Electronic Supplimentary Information for
Dynamic Constitutional Frameworks (DCFs) as nanovectors for cellular delivery of DNA

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Synthetic Methods

Step 1: The reaction between 1,3,5-benzenetrialdehyde **1**, and H_2N-PEG-NH_2 **2**, takes place in acetonitrile at room temperature in 72 hours. The reaction scheme is presented below and the ratios between the two compounds are presented in Table 1.

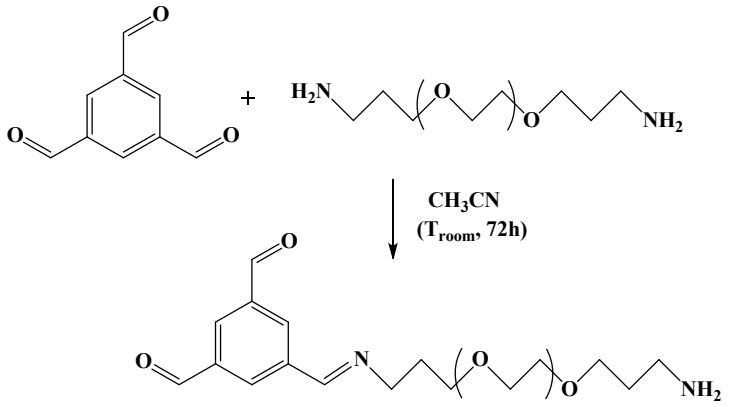
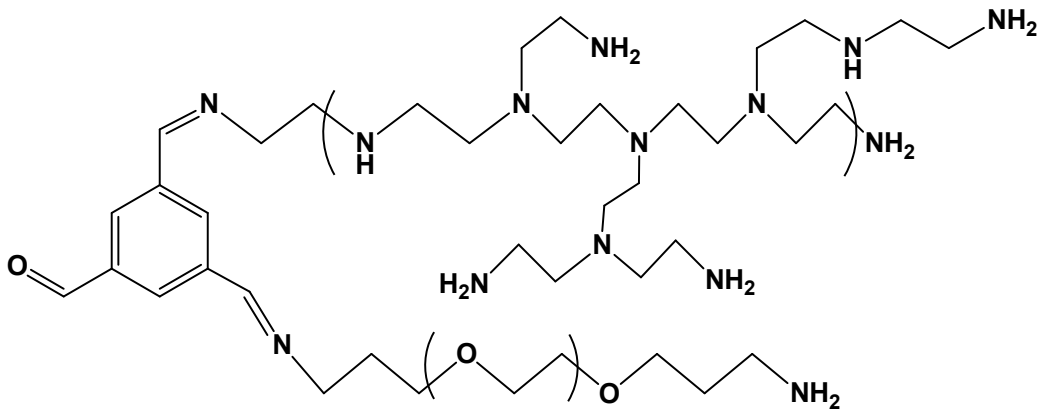


Table S1. Experimental amounts used for the synthesis in the step 1

Sample	1				2			
	Eq.	n (mmol)	m (mg)	V CH_3CN (mL)	Eq.	n (mmol)	m (mg)	V CH_3CN (mL)
A1	1	1.8582	301.4	50	1	1.8582	2788.4	100
A2	3	2.467	400	50	2	1.644	2467.0	50
A3	4	0.9263	150.2	10	3	0.6947	1042.1	20
A4	5	0.9263	150.2	10	4	0.7410	1111.6	20
A5	6	0.9263	150.3	10	5	0.7719	1158.7	20



Step 2: The unreacted aldehyde groups from the first step are here reacted with branched PEI. The reaction in deuterated water was stirred for 12 hours on a vortex, and then incubated at room temperature for 72 h. The products were analysed by NMR, in D_2O at room temperature.

^{1}H-NMR titration of mixtures of 1 and 2 with branched PEI, in D_2O.

For **1:2**=1:1 / 3:2 at low concentration of branched PEI 0.4, 0.6, 0.8 equivalents we observe the formation of a mixture of gel/ solid aggregates. We didn't succeed to disrupt this kind of aggregates by dissolving them into DMSO or MeOH, or by warming the solution, or by adding PEG-NH_2. The insolubility of the aggregates can be explained by their architecture where the TA: PEG surrounds the branched PEI and makes it insoluble. When we increase the amount of PEI, the architecture of the aggregates changes and the PEI surrounds the TA-PEG core. From the NMR analysis we see that from 1 equivalent of branched PEI all the aldehyde groups are reacted and the products can be used in DNA binding.

For **1:2**=4:3 / 5:4 / 6:5 at first we analysed all the solutions by NMR and we observed that at 0.4 equivalents all the aldehyde groups are reacted, but in time we noticed that the solutions with 0.4, 0.6 and 0.8 equivalents branched PEI precipitates in time, forming a film on the bottom of the flask.

The assignments of the signals in the NMR spectra (Figure S1-5) are the following:

- The peaks at 9.75 ppm are attributed to unreacted bis-aldehyde and monoaldehyde groups in agreement with the mono-imine and di-imine peaks, respectively, observed at 8.5-8.7 ppm.
- On the addition of PEI we observe two king of imine C<u>H</u>=N protons at 8.5 ppm attributed to PEG-N=C<u>H</u> protons and at 8.1 ppm attributed to PEI-N=CH protons

- The peaks between 8.5-7.75 ppm correspond thus to the aromatic protons of **1** with variable imino-subtitution degrees.
- The aliphatic region of spectra we may identify the methylene protons of PEI and PEG.
- - once the PEI is added at 1:1 ratios the spectra become broad probably due to specific encapsulation of aromatic residues within a highly charged shell of PEI.

Table S2 Experimental amounts used for the titration of **A1** with branched PEI, in D_2O.

A 1				**branched PEI**				**Total volume** (µL)	**Final conc.** (mg/mL)
eq	n (mmoli)	m (mg)	V (µL)	eq	n (mmoli)	m (mg)	V (µL)		
1 : 2 = 1:1	1 mL solution A1 = 0.0202 mmoli benztrialdehyde	1 mL solution = 30.04 mg A1	1000	**0**	0	0	0	1000	30.04
			1000	**0.2**	0.0040	3.23	37.8	1038	32.05
			1000	**0.4**	0.0080	6.46	75.7	1076	33.92
			1000	**0.6**	0.0121	9.69	113.6	1114	35.66
			1000	**0.8**	0.0161	12.92	151.5	1151	37.32
			1000	**1**	0.0202	16.16	189.4	1189	38.85
			1000	**1.5**	0.0303	24.24	284.2	1284	42.27
			1000	**2**	0.0404	32.32	378.9	1379	45.22
			1000	**3**	0.0606	48.48	568.4	1568	50.07

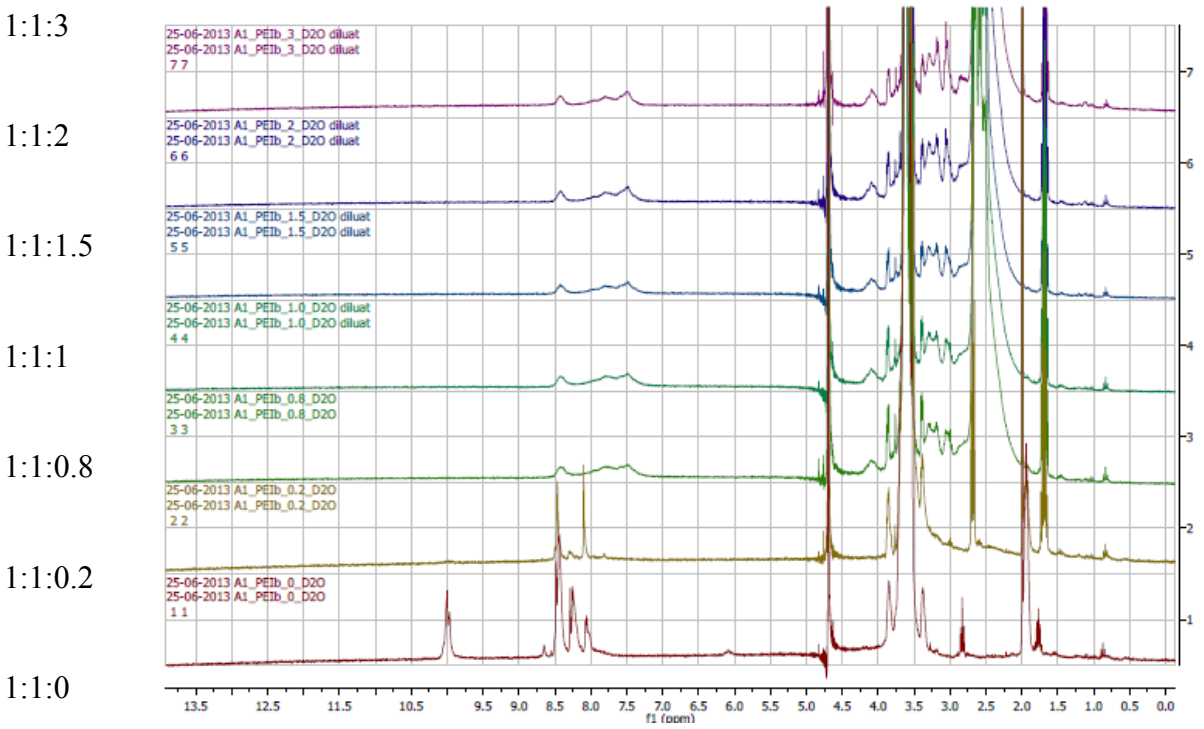
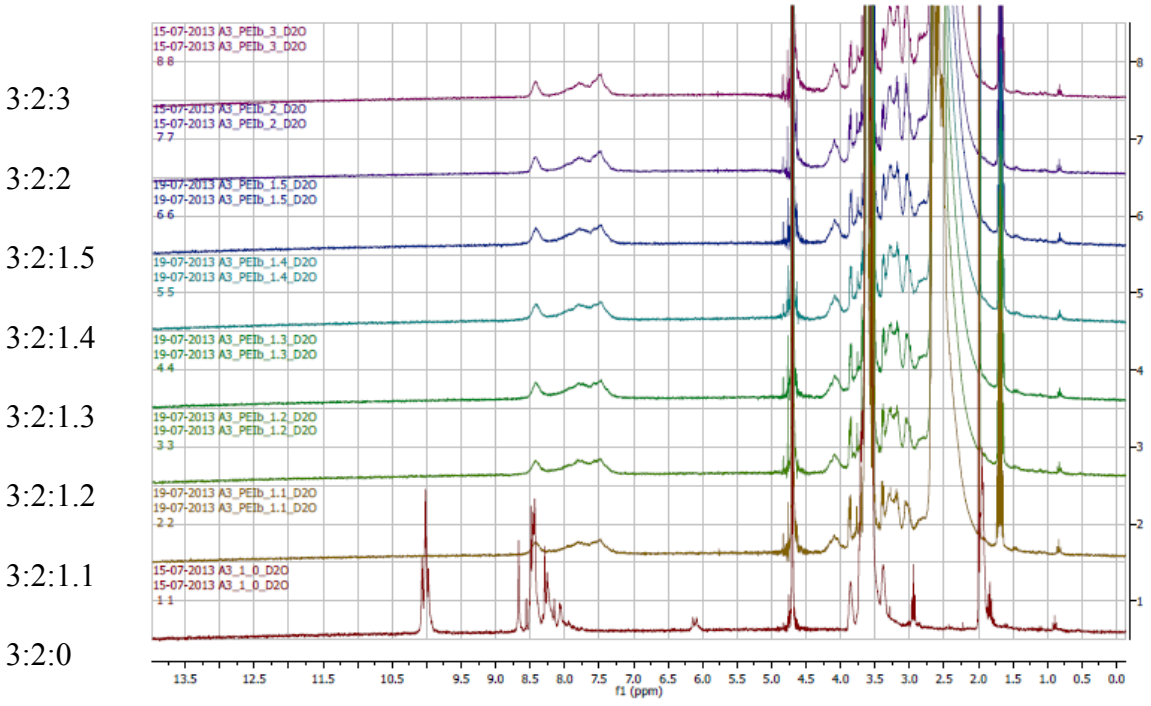


Figure S1. ^{1}H-NMR titration of **A1** with branched PEI, in D_2O.

Table S3: Experimental amounts used for the titration of **A2** with branched PEI, in D_2O.

A 2				**branched PEI**				**Total volume** (µL)	**Final conc.** (mg/ mL)
eq	n (mmoli)	m (mg)	V (µL)	eq	n (mmoli)	m (mg)	V (µL)		
1 : 2= 3:2	1 mL solution 23 = 0.01781 mmoli benztrialdehyde	1 mL solution = 20.70 mg A3	1000	**0**	0	0	0	1000	20.70
			1000	**0.2**	0.0035	2.85	26.5	1026	22.95
			1000	**0.4**	0.0071	5.69	53	1053	25.06
			1000	**0.6**	0.0106	8.54	79.5	1079	27.09
			1000	**0.8**	0.0142	11.39	106.1	1106	29.01
			1000	**1**	0.0178	14.24	132.6	1132	30.86
			1000	**1.5**	0.0267	21.37	198.9	1199	35.08
			1000	**2**	0.0356	28.49	265.2	1265	38.88
			1000	**3**	0.0534	42.74	397.9	1398	45.37



FigureS2. ^{1}H-NMR titration of **A2** with branched PEI, in D_2O.

Table S4: Experimental amounts used for the titration of **A3** with branched PEI, in D_2O.

A 3				**branched PEI**				**Total volume** (µL)	**Final conc.** (mg/mL)
eq	n (mmoli)	m (mg)	V (µL)	eq	n (mmoli)	m (mg)	V (µL)		
1 : 2= 4:3	1 mL solution A3 = 0.0077 mmoli benztrialdehyde	1 mL solution = 10.03 mg A4	1000	**0**	0	0	0	1000	10.03
			1000	**0.2**	0.0015	1.23	13.08	1013	11.11
			1000	**0.4**	0.0030	2.46	26.17	1026	12.17
			1000	**0.6**	0.0046	3.69	39.26	1039	13.20
			1000	**0.8**	0.0061	4.92	52.35	1052	14.21
			1000	**1**	0.0077	6.16	65.44	1065	15.20
			1000	**1.5**	0.0115	9.24	98.16	1098	17.55
			1000	**2**	0.0154	12.32	130.89	1131	19.76

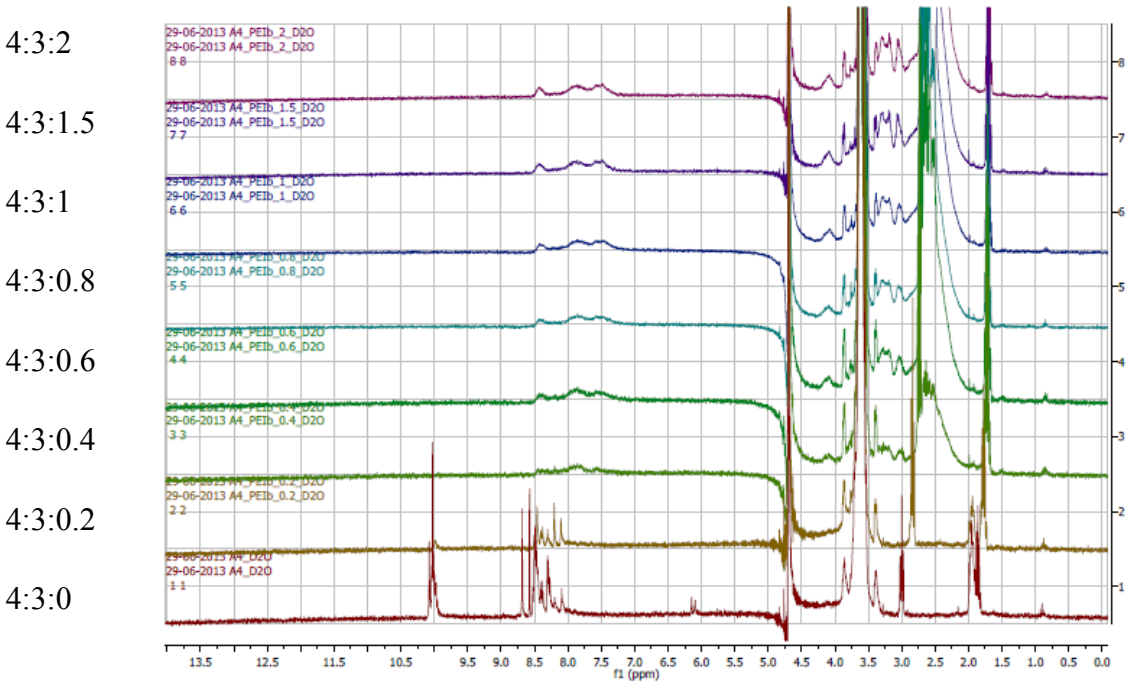


Figure S3. ^{1}H-NMR titration of **A3** with branched PEI, in D_2O.

Table S5: Experimental amounts used for the titration of **A4** with branched PEI, in D_2O.

A 4				**branched PEI**				**Total volume** (µL)	**Final conc.** (mg/mL)
eq	n (mmoli)	m (mg)	V (µL)	eq	n (mmoli)	m (mg)	V (µL)		
1 : 2= 5:4	1 mL solution A4= 0.0073 mmoli benztrialdehyde	1 mL solution = 10.06 mg A4	1000	**0**	0	0	0	1000	10.06
			1000	**0.2**	0.0014	1.16	12.39	1012	11.08
			1000	**0.4**	0.0029	2.33	24.79	1025	12.33
			1000	**0.6**	0.0043	3.50	37.19	1037	13.07
			1000	**0.8**	0.0058	4.67	49.58	1050	14.02
			1000	**1**	0.0073	5.84	61.98	1062	14.97
			1000	**1.5**	0.0109	8.76	92.97	1093	17.21
			1000	**2**	0.0146	11.68	123,96	1124	19.34

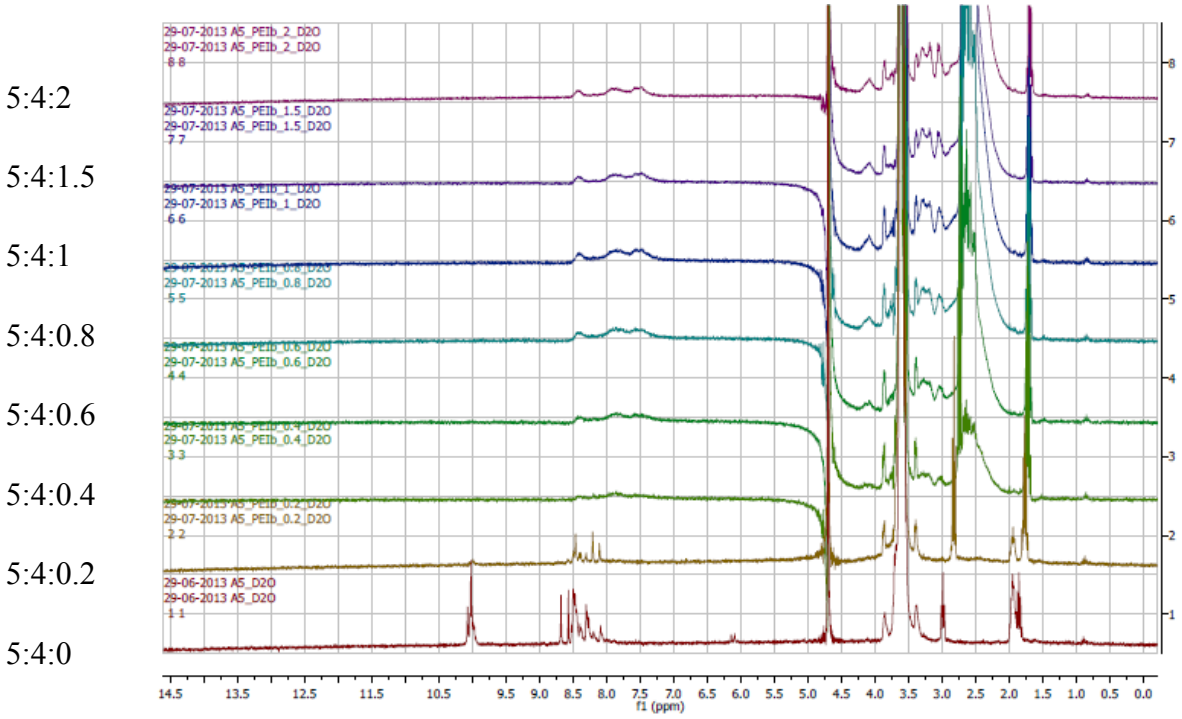


Figure S4. ^{1}H-NMR titration of **A4** with branched PEI, in D_2O.

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Table S6: Experimental amounts used for the titration of **A5** with branched PEI, in D_2O.

				branched PEI				Total volume (µL)	Final conc. (mg/mL)
eq	n (mmoli)	m (mg)	V (µL)	eq	n (mmoli)	m (mg)	V (µL)		
1 : 2 = 6:5	1 mL solution A5 = 0.0070 mmoli benztrialdehyde	1 mL solution = 10.00 mg A6	1000	**0**	0	0	0	1000	10.00
			1000	**0.2**	0.0014	1.12	11.88	1012	10.98
			1000	**0.4**	0.0028	2.24	23.77	1024	11.95
			1000	**0.6**	0.0042	3.36	35.66	1036	12.89
			1000	**0.8**	0.0056	4.48	47.55	1048	13.81
			1000	**1**	0.0070	5.6	59.44	1059	14.73
			1000	**1.5**	0.0105	8.4	89.16	1089	16.89
			1000	**2**	0.0140	11.2	118.89	1119	18.94

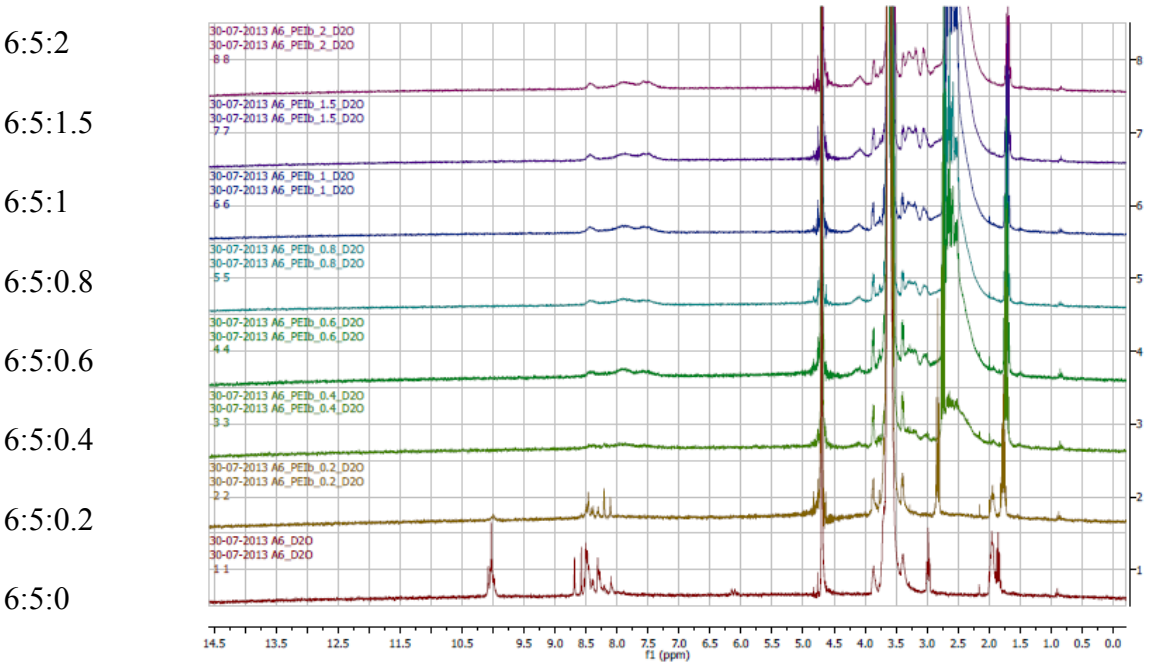

Figure S5. ^{1}H-NMR titration of **A5** with branched PEI, in D_2O.

Table S7: Experimental amounts used for Agarose gel retardation assays in the case of **A1-5** with different ratios of **3**· salmon sperm dsDNA at N/P=10

Sample		A1-PEIb			A2-PEIb			A3-PEIb
Molar ratio **Ai: 3**		1_1,5	1_2	1_3	1_1,5	1_2	1_3	1_1
	M	**1**	**2**	**3**	**4**	**5**	**6**	**7**
V buffer (µl)	3	3	3	3	3	3	3	3
V water (µl)	12	7	7	7	7	7	7	7
V sample (µl)	0	5	5	5	5	5	5	5
V DNA (µl)	5	5	5	5	5	5	5	5
V Sucroza (µl)	10	10	10	10	10	10	10	10

Sample		A3-PEIb		A4-PEIb			A5-PEIb	
Raport		1_1,5	1_2	1_1	1_1,5	1_2	1_1	1_1,5
	M	**8**	**9**	**10**	**11**	**12**	**13**	**14**
V buffer (µl)	3	3	3	3	3	3	3	3
V water (µl)	12	7	7	7	7	7	7	7
V sample (µl)	0	5	5	5	5	5	5	5
V DNA (µl)	5	5	5	5	5	5	5	5
V Sucroza (µl)	10	10	10	10	10	10	10	10

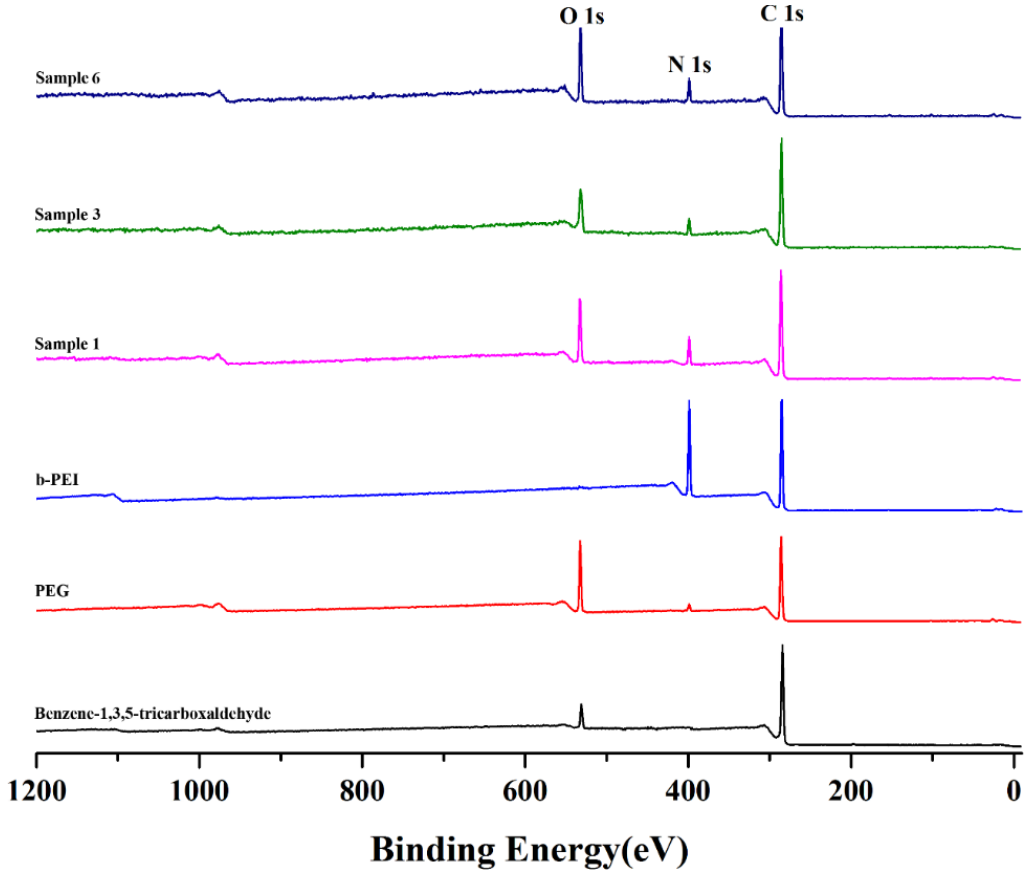

Figure S6. XPS wide scans for 1,3,5-benzenetrialdehyde, H_2N-PEG-NH_2, b-PEI, **DCF 1, 3, 6.**

Table S8. XPS experimental results for DCFxy

	Atomic concentration (%)			Mass concentration (%)		
Element	**O**	**N**	**C**	**O**	**N**	**C**
1 :1 :1.5	16.57	10.44	72.99	20.58	11.36	68.06
1 :1 :2	12.30	10.71	76.99	15.47	11.80	72.73
1 :1 :3	13.75	6.37	79.88	17.34	7.04	75.62
3 :2 :1.5	12.13	10.31	77.56	15.28	11.37	73.35
3 :2 :2	10.75	10.74	78.51	13.59	11.89	74.52
3 :2 :3	19.46	9.32	71.21	24.00	10.07	65.93
4 :3 :1	11.69	6.42	81.89	14.83	7.14	78.03
4 :3 :1.5	14.60	9.05	76.35	18.29	9.92	71.79
4 :3 :2	11.14	5.25	83.61	14.19	5.85	79.96
5 :4 :1	11.87	5.95	82.18	15.06	6.61	78.33
5 :4 :1.5	13.80	9.77	76.43	17.31	10.73	71.96
5 :4 :2	12.45	8.19	79.36	15.72	9.05	75.23
6 :5 :1	13.80	4.97	81.23	17.44	5.50	77.06
6 :5 :1.5	13.80	11.10	75.10	17.28	12.16	70.56
6 :5 :2	10.74	6.93	82.33	13.66	7.72	78.6

Table S9. Theoretical and experimental values for XPS analysis on samples **DCF1, 3 and 6**

Assuming the chemical composition based on total combination in different molar ratios of 1, 2 (considered as containing 31 ethylene glycol units) and 3 as presented in Table 1 we can calculate the theoretical values of elemental composition of resulted **DCF1, 3, 6.**

Sample	**Theoretical values (%)**			**Experimental values for the atomic composition (%)**		
	C	**N**	**O**	**C**	**N**	**O**
DCF1	69.7	16.4	13.9	72.9	10.5	16.6
DCF3	68.8	21.4	9.8	70.3	16.4	13.3
DCF6	69.7	16.5	13.8	71.2	9.3	19.5