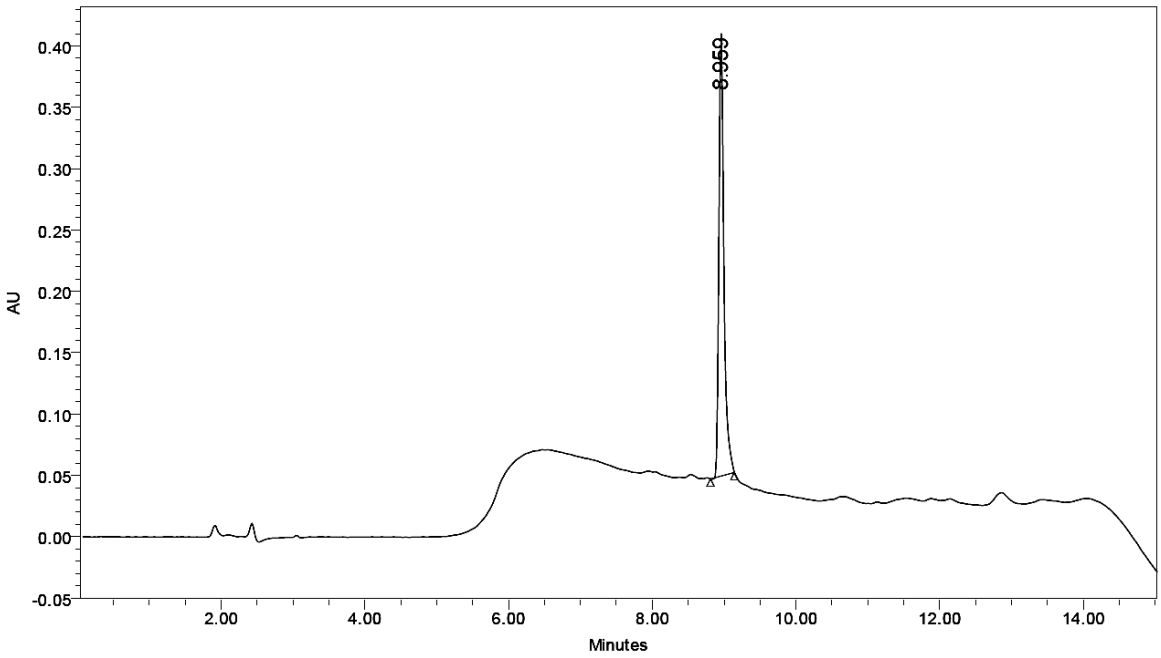
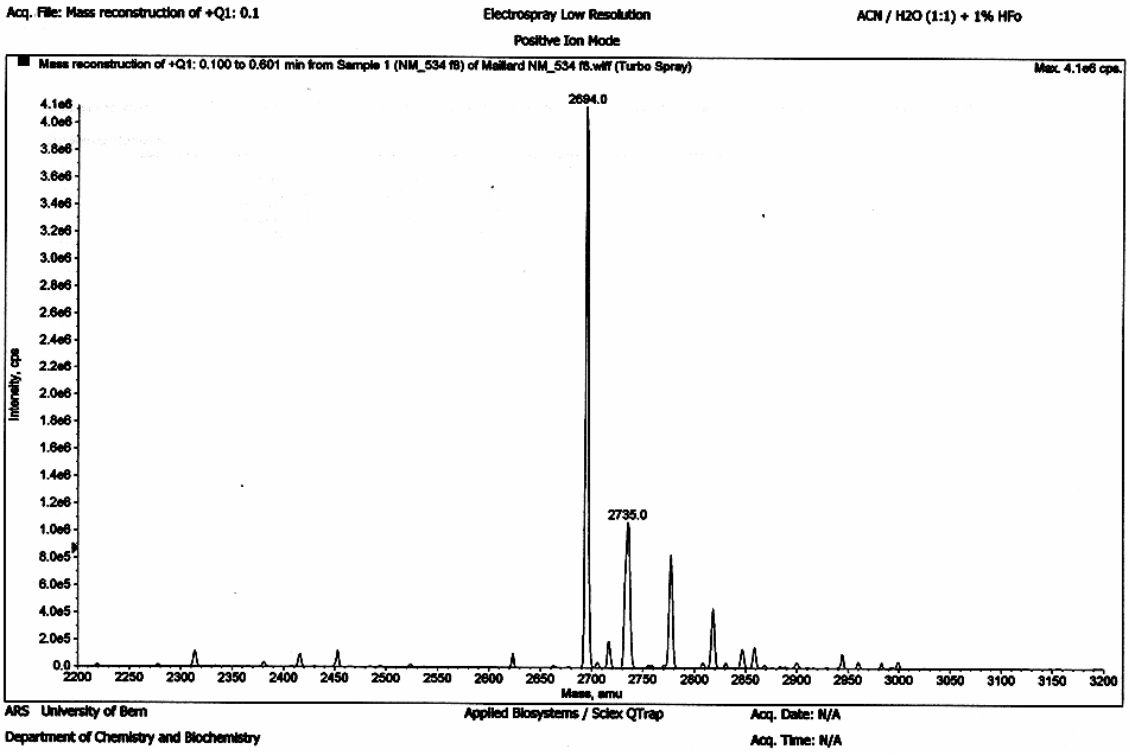
Dendrimer synthesis

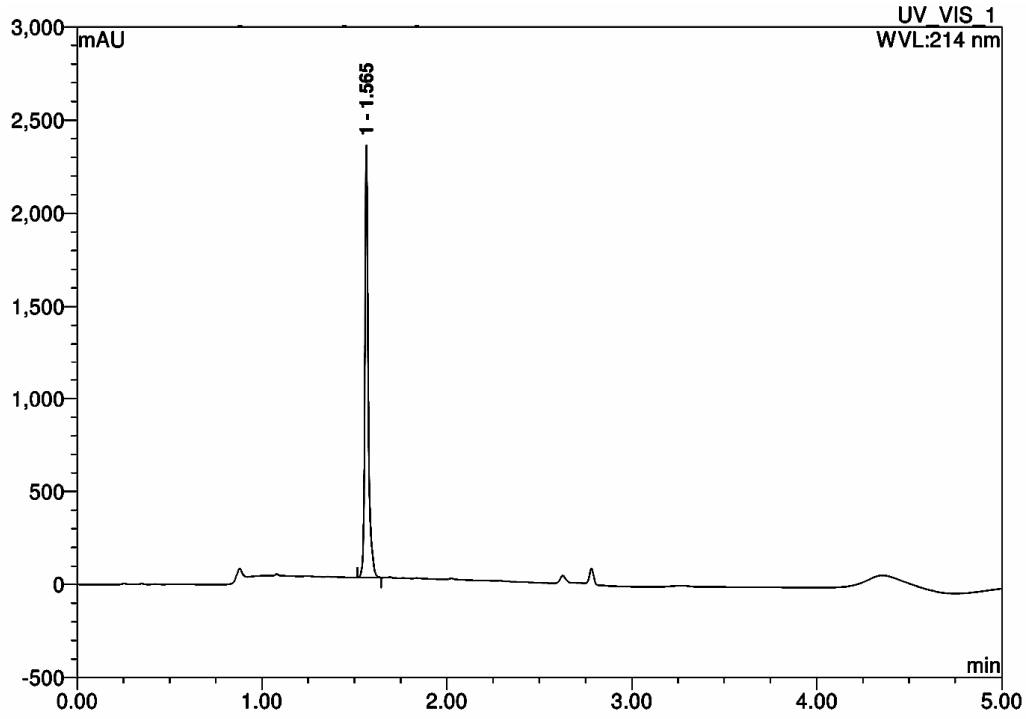
The resin (TG S RAM, or TG HL RAM) was swelled in CH_2Cl_2 and the Fmoc-protecting groups of the resin were removed with a solution of 20% piperidine in DMF (2 × 10 min). Then the resin was acylated with each amino acid, derivative or diamino acid (3 eq/G) using PyBOP (3 eq/G) and DIEA (5 eq/G) in NMP. The completion of the reaction was checked using the TNBS or chloranil test. At the end of the synthesis, the terminal amino groups were either acetylated with Ac_2O/CH_2Cl_2 (1:1) for 1 h or not acetylated. The cleavage was carried out with TFA/TIS/H_2O (94:5:1) during 4 h. The peptide was precipitated with tert-butylmethylether (MTBE) then dissolved in a H_2O/MeCN mixture. Peptides were purified by preparative RP-HPLC and obtained as TFA salts after lyophilisation. Unless not mentioned, gradient used for analytical HPLC is A/D = 100/0 to 0/100 in 10 min, 1.4 $mL \cdot min^{-1}$ or A/D = 100/0 to 0/100 in 3.5 min, 1.2 $mL \cdot min^{-1}$ or A/D = 100/0 to 0/100 in 5.5 min, 1.2 $mL \cdot min^{-1}$ or A/D = 50/50 to 0/100 in 3.2 min, 1.2 $mL \cdot min^{-1}$.

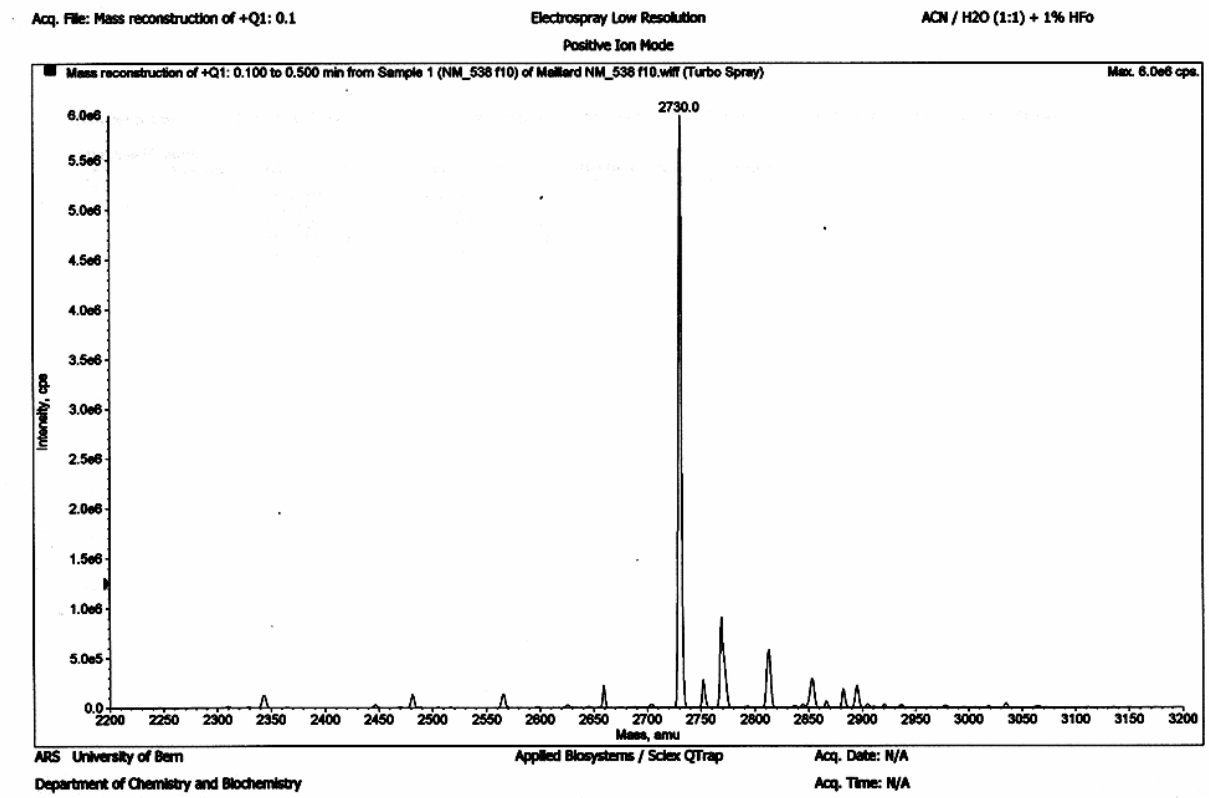
H1 (Leu)$_8$(*Lys*Leu)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **H1** was obtained as a white foamy solid after preparative RP-HPLC purification (45.6 mg, 8%). Anal. RP-HPLC: t_R = 9.0 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc. for $C_{138}H_{250}N_{31}O_{22}$ $[M+H]^+$: 2693.9, obsd: 2694.0; $[M+K]^+$: 2732.3, obsd: 2735.0.



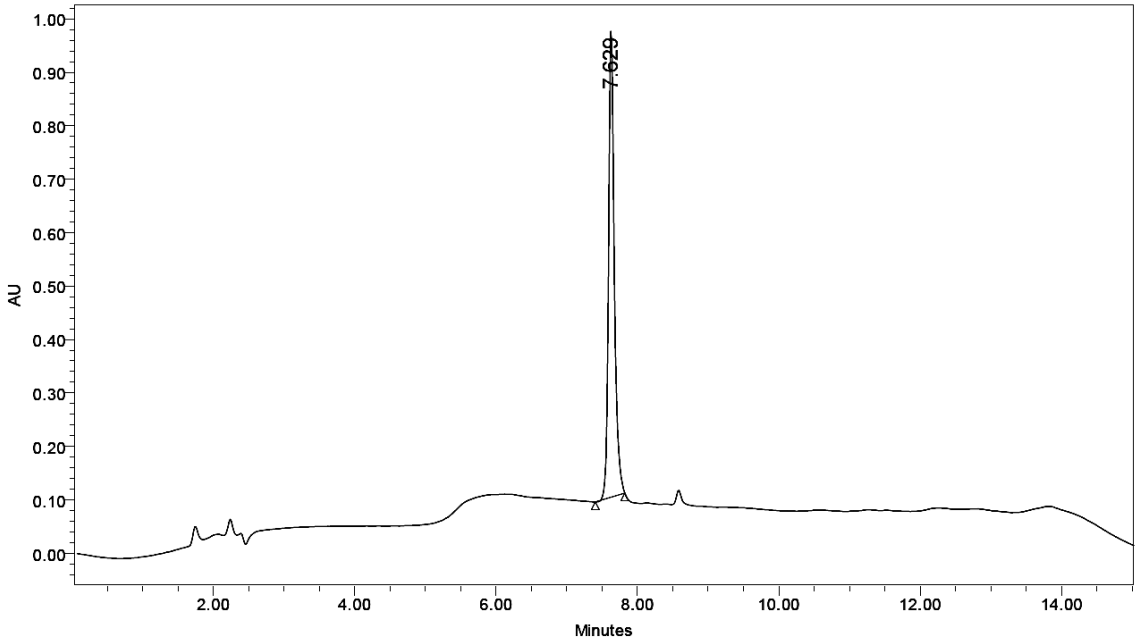


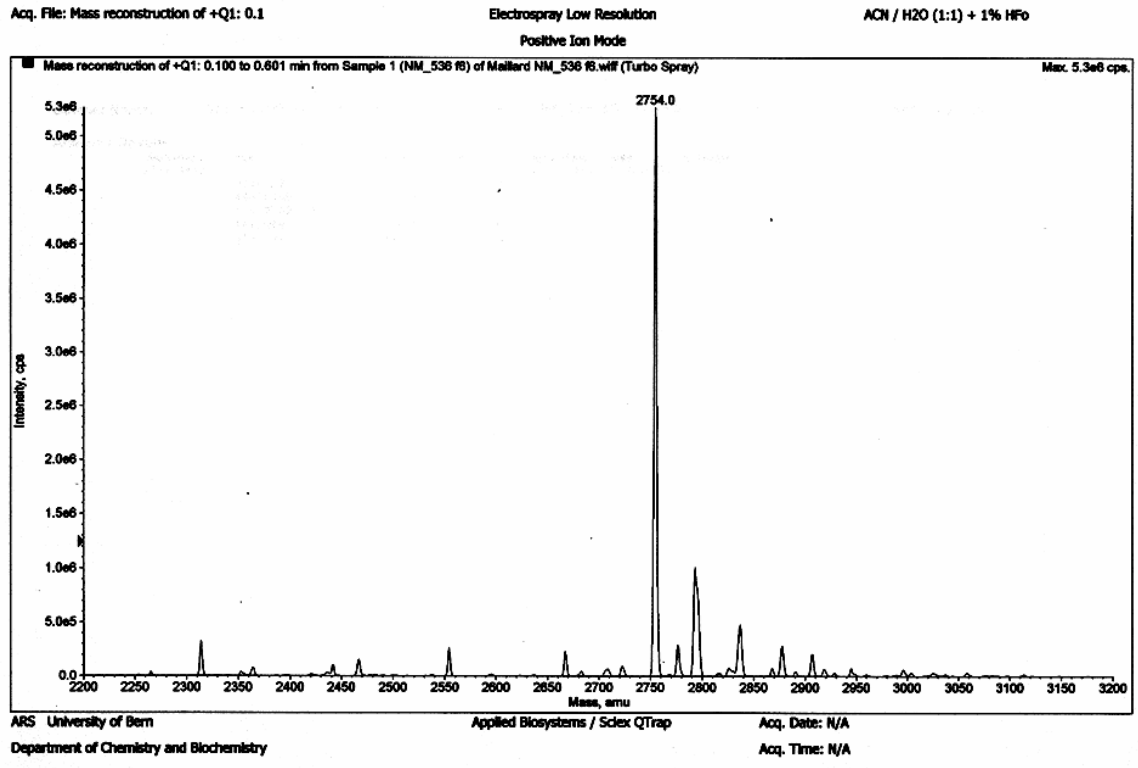
H2 (Leu)$_8$(*Lys*Phe)$_4$(*Lys*Pro)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **H2** was obtained as a white foamy solid after preparative RP-HPLC purification (62.5 mg, 14%). Anal. RP-HPLC: t_R = 1.565 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{142}H_{238}N_{31}O_{22}$ $[M+H]^+$: 2729.8, obsd: 2730.0.



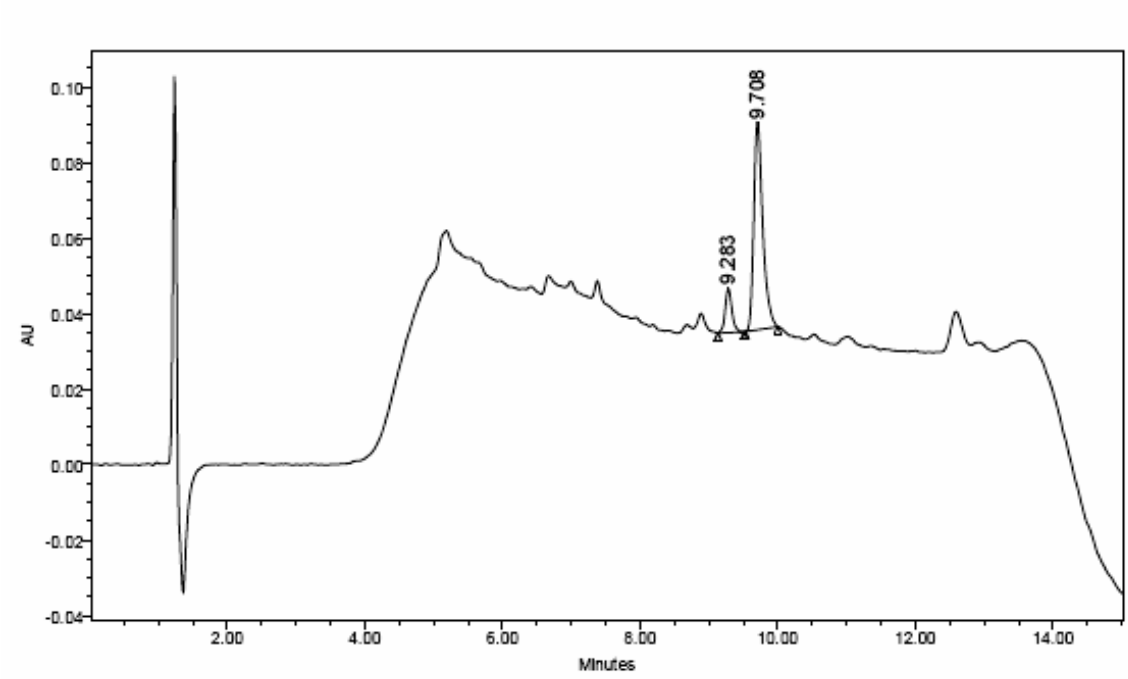


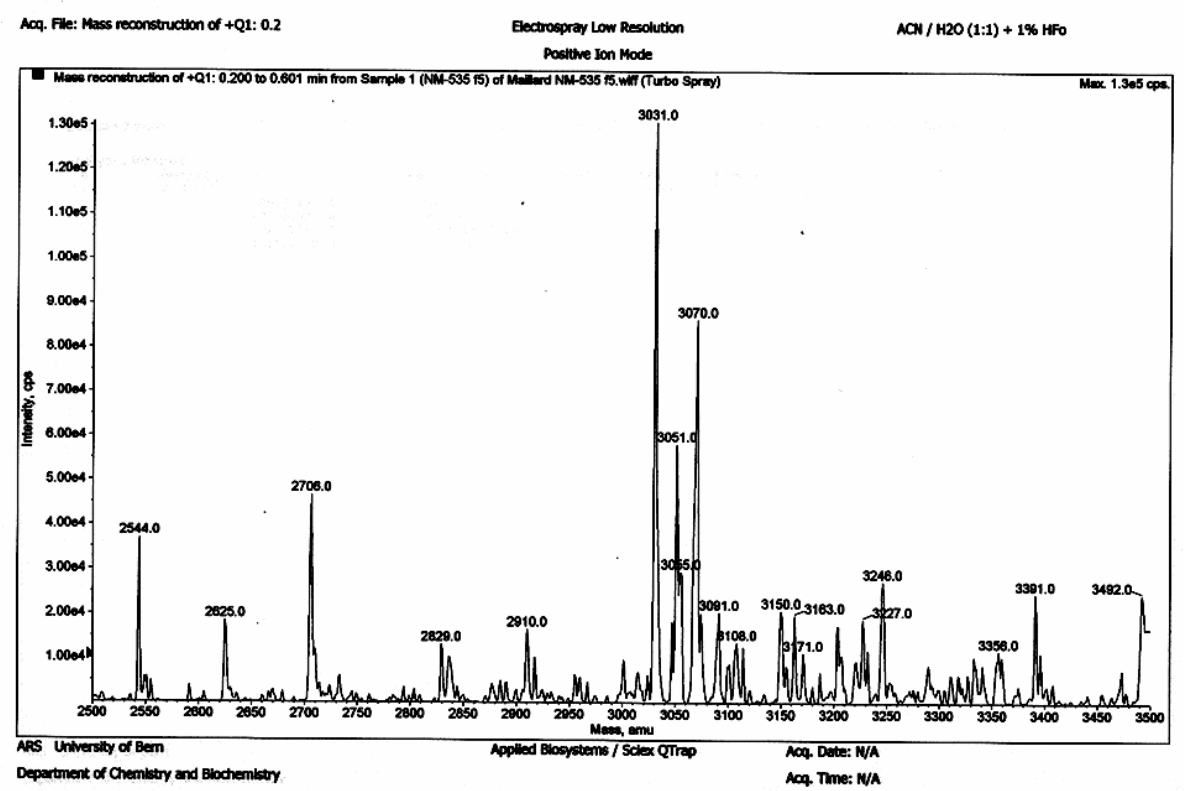
H3 (Leu)$_8$(*Lys*Gln)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (400mg, 0.24 mmol·g^{-1}), dendrimer **H3** was obtained as a white foamy solid after preparative RP-HPLC purification (73.1 mg, 12%). Anal. RP-HPLC: t_R = 7.6 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{134}H_{238}N_{35}O_{26}$ $[M+H]^+$: 2753.8, obsd: 2754.0.



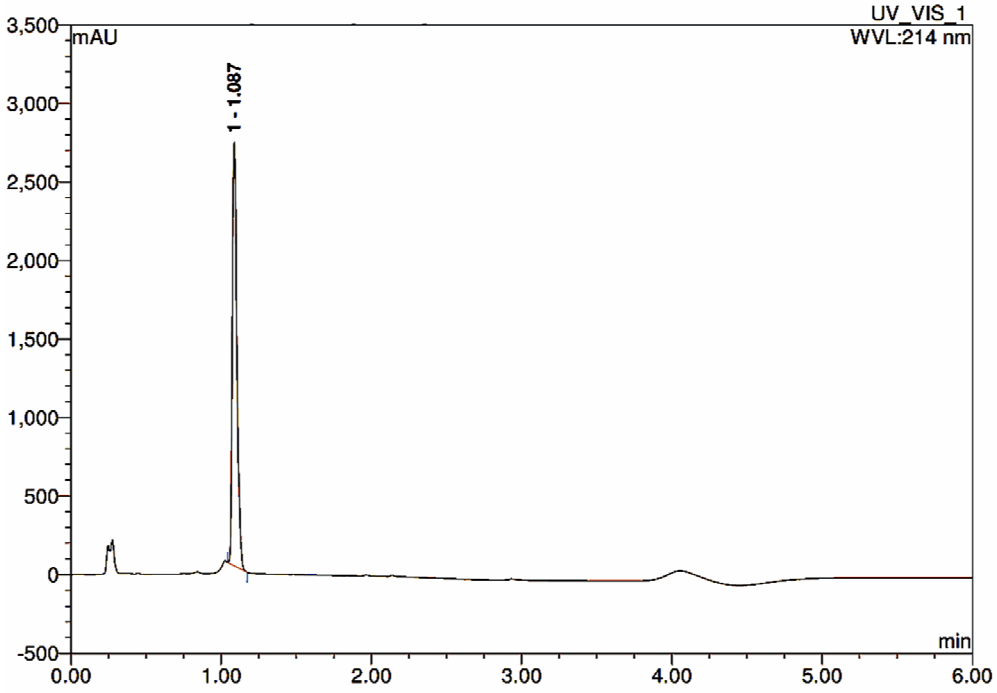


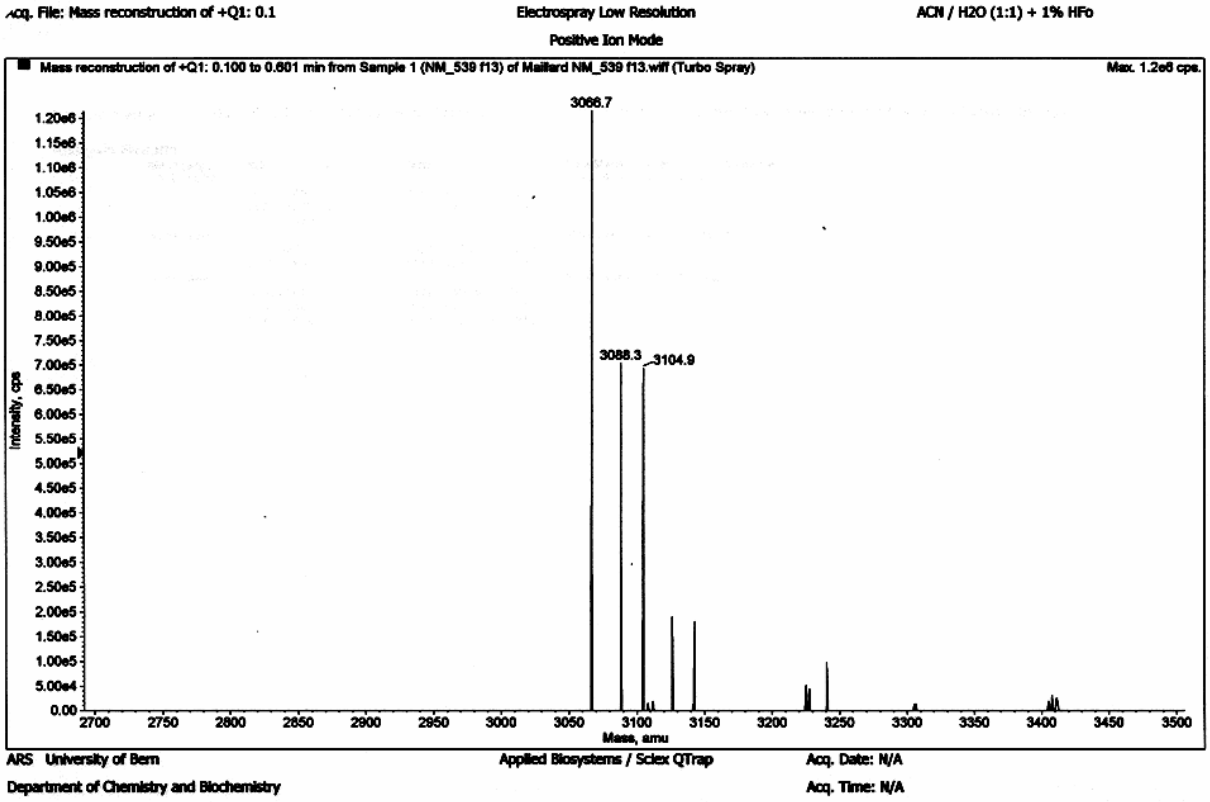
Ac1 (AcLeu)$_8$(*Lys*Leu)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (200 mg, 0.24 mmol·g^{-1}), dendrimer **Ac1** was obtained as a white foamy solid after cleavage without further purification (9.5 mg, 6%). Anal. RP-HPLC: t_R = 9.7 min (A/D = 100/0 to 0/100 in 5 min, 100D in 5 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{154}H_{266}N_{31}O_{30}$ $[M+H]^+$: 3030.0, obsd: 3031.0.



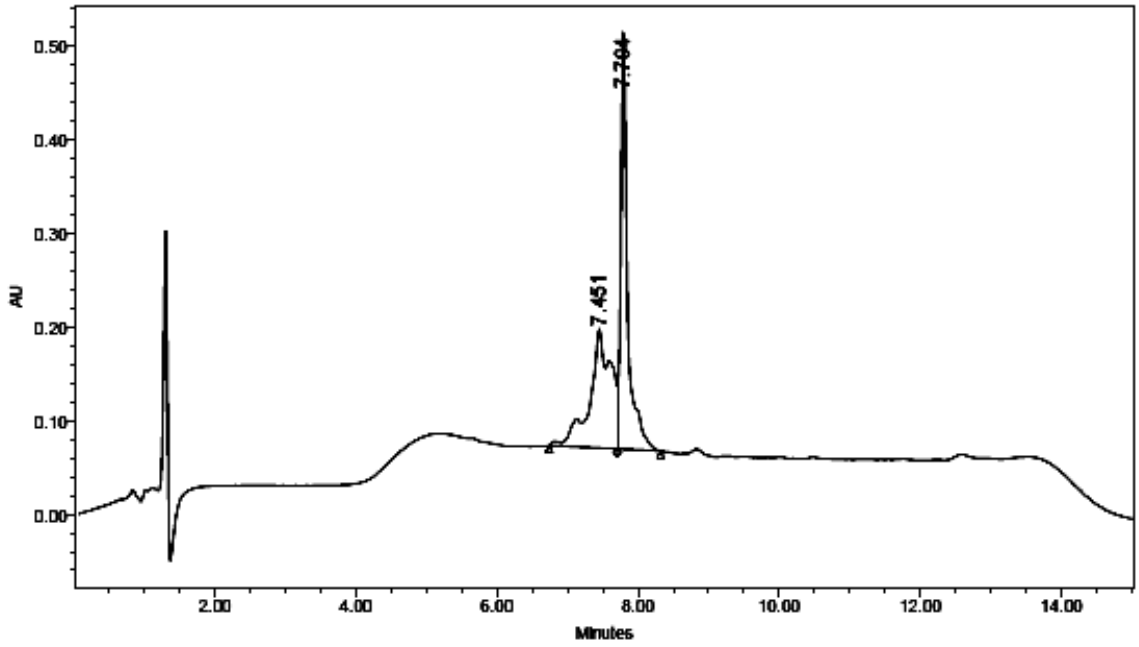


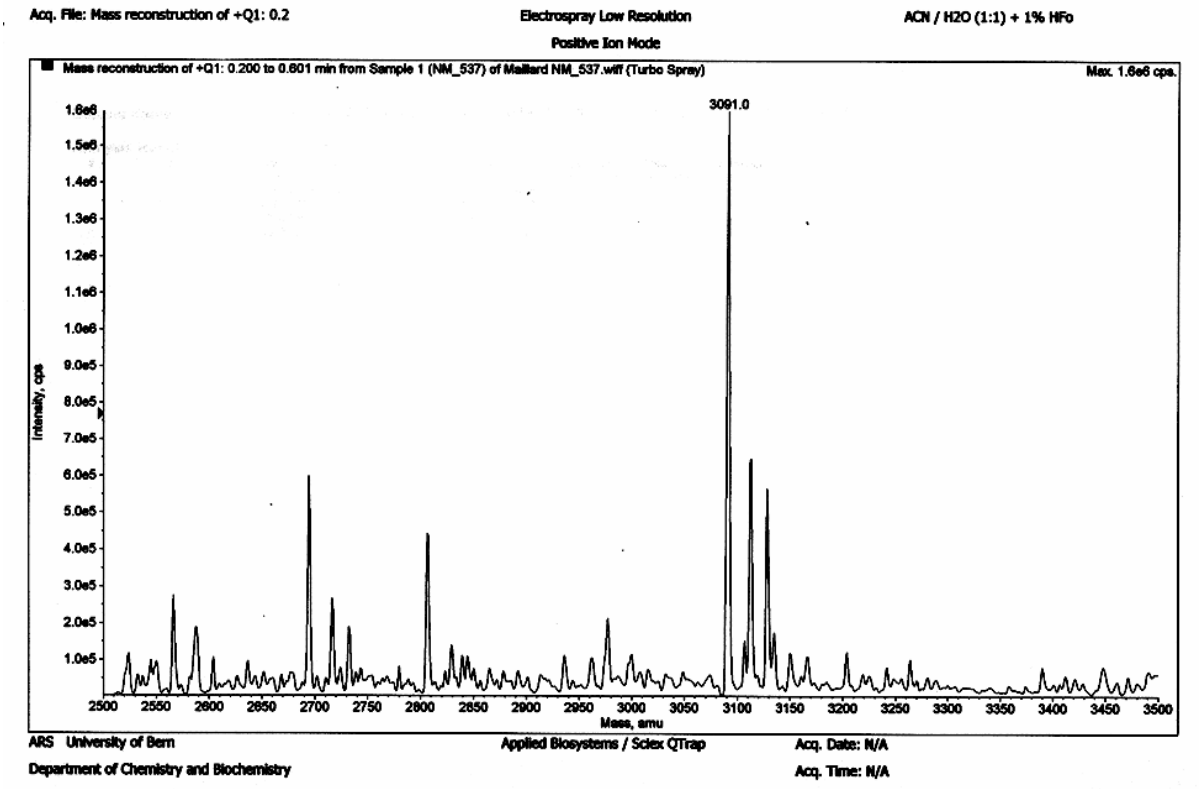
Ac2 (AcLeu)$_8$(*Lys*Phe)$_4$(*Lys*Pro)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (200 mg, 0.24 mmol·g^{-1}), dendrimer **Ac2** was obtained as a white foamy solid after preparative RP-HPLC purification (18.3 mg, 12%). Anal. RP-HPLC: t_R = 1.087 min (A/D = 100/0 to 0/100 in 5 min, 100D in 5 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{158}H_{254}N_{31}O_{30}$ $[M+H]^+$: 3065.9, obsd: 3066.7; $[M+Na]^+$: 3086.9, obsd: 3088.3; $[M+K]^+$: 3104.9, obsd: 3104.9.



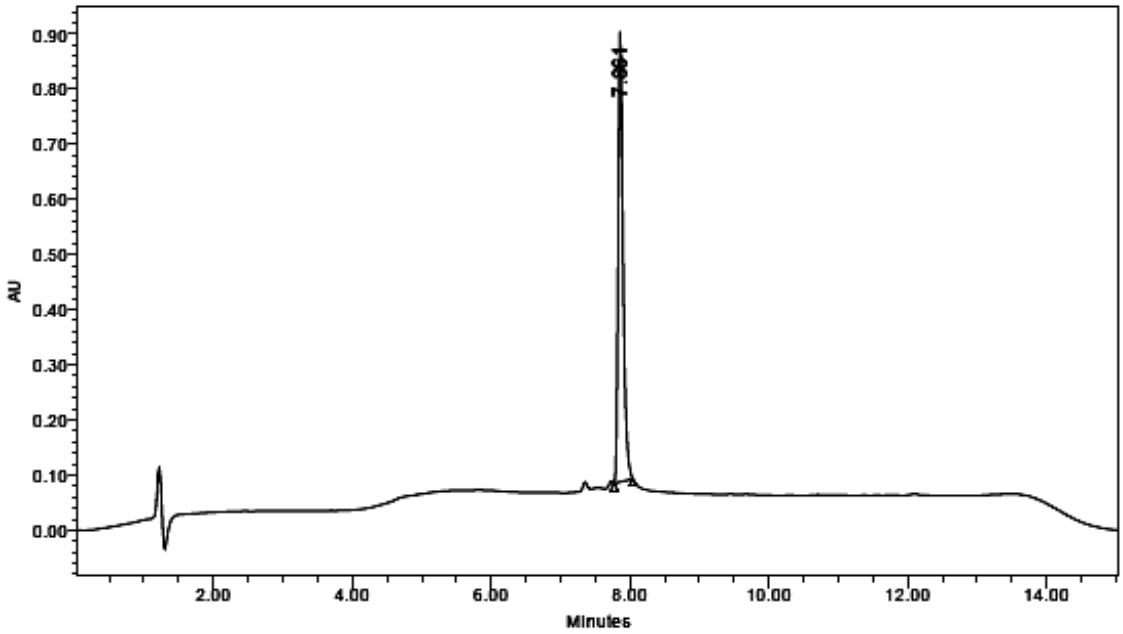


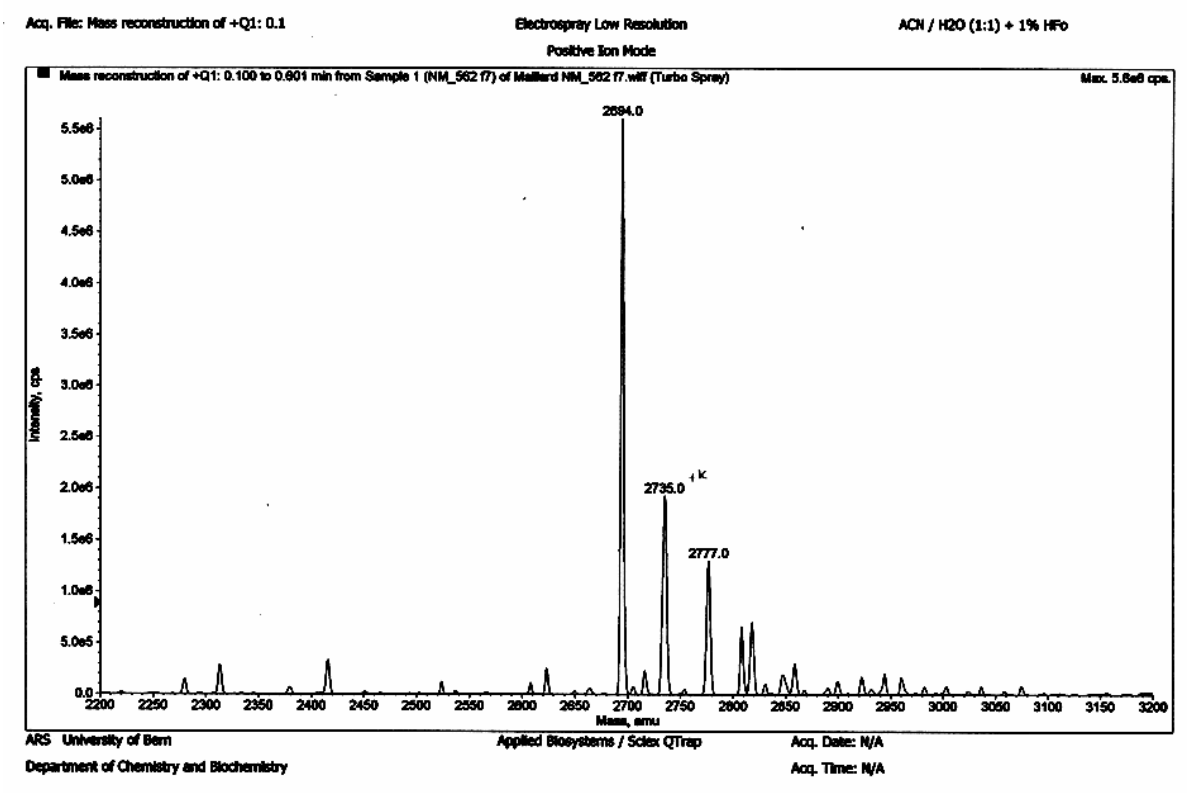
Ac3 **(AcLeu)$_8$(*Lys*Gln)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$**. From Tenta Gel S RAM® resin (200 mg, 0.24 mmol·g^{-1}), dendrimer **Ac3** was obtained as a white foamy solid after cleavage from the resin and without further purification (76.8 mg, 44%). Anal. RP-HPLC: t_R = 7,8 min (A/D = 100/0 to 0/100 in 5 min, 100D in 5 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{150}H_{254}N_{35}O_{34}$ $[M+H]^+$: 3089.9, obsd: 3091.0.



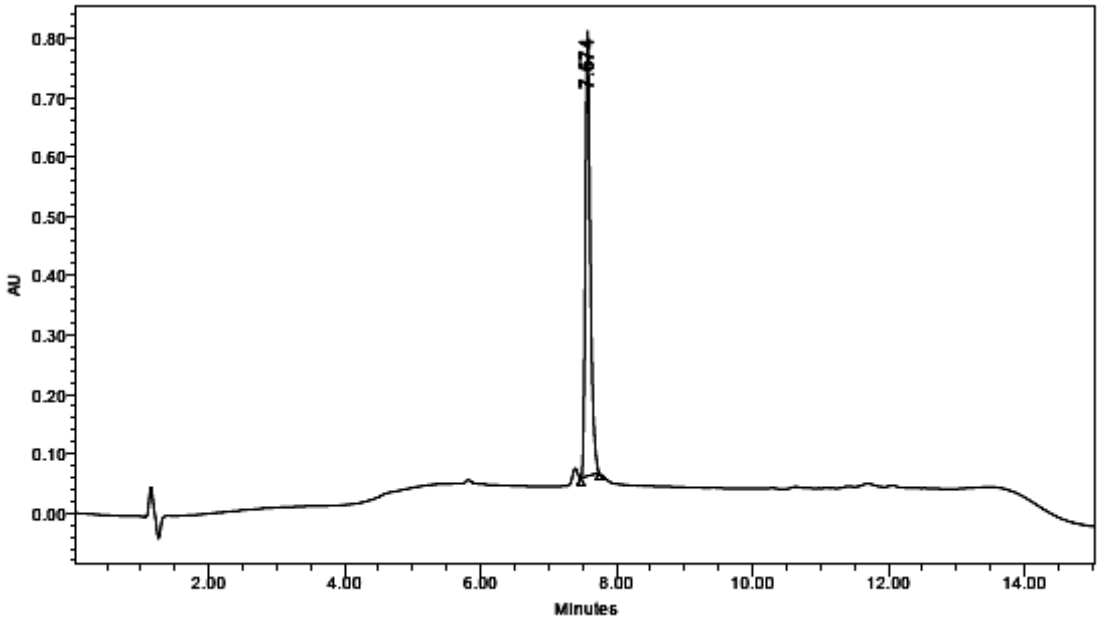


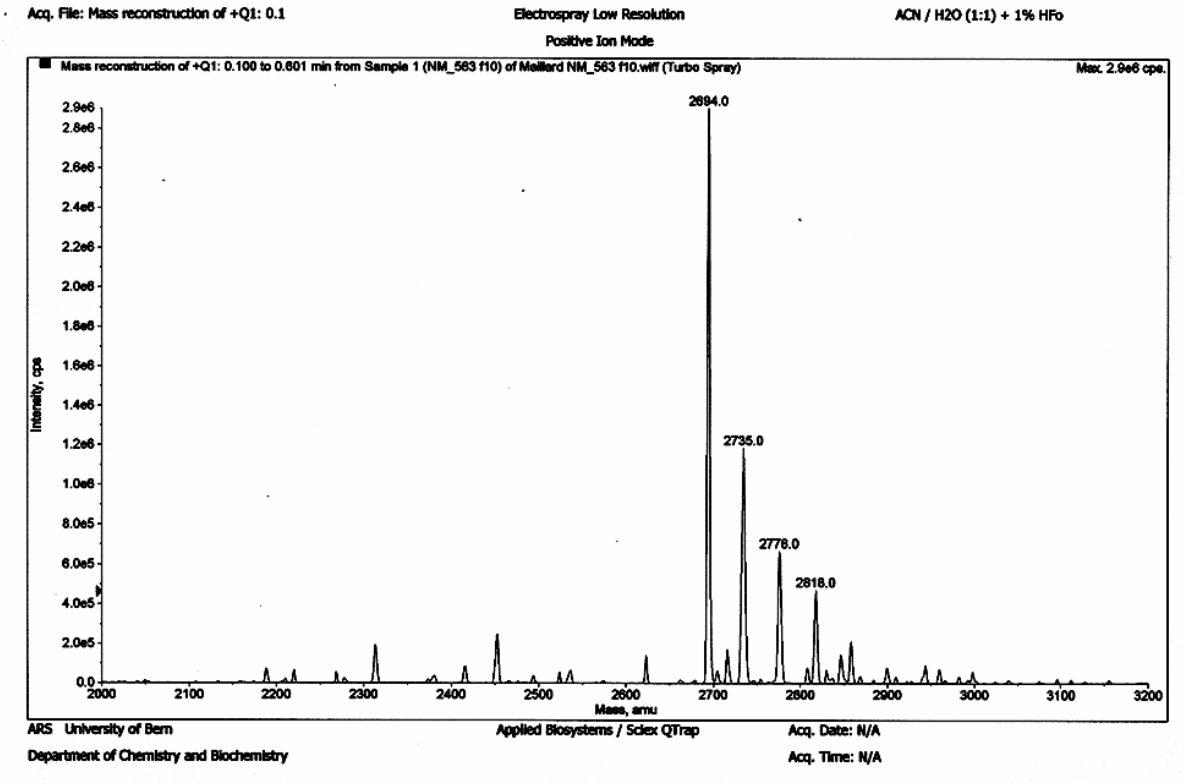
dH1 (leu)$_8$(*Lys*leu)$_4$(*Lys*phe)$_2$*Lys*lysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **dH1** was obtained as a white foamy solid after preparative RP-HPLC purification (76.2 mg, 12%). Anal. RP-HPLC: t_R = 7.9 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{138}H_{250}N_{31}O_{22}$ $[M+H]^+$: 2693.9, obsd: 2694.0; $[M+K]^+$: 2732.3, obsd: 2735.0.



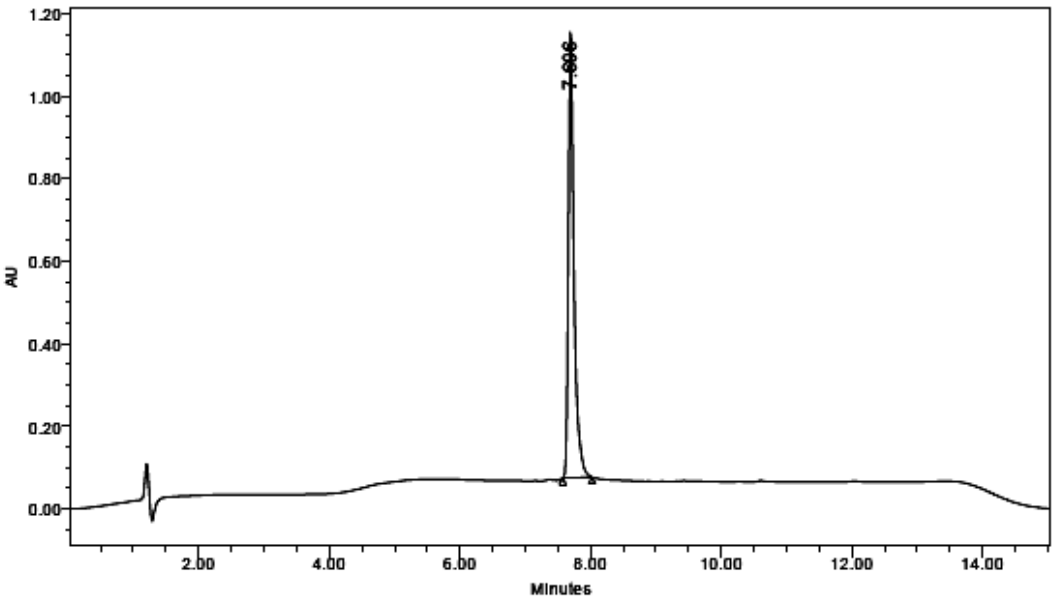


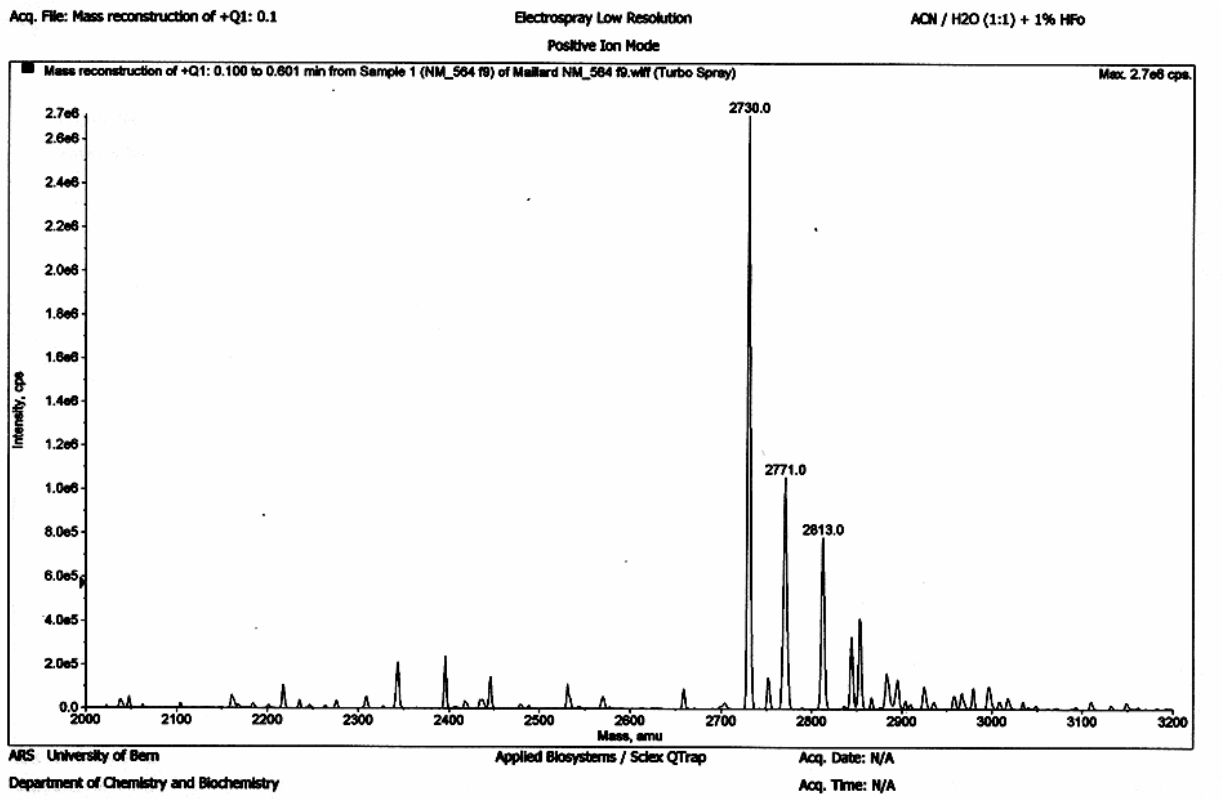
iH1 (Ile)$_8$(*Lys*Ile)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **iH1** was obtained as a white foamy solid after preparative RP-HPLC purification (33.6 mg, 1%). Anal. RP-HPLC: t_R = 7.6 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{138}H_{250}N_{31}O_{22}$ $[M+H]^+$: 2693.9, obsd: 2694.0; $[M+K]^+$: 2732.3, obsd: 2735.0.



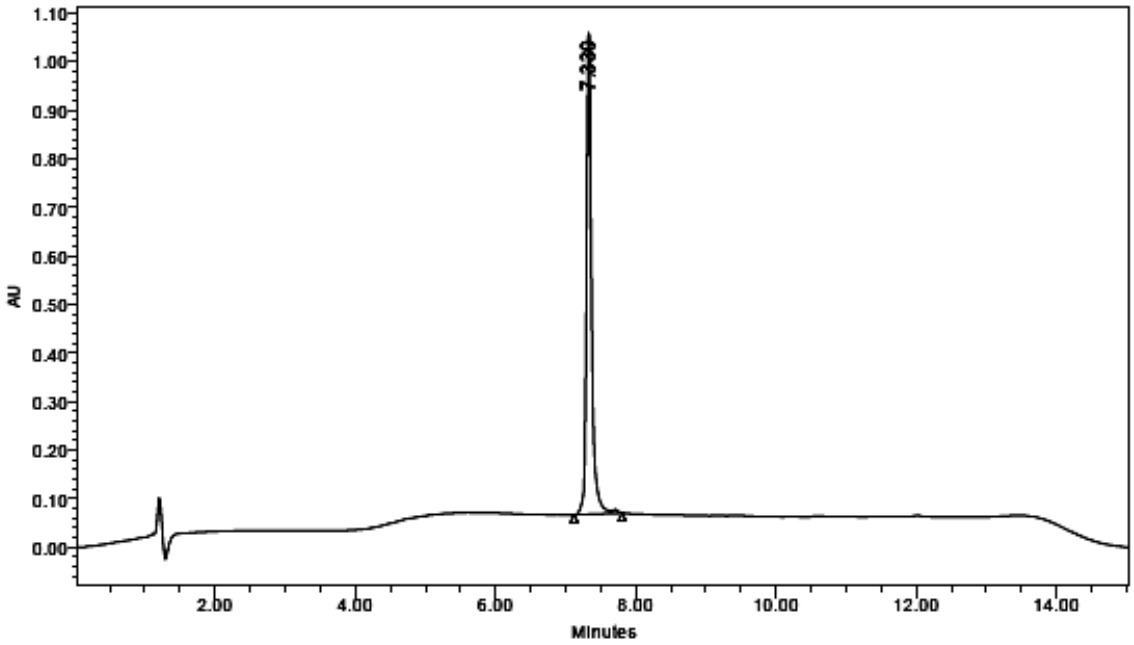


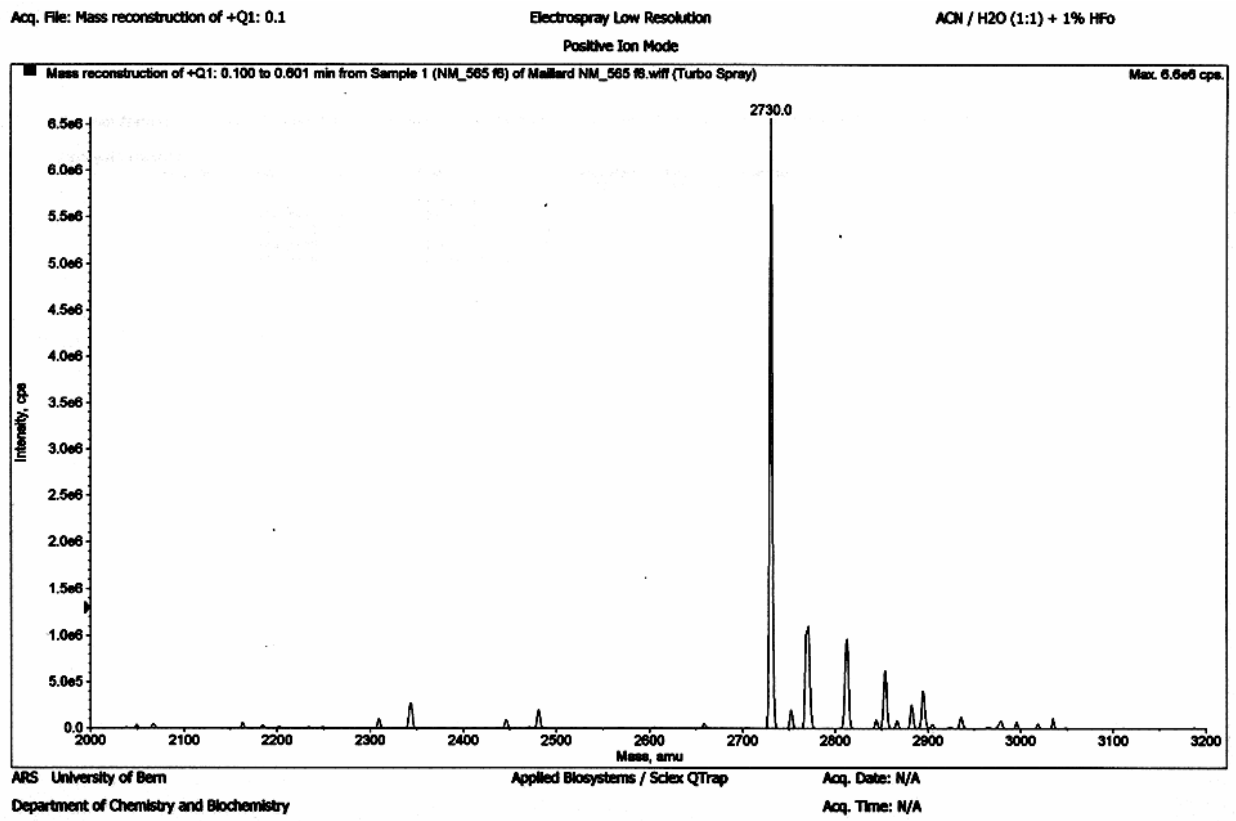
dH2 (leu)$_8$(*Lys*phe)$_4$(*Lys*pro)$_2$*Lys*lysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **dH2** was obtained as a white foamy solid after preparative RP-HPLC purification (42.0 mg, 10%). Anal. RP-HPLC: t_R = 7.7 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{142}H_{238}N_{31}O_{22}$ $[M+H]^+$: 2729.8, obsd: 2730.0; $[M+K]^+$: 2768.8, obsd: 2771.0.



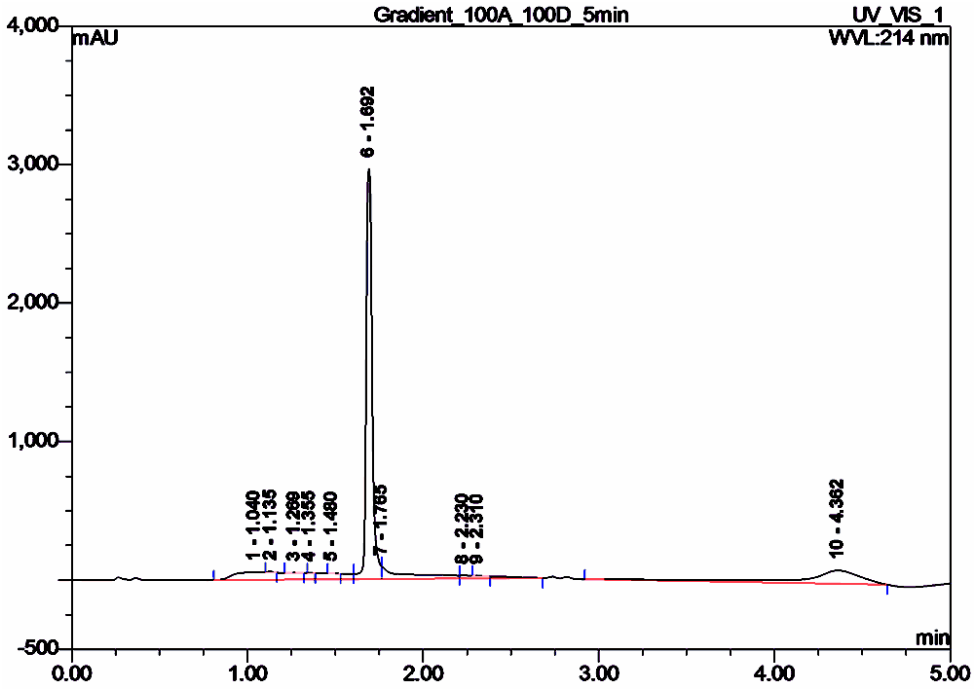


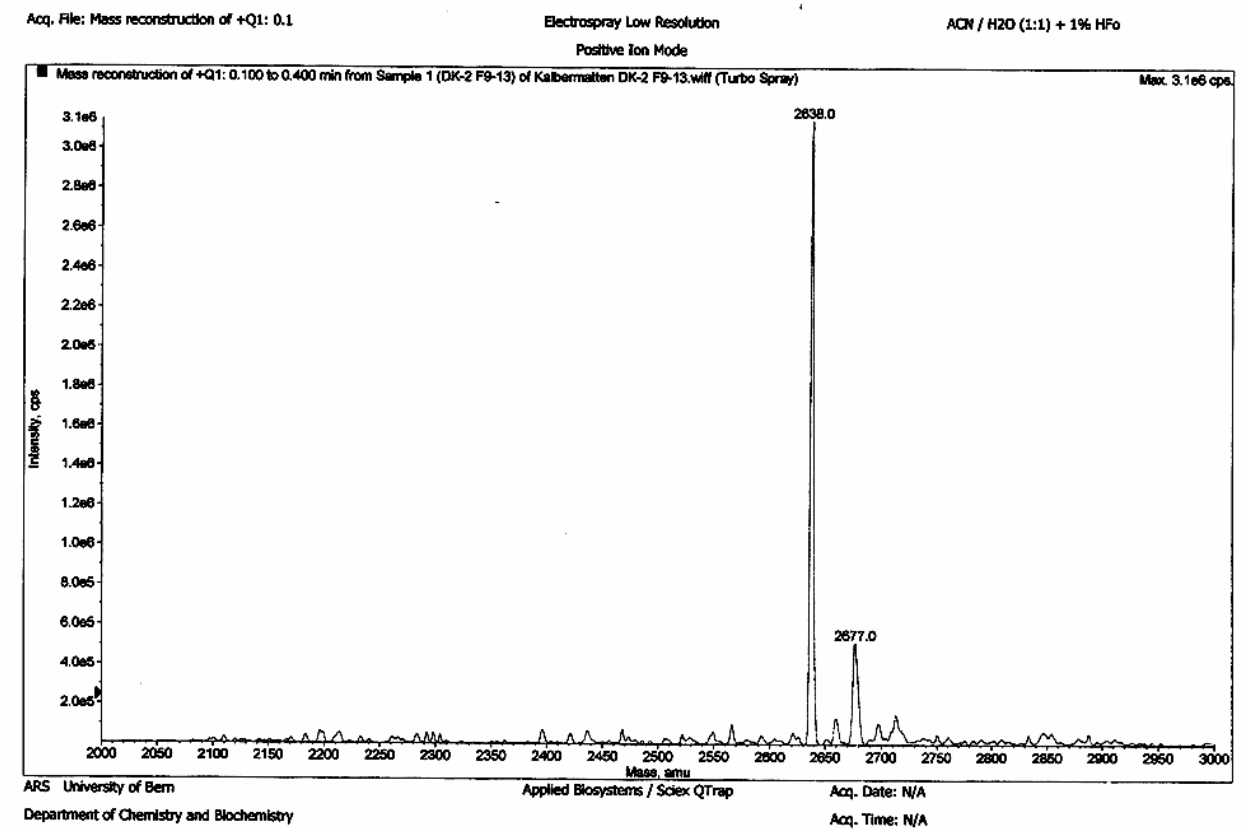
iH2 (Ile)$_8$(*Lys*Phe)$_4$(*Lys*Pro)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (400 mg, 0.24 mmol·g^{-1}), dendrimer **iH2** was obtained as a white foamy solid after preparative RP-HPLC purification (38.2 mg, 5%). Anal. RP-HPLC: t_R = 7.3 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{142}H_{238}N_{31}O_{22}$ $[M+H]^+$: 2729.8, obsd: 2730.0.



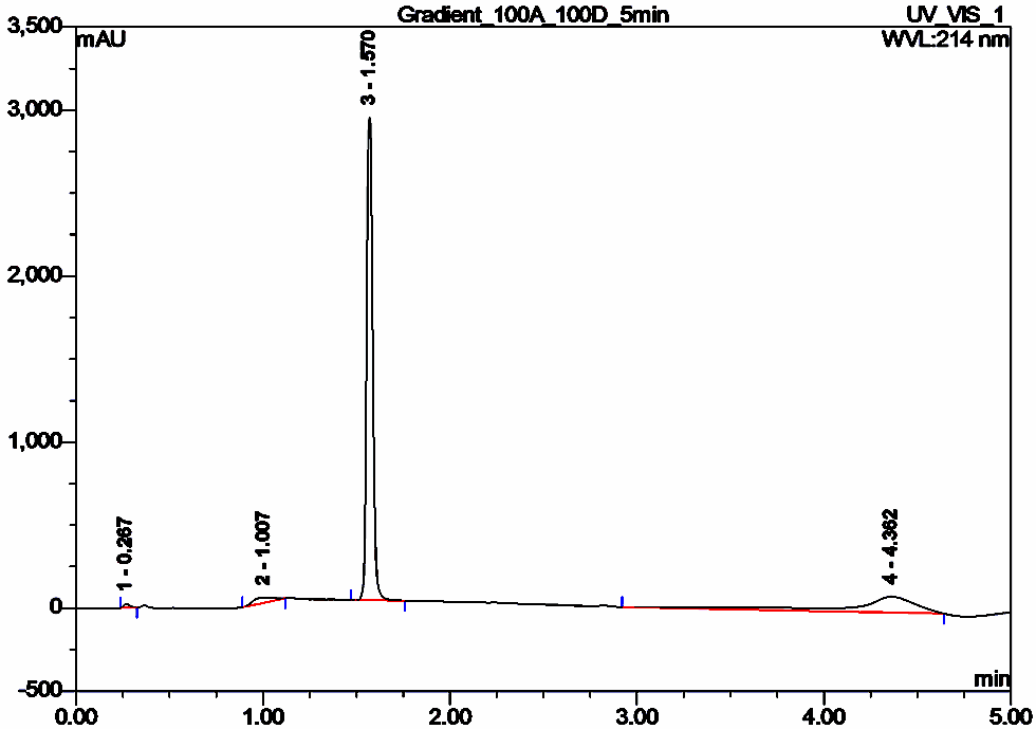


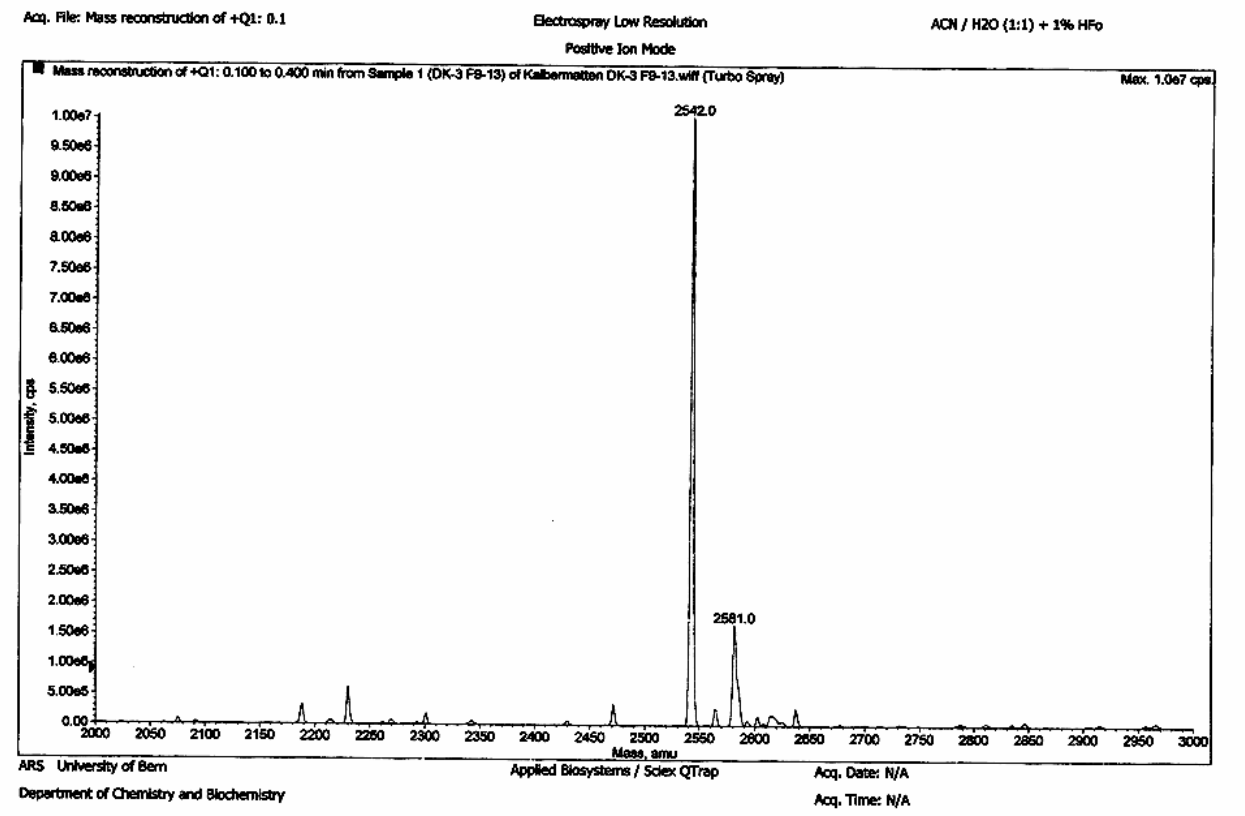
AX1 (Leu)$_8$(*Lys*Leu)$_4$(*Lys*Phe)$_2$*Lys*AlaNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.22 mmol·g^{-1}), dendrimer **AX1** was obtained as a white foamy solid after preparative RP-HPLC purification (27.2 mg, 5%). Anal. RP-HPLC: t_R = 1.692 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (ESI+) calc for $C_{135}H_{242}N_{30}O_{22}$ $[M+H]^+$: 2638.6, obsd: 2637.7, $[M+K]^+$: 2676.7, obsd: 2677.0.



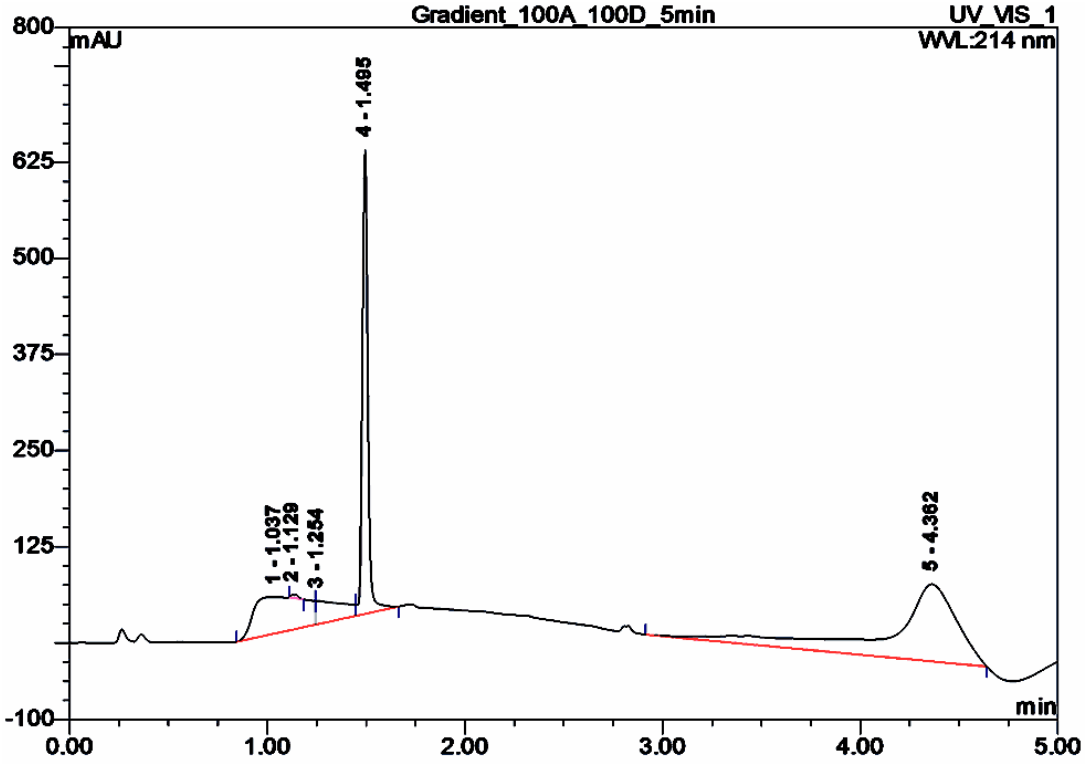


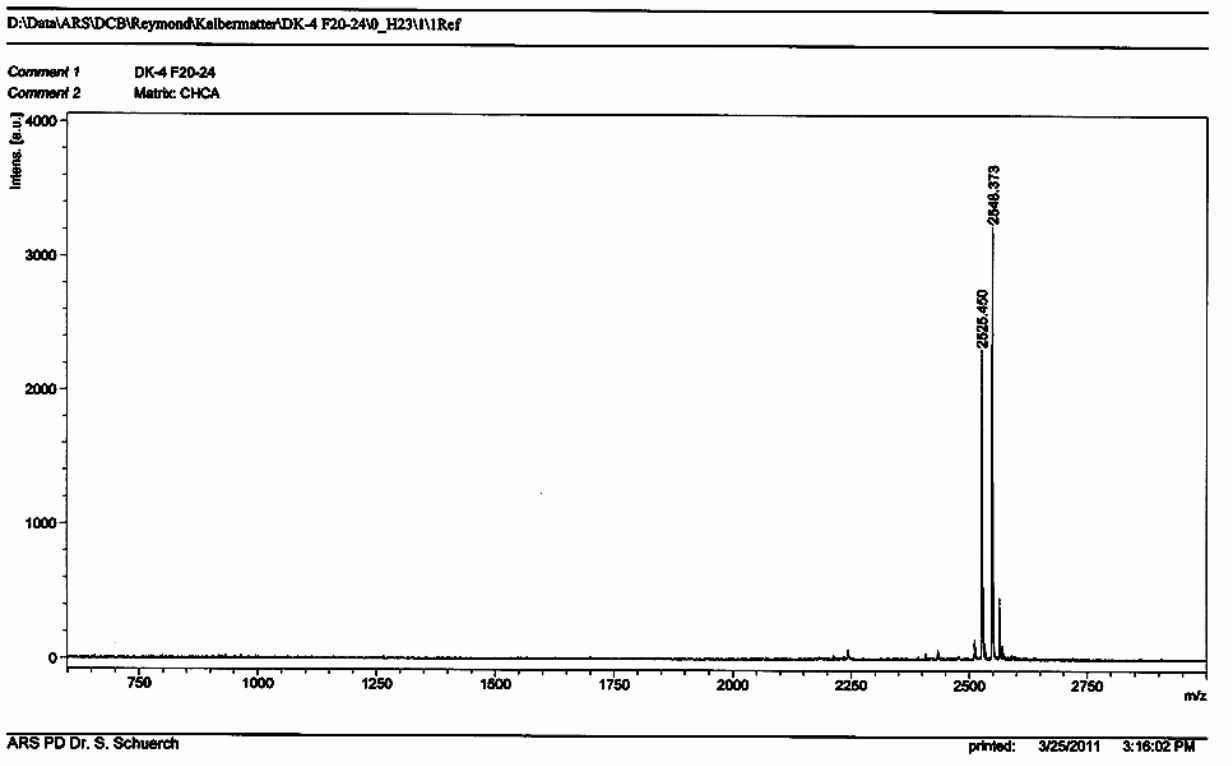
AX2 (Leu)$_8$(*Lys*Leu)$_4$(*Lys*Ala)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.22 mmol·g^{-1}), dendrimer **AX2** was obtained as a white foamy solid after preparative RP-HPLC purification (33.4 mg, 10%). Anal. RP-HPLC: t_R = 1.570 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (ESI+) calc for $C_{126}H_{241}N_{31}O_{22}$ $[M+H]^+$: 2543.5, obsd: 2542.5, $[M+K]^+$: 2581.6, obsd: 2581.0.



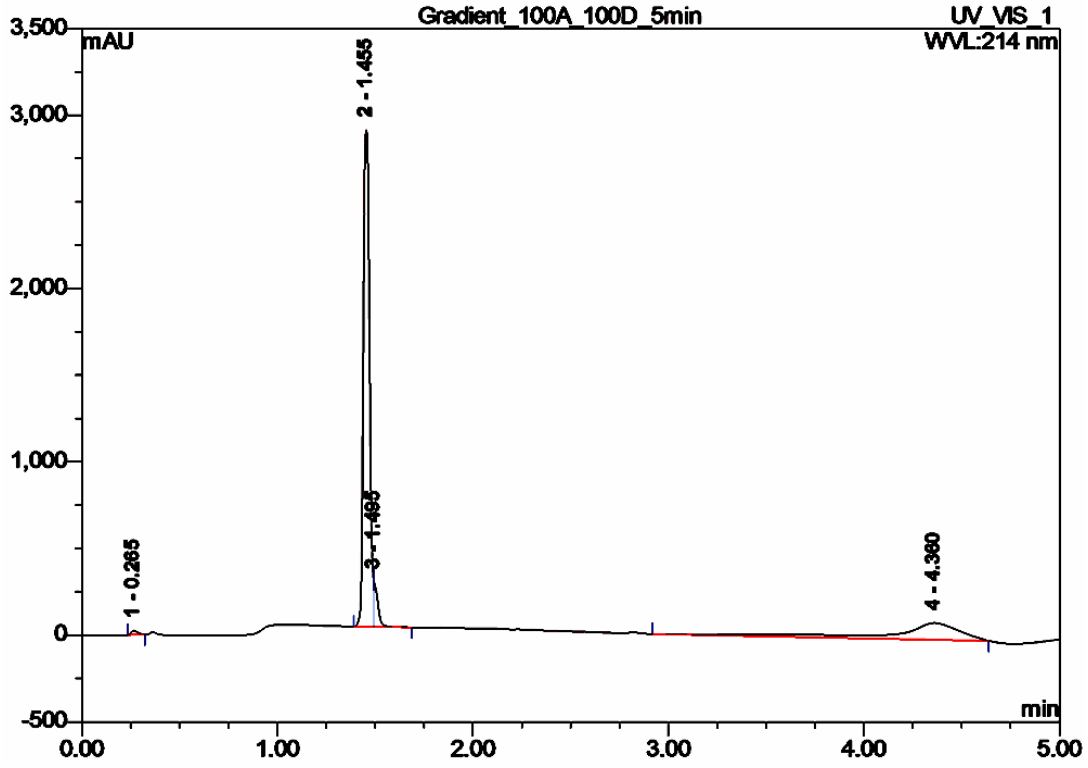


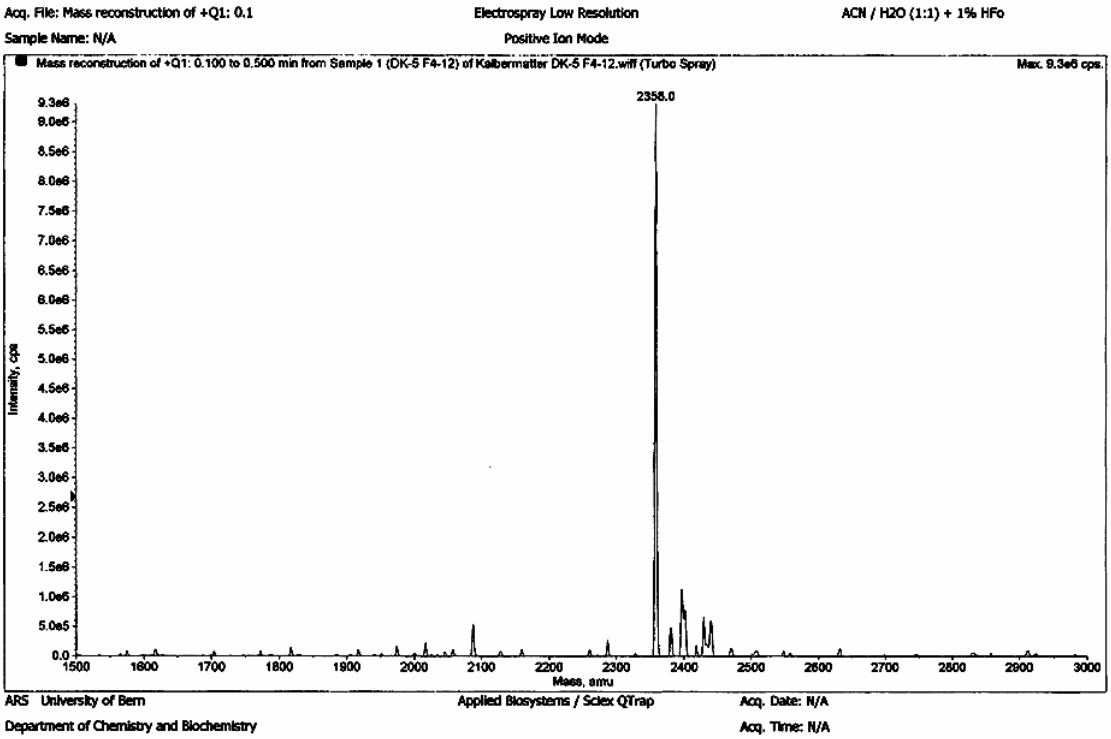
AX3 (Leu)$_8$(*Lys*Ala)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.22 mmol·g^{-1}), dendrimer **AX3** was obtained as a white foamy solid after preparative RP-HPLC purification (14.8 mg, 4%). Anal. RP-HPLC: t_R = 1.495 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (MALDI+) calc for $C_{126}H_{225}N_{31}O_{22}$ $[M+H]^+$: 2527.3, obsd: 2526.6.



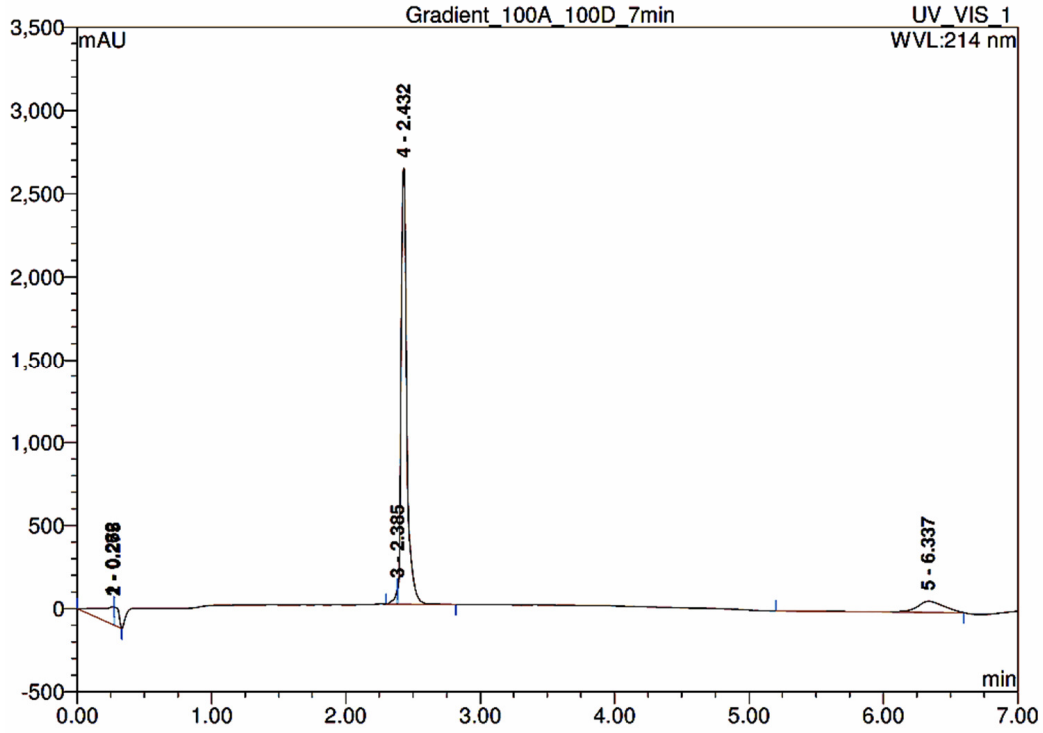


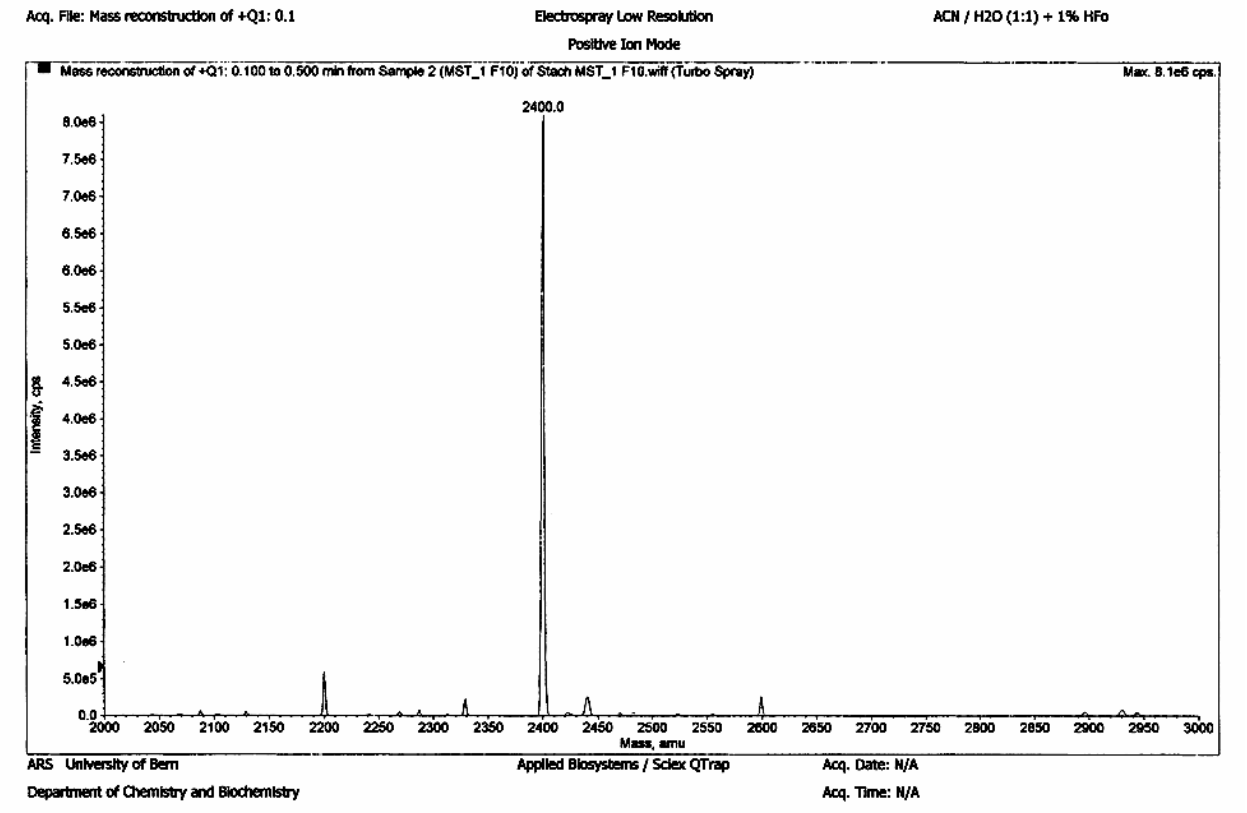
AX4 (Ala)$_8$(*Lys*Leu)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.22 mmol·g^{-1}), dendrimer **AX4** was obtained as a white foamy solid after preparative RP-HPLC purification (23.2 mg, 8%). Anal. RP-HPLC: t_R = 1.455 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (ESI+) calc for for $C_{114}H_{201}N_{31}O_{22}$ $[M+H]^+$: 2359.0, obsd: 2358.2.



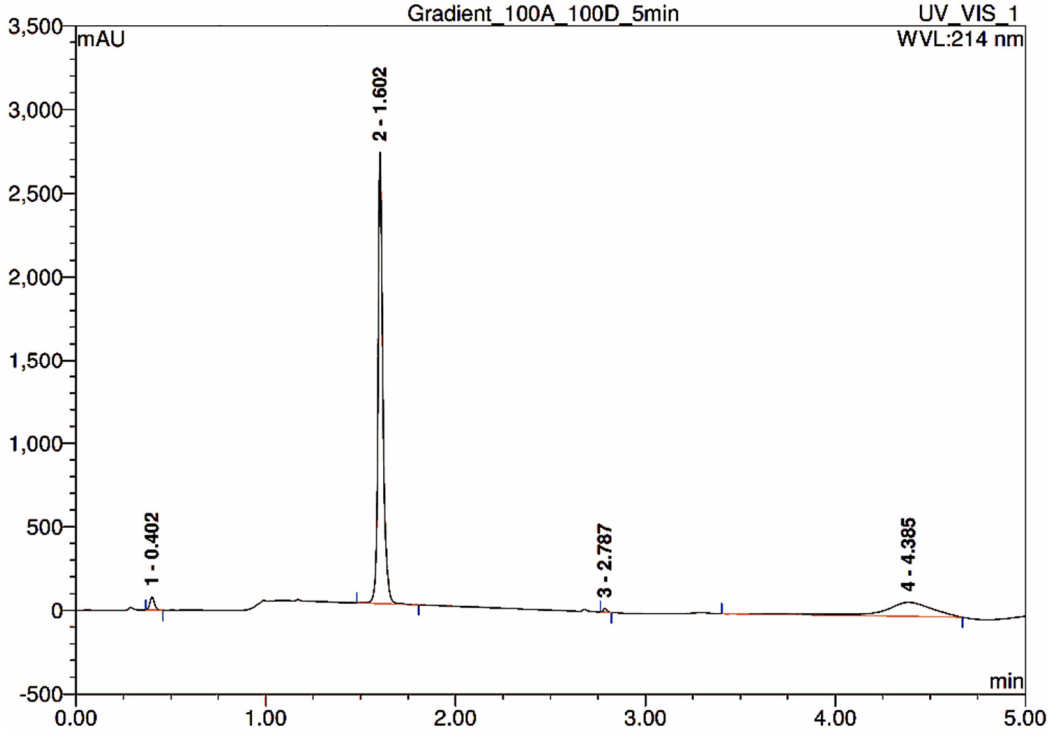


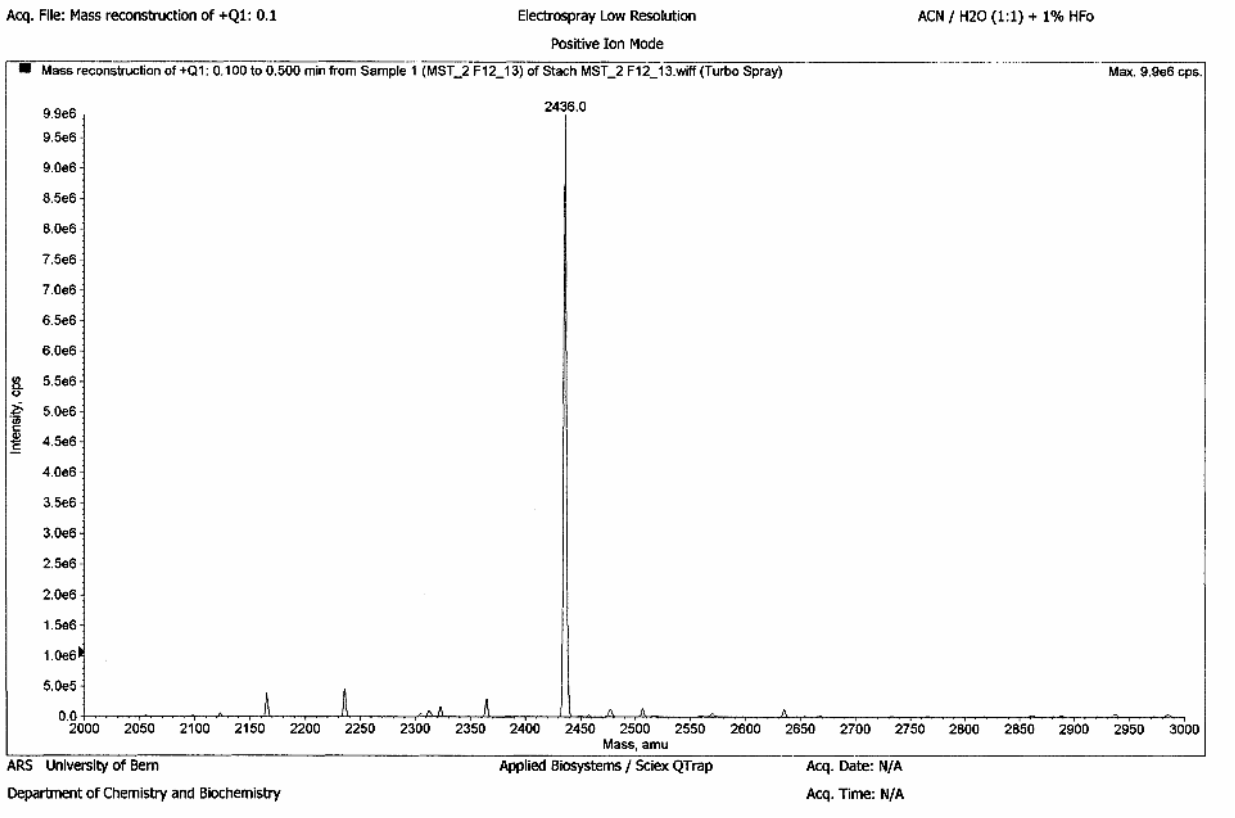
bH1 (Leu)$_8$(*Dap*Leu)$_4$(*Dap*Phe)$_2$*Dap*LysNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.24 mmol·g^{-1}), dendrimer **bH1** was obtained as a white foamy solid after preparative RP-HPLC purification (21.8 mg, 7%). Anal. RP-HPLC: t_R = 2.432 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (ESI+) calc for for $C_{117}H_{207}N_{31}O_{22}$ $[M+H]^+$: 2401.1, obsd: 2400.0.



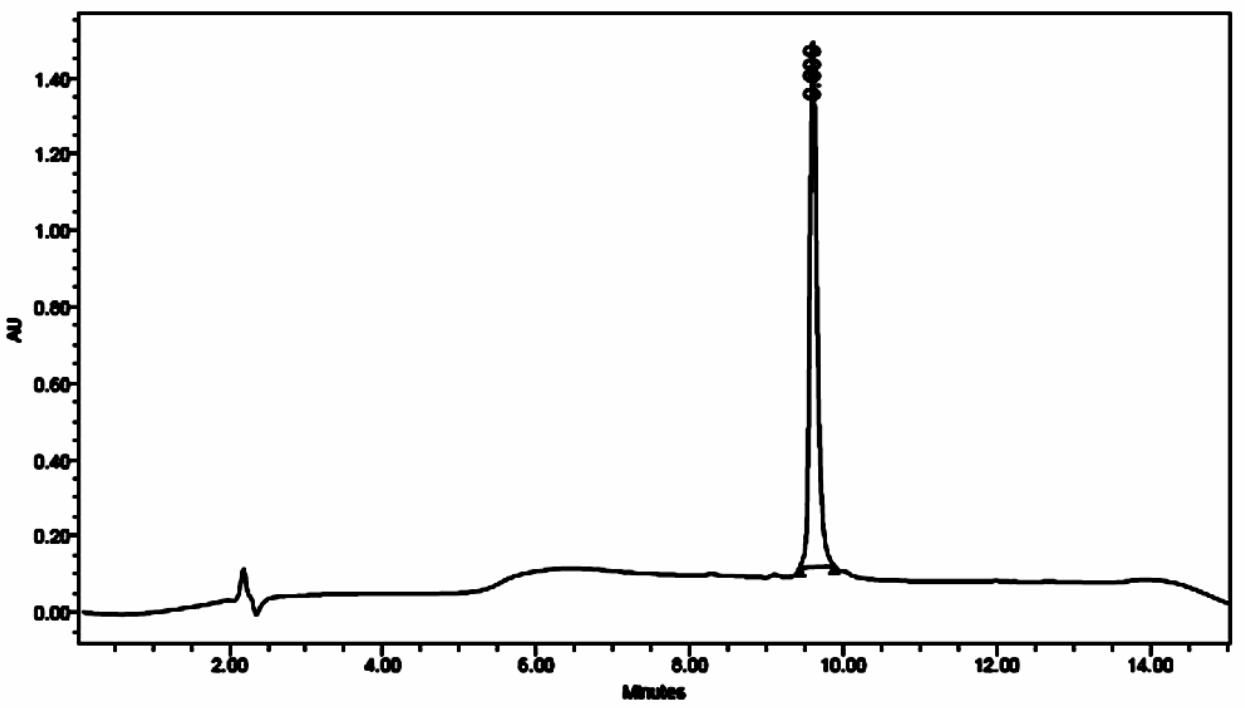


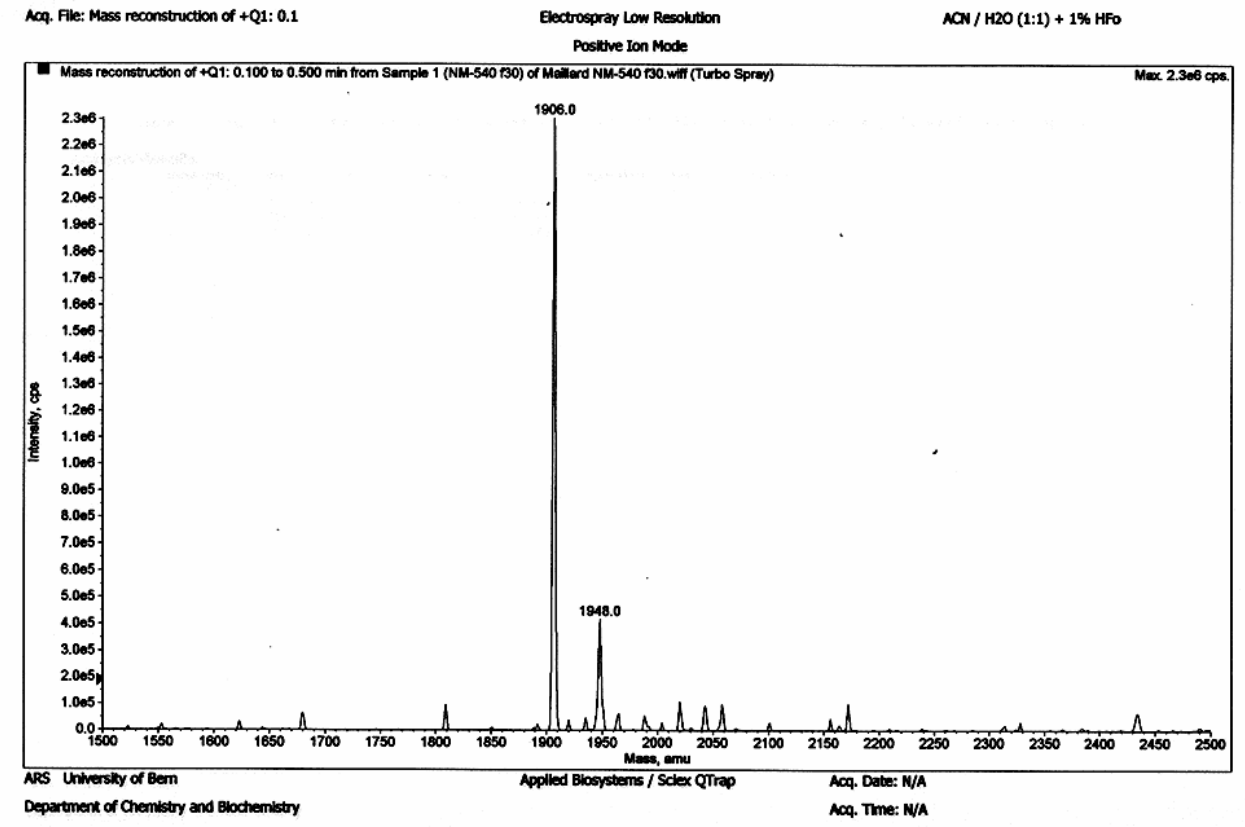
bH2 (Leu)$_8$(*Dap*Phe)$_4$(*Dap*Pro)$_2$*Dap*LysNH$_2$. From Tenta Gel S RAM® resin (300 mg, 0.24 mmol·g^{-1}), dendrimer **bH2** was obtained as a white foamy solid after preparative RP-HPLC purification (33.5 mg, 12%). Anal. RP-HPLC: t_R = 1.602 min (A/D = 100/0 to 0/100 in 2.2 min, 100D in 1.3 min, 1.2 mL·min^{-1}). MS (ESI+) calc for $C_{121}H_{195}N_{31}O_{22}$ $[M+H]^+$: 2437.1, obsd: 2436.0.





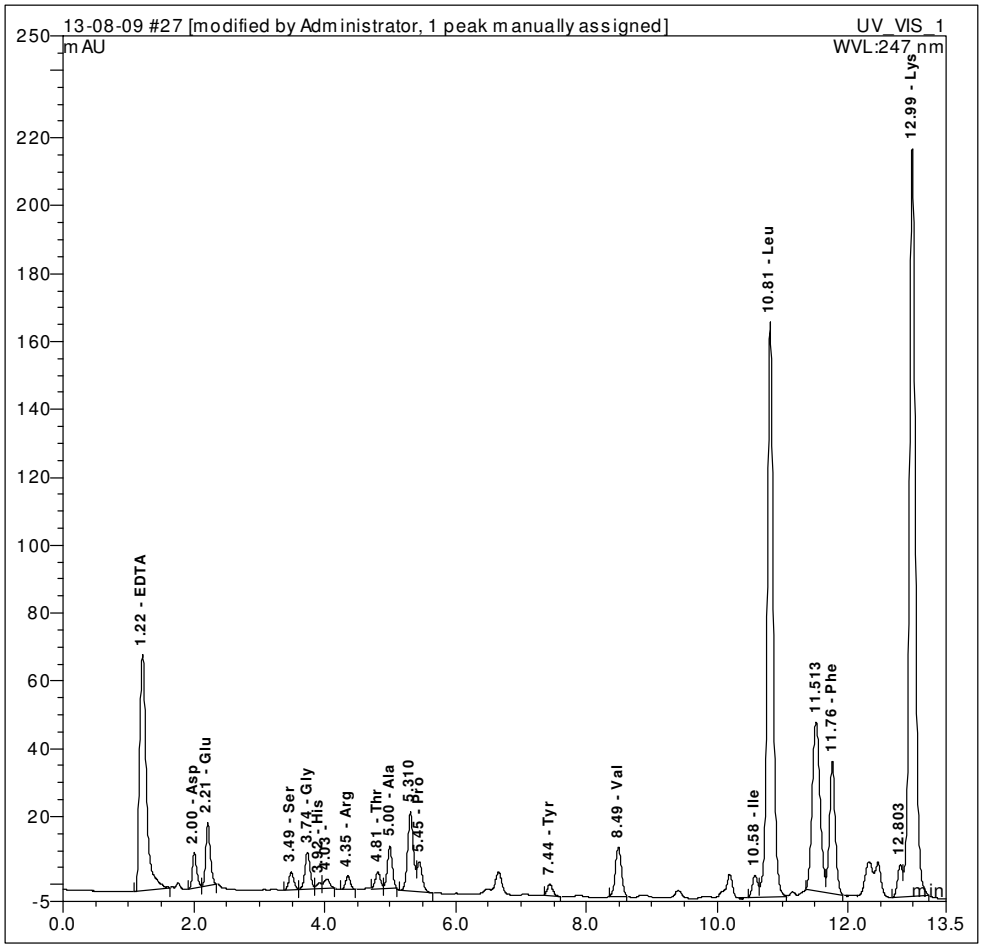
C01 Indolicidin: IleLeuProTrpLysTrpProTrpTrpProTrpArgArgNH$_2$. From Tenta Gel HL RAM® resin (600 mg, 0.39 mmol·g^{-1}), linear peptide **C01** was obtained as a white foamy solid after preparative RP-HPLC purification (47.5 mg, 9%). Anal. RP-HPLC: t_R = 9.6 min (A/D = 100/0 to 0/100 in 7 min, 100D in 3 min, 1.4 mL·min^{-1}). MS (ESI+) calc for $C_{100}H_{133}N_{26}O_{13}$ $[M+H]^+$: 1906.1, obsd: 1906.0; $[M+K]^+$: 1945.0, obsd: 1948.0.





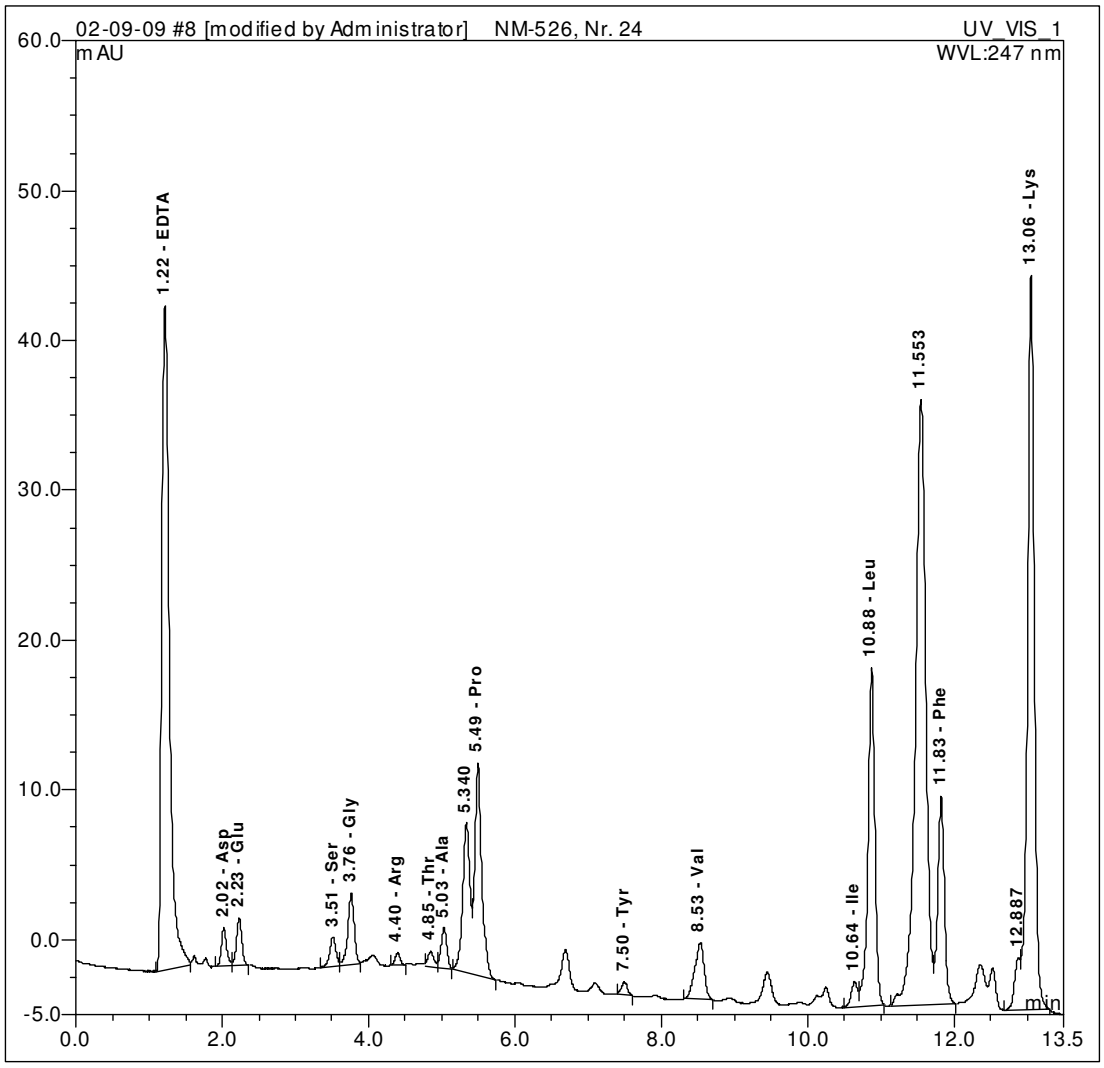
HPLC chromatograms from AAA from hit beads with clear zone to determine the sequences

H1 $(Leu)_8(LysLeu)_4(LysPhe)_2LysLysNH_2$



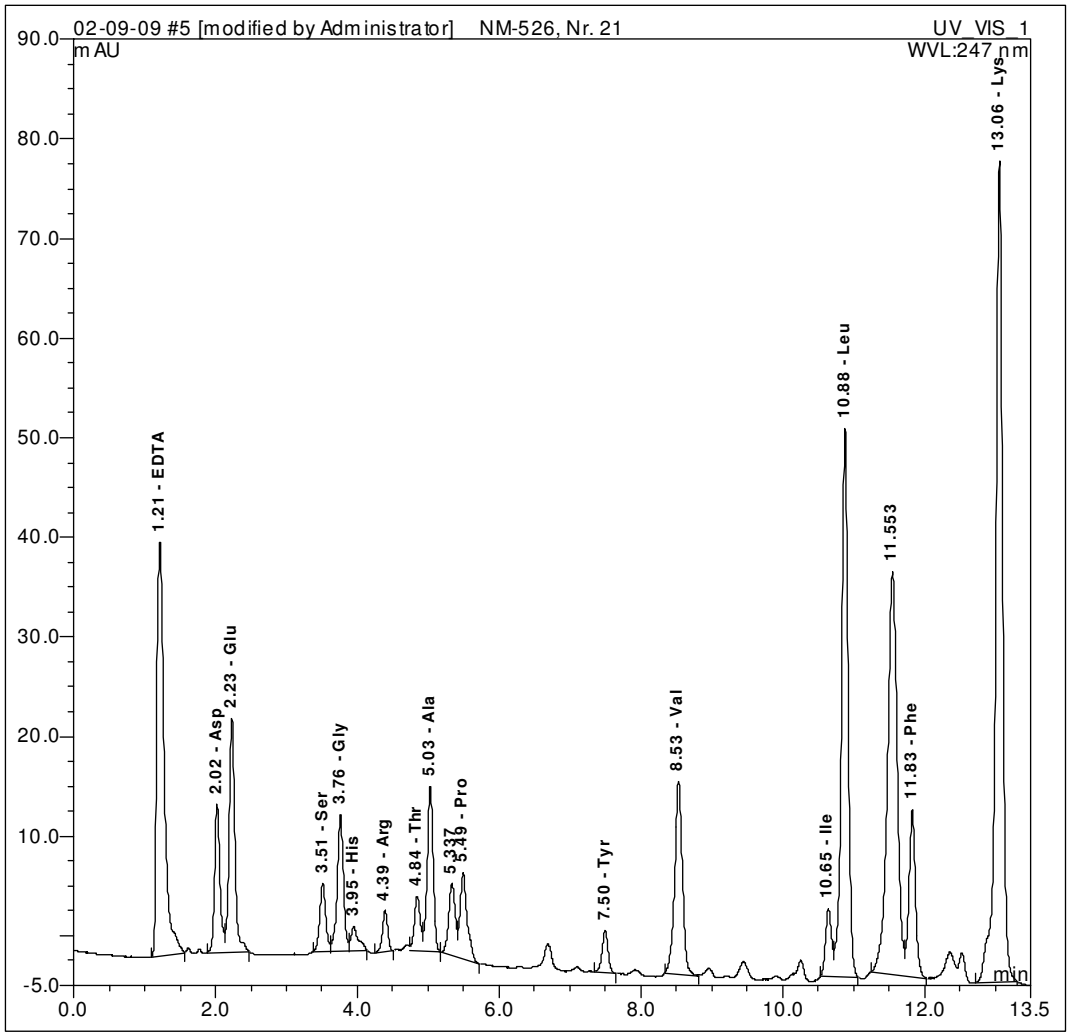
RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.22	1.21	0.109	8.48	69.48		717.0	EDTA
2.00	2.01	0.069	0.78	10.35		50.6	Asp
2.21	2.22	0.074	1.50	18.63		95.6	Glu
3.49	3.48	0.081	0.45	5.13		30.6	Ser
3.74	3.73	0.083	1.00	10.78		65.1	Gly
3.92	3.90	n.a.	0.14	1.71		10.3	His
4.35	4.34	0.078	0.32	3.83		23.4	Arg
4.81	4.80	0.083	0.45	5.00		36.1	Thr
5.00	4.99	0.082	1.10	12.50		75.7	Ala
5.45	5.44	n.a.	0.84	8.93		44.6	Pro
7.44	7.43	0.087	0.31	3.31		19.9	Tyr
8.49	8.48	0.098	1.52	14.48		86.5	Val
10.58	10.56	0.097	0.60	6.48		39.1	Ile
10.81	10.80	0.093	17.57	169.18		1067.6	Leu
11.76	11.74	0.092	3.91	38.88		230.1	Phe
12.99	12.97	0.094	23.03	220.14		787.2	Lys
Total:			61.988	598.795		3379.44	

H2 (Leu)$_8$(*Lys*Phe)$_4$(*Lys*Pro)$_2$*Lys*LysNH$_2$



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.22	1.22	0.109	5.42	44.33		386.6	EDTA
2.02	2.02	0.073	0.21	2.56		14.9	Asp
2.23	2.23	0.077	0.27	3.21		20.1	Glu
3.51	3.52	0.086	0.19	1.98		13.7	Ser
3.76	3.77	0.088	0.47	4.75		32.1	Gly
4.40	4.40	0.075	0.06	0.77		5.2	Arg
4.85	4.85	0.092	0.10	1.02		7.6	Thr
5.03	5.04	0.086	0.24	2.66		17.9	Ala
5.49	5.50	0.096	1.56	14.17		81.6	Pro
7.50	7.50	0.086	0.08	0.85		5.7	Tyr
8.53	8.55	0.138	0.56	3.79		25.7	Val
10.64	10.65	n.a.	0.17	1.68		11.4	Ile
10.88	10.88	0.094	2.43	22.52		159.9	Leu
11.83	11.83	0.095	1.48	13.86		99.9	Phe
13.06	13.06	0.095	5.36	49.01		191.1	Lys
Total:			18.616	167.162		1073.28	

H3 (Leu)$_8$(*Lys*Gln)$_4$(*Lys*Phe)$_2$*Lys*LysNH$_2$



RT min	RT (STD) min	PW(50%) min	Area mAU*min	Height mAU	n.a. n.a.	Amount pmol	Peak Name
1.21	1.22	0.107	5.08	41.59		362.7	EDTA
2.02	2.02	0.074	1.29	14.96		86.9	Asp
2.23	2.23	0.079	2.21	23.55		147.5	Glu
3.51	3.52	0.086	0.65	6.84		47.2	Ser
3.76	3.77	0.089	1.39	13.69		92.4	Gly
3.95	3.95	0.096	0.30	2.40		15.9	His
4.39	4.40	0.080	0.36	4.10		27.7	Arg
4.84	4.85	0.092	0.53	5.46		40.6	Thr
5.03	5.04	0.087	1.60	16.57		111.7	Ala
5.49	5.50	0.097	0.99	8.68		50.0	Pro
7.50	7.50	0.088	0.42	4.33		28.9	Tyr
8.53	8.55	0.112	2.46	19.43		131.6	Val
10.65	10.65	0.096	0.67	6.75		45.7	Ile
10.88	10.88	0.093	5.85	55.06		390.9	Leu
11.83	11.83	0.095	1.78	16.71		120.5	Phe
13.06	13.06	0.095	9.30	82.34		321.0	Lys
Total:			34.892	322.449		2021.17	

Biological Assays

Broth microdilution method for antimicrobial peptides. 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), microdilution broth method was used.[6] A colony of bacteria was grown in LB-medium overnight at 37°C. The concentration was quantified by measuring absorbance at 600 nm. The peptide dendrimer samples were prepared as stock solutions of 1 $mg \cdot mL^{-1}$ in H_2O or DMSO (for the non-hits **Ac1**-**Ac2**) and diluted serially by 2/3 in nutrient LB in a 96-well microtiterplate (Cornstar, polypropylene, untreated). The sample solutions (50 µL) were mixed with the diluted bacterial suspension with an OD_{600nm} = 0.001 (50 µL) and incubated at 37°C until satisfactory growth (18 - 24h). For each test, two columns of the plate were kept for sterility control (SC, broth only) and growth control (GC, broth with bacterial inoculums, no antibiotics). A solution of MTT (0.1% in H_2O) was added into each well. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the antimicrobial substance (peptide dendrimer) that inhibited visible growth of the tested bacteria (yellow) with the unaided eye. For microbiological study Indolicidin (IleLeuProTrpLysTrpProTrpTrpProTrpArgArgNH_2) and LysTyrLysLysAlaLeuLysLysLeuAlaLysLeuLeuNH_2, linear peptides, were used as references.[7]

Minimal inhibitory concentration of a series of bacterial strains. MIC values of selected peptide dendrimers were tested in Basilea Pharmaceutica Ltd. using the standard broth microdilution method as recommended by the Clinical and Laboratory Standards Institute, protocol M7-A7. The concentration range was 0.06 to 32 µl/mL. The bacterial strains used were: *Staphylococcus aureus* (ATCC25923); *Staphylococcus aureus* (887); *Staphylococcus aureus* (clinical isolate of MRSA, 42080); *Staphylococcus epidermidis* (ATCC14990); *Staphylococcus epidermidis* (J147); *Enterococcus faecalis* (ATCC29212); *Enterococcus faecalis* (Van B E808); *Enterococcus faecium* (ATCC19434); *Enterococcus faecalis* (Van B E38-10); *Escherichia coli* (ATCC25922); *Escherichia coli* (HB101(PAT266); *Escherichia coli* (DC2); *Pseudomonas aeruginosa* (ATCC27853); *Pseudomonas aeruginosa* (PAO1 HS); *Pseudomonas aeruginosa* (18S/H); *Pseudomonas aeruginosa* (K799/WT); *Pseudomonas aeruginosa* (K799/61); *Staphylococcus aureus* (ATCC29213).

Hemolysis assay. To determine the minimal haemolytic concentration (MHC) stock solutions 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 (Cornstar or Nunc, polystyrene, untreated). Human red blood cells (RBC) were obtained by centrifuging 1.5 mL of whole blood from friendly donors 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 resuspended in 10 mL of PBS at a final RBC concentration of 5%. The RBC suspension (50 µL) was added to each well and the plate was incubated at room temperature for 4 hours. Minimal haemolytic 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 RBC suspension) and a haemolytic activity control (mQ-deionized water 50 µL + 50 µL RBC suspension).[8]

Diffusion NMR measurements

Standard PGSE diffusion NMR experiments were performed using a Bruker DRX400 or DRX500 with diluted solutions (10 $mg \cdot mL^{-1}$) in D_2O or in DMSO at 303 K. The gradient with a maximum strength of $50 \cdot 10^{-4}$ $T \cdot cm^{-1}$ was calibrated using the HOD proton signal in 99.997% D_2O. The diffusion time Δ was either 100 ms or 150 ms and the gradient duration δ was either 4 ms or 7 ms. Diffusion coefficient D was derived from peak integrals or intensities using the Simfit software or Topspin Software from Bruker. The hydrodynamic radii were calculated from the diffusion coefficient D [$m^2 \cdot s^{-1}$] using the Stokes-Einstein equation $R_h = k \cdot T/6 \cdot \pi \cdot \eta \cdot D$ with Boltzmann constant $k = 1{,}380 \cdot 10^{-23}$ $J \cdot K^{-1}$, temperature T in K and viscosity η = 1.095 mPa·s for D_2O and 2.180 mPa·s for $DMSOd_6$.[9]

Table S3. Hydrodynamic radii of peptide dendrimers.

Cpd.	r_H [nm]
H1	1.30
Ac1	1.32
H3	1.34
Ac3	1.43
H2	1.40
dH1	1.43
iH1	1.38
dH2	1.40
iH2	1.38
AX1	1.26
AX2	1.26
AX3	1.23
AX4	1.18
bH1	1.15
bH2	1.18

Liposome dendrimer interactions

Preparation of *E. coli* lipid vesicles. *E. coli* lipids (*E. coli* polar lipid extract from Avanti Polar Lipids Inc., Alabaster, AL, USA) were solubilized in the detergent n-octyl-β-glucopyranoside (Anatrace, Maumee, OH, USA): 5 mg/ml *E. coli* lipids, 5% n-octyl-β-glucopyranoside, 20 mM Tris-HCl, pH 8, 150 mM NaCl, 10% glycerol, 0.01% NaN_3. This stock solution was diluted 1:5 in 20 mM Mes-NaOH, pH 6, 150 mM NaCl, 0.01% NaN_3 and dialyzed at 24°C against the same buffer. Liposomes were harvested after 5 days of dialysis.

Liposome-dendrimer interactions. A dendrimer solution (**H1** or **Ac1**; 50 µg/ml in water) was mixed with the liposome solution (1 mg/ml) at a 1:1 ratio. For the negative control, water was taken instead of a dendrimer solution. The reaction volume was 20 µl, and the final dendrimer and *E. coli* lipid concentrations were 25 µg/ml and 0.5 mg/ml, respectively. After 16 h incubation time at 24°C, electron microscopy grids were prepared.

Negative-stain transmission electron microscopy. Liposomes or liposome/dendrimer solutions were adsorbed for ~60 s to parlodion carbon-coated copper grids which were made hydrophilic by glow discharge at low pressure in air. Grids were washed with three drops of double-distilled water and stained with two drops of 0.75% uranyl formate. Electron micrographs were recorded with a Philips CM12 transmission electron microscope operated at 80 kV and equipped with a Morada CCD camera (Soft Imaging System).

Molecular Dynamic Simulations

Peptide dendrimer structure building. The starting structure for free dendrimer was built with L-amino acids using peptide building dictionary interface in maestro version 8.5 in Schrödinger suite. Parameters for branching lysine residue were obtained from natural amino acid building block based on transferability of OPLS-AA (Optimized Potentials for Liquid Simulations-All Atom)[10] force field.

Macromodel Energy Optimizations. Built free dendrimer was optimized by checking for correct bond orders, charges and performing H-bond treatment using default protocol from protein preparation wizard in maestro 8.5. The structures were subsequently minimized in macromodel (version 9.6) using steepest descent method with maximum of 500 iterations with gradient convergence threshold of 0.05 and constant dielectric with dielectric constant of 1. The potential force field used for minimizations protocol was OPLS-AA with extended cutoff values (van der Waals = 8.0Å; electrostatic = 20.0 Å; H-bond = 4.0 Å).

System Preparation for Simulations. Molecular dynamics simulation was performed using OPLS-AA force field in desmond molecular dynamics package with maestro-desmond interoperability tool, version 2.0[11] The systems were setup for explicit solvent simulations. The hydrated 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membrane used in this study consisted of 75 POPC. The solute (dendrimer **H1**:POPC) was immersed in 4794 water molecules using TIP3P water model[12] in truncated octahedron box spaced at buffer size of 1 nm from solute boundaries. The physiological salt concentration of 0.15 M was used, since dendrimer **H1** contain positive charges of +9, counterion Cl^- were added to neutralized system.

Molecular dynamic simulations. The prepared system was used for molecular dynamics simulation. MD simulation for a period of 100 ns was performed using following three steps : 1) minimization 2) equilibration and 3) production run;

*Minimization***.** The setup systems were minimized to remove close contacts between solute-solvent molecules using the LBFGS method with maximum iterations of 200 cycles and convergence threshold of 1.0 Kcal mol^{-1} $Å^{-1}$. The step size was 0.005 ps and switch criteria was 25.0 Kcal mol^{-1} $Å^{-1}$. The short range interactions used cutoff radius of 9.0 Å, whereas the long range coulombic interactions were taken into account using smooth Particle Mesh Ewald (PME) with Ewald tolerance of 1e-09 Å.

Equilibration protocol. During equilibration 2000 steps of steepest descent minimization of water molecules and ions were performed to allow water molecules to assume a lower energetic geometry, while the solute was restrained with force constant of 50 kcal/mol Å^2. The resulting systems were then subjected to 2000 steps of minimization with no restraints, reaching a root mean square gradient of 0.1 to assure the relaxation of the structure, followed by 12 ps of heating from 10 to 300 K in a constant volume ensemble with restraints on the solute heavy atoms (50 kcal/mol Å^2). This was followed by 12 ps of constant pressure unrestrained simulation at 300 K, where convergences of energies, temperature, pressure and density of the systems was monitored.

Production runs protocol. The output co-ordinates and velocities from the equilibrated run were subsequently used in productions runs over a period of 100 ns at 300 K using NPγT ensemble and surface tension of 4000 bar. Å. The bond lengths to hydrogens were constrained with a variant of the M-SHAKE algorithm[13]. To maintain 1 atm at 300 K in NPγT ensemble, the system was coupled to a Martyna-Tobias-Klein barostat[14], with relaxation time of 2 ps with isotropic coupling style and a Nose-Hoover thermostat[15] (with relaxation time of 1 ps). Long-range electrostatic interactions were modeled using a Particle Mesh Ewald method [16] . The van der Waals (VDW) interactions and real space contributions to the electrostatics were truncated at 10 Å, and estimated the long-range VDW contributions to the energy and the pressure by assuming a homogeneous distribution of VDW spheres with dispersion coefficient 69.5 kcal/mol/Å. RESPA integrator [17] with steps of 2 fs was used for bonded and short-range non bonded interactions, and 6 fs for long-range electrostatics. During the course of simulations, quality checks of system were performed by monitoring total energy profile of structures in trajectories.

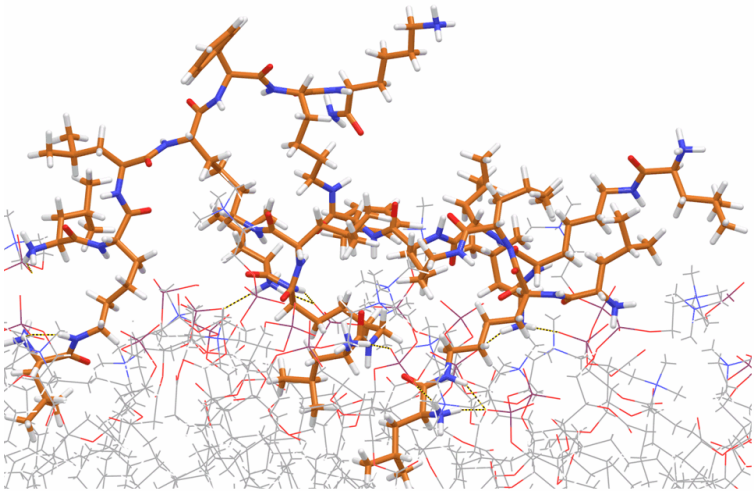


Figure S1. Molecular interaction of peptide dendrimer **H1** (orange color stick) with phosphocholine head group of POPC membrane (red color wire). H-bonds are shown in black dotted line. (+6) out of (+8) cationic Leu from dendrimer **H1** make H-bonding interaction with phosphocholine head group of POPC membrane whereas, insertion of hydrophobic aminoacid sides chains into the lipid bilayer.

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