Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Harmonized tuning of nucleic acid and lectin binding properties with multivalent cyclodextrins for macrophage-selective gene delivery

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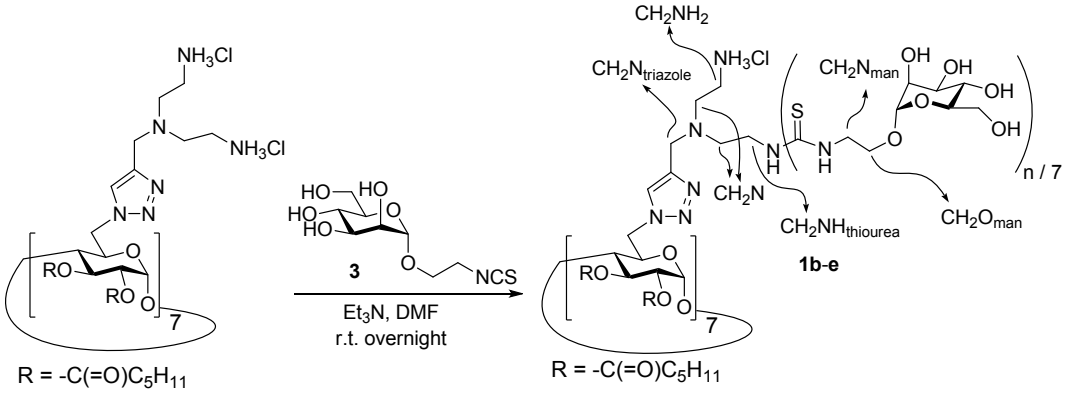
Synthesis.

*Procedure for statistical mannosylation of paCD **1a**.*



To a solution of **1a**[1] (50 mg, 12 μmol) and Et_3N (47 μL, 0.34 mmol, 2 eq) in dry DMF (5 mL), a solution of 2-isothiocyanatoethyl α-D-mannopyranoside **3**[2] (2.2 mg, 6.7 mg, 13.3 mg or 22.3 mg for 0.05, 0.15, 0.30 or 0.50 eq per primary amino group, respectively) in DMF (5 mL) was added and the solution was stirred overnight at room temperature. The solvent was removed under vacuum and the residue was purified by exclusion chromatography (Sephadex G-25) using water as eluent. The purified compound was dissolved in diluted HCl 0.1 N and freeze-dried to give a white foam in nearly quantitative yields. ^{1}H NMR (500 MHz, D_2O, 323 K) δ 8.60-8.40 (m, =CH), 5.80-5.60 (m, H-1 $_{CD}$, H-3 $_{CD}$), 5.30-5.00 (m, H-6 $_{CD}$), 5.00-4.50 (H-2 $_{CD}$, H-5 $_{CD}$, H-1_{man}), 4.20-3.60 ($CH_2N_{triazole}$, H-4 $_{CD}$, H-2_{man}, CH_2O_{man}, H-6_{man}, H-3_{man}, H-4_{man}, H-5_{man}), 3.50-2.90 ($CH_2NH_{thiourea}$, CH_2NH_2, CH_2N), 2.70-2.30 (CH_2-5 hexanoyl), 1.79 (CH_2-4 hexanoyl), 1.50 (CH_2-2,3 hexanoyl), 1.07 (CH_3 hexanoyl).

ESI-MS for **1b** (5% mannosylation extent): *m/z* unmannosylated: 735.6 $[M + 5 H]^{5+}$, 918.8 $[M + 4 H]^{4+}$, 1225 $[M + 3 H]^{3+}$; mono-mannosylated: 985.3 $[M + 4 H]^{4+}$, 1313.4 $[M + 3 H]^{3+}$; di-mannosylated: 1051.8 $[M + 4 H]^{4+}$.

ESI-MS for **1c** (15% mannosylation extent): *m/z* unmannosylated: 735.4 $[M+ 5 H]^{5+}$; di-mannosylated: 1051.3 $[M + 4 H]^{4+}$, 1401.7 $[M + 3 H]^{3+}$; tri-mannosylated: 1118.1 $[M + 4 H]^{4+}$, 1490.1 $[M+ 3 H]^{3+}$.

ESI-MS for **1d** (30% mannosylation extent): *m/z* di-mannosylated: 1051 $[M + 4 H]^{4+}$, 1400.2 $[M +3 H]^{3+}$; tri-mannosylated: 896.8 $[M + 5 H]^{5+}$, 1116.5 $[M + 4 H]^{4+}$, 1490 $[M + 3 H]^{3+}$;

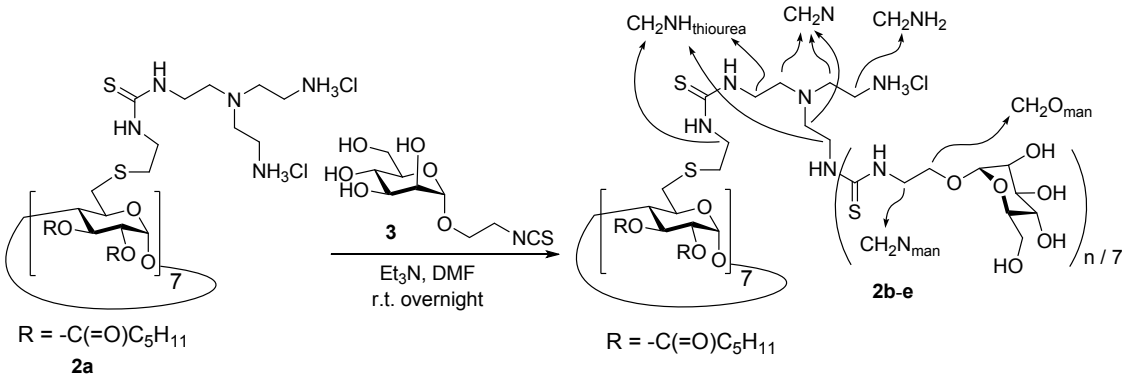
[1] A. Méndez-Ardoy, M. Gómez-García, C. Ortiz-Mellet, N. Sevillano, M. D. Girón, R. Salto, F. Santoyo-González and J. M. García Fernández, *Org. Biomol. Chem.*, 2009, **7**, 2681.

[2] C. Grabosch, K. Kolbe and T. K. Lindhorst, *ChemBioChem*, 2012, **13**, 1874.

tetra-mannosylated: 946.9 $[M + 5 H]^{5+}$, 1183.1 $[M + 4 H]^{4+}$, 1577 $[M + 3 H]^{3+}$; penta-mannosyated: m/z 1249.6 $[M + 4 H]^{4+}$, 1664.8 $[M + 3 H]^{3+}$.

ESI-MS for **1e** (50% mannosylation extent): *m/z* hexa-mannosylated: 1053.9 $[M + 5 H]^{5+}$, 1316.6 $[M + 4 H]^{4+}$, 1755.0 $[M + 3 H]^{3+}$; hepta-mannosylated: 1106.9 $[M + 5 H]^{5+}$, 1382.9 $[M + 4 H]^{4+}$; octa-mannosylated: 1159 $[M + 5 H]^{5+}$, 1449.4 $[M + 4 H]^{4+}$; nona-mannosylated: 1515.9 $[M + 4 H]^{4+}$.

*Procedure for statistical mannosylation of paCD **2a***.



To a solution of **2a**[3] (50 mg, 10.5 μmol) and Et_3N (40 μL, 0.29 μmol, 2 eq) in dry DMF (4 mL), a solution of 2-isothiocyanatoethyl α-D-mannopyranoside **3**[2] (1.96, 5.88, 11.72 or 19.52 mg for 0.05, 0.15, 0.30 or 0.50 eq per primary amino group, respectively) in DMF (5 mL) was added and the solution was stirred overnight at room temperature. The solvent was removed under vacuum and the residue was purified by exclusion chromatography (Sephadex G-25) using water as eluent. The purified compound was dissolved in diluted HCl 0.1 N and freeze-dried to give a white foam in nearly quantitative yield. Yield: 48.2, 48.6, 42.2 and 40.0 mg respectively (93, 87, 68 and 58%). ^{1}H NMR (500 MHz, 5:1 MeOD-D_2O, 313 K) δ 5.35 (nm, H-3_{CD}), 5.19 (m, H-1_{CD}), 4.87 (m, H-2_{CD} , H-1_{man}), 4.20 (m, H-5_{CD}), 4.06 (CH_2O_{man}), 3.96 (m, H-4_{CD}), 3.94 (m, H-2_{man}), 3.83-3.68 ($CH_2NH_{thiourea}$, H-3-5_{man}), 3.60 (m, CH_2N_{man}), 3.22 (CH_2NH_2), 3.00 (m, CH_2N), 2.95-2.88 (m, H-6_{CD}, CH_2S), 2.48-2.24 (CH_2-5 hexanoyl), 1.65 (CH_2-4 hexanoyl), 1.38 (CH_2-2,3 hexanoyl), 0.94 (CH_3 hexanoyl).

ESI-MS for **2b** (5% mannosylation extent): *m/z* unmannosylated: 1060.9 $[M + 4 H]^{4+}$, 1414.1 $[M + 3 H]^{3+}$, 2121.1 $[M + 2 H]^{2+}$; mono-mannosylated: 1127.3 $[M + 4 H]^{4+}$, 1502.8 $[M + 3H]^{3+}$, 2253.6 $[M + 2 H]^{2+}$; di-mannosylated: 1194.1 $[M + 4 H]^{4+}$, 1590.8 $[M + 3 H]^{3+}$.

[3] A. Díaz-Moscoso, L. Le Gourrierec, M. Gómez-García, J. M. Benito, P. Balbuena, F. Ortega-Caballero, N. Guilloteau, C. Di Giorgio, P. Vierling, J. Defaye, C. Ortiz Mellet and J. M. García Fernández, *Chem. – Eur. J.*, 2009, **15**, 12871.

ESI-MS for **2c** (15% mannosylation extent): *m/z* unmannosylated: 1061.0 $[M + 4H]^{4+}$, 1414.3 $[M + 3 H]^{3+}$; mono-mannosylated: 1127.9 $[M + 4 H]^{4+}$, 1502.5 $[M + 3 H]^{3+}$, 2254.0 $[M + 2 H]^{2+}$; di-mannosylated: 1194.1 $[M + 4 H]^{4+}$, 1591.1 $[M + 3 H]^{3+}$, 2385.5 $[M + 2 H]^{2+}$; tri-mannosylated: 1260.0 $[M + 4 H]^{4+}$, 1679.5 $[M + 3 H]^{3+}$.

ESI-MS for **2d** (30% mannosylation extent): *m/z* di-mannosylated: 955.1 $[M + 5 H]^{5+}$, 1193.5 $[M + 4 H]^{4+}$, 1591.0 $[M + 3 H]^{3+}$; tri-mannosylated: 1008.1 $[M + 5 H]^{5+}$, 1259.7 $[M + 4 H]^{4+}$, 1679.0 $[M + 3 H]^{3+}$; tetra-mannosylated: 1061.0 $[M + 5 H]^{5+}$, 1326.5 $[M + 4 H]^{4+}$, 1768.5 $[M + 3 H]^{3+}$; penta-mannosylated: 1392.7 $[M + 4 H]^{4+}$,1857.0 $[M + 3 H]^{3+}$.

ESI-MS for **2e** (50% mannosylation extent): *m/z* di-mannosylated: 1193.9 $[M + 4 H]^{4+}$, 1581.0 $[M + 3 H]^{3+}$; tri-mannosylated: 1259.6 $[M + 4 H]^{4+}$, 1679.0 $[M + 3 H]^{3+}$; tetra-mannosylated: 1326.5 $[M + 4 H]^{4+}$, 1768.5 $[M + 3 H]^{3+}$; penta-mannosylated: 1392.5 $[M + 4 H]^{4+}$, 1857.0 $[M + 3 H]^{3+}$; octa-mannosylated: 1061.5 $[M + 6 H]^{6+}$, nona-mannosylated: 1326.4 $[M + 5 H]^{5+}$.

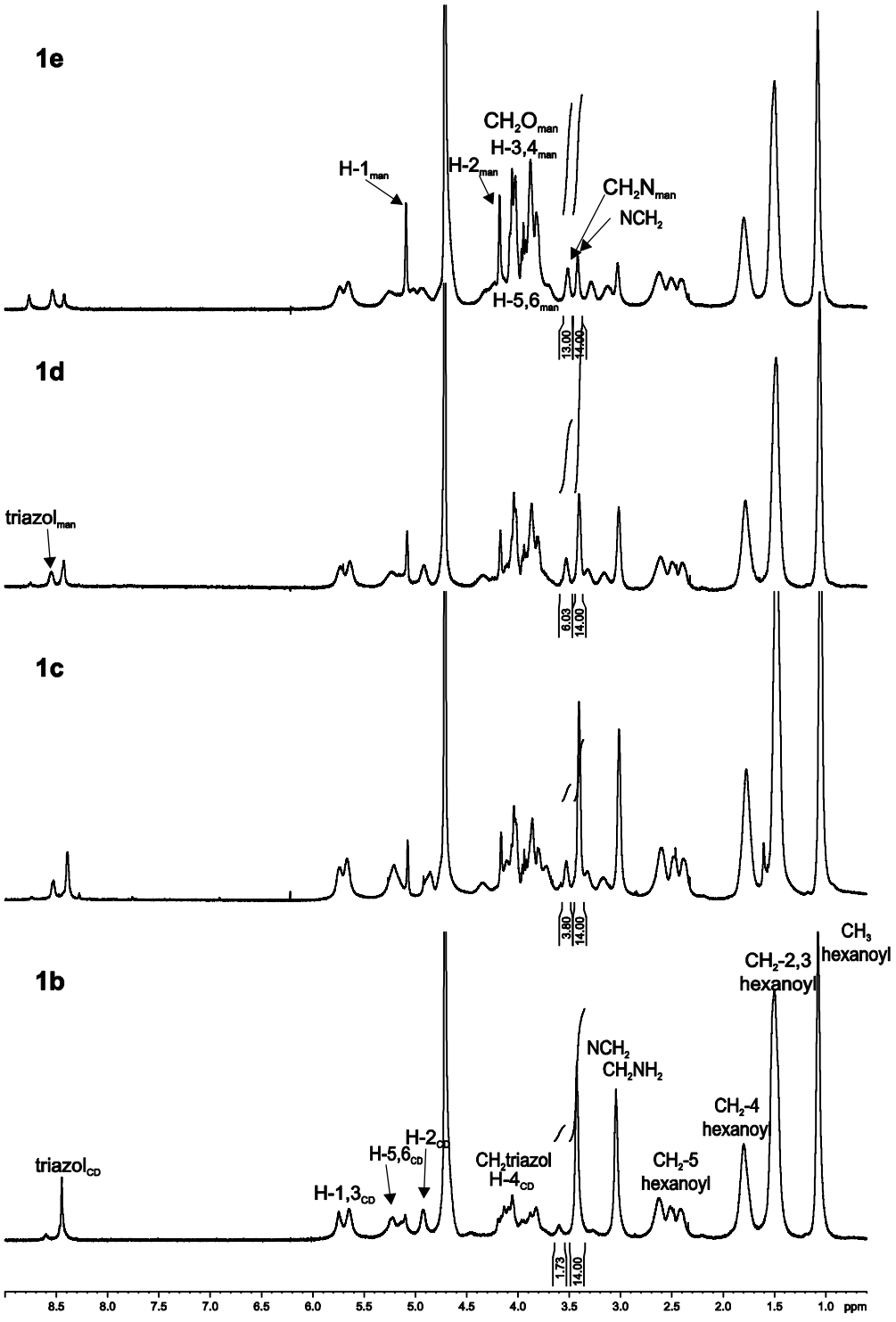


Figure S1. ^{1}H NMR (500 MHz, D_2O, 323 K) spectra of statistically mannosylated paCDs **1b**-**e** with indication of the relative CD/Man amount resulting from the integration of C*H*$_2$N vs C*H*$_2$N$_{man}$ signals.

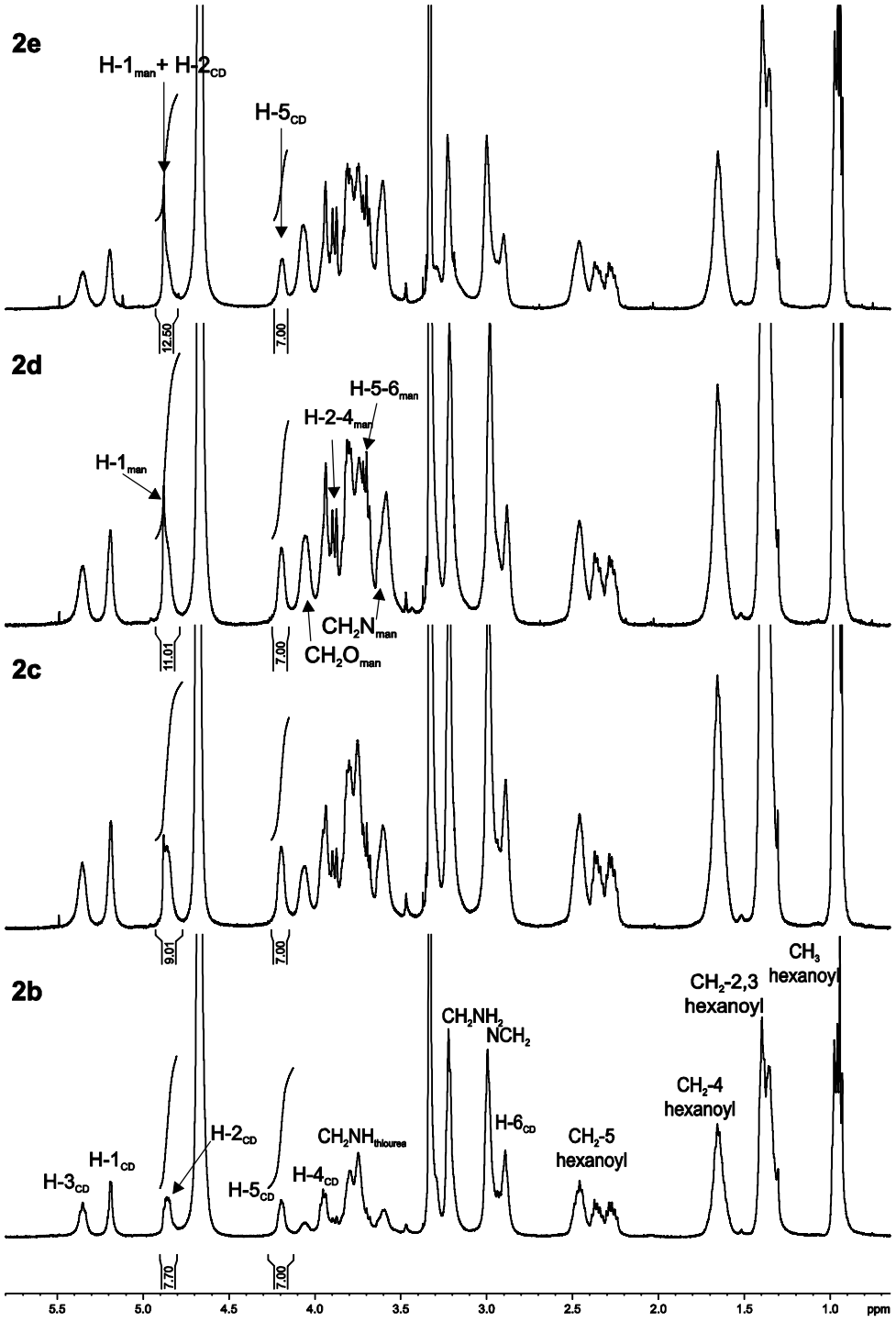


Figure S2. ^{1}H NMR (500 MHz, 5:1 MeOD-D_2O, 313 K) spectra of statistically mannosylated paCDs **2b**-**e** with indication of the relative CD/Man amount resulting from the integration of H-5_{CD} vs [H-2_{CD} + H-1_{man}] signals.

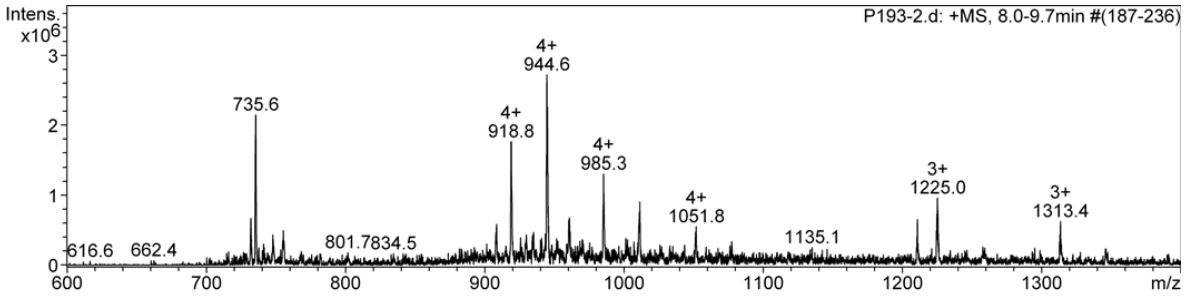


Figure S3. ESI-MS spectrum of **1b**.

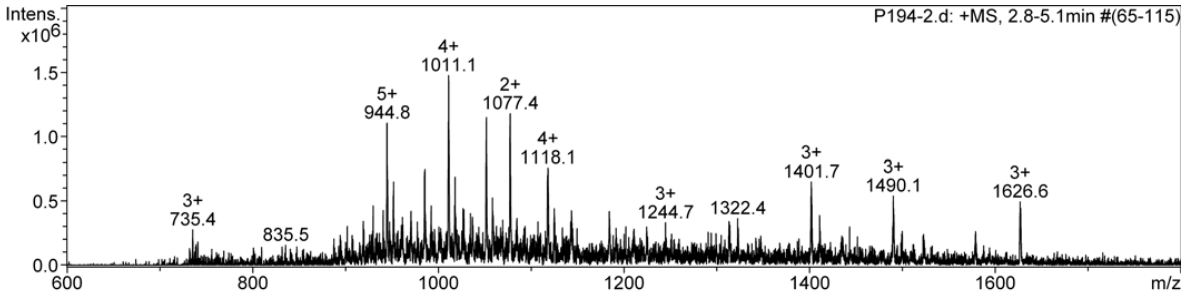


Figure S4. ESI-MS spectrum of **1c**.

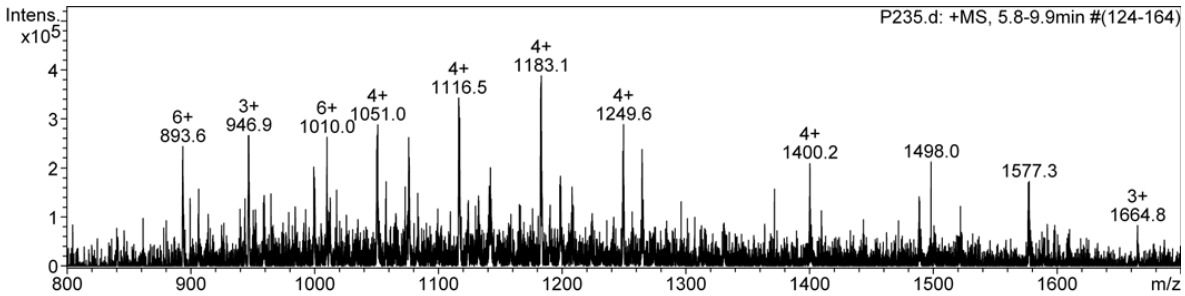


Figure S5. ESI-MS spectrum of **1d**.

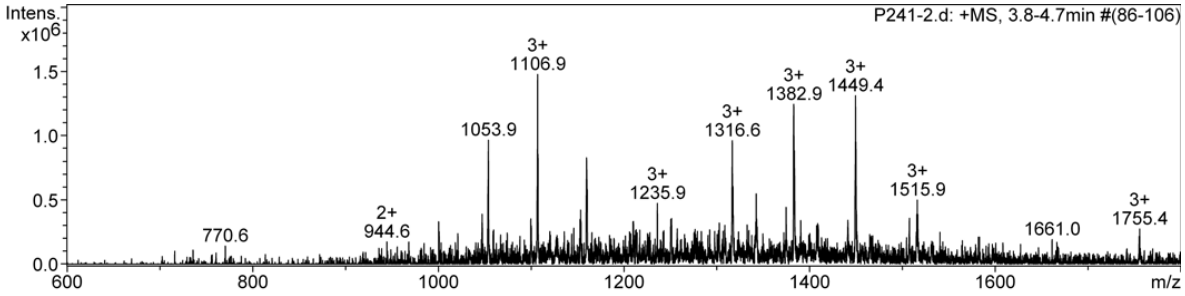


Figure S6. ESI-MS spectrum of **1e**.

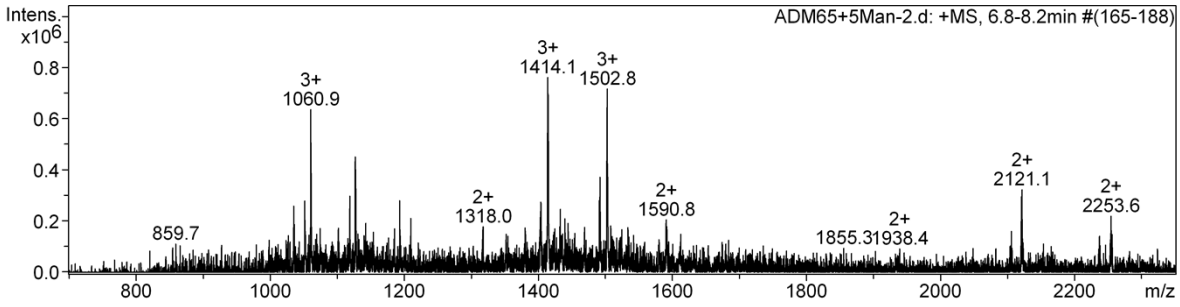


Figure S7. ESI-MS spectrum of **2b**.

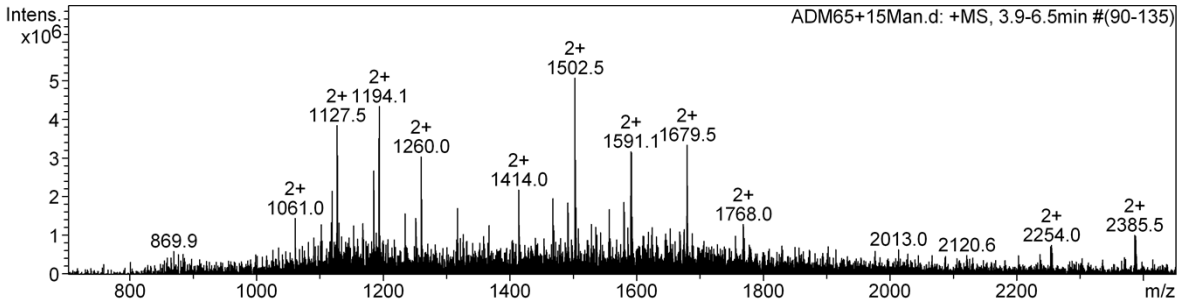


Figure S8. ESI-MS spectrum of **2c**.

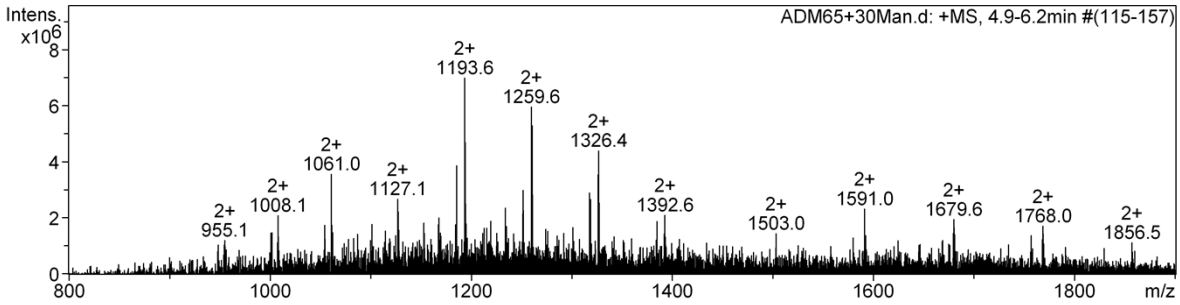


Figure S9. ESI-MS spectrum of **2d**.

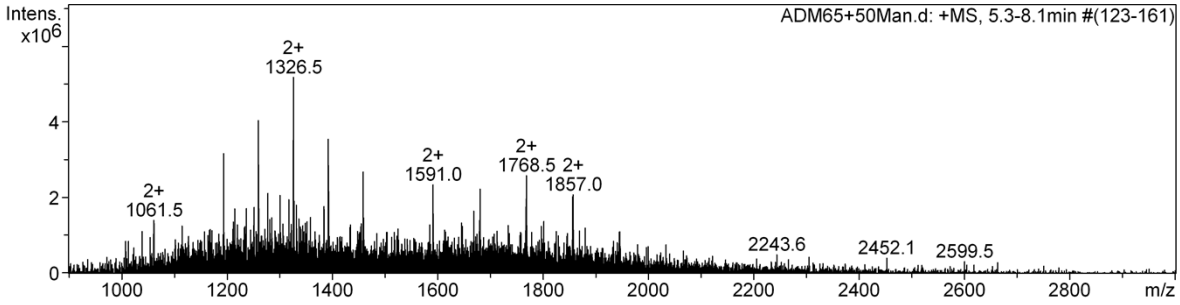


Figure S10. ESI-MS spectrum of **2e**.

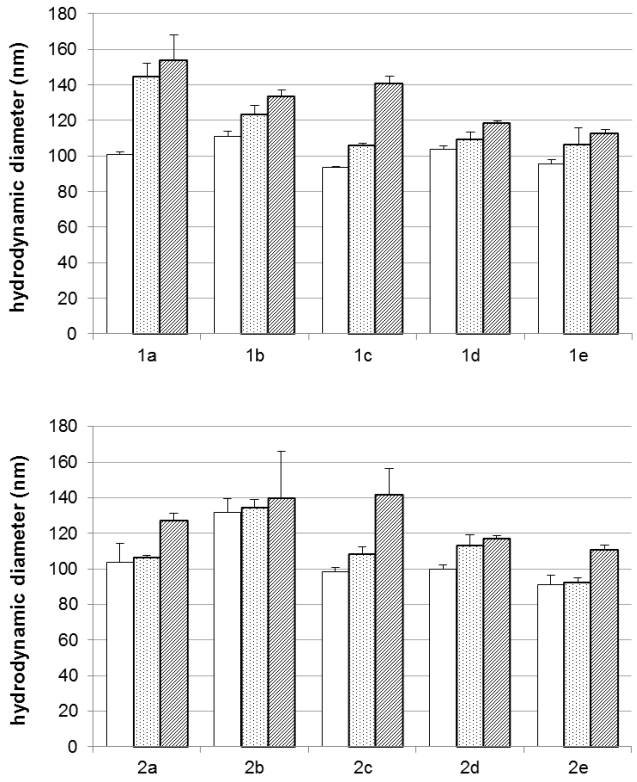


Figure S11. Hydrodynamic diameter of CDplexes (N/P 7) formulated in the absence (white bars) or in the presence of NaCl (50 or 150 mM, dotted and slashed bars, respectively) with paCD **1a** and **2a**, and their corresponding statistically mannosylated derivatives **1b**-**e** and **2b**-**e** (top and bottom panels, respectively).

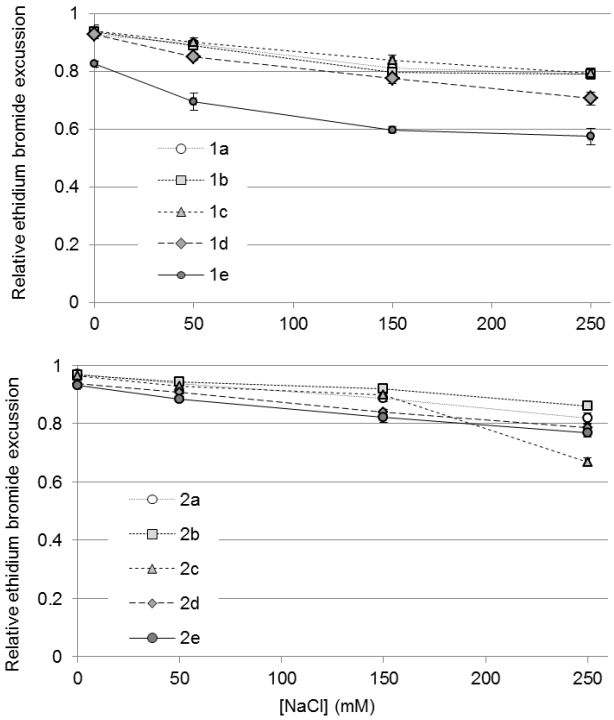


Figure S12. Ethidium bromide exclusion efficiency vs saline concentration of CDplexes (N/P ratio 7) formulated with paCD **1a** and **2a**, and their corresponding statistically mannosylated derivatives (top and bottom panels, respectively). To investigate pDNA release, the fluorescence intensity due to ethidium bromide intercalation was monitored at increasing concentration of NaCl. The ease of pDNA release is correlated with Man content, thus at 250 mM NaCl, ethidium bromide intercalation efficiency in **1e** was twice that of **1a**.

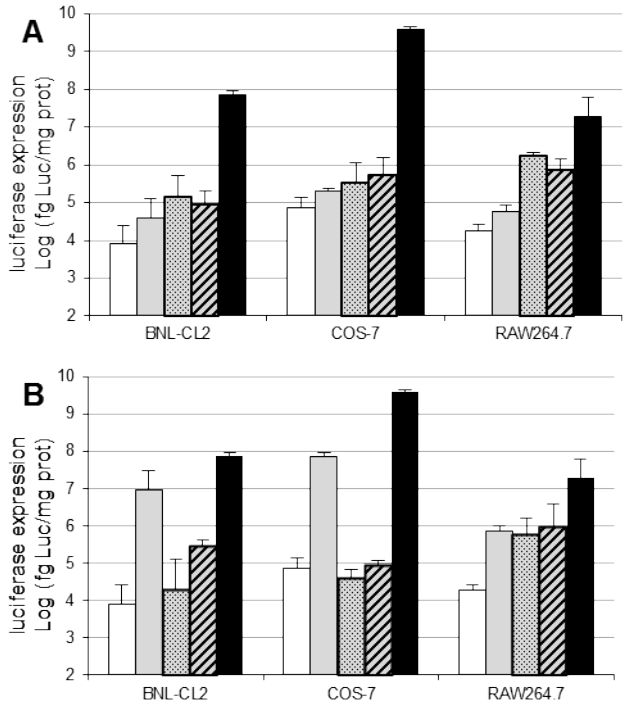


Figure S13. Transfection efficiency in terms of luciferase expression in BNL-CL2, COS-7, and RAW 264.7 cells for CDplexes formulated at N/P 5 with triazol-tethered (**1a**, grey bars; **1c**, dotted bars; **1d**, slashed bars; panel A) and thiourea-tethered (**2a**, grey bars; **2c**, dotted bars; **2d**, slashed bars; panel B) paCDs. Naked pDNA and jetPEI polyplexes (formulated at their optimal N/P 10) were used as negative and positive controls, respectively.