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Electronic Supplementary Information for:

Biophysical study of gene nanocarriers formed by anionic/zwitterionic mixed lipids and pillar[5]arene polycationic macrocycles

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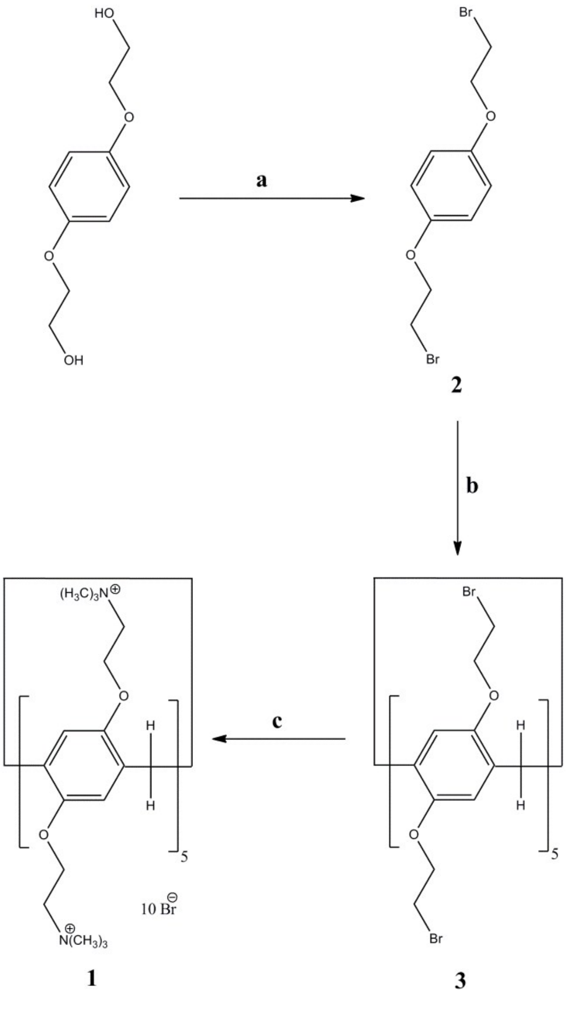
Synthesis and characterization of pillar[5]arene

Materials and methods

All reagents were commercially available and used as supplied without further purification. NMR spectra were recorded with a Varian Mercury 300 spectrophotometer or a Varian Inova 400 spectrophotometer by using deuterated solvent as the lock and the residual solvent as the internal reference. High-resolution electrospray ionization mass spectra were recorded with a Bruker Amazon ETD spectrometer equipped with ESI interface and ion trap analyzer. Thermal gravimetric analysis (TGA) was recorded with a TGA Q500 thermogravimetric analyzer.



Scheme S1 The synthetic route for the synthesis of pillar[5]arene.

The pillar[5]arene was synthesized according to the literature.[1]

a) Carbon tetrabromide (39.8 g, 120 mmol) was added to a solution of 1,4-bis(2-hydroxiethoxy)benzene (10.0 g, 50.5 mmol) and triphenylphosphine (31.5 g, 120 mmol) in 250 mL dry acetonitrile at 0 ℃ with stirring. Then, the reaction mixture was stirred for 4 h under Ar at room temperature. Finally, cold water was added to the reaction mixture to precipitate **2** (Figure S-1) as a white solid (14.4 g, 88%).

b) To a solution of **2** in dichloromethane (230 mL), paraformaldehyde (0.93 g, 30.87 mmol) and boron trifluoride diethyl etherate (4.83 g, 33.86 mmol) were added under argon atmosphere and the mixture was stirred at room temperature for 2 h. The resulting mixture was washed with water, saturated sodium bicarbonate solution and brine. Then, the solvent was removed to obtain **3** as a light yellow solid (3.37 g, 73%).

c) Compound **3** (2 g, 1.19 mmol) and trimethylamine (31-35% in ethanol, 13 mL, 48.11 mmol) were mixed in ethanol (100 mL). The solution was refluxed overnight. The resulting precipitate was collected by vacuum filtration, washed with ethanol and dried under high vacuum to obtain the pillar[5] arene **1** as a white solid (2.56 g, 95%).

The ^{1}H NMR spectrum of pillar[5]arene is shown in Figure S1. ^{1}H NMR (300 MHz, D_2O, room temperature) δ (ppm): 6.95 (s, 10H); 4.45 (s, 20H); 3.94 (s, 10H); 3.80 (s, 20H); 3.22 (s, 90H). The ^{13}C NMR spectrum of pillar[5]arene is shown in Figure S2. ^{13}C NMR (75 MHz, D_2O, room temperature) δ (ppm): 149.3, 129.9, 116.5, 64.9, 63.4, 54.1, 29.5. ESI-MS is shown in Figures S3 and S4: *m/z* $[P5+8Br^-]^{2+}$ 1055.2 (calculated: 1055.7). Thermogravimetric analysis (Figure S5) shows a 5 % (w/w) water content of pillar[5]arene, corresponding to six water molecules.

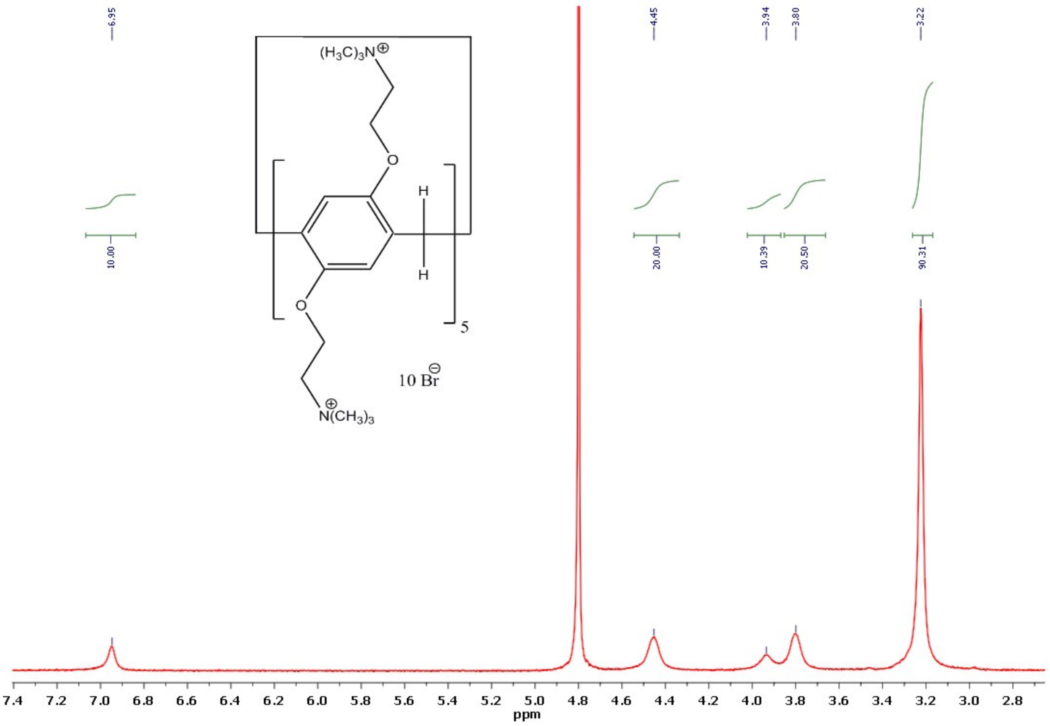


Fig. S1 ^{1}H NMR spectrum (300 MHz, D_2O, room temperature) of pillar[5]arene.

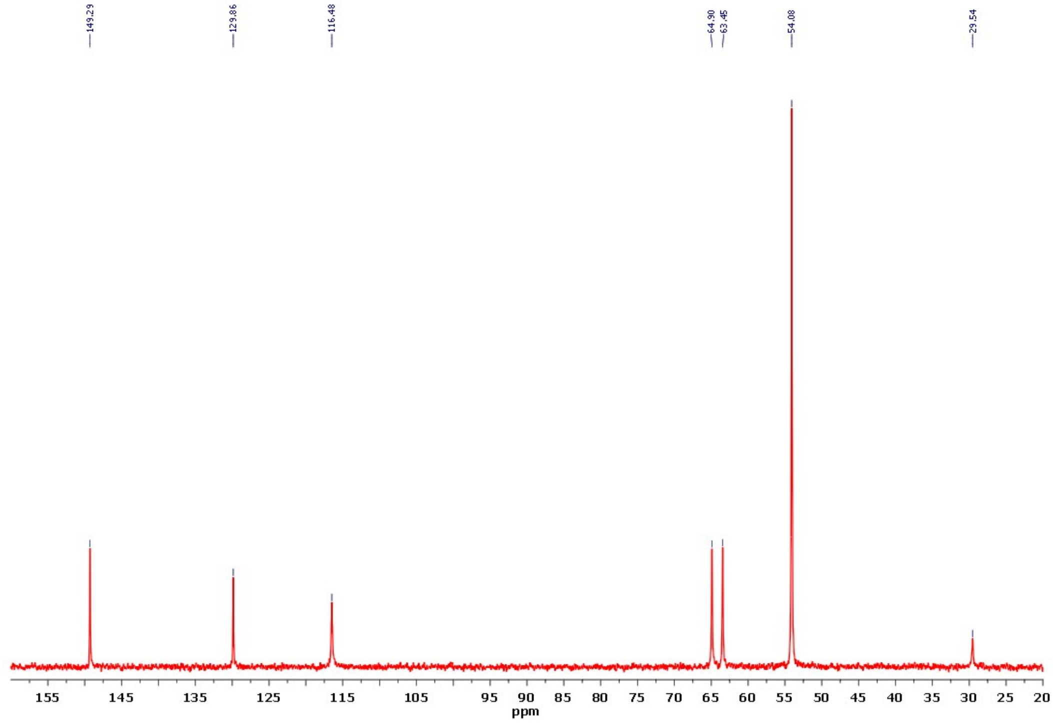


Fig. S2 ^{13}C NMR spectrum (75 MHz, D_2O, room temperature) of pillar[5]arene.

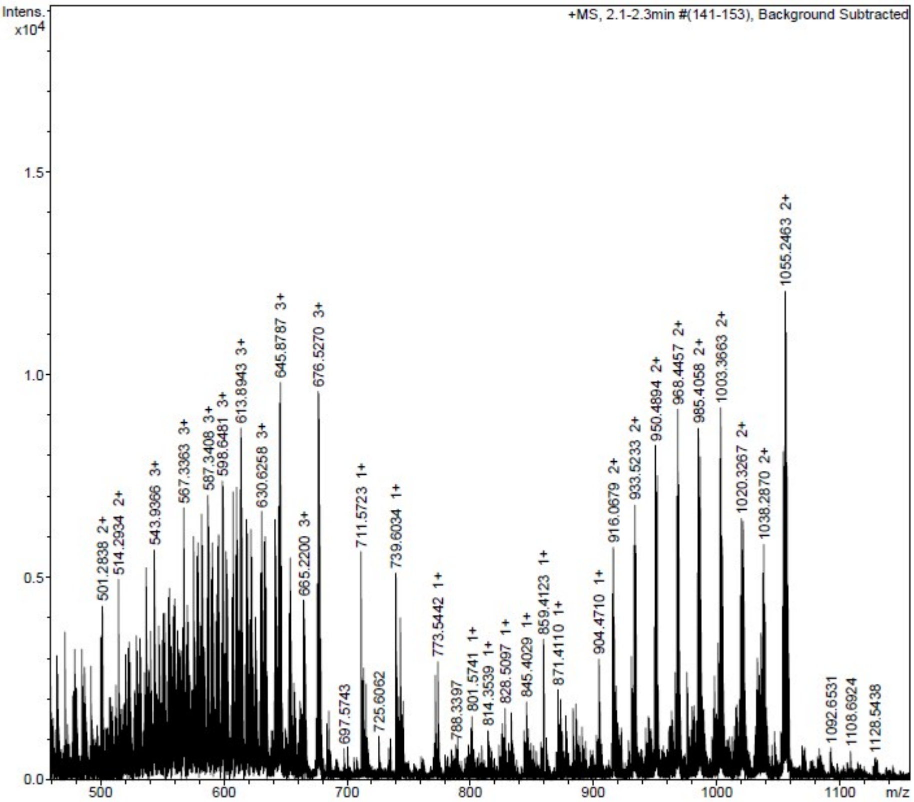


Fig. S3 Electrospray ionization mass spectrum of pillar[5]arene.

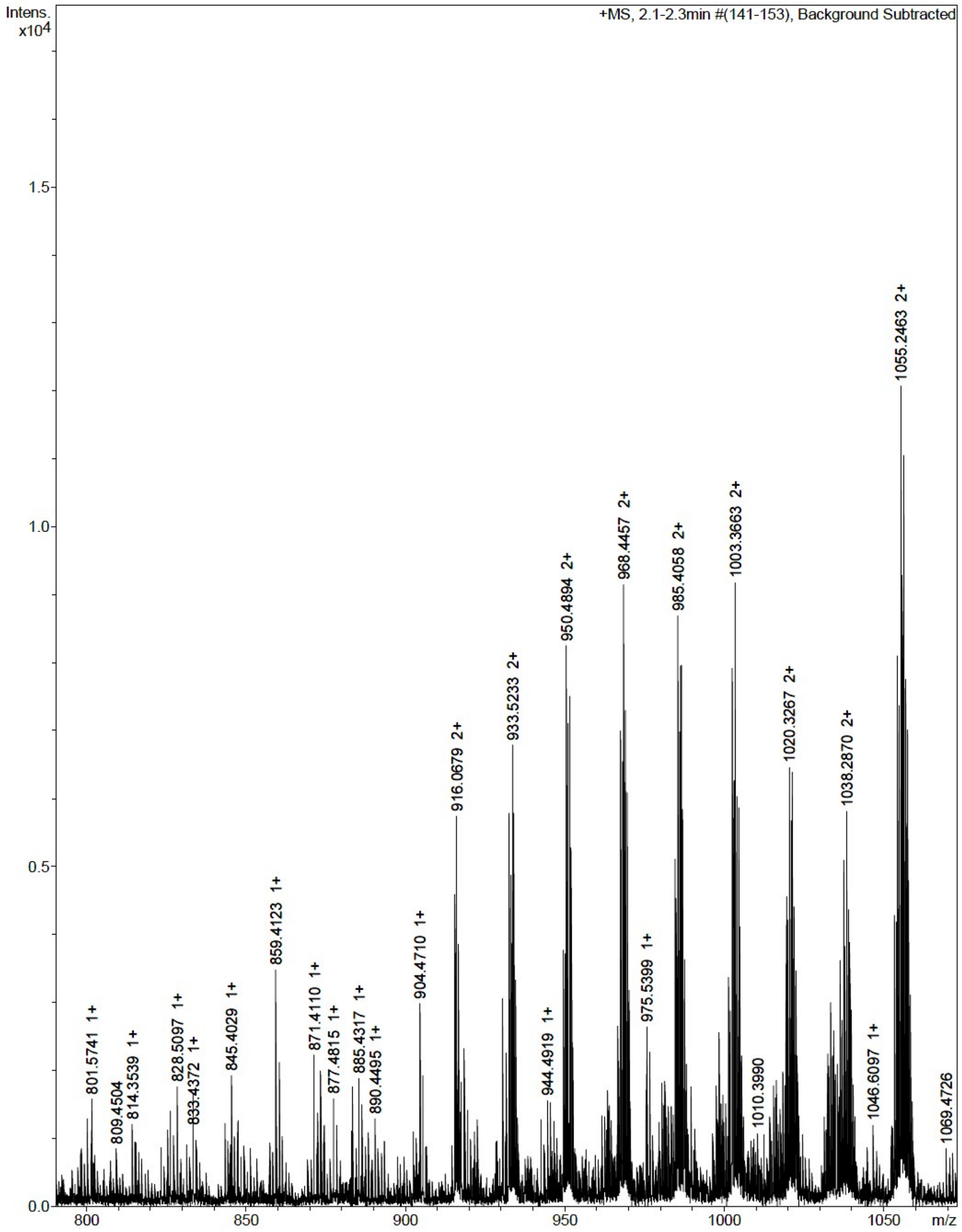


Fig. S4 High resolution electrospray ionization mass spectrum of pillar[5]arene.

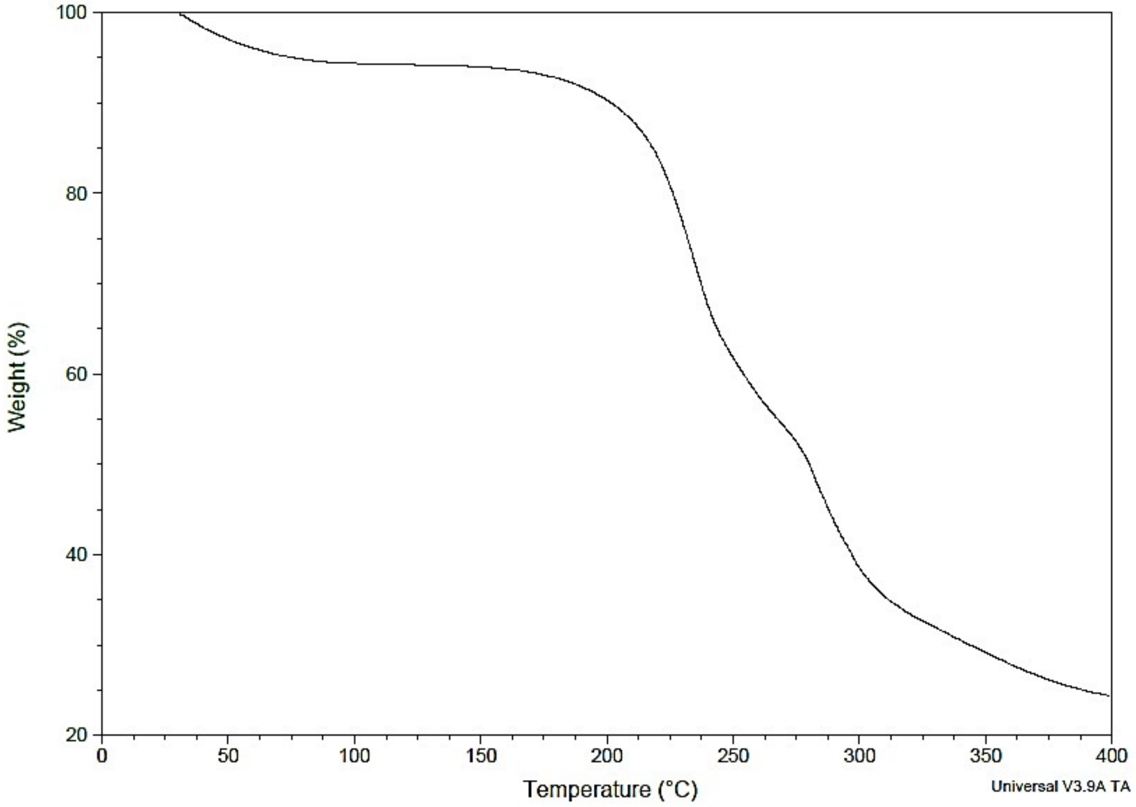


Fig. S5 Thermal gravimetric analysis of pillar[5]arene.

Table S1 Concentration of P^{10+} at which DOPG/DOPE-P^{10+}-pDNA lipoplexes reach the electroneutrality ($[P^{10+}]_\phi$) at different molar compositions (α)

α	0.2	0.5	1
$[P^{10+}]_\phi$ (μM)	3.9 ± 0.3	3.4 ± 0.3	3.2 ± 0.3

Values estimated with a 8% error

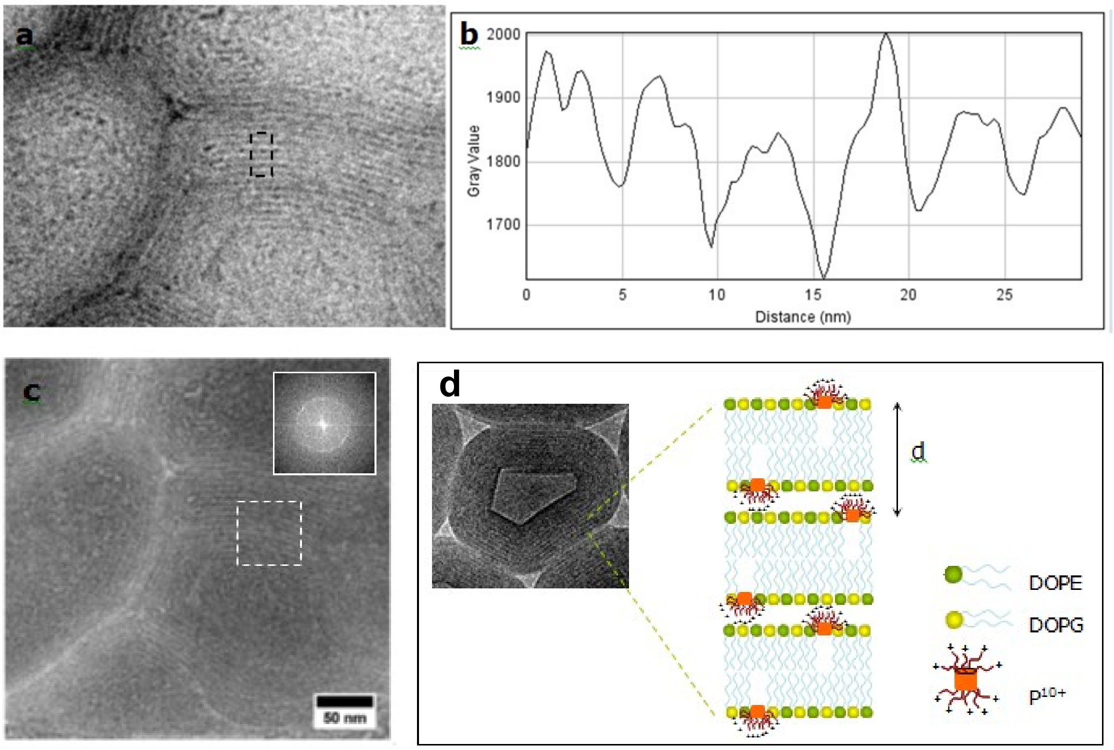


Fig. S6 A selection of cryo-TEM micrographs showing DOPG/DOPE nanoaggregates in the presence of P^{10+}, at $\alpha = 0.5$ and $[P^{10+}] = 30\ \mu M$. Panel a reports a detail extracted from the original cryo-TEM micrograph showing a zoom view of the lamellar arrangement. Panel b: plot of the grey level vs distance across the multilamellae defined by the black dashed square on panel a. The inset on panel c shows Fast Fourier Transform (FFT) profile calculated over a selected area (see dashed white square) on the original micrograph; notice that the diffraction spot corresponds to a typical lamellar pattern. Panel d shows a 2D schematic view of this lamellar organization.

Notes and References

1 Y. J. Ma, X. F. Ji, F. Xiang, X. D. Chi, C. Y. Han, J. M. He, Z. Abliz, W. X. Chen and F. H. Huang, *Chem. Commun.*, 2011, 47, 12340-12342.