

Electronic Supplementary Information

Solid-Phase Synthesis of Arginine-Based Double-Tailed Cationic Lipopeptides: Potent Nucleic Acid Carriers

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1. General Remarks

¹H NMR spectra were recorded on a Bruker DPX-500 and a Bruker DPX-400 in the deuterated solvents indicated at 298 K. Chemical shifts for proton and carbon spectra were reported on the δ scale in ppm and were referenced to residual non-deuterated solvent. All coupling constants (J values) were measured in Hz.

Low-resolution mass spectra were recorded on a VG Platform Quadrupole Electrospray Ionisation mass spectrometer. High-resolution electrospray mass spectra were recorded on a Bruker Apex III FT-ICR mass spectrometer.

IR spectra were recorded on a Bio-Rad ATR FT-IR with a golden gate accessory.

UV/VIS spectra were recorded on a Hewlett Packard HP8452A diode array spectrophotometer.

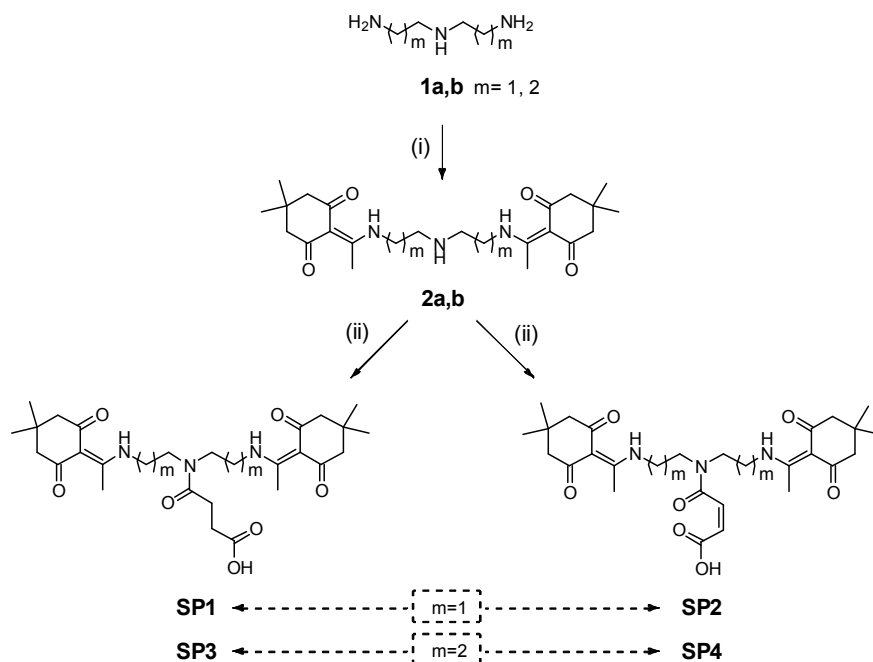
Thin layer chromatography (TLC) plate was visualised by UV light and/or stained with ninhydrin (0.3 % ninhydrin in *n*-butanol and 3% acetic acid) or permanganate solutions (KMnO₄ (3 g), K₂CO₃ (20 g), 5 % aq. NaOH solution (5 mL) and H₂O (300 mL)).

Presence/absence of free primary amines on resin-bound compounds was determined using a ninhydrin test.

Column chromatography was carried out on Sorbsil C60, 40-60 mesh silica.

Analytical RP-HPLC was performed on a HP1100 system equipped with a Phenomenex Prodigy C₁₈ reverse phase column (150 x 4.6 mm i.d.) eluting with a gradient of water/TFA (0.1%) to MeCN/TFA (0.04%) over 20 minutes with a flow rate of 1 mL/min.

2. Synthesis of Dde-protected linkers (3a,b and 4a,b)



2.1. Synthesis of SP1 and SP2

A solution of DdeOH (5g, 27 mmol) in DCM (5 mL) was added dropwise to a stirred solution of diethylenetriamine **1a** (1.34 g, 13 mmol) in DCM (10 mL). The reaction mixture was stirred for 6 h. The progress of the reaction was monitored by TLC (DCM/MeOH 9:1). After completion, the solvent was evaporated. The crude product **2a** was dissolved in DCM (5 mL) and either succinic anhydride (1.3 g, 13 mmol) or maleic anhydride (1.29 g, 13 mmol) in DCM (5 mL) was added. The resulting mixture was stirred for 8 h. The solvent was evaporated and the crude product purified by column chromatography (DCM/MeOH 15:1 as an eluent) to afford compound **3a** (3.3 g, 92%) or **4a** (3.4 g, 94%) as light yellow solids.

N,N-Bis-(2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethylamino]ethyl)succinamic acid (**SP1**) R_f = 0.6 (DCM/MeOH 9:1); m.p.= 65-68° C; IR: ν (cm^{-1}) = 2929 (O-H), 1725 (C=O), 1634 (C=C); NMR ^1H (500 MHz, CDCl_3) δ : 3.64-3.68 (m, 6H), 3.55-3.58 (m, 2H), 2.67-2.70 (m, 2H), 2.60-2.63 (m, 2H), 2.54 (s, 3H), 2.51 (s, 3H), 2.36-2.30 (m, 8H), 0.99 (s, 12H); HPLC/ELSD 3.741 min (100%); MS (ES^+): m/z (%): 532.2 (100) $[\text{M}+\text{H}]^+$; HRMS (+VE DAB) calcd for $\text{C}_{28}\text{H}_{42}\text{N}_3\text{O}_7$ 532.3022 ($[\text{M}+\text{H}]^+$), mass found m/z : 532.3022

***N,N*-Bis-(2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethylamino]ethyl)maleamic acid (SP2)** $R_f = 0.76$ (DCM/MeOH 4:1); m.p. = 78° C; IR ν (cm^{-1}) = 2929 (O-H), 1714 (C=O), 1632 (C=C); ^1H NMR (500 MHz, CDCl_3) δ : 6.60 (d, $J = 12.0$ Hz, 1H), 6.11 (d, $J = 12.0$ Hz, 1H), 3.71-3.75 (m, 2H), 3.62-3.66 (m, 4H), 3.54-3.60 (m, 2H), 2.53 (s, 3H), 2.49 (s, 3H), 2.32 (s, 4H), 2.31 (s, 4H), 0.98 (s, 6H), 0.97 (s, 6H); HPLC/ELSD 3.65 min (100%); MS (ES^+): m/z (%): 530.2 (100) $[\text{M}+\text{H}]^+$; HRMS (+VE DAB) calcd for $\text{C}_{28}\text{H}_{40}\text{N}_3\text{O}_7$ 530.2866 ($[\text{M}+\text{H}]^+$), mass found m/z : 530.2867

2.2. Synthesis of SP3 and SP4

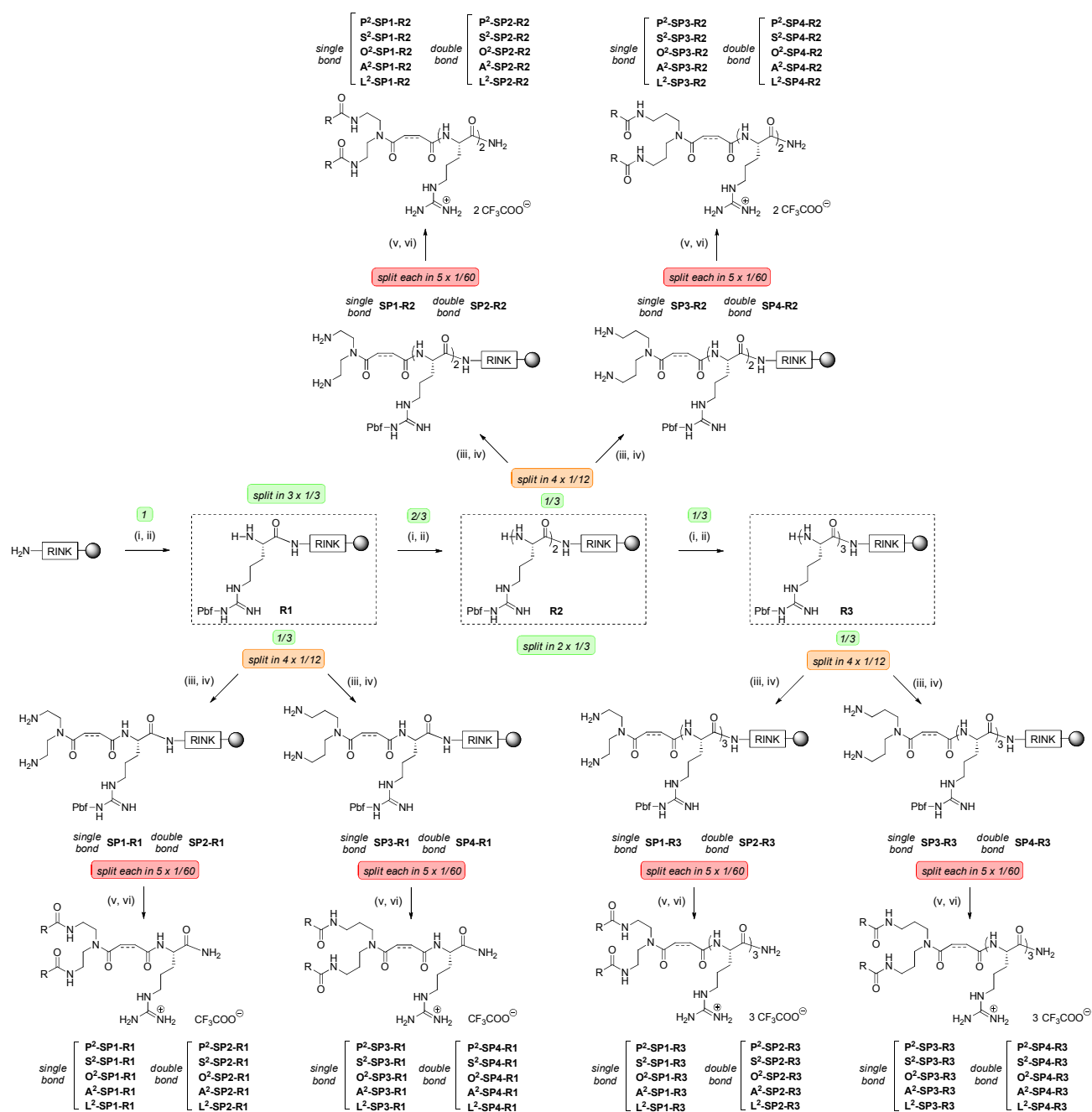
A solution of DdeOH (5g, 27 mmol) in DCM (5 mL) was added dropwise to a stirred solution of norspermidine **1b** (1.71 g, 13 mmol) in DCM (10 mL). The reaction mixture was stirred for 6 h. The progress of the reaction was monitored by TLC (DCM/MeOH 9:1). After completion, the reaction the solvent was evaporated. The crude product **2b** was dissolved in DCM (5 mL) and either succinic anhydride (1.3 g, 13 mmol) or maleic anhydride (1.29 g, 13 mmol) in DCM (5 mL) was added. The resulting mixture solution was stirred for 8 h at room temperature. The solvent was evaporated and the crude products were purified by column chromatography (DCM/MeOH 20:1 as an eluent) to afford compounds **3b** (3.6 g, 93%) or **4b** (3.5 g, 91%) as light yellow solids.

***N,N*-Bis-(2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethylamino]propyl)succinamic acid (SP3)** $R_f = 0.45$ (DCM/MeOH 9:1); m.p. 68-70° C; IR ν (cm^{-1}) = 2929 (O-H), 1725 (C=O), 1634 (C=C); ^1H -NMR (500 MHz, CDCl_3) δ : 2.63-2.67 (m, 2H), 2.53 (s, 3H), 2.49 (s, 3H), 2.32 (s, 4H), 2.31 (s, 4H), 1.85-2.05 (m, 6H), 3.34-3.44 (m, 8H), 0.98 (s, 6H), 0.97 (s, 6H); HPLC/ELSD 3.93 min (100%); MS (ES^+): m/z (%): 560.2 (100) $[\text{M}+\text{H}]^+$; HRMS (+VE DAB) calcd for $\text{C}_{30}\text{H}_{46}\text{N}_3\text{O}_7$ 560.3336 ($[\text{M}+\text{H}]^+$), mass found m/z : 560.3336.

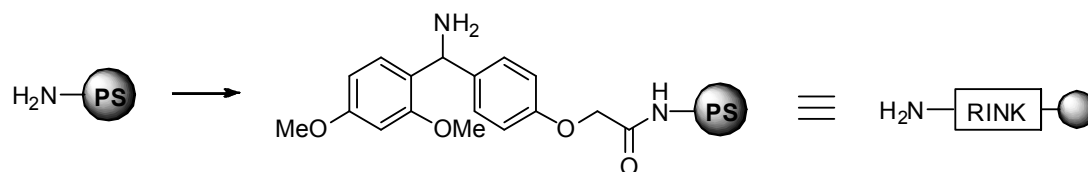
***N,N*-Bis-(2-[1-(4,4-dimethyl-2,6-dioxo-cyclohexylidene)ethylamino]propyl)maleamic acid (SP4) (4b)** $R_f = 0.4$ (DCM/MeOH 4:1); m.p. 48-50° C; IR ν (cm^{-1}) = 2956 (O-H), 1707 (C=O), 1637 (C=C); ^1H -NMR (500 MHz, CDCl_3) δ : 6.57 (d, $J = 12.0$ Hz, 1 H), 6.21 (d, $J = 12.0$ Hz, 1 H), 3.50-3.58 (m, 6H), 3.42-3.46 (m, 2H), 2.54 (s, 6H), 2.36 (d, 4H), 2.34 (d, 4H), 1.97-2.04 (m, 4H), 1.02 (s, 6H), 1.00 (s, 6H); HPLC/ELSD 3.897 min (100%); MS (ES^+): m/z (%): 558.2 (100) $[\text{M}+\text{H}]^+$; HRMS (+VE DAB) calcd for $\text{C}_{30}\text{H}_{44}\text{N}_3\text{O}_7$ 558.3179 ($[\text{M}+\text{H}]^+$), mass found m/z : 558.3175.

3. Solid-phase synthesis of arginine-containing double-tailed cationic lipids

The combinatorial strategy followed to synthesise the 60-member library is summarised below:



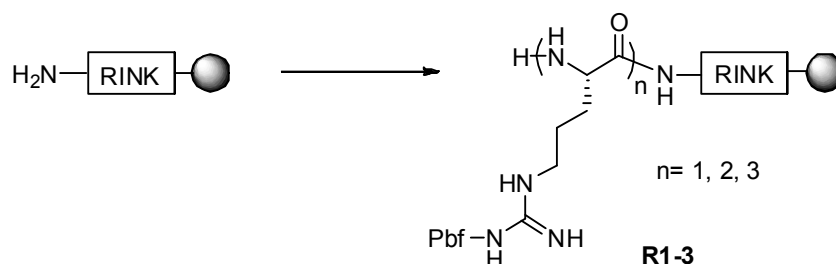
3.1. Coupling of Rink Linker to polystyrene resin



5 Fmoc-Rink-Amide Linker (3 eq., 0.3 mmol) was dissolved in DCM/DMF (2:1, 1.5 mL). DIC (3 eq., 0.3 mmol) and HOBt (3 eq., 0.3 mmol) were added and the mixture was stirred for 5 min before addition to aminomethyl polystyrene resin (loading 1.60 mmol/g, 1% DVB crosslinking, 100-200 mesh). The reaction mixture was microwave-irradiated at 60 °C for 20 min after which the resin was washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each). Next, the resin was treated with 20 % piperidine in DMF (2x 10 min) and the resin was subsequently washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each).

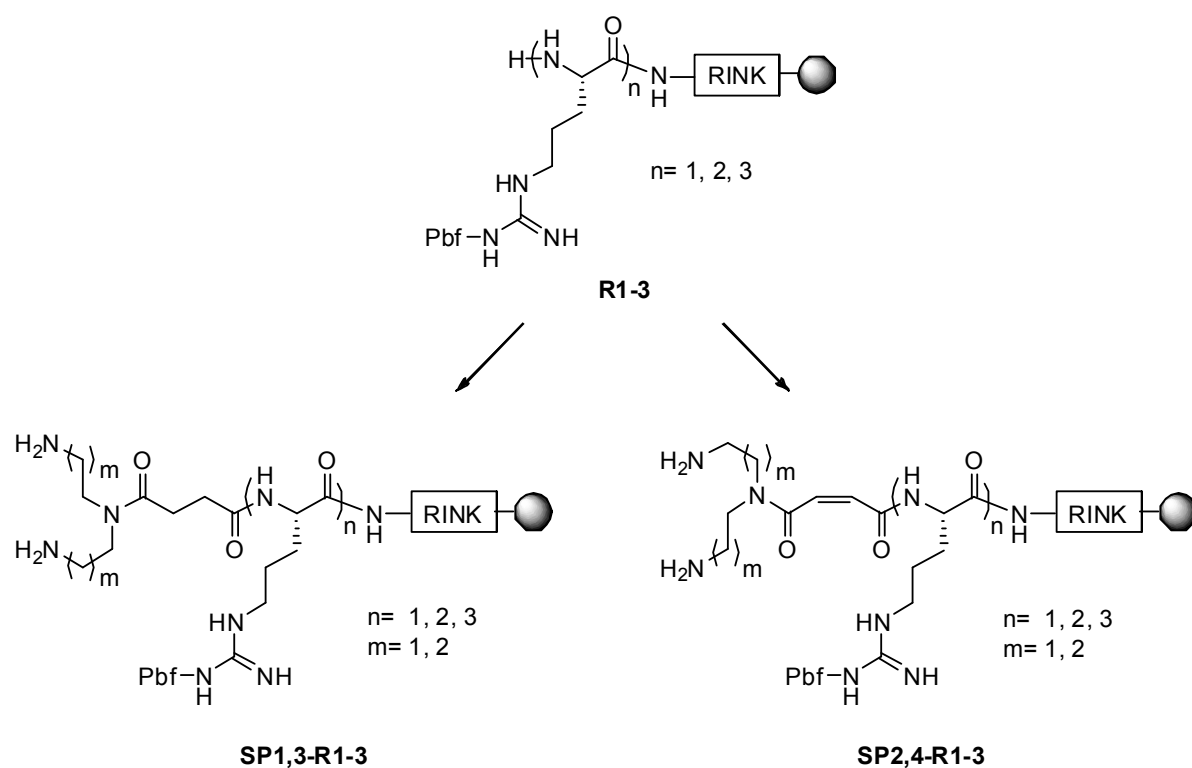
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3.2. Synthesis of arginine scaffold resins



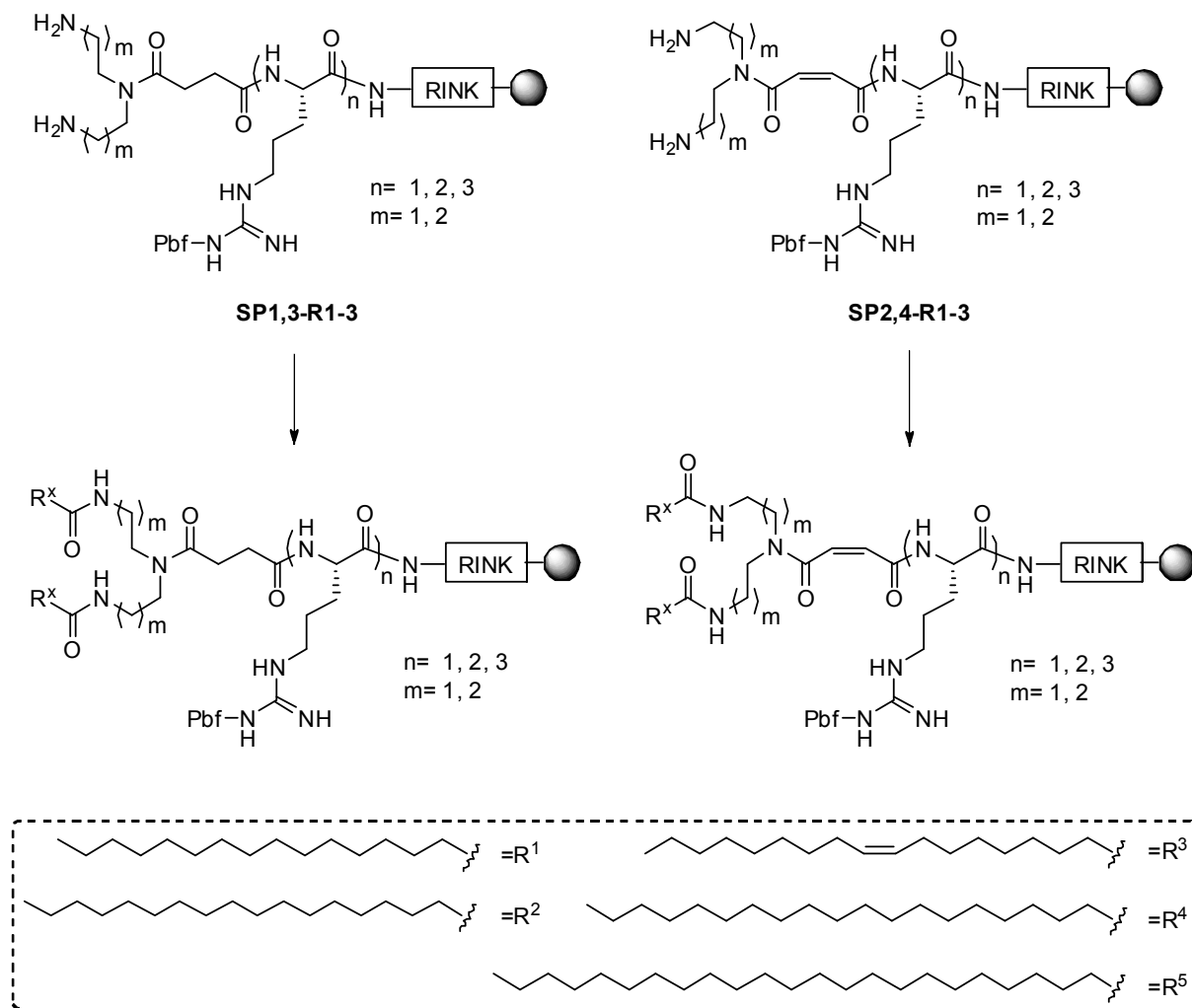
15 Mono-arginine scaffold resin (n=1) was synthesised by the coupling to the Rink resin with a solution of Fmoc-L-arginine(Pbf)-OH (3 eq., 0.3 mmol), DIC (3 eq., 0.3 mmol) and HOBt (3 eq., 0.3 mmol) in DMF/DCM (2:1, 1.5 mL). The mixture was microwave-irradiated at 60 °C for 20 min. The resulting Fmoc-protected resin was washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each) and then treated with a solution of 20 % piperidine in DMF (2 x 10 min) and washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each). Repetition of this synthetic method afforded di- and tri-arginine scaffold resins (n=2 and n=3).

3.3. Coupling of linkers to (Arg)_n scaffold resins



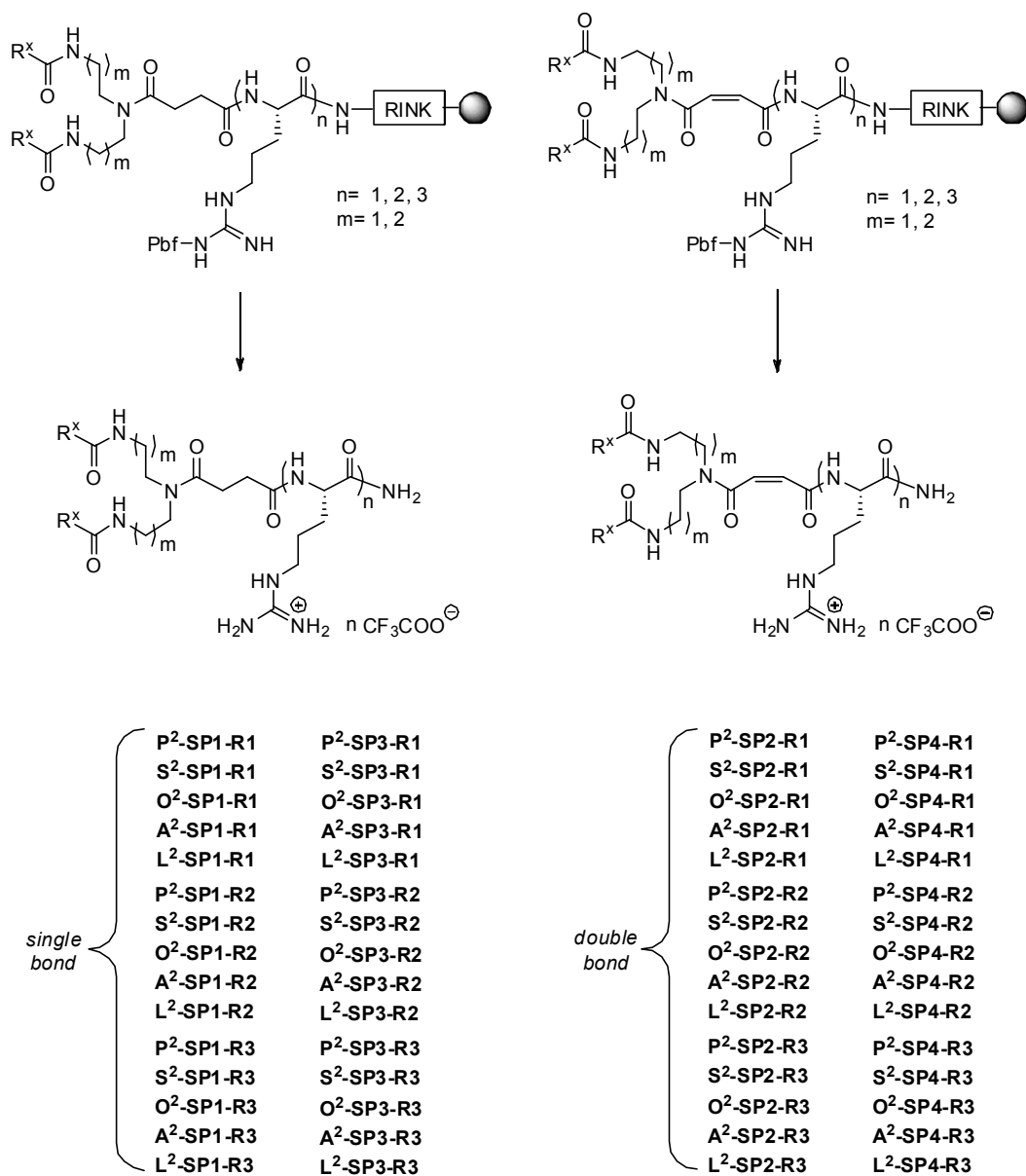
Arginine scaffold resins **R1-3** were reacted with Dde-protected polyamine linkers **SP1-4** (3 eq., 0.3 mmol) using DIC (3 eq., 0.3 mmol) and HOBt (3 eq., 0.3 mmol) in DMF/DCM (2:1, 1.5 mL). The suspensions were microwave-irradiated at 60 °C for 20 min and washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each). The resulting resins were treated with a solution of 5% hydrazine in DMF for 2 h and washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each).

3.4. Coupling of fatty acids to linker-(Arg)_n scaffold resins



- 5 Each of the polyamine resins synthesised above were split into five equal portions and reacted with a fatty acid (palmitic, stearic, oleic, arachidonic or lignoceric acid, 3 eq, 0.3 mmol) using DIC (3 eq, 0.3 mmol) and HOBT (3 eq, 0.3 mmol) in DMF/DCM (2:1, 1.5 mL). The suspensions were microwave-irradiated at 60 °C for 20 min and the resulting resins were washed with DCM, DMF, MeOH, DMF and DCM (3 x 2 mL each) and dried under vacuum.

3.5. Cleavage of cationic lipids from the resins.



- 5 The resins were pre-swollen for 15 min in DCM and filtered. A solution of TFA/TIS/H₂O (95:2.5:2.5, 2 mL) was added to the resins and the suspensions were shaken for 2 h. The solvents were removed *in vacuo*. The resulting products were redissolved in DCM (~0.5 mL) and precipitated with cold Et₂O. The resulting suspensions were centrifuged (8000 rpm for 6 min) and the solvent was removed using a pipette. If a clear precipitate was not formed immediately, the mixtures were kept at -18 °C for 1–6 h
- 10 prior to centrifugation. The desired products were further dried under vacuum for 2 h.

3.6. Synthesis of lipopeptides SP1-R2 with C14, C15 and C17 fatty acids

The lipopeptides **C14-SP1-R2**, **C15-SP1-R2** and **C17-SP1-R2** were synthesised by the same solid-phase method as described above for the exception that Oxyma was used a coupling additive instead of HOBt. The compounds were synthesised starting with 0.48 mmol of resin which was later split into three equal parts theoretically yielding 0.16 mmol of each lipopeptide.

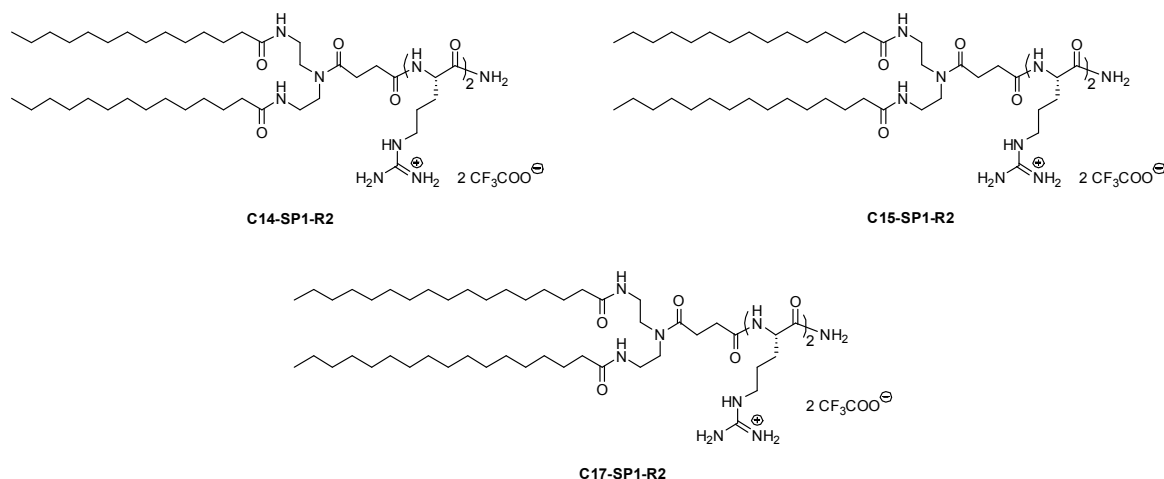


Table S1 Cationic lipids generated.

Fatty Acids		Orthogonally-Protected L-Arginine	General Formula																																																																
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C14-SP1-R2	C15-SP1-R2	C17-SP1-R2																																																																	

4. General protocol for lipoplex preparation

Cationic lipopeptides were dissolved in ethanol (1 mM) and stored at -20 °C. The corresponding quantities of cationic lipopeptide (N/P ratios 1:5 and 1:10, using 0.2 µg of DNA per experiment) were mixed at 1:1 molar ratio with DOPE (10 mg/mL in CHCl₃) in an eppendorf tube and the organic solvent removed by evaporation at 37 °C. The resultant lipid films were hydrated with 15 µL of PBS. The solutions were vortexed (10 s) and incubated at ambient temperature for 20 min. 5 µL of plasmid DNA (0.04 mg/mL in water) was subsequently added and the solution vortexed (10 s). The lipoplexes were incubated at room temperature for 20 min before being used.

5. DNA Transfection assays

5.1 Transfection of pEGFP-C1 into HeLa cells

HeLa cells were grown in RPMI supplemented with 4 mM glutamine, 10% FCS and 100 units/ml penicillin/streptomycin until 80% confluence. The cells were then suspended using trypsin/EDTA, counted, and 2×10^4 cells in 150 µL of media per well were seeded into 96 well plates and incubated overnight. The different lipoplex formulations were added to the wells. Each experiment was performed in triplicate, using Lipofectamine™ 2000 (Invitrogen) as a positive control and untreated cells as a negative control. After two days incubation at 37 °C and 5% CO₂ (the change of media after transfection was not necessary), the green fluorescent protein (GFP) expression (consequence of transfecting the pEGFP-C1) was visualised using a fluorescent microscope (Leica) and measured with a microplate reader (BioTek FLx800 microplate reader: 485/20 excitation, 530/25 emission). Hit formulations were further analysed by flow cytometry. For the analysis with BD FACSAria flow cytometer, the cells were washed twice with PBS, detached with trypsin/EDTA, harvested with 2% fetal bovine serum (FBS) in PBS, centrifuged and resuspended with 2% FBS in PBS. Transfection efficiency was measured as percentage of cells expressing EGFP. Table S2-4 shows the fluorescence analysis (microplate reader) of HeLa cells 48 h after transfection with a GFP-reporter plasmid complexed with the lipopeptides.

Hits were also analyzed using flow cytometry (results expressed as a percentage of transfected cells) and tested against the HEK293 cell line.

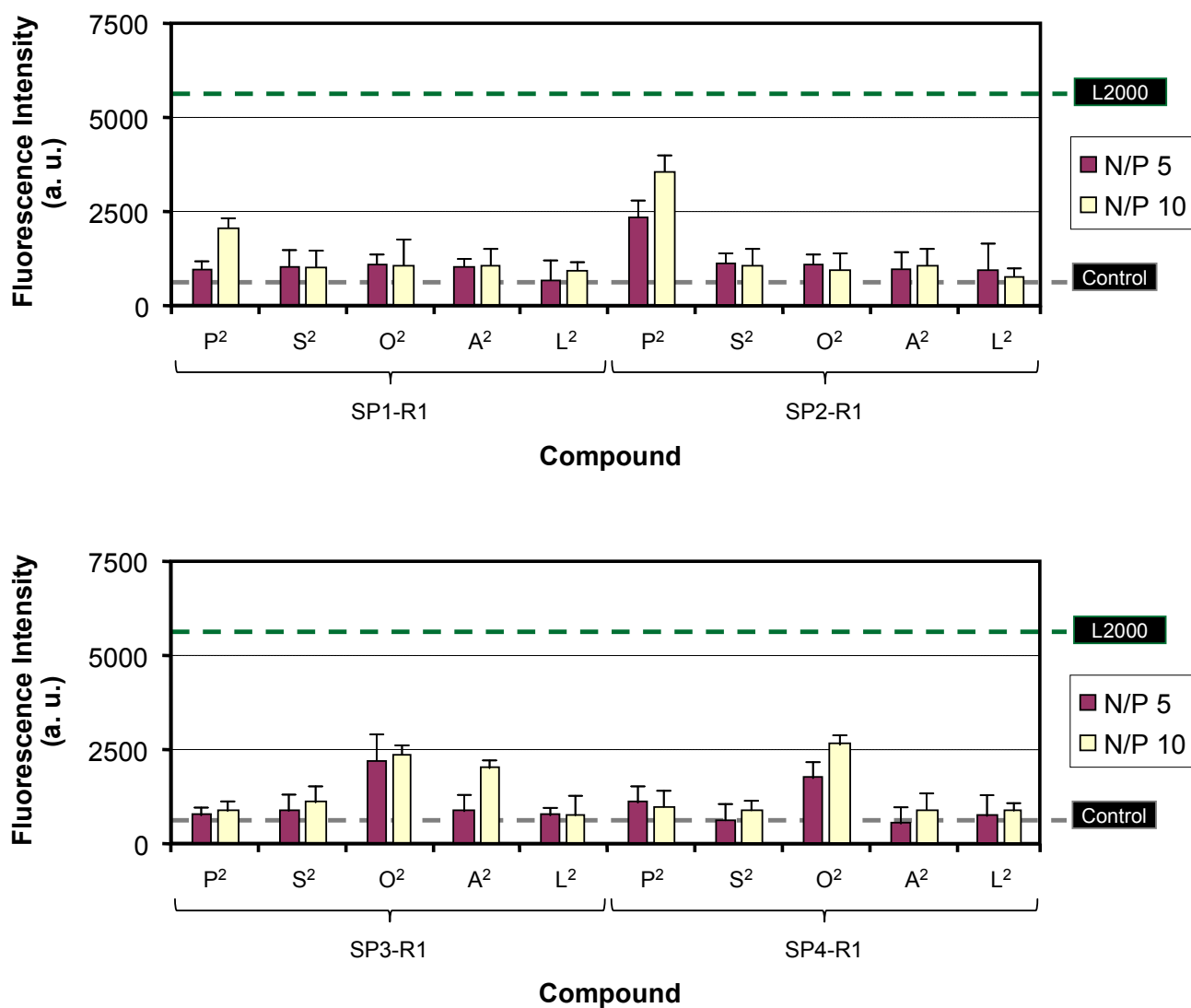


Fig S1 Transfection results with R1 lipopeptides.

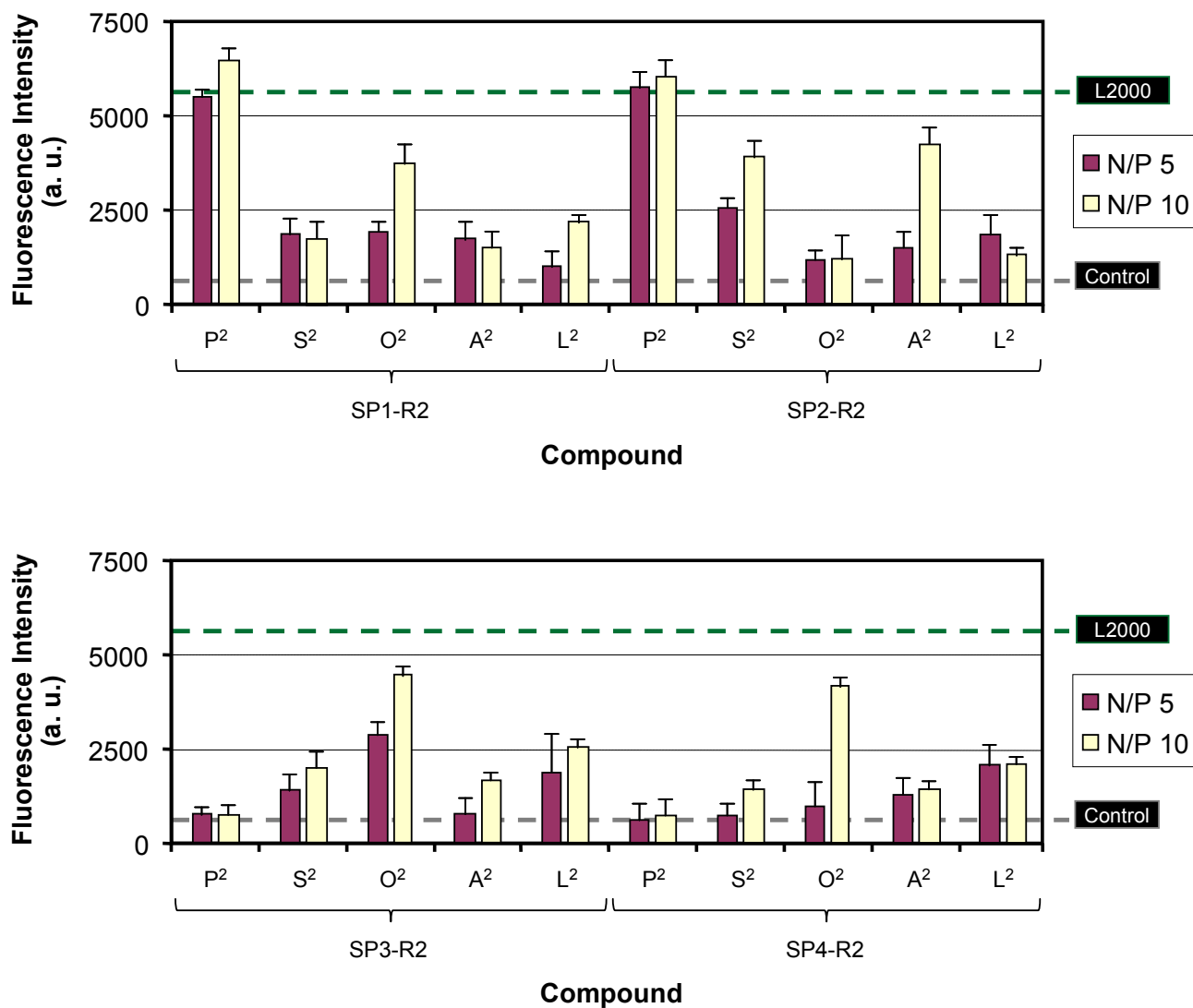


Fig S2 Transfection results with R2 lipopeptides.

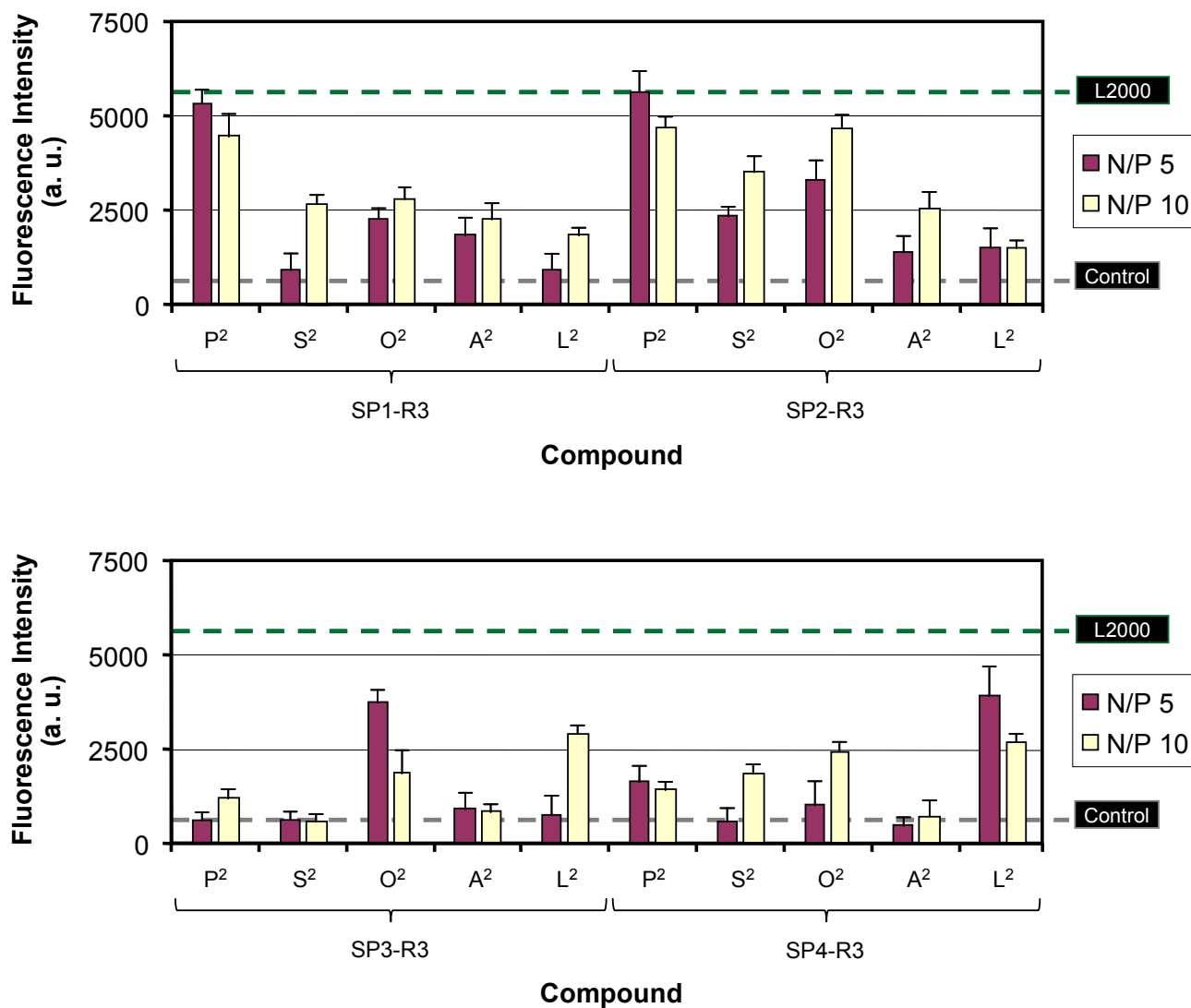


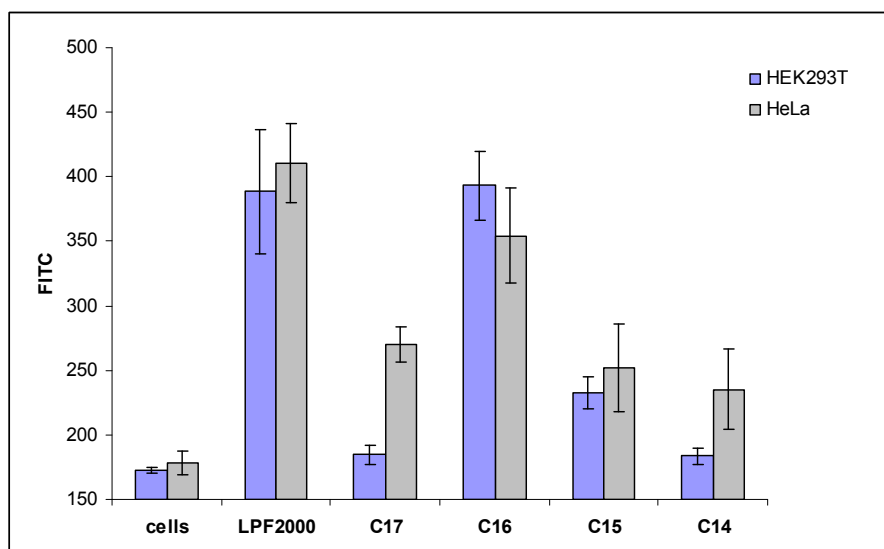
Fig S3 Transfection results with R3 lipopeptides.

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5.2 Transfection of pEGFP-C1 into HeLa and HEK293T cells with hit lipopeptides

HeLa and HEK293T cells were grown in RPMI and DMEM, respectively, supplemented with 4 mM glutamine, 10% FCS and 100units/ml penicillin/streptomycin, until 80% confluence. Experiments were performed with hit compound **P²-SP1-R2** (C16 in Fig S4) and newly synthesised derivatives **C14-SP1-R2**, **C15-SP1-R2** and **C17-SP1-R2** as described in section 5.1 and analysed by flow cytometry (Fig S4).



10 **Fig S4** Transfection results with selected compounds (y-axis= mean fluorescence in FITC channel).

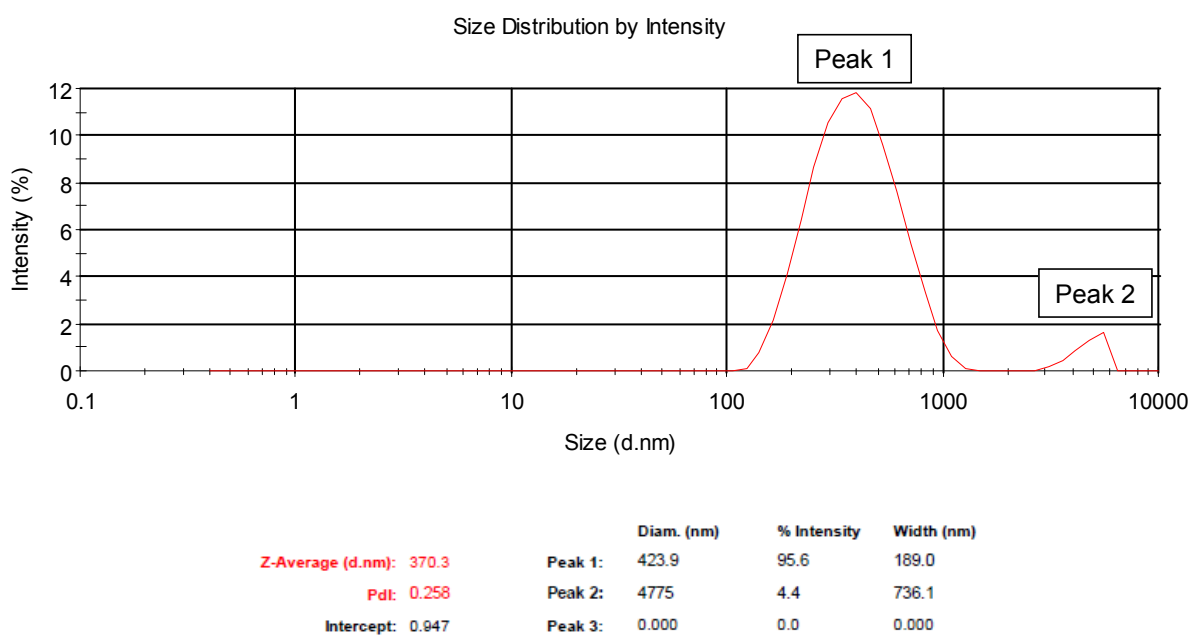
5.3. Cell viability assay of transfected cells

Twenty-four hours after the addition of transfection agents, cell viability was measured using an MTT cell proliferation assay (LGC Promochem, Middlesex, UK; absorbance was read at 570 nm), according to the manufacturer's instructions. Results indicated that none of the compounds were toxic (>90% cell viability) at the concentration used for transfection (N/P ratios 1:5 and 1:10).

6. Particle Size Measurements

Complexes of **P²-SP1-R2** / DOPE (1:1) and pEGFP-C1 at N/P ratio 10 were analysed by dynamic light scattering using a Zetasizer ZS (Malvern Ltd).

- Procedure:* Lipoplexes (**P²-SP1-R2** (N/P 10), DOPE (1:1 molar ratio), and 2 µg of pDNA in a total volume of 100 µL of PBS) were prepared as described above and then diluted with 0.9 mL of either PBS or RPMI media containing 10% foetal bovine serum. Formulations were incubated at rt for 30 min prior to analysis. Folded capillary cells (Malvern Ltd) were loaded with each formulation (1 mL) following the procedure described by the supplier and particle size distribution was determined using a Zetasizer ZS. All experiments were analysed in triplicate.
- 10 Particle size measurements in PBS (Fig S5) showed a main signal of 424 nm in diameter with a peak width of 189 nm. In the presence of serum (Fig S6), particles increased to 491 nm (peak width of 158 nm).



- 15 **Figure S5.** Representative particle size distributions of **P²-SP1-R2** / DOPE (1:1) complexed with pEGFP-C1 at N/P 10 in PBS.

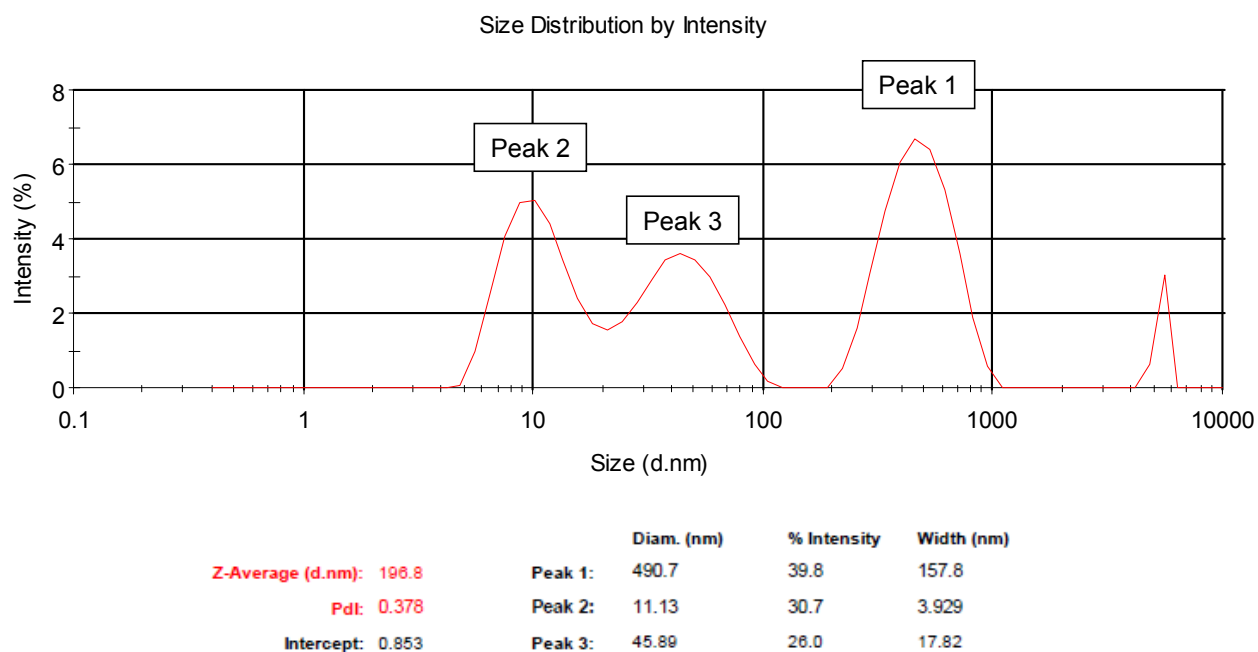
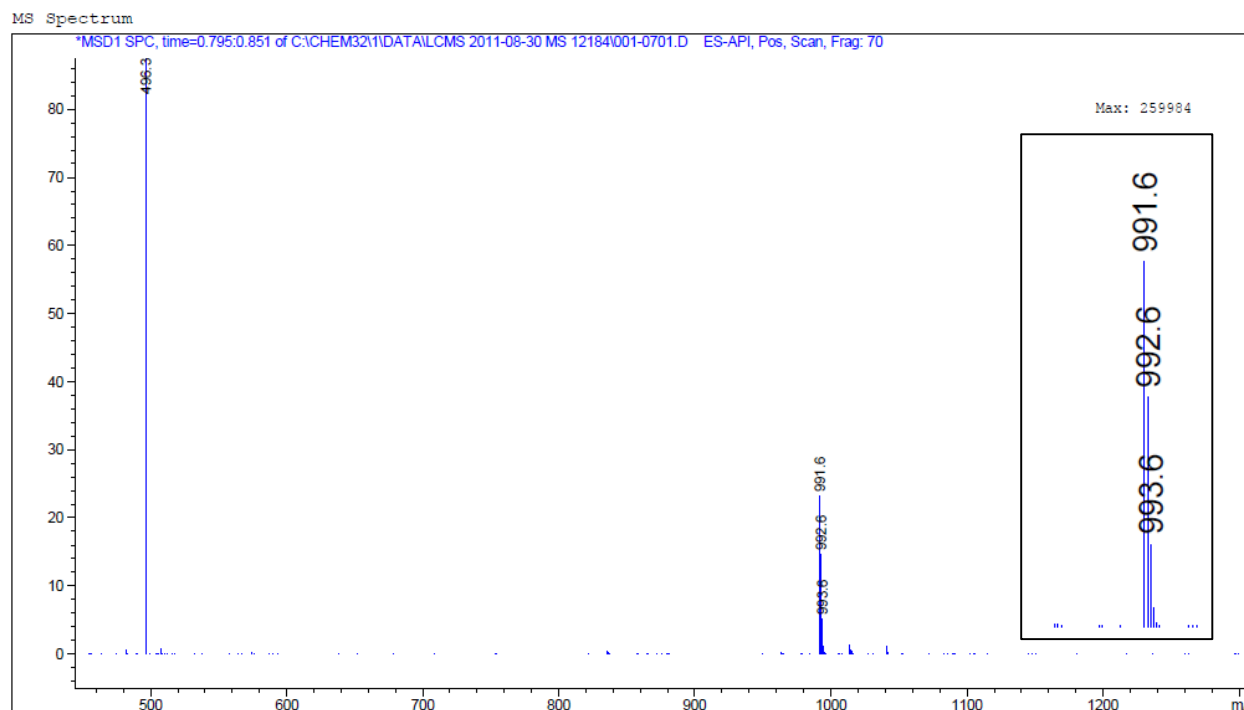


Figure S6. Representative particle size distributions of P²-SP1-R2 / DOPE (1:1) complexed with pEGFP-C1 at N/P 10 in serum-containing media. Please note that peaks 2 and 3 are signals from the serum.

7. MS spectra and HPLC trace of P²-SP1-R2



5 **Figure S7.** ESI mass spectra (positive mode) for the most active compound P²-SP1-R2.

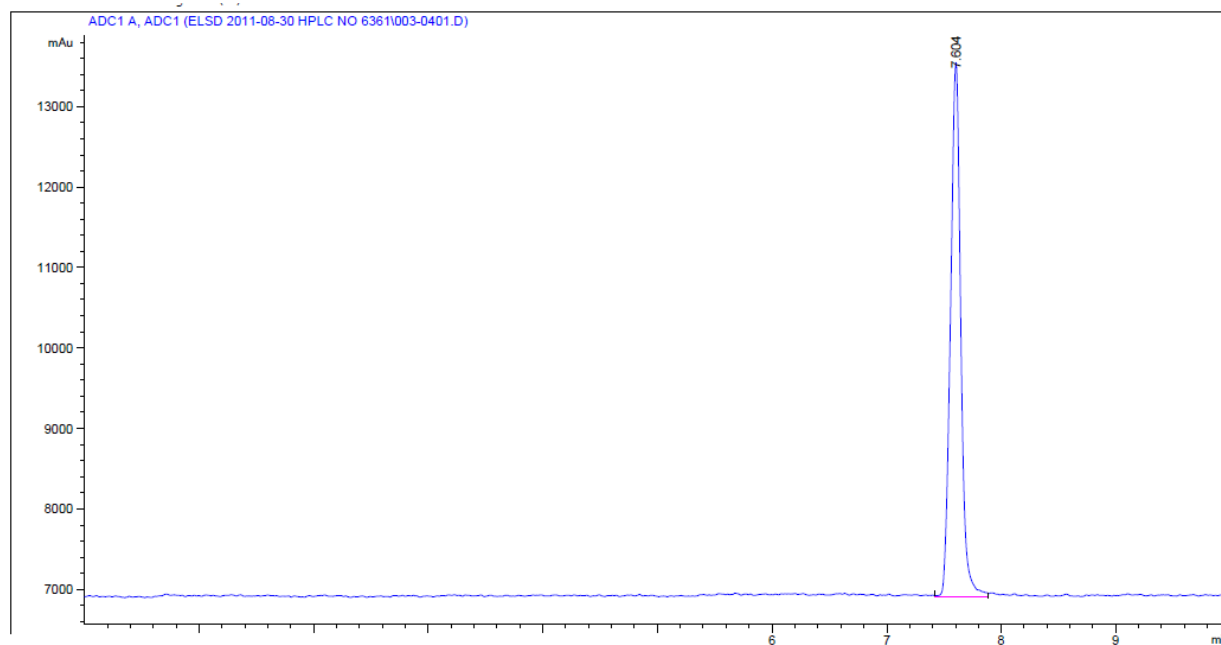
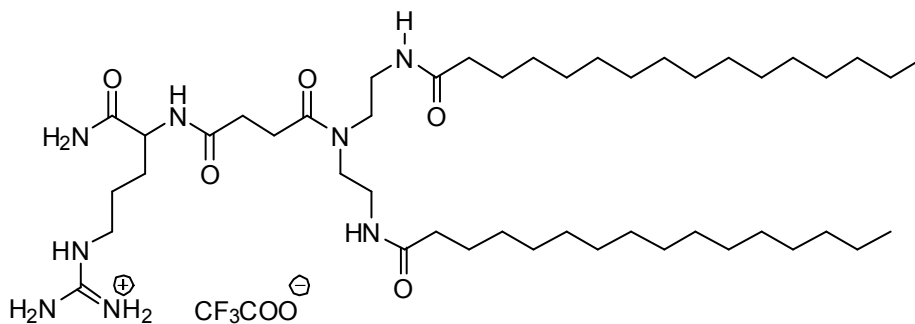


Figure S8. ELSD-HPLC chromatogram for the most active compound P²-SP1-R2.

8. Characterisation of cationic lipids.

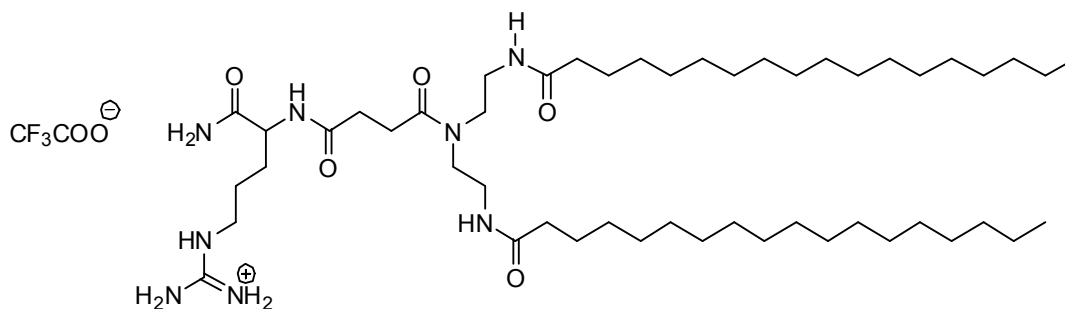
8.1. IR, H-NMR and MS characterisation of 60-member library of lipo-peptides.

4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-succinyl-L-argininamide, TFA salt (*P*²-SP1-R1)



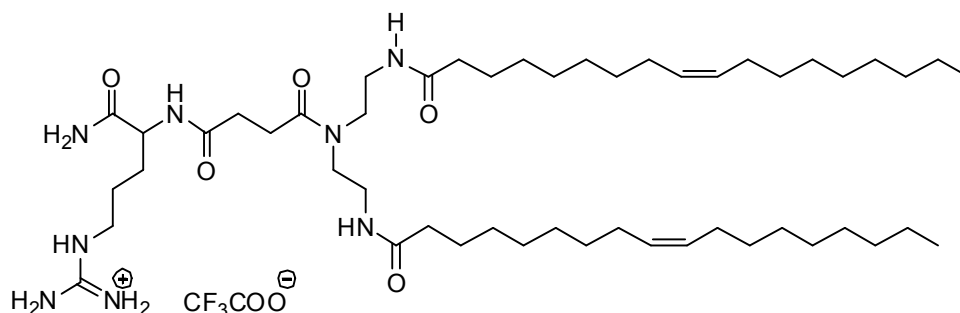
Yield: 63 mg. IR ν (cm⁻¹): 3291, 3194 (N-H), 2917 (C-H), 1627 (C=O), 1543 (N-H). ¹H NMR (360 MHz, d₆-DMSO) δ : 8.22-6.53 (m, 8H), 4.27-4.02 (m, 2H), 3.85-3.03 (m, 8H), 2.45-2.17 (m, 6H), 2.12-1.94 (m, 4H), 1.80-0.98 (m, 54H), 0.85 (t, *J* = 7 Hz, 6H); MS (ES⁺): *m/z* (%): 835.6 (100). HRMS calcd for C₄₆H₉₀N₈O₅ 835.7107 ([M+H]⁺), mass found *m/z*: 835.7083.

4-(*N,N*-Bis-[2-(stearylamo)ethyl]amino)-succinyl-L-argininamide, TFA salt (*S*²-SP1-R1)



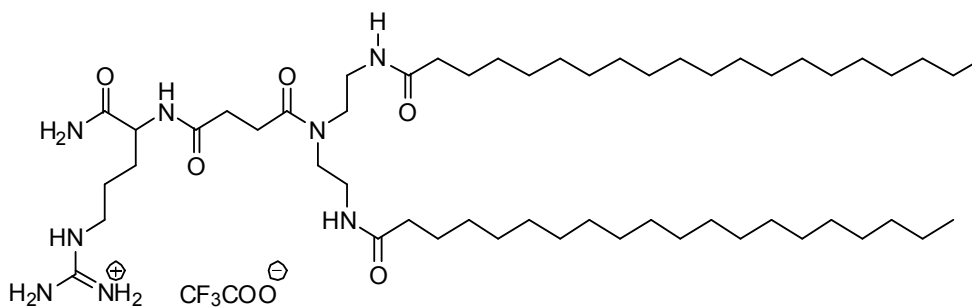
Yield: 79 mg. IR ν (cm⁻¹): 3293, 3195 (N-H), 2916 (C-H), 1639 (C=O), 1549 (N-H). ¹H NMR (360 MHz, d₆-DMSO) δ : 8.20-6.51 (m, 8H), 5.78 (s, 1H), 5.01-4.11 (m, 2H), 3.37-3.02 (m, 8H), 2.47-2.17 (m, 6H), 2.13-1.98 (m, 4H), 1.80-1.72 (m, 2H), 1.71-1.13 (m, 56H), 0.85 (t, *J* = 6.5 Hz, 6H); MS (ES⁺): *m/z* (%): 891.7 (100) [M+H]⁺. HRMS calcd for C₅₀H₉₈N₈O₅ 891.7733 ([M+H]⁺), mass found *m/z*: 891.7716.

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-succinyl-L-argininamide, TFA salt (O²-SP1-R1)



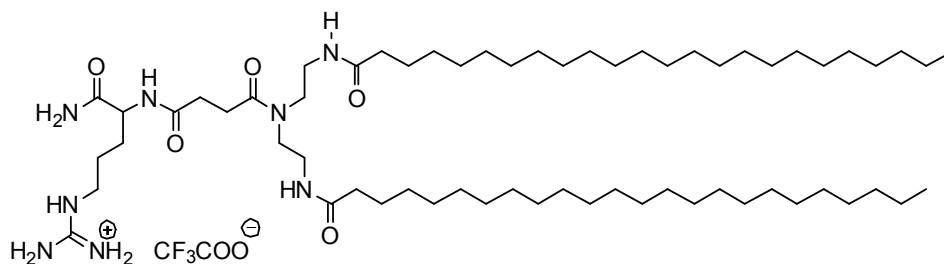
Yield: 117 mg. IR ν : 3342, 3209 (N-H), 2928 (C-H), 1627 (C=O), 1551 (N-H) cm^{-1} . ¹H NMR (360 MHz, CDCl₃) δ : 9.59-9.20 (br s, 5H), 8.08-6.26 (m, 8H), 5.34-5.20 (m, 1H), 5.03-4.91 (m, 1H), 4.39-4.19 (m, 1H), 3.64-2.98 (m, 6H), 2.73-2.39 (m, 6H), 2.23-1.79 (m, 4H), 1.70-0.92 (m, 54H), 0.91-0.73 (m, 6H); MS (ES⁺): m/z (%): 443.2 (100) [M+2H]²⁺.

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-succinyl-L-argininamide, TFA salt (A²-SP1-R1)



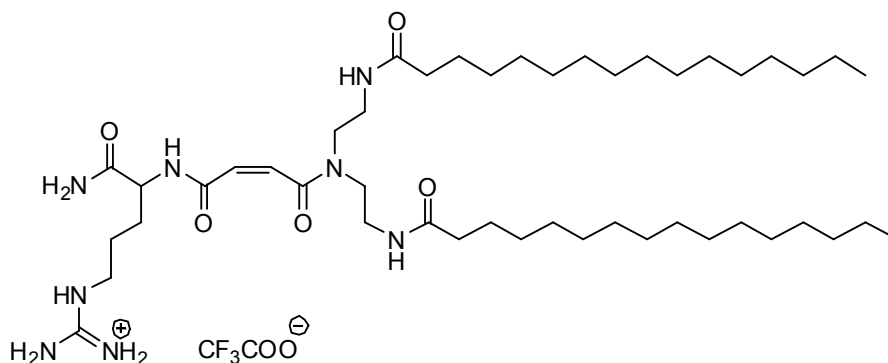
Yield: 88 mg. IR ν : 3294, 3198 (N-H), 2916 (C-H), 1638 (C=O), 1555 (N-H) cm^{-1} . ¹H NMR (360 MHz, CDCl₃) δ : 8.10-7.38 (m, 6H), 7.18-6.46 (m, 6H), 5.61-5.25 (m, 4H), 4.34-4.21 (m, 1H), 3.68-3.10 (m, 8H), 2.76-2.41 (m, 6H), 2.31-2.10 (m, 4H), 1.37-0.96 (m, 64H), 0.87 (t, $J = 6.5$ Hz, 6H); MS (ES⁺): m/z (%): 947.8 (80) [M+H]⁺. HRMS calcd for C₅₄H₁₀₆N₈O₅ 947.8358 ([M+H]⁺), mass found m/z: 947.8340.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-succinyl-L-argininamide, TFA salt (L²-SP1-R1)



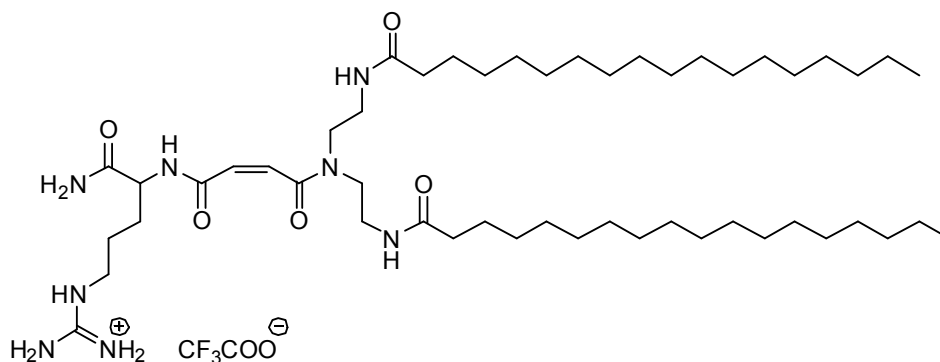
Yield: 96 mg. IR ν : 3294, 3204 (N-H), 2916 (C-H), 1640 (C=O), 1550 (N-H) cm⁻¹. ¹H NMR (500 MHz, d₆-DMSO) δ : 8.20-6.52 (m, 8H), 5.81 (s, 1H), 5.03-4.12 (m, 2H), 3.37-3.05 (m, 8H), 2.50-2.18 (m, 6H), 2.15-1.95 (m, 4H), 1.85-1.13 (m, 82H), 0.86 (t, $J = 7$ Hz, 6H); MS (ES⁺): m/z (%): 1059.9 (100) [M+H]⁺. HRMS calcd for C₆₂H₁₂₂N₈O₅ 529.9803 ([M+H]⁺), mass found m/z : 529.9814.

4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-maleyl-L-argininamide, TFA salt (P²-SP2-R1)



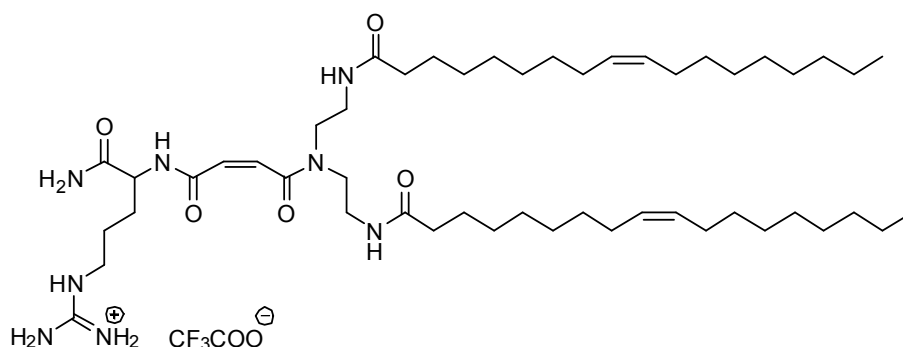
Yield: 99 mg. IR ν : 3292, 3191 (N-H), 2917 (C-H), 1633 (C=O), 1547 (N-H) cm⁻¹. ¹H NMR (360 MHz, d₆-DMSO) δ : 8.49-6.40 (m, 8H), 5.01-4.86 (m, 1H), 4.71-4.46 (m, 1H), 4.47-2.94 (m, 9H), 2.11-1.94 (m, 4H), 1.83-1.00 (br s, 56H), 0.85 (t, $J = 7$ Hz, 6H); MS (ES⁺): m/z (%): 883.7 (100) [M+H]⁺. HRMS calcd for C₄₆H₈₈N₈O₅ 416.8472 ([M+2H]²⁺), mass found m/z : 416.8460.

4-(*N,N*-Bis-[2-(stearylamido)ethyl]amino)-maleyl-L-argininamide, TFA salt (S²-SP2-R1)



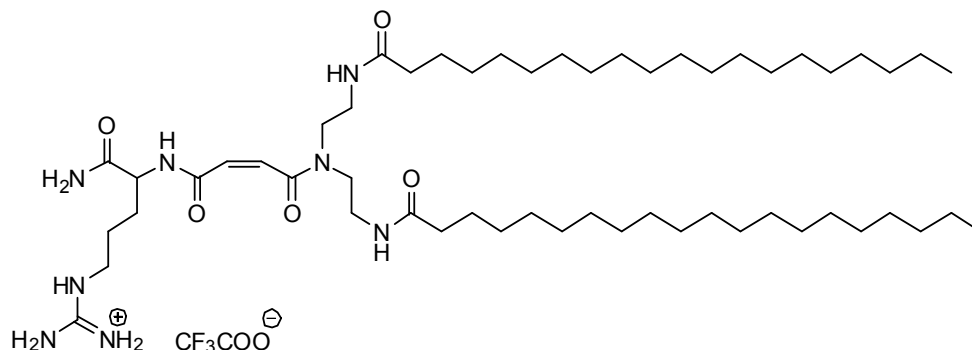
Yield: 76 mg. IR ν : 3284, 3195 (N-H), 2918 (C-H), 1635 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.45-6.60 (m, 8H), 5.92-2.96 (m, 11H), 2.37-2.24 (m, 2H), 2.14-1.93 (m, 4H), 1.83-1.00 (br s, 64H), 0.84 (t, $J = 7$ Hz, 6H); MS (ES^+): m/z (%): 889.6 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{50}\text{H}_{96}\text{N}_8\text{O}_5$ 444.8785 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 444.8796.

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-maleyl-L-argininamide, TFA salt (O²-SP2-R1)



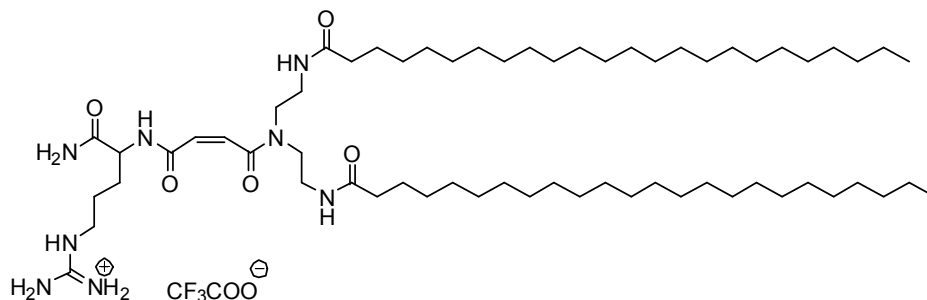
Yield: 100 mg. IR ν : 3284, 3194 (N-H), 2924 (C-H), 1633 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 8.48-6.26 (m, 12H), 5.34-5.20 (m, 1H), 5.03-4.94 (m, 1H), 4.39-4.24 (m, 1H), 3.64-2.39 (m, 12H), 2.43-0.91 (m, 60H), 0.91-0.73 (m, 6H); MS (ES^+): m/z (%): 444.2 (100) $[\text{M}+2\text{H}]^{2+}$.

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-maleyl-L-argininamide, TFA salt (A²-SP2-R1)



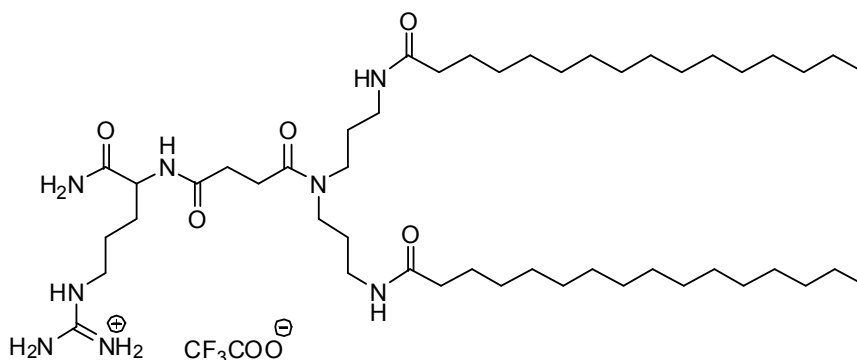
Yield: 91 mg. IR ν : 3339, 3197 (N-H), 2917 (C-H), 1638 (C=O); 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.42-6.37 (m, 9H), 5.61-2.92 (m, 8H), 2.69-2.61 (m, 2H), 2.31-2.10 (m, 2H), 2.11-1.96 (m, 4H), 1.85-0.96 (m, 72H), 0.85 (t, $J = 7$ Hz, 6H); MS (ES^+): m/z (%): 945.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-maleyl-L-argininamide, TFA salt (L²-SP2-R1)



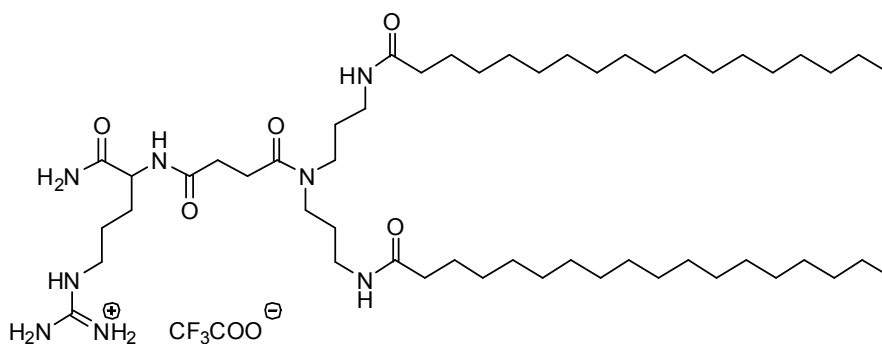
Yield: 92 mg. IR ν : 3292, 3194 (N-H), 2916 (C-H), 1635 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.68-6.51 (m, 9H), 4.74-3.79 (m, 9H), 3.18-3.06 (m, 2H), 2.11-2.03 (m, 4H), 1.80-1.64 (m, 2H), 1.62-1.37 (m, 6H), 1.27-1.01 (m, 80H), 0.85 (t, $J = 6.5$ Hz, 6H); MS (ES^+): m/z (%): 1057.8 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-succinyl-L-argininamide, TFA salt (P²-SP3-R1)



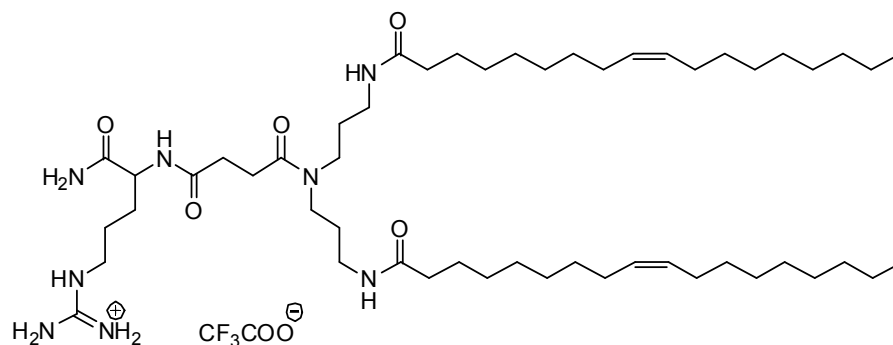
Yield: 147 mg. IR ν : 3310, 3219 (N-H), 2916 (C-H), 1637 (C=O), 1551 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.32-6.88 (m, 7H), 4.26-4.11 (m, 1H), 3.33-3.18 (m, 4H), 3.17-3.06 (m, 4H), 3.04-2.96 (m, 2H), 2.94-2.90 (m, 2H), 2.78-2.75 (m, 2H), 2.16-1.97 (m, 9H), 1.80-1.62 (m, 2H), 1.61-1.40 (m, 6H), 1.35-1.17 (m, 52H), 0.92-0.84 (m, 6H); MS (ES^+): m/z (%): 863.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{48}\text{H}_{94}\text{N}_8\text{O}_5$ 863.7420 ($[\text{M}+\text{H}]^+$), mass found m/z : 863.7427.

4-(*N,N*-Bis-[2-(stearyl)amido]propyl]amino)-succinyl-L-argininamide, TFA salt (S²-SP3-R1)



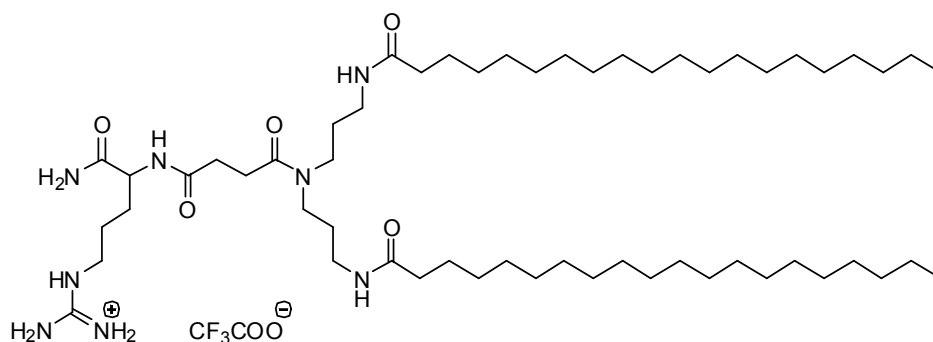
Yield: 73 mg. IR ν : 3309, 3196 (N-H), 2916 (C-H), 1637 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.25-6.91 (m, 7H), 5.08-3.73 (m, 5H), 3.29-3.17 (m, 4H), 3.01-2.95 (m, 2H), 2.92-2.89 (m, 2H), 2.72-2.59 (m, 2H), 2.44-2.29 (m, 4H), 2.11-1.99 (m, 5H), 1.81-1.62 (m, 4H), 1.60-1.43 (m, 4H), 1.34-1.16 (m, 56H), 0.91-0.84 (m, 6H); MS (ES^+): m/z (%): 919.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{52}\text{H}_{102}\text{N}_8\text{O}_5$ 919.8046 ($[\text{M}+\text{H}]^+$), mass found m/z : 919.8011.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-succinyl-L-argininamide, TFA salt (*O*²-SP3-R1)



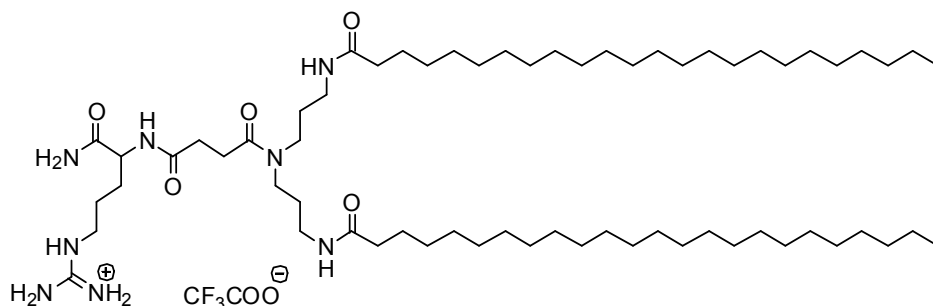
Yield: 100 mg. IR ν : 3343, 3202 (N-H), 2926 (C-H), 1623 (C=O), 1549 (N-H) cm^{-1} . ¹H NMR (360 MHz, *d*₆-DMSO) δ : 9.40-8.91 (br s, 2H), 8.10-6.02 (m, 5H), 5.29-5.20 (m, 4H), 5.01-4.88 (m, 1H), 4.32-4.13 (m, 4H), 3.43-2.75 (m, 4H), 2.69-2.37 (m, 6H), 2.20-1.94 (m, 12H), 1.84-0.87 (m, 52H), 0.86-0.64 (m, 6H); MS (ES⁺): *m/z* (%): 915.8 (100) [M+H]⁺. HRMS calcd for C₅₂H₉₈N₈O₅ 915.7733 ([M+H]⁺), mass found *m/z*: 915.7731.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-succinyl-L-argininamide, TFA salt (*A*²-SP3-R1)



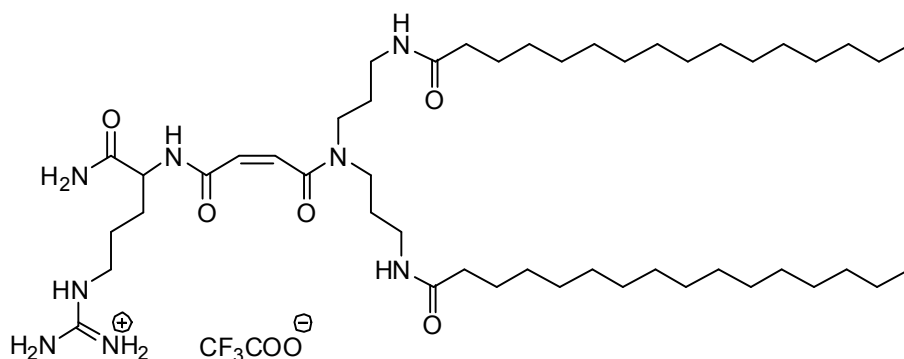
Yield: 80 mg. IR ν : 3308, 3206 (N-H), 2917 (C-H), 1637 (C=O), 1551 (N-H) cm^{-1} . ¹H NMR (360 MHz, *d*₆-DMSO) δ : 8.15-8.14 (m, 9H), 4.45-4.32 (m, 1H), 3.51-3.17 (m, 6H), 3.12-3.09 (m, 4H), 3.03-3.00 (m, 2H), 2.24-2.23 (m, 2H), 2.19-2.17 (m, 8H), 1.68-1.58 (m, 2H), 1.53-1.49 (m, 6H), 1.37-1.20 (m, 70H), 0.95-0.87 (m, 6H); MS (ES⁺): *m/z* (%): 975.7 (100) [M+H]⁺. HRMS calcd for C₅₆H₁₁₀N₈O₅ 975.8672 ([M+H]⁺), mass found *m/z*: 975.8655.

4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-succinyl-L-argininamide, TFA salt (L²-SP3-R1)



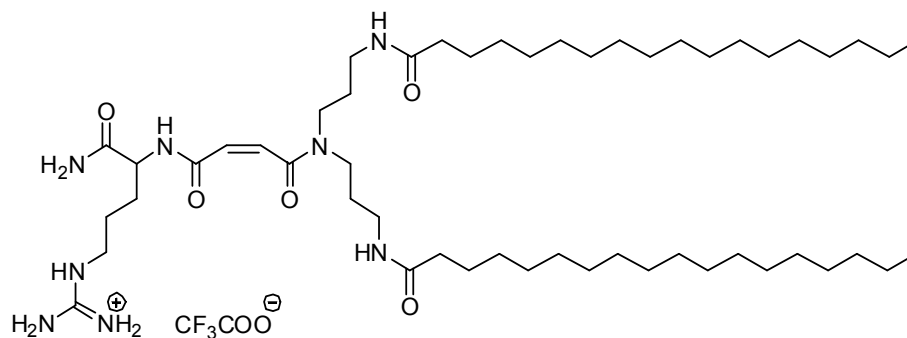
Yield: 77 mg. IR ν : 3309, 3204 (N-H), 2916 (C-H), 1637 (C=O), 1557 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.15-8.14 (m, 2H), 4.45-4.32 (m, 1H), 3.51-3.17 (m, 4H), 3.12-3.09 (m, 4H), 3.03-3.00 (m, 2H), 2.24-2.23 (m, 2H), 2.19-2.17 (m, 8H), 1.68-1.58 (m, 2H), 1.53-1.49 (m, 6H), 1.37-1.20 (m, 64H), 0.95-0.87 (m, 6H); MS (ES^+): m/z (%): 1087.9 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{64}\text{H}_{126}\text{N}_8\text{O}_5$ 362.66377 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 362.66429.

4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-maleyl-L-argininamide, TFA salt (P²-SP4-R1)



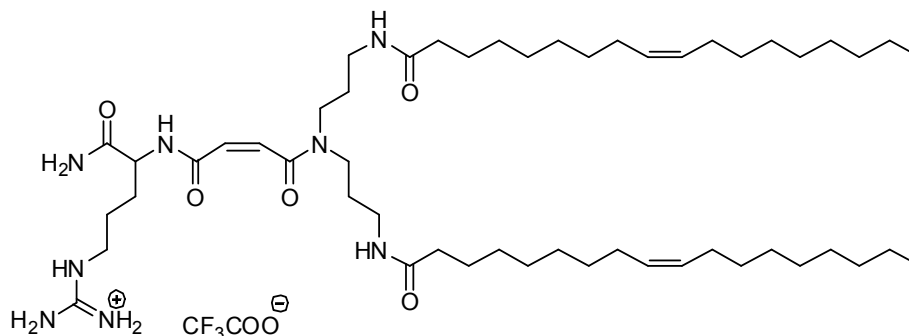
Yield: 142 mg. ν 3309, 3203 (N-H), 2917 (C-H), 1638 (C=O), 1553 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.38-8.99 (m, 2H), 8.0-7.95 (m, 3H), 7.20-7.03 (m, 4H), 4.38-4.16 (m, 5H), 3.29-2.92 (m, 4H), 2.88-2.85 (m, 2H), 2.83-2.79 (m, 12H), 2.32-1.95 (m, 2H), 1.54-1.41 (m, 8H), 1.24-1.07 (m, 52H), 0.79 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 861.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{48}\text{H}_{92}\text{N}_8\text{O}_5$ 861.72634 ($[\text{M}+\text{H}]^+$), mass found m/z : 861.72490.

4-(*N,N*-Bis-[2-(stearyl-amido)propyl]amino)-maleyl-L-argininamide, TFA salt (*S*²-SP4-R1)



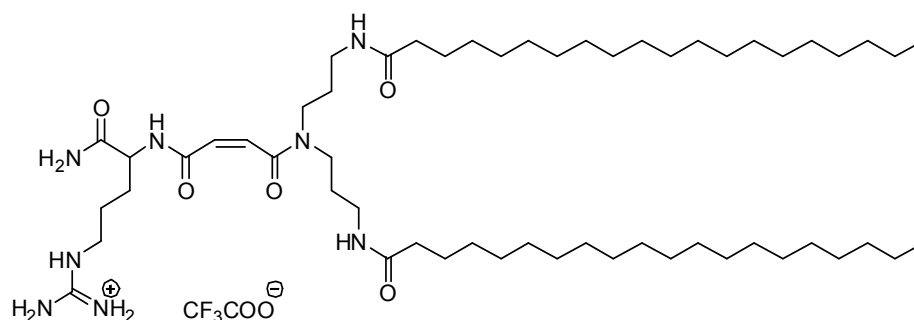
Yield: 135 mg. IR ν : 3309, 3199 (N-H), 2917 (C-H), 1636 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.23-6.32 (m, 9H), 4.48-4.35 (m, 5H), 3.48-3.00 (m, 4H), 2.97-2.92 (m, 2H), 2.36-2.06 (m, 4H), 1.68-1.54 (m, 8H), 1.53-1.45 (m, 4H), 1.41-1.07 (m, 56H), 0.93 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 917.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{52}\text{H}_{100}\text{N}_8\text{O}_5$ 917.78894 ($[\text{M}+\text{H}]^+$), mass found m/z : 917.7874.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-maleyl-L-argininamide, TFA salt (*O*²-SP4-R1)



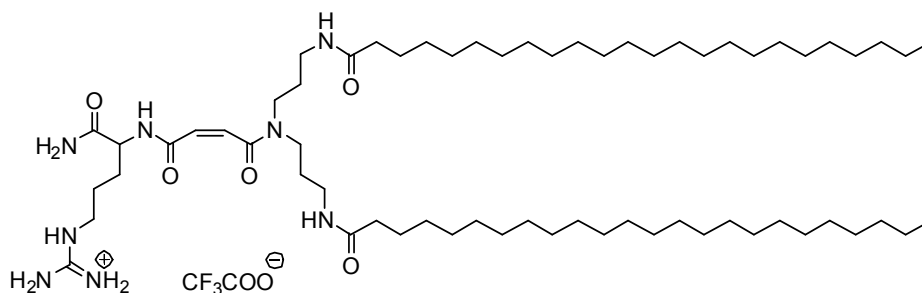
Yield: 112 mg. IR ν : 3293, 3208 (N-H), 2925 (C-H), 1625 (C=O), 1553 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.31-8.89 (br s, 2H), 8.19-6.39 (m, 6H), 5.43-5.31 (m, 4H), 4.45-4.31 (m, 1H), 3.48-2.85 (m, 10H), 2.35-1.91 (m, 12H), 1.84-0.99 (m, 52H), 0.97-0.79 (m, 6H); MS (ES^+): m/z (%): 457.5 (100) $[\text{M}+2\text{H}]^{2+}$.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-maleyl-L-argininamide, TFA salt (*A*²-SP4-R1)



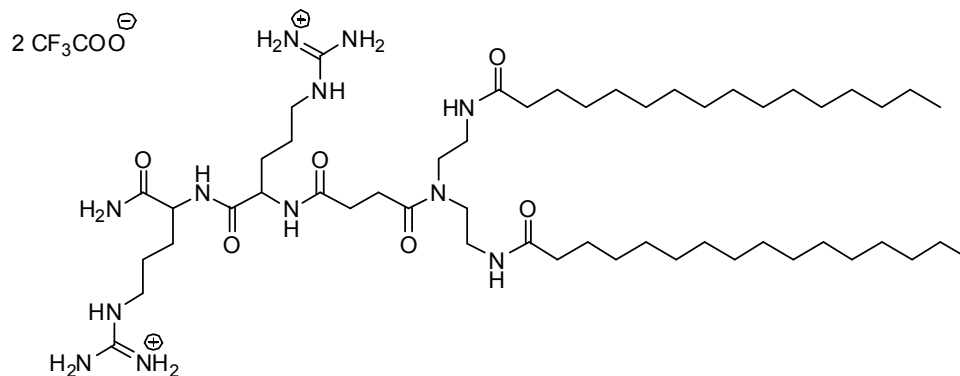
Yield: 114 mg. IR ν : 3307, 3201 (N-H), 2916 (C-H), 1638 (C=O), 1551 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.01-8.70 (br s 2H), 6.53-6.51 (m, 2H), 6.80-6.54 (m, 2H), 3.52-3.13 (5H), 2.96-2.92 (m, 4H), 2.37-2.21 (m, 2H), 2.14-2.09 (m, 4H), 1.86 (m, 4H), 1.92-1.70 (m, 2H), 1.65 (m, 2H), 1.68-1.55 (m, 4H), 1.39-1.19 (m, 64H), 0.92 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 973.8 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{56}\text{H}_{108}\text{N}_8\text{O}_5$ 486.92550 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 486.92347.

4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-maleyl-L-argininamide, TFA salt (*L*²-SP4-R1)



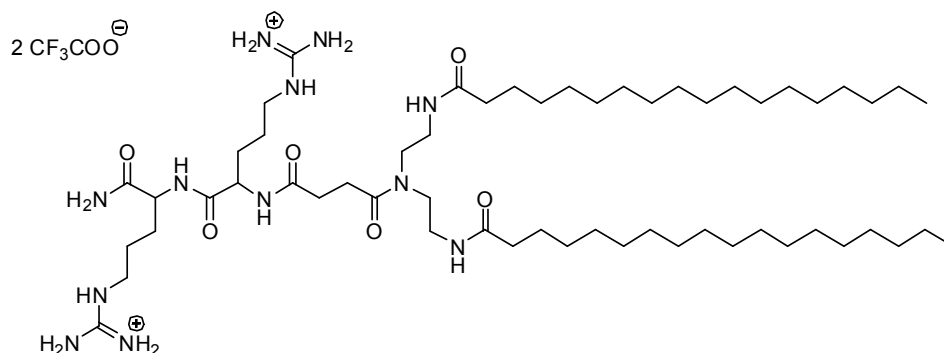
Yield: 175 mg. IR ν : 3309 (N-H), 2916 (C-H), 1637 (C=O), 1553 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.63-6.92 (m, 9H), 3.43-3.33 (m, 5H), 2.91-2.84 (m, 6H), 2.11-2.06 (m, 10H), 1.96-1.94 (m, 2H), 1.39-1.37 (m, 4H), 1.28-1.15 (m, 80H), 1.01-0.95 (m, 6H); MS (ES^+): m/z (%): 1085.7 (100) $[\text{M}+\text{H}]^+$.

**4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt
(P²-SP1-R2)**



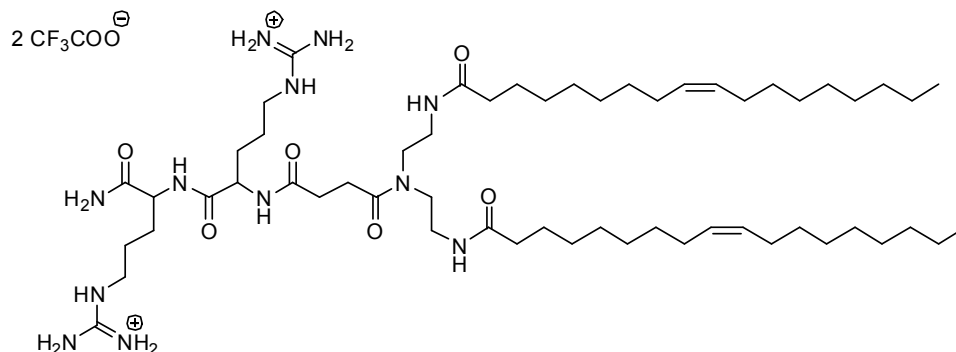
Yield: 148 mg. IR ν : 3287, 3193 (N-H), 2922 (C-H), 1630 (C=O), 1542 (N-H) cm⁻¹. ¹H NMR (360 MHz, CDCl₃) δ : 9.02-6.36 (m, 10H), 4.51-4.20 (m, 2H), 3.69-3.03 (m, 12H), 2.30-2.08 (m, 8H), 1.67-1.43 (m, 12H), 1.41-1.03 (m, 48H), 0.99-0.81 (m, 6H); MS (ES⁺): m/z (%): 496.5 (100) [M+2H]²⁺. HRMS calcd for C₅₂H₁₀₂N₁₂O₆ 991.8118 ([M+H]⁺), mass found m/z: 991.8098.

**4-(*N,N*-Bis-[2-(stearyl)amido]ethyl)amino)-succinyl-L-arginyl-L-argininamide, TFA salt
(S²-SP1-R2)**



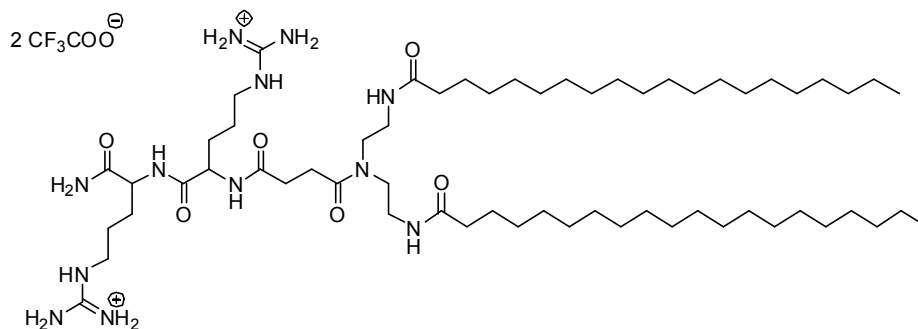
Yield: 75 mg. IR ν : 3291, 3192 (N-H), 2916 (C-H), 1628 (C=O), 1541 (N-H) cm⁻¹. ¹H NMR (360 MHz, d₆-DMSO) δ : 8.29-6.47 (m, 10H), 4.25-4.12 (m, 2H), 3.36-3.02 (m, 14H), 1.81-1.69 (m, 10H), 1.60-1.41 (m, 8H), 1.34-1.20 (m, 56H), 0.92-0.82 (m, 6H); MS (ES⁺): m/z (%): 524.5 (100) [M+2H]²⁺. HRMS calcd for C₅₆H₁₁₀N₁₂O₆ 1047.8744 ([M+H]⁺), mass found m/z: 1047.8740.

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (O²-SP1-R2)



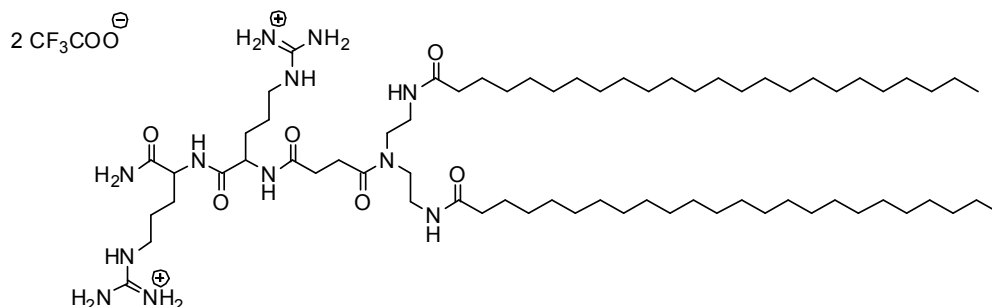
Yield: 119 mg. IR ν : 3338, 3197 (N-H), 2925 (C-H), 1628 (C=O); δ : 1547 (N-H) cm⁻¹. ¹H NMR (360 MHz, CDCl₃) δ : 9.42-8.90 (br s, 4H), 8.28-6.22 (m, 6H), 5.44-5.31 (m, 4H), 5.18-5.01 (m, 2H), 4.45-4.18 (m, 4H), 3.91-3.02 (m, 6H), 1.83-1.02 (m, 52H), 1.00-0.79 (m, 6H); MS (ES⁺): m/z (%): 522.5 (100) [M+2H]²⁺. HRMS calcd for C₅₆H₁₀₆N₁₂O₆ 521.9213 ([M+2H]²⁺), mass found m/z: 521.9182

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (A²-SP1-R2)



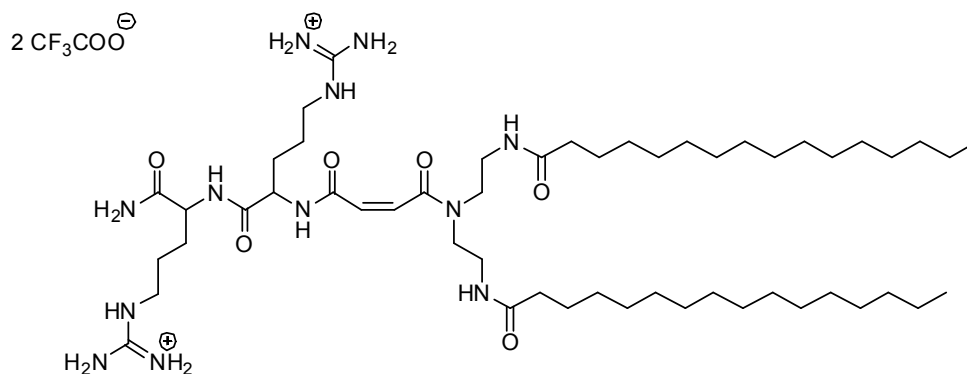
Yield: 74 mg. IR ν : 3352, 3202 (N-H), 2916 (C-H), 1628 (C=O), 1540 (N-H) cm⁻¹. ¹H NMR (500 MHz, d₆-DMSO) δ : 8.35-6.68 (m, 10H), 5.81-5.73 (m, 2H), 4.87-3.87 (m, 8H), 3.41-2.96 (m, 12H), 2.10-1.97 (m, 2H), 1.85-0.95 (m, 76H), 0.94-0.76 (m, 6H); MS (ES⁺): m/z (%): 552.5 (100) [M+2H]²⁺. HRMS calcd for C₆₀H₁₁₈N₁₂O₆ 1103.9370 ([M+H]⁺), mass found m/z: 1103.9338.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (L²-SP1-R2)



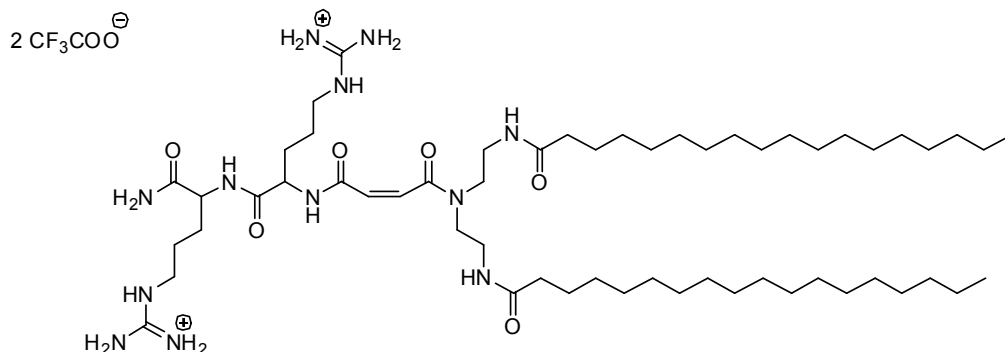
Yield: 116 mg. IR ν : 3352, 3206 (N-H), 2916 (C-H), 1628 (C=O), 1545 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.56 (m, 4H), 8.43 (m, 4H), 8.36-6.92 (m, 10H), 4.38-4.09 (m, 2H), 3.97-3.83 (m, 4H), 3.79-3.67 (m, 4H), 3.50-3.43 (m, 4H), 3.36-3.23 (m, 2H), 3.16-3.02 (m, 2H), 2.45-2.31 (m, 4H), 2.18-1.96 (m, 2H), 1.83-1.33 (m, 12H), 1.36-1.10 (m, 80H), 0.92-0.79 (m, 6H); MS (ES^+): m/z (%): 608.7 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{68}\text{H}_{134}\text{N}_{12}\text{O}_6$ 608.0308 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 608.0308.

4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (P²-SP2-R2)



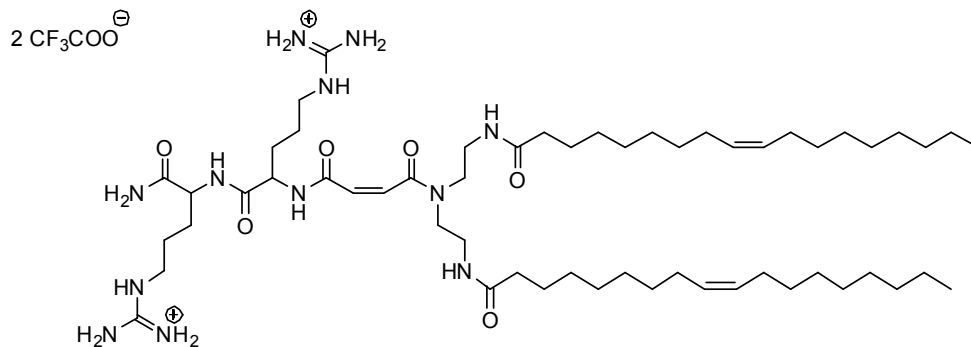
Yield: 66 mg. IR ν : 3293, 3195 (N-H), 2919 (C-H), 1634 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.46-6.52 (m, 12H), 4.22-4.11 (m, 2H), 3.57-3.45 (m, 8H), 3.16-3.00 (m, 8H), 1.87-1.63 (m, 4H), 1.57-1.37 (m, 8H), 1.33-1.14 (m, 48H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 495.5 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{52}\text{H}_{100}\text{N}_{12}\text{O}_6$ 989.7962 ($[\text{M}+\text{H}]^+$), mass found m/z : 989.7950.

4-(*N,N*-Bis-[2-(stearyl-amido)ethyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (S²-SP2-R2)



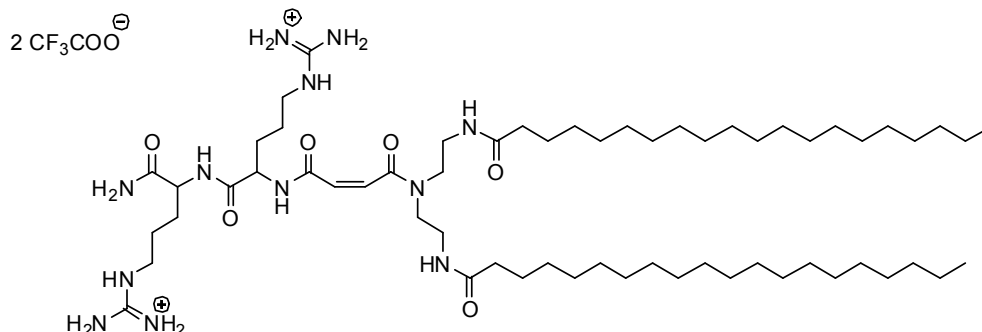
Yield: 131 mg. IR ν : 3338, 3197 (N-H), 2917 (C-H), 1633 (C=O), 1546 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.50-6.58 (m, 12H), 4.24-4.11 (m, 2H), 3.64-3.41 (m, 4H), 3.34-2.96 (m, 4H), 2.77-2.55 (m, 4H), 2.39-2.25 (m, 4H), 1.85-1.66 (m, 4H), 1.60-1.36 (m, 8H), 1.33-1.11 (m, 56H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 523.5 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{56}\text{H}_{108}\text{N}_{12}\text{O}_6$ 1045.8588 ($[\text{M}+\text{H}]^+$), mass found m/z : 1045.8577.

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (O²-SP2-R2)



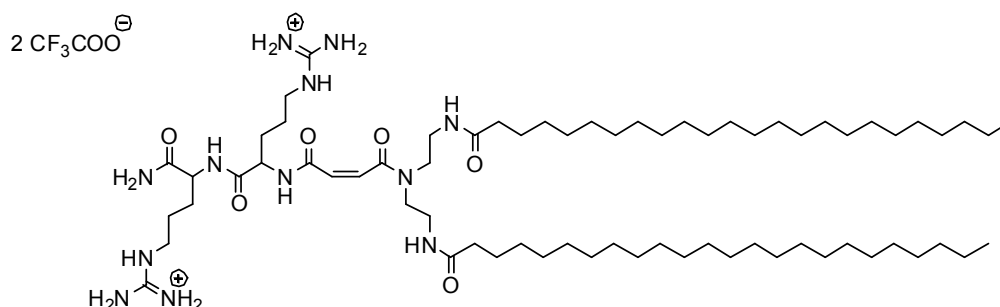
Yield: 134 mg. IR ν : 3340, 3202 (N-H), 2925 (C-H), 1633 (C=O), 1555 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.39-8.65 (br s, 4H), 8.12-6.05 (m, 8H), 5.34-4.83 (m, 4H), 4.44-3.99 (m, 2H), 3.84-3.45 (m, 8H), 3.01-2.90 (m, 4H), 2.17-1.73 (m, 16H), 1.65-0.85 (m, 48H), 0.83-0.65 (m, 6H); MS (ES^+): m/z (%): 1041.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{56}\text{H}_{104}\text{N}_{12}\text{O}_6$ 1041.8275 ($[\text{M}+\text{H}]^+$), mass found m/z : 1041.8262.

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (A²-SP2-R2)



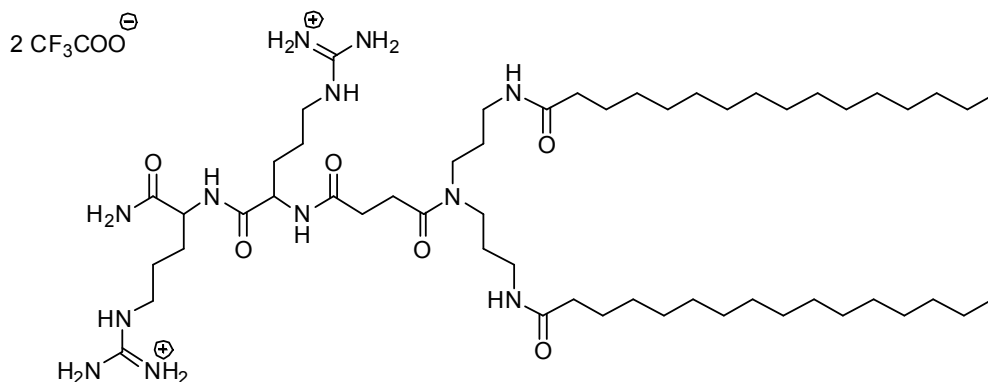
Yield: 92 mg. IR ν : 3343, 3202 (N-H), 2917 (C-H), 1632 (C=O), 1546 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.43-6.65 (m, 12H), 5.15-4.51 (m, 10H), 3.18-3.02 (m, 4H), 2.13-2.01 (m, 4H), 1.60-1.40 (m, 12H), 1.38-1.13 (m, 64H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 551.5 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{60}\text{H}_{116}\text{N}_{12}\text{O}_6$ 1101.9214 ($[\text{M}+\text{H}]^+$), mass found m/z : 1101.9230.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (L²-SP2-R2)



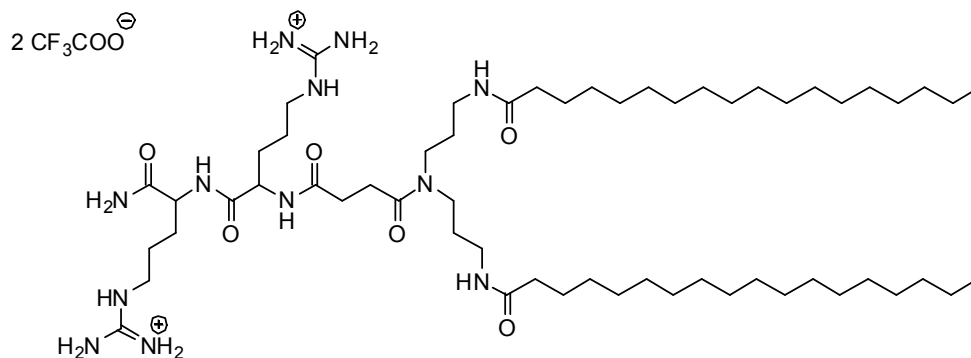
Yield: 118 mg. IR ν : 3352, 3209 (N-H), 2918 (C-H), 1636 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.64-6.75 (m, 12H), 5.11-4.53 (m, 2H), 6.18-5.07 (m, 6H), 4.25-4.10 (m, 4H), 3.17-3.00 (m, 4H), 2.18-1.95 (m, 4H), 1.79-1.37 (m, 12H), 1.36-1.16 (m, 80H), 0.94-0.83 (m, 6H); MS (ES^+): m/z (%): 607.7 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{68}\text{H}_{132}\text{N}_{12}\text{O}_6$ 607.0230 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 607.0243.

4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (P²-SP3-R2)



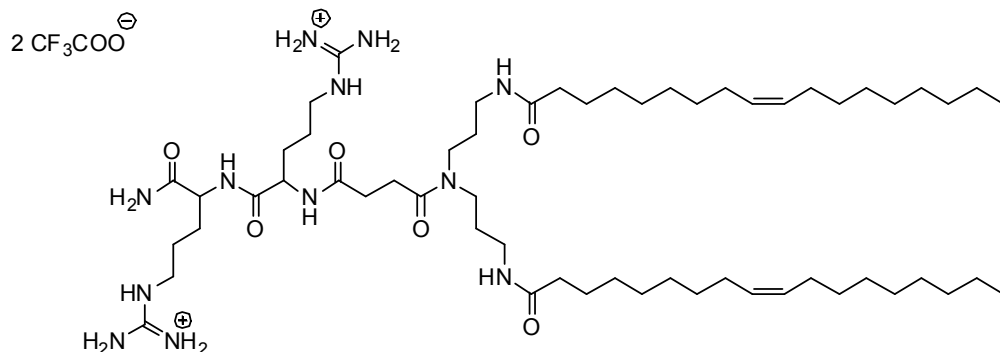
Yield: 144 mg. IR ν : 3310, 3198 (N-H), 2918 (C-H), 1630 (C=O), 1550 (N-H) cm⁻¹. ¹H NMR (360 MHz, CDCl₃) δ : 8.03-7.92 (m, 8H), 7.27-7.09 (m, 2H), 6.48-6.27 (m, 10H), 5.67-5.16 (m, 8H), 3.40-2.92 (m, 12H), 1.65-1.42 (m, 8H), 1.41-1.27 (m, 48H), 1.25-0.91 (m, 6H); MS (ES⁺): m/z (%): 510.4 (100) [M+2H]²⁺. HRMS calcd for C₅₄H₁₀₆N₁₂O₆ 510.4371 ([M+2H]²⁺), mass found m/z: 510.4363.

4-(*N,N*-Bis-[2-(stearylamo)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (S²-SP3-R2)



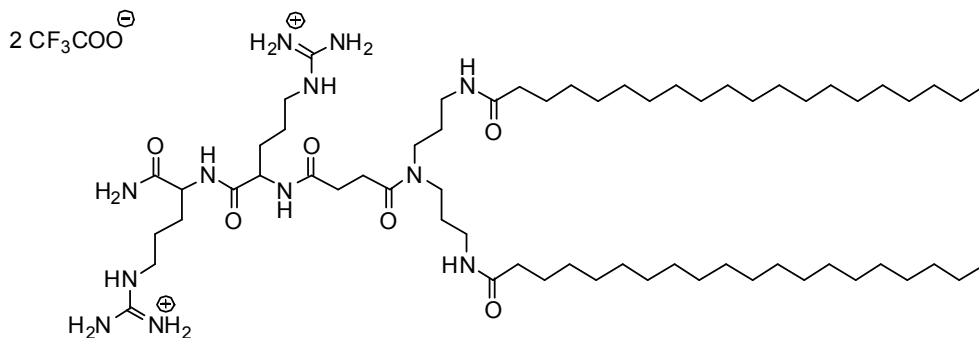
Yield: 108 mg. IR ν : 3309, 3201 (N-H), 2916 (C-H), 1637 (C=O), 1548 (N-H) cm⁻¹. ¹H NMR (360 MHz, d₆-DMSO) δ : 8.36-6.51 (m, 10H), 5.19-4.33 (m, 18H), 4.22-4.11 (m, 4H), 2.11-1.98 (m, 4H), 1.80-1.40 (m, 12H), 1.34-1.13 (m, 56H), 0.90-0.81 (m, 6H); MS (ES⁺): m/z (%): 538.5 (100) [M+2H]²⁺. HRMS calcd for C₅₈H₁₁₄N₁₂O₆ 1075.9057 ([M+H]⁺), mass found m/z: 1075.9034.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (O²-SP3-R2)



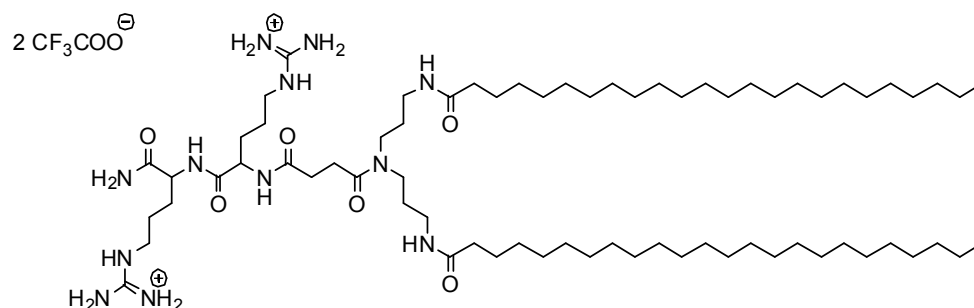
Yield: 104 mg. IR ν : 3338, 3195 (N-H), 2925 (C-H), 1625 (C=O), 1558 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.29-6.35 (m, 10H), 5.48-5.28 (m, 4H), 5.15-4.97 (m, 2H), 4.54-4.03 (m, 4H), 4.02-3.64 (m, 4H), 3.62-3.00 (m, 4H), 2.54-1.94 (m, 16H), 1.85-1.03 (m, 56H), 1.00-0.76 (m, 6H); MS (ES^+): m/z (%): 1071.6 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{58}\text{H}_{110}\text{N}_{12}\text{O}_6$ 1071.8744 ($[\text{M}+\text{H}]^+$), mass found m/z : 1071.8743.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (A²-SP3-R2)



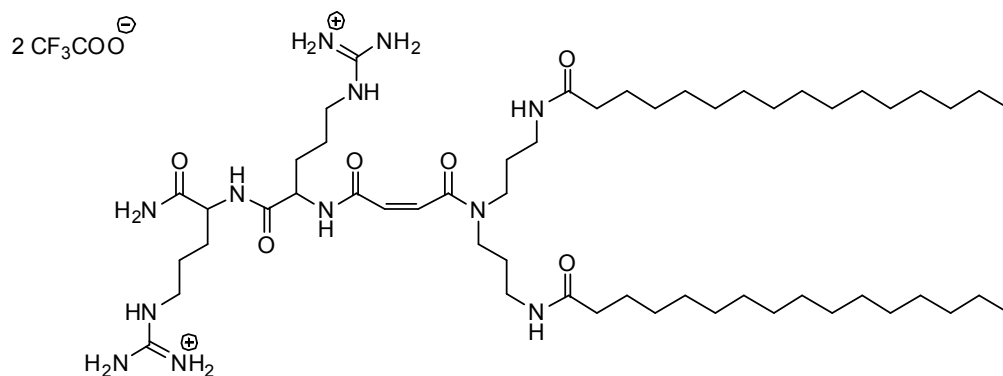
Yield: 105 mg. IR ν : 3311, 3209 (N-H), 2916 (C-H), 1637 (C=O), 1549 (N-H) cm^{-1} . ^1H NMR (500 MHz, $\text{d}_6\text{-DMSO}$) δ : 8.35-6.51 (m, 10H), 5.64-4.41 (m, 16H), 3.28-2.94 (m, 2H), 2.14-1.98 (m, 6H), 1.86-1.57 (m, 8H), 1.59-1.41 (m, 8H), 1.31-1.10 (m, 64H), 1.02-0.94 (m, 6H); MS (ES^+): m/z (%): 566.8 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{62}\text{H}_{122}\text{N}_{12}\text{O}_6$ 1131.9662 ($[\text{M}+\text{H}]^+$), mass found m/z : 1131.9683.

**4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt
(L²-SP3-R2)**



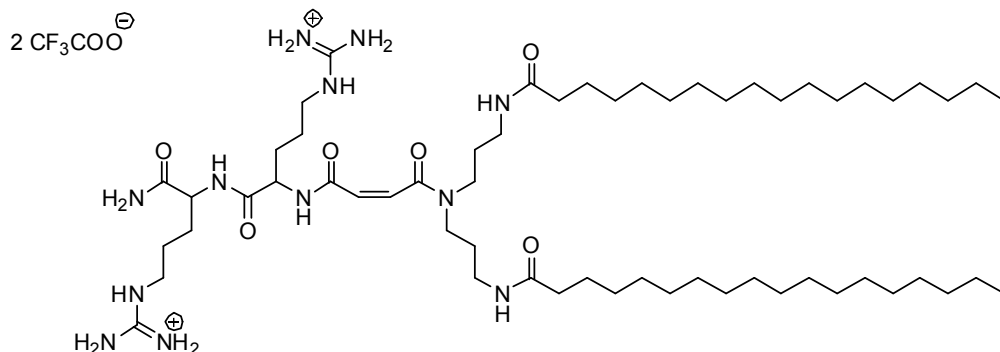
Yield: 121 mg. IR ν : 3308, 3218 (N-H), 2916 (C-H), 1638 (C=O), 1553 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.44-5.76 (m, 12H), 4.18-4.10 (m, 8H), 3.16-3.01 (m, 6H), 2.14-2.05 (m, 6H), 1.85-1.61 (m, 8H), 1.57-1.40 (m, 8H), 1.35-0.77 (m, 92H); MS (ES^+): m/z (%): 415.2 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{70}\text{H}_{138}\text{N}_{12}\text{O}_6$ 414.6975 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 414.6975.

**4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt
(P²-SP4-R2)**



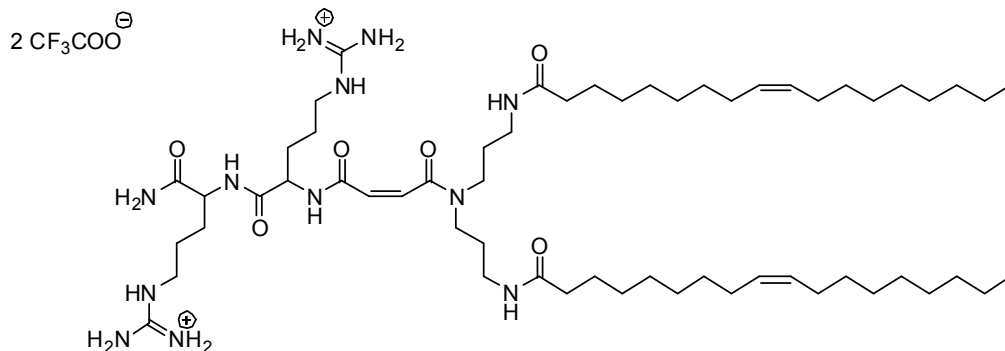
Yield: 192 mg. IR ν : 3349, 3203 (N-H), 2922 (C-H), 1657 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 8.40-5.71 (m, 12H), 4.48-4.28 (m, 2H), 3.57-3.10 (m, 4H), 3.02-2.87 (m, 8H), 2.36-2.26 (m, 4H), 1.68-1.57 (m, 8H), 1.56-1.03 (m, 56H), 0.98-0.79 (m, 6H); MS (ES^+): m/z (%): 1017.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(stearyl-amido)propyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (S²-SP4-R2)



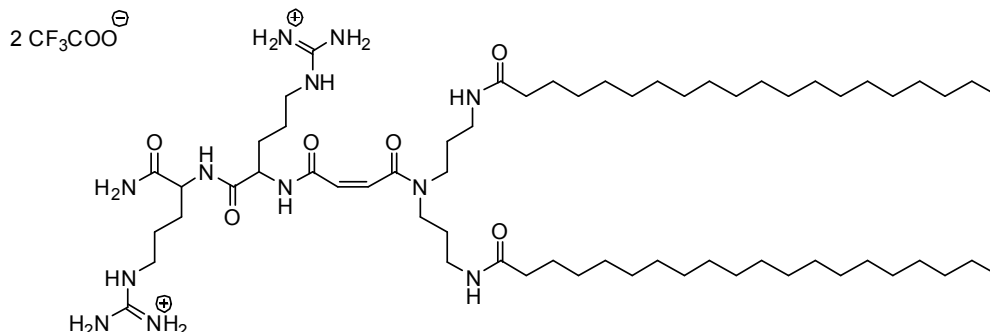
Yield: 173 mg. IR ν : 3343, 3206 (N-H), 2919 (C-H), 1655 (C=O), 1551 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.95-6.27 (m, 12H), 4.46-4.26 (m, 2H), 3.88-3.80 (m, 4H), 3.42-3.16 (m, 8H), 3.15-2.90 (m, 4H), 2.46-2.04 (m, 2H), 1.66-1.58 (m, 8H), 1.55-1.46 (m, 8H), 1.38-1.05 (m, 56H), 0.98-0.82 (m, 6H); MS (ES^+): m/z (%): 536.4 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{58}\text{H}_{112}\text{N}_{12}\text{O}_6$ 536.9448 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 536.9470.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (O²-SP4-R2)



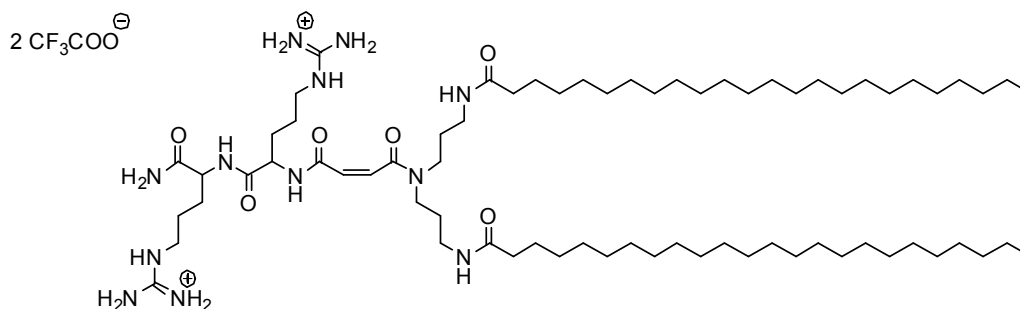
Yield: 148 mg. IR ν : 3292, 3196 (N-H), 2916 (C-H), 1626 (C=O), 1551 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 8.79-5.74 (m, 11H), 5.39-5.14 (m, 4H), 5.05-4.81 (m, 2H), 4.37-4.06 (m, 4H), 3.37-2.90 (m, 8H), 2.30-1.76 (m, 22H), 1.72-0.89 (m, 48H), 0.86-0.60 (m, 6H); MS (ES^+): m/z (%): 1069.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (A²-SP4-R2)



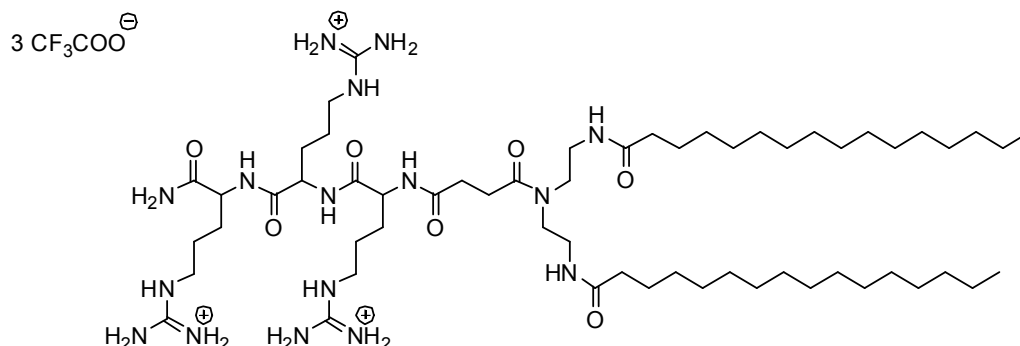
Yield: 98 mg. IR ν : 3294, 3205 (N-H), 2918 (C-H), 1628 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.48-6.62 (m, 8H), 6.40-5.12 (m, 4H), 4.23-4.11 (m, 2H), 3.29-2.85 (m, 12H), 2.11-1.97 (m, 4H), 1.79-1.39 (m, 12H), 1.38-1.02 (m, 64H), 0.91-0.78 (m, 6H); MS (ES^+): m/z (%): 1129.8 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{62}\text{H}_{120}\text{N}_{12}\text{O}_6$ 564.9760 ($[\text{M}+2\text{H}]^{2+}$), mass found m/z : 564.9761.

4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-maleyl-L-arginyl-L-argininamide, TFA salt (L²-SP4-R2)



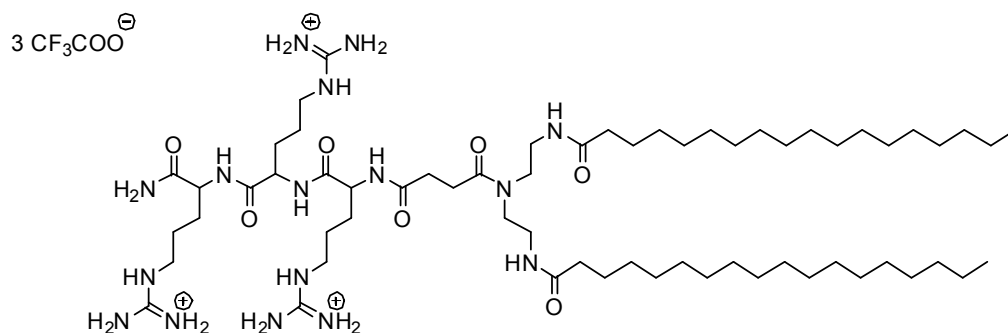
Yield: 128 mg. IR ν : 3347, 3201 (N-H), 2915 (C-H), 1653 (C=O), 1547 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.34-6.53 (m, 12H), 5.91-4.54 (m, 14H), 4.24-4.12 (m, 4H), 3.14-3.02 (m, 4H), 1.77-1.63 (m, 4H), 1.58-0.79 (m, 94H); MS (ES^+): m/z (%): 1241.2 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{70}\text{H}_{136}\text{N}_{12}\text{O}_6$ 414.0256 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 414.0235.

4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (P²-SP1-R3**)**



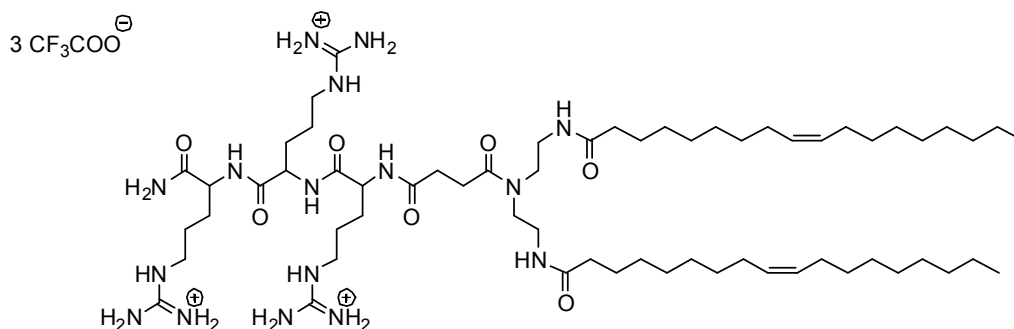
Yield: 97 mg. IR ν : 3345, 3194 (N-H), 2920 (C-H), 1633 (C=O), 1545 (N-H) cm⁻¹. ¹H NMR (360 MHz, d₆-DMSO) δ : 8.35-6.54 (m, 13H), 4.39-4.11 (m, 7H), 3.43-3.00 (m, 12H), 2.16-2.03 (m, 6H), 1.85-1.71 (m, 6H), 1.65-1.43 (m, 8H), 1.36-1.18 (m, 48H), 0.98-0.85 (m, 6H); MS (ES⁺): m/z (%): 574.5 (100) [M+2H]²⁺. HRMS calcd for C₅₈H₁₁₄N₁₆O₇ 1147.9129 ([M+H]⁺), mass found m/z: 1147.9105.

4-(*N,N*-Bis-[2-(stearyl)amido]ethyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (S²-SP1-R3**)**



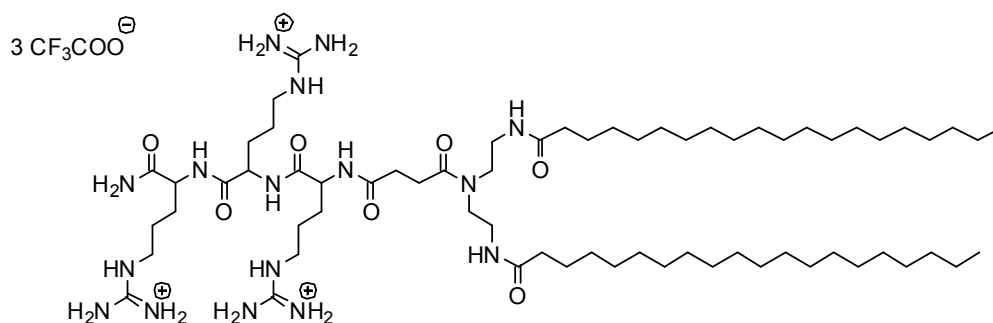
Yield: 121 mg. IR ν : 3285, 3196 (N-H), 2916 (C-H), 1627 (C=O), 1540 (N-H) cm⁻¹. ¹H NMR (360 MHz, d₆-DMSO) δ : 8.18-6.64 (m, 13H), 4.29-4.09 (m, 7H), 3.42-3.02 (m, 14H), 2.15-1.97 (m, 4H), 1.80-1.68 (m, 6H), 1.64-1.38 (m, 10H), 1.33-1.16 (m, 56H), 0.92-0.81 (m, 6H); MS (ES⁺): m/z (%): 402.0 (100) [M+3H]³⁺. HRMS calcd for C₆₂H₁₂₂N₁₆O₇ 1203.9755 ([M+2H]²⁺), mass found m/z: 1203.9718.

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (O²-SP1-R3)



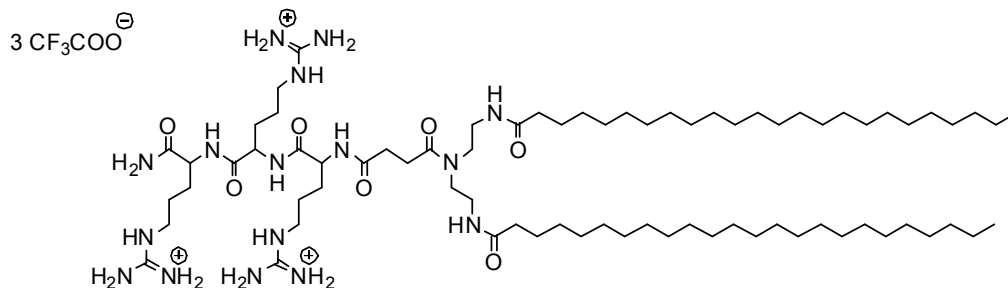
Yield: 91 mg. IR ν : 3290, 3194 (N-H), 2925 (C-H), 1625 (C=O), 1543 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.30-6.54 (m, 7H), 5.43-5.27 (m, 4H), 5.11-4.98 (m, 3H), 4.33-4.05 (m, 8H), 3.44-2.95 (m, 10H), 2.16-1.90 (m, 12H), 1.81-1.07 (m, 54H), 0.91-0.77 (m, 6H); MS (ES^+): m/z (%): 1199.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (A²-SP1-R3)



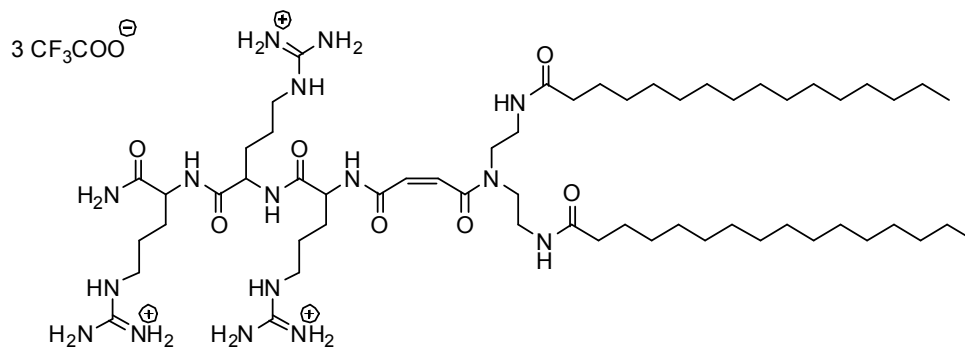
Yield: 100 mg. IR ν : 3284, 3193 (N-H), 2916 (C-H), 1628 (C=O), 1541 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.26-6.52 (m, 3H), 4.28-4.13 (m, 8H), 3.37-3.02 (m, 14H), 1.80-1.66 (m, 6H), 1.60-1.39 (m, 10H), 1.32-1.15 (m, 64H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 420.8 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{66}\text{H}_{130}\text{N}_{16}\text{O}_7$ 420.0123 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 420.0123.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (L^2 -SP1-R3)



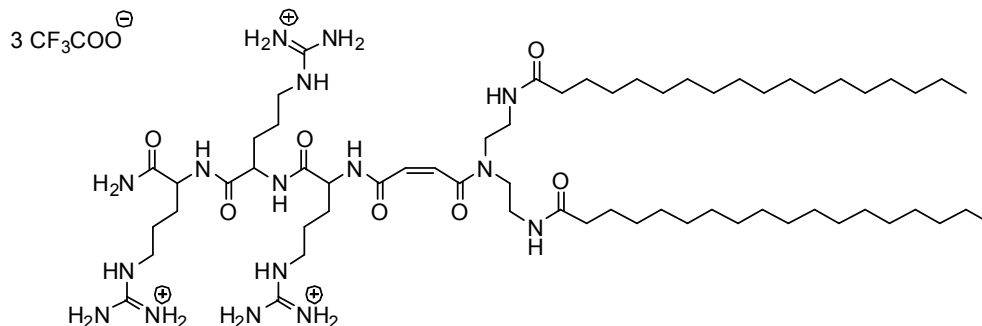
Yield: 130 mg. IR ν : 3354, 3206 (N-H), 2918 (C-H), 1654 (C=O), 1545 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.35-6.60 (m, 13H), 4.33-4.11 (m, 3H), 3.75-3.67 (m, 4H), 3.49-3.42 (m, 4H), 2.68-2.61 (m, 6H), 2.60-1.72 (m, 8H), 1.72-1.62 (m, 6H), 1.56-1.34 (m, 10H), 1.30-0.91 (m, 80H), 0.86 (t, $J = 7, 6\text{H}$); MS (ES^+): m/z (%): 1372.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(palmitoylamido)ethyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (P^2 -SP2-R3)



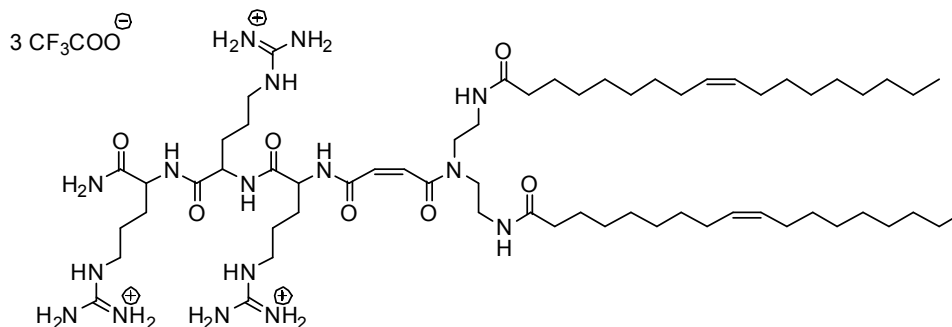
Yield: 126 mg. IR ν : 3352, 3203 (N-H), 2921 (C-H), 1634 (C=O), 1547 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.66-6.43 (m, 15H), 5.12-3.93 (m, 11H), 3.44-3.02 (m, 10H), 1.83-1.40 (m, 16H), 1.33-1.12 (m, 48H), 0.88 (t, $J = 7, 6\text{H}$); MS (ES^+): m/z (%): 573.5 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{58}\text{H}_{112}\text{N}_{16}\text{O}_7$ 1145.8973 ($[\text{M}+\text{H}]^+$), mass found m/z : 1145.8917.

4-(*N,N*-Bis-[2-(stearyl-amido)ethyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (S²-SP2-R3)



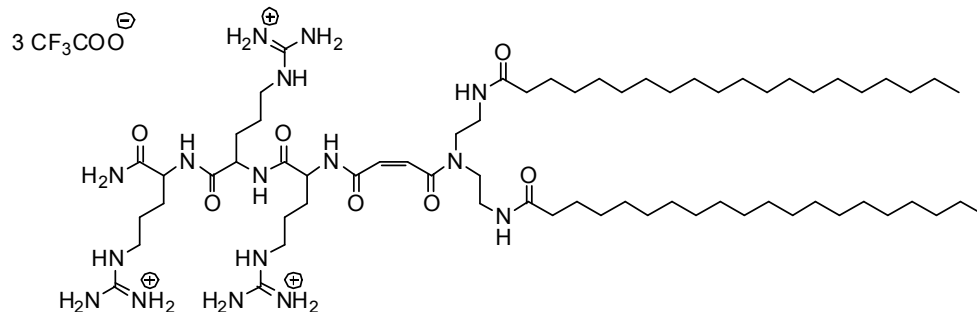
Yield: 117 mg. IR ν : 3283, 3198 (N-H), 2917 (C-H), 1628 (C=O), 1544 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.47-6.61 (m, 15H), 5.02-3.94 (m, 11H), 3.37-3.04 (m, 6H), 2.12-1.95 (m, 4H), 1.84-1.35 (m, 16H), 1.34-1.12 (m, 56H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 401.4 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{62}\text{H}_{120}\text{N}_{16}\text{O}_7$ 400.6529 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 444.8796

4-(*N,N*-Bis-[2-(oleoylamido)ethyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (O²-SP2-R3)



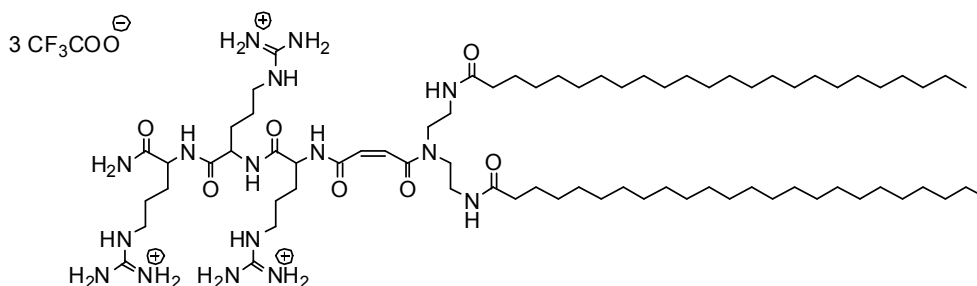
Yield: 157 mg. IR ν : 3283, 3195 (N-H), 2925 (C-H), 1633 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 8.07-7.83 (m, 7H), 7.26-6.99 (m, 1H), 6.54-6.27 (m, 1H), 5.02-4.87 (m, 7H), 3.62-3.54 (m, 14H), 2.17-1.74 (m, 21H), 1.63-1.47 (m, 10H), 1.46-0.90 (m, 40H), 0.83-0.64 (m, 6H); MS (ES^+): m/z (%): 1197.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(arachidoylamido)ethyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (A²-SP2-R3)



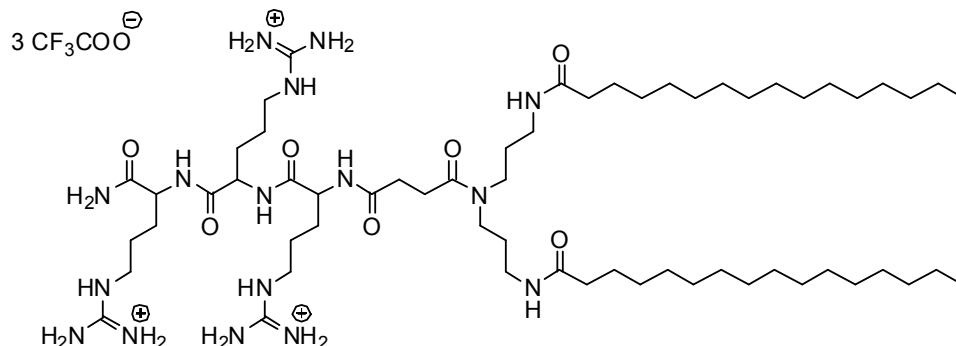
Yield: 108 mg. IR ν : 3292, 3197 (N-H), 2916 (C-H), 1632 (C=O), 1545 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.50-6.56 (m, 15H), 5.01-3.46 (m, 17H), 3.16-3.04 (m, 4H), 1.85-1.37 (m, 16H), 1.35-1.14 (m, 64H), 0.87 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 420.1 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{66}\text{H}_{128}\text{N}_{16}\text{O}_7$ 1258.0188 ($[\text{M}+\text{H}]^+$), mass found m/z : 1258.0225.

4-(*N,N*-Bis-[2-(lignocerylamido)ethyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (L²-SP2-R3)



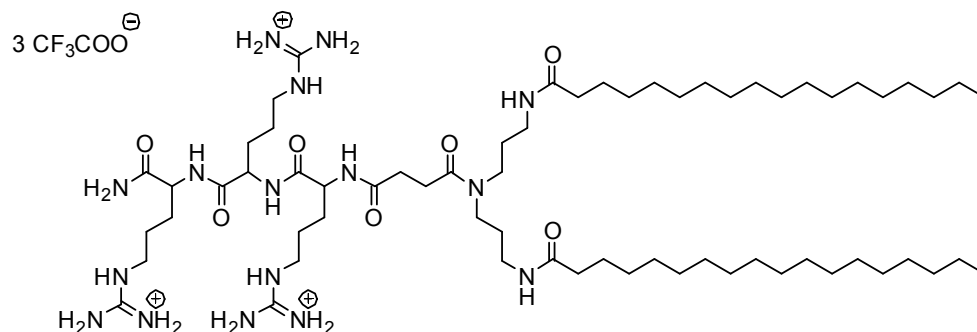
Yield: 159 mg. IR ν : 3352, 3208 (N-H), 2916 (C-H), 1655 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.28-6.63 (m, 13H), 6.32-4.92 (m, 12H), 4.34-4.11 (m, 6H), 3.15-3.00 (m, 4H), 1.79-1.40 (m, 14H), 1.39-1.05 (m, 80H), 0.86 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 1370.7 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (P²-SP3-R3)



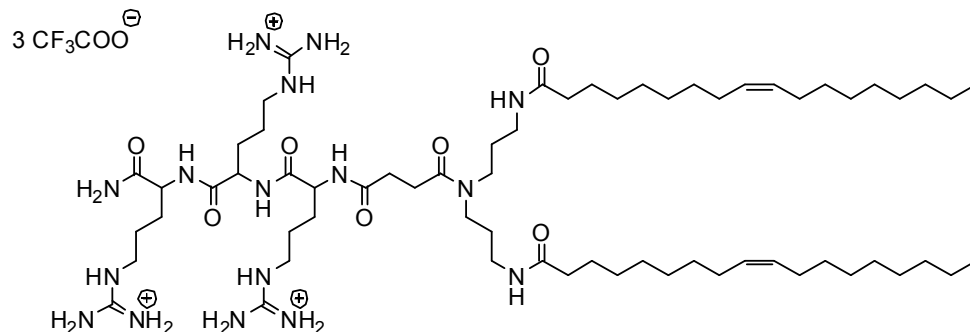
Yield: 135 mg. IR ν : 3293, 3200 (N-H), 2916 (C-H), 1638 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.33-6.70 (m, 13H), 5.98-4.99 (m, 17H), 4.34-3.97 (m, 4H), 3.26-2.88 (m, 14H), 1.78-1.34 (m, 10H), 1.31-1.01 (m, 48H), 0.85 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 1175.7 (100) $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{60}\text{H}_{118}\text{N}_{16}\text{O}_7$ 391.9810 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 391.9808.

4-(*N,N*-Bis-[2-(stearylamo)propyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (S²-SP3-R3)



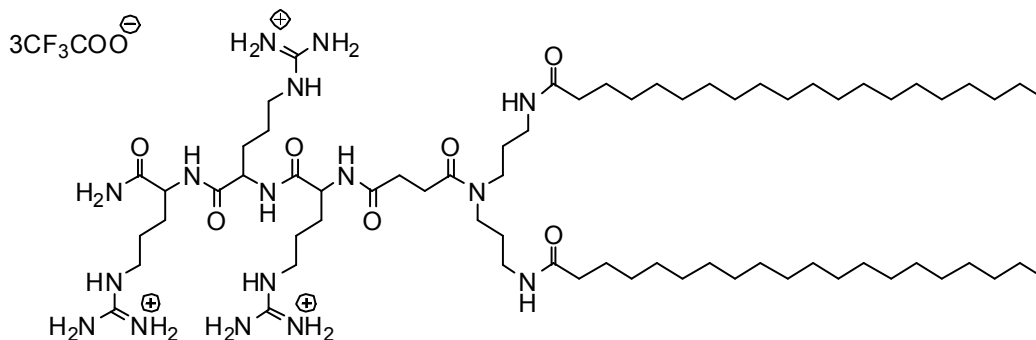
Yield: 112 mg. IR ν : 3277, 3198 (N-H), 2921 (C-H), 1634 (C=O), 1555 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.30-6.53 (m, 13H), 5.56-4.72 (m, 17H), 4.28-4.08 (m, 8H), 3.33-2.90 (m, 10H), 2.44-2.35 (m, 10H), 1.34-1.14 (m, 56H), 0.88 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 411.5 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{64}\text{H}_{126}\text{N}_{16}\text{O}_7$ 410.6686 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 410.6696.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (*O*²-SP3-R3)



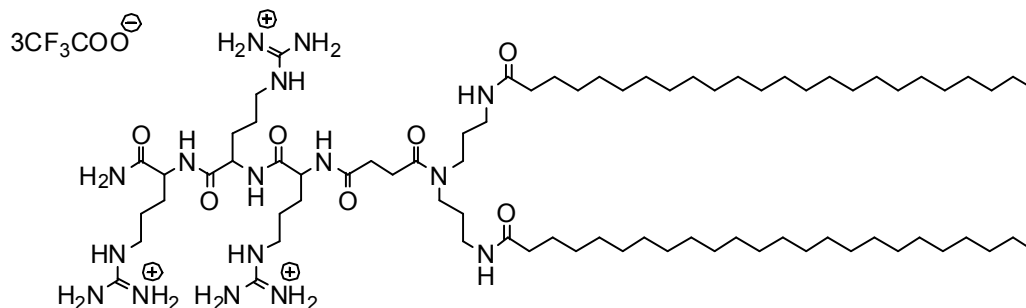
Yield: 151 mg. IR ν : 3349, 3196 (N-H), 2925 (C-H), 1627 (C=O), 1548 (N-H) cm^{-1} . ¹H NMR (360 MHz, CDCl₃) δ : 10.24-7.83 (m, 13H), 5.32-5.16 (m, 4H), 5.01-4.84 (m, 11H), 3.77-3.54 (m, 10H), 2.27-1.77 (m, 16H), 1.67-0.54 (m, 56H); MS (ES⁺): m/z (%): 409.5 (100) [M+3H]³⁺. HRMS calcd for C₆₄H₁₂₂N₁₆O₇ 409.3248 ([M+3H]³⁺), mass found m/z: 409.3261.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (*A*²-SP3-R3)



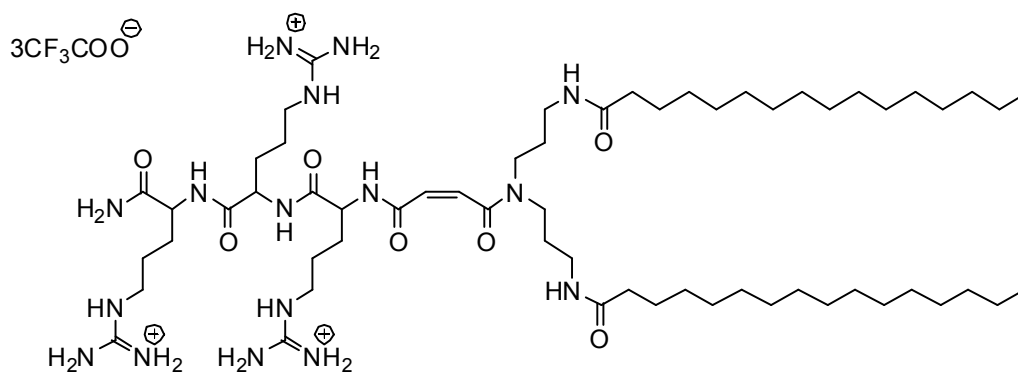
Yield: 112 mg. IR ν : 3296, 3199 (N-H), 2918 (C-H), 1639 (C=O), 1548 (N-H) cm^{-1} . ¹H NMR (500 MHz, d₆-DMSO) δ : 8.25-6.60 (m, 13H), 5.65-4.39 (m, 21H), 4.31-4.05 (m, 4H), 3.31-2.89 (m, 4H), 1.79-1.40 (m, 16H), 1.34-1.10 (m, 64H), 0.93-0.77 (m, 6H); MS (ES⁺): m/z (%): 430.0 (100) [M+3H]³⁺. HRMS calcd for C₆₈H₁₃₄N₁₆O₇ 429.3561 ([M+3H]³⁺), mass found m/z: 429.3557.

4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-succinyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (L²-SP3-R3)



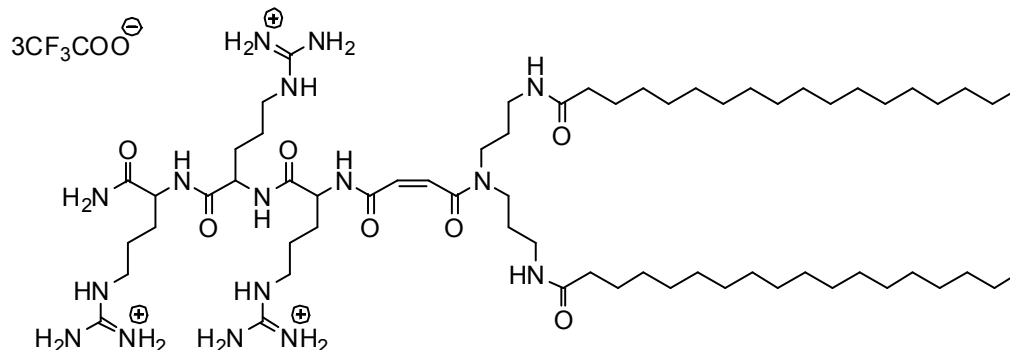
Yield: 139 mg. IR ν : 3305, 3207 (N-H), 2916 (C-H), 1638 (C=O), 1550 (N-H) cm^{-1} . ^1H NMR (500 MHz, d_6 -DMSO) δ : 8.28-6.60 (m, 13H), 6.10-4.71 (m, 19H), 4.32-4.03 (m, 2H), 3.17-2.99 (m, 8H), 1.80-1.61 (m, 6H), 1.60-1.39 (m, 10H), 1.33-1.12 (m, 80H), 0.90-0.80 (m, 6H); MS (ES^+): m/z (%): 700.5 (100) $[\text{M}+2\text{H}]^{2+}$.

4-(*N,N*-Bis-[2-(palmitoylamido)propyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (P²-SP4-R3)



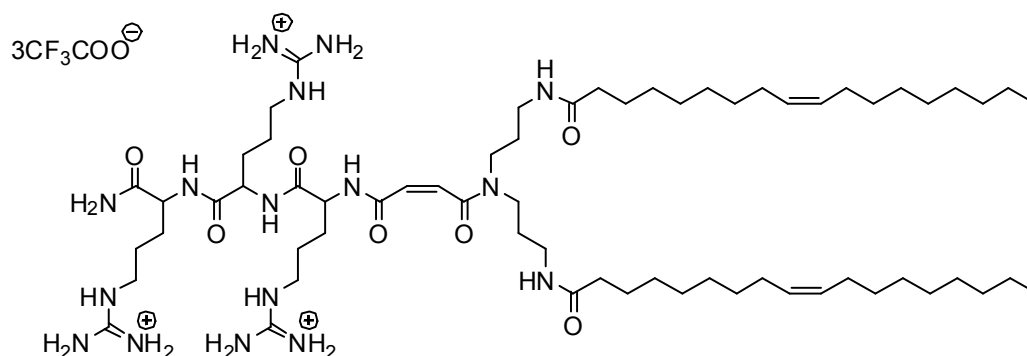
Yield: 116 mg. IR ν : 3338, 3194 (N-H), 2917 (C-H), 1627 (C=O), 1544 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 9.39-9.27 (br s, 6H), 8.57-6.52 (m, 9H), 5.44-4.34 (m, 7H), 4.29-4.04 (m, 4H), 3.30-2.89 (m, 6H), 2.71-2.58 (m, 8H), 1.82-1.31 (m, 16H), 1.34-1.05 (m, 48H), 0.85 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 588.7 (100) $[\text{M}+2\text{H}]^{2+}$.

4-(*N,N*-Bis-[2-(stearyl-amido)propyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (*S*²-SP4-R3)



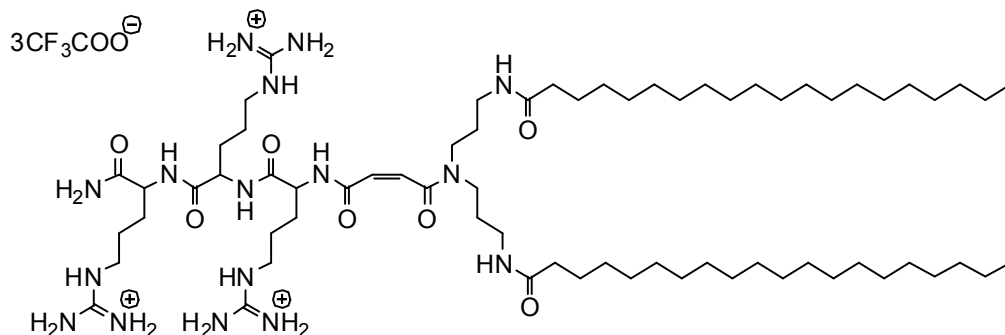
Yield: 121 mg. IR ν : 3286, 3198 (N-H), 2916 (C-H), 1627 (C=O), 1541 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 9.37-9.29 (br s, 6H), 8.47-6.57 (m, 9H), 5.28-4.34 (m, 11H), 3.22-2.91 (m, 10H), 2.07-1.96 (m, 4H), 1.84-1.35 (m, 16H), 1.31-1.12 (m, 56H), 0.85 (t, $J = 7$, 6H); MS (ES^+): m/z (%): 616.6 (100) $[\text{M}+2\text{H}]^{2+}$. HRMS calcd for $\text{C}_{64}\text{H}_{124}\text{N}_{16}\text{O}_7$ 409.9967 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 409.9986.

4-(*N,N*-Bis-[2-(oleoylamido)propyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (*O*²-SP4-R3)



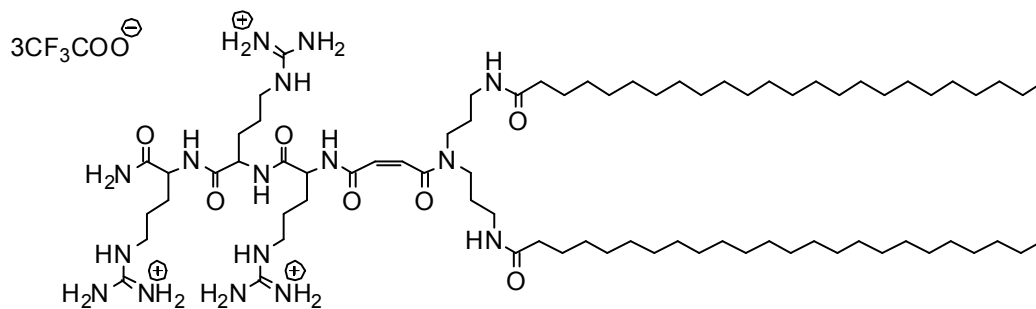
Yield: 143 mg. IR ν : 3292, 3194 (N-H), 2925 (C-H), 1626 (C=O), 1548 (N-H) cm^{-1} . ^1H NMR (360 MHz, CDCl_3) δ : 9.09-5.93 (m, 14H), 5.46-5.32 (m, 4H), 3.92-3.68 (m, 17H), 2.32-2.09 (m, 12H), 2.07-1.87 (m, 4H), 1.74-1.03 (m, 56H), 0.98-0.85 (m, 6H); MS (ES^+): m/z (%): 612.5 (100) $[\text{M}+\text{H}]^+$.

4-(*N,N*-Bis-[2-(arachidoylamido)propyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (A²-SP4-R3)



Yield: 120 mg. IR ν : 3339, 3198 (N-H), 2916 (C-H), 1651 (C=O), 1546 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.15-6.58 (m, 15H), 5.57-4.43 (m, 11H), 4.33-4.07 (m, 10H), 3.35-2.87 (m, 10H), 2.10-1.97 (m, 10H), 1.34-1.04 (m, 64H), 0.92-0.77 (m, 6H); MS (ES^+): m/z (%): 428.7 (100) $[\text{M}+3\text{H}]^{3+}$. HRMS calcd for $\text{C}_{68}\text{H}_{132}\text{N}_{16}\text{O}_7$ 428.6842 ($[\text{M}+3\text{H}]^{3+}$), mass found m/z : 428.6864.

4-(*N,N*-Bis-[2-(lignocerylamido)propyl]amino)-maleyl-L-arginyl-L-arginyl-L-argininamide, TFA salt (L²-SP4-R3)

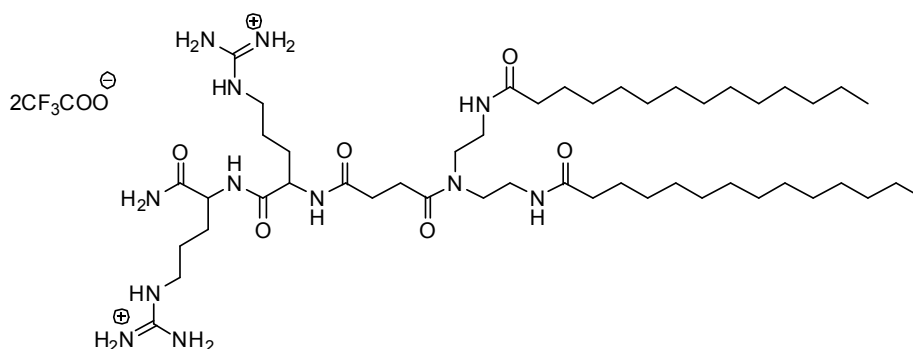


Yield: 123 mg. IR ν : 3350, 3198 (N-H), 2916 (C-H), 1634 (C=O), 1546 (N-H) cm^{-1} . ^1H NMR (360 MHz, d_6 -DMSO) δ : 8.61-6.71 (m, 15H), 5.62-4.46 (m, 11H), 3.53-2.97 (m, 14H), 2.10-1.90 (m, 6H), 1.77-1.34 (m, 10H), 1.30-1.06 (m, 80H), 0.91-0.74 (m, 6H); MS (ES^+): m/z (%): 699.5 (100) $[\text{M}+2\text{H}]^{2+}$.

8.2. IR, H-NMR and MS characterisation of 3 additional lipo-peptides synthesised.

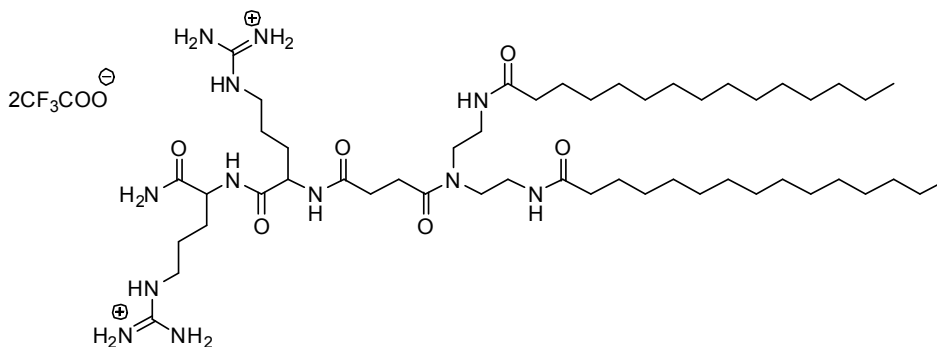
4-(*N,N*-Bis-[2-(myristoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt ... (C14-SP1-R2)

Yield: 130 mg. IR ν : 3276, 3182 (N-H), 2922, 2852 (C-H), 1627 (C=O), 1539 (N-H) cm^{-1} . ^1H NMR (400 MHz, d_6 -DMSO) δ : 9.33-7.02 (m, 16H), 4.09-4.06 (m, 2H), 3.32-3.06 (m, 12H), 2.60-2.32 (m, 4H), 2.06-2.00 (m, 4H), 1.74-1.23 (m, 52H), 0.87-0.83 (m, 6H); HRMS ESI $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{48}\text{H}_{95}\text{N}_{12}\text{O}_6$ 935.74921, found 935.7489.

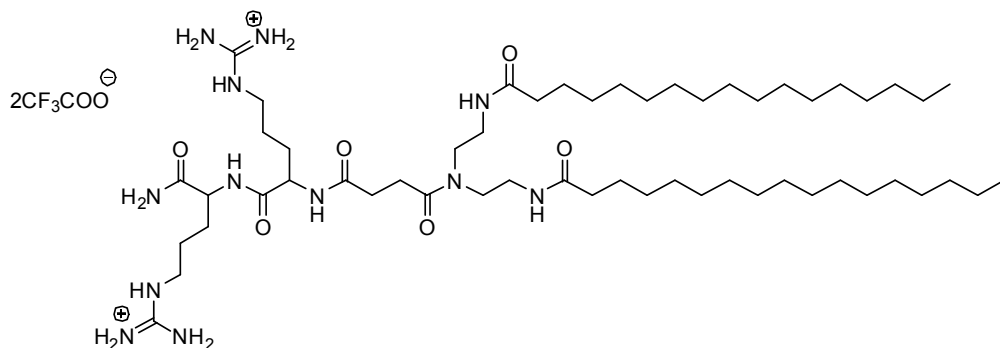


4-(*N,N*-Bis-[2-(pentadecanoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt (C15-SP1-R2)

Yield: 162 mg. IR ν : 3283, 3189 (N-H), 2922, 2853 (C-H), 1628 (C=O), 1541 (N-H) cm^{-1} . ^1H NMR (400 MHz, d_6 -DMSO) δ : 9.91-6.83 (m, 16H), 4.11-4.07 (m, 2H), 3.32-3.07 (m, 12H), 2.58-2.33 (m, 4H), 2.06-2.00 (m, 4H), 1.78-1.23 (m, 56H), 0.87-0.83 (m, 6H); HRMS ESI $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{50}\text{H}_{99}\text{N}_{12}\text{O}_6$ 963.78051, found 963.7807.



**4-(*N,N*-Bis-[2-(heptadecanoylamido)propyl]amino)-succinyl-L-arginyl-L-argininamide, TFA salt
(C17-SP1-R2)**



Yield: 155 mg. IR ν : 3274, 3183 (N-H), 2919, 2851 (C-H), 1628 (C=O), 1539 (N-H) cm⁻¹. ¹H NMR (400 MHz, d₆-DMSO) δ : 9.94-6.77 (m, 16H), 4.16-4.12 (m, 2H), 3.32-3.08 (m, 12H), 2.57-2.32 (m, 4H), 2.07-2.01 (m, 4H), 1.74-1.23 (m, 64H), 0.87-0.83 (m, 6H); HRMS [M+H]⁺ calcd for C₅₄H₁₀₇N₁₂O₆ 1019.84311, found 1019.8433.