Supplementary information

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1. SYNTHESIS and CHARACTERIZATION of the amphiphilic ionic dendrimers based on PAMAM

1.1 Synthetic procedure

The compounds were synthesized following a similar procedure to the method described by Crooks et al.,[1] which has been adapted and successfully used in previous works in our research group.[2] A scheme of this general method is depicted in Figure S1.
PAMAM 30:0

Stoichiometric proportion: 30 moles of myristic acid/1 mole of PAMAM precursor.
Yield: 100%, yellow wax.

$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm): 8.87-8.11 (m, CO-NH$^-$), 3.54-3.40 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.31-3.12 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.13-3.03 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 2.78-2.65 (NH$_3^+$-CH$_2$-CH$_2$-CO), 2.56-2.44 (m, NH$_3^+$-CH$_2$-CH$_2$-NH$_3^+$; NH-CH$_2$-CH$_2$-NH$_3^+$), 2.43-2.27 (m, NH$_3^+$-CH$_2$-CH$_2$-CO), 2.21 (t, 60H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.56 (m, 60H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$). 1.26 (m, ~600H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

FTIR (cm$^{-1}$, Nujol): 3230 (v -NHCO-), 3031 (v -NHCO-), 2941-2848 (v -NH$_3^+$; v -NH$^+$; v -CH-), 1635 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1535 ($\delta$ –NHCO-, $\nu$ as COO$^-$), 1456 ($\nu$ s COO$^-$).

PAMAM 25:5

Stoichiometric proportion: 25 moles of myristic acid/5 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.
Yield: 100%, yellow wax.

$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm): 8.80-8.08 (m, CO-NH$^-$), 3.92 (s, 10H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.66-3.58 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.57-3.52 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.52-3.42 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.37 (s, 15H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.29-3.17 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.15-3.01 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 2.81-2.60 (NH$_3^+$-CH$_2$-CH$_2$-CO), 2.60-2.47 (m, NH$_3^+$-CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$_3^+$), 2.42-2.31 (m, NH$_3^+$-CH$_2$-CH$_2$-CO), 2.28 (t, 50H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.56 (m, 50H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.26 (m, ~500H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 0.88 (t, 75H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

FTIR (cm$^{-1}$, Nujol): 3230 (v -NHCO-), 3031 (v -NHCO-), 2942-2848 (v -NH$_3^+$; v -NH$^+$; v -CH-), 1635 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1535 (v -NHCO-, $\nu$ as COO$^-$), 1456 (v s COO$^-$).
**PAMAM 20:10**

**Stoichiometric proportion:** 20 moles of myristic acid/10 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.

**Yield:** 100%, yellow viscous oil.

**$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm):** 8.76-8.08 (m, CO-NH-), 3.91 (s, 20H, -OCO-CH$_2$-(CH$_2$)$_2$O-(CH$_2$)$_2$O-CH$_3$), 3.64-3.56 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$O-CH$_2$-CH$_2$-O-CH$_3$), 3.58-3.51 (m-OCO-CH$_2$-O-CH$_2$-CH$_2$O-CH$_2$-CH$_2$-O-CH$_3$), 3.51-3.42 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.37 (s, 30H, -OCO-CH$_2$-(CH$_2$)$_2$O-(CH$_2$)$_2$O-CH$_3$), 3.30-3.16 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.13-3.00 (m, NH-CH$_2$-CH$_2$-NH$^+$), 2.80-2.60 (NH$^+$-CH$_2$-CH$_2$-CO), 2.59-2.46 (m, NH$^+$-CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$^+$), 2.44-2.29 (m, NH$^+$-CH$_2$-CH$_2$-CO), 2.24 (t, 40H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.58 (m, 40H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.26 (m, ~400H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 0.88 (t, 60H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

**FTIR (cm$^{-1}$, Nujol):** 3230 (v -NHCO-), 3035 (v -NHCO-), 2942-2850 (v -NH$^+$; v -NH$^+$; v -CH$^-$), 1681 (v -CO), 1637 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1538 (v -NHCO-, v$_{as}$ COO$^-$), 1450 (v$_s$ COO$^-$).

**PAMAM 15:15**

**Stoichiometric proportion:** 15 moles of myristic acid/15 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.

**Yield:** 100%, yellow viscous oil.

**$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm):** 8.87-8.17 (m, CO-NH-), 3.95 (s, 30H, -OCO-CH$_2$-(CH$_2$)$_2$O-(CH$_2$)$_2$O-CH$_3$), 3.67-3.59 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$O-CH$_2$-CH$_2$O-CH$_3$), 3.56-3.51 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$O-CH$_2$-CH$_2$O-CH$_3$), 3.51-3.44 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.36 (s, 45H, -OCO-CH$_2$-(CH$_2$)$_2$O-(CH$_2$)$_2$O-CH$_3$), 3.34-3.19 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.12-3.03 (m, NH-CH$_2$-CH$_2$-NH$^+$), 2.89-2.72 (NH$^+$-CH$_2$-CH$_2$-CO), 2.70-2.56 (m, NH$^+$-CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$^+$), 2.46-2.35 (m, NH$^+$-CH$_2$-CH$_2$-CO), 2.30 (t, 30H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.61 (m, 30H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.25 (m, ~300H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 0.88 (t, 45H, OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

**FTIR (cm$^{-1}$, Nujol):** 3222 (v -NHCO-), 3043 (v -NHCO-), 2941-2850 (v -NH$^+$; v -NH$^+$; v -CH$^-$), 1712 (v -CO), 1645 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1540 (v -NHCO-, v$_{as}$ COO$^-$), 1454 (v$_s$ COO$^-$).
PAMAM 10:20
Stoichiometric proportion: 10 moles of myristic acid/20 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.
Yield: 100%, yellow wax.

$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm): 8.74-8.17 (m, CO-NH-), 3.93 (s, 40H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.67-3.57 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.57-3.51 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.51-3.42 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.36 (s, 60H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.32-3.20 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.14-2.91 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 2.81-2.60 (m, NH$^+\cdot$CH$_2$-CH$_2$-CO), 2.58-2.43 (m, NH$^+\cdot$CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$^+$), 2.41-2.23 (m, NH$^+\cdot$CH$_2$-CH$_2$-CO), 2.11 (t, 10H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.51 (m, 10H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.22 (m, ~100H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 0.85 (t, 15H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

FTIR (cm$^{-1}$, Nujol): 3232 (v -NHCO-), 3035 (v -NHCO-), 2941-2848 (v -NH$_3^+$; v -NH$^+$; v -CH-), 1691 (v =CO), 1538 (v =COO$^-$).

PAMAM 5:25
Stoichiometric proportion: 5 moles of myristic acid/25 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.
Yield: 100%, yellow wax.

$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm): 8.72-8.10 (m, CO-NH-), 3.87 (s, 50H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.65-3.55 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.55-3.49 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.48-3.38 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 3.34 (s, 75H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.27-3.14 (m, NH-CH$_2$-CH$_2$-NH$^+$), 3.10-2.95 (m, NH-CH$_2$-CH$_2$-NH$_3^+$), 2.81-2.60 (NH$^+\cdot$CH$_2$-CH$_2$-CO), 2.58-2.43 (m, NH$^+\cdot$CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$^+$), 2.41-2.23 (m, NH$^+\cdot$CH$_2$-CH$_2$-CO), 2.11 (t, 10H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.51 (m, 10H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 1.22 (m, ~100H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$), 0.85 (t, 15H, 'OOC-CH$_2$-CH$_2$-(CH$_2$)$_{10}$CH$_3$).

FTIR (cm$^{-1}$, Nujol): 3245 (v -NHCO-), 3035 (v -NHCO-), 2931-2838 (v -NH$_3^+$; v -NH$^+$; v -CH-), 1631 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1538 ($\delta$ -NHCO-, v$_{as}$ COO$^-$), 1430 (v$_s$ COO$^-$).
**PAMAM 0:30**

**Stoichiometric proportion:** 30 moles of 2-(2-(2-methoxyethoxy)ethoxy)acetic acid/1 mole of PAMAM precursor.

**Yield:** 100%, yellow wax.

**$^1$H-NMR (CDCl$_3$, 400MHz), $\delta$ (ppm):** 8.74-8.13 (m, CO-NH-), 3.90 (s, 60H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.67-3.56 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.55-3.50 (m, -OCO-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-CH$_2$-O-CH$_3$), 3.50-3.41 (m, -CO-NH-CH$_2$-CH$_2$-NH$_3^+$), 3.35 (s, 90H, -OCO-CH$_2$-(CH$_2$)$_2$-O-(CH$_2$)$_2$-O-CH$_3$), 3.29-3.19 (m, -CO-NH-CH$_2$-CH$_2$-NH$^+$), 3.12-3.00 (m, -CO-NH-CH$_2$-CH$_2$-NH$_3^+$), 2.87-2.60 (NH$^+$-CH$_2$-CH$_2$-CO), 2.59-2.51 (m, NH$^+$-CH$_2$-CH$_2$-NH$^+$; NH-CH$_2$-CH$_2$-NH$^+$), 2.43-2.30 (m, NH$^+$-CH$_2$-CH$_2$-CO).

**FTIR (cm$^{-1}$, Nujol):** 3232 (v -NHCO-), 3043 (v -NHCO-), 2908-2813 (v -NH$_3^+$; v -NH$^+$; v -CH-), 1619 (v -NHCO-; $\delta$ NH$_3^+$, $\delta$ NH$^+$), 1538 (v$_{as}$ COO’), 1431 (v$_s$ COO’).
1.2. Dendrimers characterization

Figure S2 represents the FT-IR spectrum of compound PAMAM 20:10 compared with the spectra of their precursor compounds: Second generation of non-functionalized PAMAM dendrimer, myristic acid and 2-(2-(2-methoxyethoxy)ethoxy)acetic acid.

Figure S2: FT-IR spectra of compounds PAMAM 20:10, second generation non-functionalized PAMAM dendrimer, myristic acid and 2-(2-(2-methoxyethoxy)ethoxy)acetic acid.
Figure S3 represents the $^1$H-NMR spectrum of PAMAM 2G 20:10 with the corresponding peak assignation as an example of an ionic co-dendrimer bearing both myristic acid and 2-(2-(2-methoxyethoxy)ethoxy)acetic acid.

Figure S3: $^1$H-NMR spectrum (400MHz, CDCl$_3$) of PAMAM 2G 20:10. The chemical structures of PAMAM, myristic acid and 2-(2-(2-methoxyethoxy)ethoxy)acetic acid and their corresponding peak assignation are indicated in the figure.
1. THERMAL and LIQUID CRYSTAL PROPERTIES.

Table S1 gathers data obtained by thermogravimetric analysis (TGA). For each compound, the temperature at which the mass loss is 5% in weight ($T_{5\%}$), the maximum temperature ($T_{\text{max}}$), and the onset temperature ($T_{\text{onset}}$) relative to the decomposition process (obtained by the first derivative of the TGA curve or DTGA), and the mass residue (given as %) at 600°C (Res 600) are reported. The decomposition process is not represented by a single but by several decomposition processes.

Table S1: TGA data of the ionic PAMAM derivatives

<table>
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<th>Compound</th>
<th>$T_{5%}$</th>
<th>$T_{\text{max}}$</th>
<th>$T_{\text{onset}}$</th>
<th>Res 600°C (%)</th>
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</thead>
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<td>297</td>
<td>212</td>
<td>1.7</td>
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<td>287</td>
<td>212</td>
<td>1.3</td>
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<td>295</td>
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</tbody>
</table>

Figure S4: Thermograms of the PAMAM derivatives.
Figure S5: DSC curves of the PAMAM derivatives, relative to the second heating-cooling cycle performed at 10°C/min.
Figure S6: Diffractogram recorded (exposition time 1h 40min) for compound PAMAM 2G 15:15 at 40°C consistent with the proposed lamellar phase.
3. PREPARATION OF NANOAGGREGATES

All the ionic compounds were dissolved in 0.5 mL of dichloromethane, and 1 mL of milli-Q water was added. The amount of each compound was the corresponding to a final concentration of 0.5 mM in water. The mixture was shaken in an orbital shaker to open air at room temperature until the complete evaporation of the organic fraction. The insoluble fraction corresponding to myristic acid was eliminated washing twice the water solution of the aggregates with dichloromethane in order to dissolve the solid precipitate. The organic fraction was removed and put in a new vial with known weight; this vial was dried under vacuum for 3 days at 50°C until its weight remained constant. By weight difference, the quantity of precipitate was calculated. The $^1$H-NMR spectrum in figure S7 shows that the precipitate consisted almost exclusively on lipophilic chains, i.e. myristic acid. Only very weak signals corresponding to the hydrophilic acid chains were present.

![1H-NMR spectrum](image)

Figure S7: $^1$H-NMR spectrum (400MHz, CDCl$_3$) of the solid residue of compound PAMAM 2G 10:20. The assignation of the signals has been previously explained in this section.
4. MORPHOLOGICAL STUDIES

In the sample of compound **PAMAM 2G 20:10** (Figure S9), the last one (in lipophilic order) of the series in which it is still possible to observe a multi-layer morphology in the nanospheres (pointed out with a green arrow in figure S9 left), few homogeneous nanospheres without layered structure are present (pointed out with an orange arrow in figure S9 left). Moreover, some of the aggregates are no longer well-defined objects but fused or trapped on a material tangle.

Figure S8: TEM images relative to ionic PAMAM derivatives.
Figure S9: TEM images of compound **PAMAM 2G 20:10**. Left, presence of different types of aggregates: homogeneous nanospheres (orange), multi-layered nanospheres (green). Right, aggregates forming a material tangle.
5. CYTOTOXICITY OF THE IONIC DENDRIMES

DMEM (Dulbecco’s Modified Eagle’s Medium, 4.5g/l glucose), DPBS (Dulbecco’s Phosphate Buffered Saline), FBS (Fetal bovine serum) and Alamar Blue reagent were purchased from Gibco. Penicillin/Streptomycin (5000 U/ml), Amphotericin B (250µg/ml) and Trypsin (Trypsin-Versene 10X) were obtained from Lonza.

HeLa cells (Homo sapiens cervix adenocarcinoma, Cancer Research UK, Cell Lines Services) were cultured in DMEM containing 4.5 g/l D-Glucose, and 10% FBS, 1% penicillin/streptomycin and 1% amphotericin B were added to complement the media. Cells were seeded at 5x10³ cells/well in 96 well-plates. After 24 hours, medium was removed and 0.25 mg/mL of PAMAM amphiphilic dendrimers were added to cell media were added. All experiments were carried out in triplicate. The culture medium with the amphiphilic dendrimers was removed after 24, 48 or 72 hours of incubation and replaced by fresh culture medium and 10% of Alamar Blue dye solution. After 3 h incubation at 37ºC, fluorescence was read at 530/590 (excitation/emission) on a Synergy HT plate reader (BioTek, USA). Untreated cells incubated with medium without dendrimers were used as control. Cytotoxicity was expressed as relative viability of cells compared to control cells incubated with culture medium only (considered as 100% viability).
6. ENCAPSULATION OFPLITIDEPSIN

The encapsulation procedure was performed employing the oil-in-water method explained above and adapted for this specific procedure. In order to exploit the complete payload capacity of the aggregates, a concentration of Plitidepsin of 1 mM, which is twice the concentration of the ionic dendrimer, was chosen to perform the encapsulation.

The ionic dendrimer and Plitidepsin were dissolved in separated vials in 0.5 mL of dichloromethane. The quantity of each component was chosen in order to obtain 1.0 mL of water solution with final concentration 0.5 mM of the dendrimeric derivative and 1 mM of Plitidepsin (1.11 mg, M 1110.34).

The two organic solutions were mixed together and stirred for few minutes in a closed vial to allow the complete stabilization of the solution. 1.0 mL of Milli-Q water was gently added and the open vial was shaken in an orbital shaker at room temperature till complete evaporation of the organic solvent.

After the complete evaporation of the organic fraction, a solid precipitate appeared. The exact concentration of Plitidepsin in solution was established by HPLC using a standard procedure developed by PharmaMar (See Fig S10 as an example). In order to establish the real concentration in solution of the ionic dendrimer, the water solution of the host-guest complex was washed twice with dichloromethane to dissolve the solid precipitate. The organic fraction was removed and put in a new vial with known weight; this vial was dried under vacuum for 3 days at 50°C until its weight remained constant. By weight difference, the quantity of precipitate was calculated.

Since the concentration of Plitidepsin in solution was established by HPLC, its amount in the solid precipitate was calculated by difference with the initial amount of Plitidepsin (1.11 mg). Knowing the global amount of the precipitate and the fraction of Plitidepsin, the quantity of amphiphile in the precipitate and, consequently its concentration in water was calculated.
Figure S10: HPLC Chromatogram for Plitidepsin and PAMAM 2G (15:15) encapsulating Plitidepsin.
7. References