Electronic Supplementary Information for the paper

Structure-delivery relationships of lysine-based gemini surfactants and their lipoplexes

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The Electronic Supplementary Information contains information on the synthesis and characterization of various lysine-based surfactants.

S.1. General considerations on the synthesis
S.2. Preparation of the ‘half-gemini’ classical surfactant Oleoyl-Lys(H)-NHCH2CH3.HCl. 10
S.3. Incorporation of the diamine spacer (step iv of Scheme 1) to give (Z-Lys(Boc)-NH)2(CH2)n. 10
S.4. Z-Deprotection/Acylation (step v-vi of Scheme 1) to give (Oleoyl-Lys(Boc)-NH)2(CH2)n. 10
S.5. Boc-Deprotection (step iii of Scheme 1) to give (Oleoyl-Lys(H)-NH)2(CH2)n·2HCl. 10
S.6. Preparation of the enantiomer (Oleoyl-D-Lys(H)-NH)2(CH2)6·2HCl. 15
S.7. Preparation of the acyl analogues of (Oleoyl-Lys(H)-NH)2(CH2)6·2HCl. 15

S.1. General considerations on the synthesis:
The synthesis of the surfactants was accomplished according to the procedure outlined in Scheme 1 in the Results and Discussion section of the manuscript.

For the synthesis of the two amide bonds, carbodiimide/HOBt couplings as applied in modern solution phase peptide synthesis, were used [1]. Ethylene dimethylaminopropyl carbodiimide•HCl (EDC) was preferred over the more frequently used dicyclohexyl carbodiimide (DCC) as the coupling reagent because it is water-soluble and can be removed by acidic extraction. HOBt (1-hydroxybenzotriazole) was added both as a catalyst for the coupling reaction and to suppress epimerization [2]. It can be removed using basic extraction as was also anticipated for Z-Lys(Boc)-OH and oleic acid. Because the products of the amide couplings (Z-Lys(Boc)-NH)2(CH2)n and target product (R1(CO)-Lys(Boc)-NH)2(CH2)n cannot be protonated or deprotonated by weak acids or weak bases, they are expected to remain in the organic layer during extractions, so that it should be possible to do all purifications by extractions only.

The synthesis of product (Z-Lys(Boc)-NH)2(CH2)n was performed using ethyl acetate as the solvent because of its convenience in extractions and because it readily dissolves all reagents; it was saturated with water before use in order to dissolve the ethylenedimethylaminopropyl urea (EDU) that is formed during the reaction. Without the water, the EDU was found to give a very inconvenient yellow chewing gum-like substance that obstructed stirring of the reaction mixture and was difficult to remove. The reaction was monitored by TLC and when all diamine had reacted (after 1 night), a white precipitate had appeared that was found to be the desired product. After extensive aqueous extractions of the suspension, the product was filtered and dried in the desiccator. Unprecipitated product that
remained in the filtrate was discarded for convenience because it still contained unreacted Z-Lys(Boc)-OH.

For the selective deprotection of the α-amine function (step vi), it proved to be difficult to dissolve the product \((Z\text{-Lys(Boc)-NH})_2(CH_2)_n\) completely in methanol. Therefore DMF was chosen as the solvent, even though it is not common in hydrogenation reactions using Pd/C as a catalyst. The deprotection of the α-amine of the lysines could be monitored very well with TLC, due to the large difference in Rf values and the convenient staining with ninhydrine. Typically the reaction had finished after 1.5 h. After removing the Pd/C catalyst from the DMF by filtration, the oleic acid could be coupled using EDC and HOBt to obtain \((R^1(CO)\text{-Lys(Boc)-NH})_2(CH_2)_n\). Again, this reaction could be monitored very well with TLC and had finished after overnight. After aqueous extractions to remove all reagents, using chloroform as the organic phase, it was observed by NMR that some residual peaks of reagents were still present, indicating that the basic extractions had not been efficient enough. Because purification of the target product was expected to be very difficult due to its amphiphilic character, it was decided to do an additional purification of \((R^1(CO)\text{-Lys(Boc)-NH})_2(CH_2)_n\) by column chromatography. This removed all side products, but in some cases a small amount of unreacted oleic acid remained. A small contamination of this acid remained after column chromatography and could not be avoided, even by changing the small 10% excess of oleic acid into a 10% deficit.

The \(^1\text{H-NMR}\) spectra of \((\text{Oleoyl-Lys(H)-NH})_2(CH_2)_6\) and its intermediates could be assigned using COSY and NOESY techniques, and the presence of the chiral centers resulted in some special features for \((\text{Oleoyl-Lys(H)-NH})_2(CH_2)_6\). Because of their proximity to the chiral centers, the β-CH₂ protons of the Lys residues were chemically non-equivalent (ABX system, not considering the additional coupling with the γ-CH₂ protons), as expected. More remarkably, the protons of the 1,6-CH₂ groups of the diamine and the β-CH₂ group of the oleic acid also appeared as diastereotopic atoms, probably as a result of the formation of an intramolecular H-bond between the N-H of the diamine to the C=O of the oleic acid residue (7-membered ring). It is of interest to compare this result with that obtained for the ‘half-gemini’ \((\text{Oleoyl-Lys(H)-NH}CH_2CH_3\), a classical (non-dimeric) surfactant obtained by reaction of Z-Lys(Boc)-OH with ethylamine instead of diamine. For this compound, the lysine β-CH₂ protons appeared as diastereotopic in \(^1\text{H-NMR}\), but those in the oleoyl and ethyl groups did not.

In the same way as described above for \((\text{Oleoyl-Lys(H)-NH})_2(CH_2)_6\), the analogue compounds with spacer lengths 2, 3, 4, 5, 7, and 8 instead of 6 were also prepared, starting from protected enantiopure L-amino acids, and using the appropriate diamine in step iv. The enantiomer \((\text{Oleoyl-D-Lys(H)-NH})_2(CH_2)_6\) was prepared starting from protected D-lysine.

\S.2. Preparation of the ‘half-gemini’ classical surfactant Oleoyl-Lys(H)-NHCH₂CH₃.HCl.

As for \( (Z\text{-Lys}(Boc)-NH)_{2}(CH_2)_6 \) starting from \( Z\text{-Lys}(Boc)-OH \).DCHA salt (1.53 g, 2.72 mmol) yielding as intermediate product 902.9 mg \( Z\text{-Lys}(Boc)-OH \) (2.373 mmol, 87.3%), which was further reacted with CH\(_3\)CH\(_2\)NH\(_2\).HCl (207.7 mg, 2.55 mmol), DIPEA (430 μl, 2.50 mmol), HOBt.H\(_2\)O (405.1 mg, 2.65 mmol), EDC.HCl (483.9 mg, 2.52 mmol). \( Z\text{-Lys}(Boc)-NHCH_2CH_3 \) was obtained as a white solid. Yield: 749 mg (MW = 407.50, 78%) R\(_f\)=0.63 (CHCl\(_3\):MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl\(_3\)) δ: 7.33 (m, 5H, Z Ar), 6.05 (br s, 1H, di amine NH), 5.45 (br s, 1H, lysine α-NH), 5.10 (s, 2H, Z CH\(_2\)), 4.58 (br s, 1H, lysine ε-NH), 4.09 (m, 1H, lysine α-CH\(^*\)), 3.28 (m, 2H, amine CH\(_2\)), 3.11 (m, 2H, lysine ε-CH\(_2\)), 1.85 (m, 1H, lysine β-CH\(_2\)H\(_2\)), 1.66 (m, 1H, lysine β-CH\(_2\)H), 1.49-1.37 (s, 9H, Boc CH\(_3\)), (t, 2H, lysine δ-CH\(_2\)); (t, 2H, lysine γ-CH\(_2\)); 1.13 (t, 3H, amine CH\(_3\)), 13C NMR (300 MHz, CDCl\(_3\)) δ: 171.4, 156.2, 136.2, 128.5, 128.1, 67.0, 54.9, 39.8, 34.4, 32.1, 29.6, 28.4, 22.4, 14.7. LCQ (ESI) calculated (C\(_{21}\)H\(_{33}\)N\(_3\)O\(_5\)): 407.2, found: 408.2 (M+H\(^+\)), 815.2 (2M+H\(^+\)), 837.1 (2M+Na\(^+\)).

S.2.2. Synthesis of Oleoyl-Lys(Boc)-NHCH\(_2\)CH\(_3\).

As for \( (Oleoyl-Lys(Boc)-NH)_{2}(CH_2)_6 \) (section 2.2.2) starting from \( Z\text{-Lys}(Boc)-NHCH_2CH_3 \) (252 mg, 0.618 mmol) and Pd/C catalyst (103 mg) in 75 mL DMF, oleic acid (154 μl, 0.486 mmol), HOBt.H\(_2\)O (127 mg, 0.829 mmol), EDC. HCl (132 mg, 0.689 mmol). Oleoyl-Lys(Boc)-NHCH\(_2\)CH\(_3\) was obtained as a white solid. Yield: 216 mg (MW = 537.82, 65.0%) R\(_f\)= 0.88 (CHCl\(_3\):MeOH = 5:1 v/v). Intermediate H-Lys(Boc)-NHCH\(_2\)CH\(_3\) R\(_f\)= 0.40 (BAW). 1H NMR (300 MHz, CDCl\(_3\)) δ: 6.19 (br m, 1H, amine NH), 5.34 (m, 2H, oleoyl CH =CH), 4.60 (br s, 1H, lysine ε-NH), 4.35 (q, 1H, lysine α-CH\(^*\)), 3.28 (dq, 2H, amine CH\(_2\)), 3.09 (m, 2H, lysine ε-CH\(_2\)), 2.32 (t, trace, 20 oleic acid α-CH\(_2\)), 2.24 (t, 2H, oleoyl α-CH\(_2\)), 2.02 (m, 4H, oleoyl CH\(_2\)-CH=CH), 1.83 (m, 1H, lysine β-C\(_3\)H\(_2\)); (m, NH\(_3^+\)-CH\(_2\)-C\(_3\)H\(_2\)), 1.14 (t, 3H, amine CH\(_3\)), 1.28-1.31 (m, 20H, oleoyl CH\(_2\)); (m, 2H, lysine γ-CH\(_2\)); 1.14 (t, 3H, amine CH\(_3\)), 0.88 (t, 3H, oleoyl CH\(_3\)). LCQ (ESI) calculated (C\(_{31}\)H\(_{59}\)N\(_3\)O\(_4\)): 537.4, found: 538.1 (M+H\(^+\)), 1075.1 (2M+H\(^+\)), 1129.5, 1151.7.

S.2.3. Synthesis of Oleoyl-Lys(H)-NHCH\(_2\)CH\(_3\).HCl.

As for \( (Oleoyl-Lys(H)-NH)_{2}(CH_2)_6\)\(_2\)HCl (section 2.2.3) without the final chromatography step, starting from Oleoyl-Lys(Boc)-NHCH\(_2\)CH\(_3\) (216 mg, 0.40 mmol). Oleoyl-Lys(H)NHCH\(_2\)CH\(_3\).HCl was obtained as a white solid. Yield: 177 mg (MW = 474.16, 93%) R\(_f\)= 0.28 (CHCl\(_3\):MeOH = 5:1 v/v). 1H NMR (300 MHz, DMSO-d\(_6\)) δ: 5.34 (m, 2H, CH=CH\(_3\)), 4.33 (q, J = 5.71 Hz, 1H, CO-CH\(^*\)-NH), 3.38 (m, 2H, NH-CH\(_2\)-CH\(_3\)), 3.23 (m, 2H, NH\(^+\)-CH\(_2\)-CH\(_3\)), 2.24 (t, J = 7.66 Hz, 2H, NH-CO-CH\(_3\)), 2.02 (m, 4H, CH=CH-CH\(_3\)), 1.55-1.80 (m, 2H, CO-CH\(_2\)-CH\(_2\)); (m, NH\(^+\)-CH\(_2\)-CH\(_2\)); (m, 2H, NH-C\(_3\)-CH\(_2\)); 1.20-1.45 (m, 20H, CH\(_2\)-Alkyl tail); (m, 4H, NH-CH\(^*\)-CH\(_2\)-CH\(_3\)), 1.14 (t, J = 7.36 Hz, 3H, NH-CH\(_3\)-CH\(_3\)), 0.88 (t, J = 6.76 Hz, 3H, CH\(_3\)-Alkyl tail). 13C NMR (300 MHz, DMSO-d\(_6\)) δ: 172.0, 171.3, 129.5, 52.2, 35.1, 33.3, 31.2, 29.0, 28.6, 28.5, 26.5, 25.2, 22.0, 14.6, 13.9. LCQ (ESI) calculated (C\(_{26}\)H\(_{52}\)N\(_3\)O\(_2\)): 437.8, found: 438.3 (M+H\(^+\)), 875.1 (2M+H\(^+\)).
S.3. Incorporation of the diamine spacer (step iv of Scheme 1) to give (Z-Lys(Boc)-NH)2(CH2)n.

S.3.2. Synthesis of (Z-Lys(Boc)-NH)2(CH2)2.

As for (Z-Lys(Boc)-NH)2(CH2)6 (section 2.2.1), with Z-Lys(Boc)-OH.DCHA salt (1.5155 g, 2.7075 mmol) yielding 0.8977 g Z-Lys(Boc)OH (2.360 mmol, 87.2%), ethylenediamine (0.0709 mL, 1.0618 mmol), HOBt.H2O (0.4332 g, 2.8315 mmol), EDC.HCl (0.4507 g, 2.3596 mmol) in 20 mL ethyl acetate saturated with H2O. (Z-Lys(Boc)-NH)2(CH2)2 was obtained as a white solid. Yield: 1.0980 g (MW = 784.94, 69.41 %) Rf = 0.57 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, DMSO-d6) δ: 8.06 (br s, 2H, NH), 7.47 (m, 10H, Z: Ar); (br s, 2H, NH), 6.86 (br t, 2H, NH), 5.13 (m, 4H, Z CH2), 4.00 (q, 2H, lysine α-H*), 3.21 (br s, 4H, diamine 1,2-CH2), 2.99 (q, 4H, lysine ε-CH2), 1.69 (br m, 2H, lysine β-CHH), 1.62 (br m, 2H, lysine β-CHH), 1.48 (s, 18H, Boc CH3), 1.27 (m, 4H, lysine γ-CH2). LCQ (ESI) calculated (C40H60N6O10): 784.4, found: 785.1 (M+H)+, 801.9 (M+NH4)+, 807.3 (M+Na)+.

S.3.3. Synthesis of (Z-Lys(Boc)-NH)2(CH2)3.

As for (Z-Lys(Boc)-NH)2(CH2)6 (section 2.2.1), with Z-Lys(Boc)-OH.DCHA salt (1.3081 g, 2.329 mmol) yielding 920 mg Z-Lys(Boc)OH (2.418 mmol, 103%), 1,3-diaminopropane (83.5 μL, 1.00 15 mmol), HOBt.H2O (337.7 mg, 2.205 mmol), EDC.HCl (428.3 mg, 2.234 mmol) in 20 mL ethyl acetate saturated with H2O. (Z-Lys(Boc)-NH)2(CH2)3 was obtained as a white solid. Yield: 490 mg (MW = 798.97, 61.31%) Rf = 0.66 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) δ: 7.33 (s, 10H, Z Ar), 6.84 (br s, 2H, diamine NH), 5.56 (br s, 2H, lysine α-NH), 5.08 (s, 4H, Z CH2), 4.56 (s, 2H, Boc NH), 4.11 (m, 2H, Lysine α-CH*), 3.35 (m, 2H, diamine 1,3-CH2), 3.08 (m, 4H, diamine 1,3-CH2); (m, 4H, lysine ε-CH2), 1.81 (m, 2H, lysine β-CHH), 1.62 (m, 2H, lysine β-CHH), 1.41 (s, 18H, Boc NH), 1.53-1.33 (m, 4H, lysine δ-CH2), (m, 4H, lysine γ-CH2); (m, diamine 2-CH2). 13C NMR (300 MHz, CDCl3) δ: 172.4, 156.2, 136.2, 128.5, 128.0, 79.2, 67.0, 55.1, 39.8, 36.6, 31.8, 29.6, 28.4, 22.5. LCQ (ESI) calculated (C41H62N6O10): 798.5, found: 799.5 (M+H)+, 821.5 (M+Na), 1619.0 (2M+Na)+.

S.3.4. Synthesis of (Z-Lys(Boc)-NH)2(CH2)4.

As for (Z-Lys(Boc)-NH)2(CH2)6 (section 2.2.1) with Z-Lys(Boc)-OH.DCHA salt (1.318 g, 2.346 mmol) yielding 811.2 mg Z-Lys(Boc)OH (2.135 mmol, 91.2%), 1,4-diaminobutane (94.1 mg, 1.07 mmol), HOBt.H2O (323.5 mg, 2.11 mmol), EDC.HCl (408.6 mg, 2.131 mmol) in 20 mL ethyl acetate saturated with H2O. (Z-Lys(Boc)-NH)2(CH2)4 was obtained as a white solid. Yield: 539.2 mg (MW = 812.99, 66.3%) Rf = 0.59 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) δ: 7.32 (s, 10H, Z Ar), 6.69 (br s, 2H, diamineNH), 5.71 (br s, 2H, lysine α-NH), 5.07 (s, 4H, Z CH2), 4.70 (s, 2H, lysine ε-NH), 4.13 (m, 2H, lysine α-CH*), 3.27 (m, 2H, diamine 1,4-CHH), 3.09 (m, 4H, diamine 1,4-CHH); (m, 4H, lysine ε-CH2), 1.80 (m, 2H, lysine β-CHH), 1.70 (s, 2H, lysine β-CHH), 1.45 (s, 18H, Boc CH3), 1.29-1.35 (s, 4H, lysine δ-CH2); (m, 4H, lysine γ-CH2); (t, 4H, diamine 2,3-CH2), 1.25 t. 13C
NMR (300 MHz, CDCl 3) δ: 171.8, 155.8, 135.8, 128.0, 127.5, 78.7, 66.5, 54.4, 39.5, 38.4, 31.8, 29.0, 28.0, 25.9, 22.1. LCQ (ESI) calculated (C 42H64N6O10): 812.5, found: 813.5 (M+H)+, 835.5 (M+Na)+, 1647.1 (2M+Na)+.

S.3.5. Synthesis of \((Z\text{-Lys(Boc)-NH})_2(CH_2)_5\).

As for \((Z\text{-Lys(Boc)-NH})_2(CH_2)_6\) (section 2.2.1), with Z-Lys(Boc)-OH.DCHA salt (3.1104 g, 5.537 mmol) yielding 1.985 g Z-Lys(Bo c)OH (5.21 mmol, 94.3%), 292.6 μl 1,5-diaminopentane (2.50 mmol), 952.1 mg HOBt.H 2O (6.22 mmol), 1.0621 g EDC.HCl (5.54 mmo l) in 20 mL ethyl acetate saturated with H 2O. \((Z\text{-Lys(Boc)-NH})_2(CH_2)_5\) was obtained as a white solid. Yield: 2.223 g (MW = 827.02, 84.9%), R f = 0.65 (CHCl 3:MeOH=5:1 v/v). 1H NMR (300 MHz, CDCl 3) δ: 7.28 (s, 10H, Z Ar), 6.68 (br s, 2H, diamine NH), 5.93 (br s, 2H, lysine NH), 5.03 (br s, 4H, Z CH 2), 4.73 (br s, 2H, 10 lysine ε-NH), 4.16 (m, 2H, lysine α-CH*), 3.34 (br m, 2H, diamine 1,5-C H H), 3.10 (br m, 2H, diamine 1,5-CH H), 1.81 (m, 4H, lysine ε-CH2), 1.48 (m, 4H, lysine ε-CH2), 1.43 (s, 18H, Boc CH 3), 1.24 (m, 2H, diamine 3-CH2). 13C NMR (300MHz, CDCl 3) δ: 172.3, 156.6, 156.2, 136.2, 128.5, 127.4, 78.6, 66.4, 54.4, 39.5, 38.7, 31.9, 29.1, 28.0, 25.6, 22.1. LCQ (ESI) calculated (C 32H66N6O10): 854.5, found: 855.5 (M+H)+, 877.5 (M+Na)+, 1731.1 (2M+Na)+.

S.3.7. Synthesis of \((Z\text{-Lys(Boc)-NH})_2(CH_2)_7\).

As for \((Z\text{-Lys(Boc)-NH})_2(CH_2)_6\) (section 2.2.1), with Z-Lys(Boc)-OH.DCHA salt (1.297 g, 2.31 mmol) yielding 814.2 mg Z-Lys( Boc)OH (2.14 mmol, 92.6%), 1,7-diaminoheptane (132.0 mg, 1.0 mmol), HOBt.H 2O (352.2 mg, 2.3 mmol), EDC.HCl (480.1 mg, 2.5 mmol) in 20 mL ethyl acetate saturated with H 2O. \((Z\text{-Lys(Boc)-NH})_2(CH_2)_7\) was obtained as a white solid. Yield: 242.6 mg (MW = 855.07, 28.7%) R f = 0.75 (CHCl 3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl 3) δ: 7.32 (s, 10H, Z Ar), 6.52 (br s, 2H, diamine NH), 5.78 (br d, 2H, lysine α-NH), 5.07 (s, 4H, Z CH 2), 4.68 (br s, 2H, lysine ε-NH), 4.15 (m, 2H, lysine α-CH*), 3.27 (m, 2H, diamine 1,7-C H H), 3.16 (m, 2H, diamine 1,7-CH H), 3.07 (m, 4H, lysine ε-CH2), 1.79 (m, 2H, lysine β-CH H), 1.43 (s, 2H, diamine 3-CH2). 13C NMR (300MHz, CDCl 3) δ: 171.6, 156.0, 155.7, 135.8, 127.4, 78.6, 66.4, 54.4, 39.5, 38.7, 31.9, 29.1, 28.0, 25.6, 22.1. LCQ (ESI) calculated (C 45H70N6O10): 882.5, found: 882.5 (M+H)+, 849.5 (M+Na)+, 1675.0 (2M+Na)+.

S.3.8. Synthesis of \((Z\text{-Lys(Boc)-NH})_2(CH_2)_8\).

As for \((Z\text{-Lys(Boc)-NH})_2(CH_2)_6\) (section 2.2.1) with Z-Lys(Boc)-OH.DCHA salt (5.07 g, 9.03 mmol) yielding 2.7900 g Z-Lys(Boc)OH (7.33 mmol, 81%), 1,8-diamino-octane (483.7 mg, 3.35 mmol), HOBt.H 2O (1.1204 g, 7.32 mmol), EDC.HCl (1.4093 g, 7.35 mmol) in 20 mL ethyl acetate saturated with H 2O. \((Z\text{-Lys(Boc)-NH})_2(CH_2)_8\) was obtained as a white solid. Yield: 2.0822 g (MW = 869.10, 71.6 %) R f = 0.67 (CHCl 3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl 3) δ: 7.33 (s, 10H, Z Ar), 6.68 (br s, 2H, diamine NH), 5.93 (br s, 2H, lysine NH), 5.03 (br s, 4H, Z CH 2), 4.73 (br s, 2H, lysine ε-NH), 4.16 (m, 2H, lysine α-CH*), 3.34 (br m, 2H, diamine 1,8-C H H), 3.10 (br m, 2H, diamine 1,8-CH H), 1.24 (m, 2H, diamine 3-CH2). 13C NMR (300MHz, CDCl 3) δ: 172.3, 156.6, 156.2, 135.8, 128.5, 128.0, 79.1, 67.0, 54.9, 39.9, 38.7, 32.0, 29.5, 28.4, 22.6. LCQ (ESI) calculated (C 43H70N6O10): 884.5, found: 885.5 (M+H)+, 849.5 (M+Na)+, 1731.1 (2M+Na)+.
Ar), 6.34 (br s, 2H, diamine NH), 5.59 (br s, 2H, lysine α-NH), 5.08 (s, 4H, Z CH2), 4.65 (s, 2H, lysine ε-NH), 4.12 (m, 2H, lysine α-CH*), 3.26 (m, 2H, diamine 1,8-CH2H), 3.16 (m, 2H, diamine 1,8-CHH), 3.07 (m, 4H, lysine ε-CH2), 1.80 (m, 2H, lysine β-CHH), 1.64 (m, 2H, lysine β-CHH), 1.42 (s, 18H, Boc: CH3), 1.31-1.53 (m, 4H, lysine δ-CH2); (m, 4H, lysine γ-CH2); (m, 4H, diamine 2,7-CH2), 1.26 (br s, 8H, diamine 3,4,5,6-CH2). LCQ (ESI) calculated (C46H72N6O10): 868.5, found: 869.6 (M+H)+, 891.6 (M+Na)+, 1760.1 (2M+Na)+.

S.4.n. Z-Deprotection/Acylation (step v-vi of Scheme 1) to give (Oleoyl-Lys(Boc)-NH)2(CH2)n.

S.4.2. Synthesis of (Oleoyl-Lys(Boc)-NH)2(CH2)2.

As for (Oleoyl-Lys(Boc)-NH)2(CH2)6 (section 2.2.2) starting from Z-Lys(Boc)NH)2(CH2)2 (0.3018 g, 0.3845 mmol), Pd/C catalyst (0.1 g) in 50 mL DMF, oleic acid (0.2441 mL, 0.7690 mmol), HOBt.H2O (0.1412 g, 0.9227 mmol) and EDC.HCl (0.1616 g, 0.8459 mmol). (Oleoyl-Lys(Boc)-NH)2(CH2)2 was obtained as a white solid. Yield: 0.2296 g (MW = 1045.57, 57.11 %) Rf = 0.72 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) δ: 7.06 (br s, 2H, diamine NH), 6.58 (br s, 2H, lysine α-NH), 5.34 (m, 4H, lysine CH=CH), 4.83 (br s, 2H, lysine ε-NH), 4.30 (m, 2H, lysine α-CH*), 3.46 (m, 2H, lysine β-CH/H), 3.29 (m, 2H, lysine β-CHH), 3.11 (m, 4H, diamine 1,2-CH2), 2.33 (t, 150.48H, oleic acid α-CH2), 2.20 (t, 4H, oleoyl α-CH2), 2.00 (br q, 8H, oleoyl C1H2-CH=CH), 1.79 (br m, 2H, lysine β-C1H2), 1.60 (br m, 2H, lysine β-CHH); (br m, 4H lysine δ-CH2), 1.57-1.38 (m, 4H, oleoyl β-CH2); (s, 18H, Boc CH3), 1.28 (br m, 4H, lysine γ-CH2) (br m, 40H, oleoyl CH2), 0.90 (t, 6H, oleoyl CH3). LCQ (ESI) calculated (C60H112N6O8): 1044.9, found: 1045.4 (M+H)+, 1067.7 (M+Na)+.

S.4.3. Synthesis of (Oleoyl-Lys(Boc)-NH)2(CH2)3.

As for (Oleoyl-Lys(Boc)-NH)2(CH2)6 (section 2.2.2) starting from Z-Lys(Boc)NH)2(CH2)3 (301 mg, 0.377 mmol), Pd/C catalyst (71 mg) in 75 mL DMF, oleic acid (228 μl, 0.719 mmol), HOBt.H2O (160 mg, 1.045 mmol) and EDC.HCl (161 mg, 0.83 mmol). (Oleoyl-Lys(Boc)-NH)2(CH2)3 was obtained as a white solid. Yield: 207.7 mg (MW = 1059.59, 52 %) Rf = 0.72 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) δ: 7.18 (br s, 2H, diamine NH), 6.35 (br d, 2H, lysine α-NH), 5.34 (m, 4H, lysine ε-CH2), 4.74 (s, 2H, lysine ε-NH), 4.34 (q, 2H, lysine α-CH*), 3.44 (m, 2H, diamine 1,3CHH), 3.11 (m, 2H, diamine 1,3CHH) (m, 4H, lysine ε-CH2), 2.34 (t, 0.05H, oleic acid α-CH2), 2.27 (t, 4H, oleoyl α-CH2), 2.00 (br q, 8H, oleoyl CH2-CH=CH), 1.79 (br m, 2H, lysine β-CH/H), 1.60 (br m, 2H, lysine β-CHH); (br m, 4H lysine δ-CH2), 1.57-1.38 (m, 4H, oleoyl β-CH2); (s, 18H, Boc CH3), 1.28 (br m, 4H, lysine γ-CH2) (br m, 40H, oleoyl CH2), 0.90 (t, 6H, oleoyl CH3). LCQ (ESI) calculated (C61H114N6O8): 1058.8, found: 1059.5 (M+H)+, 1081.7 (M+Na)+.

S.4.4. Synthesis of (Oleoyl-Lys(Boc)-NH)2(CH2)4.

As for (Oleoyl-Lys(Boc)-NH)2(CH2)6 (section 2.2.2) starting from Z-Lys(Boc)NH)2(CH2)4 (225.6 mg, 0.277 mmol), Pd/C catalyst (0.10 g) in 75 mL DMF, oleic acid (0.2300 mL, 1.2246 mmol),
S.4.5. Synthesis of \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_4\). 

As for \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_6\) (section 2.2.2) starting from \(Z\text{-Lys(Boc)NH})_2(\text{CH}_2)_7\) (0.4911 g, 0.5938 mmol), Pd/C catalyst (0.10 g) in 50 mL DMF, oleic acid (0.4600 mL, 1.4492 mmol), HOBt.H2O (0.7360 g, 4.8105 mmol), EDC.HCl (0.6900 g, 3.6126 mmol). \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_4\) was obtained as a white solid. Yield: 0.260 g (MW = 1087.65, 40.25%) \(R_f = 0.76 \) (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) \(\delta\): 7.06 (br m, 2H, diamine NH), 6.96 (br d, 2H, lysine \(\alpha\)-NH), 5.34 (m, 4H, oleoyl CH=CH), 4.87 (m, 2H, lysine \(\varepsilon\)-NH), 4.47 (q, 2H, lysine \(\alpha\)-CH*), 3.42 (m, 2H, diamine 1,5-CH2H), 3.10 (m, 2H, diamine 1,5-CH2H); (m, 4H, lysine \(\varepsilon\)-CH2), 2.17 (dt, 4H, oleoyl \(\alpha\)-CH2), 2.00 (q, 4H, oleoyl C3H2-CH=CH), 1.83 (m, 2H, lysine \(\beta\)-CH2H); (m, 4H, oleoyl \(\beta\)-CH2), 1.43 (m, 18H, Boc CH3); (M, 4H, diamine 2,4-CH2), 1.27 (br m, 40H, oleoyl CH2); (m, 2H, diamine 3-CH2), 0.88 (t, 6H, oleoyl CH3). LCQ (ESI) calculated (C63H118N6O8): 1086.9, found: 1087.5 (M+H)+, 1109.7 (M+Na)+. 

S.4.7. Synthesis of \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_7\).

As for \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_8\) (section 2.2.2) starting from \(Z\text{-Lys(Boc)NH})_2(\text{CH}_2)_7\) (0.1951 g, 0.2282 mmol), Pd/C catalyst (0.10 g) in 50 mL DMF, oleic acid (0.1449 mL, 0.4546 mmol), HOBt.H2O (0.0838 g, 0.5477 mmol) and EDC.HCl (0.0959 g, 0.5020 mmol). \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_7\) was obtained as a white solid. Yield: 0.1785 g (MW = 1115.70, 70.11%) \(R_f = 0.78 \) (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) \(\delta\): 7.42 (br s, 2H, diamine NH), 6.95 (br d, 2H, lysine \(\alpha\)-NH), 5.34 (m, 4H, oleoyl CH=CH), 4.87 (br m, 2H, lysine \(\varepsilon\)-NH), 4.59 (q, 2H, lysine \(\alpha\)-CH*), 3.39 (m, 2H, diamine 1,7-CH2H), 3.08 (m, 2H, diamine 1,7-CH2H); (m, 4H, lysine \(\varepsilon\)-CH2), 2.33 (t, 0.37H, oleic acid \(\alpha\)-CH2), 2.17 (m, 4H, oleoyl \(\alpha\)-CH2), 2.00 (q, 8H, oleoyl CH2-CH=CH), 1.78 (m, 2H, lysine \(\beta\)-CH2H); (m, 4H, lysine \(\delta\)-CH2), 1.55-1.39 (m, 4H,diamine 2,5-CH2); (m, 4H, oleoyl \(\beta\)-CH2); (m, 18H, Boc CH3); (M, 4H, diamine 3,4,5-CH2), 0.88 (t, 6H, oleoyl CH3). LCQ (ESI) calculated (C65H122N6O8): 1114.932, found: 1115.6 (M+H)+, 1137.7 (M+Na)+.

S.4.8. Synthesis of \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_8\).

As for \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_8\) (section 2.2.2) starting from \(Z\text{-Lys(Boc)NH})_2(\text{CH}_2)_8\) (499 mg, 0.575 mmol), Pd/C catalyst (81 mg) in 75 mL DMF, oleic acid (228 \(\mu\)l, 0.715 mmol), HOBt.H2O (804 mg, 5.255 mmol) and EDC.HCl (499 mg, 2.613 mmol). \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_8\) was obtained as a white solid. Yield: 665 mg (MW = 1129.73 g/mol, 107%) \(R_f = 0.80 \) (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, CDCl3) \(\delta\): 6.90 (br t, 2H, diamine NH), 6.50 (br d, 2H, lysine \(\alpha\)-NH), 5.34 (m, 4H, lysine CH=CH), 4.76 (br s, 2H, lysine \(\varepsilon\)-NH), 4.47 (q, 2H, lysine \(\alpha\)-CH*), 3.39 (m, 2H,
diamine 1,8-CH\textsubscript{2}H), 3.09 (m, 2H, diamine 1,8-CH\textsubscript{2}H), 1.77 (m, 2H, lysine β-CH\textsubscript{2}H), 1.62 (m, 2H, lysine β-CH\textsubscript{2}H), 1.55-1.39 (m, 4H, diamine 2,7-CH\textsubscript{2}), (m, 4H, oleoyl β-CH\textsubscript{2}); (m, 18H, Boc CH\textsubscript{3}), 1.28-1.31 (br m, 40H, oleoyl CH\textsubscript{2}); (br m, 4H, diamine 3,4,5,6-CH\textsubscript{2}), 0.88 (t, 6H, oleoyl CH\textsubscript{3}). LCQ (ESI) calculated (C\textsubscript{66}H\textsubscript{124}N\textsubscript{6}O\textsubscript{8}): 1128.9, found: 1129.6 (M+H)+, 1151.8 (M+Na)+.

S.5.n. Boc-Deprotection (step iii of Scheme 1) to give (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{n}.2HCl.

S.5.2. Synthesis of (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{2}.2HCl.

As for (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{6}.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{2} (0.1641 g, 0.1569 mmol). (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{2}.2HCl was obtained as a white solid. Yield: 0.1113 g (Mw = 918.26, 77.23%) Rf = 0.09 (CHCl\textsubscript{3}:MeOH = 5:1 v/v). \textsuperscript{1}H NMR (300 MHz, DMSO-d\textsubscript{6}) δ: 7.95 (m, 10H, NH), 5.32 (m, 4H, oleoyl CH=CH), 4.18 (m, 2H, lysine α-CH\textsuperscript{*}), 3.10 (q, 4H, diamine 1,2-CH\textsubscript{2}), 2.73 (q, 4H, lysine ε-CH\textsubscript{2}), 2.18 (t, oleic acid α-CH\textsubscript{2}), 2.13 (t, 4H, oleoyl α-CH\textsubscript{2}), 1.98 (m, 8H, oleoyl CH\textsubscript{2}-CH=CH), 1.42-1.64 (m, 4H, lysine δ-CH\textsubscript{2}); (m, 4H, oleoyl β-CH\textsubscript{2}); (m, 4H, lysine β-CH\textsubscript{2}); 0.85 (t, 6H, oleoyl CH\textsubscript{3}). \textsuperscript{13}C NMR (300MHz, DMSO-d\textsubscript{6}) δ: 172.2, 171.8, 129.5, 52.6, 52.3, 51.1, 33.2, 31.2, 29.0, 28.5, 26.5, 25.1, 24.3, 22.0, 13.9. LCQ (ESI) calculated (C\textsubscript{50}H\textsubscript{98}Cl\textsubscript{2}N\textsubscript{6}O\textsubscript{4}): 844.7, found: 845.6 (M+H)+, 423.4 (M+2H)\textsuperscript{2+}.

S.5.3. Synthesis of (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{3}.2HCl.

As for (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{6}.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{3} (166.06 mg, 0.157 mmol). (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{3}.2HCl was obtained as a white solid. Yield: 132 mg (MW = 932.28, 98%) Rf = 0.32 (CHCl\textsubscript{3}:MeOH = 5:1 v/v). \textsuperscript{1}H NMR (300 MHz, DMSO-d\textsubscript{6}) δ: 7.92 (m, 4H, amide NH), 7.77 (m, 6H, lysine NH\textsubscript{3}), 5.36 (m, 4H, oleoyl CH=CH), 4.18 (m, 2H, lysine α-CH\textsuperscript{*}), 3.09 (q, 4H, diamine 1,3-CH\textsubscript{2}), 2.79 (q, 4H, lysine ε-CH\textsubscript{2}), 2.19 (t, 4H, oleoyl α-CH\textsubscript{2}), 2.05 (m, 8H, oleoyl CH\textsubscript{2}-CH=CH), 1.48-1.65 (m, 2H, diamine 2 CH\textsubscript{2}); (m, 4H, lysine δ-CH\textsubscript{2}); (m, 4H, oleoyl β-CH\textsubscript{2}); (m, 4H, lysine β-CH\textsubscript{2}); 1.23-1.40 (br s, 40H, oleoyl CH\textsubscript{2}); (br s, 4H, γ-CH\textsubscript{2}); 0.85 (t, 6H, oleoyl CH\textsubscript{3}). \textsuperscript{13}C NMR (300MHz, DMSO-d\textsubscript{6}) δ: 172.1, 171.8, 129.5, 52.6, 52.3, 51.1, 33.2, 31.2, 29.0, 28.5, 26.5, 25.1, 24.3, 22.0, 13.9. LCQ (ESI) calculated (C\textsubscript{51}H\textsubscript{100}N\textsubscript{6}O\textsubscript{4}): 858.8, found: 859.7 (M+H)+, 432.4 (M+2H)\textsuperscript{2+}.

S.5.4. Synthesis of (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{4}.2HCl.

As for (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{6}.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{4} (0.1289 g, 0.1201 mmol). (Oleoyl-Lys(H)-NH\textsubscript{2})(CH\textsubscript{2})\textsubscript{4}.2HCl was obtained as a white solid. Yield: 0.0987 g (MW = 946.31, 86.88%) Rf = 0.10 (CHCl\textsubscript{3}:MeOH = 5:1 v/v). \textsuperscript{1}H NMR (300 MHz, DMSO-d\textsubscript{6}) δ: 7.86 (m, 4H, amide NH), 7.81 (m, 6H, lysine NH\textsubscript{3}), 5.30 (m, 4H, oleoyl CH=CH), 4.14 (m, 2H, lysine α-CH\textsuperscript{*}), 3.00 (q, 4H, diamine 1,4-CH\textsubscript{2}),
2.70 (q, 4H, lysine ε-CH2), 2.11 (t, 4H, oleoyl α-CH2), 2.96 (m, 8H, oleoyl CH2-CH=CH), 1.40-1.62 (m, 2H, diamine 2,3-CH2); (m, 4H, lysine δ-CH2); (m, 4H, oleoyl β-CH2); (m, 4H, lysine β-CH2); 1.34 (m, 4H, NH-CH*-C6H5), 1.10-1.26 (br s, 40H, oleoyl CH2); (br s, 4H, lysine γ-CH2), 0.80 (t, 3H, oleoyl CH3). 13C NMR (300 MHz, DMSO-d6) δ: 172.1, 171.5, 129.6, 52.2, 35.1, 31.5, 31.2, 29.0, 28.8, 28.6, 26.5, 25.2, 22.3, 22.0, 13.9. LCQ (ESI) calculated (C52H100N6O4): 872.78, found: 873.7 (M+H)+ and 437.5 (M+2H)+.

S.5.5. Synthesis of (Oleoyl-Lys(H)-NH)2(CH2)5.2HCl.

As for (Oleoyl-Lys(H)-NH)2(CH2)6.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH)2(CH2)5 (0.1032 g, 0.0949 mmol). (Oleoyl-Lys(H)-NH)2(CH2)5.2HCl was obtained as a white solid. Yield: 0.0976 g (MW = 960.34, 107.11%) Rf = 0.09 (CHCl3:MeOH = 5:1 v/v, ninhydrine). 1H NMR (300 MHz, DMSO-d6) δ: 7.86 (m, 10H, NH), 5.32 (m, 4H, lysine CH=CH), 4.17 (m, 2H, lysine α-CH*), 3.01 (q, 4H, diamine 1,5-CH2), 2.72 (q, 4H, lysine ε-CH2), 2.11 (t, 4H, oleoyl α-CH2), 1.97 (m, 8H, oleoyl CH2-CH=CH), 1.50 (m, 4H, diamine 2,4-CH2); (m, 4H, lysine β-CH2); (m, 4H, oleoyl β-CH2), 1.18-1.41 (br s, 40H, oleoyl CH2); (br s, 2H, diamine 3-CH2); (br s, 4H, γ-CH2), 0.86 (t, 6H, oleoyl CH3). 13C NMR (300 MHz, DMSO-d6) δ: 172.1, 171.4, 129.5, 52.2, 35.1, 31.5, 31.2, 29.1, 29.0, 28.6, 26.6, 25.2, 23.6, 22.3, 22.0, 13.9. LCQ (ESI) calculated (C53H102N6O4): 886.80, found: 910.8 (M+Na)+, 887.7 (M+H)+, 444.5 (M+2H)2+.

S.5.7. Synthesis of (Oleoyl-Lys(H)-NH)2(CH2)7 .2HCl.

As for (Oleoyl-Lys(H)-NH)2(CH2)6.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH)2(CH2)7 (0.1383 g, 0.1240 mmol). (Oleoyl-Lys(H)-NH)2(CH2)7.2HCl was obtained as a white solid. Yield: 0.1159 g (MW = 988.39, 94.60%) Rf = 0.10 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, DMSO-d6) δ: 7.83 (m, 10H, NH), 5.32 (m, 4H, oleoyl CH=CH), 4.17 (m, 2H, lysine α-CH*), 3.02 (q, 4H, diamine 1,7-CH2), 2.73 (m, 4H, lysine ε-CH2), 2.11 (t, 4H, oleoyl α-CH2), 2.00 (m, 8H, oleoyl CH2-CH=CH), 1.42-1.67 (m, 4H, diamine 2,7-CH2); (m, 4H, lysine δ-CH2); (m, 4H, oleoyl β-CH2); (m, 4H, lysine β-CH2), 1.24 (m+s, 40H, oleoyl CH2); (s+m, 6H, 3,4,5-diamine 3,4,5-CH2); (s+m, 4H, lysine γ-CH2), 0.85 (t, 6H, oleoyl CH3). 13C NMR (300 MHz, DMSO-d6) δ: 172.0, 171.4, 129.5, 52.2, 35.1, 31.4, 31.2, 29.0, 28.8, 28.6, 26.5, 26.2, 22.3, 22.0, 13.9. LCQ (ESI) calculated (C55H106N6O4): 914.83, found: 915.7 (M+H)+ and 458.5 (M+2H)2+.

S.5.8. Synthesis of (Oleoyl-Lys(H)-NH)2(CH2)8 .2HCl.

As for (Oleoyl-Lys(H)-NH)2(CH2)6.2HCl (section 2.2.3) without the final chromatography step, starting from (Oleoyl-Lys(Boc)-NH)2(CH2)8 (335 mg, 0.297 mmol) was obtained as a white solid. Yield: 309 mg (MW = 1002.42, 96.4 %) Rf = 0.22 (CHCl3:MeOH = 5:1 v/v). 1H NMR (300 MHz, DMSO-d6) δ: 7.85-8.05 (br m, 10H, NH), 5.36 (m, 4H, oleoyl CH=CH), 4.17 (m, 2H, lysine α-CH*), 3.05 (m, 4H, oleoyl β-CH2), 2.75 (m, 4H, lysine ε-CH2), 2.15 (t, 4H, oleoyl α-CH2), 2.00 (m, 8H, oleoyl CH2-CH=CH), 1.42-1.67 (m, 4H, diamine 2,7-CH2); (m, 4H, lysine δ-CH2); (m, 4H, oleoyl β-CH2);
(m, 4H, lysine β-CH₂); 1.42 (m, 4H, NH-CH²-CH₂), 1.18-1.30 (m, 40H, oleoyl CH₂); (m, 8H, diamine 3,4,5,6-CH₂); 0.89 (t, 3H, oleoyl CH₃). 13C NMR (300 MHz, DMSO-d₆) δ: 172.2, 171.5, 129.5, 52.3, 48.5, 35.1, 31.2, 28.8, 26.5, 26.2, 25.24, 22.3, 22.0, 13.8. LCQ (ESI) calculated (C₅₆H₁₀₈N₆O₄): 928.8, found: 929.7 (M+H)+, 465.5 (M+2H)²⁺.

S.6. Preparation of the enantiomer (Oleoyl-D-Lys(H)-NH)₂(CH₂)₆·2HCl.

S.6.1. Synthesis of (Z-D-Lys(Boc)-NH)₂(CH₂)₆.

As for (Oleoyl-Lys(Boc)-NH)₂(CH₂)₆ starting from Z-D-Lys(Boc)OH.DCHA (3.137 g, 5.58 mmol), yielding 1.5707 g Z-D-Lys(Boc)-OH (4.13 mmol), and 1,6-diaminohexane (223.3 mg, 1.92 mmol), HOBT.H₂O (708.3 mg, 4.63 mmol), EDC.HCl (895.9 mg, 4.67 mmol). (Z-D-Lys(Boc)-NH)₂(CH₂)₆ was obtained as a white solid. Yield: 1.1563g (MW=841.04, 71.6 %) Rₐ=0.62. ¹H NMR (300MHz, CDCl₃) δ: 7.30 (s, 10H, Z Ar), 6.54 (br s, 2H, diamine NH), 5.06 (s, 4H, Z CH₂), 4.69 (br s, 2H, lysine ε-NH), 4.13 (m, 2H, lysine α-CH²), 3.28 (m, 2H, diamine 1,6-C₂H₆), 3.19 (m, 2H, diamine 1,6-CH₂), 3.08 (m, 4H, lysine ε-CH₂), 1.79 (m, 2H, lysine β-CH₂), 1.73 (m, 2H, lysine β-CH₂); (m, 4H, lysine δ-CH₂); (m, 4H, oleoyl β-CH₂), 1.58-1.42 (m, 30H, diamine 2,5-CH₂), 1.44 (s, 18H, Boc CH₂), 1.28-1.31 (m, 4H, lysine γ-CH₂); (m, 4H, oleoyl CH₂); (m, 2H, diamine 3,4-CH₂), 0.88 (t, 6H, oleoyl: CH₃). ¹³C NMR (300MHz, DMSO-d₆) δ: 174.4, 172.1, 171.5, 129.5, 52.3, 35.1, 31.4, 31.2, 28.9, 28.6, 28.5, 26.5, 25.9, 25.2, 24.4, 22.3, 22.0, 13.9. LCQ (ESI) calculated (C₅₆H₁₀₈N₆O₄): 900.8, found: 901.7 (M+H)+ and 451.4 (M+2H)²⁺.

S.6.2. Synthesis of (Oleoyl-D-Lys(Boc)-NH)₂(CH₂)₆.

As for (Oleoyl-Lys(Boc)-NH)₂(CH₂)₆, starting from (Z-D-Lys(Boc)-NH)₂(CH₂)₆. In this case the hydrogenation step (v) went less smoothly than for the L-enantiomer, for unknown reasons. The product was directly used for the next step.

S.6.3. Synthesis of (Oleoyl-D-Lys(H)-NH)₂(CH₂)₆·2HCl.

As for (Oleoyl-Lys(H)-NH)₂(CH₂)₆·2HCl (section 2.2.3) starting from (Oleoyl-D-Lys(Boc)-NH)₂(CH₂)₆ (0.0685g, 0.0622mmols). No precipitate was formed during workup. (Oleoyl-D-Lys(H)-NH)₂(CH₂)₆·2HCl was obtained as a white solid. Yield: 0.0612g (MW=974.36, 101.02%) Rₐ=0.09. ¹H NMR (300MHz, DMSO-d₆) δ: 6.79 (br t, 2H, diamine NH), 6.65 (br d, 2H, lysine α-NH), 5.32 (m, 4H, oleoyl CH=CH), 4.75 (br t, 2H, lysine ε-NH), 4.44 (q, 2H, lysine α-CH²), 3.37 (m, 2H, diamine 1,6-C₂H₆), 3.10 (m, 2H, diamine 1,6-CH₂); (m, 4H, oleoyl CH₂), 2.17 (m, 4H, oleoyl α-CH₂), 2.00 (m, 8H, oleoyl CH₂-CH=CH), 1.80 (m, 2H, lysine β-CH₂); (m, 2H, lysine β-CH₂); (m, 4H, lysine δ-CH₂); (m, 4H, oleoyl β-CH₂), 1.58-1.42 (m, 4H, diamine 2,5-CH₂), 1.44 (s, 18H, Boc CH₂), 1.28-1.31 (m, 4H, lysine γ-CH₂); (m, 4H, oleoyl CH₂); (m, 2H, diamine 3,4-CH₂), 0.88 (t, 6H, oleoyl: CH₃). ¹³C NMR (300MHz, DMSO-d₆) δ: 174.4, 172.1, 171.5, 129.5, 52.3, 35.1, 33.6, 31.4, 31.2, 28.9, 28.6, 28.5, 26.5, 25.9, 25.2, 24.4, 22.3, 22.0, 13.9. LCQ (ESI) calculated (C₅₆H₁₀₈N₆O₄): 900.8, found: 901.7 (M+H)+ and 451.4 (M+2H)²⁺.
S. 7. Preparation of the acyl analogues of \((\text{Oleoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\).

S. 7.1. Synthesis of \((\text{Stearoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\).

As for compound \((\text{Oleoyl-Lys(Boc)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) (section 2.2.2) with \((\text{Z-Lys(Boc)-NH})_2(\text{CH}_2)_6\) (395.7 mg, 0.427 mmol), Pd/C catalyst (100 mg), HOBt.H\(_2\)O (188.4 mg, 1.230 mmol), stearic acid (307.8 mg, 1.08 mmol), EDC.HCl (240.7 mg, 1.256 mmol). The intermediate Boc-protected gemini was not characterized but deprotection was continued as for compound (3.2.3). After Boc deprotection using a 2M HCl in EtOAc solution, a white precipitate was formed that could be isolated by centrifugation and drying in a desiccator in vacuo. \((\text{Stearoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) was obtained as a white solid. Yield: 137.6 mg (MW = 978.39, 33%). \(^1\)H NMR (300 MHz, CDCl\(_3/\text{CD}_3\)OD) \(\delta\): 7.28-7.85 (m, 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 3.33 (m, 2H, ?), 3.22 (q, 4H, diamine 1,6-CH\(_2\)), 2.91 (q, 4H, lysine \(\varepsilon\)-CH\(_2\)), 2.23 (t, 4H, alkyl \(\alpha\)-CH\(_2\)), 1.39-1.84 (m, 4H, diamine 2,5-CH\(_2\)); (m, 4H, lysine \(\delta\)-CH\(_2\)); (m, 4H, lysine \(\beta\)-CH\(_2\)); 1.22-1.39 (m, 68H, alkyl CH\(_2\)); (m, 4H, diamine 3,4-CH\(_2\)); (m, 4H, lysine \(\gamma\)-CH\(_2\)); 0.88 (t, 6H, alkyl CH\(_3\)). LCQ Calculated mass (C\(_{54}\)H\(_{108}\)N\(_6\)O\(_4\)) 904.84, found: 906.0 (M+H\(^+\)), 554.0 (2M+H\(^+\)).

S. 7.2. Synthesis of \((\text{Palmitoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\).

As for \((\text{Stearoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) with \((\text{Z-Lys(Boc)-NH})_2(\text{CH}_2)_6\) (236.6 mg, 0.281 mmol), Pd/C catalyst (100 mg). HOBt.H\(_2\)O (101.4 mg, 0.662 mmol), palmitic acid (C\(_{15}\)H\(_{31}\)COOH, 170.5 mg, 0.665 mmol), EDC.HCl (137.6 mg, 0.718 mmol). \((\text{Palmitoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) was obtained as a white solid. Yield: 164.0 (MW = 922.29, 64.2%). \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\): 7.82-7.89 (m, 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 2.99 (q, 4H, diamine 1,6-CH\(_2\)), 2.70 (q, 4H, lysine \(\varepsilon\)-CH\(_2\)), 2.09 (t, 4H, alkyl \(\alpha\)-CH\(_2\)), 1.39-1.63(m, 4H, diamine 2,5-CH\(_2\)); (m, 4H, lysine \(\delta\)-CH\(_2\)); (m, 4H, lysine \(\beta\)-CH\(_2\)); 1.30-1.39 (m, 4H, alkyl \(\beta\)-CH\(_2\)); 1.15-1.30 (m, 52H , alkyl CH\(_2\)); (m, 4H, diamine 3,4-CH\(_2\)); (m, 4H, lysine \(\gamma\)-CH\(_2\)); 0.83 (t, 6H, alkyl CH\(_3\)). LCQ Calculated mass (C\(_{50}\)H\(_{101}\)N\(_6\)O\(_4\)) +) : 849.78, found: 850.0 (M+H\(^+\)), 426.0 (M+2H\(^+\)).

S. 7.3. Synthesis of \((\text{Myristoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\).

As for \((\text{Stearoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) with \((\text{Z-Lys(Boc)-NH})_2(\text{CH}_2)_6\) (210.3 mg, 0.250 mmol), Pd/C catalyst (100 mg). HOBt.H\(_2\)O (92.0 mg, 0.601 mmol), myristic acid (C\(_{14}\)H\(_{27}\)COOH, 143 mg, 0.626 mmol), EDC.HCl (113.1 mg, 0.590 mmol). \((\text{Myristoyl-Lys(H)-NH})_2(\text{CH}_2)_6\cdot2\text{HCl}\) was obtained as a white solid. Yield: 210.3 mg (MW = 866.18, 97.1%). \(^1\)H NMR (300 MHz, DMSO-d\(_6\)) \(\delta\): 7.81-7.88 (m, 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 2.90 (q, 4H, diamine 1,6-CH\(_2\)), 2.70 (q, 4H, lysine \(\varepsilon\)-CH\(_2\)), 2.08 (t, 4H, alkyl \(\alpha\)-CH\(_2\)), 1.38-1.64(m, 4H, diamine 2,5-CH\(_2\)); (m, 4H, lysine \(\delta\)-CH\(_2\)); (m, 4H, lysine \(\beta\)-CH\(_2\)); 1.28-1.38 (m, 4H, alkyl \(\beta\)-CH\(_2\)); 1.09-1.28 (m, 44H , alkyl CH\(_2\)); (m, 4H, diamine 3,4-CH\(_2\)); (m, 4H, lysine \(\gamma\)-CH\(_2\)); 0.83 (t, 6H, alkyl CH\(_3\)). LCQ Calculated mass (C\(_{46}\)H\(_{93}\)N\(_6\)O\(_4\)) - : 793.73, found: 793.9 (M+H\(^+\)), 397.8 (M+2H\(^+\)).
S.7.4. Synthesis of \((\text{Lauroyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\).

As for \((\text{Stearoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) with \((Z\text{-Lys(Boc)}\text{-NH})_2(\text{CH}_2)_6\) (251.0 mg, 0.298 mmol), Pd/C catalyst (100 mg), HOBt.H_2O (184.2, 1.204 mmol), lauric acid (C_{11}H_{23}COOH, 144.9 mg, 0.72 3mmol), EDC.HCl (138.5 mg, 0.720 mmol). \((\text{Lauroyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) was obtained as a white solid. Yield: 138.8 mg (MW= 810.08, 57.1%). 1H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\): 7.81-7.88 (m, 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 2.99 (q, 4H, diamine 1,6-CH2), 2.70 (q, 4H, lysine \(\varepsilon\)-CH2), 2.08 (t, 4H, alkyl \(\alpha\)-CH2), 1.39-1.63 (m, 4H, diamine 2,5-CH2); (m, 4H, lysine \(\delta\)-CH2); (m, 4H, lysine \(\beta\)-CH2); 1.30-1.39 (m, 4H, alkyl \(\beta\)-CH2), 1.12-1.30 (m, 36H , alkyl CH2); (m, 4H, diamine 3,4-CH2); (m, 4H, lysine \(\gamma\)-CH2), 0.83 (t, 6H, alkyl CH3). LCQ Calculated mass (C_{42}H_{85}N_{6}O_{4}^{+}): 737.66, found: 737.7 (M+H)^{+}, 369.6 (M+2H)^{+}.

S.7.5. Synthesis of \((\text{Caprinoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\).

As for \((\text{Stearoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) with \((Z\text{-Lys(Boc)}\text{-NH})_2(\text{CH}_2)_6\) (250 mg, 0.297 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (188.4, 1.230 mmol), capric acid (C_{9}H_{19}COOH, 105.7 mg, 0.614 mmol), EDC.HCl (138.0 mg, 0.720 mmol). \((\text{Caprinoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) was obtained as a white solid. Yield: 146.3mg (MW = 753.97, 64.6%). 1H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\): 7.83-7.89 (m, 15 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 2.99 (q, 4H, diamine 1,6-CH2), 2.70 (q, 4H, lysine \(\varepsilon\)-CH2), 2.08 (t, 4H, alkyl \(\alpha\)-CH2), 1.39-1.64 (m, 4H, diamine 2,5-CH2); (m, 4H, lysine \(\delta\)-CH2); (m, 4H, lysine \(\beta\)-CH2); 1.30-1.39 (m, 4H, alkyl \(\beta\)-CH2), 1.14-1.30 (m, 28H , alkyl CH2); (m, 4H, diamine 3,4-CH2); (m, 4H, lysine \(\gamma\)-CH2), 0.83 (t, 6H, alkyl CH3). LCQ Calculated mass (C_{38}H_{77}N_{6}O_{4}^{+}): 681.60, found: 681.7 (M+H)^{+}, 341.5 (M+2H)^{+}.

S.7.6. Synthesis of \((\text{Capryloyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\).

As for \((\text{Stearoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) with \((Z\text{-Lys(Boc)}\text{-NH})_2(\text{CH}_2)_6\) (250 mg, 0.297 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (188.4, 1.230 mmol), caprylic acid (C_{7}H_{15}COOH, 88.7 mg, 0.615 mmol), EDC.HCl (138.7 mg, 0.724 mmol). \((\text{Capryloyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) was obtained as a white solid. Yield: 131.4 mg (MW = 69.86 , 62.6%). 1H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\): 7.83-7.92 (m, 4H, NH), 4.15 (q, 2H, lysine \(\alpha\)-CH*), 2.99 (q, 4H, diamine 1,6-CH2), 2.70 (q, 4H, lysine \(\varepsilon\)-CH2), 2.09 (t, 4H, alkyl \(\alpha\)-CH2), 1.39-1.64 (m, 4H, diamine 2,5-CH2); (m, 4H, lysine \(\delta\)-CH2); (m, 4H, lysine \(\beta\)-CH2); 1.30-1.39 (m, 4H, alkyl \(\beta\)-CH2), 1.14-1.30 (m, 20H , alkyl CH2); (m, 4H, diamine 3,4-CH2); (m, 4H, lysine \(\gamma\)-CH2), 0.83 (t, 6H, alkyl CH3). LCQ Calculated mass (C_{34}H_{69}N_{6}O_{4}^{+}): 625.54, found: 625.7 (M+H)^{+}, 313.5 (M+2H)^{+}.

S.7.8. Synthesis of \((\text{Elaidoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\).

As for \((\text{Stearoyl-Lys(H)}\text{-NH})_2(\text{CH}_2)_6\text{.2HCl}\) with \((Z\text{-Lys(Boc)}\text{-NH})_2(\text{CH}_2)_6\) (249.5 mg, 0.297 mmol), Pd/C catalyst (57.9 mg), HOBt.H_2O (160.2 mg, 1.339 mmol), elaidic acid (190.2 mg, 0.673 mmol), EDC.HCl (146.4 mg, 0.762 mmol). Reaction not complete after one night, added: HOBt.H_2O
(45.7 mg, 0.298 mmol), elaidic acid (62.9 mg, 0.223 mmol), EDC.HCl (38.3 mg, 0.200 mmol)
Continued as for (Oleoyl-Lys(H)-NH)2(CH2)6.2HCl (section 2.2.3). Recrystallized from MeOH.
(Elaidoyl-Lys(H)-NH)2(CH2)6.2HCl was obtained as a yellow solid. Yield: 157.2 (MW = 974.36, 54%).
1H NMR (300 MHz, CDCl3/CD3OD) δ: 7.71, 7.60, 7.34 (m, 1.6H, NH), 5.25 (m, 4H, elaidyl CH=CH), 4.24 (q, 2H, lysine α-CH*), 3.05 (q, 4H, diamine 1,6-CH2), 2.78 (q, 4H, lysine ε-CH2), 2.11 (t, 4H, oleoyl α-CH2), 1.83 (m, 8H, elaidyl CH2-CH=CH), 1.60-1.31 (m, 4H, diamine 2,5-CH2); (m, 4H, lysine δ-CH2); (m, 4H, oleoyl β-CH2); (m, 4H, lysine β-CH2), 1.28-1.31 (m, 40H, oleoyl: CH2); (m, 4H, diamine 3,4-CH2); (m, 4H, lysine γ-CH2), 0.74 (t, 6H, oleoyl CH3). LCQ Calculated mass (C54H104N6O4) 900.81, found: 902.0 (M+H)+, 452.0 (2M+H)+. MALDI-TOF: 792.43 (small), 901.55 (small, M), 923.61(M+Na)+.

S.7.9. Synthesis of (Linoleoyl-Lys(H)-NH)2(CH2)6.2HCl.

As for (Stearoyl-Lys(H)-NH)2(CH2)6.2HCl with (Z-Lys(Boc)-NH)2(CH2)6 (348.5 mg, 0.414 mmol), Pd/C catalyst (78 mg), HOBt.H2O (205.1 mg, 1.339 mmol), linoleic acid (310.6 μl, 1.000 mmol), EDC.HCl (202.2 mg, 1.055 mmol). Reaction not complete after one night, added: linoleic acid (150 μl, 0.483 mmol) and EDC.HCl (101.1 mg, 0.502 mmol). Yield: 671.6 mg (MW = 1097.64, 147.8%). Rf=0.89 (MeOH:CHCl3 = 5:1 v/v). Reprecipitated in ether from MeOH. (Linoleoyl-Lys(H)-NH)2(CH2)6.2HCl was obtained as a yellow solid. Yield: 72.6 (MW = 970.33, 18%). 1H NMR (300 MHz, CDCl3/CD3OD) δ: no clear amide or ammonium signals, 5.32 (br m, less then 8H, CH=CH), 4.33 (t, 2H, lysine α-CH*), 3.18 (br m, 4H, lysine ε-CH2), 2.90 (br t, 4H, CH=CHCH2CH=CH), 2.77 (t, 4H, diamine 1,6-CH2), 2.25 (t, 8H, CH2-CH=CH), 2.06 (t, 4H, linoleoyl α-CH2), 1.85-1.40 (m, 4H, lysine δ-CH2); (m, 4H, diamine 2,5-CH2); (m, 4H, linoleoyl β-CH2); (m, 4H, lysine β-CH2), 1.32 (m, 24H, linoleoyl CH2); (m, 4H, diamine 3,4-CH2); (m, 4H, lysine γ-CH2), 0.97 (t, 6H, linoleoyl CH3).