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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.

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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)$_2$(CH$_2$)$_n$** and target product **(R^1(CO)-Lys(Boc)-NH)$_2$(CH$_2$)$_n$** 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)$_2$(CH$_2$)$_n$** 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-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 R_f 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)-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)-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}H-NMR spectra of **(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 **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$**. Because of their proximity to the chiral centers, the β-CH_2 protons of the Lys residues were chemically non-equivalent (ABX system, not considering the additional coupling with the γ-CH_2 protons), as expected. More remarkably, the protons of the 1,6-CH_2 groups of the diamine and the β-CH_2 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' **(Oleoyl-Lys(H)-NHCH$_2$CH$_3$**, a classical (non-dimeric) surfactant obtained by reaction of Z-Lys(Boc)-OH with ethylamine instead of diamine. For this compound, the lysine β-CH_2 protons appeared as diastereotopic in ^{1}H-NMR, but those in the oleoyl and ethyl groups did not.

In the same way as described above for **(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 (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)-NHCH$_2$CH$_3$.HCl.**

S.2.1. Synthesis of **Z-Lys(Boc)-NHCH$_2$CH$_3$**.

As for **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** starting from **Z-Lys(Boc)-OH.DCHA** salt (1.53 g, 2.72 mmol) yielding as intermediate product 902.9 mg **Z- Lys(Boc)-OH** (2.373 mmol, 87.3%), which was further reacted with $CH_3CH_2NH_2$.HCl (207.7 mg, 2.55 mmol), DIPEA (430 μl, 2.50 mmol), HOBt.H_2O (405.1 mg, 2.65 mmol), EDC.HCl (483.9 mg, 2.52 mmol). **Z-Lys(Boc)-NHCH$_2$CH$_3$** was obtained as a white solid. Yield: 749 mg (MW = 407.50, 78%) R_f=0.63 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (m, 5H, Z Ar), 6.05 (br s, 1H, diamine NH), 5.45 (br s, 1H, lysine α-N*H*), 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$\underline{H}$), 1.66 (m, 1H, lysine β-C$\underline{H}$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), ^{13}C 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 ($C_{21}H_{33}N_3O_5$): 407.2, found: 408.2 (M+H$^+$, 430.3 (M+Na$^+$), 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-Lys(Boc)-NHCH$_2$CH$_3$** (252 mg, 0.618 mmol) and Pd/C catalyst (103 mg) in 75 mL DMF, oleic acid (154 μl, 0.486 mmol), HOBt.H_2O (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). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.19 (br m, 1H, amine NH); (br m, 1H, lysine α-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, oleic acid α-CH_2), 2.20 (t, 2H, oleoyl α-CH_2), 2.02 (m, 4H, oleoyl CH_2-CH=CH), 1.83 (m, 1H, lysine β-C$\underline{H}$H), 1.64 (m, 1H, lysine β-CH$\underline{H}$); (m, 1H, lysine δ-CH_2), 1.56-1.45 (m, 2H, oleoyl β-CH_2); 1.43 (s, 9H, Boc 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 ($C_{31}H_{59}N_3O_4$): 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$_2$)$_6$.2HCl** (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). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 5.34 (m, 2H, C*H*=C*H*), 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_3^+-CH_2), 2.24 (t, J = 7.66 Hz, 2H, NH-CO-CH_2), 2.02 (m, 4H, CH=CH-CH_2), 1.55-1.80 (m, 2H, CO-CH_2-CH_2); (m, NH_3^+-CH_2-CH_2); (m, 2H, NH-CH^*-CH_2), 1.20-1.45 (m, 20H, CH_2-Alkyl tail); (m, 4H, NH-CH^*-CH_2-CH_2), 1.14 (t, J = 7.36 Hz, 3H, NH-CH_2-CH_3), 0.88 (t, J = 6.76 Hz, 3H, CH_3-Alkyl tail). ^{13}C 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 ($C_{26}H_{52}N_3O_2$): 437.8, found: 438.3 (M+H)$^+$, 875.1 (2M+H)$^+$.

S.3.n. Incorporation of the diamine spacer (step iv of Scheme 1) to give **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_n$.**

S.3.2. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_2$**.

As for **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (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.H_2O (0.4332 g, 2.8315 mmol), EDC.HCl (0.4507 g, 2.3596 mmol) in 20 mL ethyl acetate saturated with H_2O. **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_2$** was obtained as a white solid. Yield: 1.0980 g (MW = 784.94, 69.41 %) R_f = 0.57 ($CHCl_3$:MeOH = 5:1 v/v) .^{1}H NMR (300 MHz, DMSO-*d$_6$*) 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 CH$_2$), 4.00 (q, 2H, lysine α-H*), 3.21 (br s, 4H, diamine 1,2-CH$_2$), 2.99 (q, 4H, lysine ε-CH$_2$), 1.69 (br m, 2H, lysine β-C*H*H), 1.62 (br m, 2H, lysine β-CH*H*), 1.48 (s, 18H, Boc CH$_3$), 1.44 (m, 4H, lysine δ-CH$_2$), 1.27 (m, 4H, lysine γ-CH$_2$). LCQ (ESI) calculated ($C_{40}H_{60}N_6O_{10}$): 784.4, found: 785.1 $(M+H)^+$, 801.9 $(M+NH_4)^+$, 807.3 $(M+Na)^+$.

S.3.3. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_3$**.

As for **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (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 mmol), HOBt.H_2O (337.7 mg, 2.205 mmol), EDC.HCl (428.3 mg, 2.234 mmol) in 20 mL ethyl acetate saturated with H_2O. **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_3$** was obtained as a white solid. Yield: 490 mg (MW = 798.97, 61.31%) R_f = 0.66 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (s, 10H, Z Ar), 6.84 (br s, 2H, diamine N*H*), 5.56 (br s, 2H, lysine α-NH), 5.08 (s, 4H, Z CH$_2$), 4.56 (s, 2H, Boc NH), 4.11 (m, 2H, Lysine α-CH*), 3.35 (m, 2H, diamine 1,3-C*H*H), 3.08 (m, 2H, diamine 1,3-CH*H*), 3.08 (m, 4H, lysine ε-CH$_2$), 1.81 (m, 2H, lysine β-C*H*H), 1.62 (m, 2H, lysine β-CH*H*), 1.41 (s, 18H, Boc CH$_3$), 1.53-1.33 (m, 4H, lysine δ-CH$_2$), (m, 4H, lysine γ-CH$_2$); (m, diamine 2-CH$_2$). ^{13}C NMR (300 MHz, $CDCl_3$) 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 ($C_{41}H_{62}N_6O_{10}$): 798.5, found: 799.5 $(M+H)^+$, 821.5 (M+Na)), 1619.0 $(2M+Na)^+$.

S.3.4. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_4$**.

As for **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (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.H_2O (323.5 mg, 2.11 mmol), EDC.HCl (408.6 mg, 2.131 mmol) in 20 mL ethyl acetate saturated with H_2O. **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_4$** was obtained as a white solid. Yield: 539.2 mg (MW = 812.99, 66.3%) R_f = 0.59 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) 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 CH$_2$), 4.70 (s, 2H, lysine ε-NH), 4.13 (m, 2H, lysine α-CH*), 3.27 (m, 2H, diamine 1,4-CH*H*), 3.09 (m, 4H, diamine 1,4-C*H*H); (m, 4H, lysine ε-CH$_2$), 1.80 (m, 2H, lysine β-CH*H*), 1.70 (s, 2H, lysine β-C*H*H), 1.45 (s, 18H, Boc CH$_3$), 1.29-1.35 (s, 4H, lysine δ-CH$_2$); (m, 4H, lysine γ-CH$_2$); (t, 4H, diamine 2,3-CH$_2$), 1.25 t. ^{13}C

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 ($C_{42}H_{64}N_6O_{10}$): 812.5, found: 813.5 $(M+H)^+$, 835.5 $(M+Na)^+$, 1647.1 $(2M+Na)^+$.

S.3.5. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_5$**.

As for **(Z-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(Boc)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 mmol) in 20 mL ethyl acetate saturated with H_2O. **(Z-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). ^{1}H 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, lysine ε-NH), 4.16 (m, 2H, lysine α-CH*), 3.34 (br m, 2H, diamine 1,5-C$\underline{H}$H), 3.10 (br m, 2H, diamine 1,5-CH$\underline{H}$); (m, 4H, lysine ε-CH_2), 1.81 (m, 2H, lysine β-CH$\underline{H}$), 1.70; (s, 4H, lysine (m, 2H, lysine β-C$\underline{H}$H), 1.48 (m, 4H, lysine δ-CH_2), 1.43 (s, 18H, Boc CH_3); (m, 4H, lysine γ-CH_2); (m, 4H, diamine 2,4-CH_2), 1.24 (m, 2H, diamine 3-CH_2). ^{13}C NMR (300MHz, $CDCl_3$) δ: 172.3, 156.6, 156.2, 136.2, 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 ($C_{32}H_{66}N_6O_{10}$): 826.5, found: 827.5 $(M+H)^+$, 849.5 $(M+Na)^+$, 1675.0 $(2M+Na)^+$.

S.3.7. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_7$**.

As for **(Z-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-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). H 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$\underline{H}$H), 3.16 (m, 2H, diamine 1,7-CH$\underline{H}$), 3.07 (m, 4H, lysine ε-CH_2), 1.79 (m, 2H, lysine β-C$\underline{H}$H), 1.64 (m, 2H, lysine β-CH$\underline{H}$), 1.41 (s, 18H, Boc CH_3), 1.29-1.35; (s, 4H, diamine 2,6-CH_2) (m, 4H, lysine γ-CH_2); (m, 4H, lysine δ-CH_2), 1.28 (s, 6H, diamine 3,4,5-CH_2). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 171.6, 156.0, 155.7, 135.8, 128.0, 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 ($C_{45}H_{70}N_6O_{10}$): 854.5, found: 855.5 $(M+H)^+$, 877.5 $(M+Na)^+$, 1731.1 $(2M+Na)^+$.

S.3.8. Synthesis of **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_8$**.

As for **(Z-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-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). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33 (s, 10H, Z

Ar), 6.34 (br s, 2H, diamine NH), 5.59 (br s, 2H, lysine α-NH), 5.08 (s, 4H, Z CH_2), 4.65 (s, 2H, lysine ε-NH), 4.12 (m, 2H, lysine α-CH*), 3.26 (m, 2H, diamine 1,8-C$\underline{H}$H), 3.16 (m, 2H, diamine 1,8-CH$\underline{H}$), 3.07 (m, 4H, lysine ε-CH_2), 1.80 (m, 2H, lysine β-C$\underline{H}$H), 1.64 (m, 2H, lysine β-CH$\underline{H}$), 1.42 (s, 18H, Boc: CH_3), 1.31-1.53 (m, 4H, lysine δ-CH_2); (m, 4H, lysine γ-CH_2); (m, 4H, diamine 2,7-CH_2), 1.26 (br s, 8H, diamine 3,4,5,6-CH_2). LCQ (ESI) calculated ($C_{46}H_{72}N_6O_{10}$): 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)$_2$(CH$_2$)$_n$.**

S.4.2. Synthesis of **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_2$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_2$** (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.H_2O (0.1412 g, 0.9227 mmol) and EDC.HCl (0.1616 g, 0.8459 mmol). **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_2$** was obtained as a white solid. Yield: 0.2296 g (MW = 1045.57, 57.11 %) R_f = 0.72 ($CHCl_3$:MeOH = 5:1 v/v). ^1H NMR (300 MHz, $CDCl_3$) 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-C$\underline{H}$H), 3.29 (m, 2H, lysine β-CH$\underline{H}$), 3.11 (m, 4H, diamine 1,2-CH_2), 2.33 (t, 0.48H, oleic acid α-CH_2), 2.20 (t, 4H, oleoyl α-CH_2), 2.00 (br q, 8H, oleoyl C$\underline{H}_2$-CH=CH), 1.79 (br m, 2H, lysine β-C$\underline{H}$H), 1.60 (br m, 2H, lysine β-CH$\underline{H}$); (br m, 4H lysine δ-CH_2), 1.57-1.38 (m, 4H, oleoyl β-CH_2); (s, 18H, Boc CH_3), 1.28 (br m, 4H, lysine γ-CH_2) (br m, 40H, oleoyl CH_2), 0.90 (t, 6H, oleoyl CH_3). LCQ (ESI) calculated ($C_{60}H_{112}N_6O_8$): 1044.9, found: 1045.4 $(M+H)^+$, 1067.7 $(M+Na)^+$.

S.4.3. Synthesis of **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_3$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_3$** (301 mg, 0.377 mmol), Pd/C catalyst (71 mg) in 75 mL DMF, oleic acid (228 µl, 0.719 mmol), HOBt.H_2O (160 mg, 1.045 mmol) and EDC.HCl (161 mg, 0.83 mmol). **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_3$** was obtained as a white solid. Yield: 207.7 mg (MW = 1059.59, 52 %) R_f = 0.72 ($CHCl_3$:MeOH = 5:1 v/v). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.18 (br s, 2H, diamine NH), 6.35 (br d, 2H, lysine α-NH), 5.34 (m, 4H, lysine CH=CH), 4.74 (s, 2H, lysine ε-NH), 4.34 (q, 2H, lysine α-CH*), 3.44 (m, 2H, diamine 1,3C$\underline{H}$H), 3.11 (m, 2H, diamine 1,3CH$\underline{H}$) (m, 4H, lysine ε-CH_2), 2.34 (t, 0.05H, oleic acid α-CH_2), 2.27 (t, 4H, oleoyl α-CH_2), 2.00 (br q, 8H, oleoyl CH_2-CH=CH), 1.82 (m, 2H, lysine β-C$\underline{H}$H), 1.61 (m, 4H, lysine β-CH$\underline{H}$); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2), 1.49 (t, 2H diamine 2-CH_2), 1.43 (s, 18H, Boc CH_3), 1.28-1.31 (br m, 40H, oleoyl CH_2); (m, 4H, lysine γ-CH_2), 0.88 (t, 6H, oleoyl CH_3). LCQ (ESI) calculated ($C_{61}H_{114}N_6O_8$): 1058.8, found: 1059.5 $(M+H)^+$, 1081.7 $(M+Na)^+$.

S.4.4. Synthesis of **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_4$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_4$** (225.6 mg, 0.277 mmol), Pd/C catalyst (0.10 g) in 75 mL DMF, oleic acid (0.2300 mL, 1.2246 mmol), HOBt.H_2O (0.3680 g, 2.4052 mmol), EDC.HCl (0.3450 g, 1.8063 mmol). **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_4$** was obtained as a white solid with MW = 1073.02 and R_f = 0.55 ($CHCl_3$:MeOH = 5:1). The product was used in the next step.

S.4.5. Synthesis of **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_5$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_5$** (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.H_2O (0.7360 g, 4.8105 mmol), EDC.HCl (0.6900 g, 3.6126 mmol). **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_5$** was obtained as a white solid. Yield: 0.260 g (MW= 1087.65, 40.25%) R_f = 0.76 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.06 (br m, 2H, diamine NH), 6.96 (br d, 2H, lysine α-NH), 5.34 (m, 4H, oleoyl CH=CH), 4.87 (m, 2H, lysine ε-NH), 4.47 (q, 2H, lysine α-CH*), 3.42 (m, 2H, diamine 1,5-C*H*H), 3.10 (m, 2H, diamine 1,5-CH*H*); (m, 4H, lysine ε-CH_2), 2.17 (dt, 4H, oleoyl α-CH_2), 2.00 (q, 4H, oleoyl C*H*$_2$-CH=CH), 1.83 (m, 2H, lysine β-C*H*H), 1.75-1.48 (m, 2H, lysine β-CH*H*); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2), 1.43 (m, 18H, Boc CH_3); (M, 4H, diamine 2,4-CH_2), 1.27 (br m, 40H, oleoyl CH_2); (m, 2H, diamine 3-CH_2), 0.88 (t, 6H, oleoyl CH_3). LCQ (ESI) calculated ($C_{63}H_{118}N_6O_8$): 1086.9, found: 1087.5 $(M+H)^+$, 1109.7 $(M+Na)^+$.

S.4.7. Synthesis of **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_7$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_7$** (0.1951 g, 0.2282 mmol), Pd/C catalyst (0.1g) in 50 mL DMF, oleic acid (0.1449 mL, 0.4546 mmol), HOBt.H_2O (0.0838 g, 0.5477 mmol) and EDC.HCl (0.0959 g, 0.5020 mmol). **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_7$** was obtained as a white solid. Yield: 0.1785 g (MW = 1115.70, 70.11 %) R_f = 0.78 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.42 (br s, 2H, diamine NH), 6.95 (br d, 2H, lysine α-NH), 5.34 (m, 4H, oleoyl CH=CH), 4.87 (br m, 2H, lysine ε-NH), 4.59 (q, 2H, lysine α-CH*), 3.39 (m, 2H, diamine 1,7-C*H*H), 3.08 (m, 2H, diamine 1,7-CH*H*); (m, 4H, lysine ε-CH_2), 2.33 (t, 0.37H, oleic acid α-CH_2), 2.17 (m, 4H, oleoyl α-CH_2), 2.00 (q, 8H, oleoyl C*H*$_2$-CH=CH), 1.78 (m, 2H, lysine β-C*H*H), 1.61 (m, 2H, lysine β-C*H*H); (m, 4H, lysine δ-CH_2), 1.55-1.39 (m, 4H,diamine 2,5-CH_2); (m, 4H, oleoyl β-CH_2); (m, 18H, Boc CH_3), 1.39-1.20 (br m, 40H, oleoyl CH_2); (br m, 4H, diamine 3,4,5-CH_2), 0.88 (t, 6H, oleoyl CH_3). LCQ (ESI) calculated ($C_{65}H_{122}N_6O_8$): 1114.932, found: 1115.6 $(M+H)^+$, 1137.7 $(M+Na)^+$.

S.4.8. Synthesis of **(Oleoyl–Lys(Boc)-NH)$_2$(CH$_2$)$_8$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) starting from **Z-Lys(Boc)NH)$_2$(CH$_2$)$_8$** (499 mg, 0.575 mmol), Pd/C catalyst (81 mg) in 75 mL DMF, oleic acid (228 µl, 0.715 mmol), HOBt.H_2O (804 mg, 5.255 mmol) and EDC.HCl (499 mg, 2.613 mmol). **(Oleoyl–Lys(Boc)-NH)$_2$(CH$_2$)$_8$** was obtained as a white solid. Yield: 665 mg (MW = 1129.73 g/mol, 107%) R_f = 0.80 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.90 (br t, 2H, diamine NH), 6.50 (br d, 2H, lysine α-NH), 5.34 (m, 4H, lysine CH=CH), 4.76 (br s, 2H, lysine ε-NH), 4.47 (q, 2H, lysine α-CH*), 3.39 (m, 2H, diamine 1,8-C$\underline{H}$H), 3.09 (m, 2H, diamine 1,8-CH$\underline{H}$) (m, 4H, lysine ε-CH_2), 2.22 (dt, 4H, oleoyl α-CH_2), 2.00 (q, 8H, lysine CH_2-CH=CH), 1.77 (m, 2H, lysine β-C$\underline{H}$H), 1.62 (m, 2H, lysine β-CH$\underline{H}$) (m, 4H, lysine δ-CH_2), 1.55-1.39 (m, 4H, diamine 2,7-CH_2); (m, 4H, oleoyl β-CH_2); (m, 18H, Boc CH_3), 1.28-1.31 (br m, 40H, oleoyl CH_2); (br m, 4H, diamine 3,4,5,6-CH_2), 0.88 (t, 6H, oleoyl CH_3). LCQ (ESI) calculated ($C_{66}H_{124}N_6O_8$): 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)$_2$(CH$_2$)$_n$ .2HCl**.

S.5.2. Synthesis of **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_2$ .2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_2$** (0.1641 g, 0.1569 mmol). **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_2$ .2HCl** was obtained as a white solid. Yield: 0.1113 g (Mw = 918.26, 77.23%) $R_f = 0.09$ ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, DMSO-*d$_6$*) δ: 7.95 (m, 10H, NH), 5.32 (m, 4H, oleoyl CH=CH), 4.14 (m, 2H, lysine α-CH*), 3.10 (q, 4H, diamine 1,2-CH_2), 2.73 (q, 4H, lysine ε- CH_2), 2.18 (t, oleic acid α-CH_2), 2.13 (t, 4H, oleoyl α-CH_2), 1.98 (m, 8H, oleoyl CH_2-CH=CH), 1.42-1.64 (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2); (m, 4H, lysine β-CH_2); 1.24 (br s, 40H, oleoyl CH_2); (br s, 4H, lysine γ-CH_2); (br s, 4H, γ-CH_2), 0.85 (t, 6H, oleoyl CH_3). ^{13}C NMR (300MHz, DMSO-*d$_6$*) δ: 172.2, 171.8, 129.5, 52.6, 52.3, 51.1, 35.1, 33.2, 31.2, 29.0, 28.5, 26.5, 25.1, 24.3, 22.0, 13.9. LCQ (ESI) calculated ($C_{50}H_{98}Cl_2N_6O_4$): 844.7, found: 845.6 $(M+H)^+$, 423.4 $(M+2H)^{2+}$.

S.5.3. Synthesis of **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_3$.2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_3$** (166.06 mg, 0.157 mmol). **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_3$.2HCl** was obtained as a white solid. Yield: 132 mg (MW = 932.28, 98%) $R_f = 0.32$ ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, DMSO-*d$_6$*) δ: 7.92 (m, 4H, amide NH), 7.77 (m, 6H, lysine NH_3), 5.36 (m, 4H, oleoyl CH=CH), 4.18 (m, 2H, lysine α-CH*), 3.09 (q, 4H, diamine 1,3 CH_2), 2.79 (q, 4H, lysine ε-CH_2), 2.19 (t, 4H, oleoyl α-CH_2), 2.05 (m, 8H, oleoyl CH_2-CH=CH), 1.48-1.65 (m, 2H, diamine 2 CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2); (m, 4H, lysine β-CH_2); 1.42 (m, 4H, NH-CH*-C*H$_2$*), 1.25-1.40 (br s, 40H, oleoyl CH_2); (br s, 4H, lysine γ-CH_2), 0.89 (t, 3H, oleoyl CH_3). ^{13}C NMR (300 MHz, DMSO-*d$_6$*) δ: 172.1, 171.6, 129.6, 52.3, 31.4, 31.2, 29.0, 28.7, 28.5, 26.5, 22.3, 22.0, 13.9. LCQ (ESI) calculated ($C_{51}H_{100}N_6O_4$): 858.8, found: 859.7 $(M+H)^+$, 430.4 $(M+2H)^{2+}$.

S.5.4. Synthesis of **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_4$ .2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_4$** (0.1289 g, 0.1201 mmol). **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_4$ .2HCl** was obtained as a white solid. Yield: 0.0987 g (MW = 946.31, 86.88%) $R_f = 0.10$ ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, DMSO-*d$_6$*): 7.86 (m, 4H, amide NH), 7.81 (m, 6H, lysine NH_3), 5.30 (m, 4H, oleoyl CH=CH), 4.14 (m, 2H, lysine α-CH*), 3.00 (q, 4H, diamine 1,4-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.11 (t, 4H, oleoyl α-CH_2), 2.96 (m, 8H, oleoyl CH_2-CH=CH), 1.40-1.62 (m, 2H, diamine 2,3-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2); (m, 4H, lysine β-CH_2); 1.34 (m, 4H, NH-CH^*-CH_2), 1.10-1.26 (br s, 40H, oleoyl CH_2); (br s, 4H, lysine γ-CH_2), 0.80 (t, 3H, oleoyl CH_3). ^{13}C 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 ($C_{52}H_{100}N_6O_4$): 872.78, found; 873.7 $(M+H)^+$ and 437.5 $(M+2H)^+$.

S.5.5. Synthesis of **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_5$. 2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_5$** (0.1032 g, 0.0949 mmol). **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_5$. 2HCl** was obtained as a white solid. Yield: 0.0976 g (MW = 960.34, 107.11%) R_f = 0.09 ($CHCl_3$:MeOH = 5:1 v/v, ninhydrine). ^{1}H 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-CH_2), 2.72 (q, 4H, lysine ε-CH_2), 2.11 (t, 4H, oleoyl α-CH_2), 1.97 (m, 8H, oleoyl CH_2-CH=CH), 1.50 (m, 4H, diamine 2,4-CH_2); (m, 4H, lysine β-CH_2); (m, 4H, oleoyl β-CH_2), 1.18-1.41 (br s, 40H, oleoyl CH_2); (br s, 2H, diamine 3-CH_2); (br s, 4H, γ-CH_2), 0.86 (t, 6H, oleoyl CH_3). ^{13}C 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 ($C_{53}H_{102}N_6O_4$): 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)$_2$(CH$_2$)$_7$ .2HCl** .

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_7$** (0.1383 g, 0.1240 mmol). **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_7$.2HCl** was obtained as a white solid. Yield: 0.1159 g (MW = 988.39, 94.60%) R_f = 0.10 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, DMSO-*d6*) δ=7.83(m, 10H, NH), 5.32 (m, 4H, oleyl CH=CH), 4.17 (m, 2H, lysine α-CH^*), 3.02 (q, 4H, diamine 1,7-CH_2), 2.73 (q, 4H, lysine ε-CH_2), 2.11 (t, 4H, oleoyl α-CH_2), 1.98 (m, 8H, oleoyl CH_2-CH=CH), 1.52-1.47 (m, 4H, siamine 2,6-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2); (m, 4H, lysine β-CH_2), 1.24 (m+s, 40H, oleoyl CH_2);(s+m, 6H, diamine 3,4,5-CH_2); (s+m, 4H, lysine γ-CH_2); 0.85 (t, 6H, oleoyl CH_3). ^{13}C NMR (300MHz, 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, 25.2, 22.3, 22.0, 13.9. LCQ (ESI) calculated ($C_{55}H_{106}N_6O_4$) 914.83, found: 915.7 $(M+H)^+$ and 458.5 $(M+2H)^{2+}$.

S.5.8. Synthesis of **(Oleoyl–Lys(H)-NH)$_2$(CH$_2$)$_8$.2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) without the final chromatography step, starting from **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_8$** (335 mg, 0.297 mmol) was obtained as a white solid. Yield: 309 mg (MW = 1002.42, 96.4 %), R_f = 0.22 ($CHCl_3$:MeOH = 5:1 v/v). ^{1}H NMR (300 MHz, DMSO) δ: 7.85-8.05 (br m, 10H, NH), 5.36 (m, 4H, lysine CH=CH), 4.20 (m, 2H, lysine α-CH^*), 3.05 (m, 4H, diamine 1,8-CH_2), 2.75 (m, 4H, lysine ε-CH_2), 2.15 (t, 4H, oleoyl α-CH_2), 2.00 (m, 8H, loeoyl CH_2-CH=CH), 1.42-1.67 (m, 4H, diamine 2,7-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2);

(m, 4H, lysine β-CH_2); 1.42 (m, 4H, NH-CH*-C*H*$_2$), 1.18-1.30 (m, 40H, oleoyl CH_2); (m, 8H, diamine 3,4,5,6-CH_2); (m, 4H, lysine γ-CH_2), 0.89 (t, 3H, oleoyl CH_3). ^{13}C NMR (300 MHz, DMSO-*d*$_6$) 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 ($C_{56}H_{108}N_6O_4$): 928.8, found: 929.7 $(M+H)^+$, 465.5 $(M+2H)^{2+}$.

S.6. Preparation of the enantiomer **(Oleoyl-D-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl.**

S.6.1. Synthesis of **(Z-D-Lys(Boc)-NH)$_2$(CH$_2$)$_6$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** 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.H_2O (708.3 mg, 4.63 mmol), EDC.HCl (895.9 mg, 4.67 mmol). **(Z-D-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** was obtained as a white solid. Yield: 1.1563g (MW=841.04, 71.6 %) R_f=0.62 ($CHCl_3$:MeOH=5:1 v/v). ^1H NMR (300MHz, $CDCl_3$) δ: 7.30 (s, 10H, Z Ar), 6.54 (br s, 2H, diamine N*H*), 5.84 (br s, 2H, lysine α-NH), 5.06 (s, 4H, Z CH_2), 4.69 (br s, 2H, lysine ε-NH), 4.13 (m, 2H, lysine α-CH*), 3.28 (m, 2H, diamine 1,6-C*H*H), 3.19 (m, 2H, diamine 1,6-CH*H*), 3.08 (m, 4H, lysine ε-CH_2), 1.79 (m, 2H, lysine β-C*H*H), 1.67 (m, 2H, lysine β-CH*H*), 1.43 (s, 18H, boc CH_3), 1.54-1.20 (m, 8H, diamine 2,3,4,5-CH_2); (m, 4H, lysine: γ-CH_2) (m, 4H, lysine δ-CH_2). ^{13}C NMR (300MHz, DMSO-*d*$_6$) δ: 171.8, 156.0, 155.7, 135.7, 127.9, 127.4, 78.6, 76.6 (t), 66.4, 54.5, 39.5, 37.9, 31.7, 29.0, 28.4, 27.9, 24.7, 22.1.

S.6.2. Synthesis of **(Oleoyl-D-Lys(Boc)-NH)$_2$(CH$_2$)$_6$**.

As for **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$**, starting from **(Z-D-Lys(Boc)-NH)$_2$(CH$_2$)$_6$**. 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)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3) starting from **(Oleoyl-D-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (0.0685g, 0.0622mmols). No precipitate was formed during workup. **(Oleoyl-D-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 0.0612g (MW=974.36, 101.02%) R_f=0.09 ($CHCl_3$:MeOH=5:1v/v) with some impurities. ^1H NMR (300 MHz, DMSO-*d*$_6$) 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-CH*), 3.37 (m, 2H, diamine 1,6-C*H*H), 3.10 (m, 2H, diamine 1.6-CH*H*); (m, 4H, lysine ε-CH_2), 2.17 (m, 4H, oleoyl α-CH_2), 2.00 (m, 8H, oleoyl C*H*$_2$-CH=CH), 1.80 (m, 2H, lysine β-C*H*H), 1.73-1.58 (m, 2H, lysine β-CH*H*); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2), 1.58-1.42 (m, 4H, diamine 2,5-CH_2), 1.44 (s, 18H, Boc CH_3), 1.28-1.31 (m, 4H, lysine γ-CH_2); (m, 40H, oleoyl CH_2); (m, 2H, diamine 3,4-CH_2), 0.88 (t, 6H, oleoyl: CH_3). ^{13}C NMR (300 MHz, DMSO-*d*$_6$) 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 ($C_{54}H_{106}N_6O_4$): 900.8, found: 901.7 $(M+H)^+$ and 451.4 $(M+2H)^{2+}$.

S. 7. Preparation of the acyl analogues of **(Oleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

S.7.1. Synthesis of **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for compound **(Oleoyl-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (section 2.2.2) with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (395.7 mg, 0.427 mmol), Pd/C catalyst (100 mg), HOBt.H_2O (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*. **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 137.6 mg (MW = 978.39, 33%). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) δ: 7.28-7.85 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 3.33 (m, 2H, ?), 3.22 (q, 4H, diamine 1,6-CH_2), 2.91 (q, 4H, lysine ε-CH_2), 2.23 (t, 4H, alkyl α-CH_2), 1.39-1.84 (m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.22-1.39 (m, 68H, alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2); 0.88 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{54}H_{108}N_6O_4$) 904.84, found: 906.0 ($M+H^+$), 554.0 ($2M+H^+$).

S.7.2. Synthesis of **(Palmitoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (236.6 mg, 0.281 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (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). **(Palmitoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 164.0 (MW = 922.29, 64.2%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.82-7.89 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 2.99 (q, 4H, diamine 1,6-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.09 (t, 4H, alkyl α-CH_2), 1.39-1.63(m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.30-1.39 (m, 4H, alkyl β-CH_2), 1.15-1.30 (m, 52H , alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.83 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{50}H_{101}N_6O_4^+$) : 849.78, found: 850.0 $(M+H)^+$, 426.0 $(M+2H)^+$.

S.7.3. Synthesis of **(Myristoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (210.3 mg, 0.250 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (92.0 mg, 0.601 mmol), myristic acid ($C_{13}H_{27}COOH$, 143 mg, 0.626 mmol), EDC.HCl (113.1 mg, 0.590 mmol). **(Myristoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 210.3mg (MW = 866.18, 97.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.81-7.88 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 3.00 (q, 4H, diamine 1,6-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.08 (t, 4H, alkyl α-CH_2), 1.38-1.64(m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.28-1.38 (m, 4H, alkyl β-CH_2), 1.09-1.28 (m, 44H , alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.83 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{46}H_{93}N_6O_4^+$): 793.73, found: 793.9 ($M+H^+$), 397.8 ($M+2H^+$).

S.7.4. Synthesis of **(Lauroyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(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). **(Lauroyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 138.8 mg (MW= 810.08, 57.1%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.81-7.88 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 2.99 (q, 4H, diamine 1,6-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.08 (t, 4H, alkyl α-CH_2), 1.39-1.63 (m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.30-1.39 (m, 4H, alkyl β-CH_2), 1.12-1.30 (m, 36H , alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.83 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{42}H_{85}N_6O_4^+$): 737.66, found: 737.7 $(M+H)^+$, 369.6 $(M+2H)^+$.

S.7.5. Synthesis of **(Caprinoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (250 mg, 0.297 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (188.4, 1.230 mmol), capric acid ($C_9H_{19}COOH$, 105.7 mg, 0.614 mmol), EDC.HCl (138.0 mg, 0.720 mmol). **(Caprinoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 146.3mg (MW= 753.97, 64.6%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.83-7.89 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 2.99 (q, 4H, diamine 1,6-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.08 (t, 4H, alkyl α-CH_2), 1.39-1.64 (m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.30-1.39 (m, 4H, alkyl β-CH_2), 1.14-1.30 (m, 28H , alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.83 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{38}H_{77}N_6O_4^+$): 681.60, found: 681.7 $(M+H)^+$, 341.5 $(M+2H)^+$.

S.7.6. Synthesis of **(Capryloyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (250 mg, 0.297 mmol), Pd/C catalyst (100 mg). HOBt.H_2O (188.4 mg, 1.230 mmol), caprylic acid ($C_7H_{15}COOH$, 88.7 mg, 0.615 mmol), EDC.HCl (138.7 mg, 0.724 mmol). **(Capryloyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a white solid. Yield: 131.4 mg (MW = 69.86 , 62.6%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.83-7.92 (m, 4H, NH), 4.15 (q, 2H, lysine α-CH*), 2.99 (q, 4H, diamine 1,6-CH_2), 2.70 (q, 4H, lysine ε-CH_2), 2.09 (t, 4H, alkyl α-CH_2), 1.39-1.63(m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, lysine β-CH_2); 1.30-1.39 (m, 4H, alkyl β-CH_2), 1.15-1.30 (m, 20H , alkyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.83 (t, 6H, alkyl CH_3). LCQ Calculated mass ($C_{34}H_{69}N_6O_4^+$): 625.54, found: 625.7 $(M+H)^+$, 313.5 $(M+2H)^+$.

S.7.8. Synthesis of **(Elaidoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(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)$_2$(CH$_2$)$_6$.2HCl** (section 2.2.3). Recrystallized from MeOH. **(Elaidoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a yellow solid. Yield: 157.2 (MW = 974.36, 54%). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) 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-CH_2), 2.78 (q, 4H, lysine ε-CH_2), 2.11 (t, 4H, oleoyl α-CH_2), 1.83 (m, 8H, elaidyl CH_2-CH=CH), 1.60-1.31 (m, 4H, diamine 2,5-CH_2); (m, 4H, lysine δ-CH_2); (m, 4H, oleoyl β-CH_2); (m, 4H, lysine β-CH_2), 1.28-1.31 (m, 40H, oleoyl: CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.74 (t, 6H, oleoyl CH_3). LCQ Calculated mass ($C_{54}H_{104}N_6O_4$) 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)$_2$(CH$_2$)$_6$.2HCl**.

As for **(Stearoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** with **(Z-Lys(Boc)-NH)$_2$(CH$_2$)$_6$** (348.5 mg, 0.414 mmol), Pd/C catalyst (78 mg), HOBt.H_2O (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%). R_f=0.89 (MeOH:$CHCl_3$ = 5:1 v/v). Reprecipitated in ether from MeOH. **(Linoleoyl-Lys(H)-NH)$_2$(CH$_2$)$_6$.2HCl** was obtained as a yellow solid. Yield: 72.6 (MW = 970.33, 18%). ^{1}H NMR (300 MHz, $CDCl_3/CD_3OD$) 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-CH_2), 2.90 (br t, 4H, CH=CHCH_2CH=CH), 2.77 (t, 4H, diamine 1,6-CH_2), 2.25 (t, 8H, CH_2-CH=CH), 2.06 (t, 4H, linoleoyl α-CH_2), 1.85-1.40 (m, 4H, lysine δ-CH_2); (m, 4H, diamine 2,5-CH_2); (m, 4H, linoleoyl β-CH_2); (m, 4H, lysine β-CH_2), 1.32 (m, 24H, linoleoyl CH_2); (m, 4H, diamine 3,4-CH_2); (m, 4H, lysine γ-CH_2), 0.97 (t, 6H, linoleoyl CH_3).