Supporting Information

Dendrimeric Amide- and Carbamate-linked Lysine-based Efficient Molecular Transporters

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General Information

All reagents were purchased from Sigma-Aldrich. DMF, CH_2Cl_2 (dichloromethane), pyridine were dried over P_2O_5, CaH_2 and KOH respectively. DMF, CH_2Cl_2 were stored by adding 4 Å molecular sieves. Column chromatography was performed for purification of compounds on silica gel (100- 200 mesh or 60-120 mesh, Merck). TLCs were performed on Merck 5554 silica 60 pre-coated aluminium sheets. TLCs were performed using petroleum ether-ethyl acetate solvent systems. Compounds were visualized under UV light and/or by spraying with Ninhydrin reagent and heating. ^1H and ^{13}C NMR (200 MHz) spectra were recorded on a Bruker ACF 200 spectrometer fitted with an Aspect 3000 computer and all the chemical shifts (ppm) are referred to internal TMS for ^1H and chloroform-d for ^{13}C NMR. ^1H NMR data are reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; br s, broad singlet; m, multiplet and/ or multiple resonance), number of protons. MALDI-TOF spectra were obtained from a Voyager-De-STR (Applied Biosystems). CHCA (α-Cyano-4-hydroxycinnamic acid) matrix was used to analyze MALDI-TOF samples. UV absorbance studies were performed on a Varian Cary 300 UV-VIS spectrophotometer. Circular Dichroism (CD) analyses were performed on a JASCO J-815 spectrophotometer, and spectra were recorded at 25 °C in a 2 mm path length cuvette as accumulations of 3 scans using a scan speed of 200 nm/min, data pitch of 1.0 or 0.1 nm, slit width of 500 μm, and D.I.T. of 1s. The spectra were smoothened and plotted as CD (mdeg) Vs wavelength.

Experimental procedures

1,5(*S*)-di-(*tert*-butyloxycarbonyl-amino)-6-hydroxyhexane 2a In a dry 100 mL round bottom flask with magnetic stir bar, under argon atmosphere, $NaBH_4$ (1.13 g, 29.73 mmol) was added in 3:4 mixture of solvents THF: EtOH (40 mL). To this, slowly, LiCl (1.39 g, 33.09 mmol) was added at ice-cold conditions. The reaction mixture was stirred for 30 min for *in situ* generation of $LiBH_4$. The compound **1a** (3 gm, 8.3 mmol) dissolved in ethanol (15 mL) was added drop-wise. The reaction mixture was stirred for a further 3 h and then allowed to warm to room temperature. The reaction was monitored by TLC. Solvents were evaporated *in vacuo* and the crude residue re-dissolved in water. The reaction mixture was neutralized by adding aq. NH_4Cl, followed by extraction of the water layer by ethyl acetate (3 x 20 mL). Brine washes (3 x 25 mL) were given followed by separation of the layers. The combined organic layer was dried over Na_2SO_4. Solvents were removed *in vacuo* and the crude

compound was purified by silica gel column chromatography (50% pet. ether/EtOAc) to give title compound **2a** (2.45 g, 90%) as a white foamy solid.

^{1}H NMR (200 MHz, D_2O) δ: 5.03 (br s, 1H), 4.86 (br s, 1H), 3.59 (m, 3H), 3.11 (m, 2H), 1.44 (m, 24H).

^{13}C NMR (125 MHz, D_2O) δ: 156.4., 79.6, 79.3, 65.5, 52.6, 39.7, 30.7, 30.0, 28.4, 22.7.

^{13}C DEPT (125 MHz, D_2O) δ: *positive peaks*: 52.6, 28.4; *negative peaks*: 65.5, 39.7, 30.7, 30.0, 22.7.

HRMS (ESI): *m/z* calculated for $C_{16}H_{32}O_5N_2Na$: 355.2203, found: 355.2206

1,5(*S*)-di-(*tert*-buyloxycarbonyl-amino)-6-(((4-nitrophenyloxy)carbonyl)oxy)hexane 3a
In a dry 100 mL round bottom flask equipped with magnetic bar, alcohol **2a** (2.1 g, 6.0 mmol) was dissolved in dry CH_2Cl_2 (10 mL). In ice-cold conditions and under argon atmosphere, dry pyridine (0.97 mL, 12.27 mmol) was added by syringe and the reaction mixture stirred for 30 min at 0°C. *p*-Nitrophenyl chloroformate (1.46 g, 7.26 mmol) was added to the reaction under anhydrous conditions and the mixture was stirred for 3h. The solvent was evaporated at room temperature *in vacuo* to give the crude product, which was further purified by flash silica gel column chromatography (15% EtOAc/Pet-ether) to give title compound **3a** (2.51 g, 80) as a white foamy solid.

^{1}H NMR (200 MHz, D_2O) δ: 8.26 (d, J = 9.1 Hz, 2H), 7.38 (d, J = 9.1 Hz, 2H), 4.62 (m, 2H), 4.20 (m, 2H), 3.96 (m, 1H), 3.12 (m, 2H), 1.45 (m, 24H)

^{13}C NMR (125 MHz, D_2O) δ: 156.1, 155.5, 152.5, 145.4, 125.3, 121.7, 79.8, 79.2, 70.9, 49.2, 40.0, 30.9, 29.8, 28.3, 22.8

^{13}C DEPT (125 MHz, D_2O) δ: *positive peaks*: 125.3, 121.8, 49.2, 28.4; *negative peaks*: 70.9, 40.0, 30.9, 29.8, 22.8.

HRMS (ESI): *m/z* calculated for $C_{23}H_{35}O_9N_3Na$: 520.2266, found: 520.2271

Complex Preparation. Dendrimer−pDNA complexes were prepared at charge ratio 10, where charge ratio or N/P ratio refers to the ratio of the total number of positively charged nitrogens of the dendrimer to the total number of negative charges of the phosphates of the

pDNA in solution and is designated as Z (±). This charge ratio was chosen since maximum transfection efficiency was previously observed under these conditions.[1] Briefly, pDNA was diluted in Milli-Q to working concentrations of 20 ng/μL (for biophysical studies) and 40 ng/μL (for all cellular uptake and transfection studies) and 100 μL of this solution was added in a drop-wise manner to appropriate dilution of dendrimer in Milli-Q water (100 μL). The final concentration of dendrimer in 200 μL of complex for biophysical and cellular uptake studies was 0.038 mM and 0.076 mM respectively, and that of dendrimer was 0.037 mM and 0.074 mM respectively. The mixture was kept undisturbed for 1 h or overnight, as described, at room temperature to allow formation of a stable complex.

Cellular Uptake Studies: CHO-K1 and HaCaT cells were seeded in a 24 well plate at a density of 40,000 & 60,000 cells per well respectively. After 24 h when 70 % confluency was attained, 300 μl of serum free media i.e; OPTI-MEM (Invitrogen) was added to each well. Following this,100 μl of bare carboxyfluorescein-labeled peptides (5 μM) were added. After 4 h incubation at 37 °C the cells were washed with 400 μl of 1X ice-cold PBS containing 1 mg/ml heparin salt followed by 400 μl of 0.4 % trypan blue wash to quench extracellular fluorescence. Following this, cells were trypsinized by 100 μl 0.25 % trypsin and centrifuged at 2500 rpm for 10 min at 4 °C. The cellular pellet obtained was resuspended in 200 μl ice-cold 1X PBS & kept on ice. Flow cytometry was immediately performed using BD Accuri C6 flow cytometer.

Transfection Study: CHO-K1and HaCaT cells were seeded in a 24 well plate and after 24 h, when 70 % of confluency was attained, 300 μl of serum-free media, i.e., OPTI-MEM (Invitrogen) was added to each well. pDNA-peptide complexes were added to each well at charge ratio 10. After 4 h, media was changed and supplemented with growth media. After 24 h of addition of the complexes, the media was discarded and the cells were rinsed with 1X PBS. Following this, 1X lysis buffer was added and the lysed cells were centrifuged at 7000 rpm for 15 min at 4 °C. After this, supernatants were collected and 50 μl of protein was mixed with 50 μl of luciferase substrate and kept in ice. Luminescence was measured using PerkinElmer top count NXT Microplate Scintillation and Luminescence Counter. Luciferase activity was normalized with total protein content of the cells estimated using BCA protein assay.

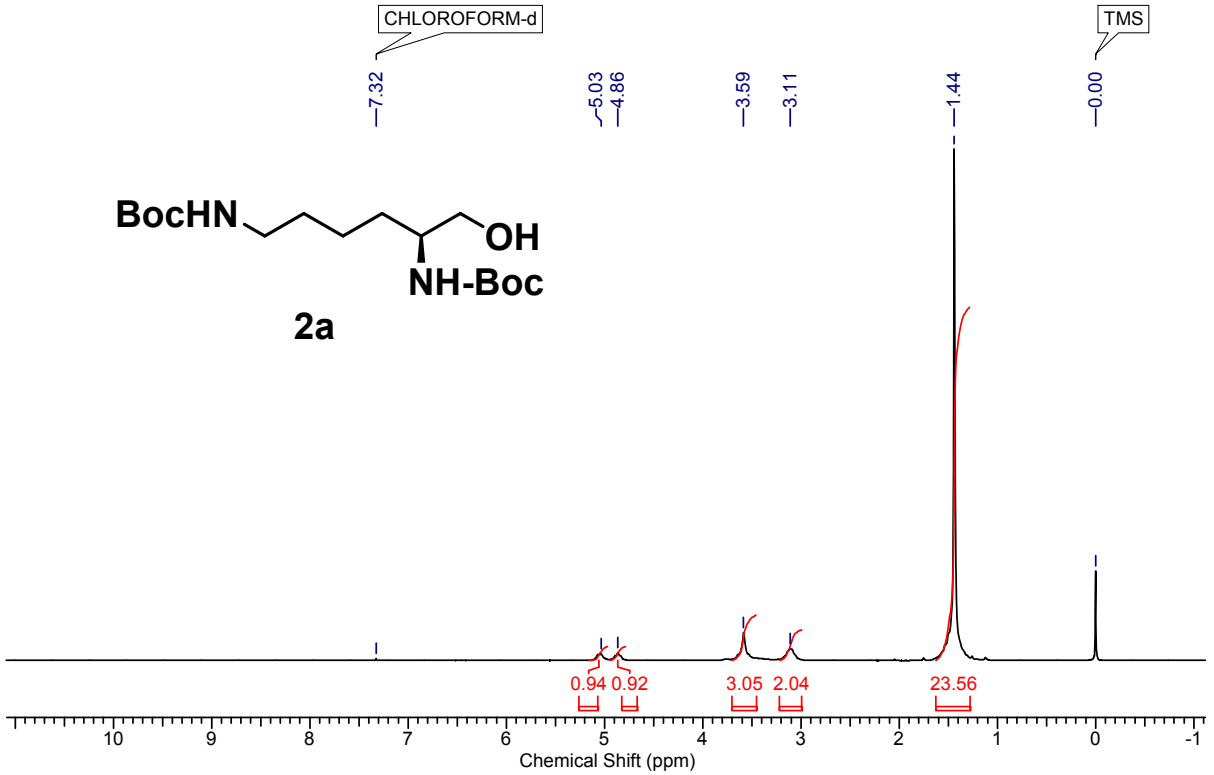
Electrophoretic Mobility Shift Assay: For electrophoretic mobility shift assay to analyze complexation of DNA by peptides, complexes were prepared at different charge ratios (from

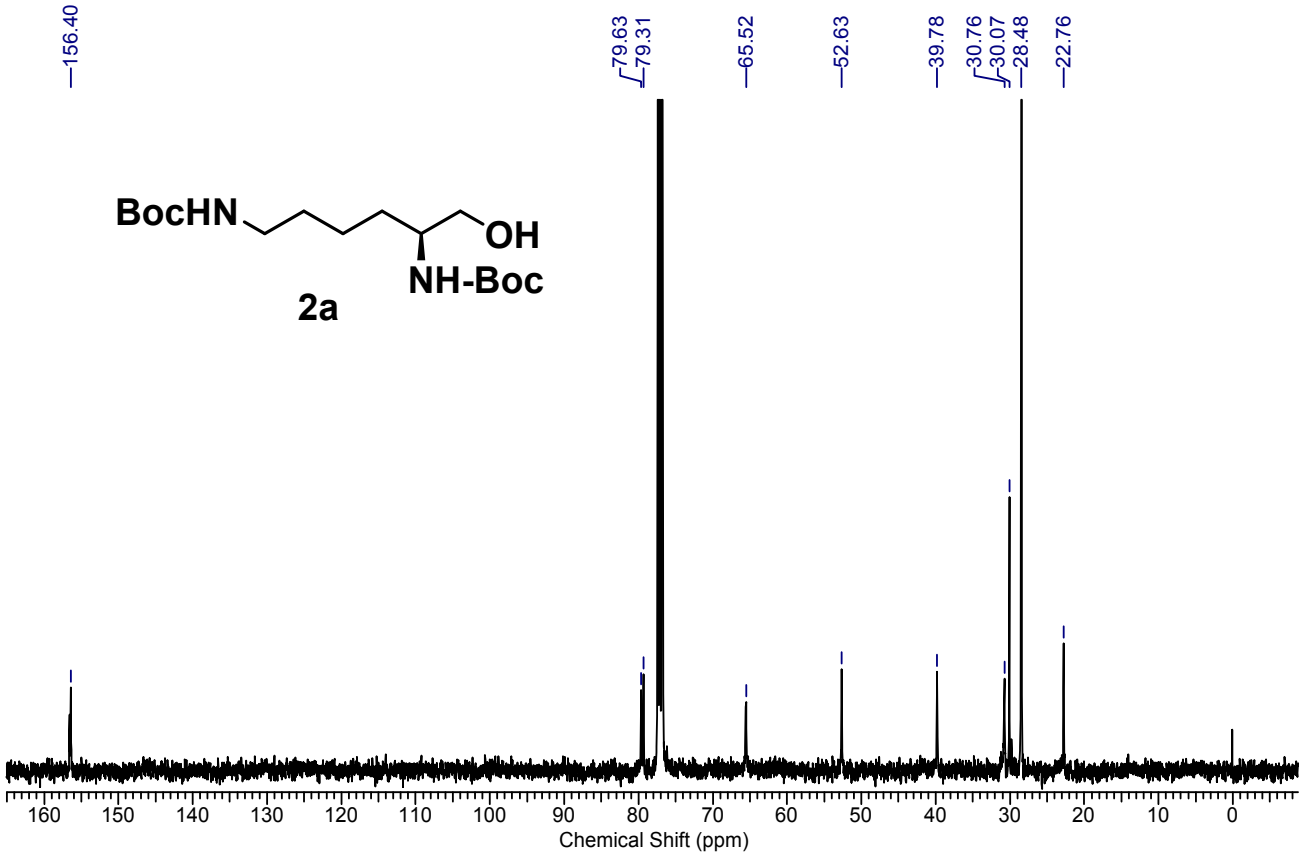
1 to 10) as previously described, using 20 ng/µL PMIR plasmid DNA stock. After 30 min of incubation, complexes were loaded on a 1 % agarose gel containing ethidium bromide. 20 µL of the complex containing 200 ng of DNA was loaded in each case and electrophoresis was carried out at 120 V in 1X TAE buffer (pH 7.4) for 45 min. The mobility of the complex was compared with the mobility of the uncomplexed plasmid DNA. In order to analyze the stability of complexes against anionic challenge, the complexes were prepared at Z (±) of 10, incubated for 30 min or overnight at room temperature, and then treated with increasing amounts of anionic agent, heparin (H3149-100KU), in wt/wt (heparin/peptide) ratios ranging from 0.1:1 to 10:1. 20 µL of complex in each case. The complexes were further incubated for 30 min and electrophoresis was carried out on a 1 % agarose gel. The amount of the DNA released from the complexes was compared with the uncomplexed pDNA band.

Cellular Viability Assay: Cells were seeded in a 96 well plate and after 24 h, when 70 % confluency was attained, complete growth media was replaced by 50 µl serum free media i.e., OPTI-MEM. After this 50 µl of complexes and the bare peptides (final concentration of 5 µM) were added to the serum free media. After 4 h or 24 h respectively (depending on what time point the toxicity measurement was carried out), the serum-free media containing bare peptides and complexes were completely removed and the cells were supplemented with complete growth media.100 µl of MTT (1 mg/ml) was added to the cells & incubated for 4 h. The purple formazan product formed was dissolved in 100 µl of DMSO, and absorbance was measured using ELISA reader at a wavelength of 540 nm and a reference wavelength of 620 nm. Percentage viable cells were expressed considering the untreated cells as 100 % viable.

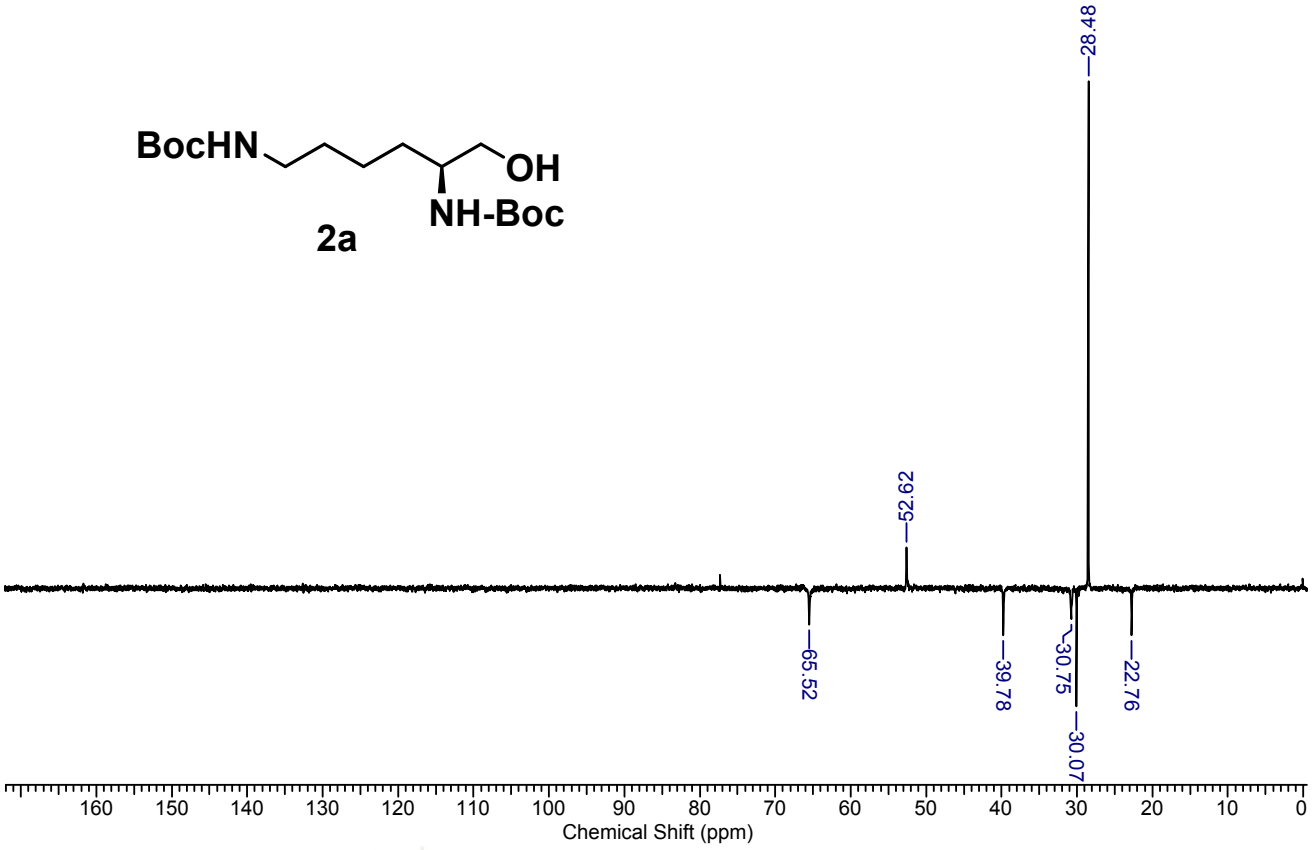
Cellular uptake of peptides

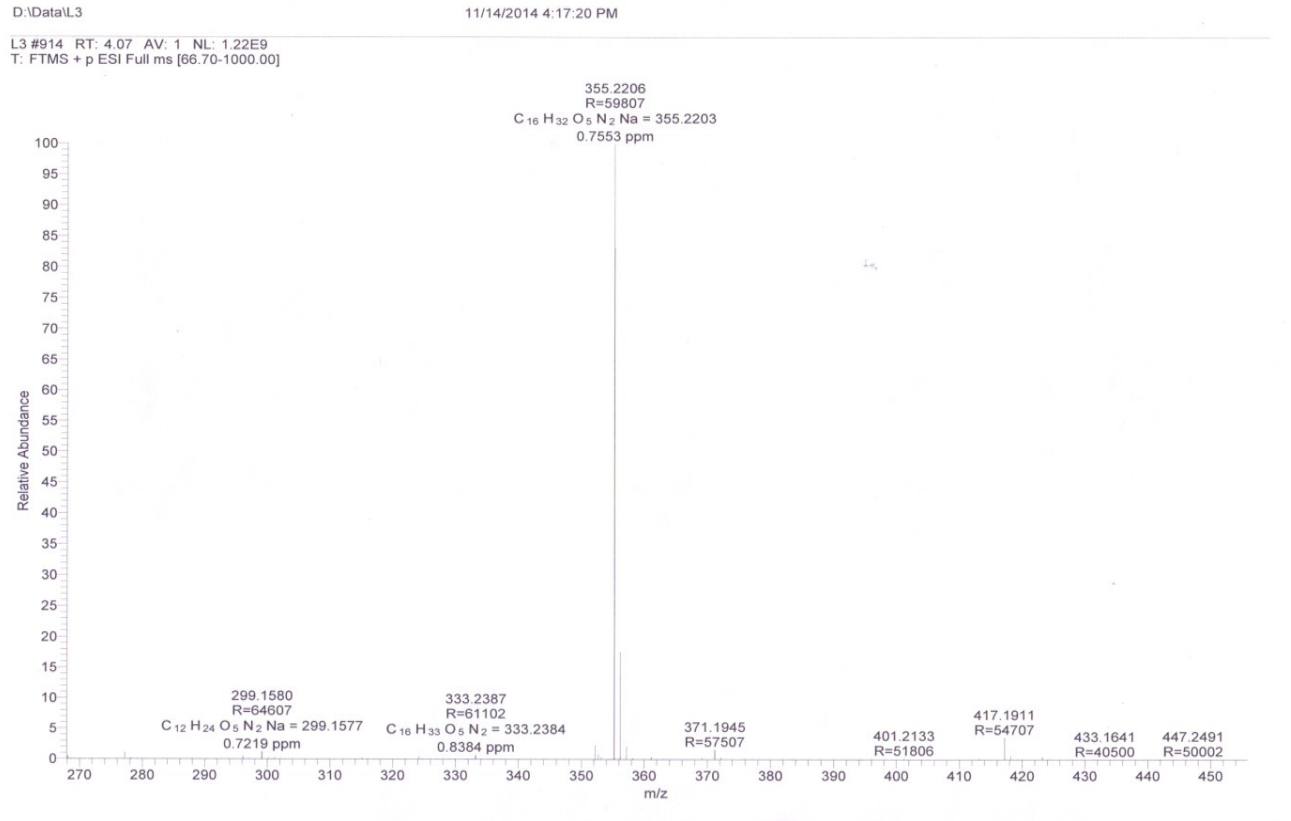
HaCaT cells were seeded in a 24 well plate at density of 30,000 cells per well. After 24 h, when cells were 70-80 % confluent, complete media was replaced with 300 µl of opti-MEM (Invitrogen) in each well. Following this, 100 µl of *cf*-labelled peptides (final concentration 5 µM per well) were added to the cells, After 4 h of incubation at 37 ˚C, cells were washed with 1X PBS followed by fixation using 4 % PFA. The cells were then washed with 1X PBS and stained with Hoechst for 20 min. The cells were washed again with 1X PBS and finally imaged using FLoid cell imaging station (life techonologies).



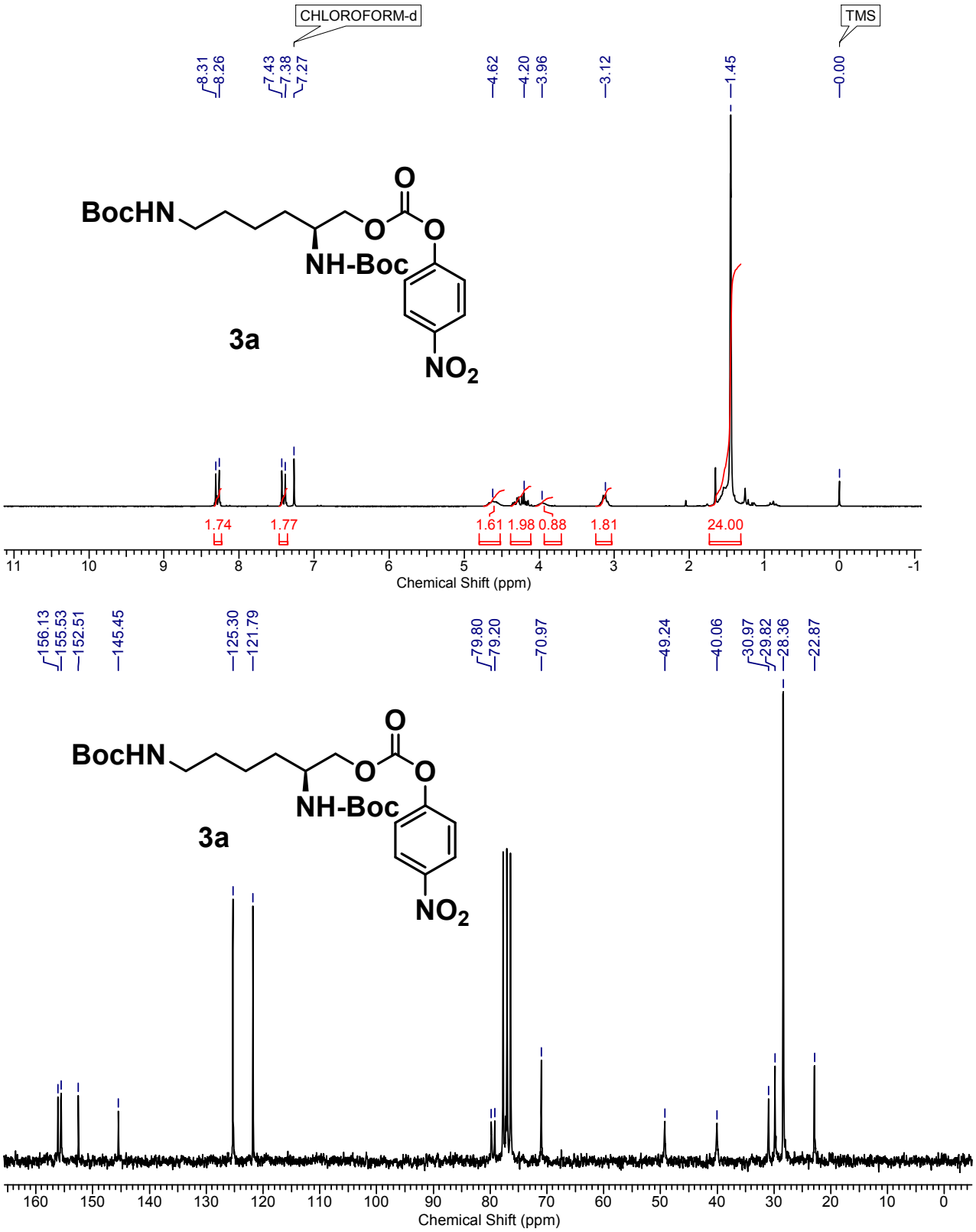


^{1}H NMR and ^{13}C NMR spectra of compound **2a**

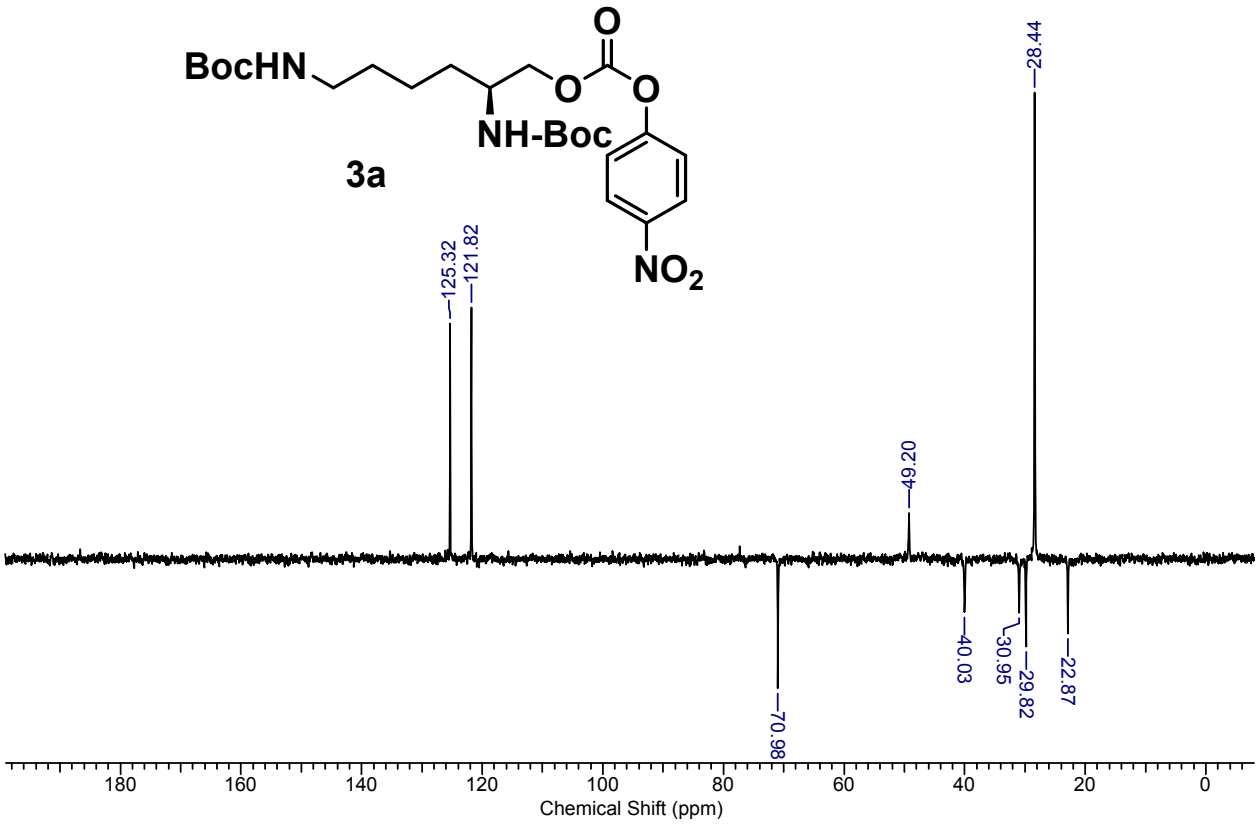


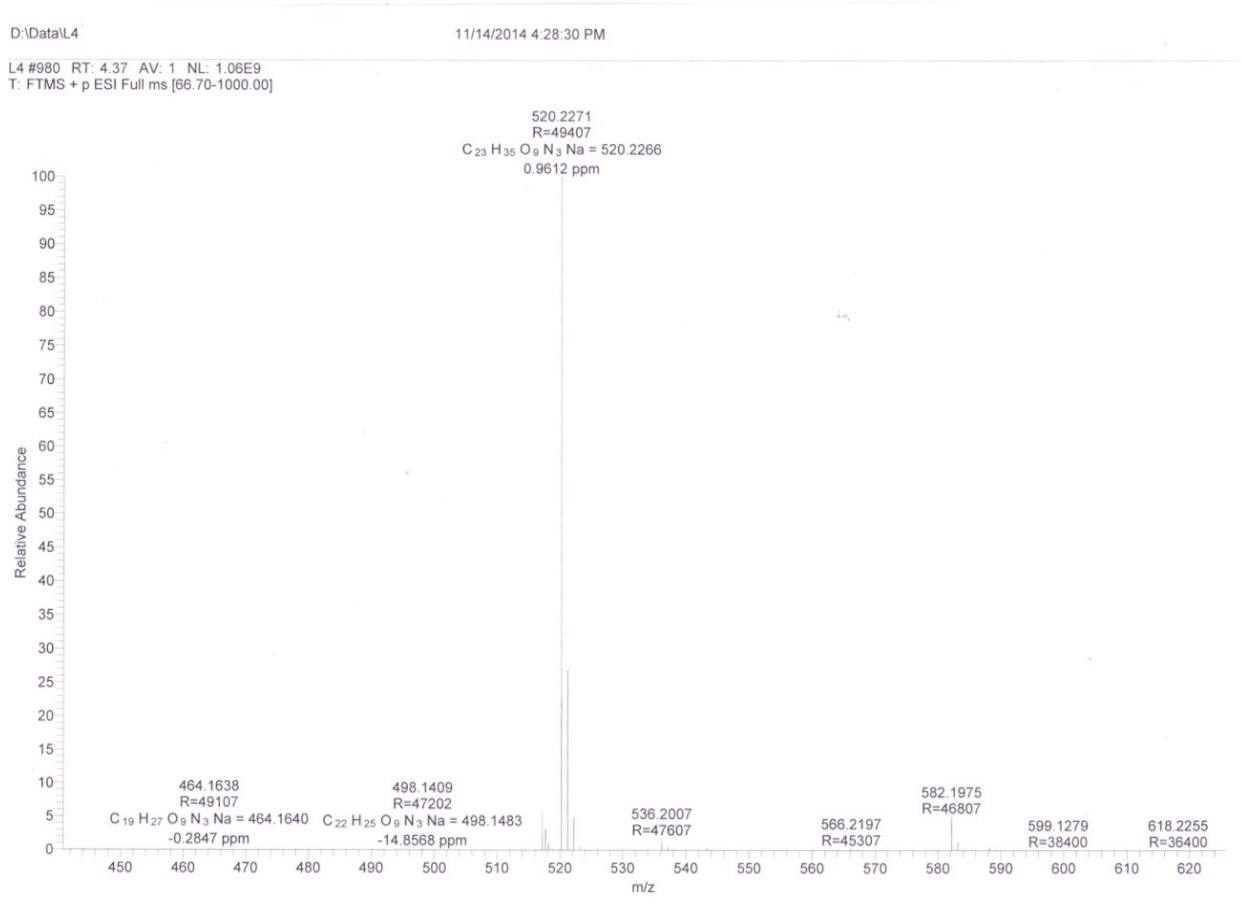


DEPT NMR and HRMS spectra of compound **2a**



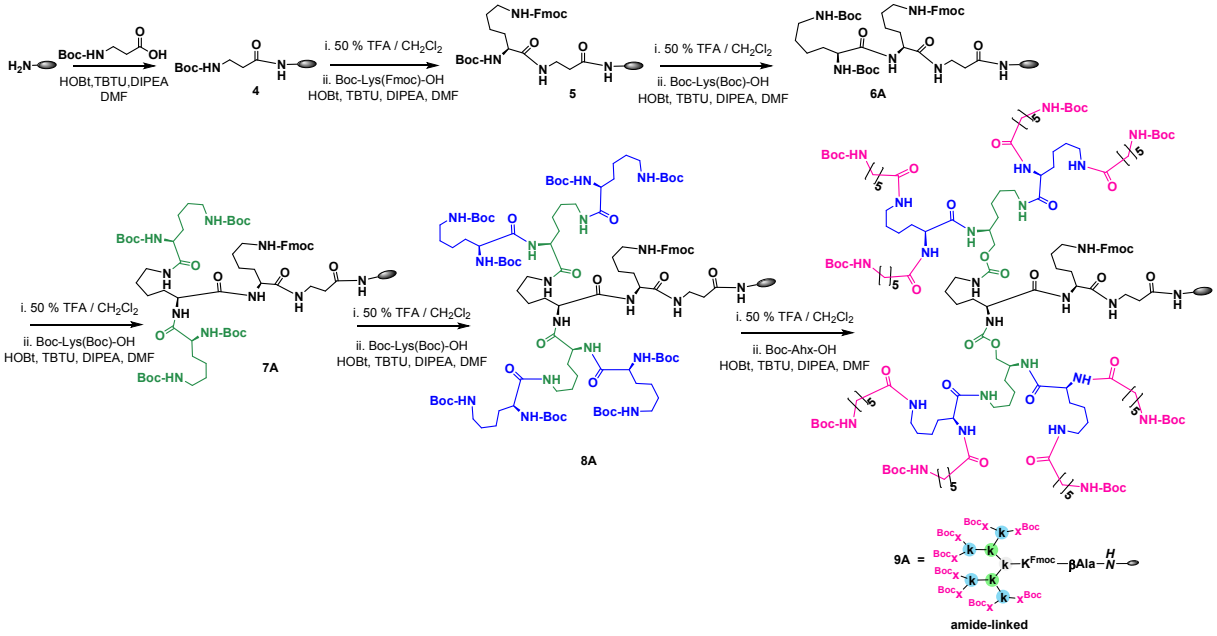
^{1}H NMR and ^{13}C NMR spectra of compound **3a**





DEPT NMR and HRMS spectra of compound **3a**

Scheme S1. Synthesis of amide-linked lysine dendrimer precursor **9A**.



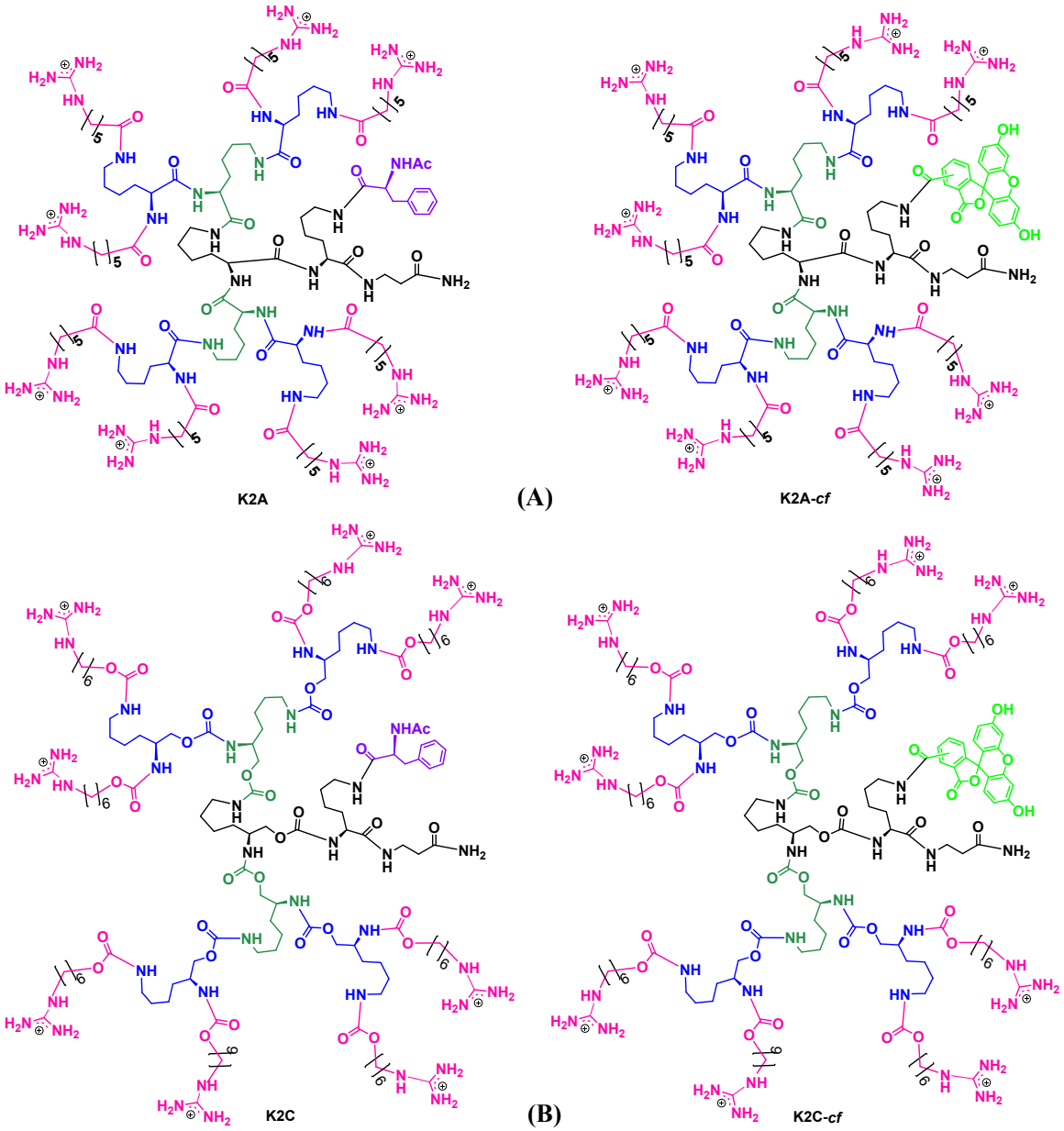
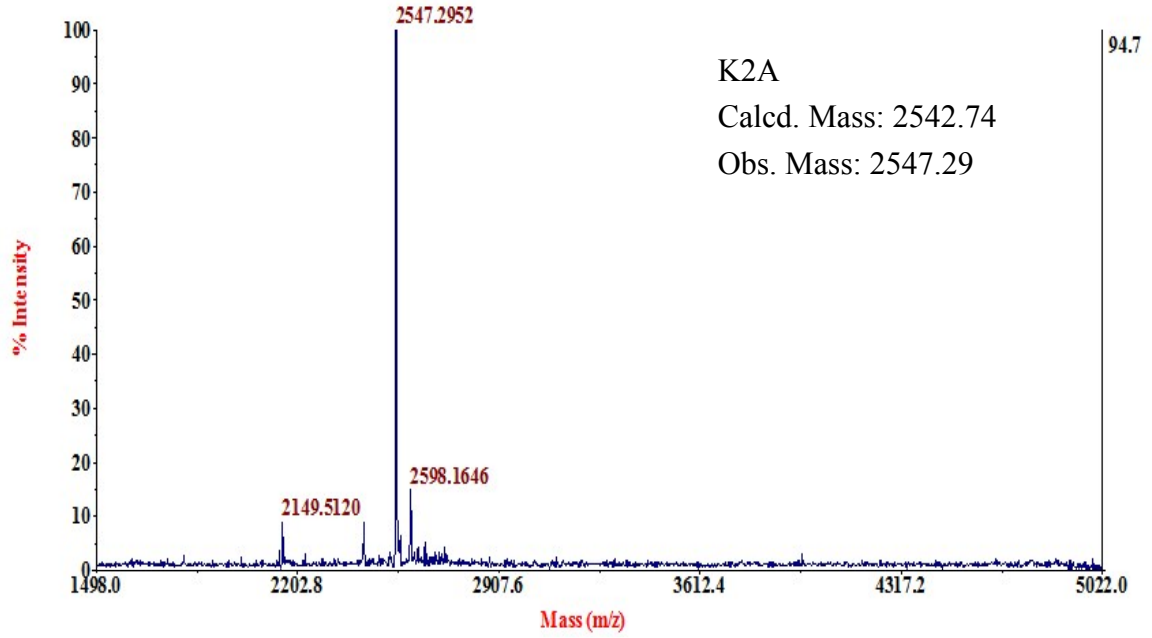
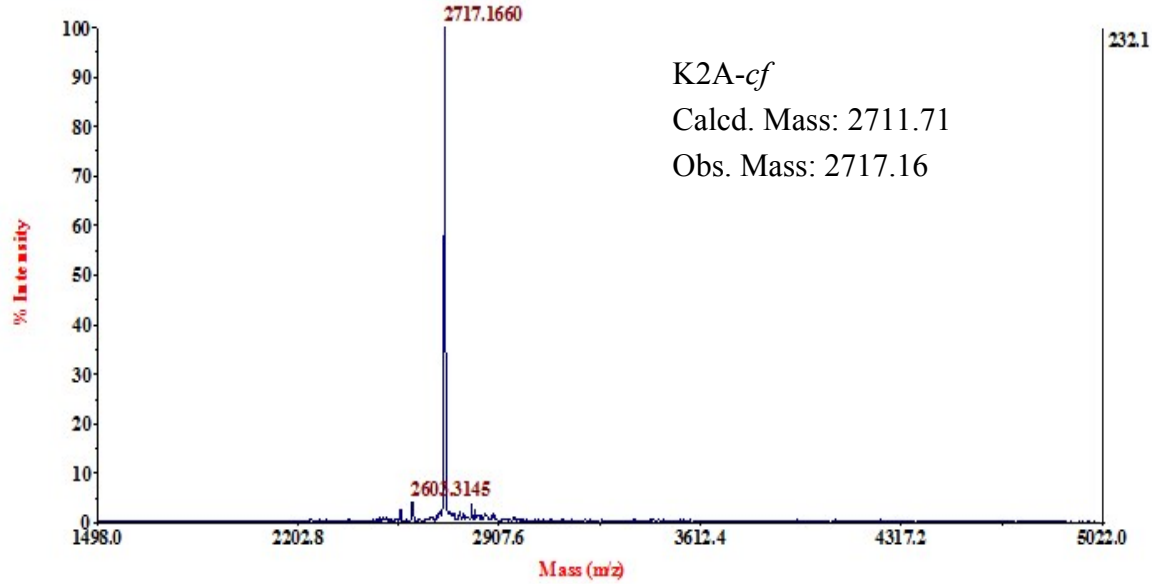


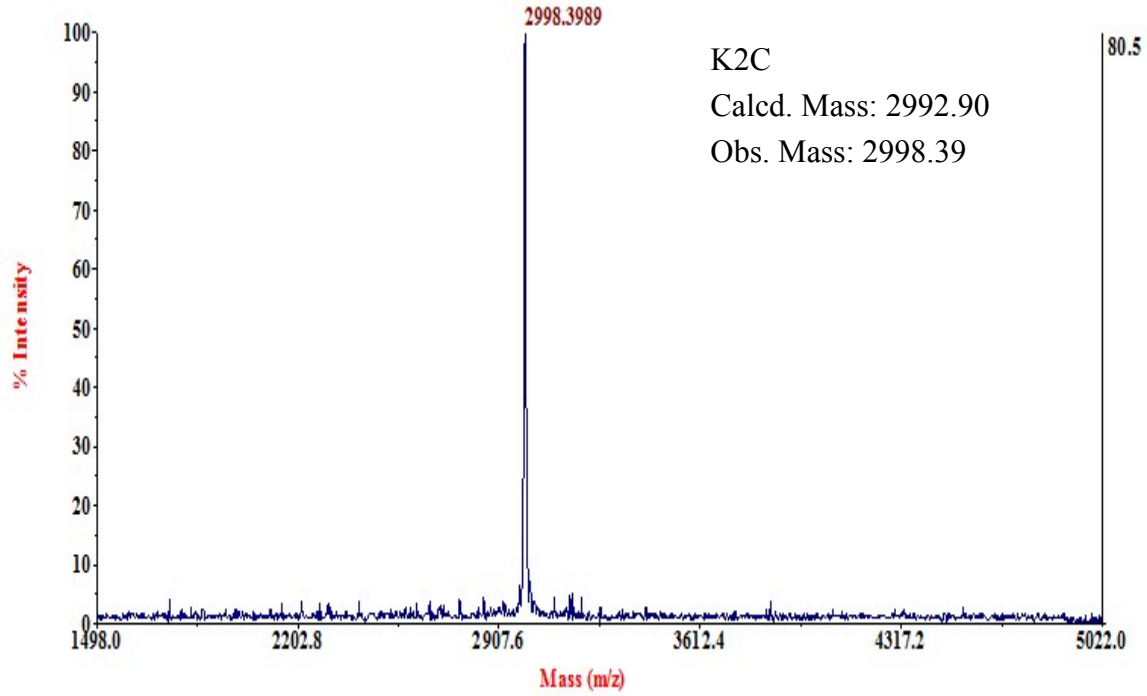
Figure S1. Chemical structures of (A) amide-linked dendrimers, K2A and K2A-*cf* and (B) carbamate-linked dendrimers, K2C and K2C-*cf*.



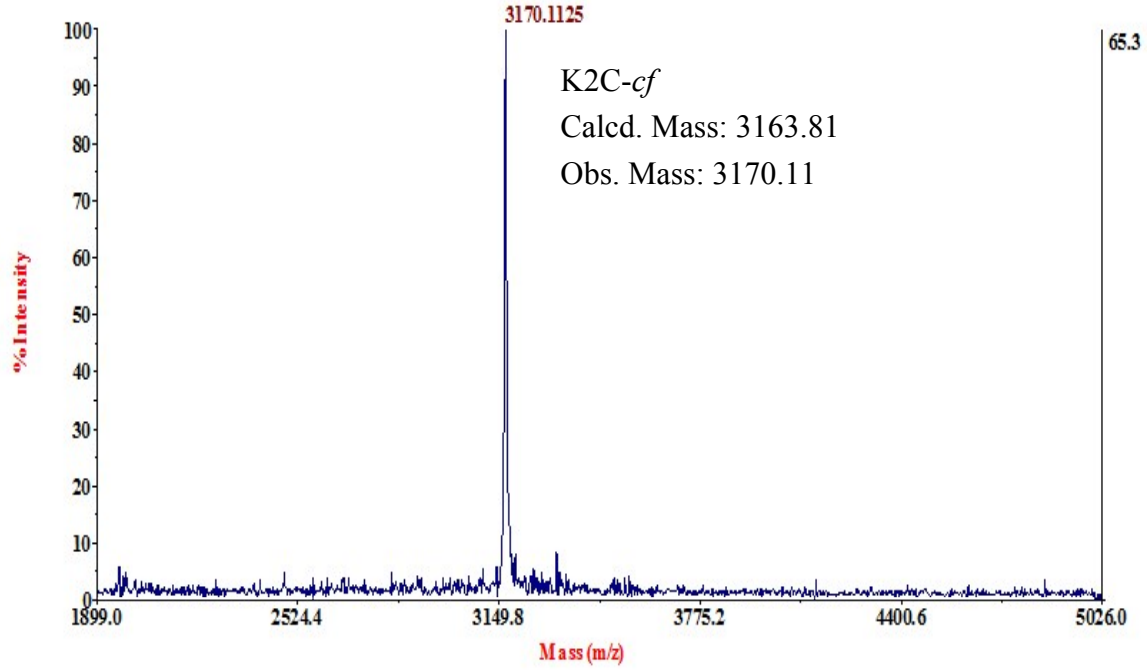
MALDI-ToF mass spectrum of K2A



MALDI-ToF mass spectrum of K2A-*cf*.



MALDI-ToF mass spectrum of K2C.



MALDI-ToF mass spectrum of K2C-*cf*.

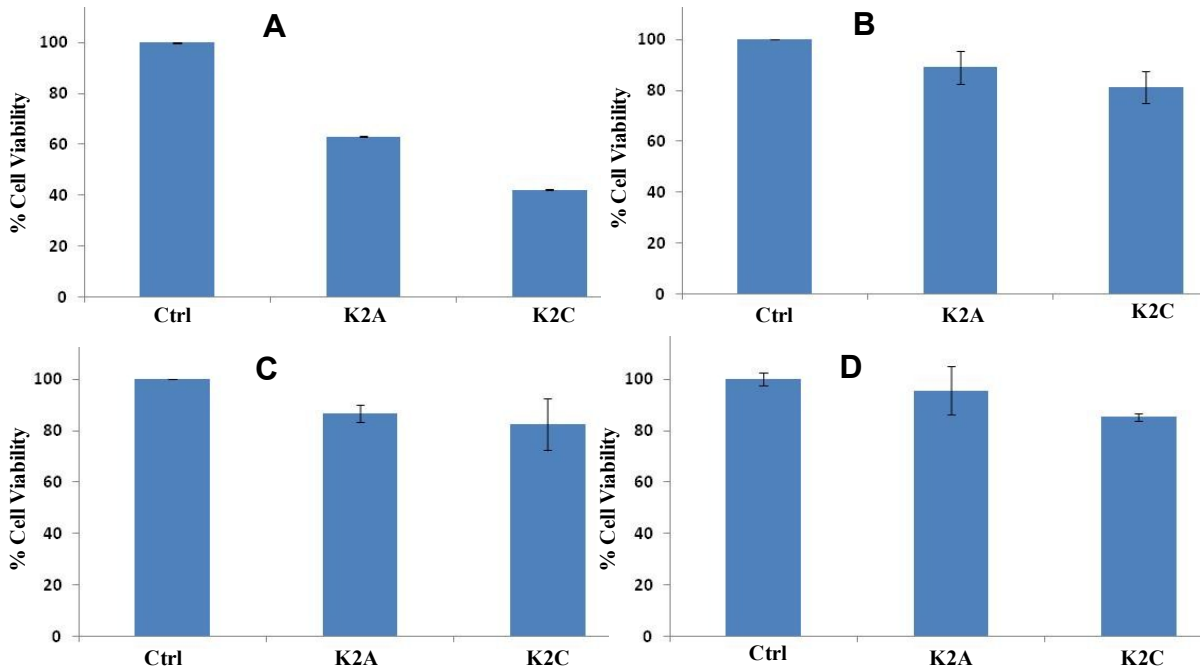


Figure S2. Effect of dendrimers (left panels) and dendrimer-pDNA complexes (right panels) on cell viability after 24 h in CHO-K1 (A and B respectively) and HaCaT cells (C and D respectively).

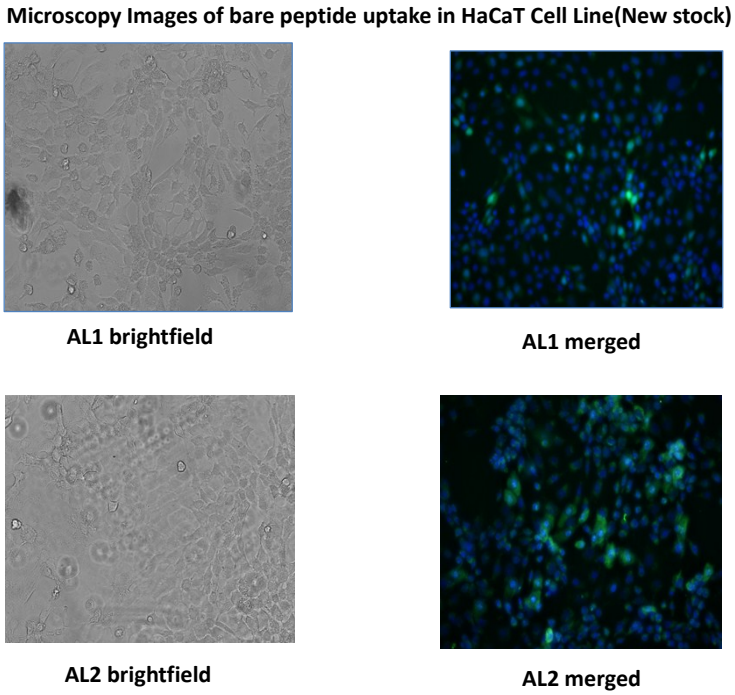


Figure S3. Fluorescence microscopy images of HaCaT cells treated with K2A-*cf* or K2C-*cf* for 4 h. Panel A: brightfield images, panel B: merged.

References

1. Patil, K. M.; Naik, R. J.; Rajpal; Fernandes, M.; Ganguli, M.; Kumar, V. A. *J. Am. Chem. Soc*. **2012**, *134*, 7196−7199.