Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental materials to:

In silico study of PEI-PEG-squalene-dsDNA polyplex formation: The delicate role of PEG length to the binding of PEI to DNA

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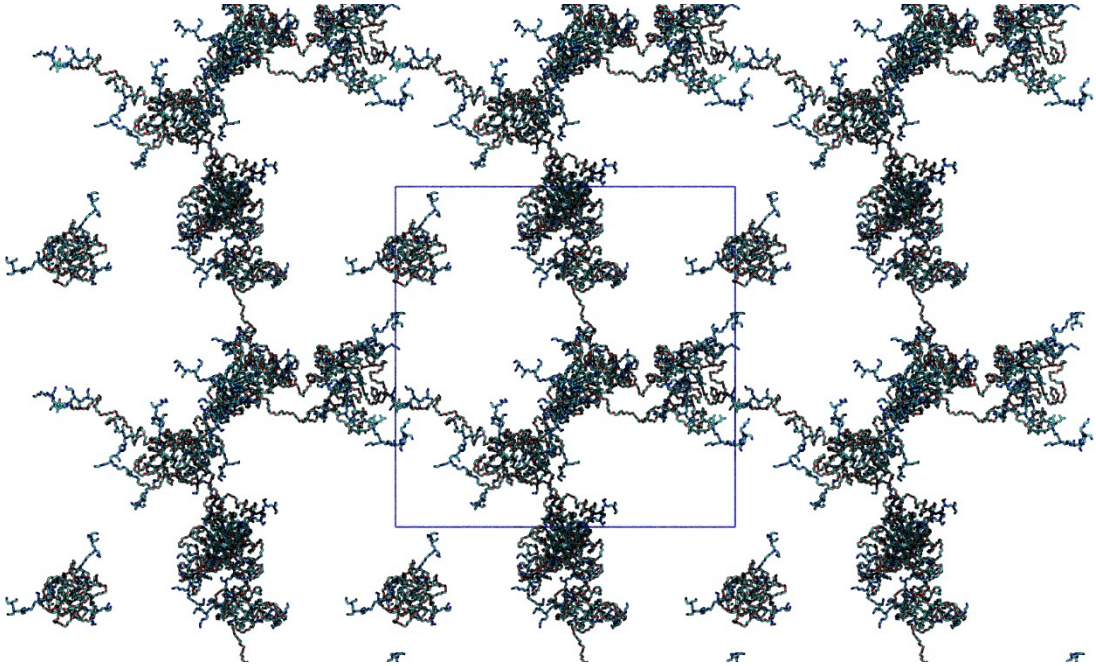

Figure S1. Snapshot depicting the trans-PBC network formed when starting from an extended conformation of the Vector 1500. The square represents the simulation box.

1) The synthesis of 1,1',2-tris-norsqualene aldehyde:

1,1',2-Tris-norsqualene aldehyde (SQ-CHO; **1**) synthesis was achieved in three steps using previously published protocols (17,70,71). Briefly, in the first step, 2-hydroxy-3-bromosqualene was obtained from reaction of squalene with N-bromosuccinimide in tetrahydrofuran at 0 °C, for 90 minutes, followed by its treatment with potassium carbonate in methanol, at room temperature (24 °C), for 2 hours, to obtain 2,3-oxidosqualene. In the last step, 2,3-oxidosqualene was reduced to SQ-CHO with periodic acid in water-dioxane solution, at room temperature (24 °C), in 2 hours. SQ-CHO **1** obtained in 7 % overall yield was purified by "flash chromatography", using 230-400 mesh silicagel as stationary phase and a mixture of methylene chloride and n-hexane in 1:1 volumetric ratio as eluent. The chemical structure of pure SQ-CHO, as a pale-yellow oil was confirmed by ^{1}H-NMR (Figure S2) and ^{13}C-NMR (Figure S3). **^{1}H-NMR** (400 MHz, $CDCl_3$, TMS) δ (ppm) = 9.75 (t, J = 1.8 Hz, 1H), 5.16 – 5.09 (m, 5H), 2.50 (t, J = 7.5 Hz, 2H), 2.31 (t, J = 7.5 Hz, 2H), 2.12 – 1.98 (m, 16H), 1.68 (s, 3H), 1.60 (s, 16H). **^{13}C-NMR** (101 MHz, $CDCl_3$, TMS) δ (ppm) 202.69 (-CH=O), 135.14 (CH_3-C=C-), 134.90 (CH_3-C=C-), 134.78(CH_3-C=C-), 132.84 (CH_3-C=C-), 131.24 ((CH_3)$_2$C=), 125.43 (-CH=), 124.53 (-CH=), 124.40 (-CH=), 124.25 (-CH=), 42.15 (-CH_2-), 39.72 (-CH_2-), 39.52 (-CH_2-), 31.85 (-CH_2-), 28.24 (-CH_2-), 26.76 (-CH_2-), 26.65 (-CH_2-), 26.54 (-CH_2-), 25.69 (-CH_3), 17.67 (-CH_3), 16.08 (-CH_3), 16.04 (-CH_3), 15.99 (-CH_3).

2) The synthesis of PEGylated squalene

The synthesis of PEGylated squalene (SQ-PEG-NH_2; **2**) was performed using H_2N-PEG-NH_2 of three different lengths (molecular weights of 544 Da, 1500 Da, and 3000 Da), according previously described protocols (17,18,58).

a) SQ-PEG-NH_2 with H_2N-PEG-NH_2 of 544 Da

SQ-CHO (41 mg, 0.108 mmol, 1 equiv.) was solubilized in dry acetonitrile (5 mL) and H_2N-PEG-NH_2 (MW: 544 Da) (59 mg, 0.108 mmol, 1 equiv.) was added to the obtained solution. The reaction mixture was magnetically stirred at room temperature (24 °C) for 48 hours under nitrogen atmosphere. The product SQ-PEG-NH_2-0.5 (**2a**) was obtained in a pure form and used further without purification. **^{1}H-NMR** (400 MHz, $CDCl_3$, TMS) δ (ppm) = 7.65 (1H, t, J=4.8, -SQ-C***H***=N), 5.09 (5H, m, SQ-C***H***=C-), 3.80 (2H, m, PEG-C***H***$_2$-NH_2), 3.70 (2H, m, PEG-C***H***$_2$-CH_2-NH_2), 3.64 (40 H, m, SQ-O-C***H***$_2$-C***H***$_2$-O-), 3.52 (2H, t, PEG-C***H***$_2$-N=CH-), 3.47 (2H, m, PEG-O-C***H***$_2$-CH_2-N=), 2.87 (2H, m, PEG-N***H***$_2$), 2.01 (16H, m, SQ-C***H***$_2$-C***H***$_2$-), 1.67 (3H, m, SQ-C***H***$_3$), 1.59 (12H, m, SQ=C(C***H***$_3$)-), 1.56 (2H, m, SQ-CH_2-C***H***$_2$-CH=) (Figure S4). **^{13}C-NMR** (101 MHz, $CDCl_3$, TMS) δ (ppm) = 166.86 (-***C***H=N-), 135.13 (-***C***(CH_3)=C-), 134.9 (-***C***(CH_3)=C-), 131.25 (=***C***(CH_3)$_2$), 125.04 (-***C***H=C(CH_3)$_2$) 124.4 (-***C***H=C(CH_3)-), 124,26 (-***C***H=C(CH_3)-), 73.07 (-O-***C***H$_2$-CH_2-), 70.56 (O-***C***H$_2$-CH_2-N=), 70.27 (-***C***H$_2$-O-), 60.69 (-***C***H$_2$-N=), 41.72 (-***C***H$_2$-NH_2), 39.73 (-***C***H$_2$-CH_2-), 35.9 (-***C***H$_2$-C(CH_3)=), 34.47 (-***C***H$_2$-CH=N-), 28.27 (-***C***H$_2$-CH=C(CH_3)$_2$), 26.76 (-***C***H$_2$-CH_2-), 26.66 (-CH_2-***C***H$_2$-), 25.71 (=C(***C***H$_3$)$_2$), 17.69 (=C(***C***H$_3$)$_2$), 16.06 (-C(***C***H$_3$)=) (Figure S5).

b) SQ-PEG-NH_2 with H_2N-PEG-NH_2 of 1500 Da

SQ-CHO (20 mg, 0.053 mmol, 1 equiv) was solubilized in dry acetonitrile (5 mL) and H_2N-PEG-NH_2 (MW: 1500 Da) (80 mg, 0.053 mmol, 1 equiv) was added to the obtained solution. The reaction mixture was magnetically stirred at room temperature (24 °C) for 48 hours under nitrogen atmosphere. The product SQ-PEG-NH_2-1.5 (**2b**) was obtained in a pure form and used further without purification. **^{1}H-NMR** (400 MHz, $CDCl_3$, TMS) δ (ppm) = 7.62 (1H, t, J=4.8, -SQ-C***H***=N), 5.09 (5H, m, SQ-C***H***=C-), 3.80 (2H, m, PEG-C***H***$_2$-NH_2), 3.71 (2H, m, PEG-C***H***$_2$-CH_2-NH_2), 3.63 (120 H, m, PEG-O-C***H***$_2$-C***H***$_2$-O-), 3.55 (2H, t, PEG-C***H***$_2$-N=CH-), 3.46 (2H, m, PEG-O-C***H***$_2$-CH_2-N=), 2.82 (2H, m, PEG-N***H***$_2$), 2.01 (16H, m, SQ-C***H***$_2$-C***H***$_2$-), 1.66 (3H, m, SQ-C***H***$_3$), 1.58 (12H, m, SQ=C(C***H***$_3$)-), 1.56 (2H, m, SQ-CH_2-C***H***$_2$-CH=) (Figure S6). **^{13}C-NMR** (101 MHz, $CDCl_3$,

TMS) δ (ppm) = 165.23 (-***C***H=N-), 135.11 (-***C***(CH_3)=C-), 134.88 (-***C***(CH_3)=C-), 131.21 (=***C***(CH_3)$_2$), 125.02 (-***C***H=C(CH_3)$_2$) 124.41 (-***C***H=C(CH_3)-), 124,27 (-***C***H=C(CH_3)-), 72.57 (-O-***C***H_2-CH_2-), 70.57 (O-***C***H_2-CH_2-N=), 70.08 (-***C***H_2-O-), 61.72 (-***C***H_2-N=), 57.88 (-***C***H_2-NH_2), 39.72 (-***C***H_2-CH_2-), 35.95 (-***C***H_2-C(CH_3)=), 34.34 (-***C***H_2-CH=N-), 28.27 (-***C***H_2-CH=C(CH_3)$_2$), 26.77 (-***C***H_2-CH_2-), 26.66 (-CH_2-***C***H_2-), 25.68 (=C(***C***H_3)$_2$), 17.67 (=C(***C***H_3)$_2$), 16.04 (-C(***C***H_3)=) (Figure S7).

c) SQ-PEG-NH_2 with H_2N-PEG-NH_2 of 3000 Da

SQ-CHO (11 mg, 0.030 mmol, 1 equiv.) was solubilized in dry acetonitrile (5 mL) and H2N-PEG-NH2 (MW: 3000 Da) (89 mg, 0.030 mmol, 1 equiv.) was added to the obtained solution. The reaction mixture was magnetically stirred at room temperature (24 °C) for 48 hours under nitrogen atmosphere. The product SQ-PEG-NH2-3 (2c) was obtained in a pure form and used further without purification. 1H-NMR (400 MHz, CDCl3, TMS) δ (ppm) = 7.65 (1H, t, J=4.8, SQ-CH=N), 5.09 (5H, m, SQ-CH=C-), 3.80 (2H, m, PEG-CH2-NH2), 3.71 (2H, m, PEG-C***H***$_2$-CH_2-NH_2), 3.63 (264H, m, PEG-O-C***H***$_2$-C***H***$_2$-O-), 3.54 (2H, t, PEG-C***H***$_2$-N=CH-), 3.45 (2H, m, PEG-O-C***H***$_2$-CH_2-N=), 2.89 (2H, m, PEG-N***H***$_2$), 2.05 (16H, m, SQ-C***H***$_2$-C***H***$_2$-), 1.66 (3H, m, SQ-C***H***$_3$), 1.58 (12H, m, SQ=C(C***H***$_3$)-), 1.56 (2H, m, SQ-CH_2-C***H***$_2$-CH=) (Figure S8). **^{13}C-NMR** (101 MHz, $CDCl_3$, TMS) δ (ppm) = 166.80 (-***C***H=N-), 135.11 (-***C***(CH_3)=C-), 134.88 (-***C***(CH_3)=C-), 131.21 (=***C***(CH_3)$_2$), 124.98 (-***C***H=C(CH_3)$_2$) 124.41 (-***C***H=C(CH_3)-), 124,27 (-***C***H=C(CH_3)-), 72.68 (-O-***C***H_2-CH_2-), 70.58 (O-***C***H_2-CH_2-N=), 70.26 (-***C***H_2-O-), 61.73 (-***C***H_2-N=), 41.66 (-***C***H_2-NH_2), 39.72 (-***C***H_2-CH_2-), 35.88 (-***C***H_2-C(CH_3)=), 34.46 (-***C***H_2-CH=N-), 28.28 (-***C***H_2-CH=C(CH_3)$_2$), 26.77 (-***C***H_2-CH_2-), 26.67 (-CH_2-***C***H_2-), 25.68 (=C(***C***H_3)$_2$), 17.67 (=C(***C***H_3)$_2$), 16.05 (-C(***C***H_3)=) (Figure S9).

3) The synthesis of p-methyl-2,6-diformylphenol (FDA2):

The synthesis was achieved according to Lindoy modified protocol (72). Briefly, into a 100 mL two neck round bottom flask, *p*-cresol (2 g, 18.5 mmol) and hexamethylenetetramine (5.19 g, 36.7 mmol) were solubilized in anhydrous TFA (30 mL), under nitrogen atmosphere, and the resulting yellow solution was refluxed for 48h under magnetic stirring and nitrogen atmosphere, till the solution color changed to orange. The end of reaction was visualized with STLC plates F254, eluted with dichloromethane which showed at 254 nm the disappearance of reactant spot and appearance of new spots at low Rf values. Reaction mixture was poured in cold 4M HCl solution (100 mL), magnetic stirred for 20 minutes, and extracted with DCM (3 x 100 mL). The organic layer was washed with double distilled water (2 x 100 mL), saturated brine (2 x 100 mL), dried on anhydrous $NaSO_4$, filtered and evaporated to dryness *in vacuo* to give a yellow crystalline residue. The product was purified by "flash chromatography" using 230 – 400 mesh silica gel as stationary phase and a mixture of hexane:ethyl acetate (20:1) as eluent. **FDA2** was obtained as crystalline yellow needles (1.51 g, yield 50%), **mp:** 129.9 – 130.2 ^{o}C, (literature mp: 132-133 ^{o}C (73) and 130 ^{o}C (74)). **^{1}H-NMR** ($CDCl_3$, 400 MHz, TMS) δ (ppm) = 11.46 (s, 1H, -O*H*), 10.22 (s. 2H, -C*H*O), 7.77 (s, 2H, Ar-*H*), 2.39 (s, 3H, -CH_3) (Figure S10); **^{13}C-NMR** ($CDCl_3$, 101 MHz) δ (ppm) = 192.31 (-*C*HO), 161.79 (arom. *C*-2), 138.01 (arom. *C*-4,6), 129.54 (arom. *C*-5), 122.9 (arom. *C*-1,3), 20.10 (-*C*H_3) (Figure S11), **FT-IR (KBr) (cm^{-1}):** 3445.05 (alcohols O-H), 2923.18 (aliphatic –CH_3), 2869.38 (formyl C-H), 1682.86 (formyl C=O), 1666.94 (formyl C=O associated with OH), 1603.3 (aromatic C-C stretching), 1215.23 (phenolic C-O), 961.74 (aromatic C-H bending) (Figure S12).

4) Synthesis of intermediary systems

The synthesis of intermediary systems **3** was conducted using the protocols previously described in literature (15,16,18) and briefly described below for each system.

a) Synthesis of **3a** intermediary system

FDA2 (0.014 g, 0.09 mmol) was dissolved in a small volume of DCM (1-2 mL) and added dropwise to previously obtained **2a** (0.08 g, 0.09 mmol) in acetonitrile. With the addition of **FDA2** to the **2a** solution, a change of colour was observed, suggesting the formation of imine linkage. The obtained yellow mixture was allowed to stir for 48h at room temperature (24 °C), under nitrogen atmosphere. The product **3a**, as intense yellow oil, was obtained in a pure form, as shown by H-NMR (Figure S13), and further used without purification.

b) Synthesis of **3b** intermediary system

FDA2 (0.007 g, 0.04 mmol) was dissolved in a small volume of DCM (1-2 mL) and added dropwise to previously obtained **2b** (0.08 g, 0.04 mmol) in acetonitrile solution. With the addition of **FDA2** to **2b** solution a change of colour was observed, suggesting the formation of reversible imine linkage. The obtained yellow mixture was allowed to stir for 48h at room temperature (24 °C), under nitrogen atmosphere. The product **3b**, as intense yellow oil, was obtained in a pure form, as shown by H-NMR (Figure S14), and further used without purification.

c) Synthesis of **3c** intermediary system

FDA2 (0.004 g, 0.02 mmol) was dissolved in a small volume of DCM (1-2 mL) and added dropwise to previously obtained **2c** (0.08 g, 0.02 mmol) in acetonitrile. With the addition of **FDA2** over **2c** solution, a change of colour was observed, indicating the formation of reversible imine linkage. The obtained yellow mixture was allowed to stir for 48h at room temperature (24 °C), under nitrogen atmosphere. The product **3c**, as intense yellow oil, was obtained in a pure form, as shown by ^{1}H-NMR (Figure S15), and further used without purification.

5) Synthesis of the vectors

The synthesis of vectors (VECTOR 500, VECTOR 1500, VECTOR 3000) was accomplished according a protocol that we have described earlier (15,16,18).

a) Synthesis of VECTOR 500

3a (0.05 g, 0.047 mmol) was solubilized in water (2-3 mL) and 1 mL of aqueous solution of ***b*-PEI800** (0.037 g, 0.047 mmol) was added. The reaction mixture was stirred magnetically for 48 hours at room temperature (24 °C) and was considered completed when ^{1}H-NMR spectra showed the total consumption of carbonyl group at 10.53 ppm (Figure S16). VECTOR 500 was obtained as yellow solution, stored at 2-4 °C, and used further without purification. For the full solvation of VECTOR 500, it was necessary to add 10% DMSO.

b) Synthesis of VECTOR 1500

3b (0.05 g, 0.025 mmol) was solubilized in water (2-3 mL) and 1 mL of aqueous solution of *b*-PEI800 (0.020 g, 0.025 mmol) was added. The reaction mixture was stirred magnetically for 48 hours at room temperature (24 °C) and was considered completed when ^{1}H-NMR spectra showed the total consumption of carbonyl group at 10.53 ppm. VECTOR 1500 was obtained as yellow solution, stored at 2-4 °C, and used without further purification.

c) Synthesis of VECTOR 3000

3c (0.05 g, 0.014 mmol) was solubilized in water (2-3 mL), and 1 mL of aqueous solution of *b*-PEI800 (0.011 g, 0.014 mmol) was added. The reaction mixture was stirred magnetically for 48 hours at room

temperature (24 °C), and was considered completed when ^{1}H-NMR spectra showed the total consumption of carbonyl group at 10.53 ppm. VECTOR 3000 was obtained as yellow aqueous solution, stored at 2-4 °C, and used without further purification.

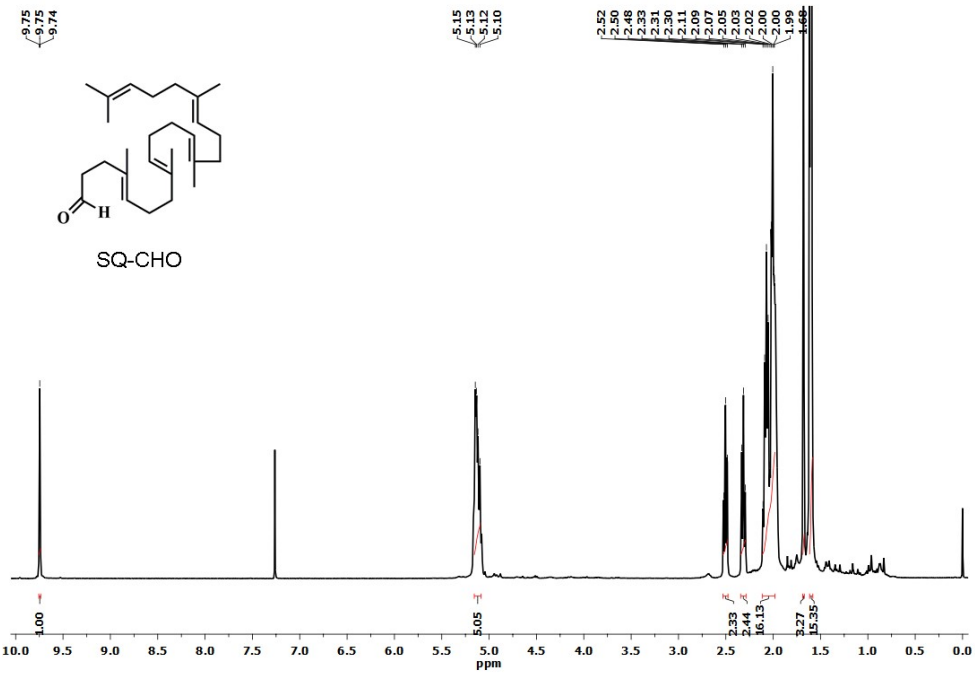

Figure S2. ^{1}H-NMR ($CDCl_3$, 400 MHz) of SQ-CHO.

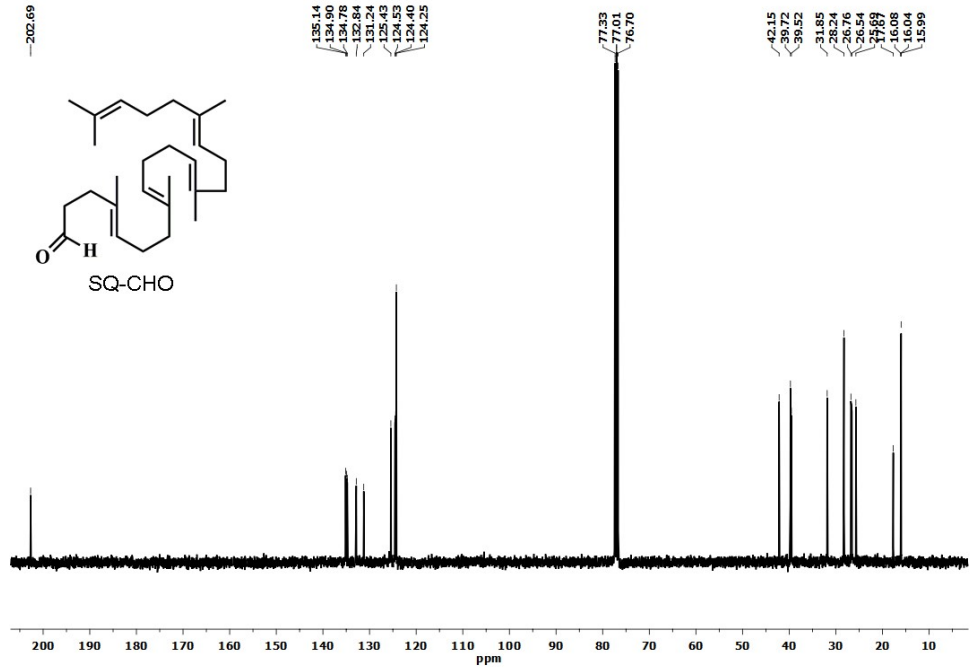

Figure S3. ^{13}C-NMR ($CDCl_3$, 100 MHz) of SQ-CHO.

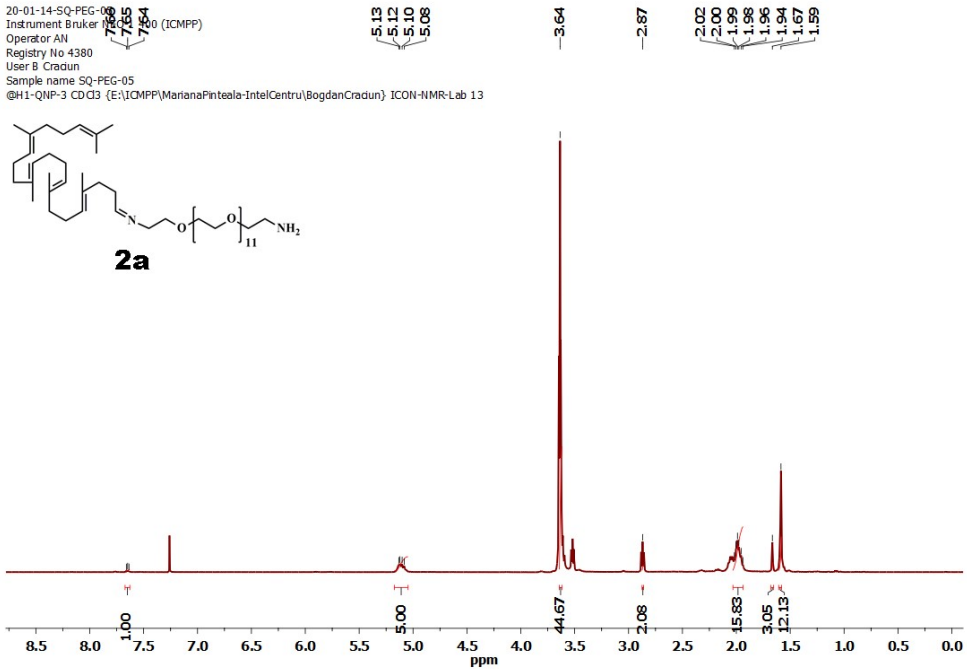

Figure S4. ^{1}H-NMR ($CDCl_3$, 400 MHz) of 2a.

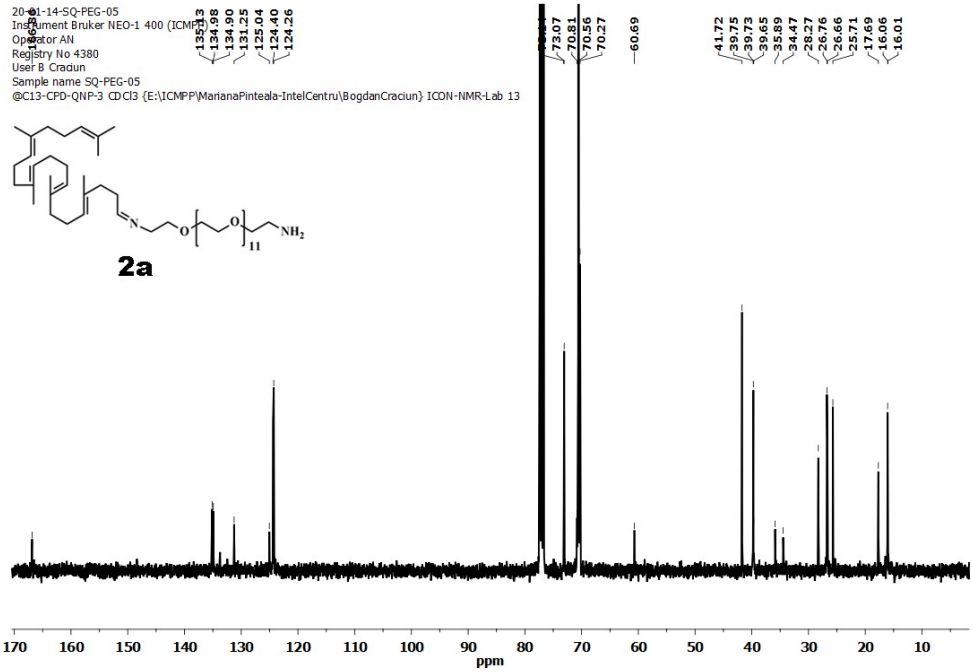

Figure S5. ^{13}C-NMR ($CDCl_3$, 100 MHz) of 2a.

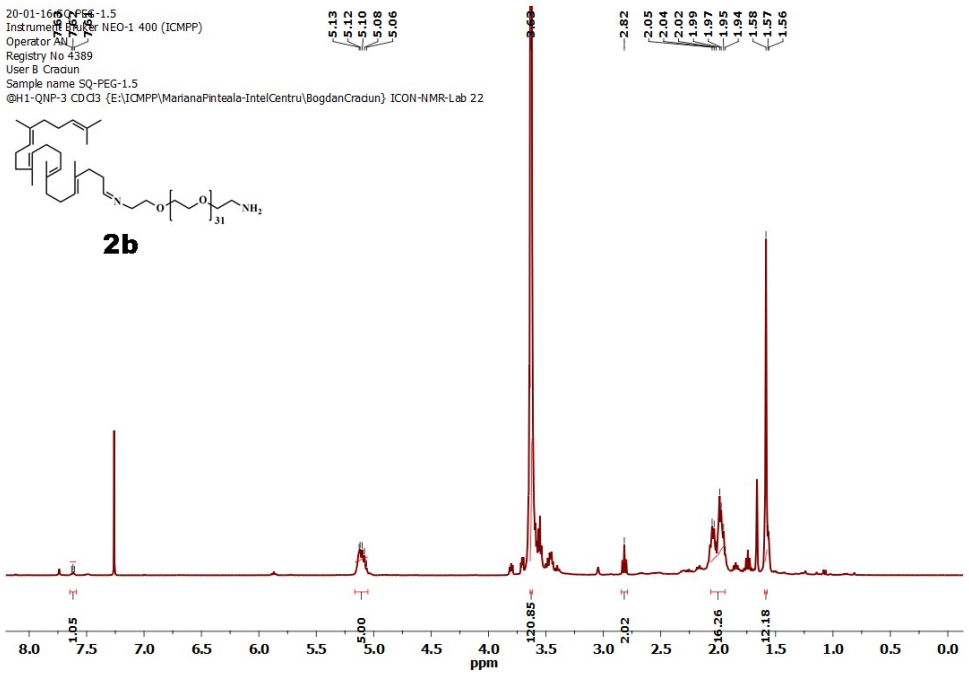

Figure S6. ^{1}H-NMR ($CDCl_3$, 400 MHz) of 2b.

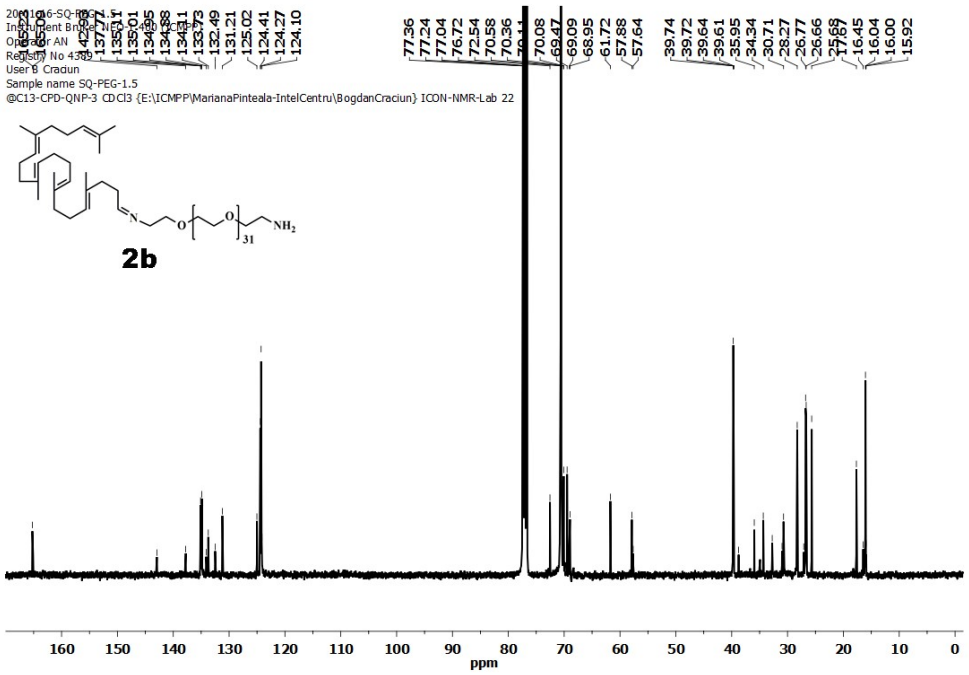

Figure S7. ^{13}C-NMR ($CDCl_3$, 100 MHz) of 2b.

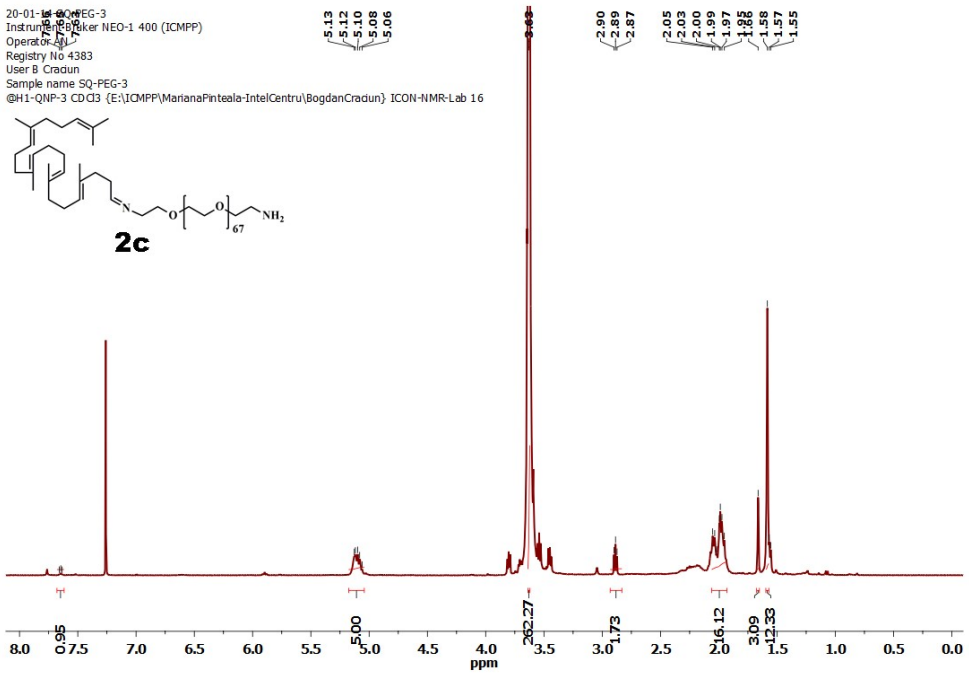

Figure S8. ^{1}H-NMR ($CDCl_3$, 400 MHz) of 2c.

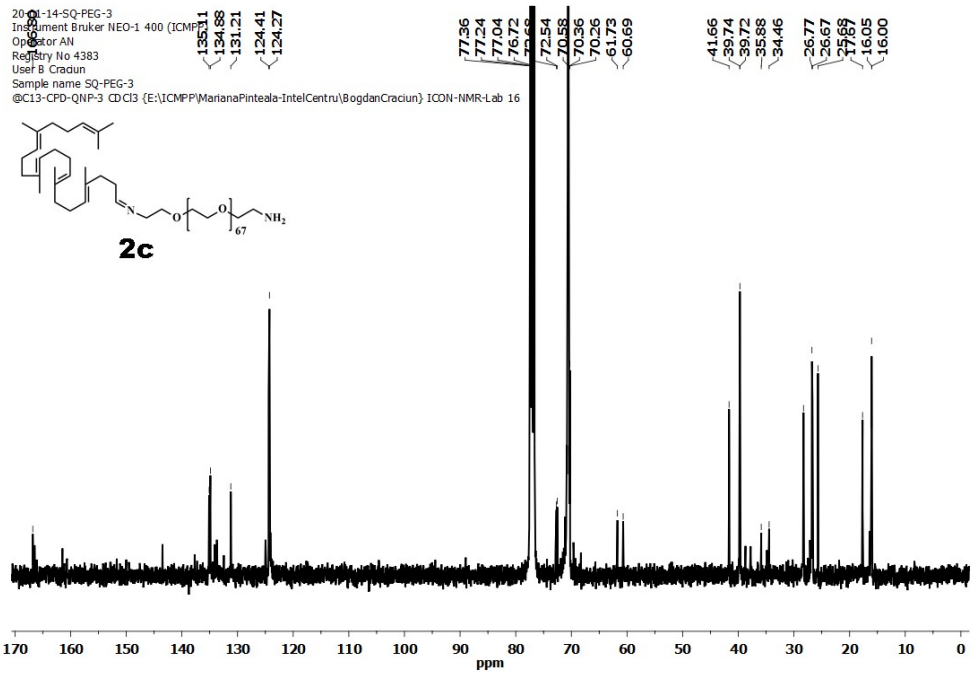

Figure S9. ^{13}C-NMR ($CDCl_3$, 100 MHz) of 2c.

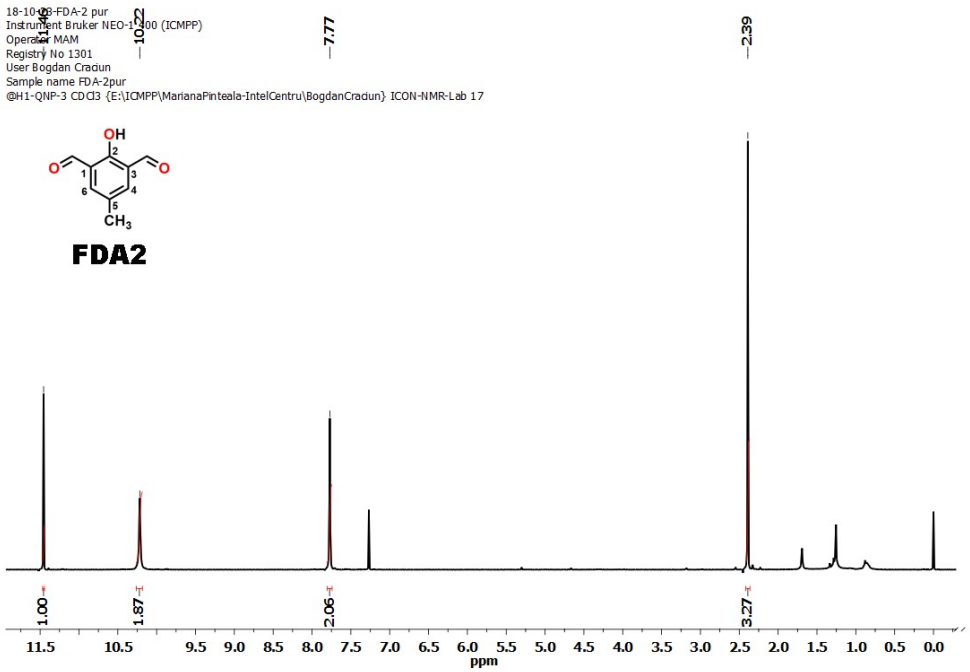

Figure S10. ^{1}H-NMR ($CDCl_3$, 400 MHz) of FDA2.

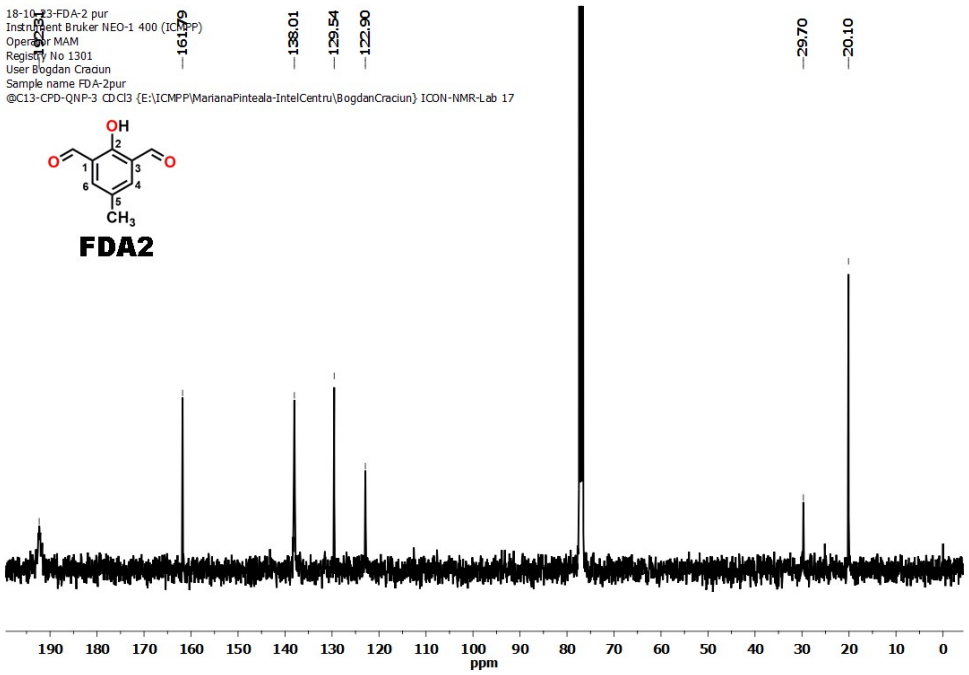

Figure S11. ^{13}C-NMR ($CDCl_3$, 100 MHz) of FDA2.

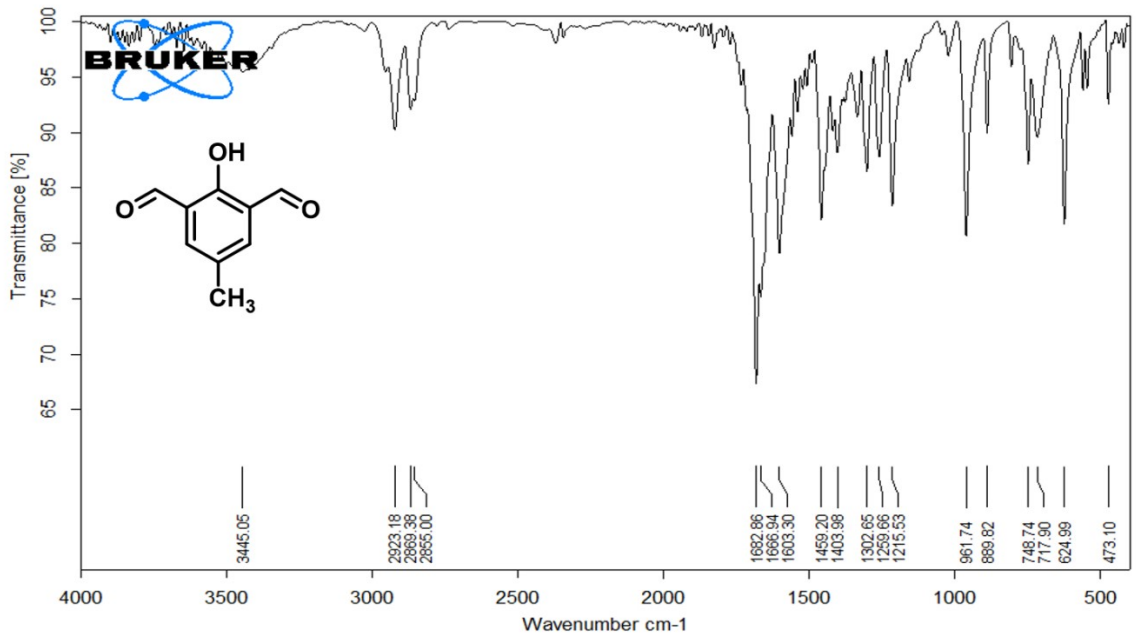

Figure S12. FTIR (KBr) of FDA2.

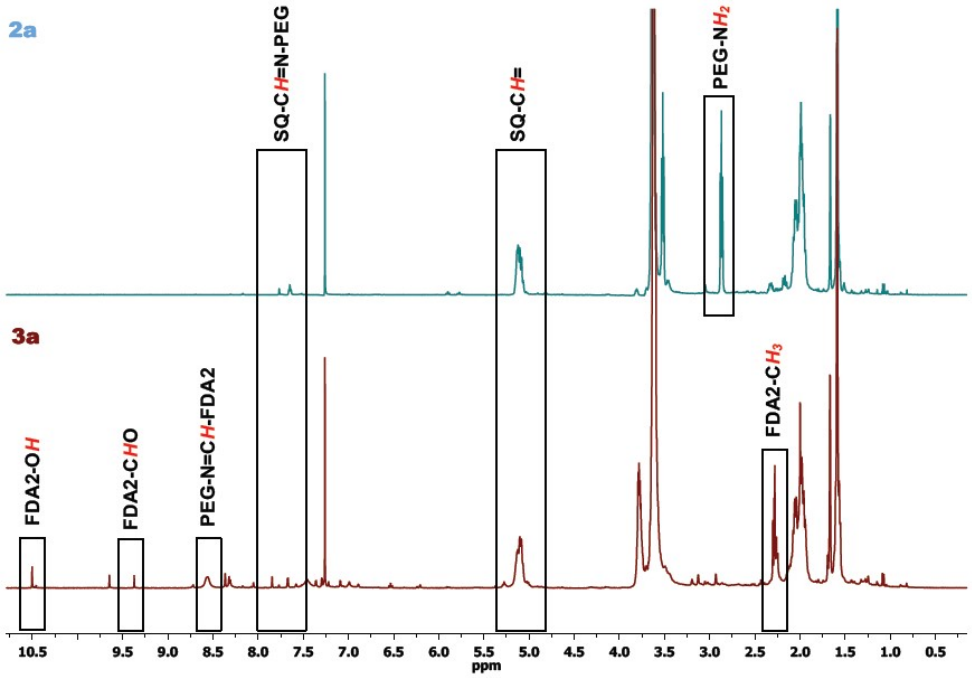

Figure S13. ^{1}H-NMR ($CDCl_3$, 400 MHz) overlaid spectra of 2a and 3a.

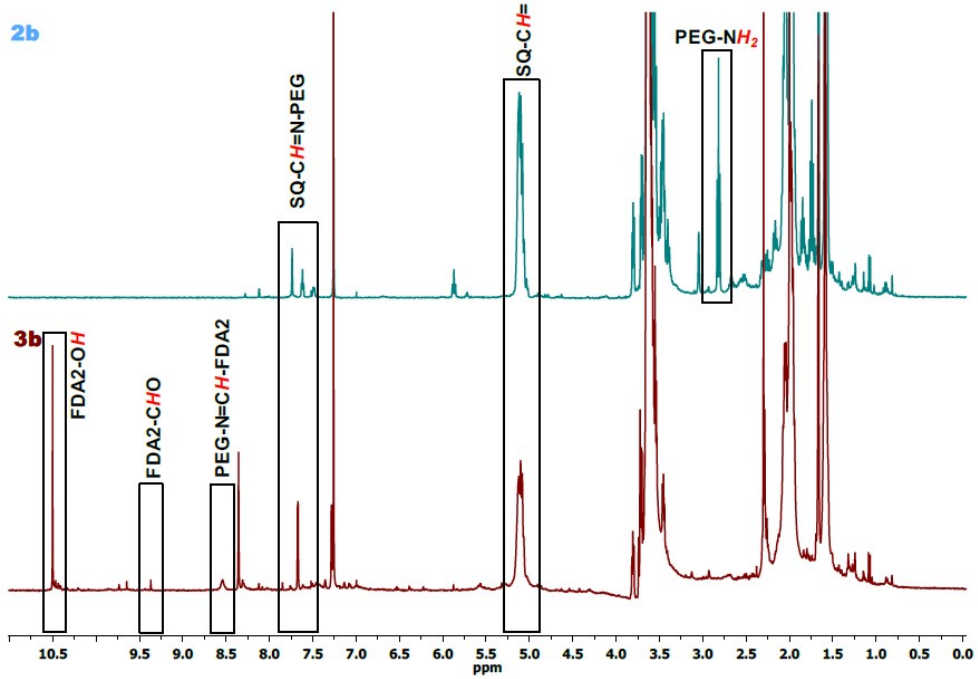

Figure S14. ^{1}H-NMR ($CDCl_3$, 400 MHz) overlaid spectra of 2b and 3b.

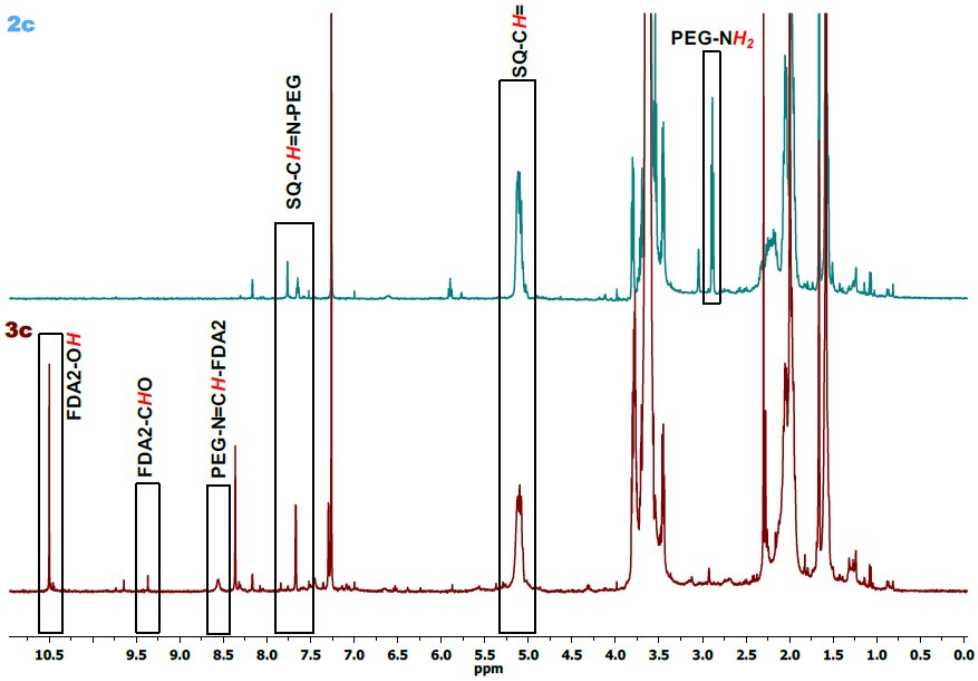

Figure S15. ^{1}H-NMR ($CDCl_3$, 400 MHz) overlaid spectra of 2c and 3c.

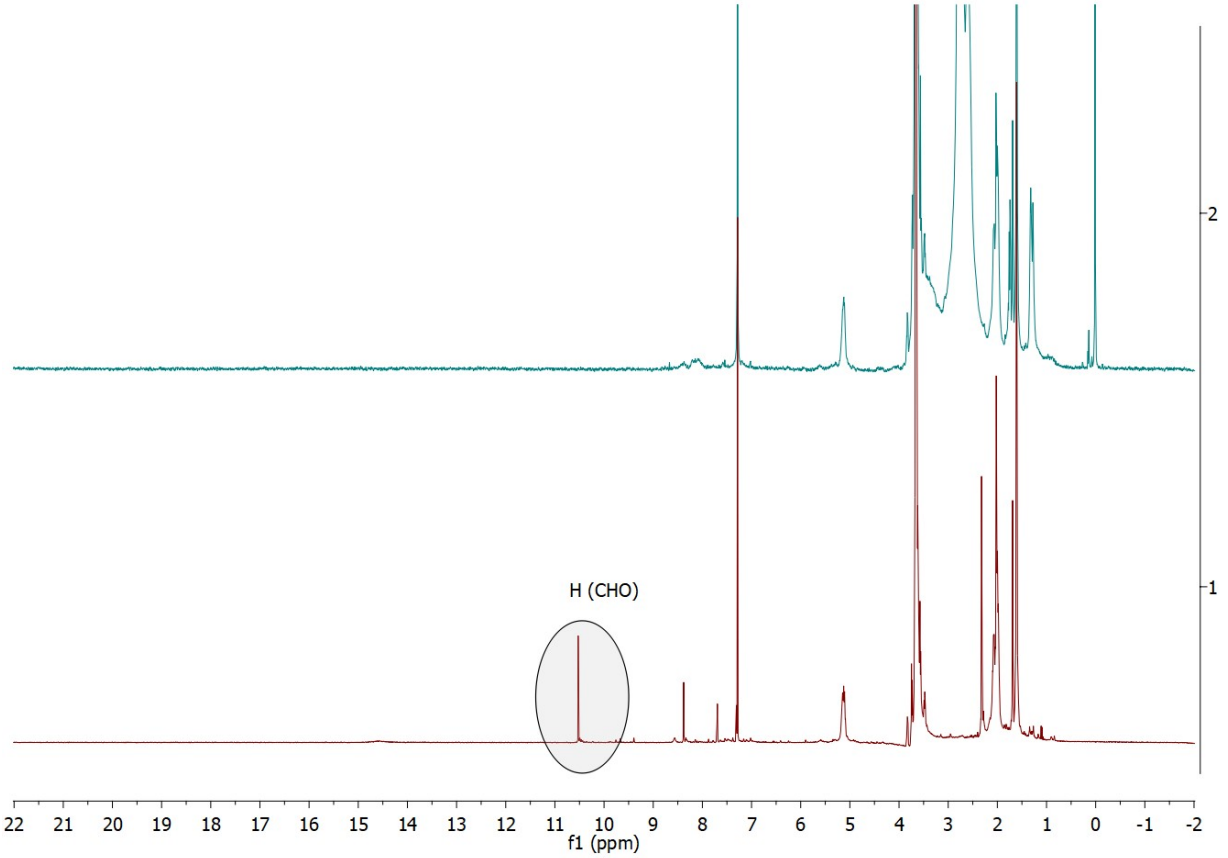

Figure S16 H-NMR of Vector 1500 (up) and 3c (down) in CDCl3. The consumption of CO group indicating the completion of the reaction and the formation of Vector 1500.

MALDI-TOF analyses:

MALDI-TOF analyses were performed for the following samples: PEI800 (figure 1.), PEG 500 (figure 2.), PEG 1500 (figure 3.), PEG 3000 (figure 4.), VECTOR 500 (figure 5), VECTOR 1500 (figure 6.) and VECTOR 3000 (figure 7.) using 2,5-Dihydroxybenzoic acid (DHB) or α-Cyano-4-hydroxycinnamic acid (CCA) as matrix.

From the included spectra we observed that PEI 800 as well as PEG sequences have high polydispersity, therefore, the MALDI analysis for VECTOR 500, VECTOR 1500 and VECTOR 3000 is not very conclusive especially for VECTOR 500 where the very low peak at highest m/z value (2187 m/z) could be attributed to a large PEI with a large PEG or a PEI with two small PEG (here large and small refer to the extremities of the polydispersity). Differently, VECTOR 1500 and VECTOR 3000 do not have any peaks over 3000 m/z, and 4500 m/z respectively, thus ruling out the possibility that two pegylated squalene molecules could be covalently bound to a single PEI. In conclusion, the range of the molecular masses obtained by MALDI are in a reasonable agreement with the calculated masses for the vectors with a ratio of 1:1 PEG:PEI.

MALDI-TOF sample preparation

Table S1. Sample deposition on MALDI plate.

Position	*Matrix*	*Volume*	*Sample*	*Volume*	*Sample preparation*	*calculated MASS*
E3	DHB	1 µL	MD1	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	1890
E4	DHB	1 µL	MD2	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	2850
E5	DHB	1 µL	MD3	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	4350
E8	DHB	1 µL	MD1	1.5 µL	matrix deposited, air dried, sample deposited, air dried 24h	1890
E9	DHB	1 µL	MD2	1.5 µL	matrix deposited, air dried, sample deposited, air dried 24h	2850
E10	DHB	1 µL	MD3	1.5 µL	matrix deposited, air dried, sample deposited, air dried 24h	4350
E13	DHB	1 µL	MD1	1 µL	matrix mixed with the sample and deposited, air dried for 24 h	1890
E14	DHB	1 µL	MD2	1 µL	matrix mixed with the sample and deposited, air dried for 24 h	2850
E15	DHB	1 µL	MD3	1 µL	matrix mixed with the sample and deposited, air dried for 24 h	4350
E21	DHB	1 µL	MD1	1.5 µL	matrix mixed with the sample and deposited, air dried for 24 h	1890
E22	DHB	1 µL	MD2	1.5 µL	matrix mixed with the sample and deposited, air dried for 24 h	2850
E23	DHB	1 µL	MD3	1.5 µL	matrix mixed with the sample and deposited, air dried for 24 h	4350
G3	CCA	1 µL	MD1	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	1890
G5	CCA	1 µL	MD3	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	4350
G6	CCA	1 µL	PEI 800	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H2	CCA	1 µL	PEI 800	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H3	CCA	1 µL	PEI 800	1.5 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H4	CCA	1 µL	PEI 800	2 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H6	DHB	1 µL	PEI 800	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H7	DHB	1 µL	PEI 800	1.5 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
H8	DHB	1 µL	PEI 800	2 µL	matrix deposited, air dried, sample deposited, air dried 24h	800
G12	CCA	1 µL	PEG 500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	500
G13	CCA	1 µL	PEG 1500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	1500
G14	CCA	1 µL	PEG 3000	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	3000
G16	DHB	1 µL	PEG 500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	500
G17	DHB	1 µL	PEG 1500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	1500
G18	DHB	1 µL	PEG 3000	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	3000
F21	DHB	1 µL	PEG 500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	500
F22	DHB	1 µL	PEG 1500	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	1500
F23	DHB	1 µL	PEG 3000	1 µL	matrix deposited, air dried, sample deposited, air dried 24h	3000

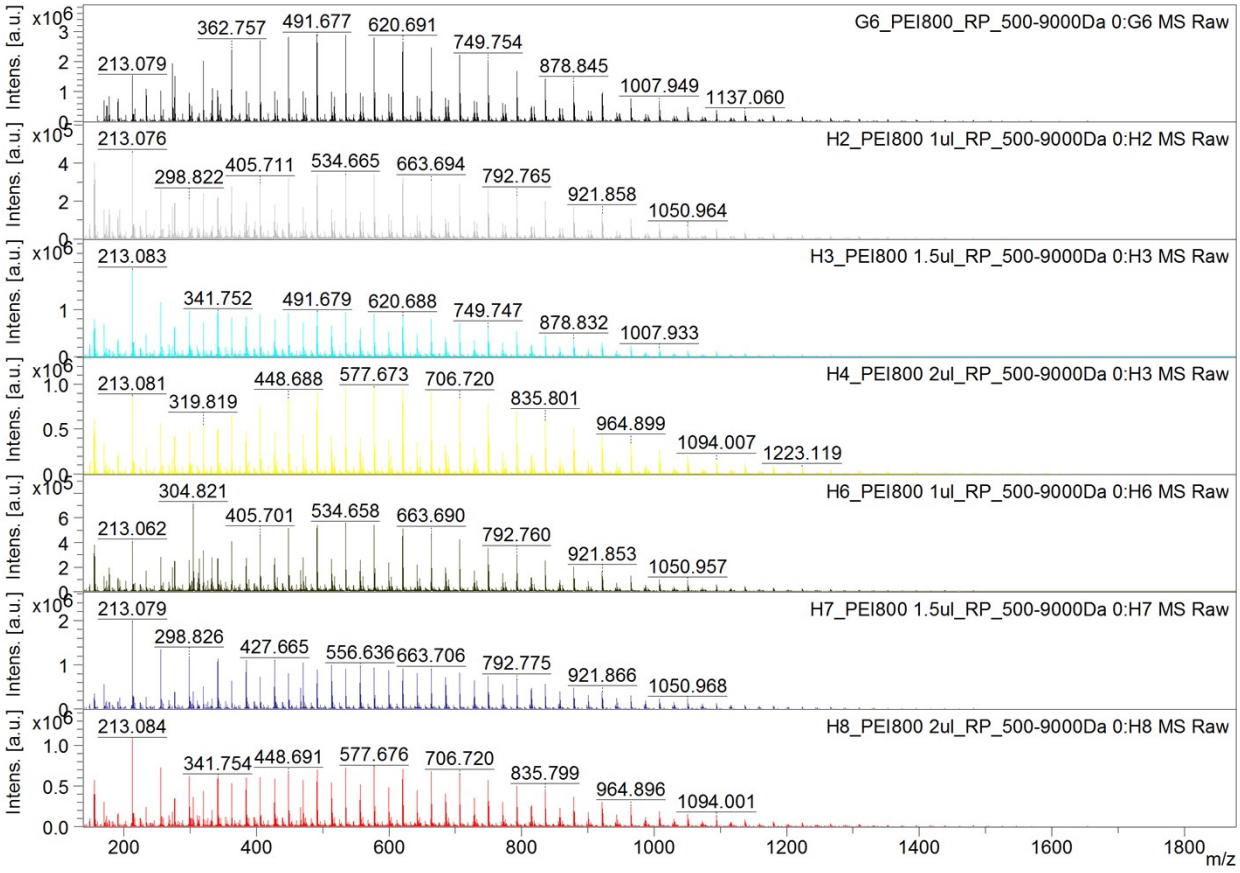

Figure S17. . MALDI-TOF for PEI 800 MS (PEI 800) calculated=800. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

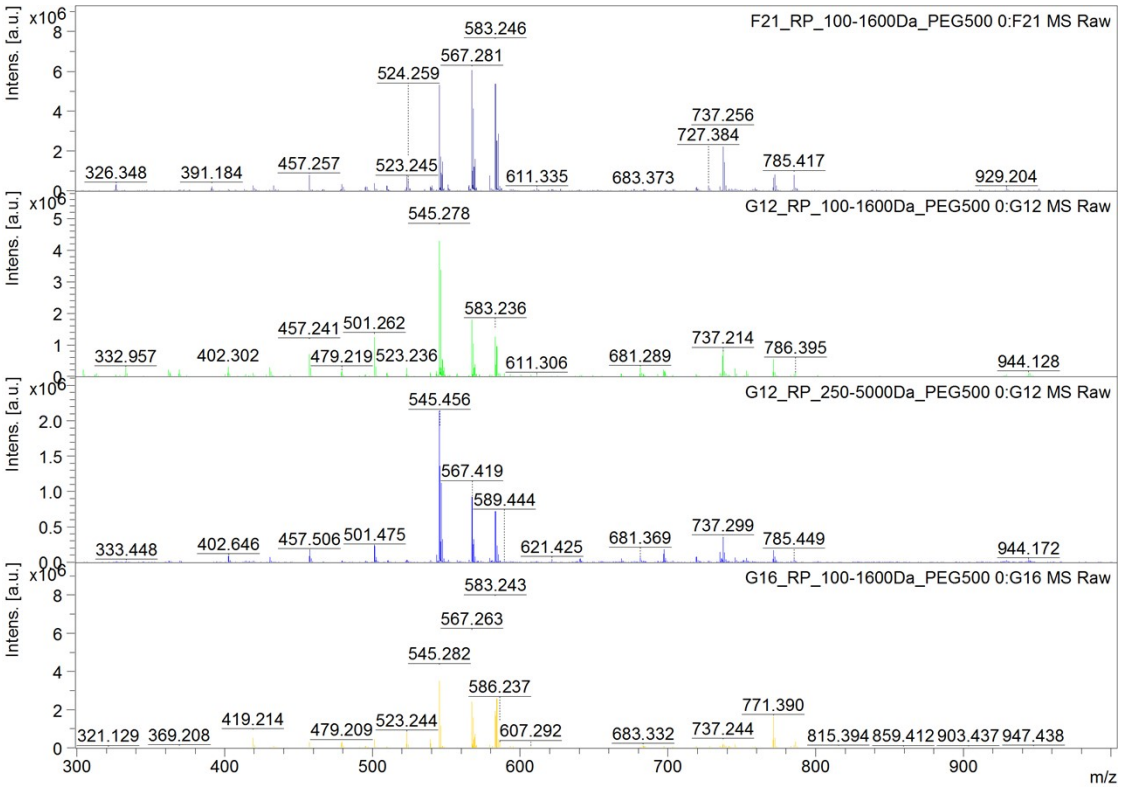

Figure S18. MALDI TOF analysis of PEG 500, MS (PEG 500) calculated=500. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

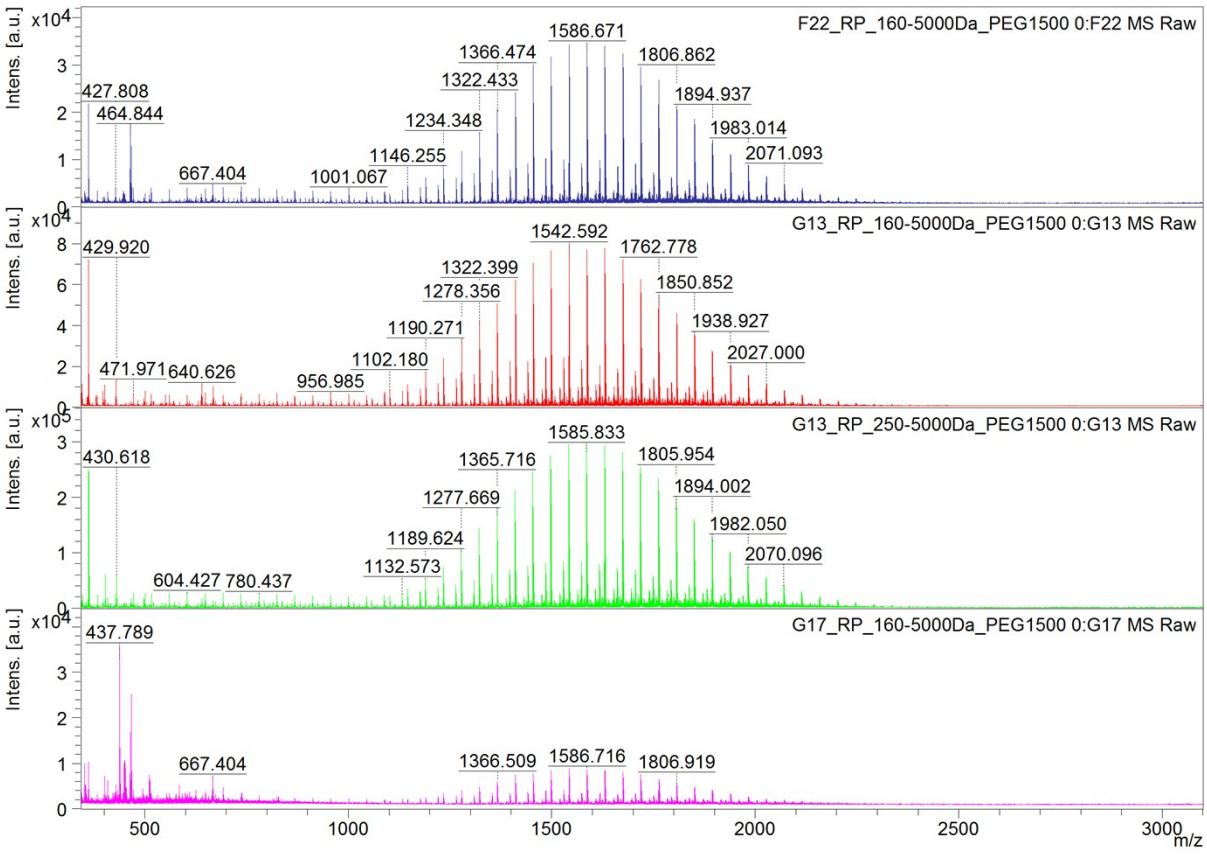

Figure S19. MALDI TOF analysis for PEG 1500. MS (PEG 1500) calculated=1500. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

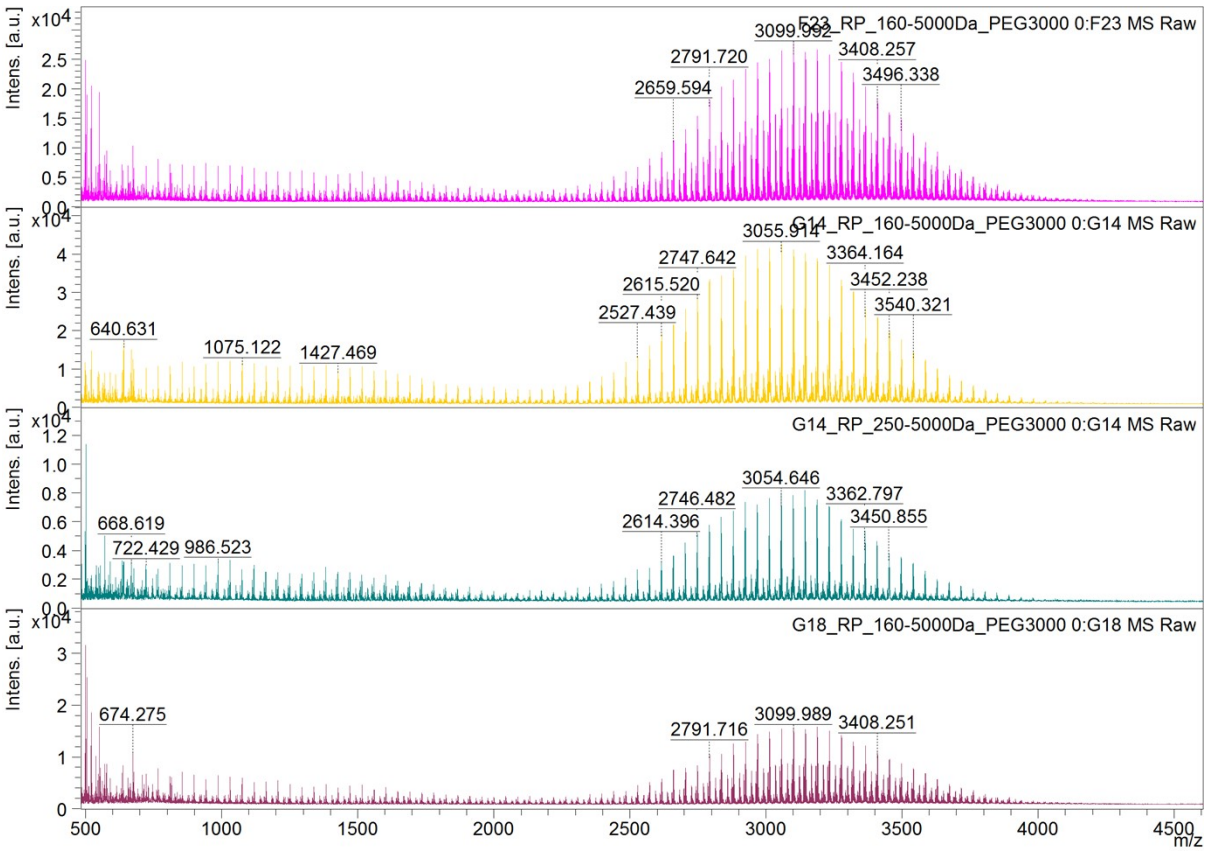

Figure S20. MALDI TOF for PEG 3000. MS (PEG 3000) calculated=3000. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

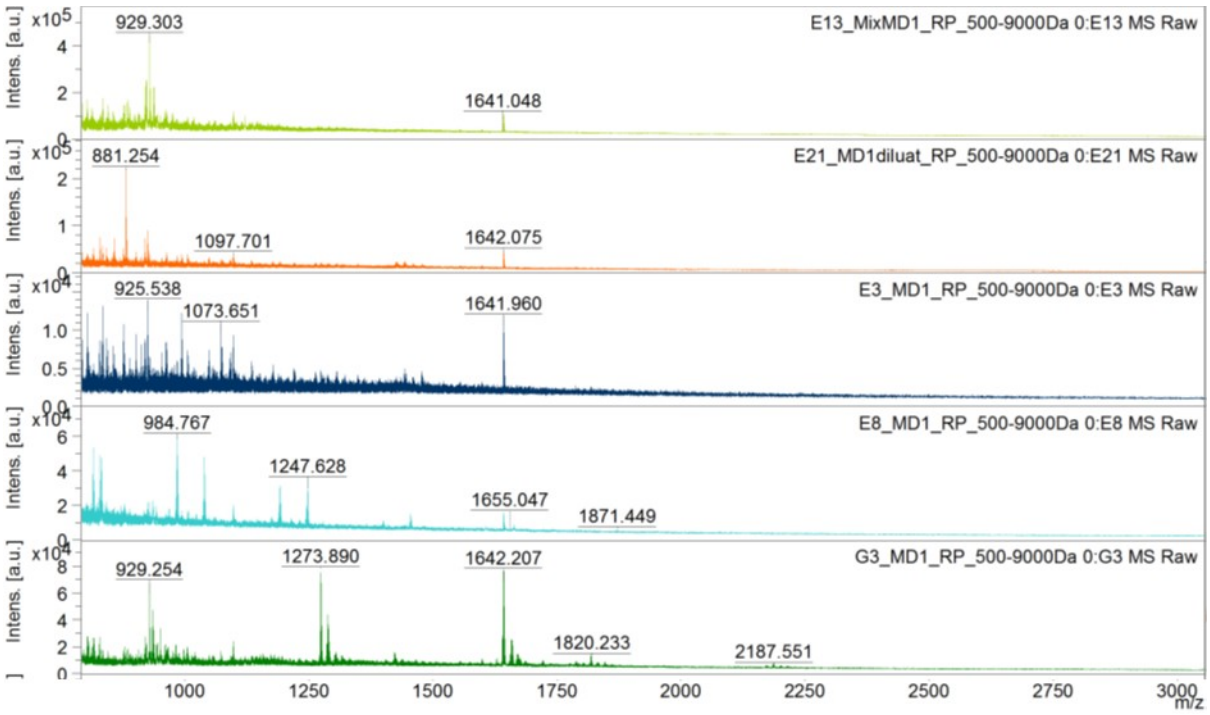

Figure S21. MALDI-TOF characterization of VECTOR 500 (code name MD1). MS (VECTOR 500) calculated=1890. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

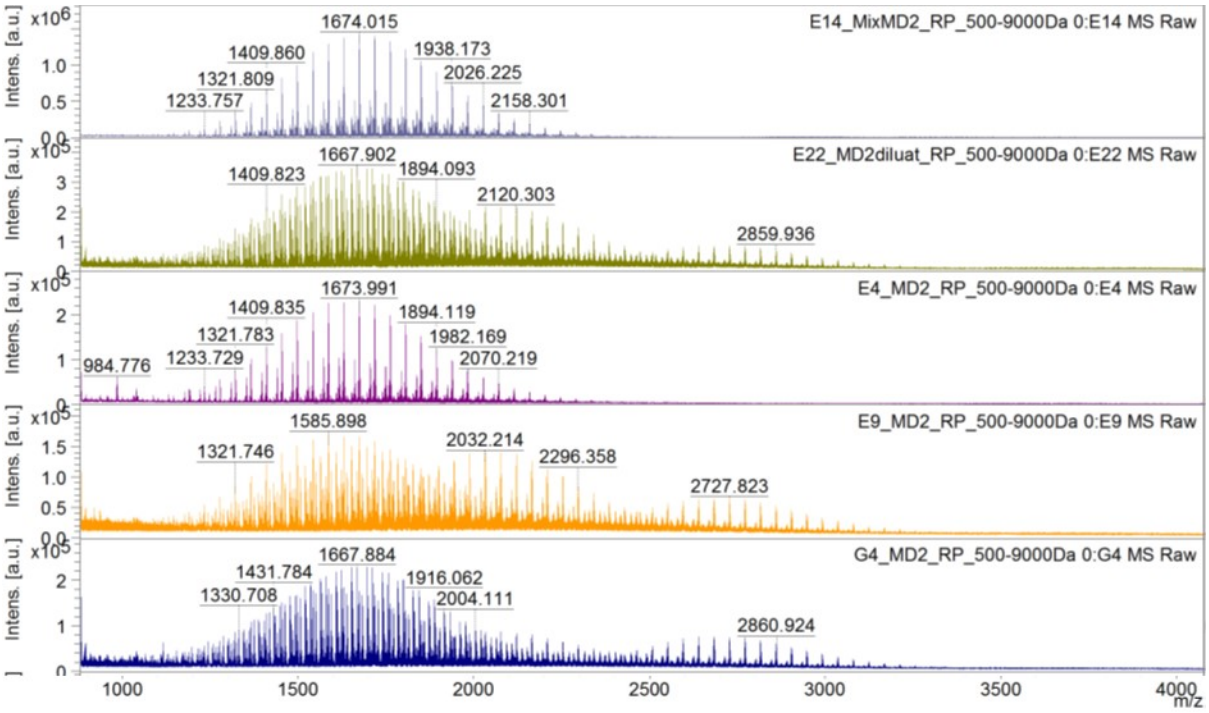

Figure S22. MALDI-TOF characterization of VECTOR 1500 (code name MD2). MS (VECTOR 1500) calculated=2850. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

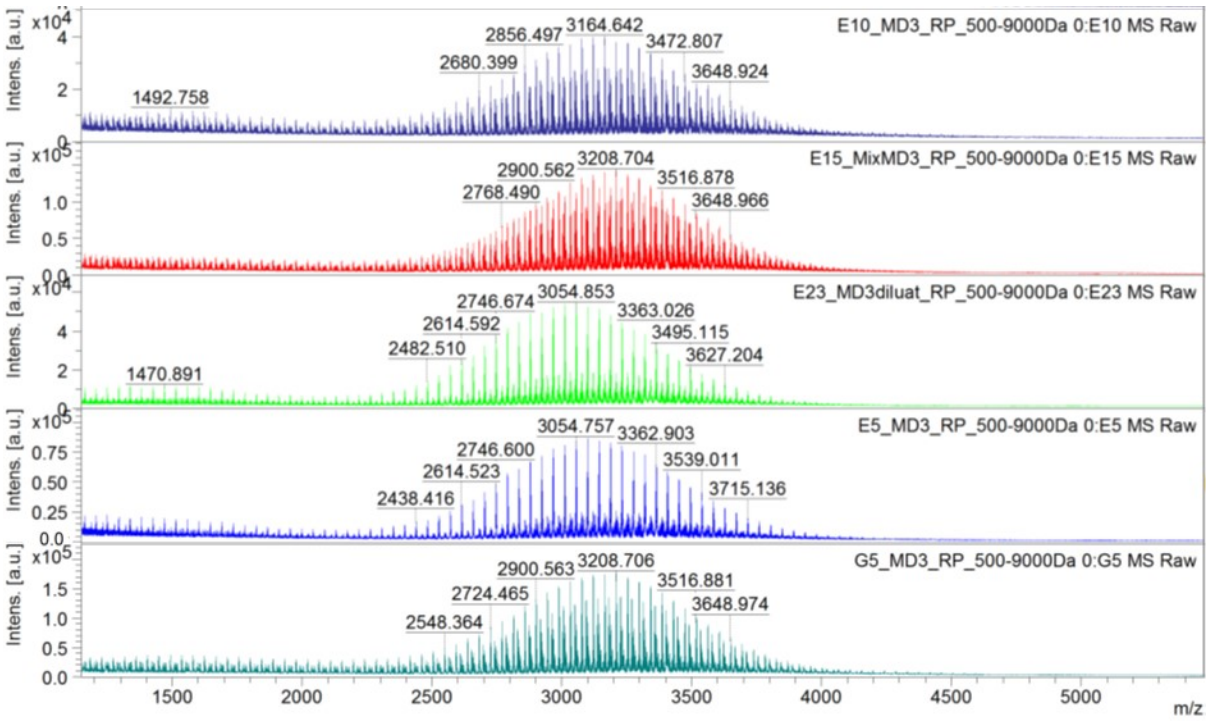

Figure S23. MALDI-TOF characterization of VECTOR 3000 (code name MD3). MS (VECTOR 3000) calculated=4350. The initial letters in the writing on the top of each spectra refers to the code indicating the sample preparation method, as specified in Table S1, where the codes are indicated in the first column.

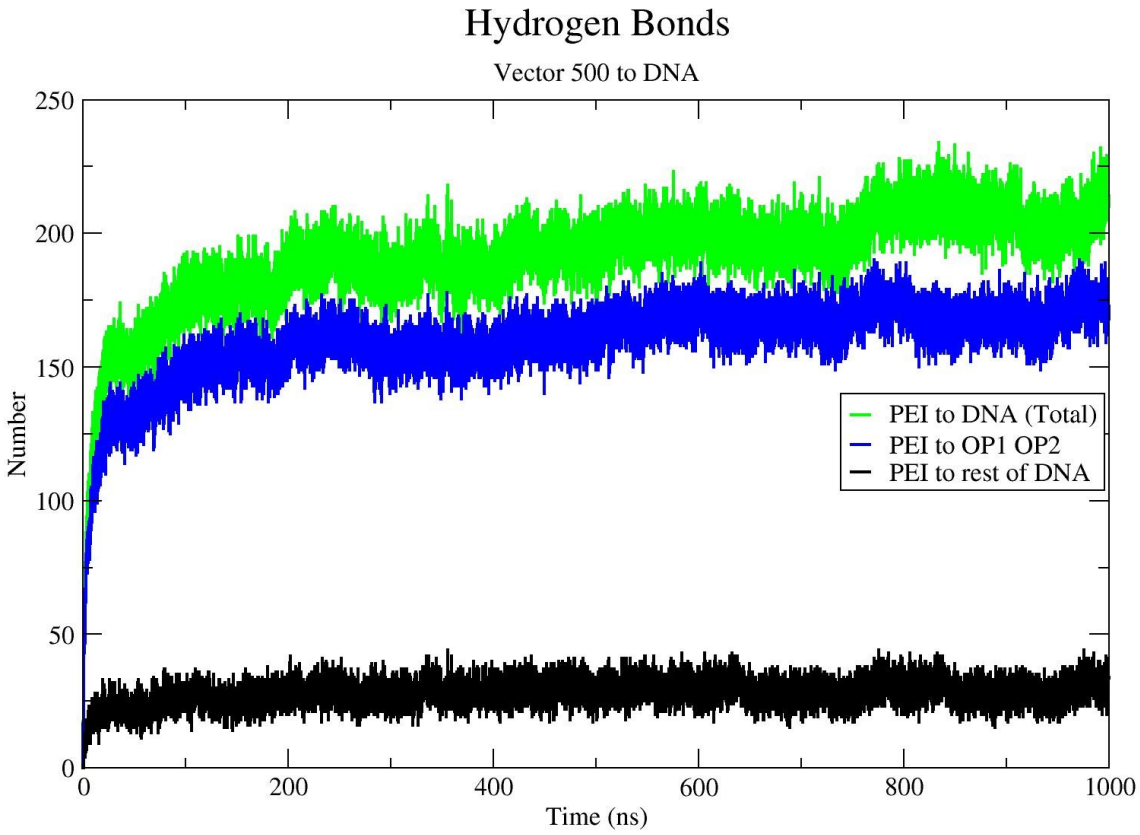

Figure S24. Graphical representation of the evolution of hydrogen bonds between the PEI from VECTOR 500 and different parts of DNA.

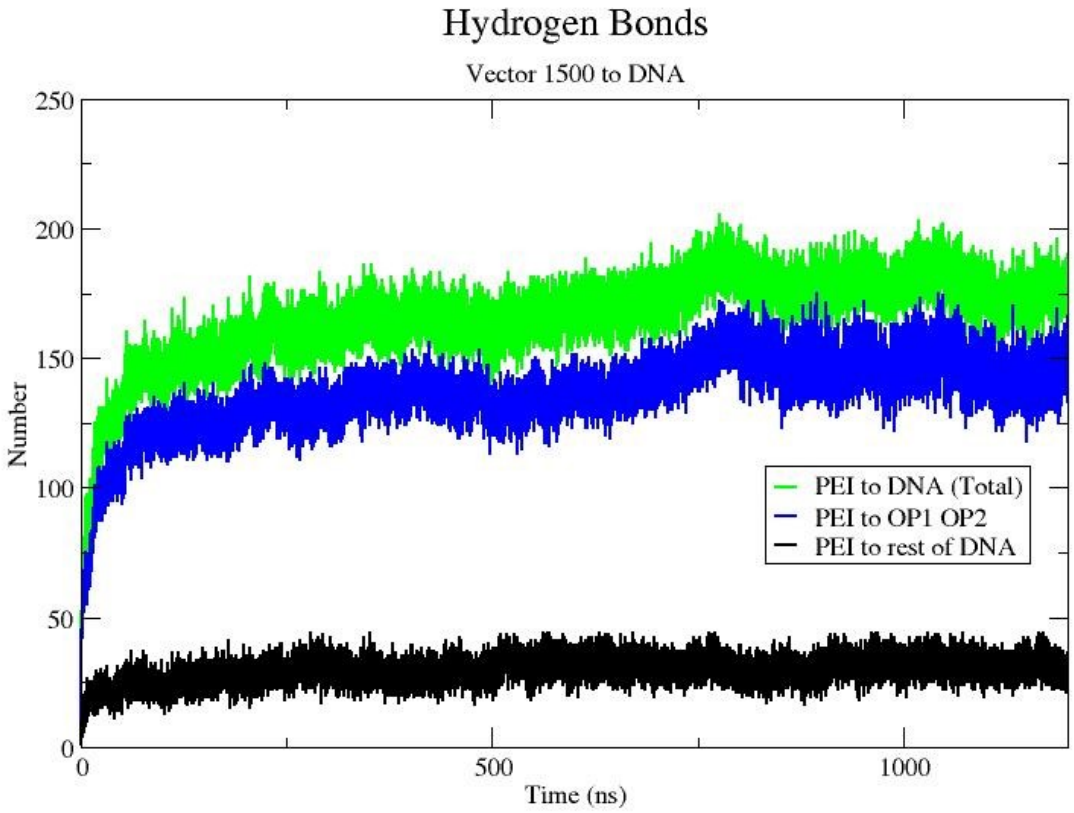

Figure S25. Graphical representation of the evolution of hydrogen bonds between the PEI from VECTOR 1500 and different parts of DNA.

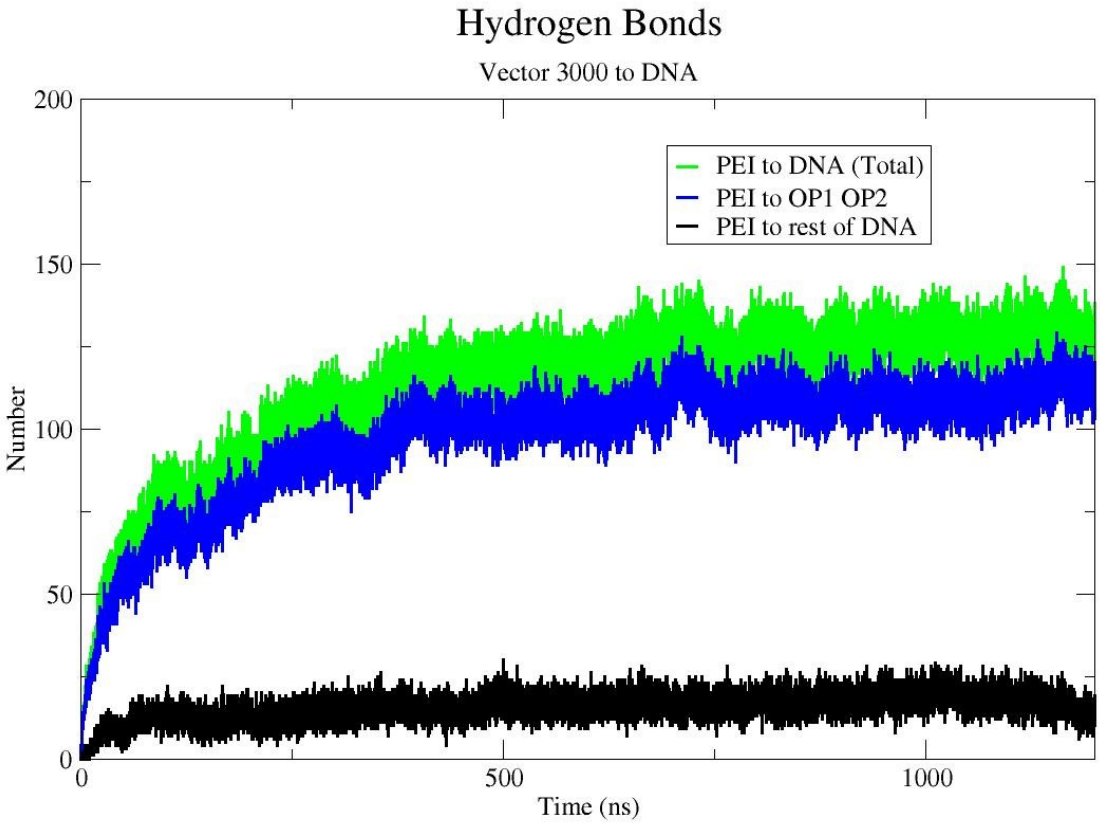

Figure S26. Graphical representation of the evolution of hydrogen bonds between the PEI from VECTOR 3000 and different parts of DNA.