Supporting Information

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Mannosylated Cationic Copolymers for Gene Delivery to Macrophages

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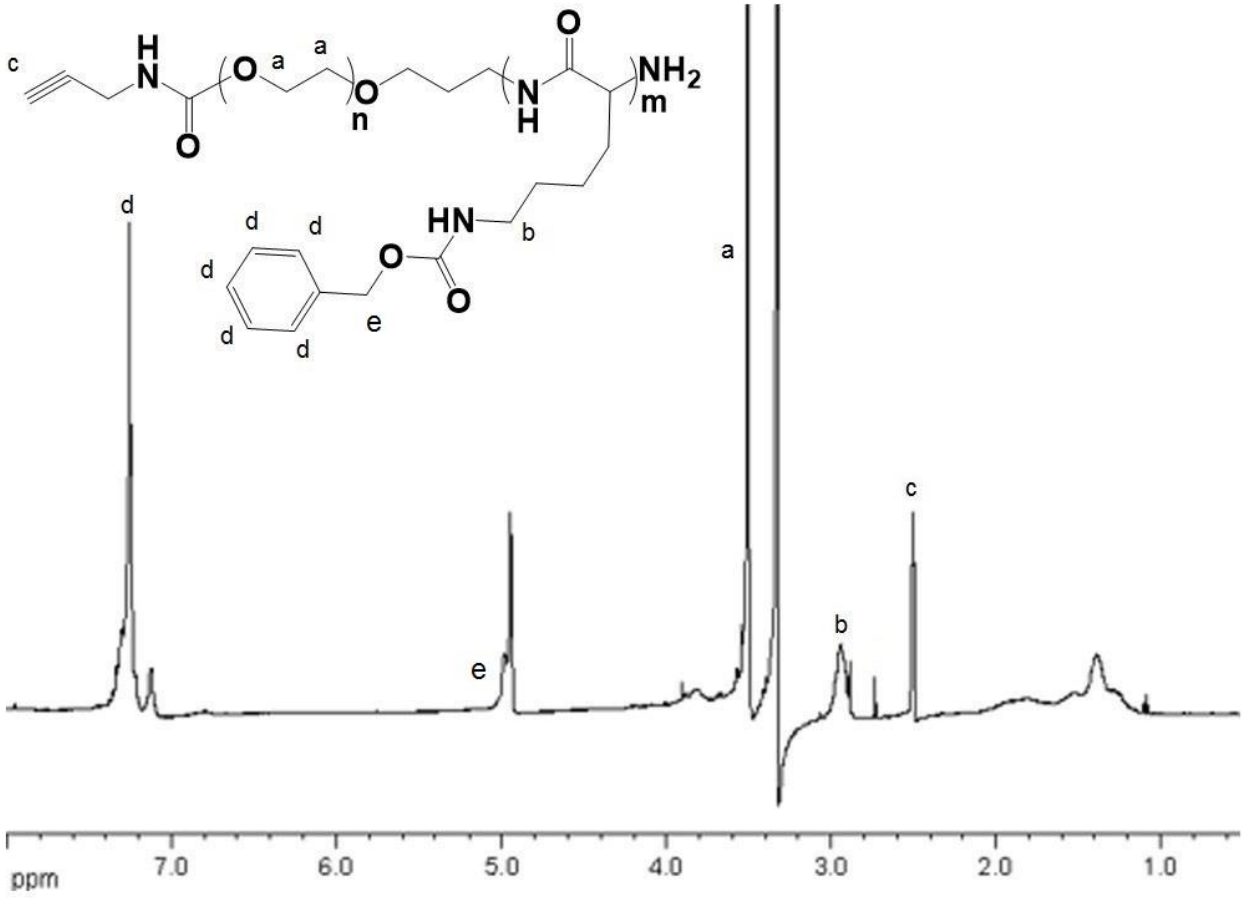

Figure S1. The ^{1}H-NMR spectra of propargyl-PEG$_{114}$-*b*-PLL(Z)$_{62}$ (DMSO-d$_6$, 400 MHz, 25 °C).

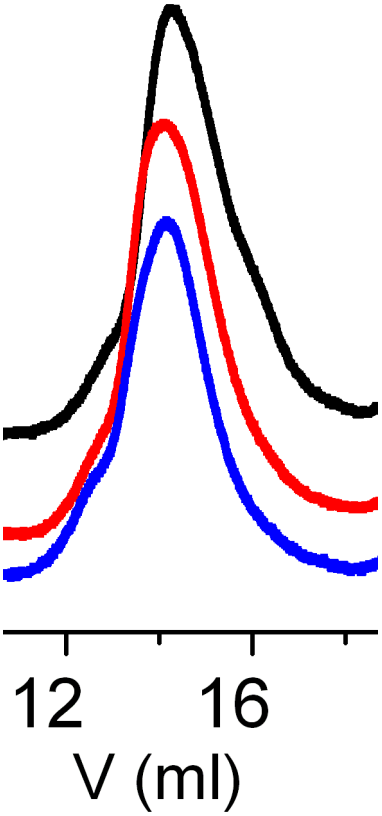

Figure S2. GPC chromatograms of propargyl-PEG$_{114}$-*b*-PLL(Z)$_{62}$ (black), propargyl-PEG$_{114}$-*b*-PLL(Z)$_{150}$ (red), propargyl-PEG$_{114}$-*b*-PLL(Z)$_{206}$ (blue) in DMF containing 1% LiBr obtained at 45 °C at a flow rate of 1.0 mL/min using the refractive index detection.

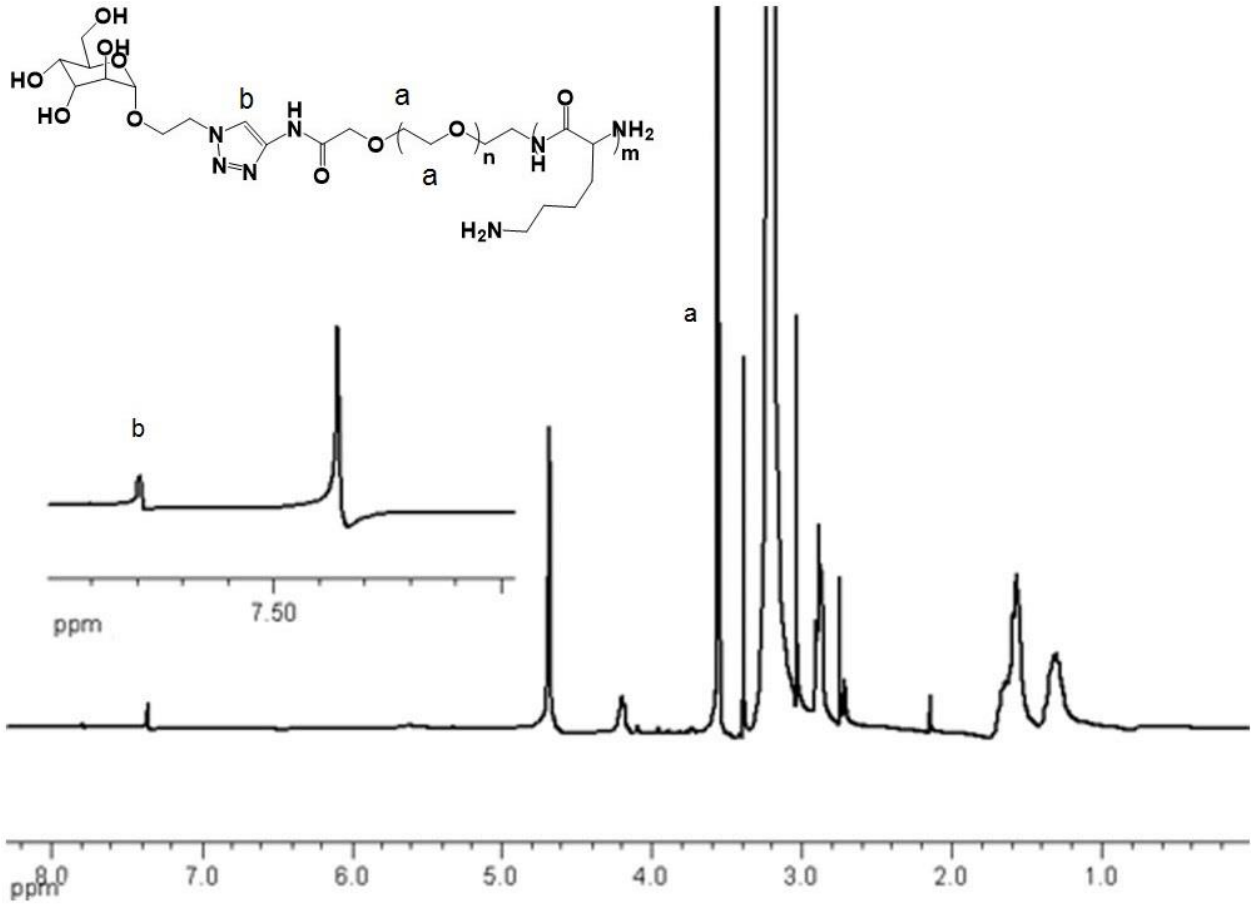

Figure S3. The ^{1}H-NMR spectra of Man-PEG$_{114}$-*b*-PLL$_{62}$ (D_2O, 400 MHz, 25 °C).

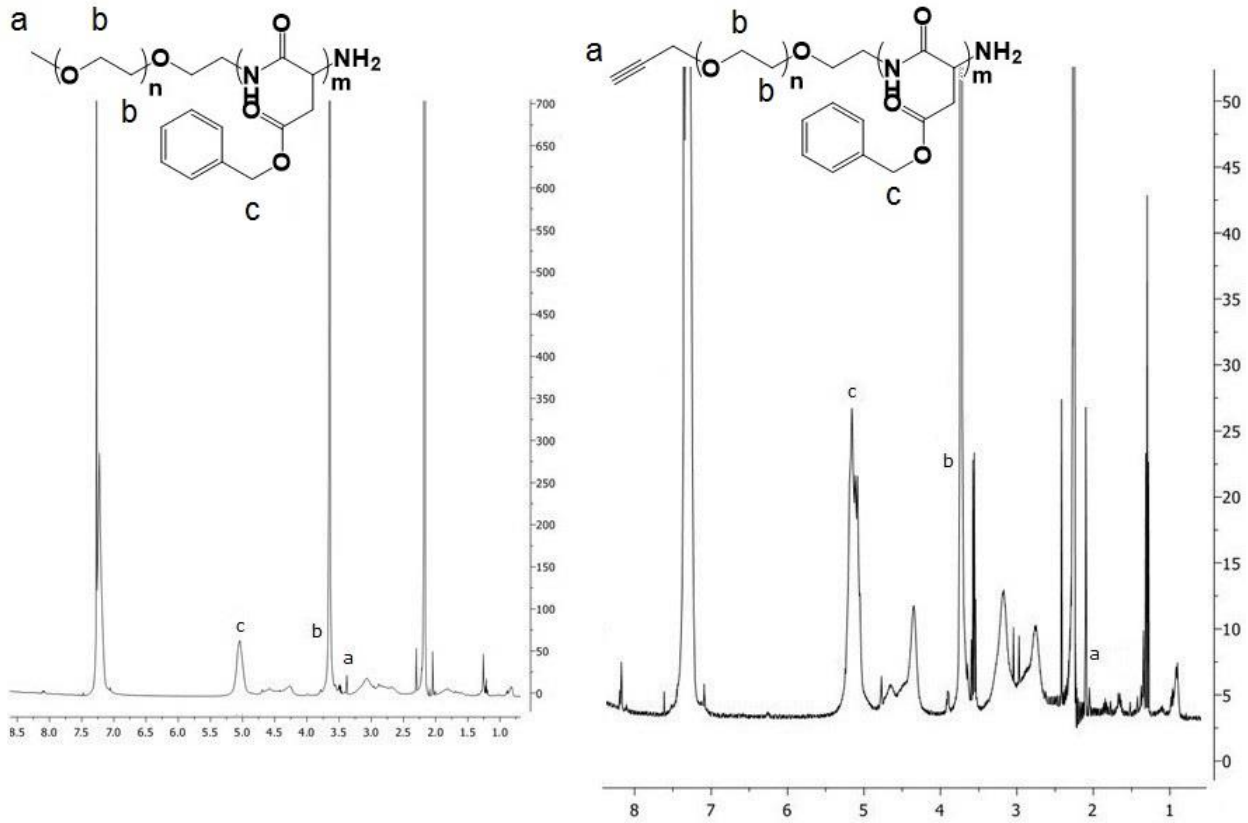

Figure S4. ^{1}H NMR spectra of mPEG-*b*-PBLA (left) and alkyne-PEG-*b*-PBLA (right) ($CDCl_3$, 400 MHz, 25 °C).

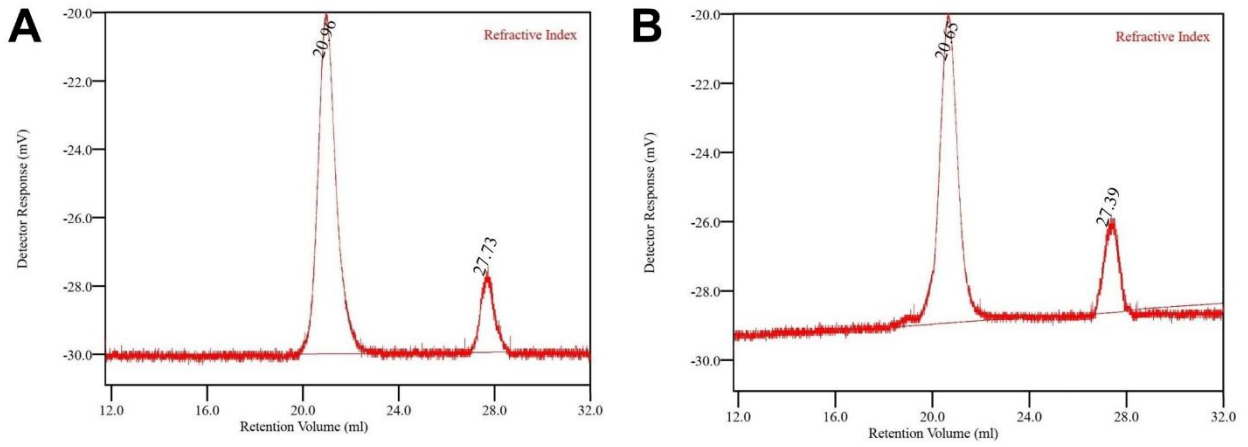

Figure S5. GPC chromatograms of **(A)** mPEG-*b*-pBLA and **(B)** alkyne-PEG-*b*-pBLA in DMF containing 1% LiBr obtained at 45 °C at a flow rate of 1.0 mL/min using the refractive index detection.

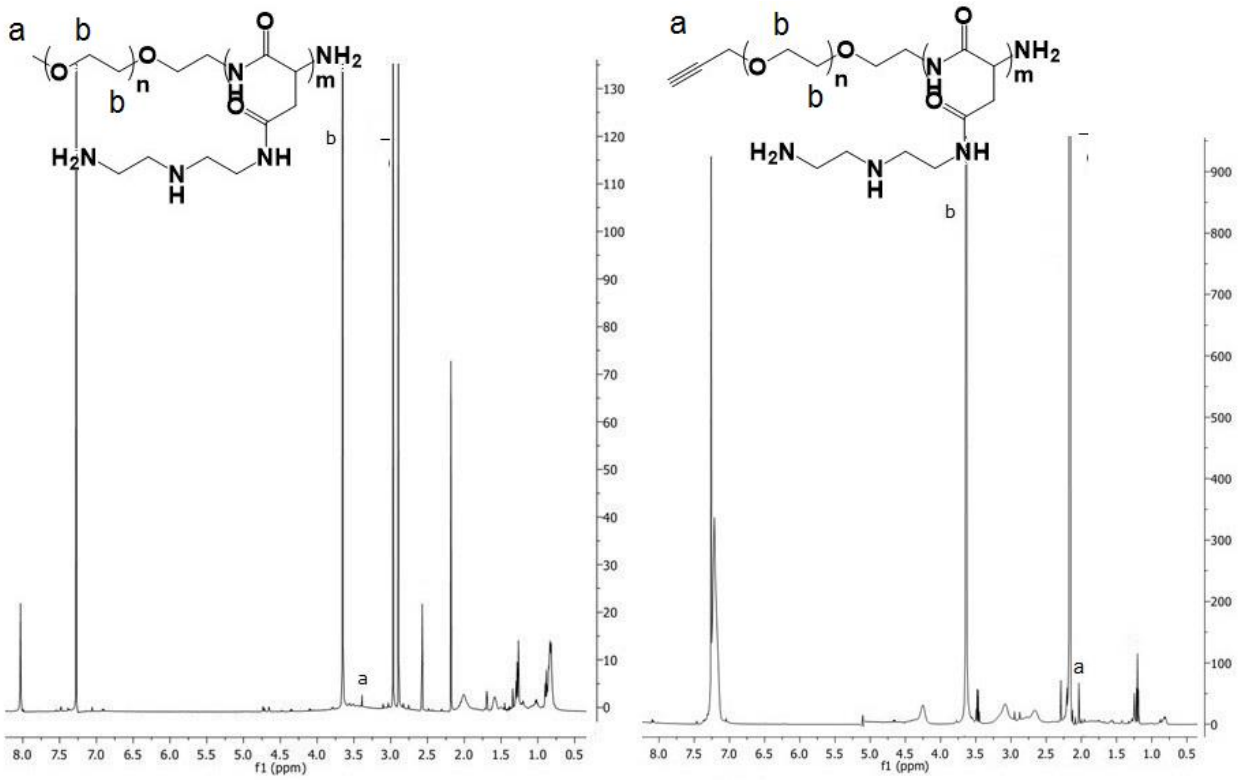

Figure S6. ^{1}H NMR spectra of mPEG-*b*-pAsp(DET) (left) and alkyne-PEG-*b*-pAsp(DET) (right) ($CDCl_3$, 400 MHz, 25 °C).

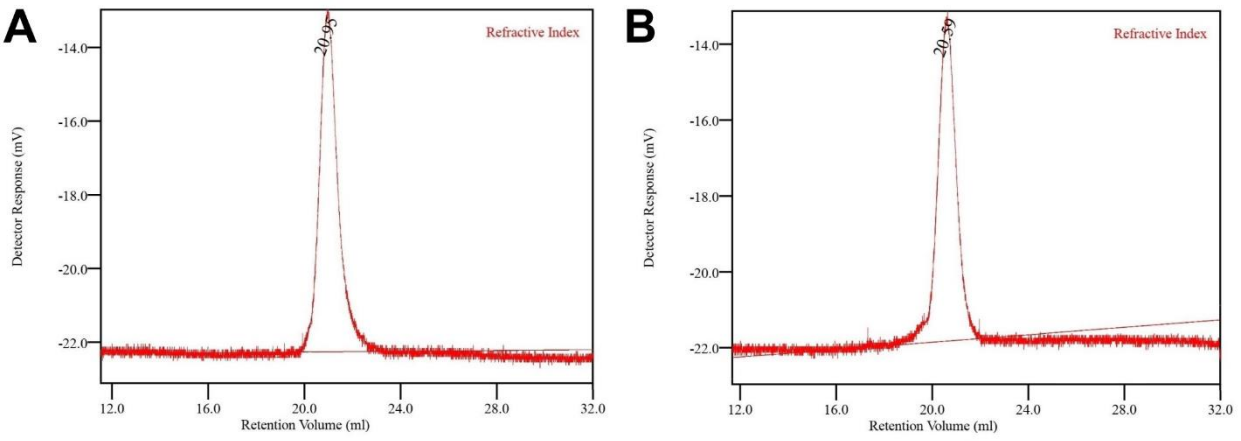

Figure S7. GPC chromatograms of (A) mPEG-*b*-pAsp(DET) and (B) alkyne-PEG-*b*-pAsp(DET) in DMF containing 1% LiBr obtained at 45 °C at a flow rate of 1.0 mL/min using the refractive index detection.

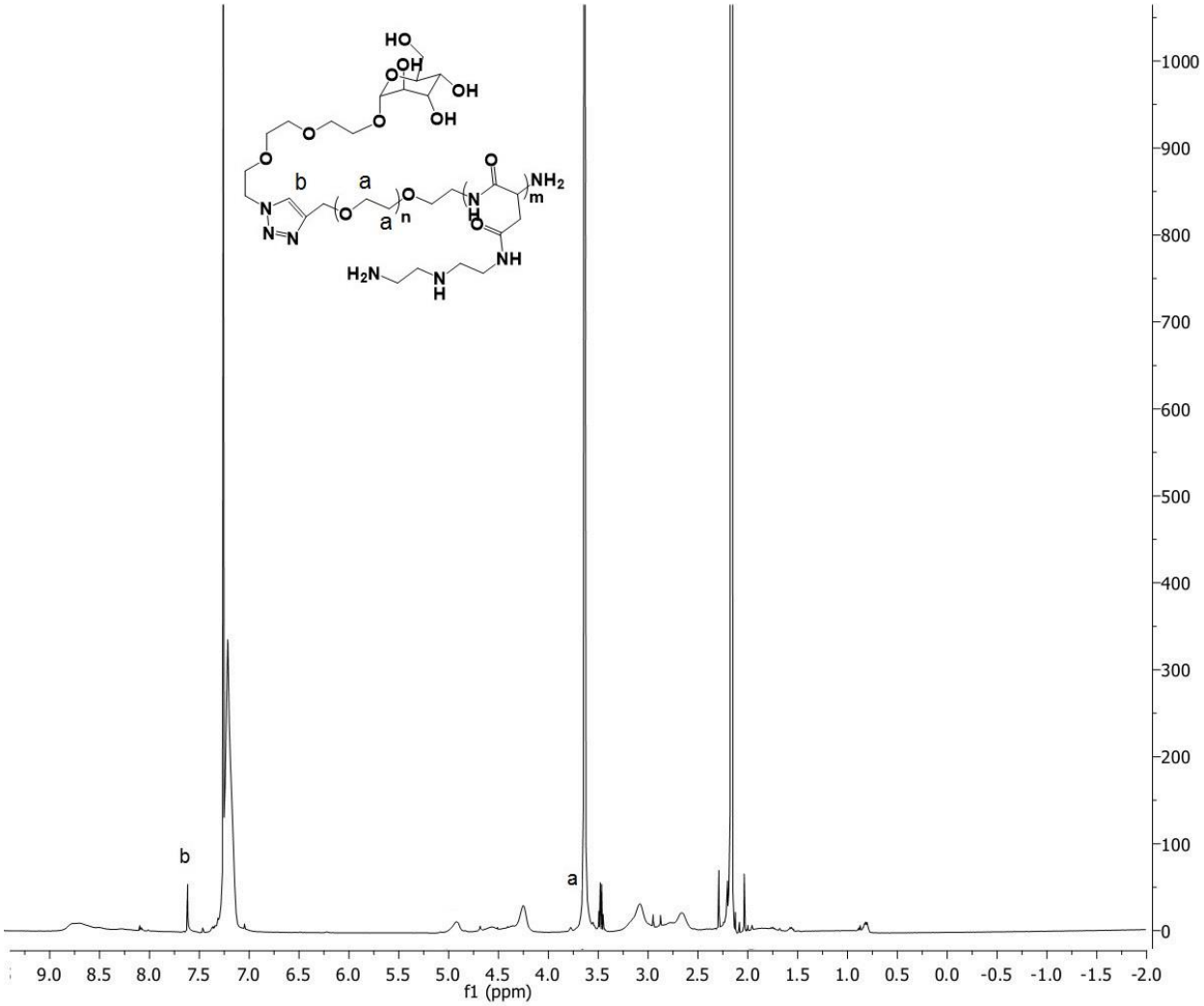

Figure S8. ^{1}H NMR spectra of Man-PEG-*b*-pAsp(DET) ($CDCl_3$, 400 MHz, 25 °C).

Table S1. Characterization of polyplexes prepared using different PEG-*b*-PLL copolymers. PEG tethering density was calculated by dividing the number of PEG chains per pDNA molecule in a hypothetical stoichiometric complex by the surface area of the naked pDNA [for gWiz-GFP, 5757 bp: $2\pi \times 1$ (nm) $\times$ 0.338 (nm/bp) $\times$ 5757 (bp) = 12,226.2 (nm^2)]. The reduced tethering density (RTD) was calculated as $\pi R_g^2 \sigma$. Radius of gyration (R_g, nm) of PEG was estimated according to the following equation: $R_g = 0.181 \times (\text{DP of PEG})^{0.58} = 2.82$ (nm).

Polymer used	PEG tethering density, σ (chain/nm^2)	RTD, $\pi R_g^2 \sigma$
PEG_{114}-*b*-PLL_{62}	0.046	1.141
PEG_{114}-*b*-PLL_{150}	0.019	0.471
PEG_{114}- *b*-PLL_{206}	0.014	0.343

Table S2. Transfection efficiency of gWiz-Luc pDNA containing polyplexes, formed from PEG-*b*-pAsp(DET) cationic copolymers. The concentrations of pDNA were 4.53 μg/ml for both targeted and non-targeted. Polyplexes were prepared at various N/P and were incubated with the cells for various period. Relative Luminescent Units (RLU) were normalized on protein content. Values are mean ± SEM (n = 3).

Polyplexes type		RLU/mg protein		
Polymer used	N/P	4 h incubation	8 incubation	24 incubation
mPEG-*b*-pAsp(DET)	1	18763 ± 1635	23408 ± 3043	42851 ± 3686
mPEG-*b*-pAsp(DET)	8	33007 ± 2648	24499 ± 11842	194067 ± 97037
mPEG-*b*-pAsp(DET)	20	887248 ± 252252	168536 ± 64998	364766 ± 187237
Man-PEG-*b*-pAsp(DET)	1	68923 ± 8784	164679 ± 18158	389330 ± 110,921
Man-PEG-*b*-pAsp(DET)	8	866634 ± 84233	10196817 ± 1803821	23372325 ± 3155373
Man-PEG-*b*-pAsp(DET)	20	14325463 ± 1506281	79739546 ± 2087039	104834769 ± 16372866

Table S3. Extent of enhancement of transfection as a result of attachment of the mannose targeting moieties to the PEG-*b*-pAsp(DET)/pDNA polyplexes. For every N/P ratio and time point data are presented as the ratio of luciferase expression levels (RLU/mg protein) for the targeted vs. non-targeted polyplexes. Values are mean ± SEM (n = 3).

N/P	Times increase targeted vs. non-targeted polyplex		
	4 h incubation	8 incubation	24 incubation
1	3.7 ± 0.1	26.2 ± 0.3	16.7 ± 2.3
8	7.0 ± 0.1	469.8 ± 128.8	524.5 ± 150.3
20	8.9 ± 1.2	139.9 ± 45.5	335.0 ± 108.8

Table S4. The protein concentration (mg/ml) determined using BCA assay after treating IC-21 cells transfected using PEG-*b*-pAsp(DET)/pDNA with lysis buffer for 2 hrs. Values are mean ± SEM (n = 3).

Polyplexe type		Incubation time, hrs		
Polymer used	N/P	4 h incubation	8 incubation	24 incubation
mPEG-*b*-pAsp(DET)	1	2.19 ± 0.08	2.05 ± 0.34	0.80 ± 0.15
mPEG-*b*-pAsp(DET)	8	2.06 ± 0.07	2.47 ± 0.44	0.81 ± 0.16
mPEG-*b*-pAsp(DET)	20	1.08 ± 0.86	1.85 ± 0.21	0.86 ± 0.13
Man-PEG-*b*-pAsp(DET)	1	1.93 ± 0.24	1.89 ± 0.22	0.89 ± 0.09
Man-PEG-*b*-pAsp(DET)	8	1.79 ± 0.18	2.05 ± 0.31	0.85 ± 0.16
Man-PEG-*b*-pAsp(DET)	20	1.72 ± 0.24	1.91 ± 0.26	0.92 ± 0.11