Supplementary Information

Synthesis of Diblock/Statistical Cationic Glycopolymers with Pendant Galactose and Lysine Moieties: Gene Delivery Application and Intracellular Behaviors

Jingjing Sun, Ruilong Sheng*, Ting Luo, Zhao Wang, Hui Li and Amin Cao*

1. Key Laboratory of Synthetic and Self-assembly Chemistry for Organic Functional Molecules, Shanghai Institute of Organic Chemistry, CAS. Lingling Road 345, Shanghai, 200032, China.
2. Department of Chemistry, Université de Montréal, Succursale Centre-ville, Montreal, Quebec, H3C3J7, Canada.

Scheme S1. Synthesis of the glycomonomer 6-O-methacryloyl-1,2,3,4-di-O-isopropylidene-galactopyranose (MAIGal)

Figure S1. a) $^1$H NMR spectrum of the galactose methylacylate monomer (MAIGal) in CDCl$_3$. b) $^{13}$C NMR spectrum of the galactose methylacylate monomer (MAIGal) in CDCl$_3$

Figure S2. $^1$H NMR spectra of the statistical copolymer P (HMLBoc$_{40}$-st-MAIGal$_{13}$) (a) and BOC-deprotected cationic polymer P (HML$_{40}$-st-MAGal$_{13}$) (b) in DMSO-$d_6$

Figure S3. FT-IR spectra of the statistical copolymer P (HMLBoc$_{40}$-st-MAIGal$_{13}$) (a) and BOC-deprotected cationic polymer P(HML$_{40}$-st-MAGal$_{13}$) (b)

Figure S4. TEM images of the PHML$_{40}$, PHML$_{40}$-b-PMAGal$_3$ and P(HML$_{40}$-st-MAGal$_{13}$)/pDNA polyplexes (N/P=40)

Figure S5. Fluorescence microscopic images (400×) of the localization of Cy3-labeled pDNA in H1299 cells recorded after 6 h gene transfection by P(HML$_{40}$-st-MAGal$_{13}$) vector in the presence of 10% FBS (Green: Lysotracker labeled lysosomes; Red: Cy3 labeled pDNA; Blue: DAPI stained cell nuclei)
S1. Synthesis of the glycomonomer 6-O-methacryloyl-1, 2, 3, 4-di-O-isopropylidene-galactopyranose (MAIGal)

Firstly, the hydroxy groups of D-galactose were protected by condensation with acetone in the presence of concentrated H$_2$SO$_4$ (98%) and anhydrous CuSO$_4$, then the protected D-galactose was further esterified with methacryloyl chloride at 0 °C. The solvent was evaporated to afford crude product, finally, pure product of MAIGal was purified via column chromatography and obtained as white solid. Yield of the two steps: 57 %.

$^1$H NMR (CDCl$_3$, δ in ppm): 6.13 (m, 1H, =CHH), 5.58 (m, 1H, =CHH), 5.53 (d, 1H, Galactopyranose (Gal)$-H$ at 1 position), 4.61 (m, 1H, Gal$-H$ at 3 position), 4.30 (m, 4H, Gal$-H$ at 2, 4 and 6 position), 4.05 (m, 1H, Gal$-H$ at 5 position), 1.93 (s, 3H, CH$_3$CR=CH$_2$), 1.53-1.33 (m, 12H, (CH$_3$)$_2$COO).

$^{13}$C NMR (CDCl$_3$, δ in ppm): 167.2, 136.1, 125.8, 109.5, 108.8, 96.3, 72.1, 70.7, 70.5, 66.1, 63.6, 25.9, 25.0, 24.4, 18.3. FTIR (in cm$^{-1}$): 2978, 2927, 1715, 1385, 1258, 1210, 1176, 1164, 1112, 1064, 1006, 936, 901, 865.

ESI-MS [M+H$^+$] (in m/z): 329.1 (Cal: 329.2).
Figure S1 a) $^1$H NMR spectrum of the galactose methylacylate monomer (MAIGal) in CDCl$_3$

Figure S1 b) $^{13}$C NMR spectrum of the galactose methylacylate monomer (MAIGal) in CDCl$_3$
Figure S2. $^1$H NMR spectra of the statistical copolymer P (HMLBoc$_{40}$-st-MAIGal$_{13}$) (a) and BOC-deprotected cationic polymer P(HML$_{40}$-st-MAGal$_{13}$) (b) in DMSO-$d_6$. 
Figure S3. FT-IR spectra of the statistical copolymer $\text{P(HMLBoc}_{40}\text{-st-MAIGal}_{13})$ (a) and BOC-deprotected cationic polymer $\text{P(HML}_{40}\text{-st-MAGal}_{13})$ (b).
Figure S4. TEM images of the PHML$_{40}$, PHML$_{40}$-b-PMAGal$_3$ and P(HML-st-MAGal$_4$)/pDNA polyplexes (N/P=40)
**Figure S5.** Fluorescence microscopic images (400×) of the localization of Cy3-labeled pDNA in H1299 cells recorded after 6 h gene transfection by P(HML$_{40}$-st-MAGa$l_4$) vector in the presence of 10% FBS (Green: Lysotracker labeled lysosomes; Red: Cy3-labeled pDNA; Blue: DAPI stained cell nuclei)