[Supporting Information]

"Synthesis and Characterization of Head-Tail Type Polycation Block Copolymer as Non-Viral Gene Vector"

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Synthesis and characterization of head-tail type polycation block copolymer

Synthesis of PAMAM dendron.

The PAMAM dendron block was synthesized by repetitive and exhaustive Michael additions with methyl acrylate using tert-butyl N-(2-aminoethyl)carbamate] and subsequent exhaustive amidations with ethylenediamine as follows:

Tert-butyl N-(2-aminoethyl)carbamate] (4.889 g, 3.05×10^{-2} mol) was dissolved in distilled methyl acrylate (300 mL, 3.35 mol) and the mixture was refluxed under an argon atmosphere. After 7 days, the unreacted methyl acrylate was removed under vacuum, and the residue was chromatographed on silica gel using a mixed solvent ($CHCl_3$: MeOH = 10 : 1 vol.) as the eluent. Boc-PAMAM dendron (generation 0.5) was thus obtained.

^{1}H NMR ($CDCl_3$): δ1.45 (s, (C**H**$_3$)$_3$OCO-), 2.44 (t, -C**H**$_2$COO-), 2.53 (t, -C**H**$_2$N(CH$_2$)$_2$-), 2.76 (t, -C**H**$_2$CH$_2$CO-), 3.20 (m, -OCONHC**H**$_2$-), 3.69 (s, -OC**H**$_3$)

The Boc-PAMAM dendron (generation 0.5) (3.38 g, 1.02×10^{-2} mol) was dissolved in distilled methanol (6 mL). The mixture was added to distilled ethylenediamine (300 mL, 4.50 mol) containing sodium cyanide (100 mg, 2.04×10^{-3} mol) and stirred at 40 ^{o}C under an argon atmosphere. After 5 days, the methanol and unreacted ethylenediamine were removed from the reaction mixture under vacuum. The obtained product was purified through a Sephadex LH-20 column using methanol as the eluent. The

Boc-PAMAM dendron (generation 1.0) was thus obtained.

^{1}H NMR (D_2O): δ1.40 (s, (C**H**$_3$)$_3$OCO-), 2.40 (t, -C**H**$_2$CONH-), 2.57 (t, -C**H**$_2$N(CH$_2$)$_2$-), 2.70 (t, -C**H**$_2$CH$_2$CO-), 2.79 (t, -OCONHC**H**$_2$-), 3.22 (t, -CH$_2$CONHC**H**$_2$-)

The Boc-PAMAM dendron (generation 1.0) (2.75 g, 7.08×10^{-3} mol) dissolved in methanol (6 mL) was added to distilled methyl acrylate (450 mL, 5.02 mol). This mixture was stirred at 35 ^{o}C for 4 days under an argon atmosphere. The methanol and unreacted ethylenediamine were removed under vacuum. The obtained product was purified through a silica gel column using the mixed solvent (CHCl3 : MeOH = 10 : 1 vol.) as the eluent and through a Sephadex LH-20 column using methanol as the eluent. The Boc-PAMAM dendron (generation 1.5) was thus obtained.

^{1}H NMR ($CDCl_3$): δ1.43 (s, (C**H**$_3$)$_3$OCO-), 2.35 (t, -C**H**$_2$CONH-), 2.44 (t, -C**H**$_2$COO-), 2.56 (t, -C**H**$_2$N(CH$_2$)$_2$-), 2.76 (m, -C**H**$_2$CH$_2$CO-), 3.19 (m, -OCONHC**H**$_2$-), 3.31 (br, -CH$_2$CONHC**H**$_2$-), 3.68 (s, -OC**H**$_3$)

The Boc-PAMAM dendron (generation 1.5) (2.10 g, 2.87×10^{-3} mol) dissolved in methanol (6 mL) was added to distilled ethylenediamine (300 mL, 4.50 mol) containing sodium cyanide (90 mg, 1.84×10^{-3} mol) and stirred at 45 ^{o}C under an argon atmosphere. After 3 days, the methanol and unreacted ethylenediamine were removed from the reaction mixture under vacuum. The obtained product was purified through a Sephadex LH-20 column using methanol as the eluent. The Boc-PAMAM dendron (generation 2.0) was thus obtained.

^{1}H NMR (D_2O): δ1.44 (s, (C**H**$_3$)$_3$OCO-), 2.45 (m, -C**H**$_2$CONH-), 2.63 (m, -C**H**$_2$N(CH$_2$)$_2$-), 2.74 (t, -C**H**$_2$CH$_2$CO-), 2.83 (m, -C**H**$_2$NH$_2$), 3.16 (m, -OCONHC**H**$_2$-), 3.26 (m, -CH$_2$CONHC**H**$_2$-)

The Boc-PAMAM dendron (generation 2.0) (2.00 g, 2.37×10^{-3} mol) dissolved in methanol (4 mL) was added to distilled methyl acrylate (500 mL, 5.58 mol). This mixture was stirred at 35 ^{o}C for 3 days under an argon atmosphere. The methanol and unreacted ethylenediamine were then removed under vacuum. The obtained product was purified through a Sephadex LH-20 column using methanol as the eluent. The Boc-PAMAM dendron (generation 2.5) was thus obtained.

^{1}H NMR ($CDCl_3$): δ1.42 (s, (C**H**$_3$)$_3$OCO-), 2.41 (m, -C**H**$_2$CONH-), 2.56 (m, -C**H**$_2$N(CH$_2$)$_2$-), 2.78 (m, -C**H**$_2$CH$_2$CO-), 3.16 (br, -OCONHC**H**$_2$-), 3.29 (br, -CH$_2$CONHC**H**$_2$-), 3.65 (s, -OC**H**$_3$)

The Boc-PAMAM dendron (generation 2.5) (3.27 g, 2.13×10^{-3} mol) dissolved in methanol (4 mL) was added to distilled ethylenediamine (200 mL, 3.00 mol) containing sodium cyanide (60 mg, 1.20×10^{-3} mol) and stirred at 45 ^{o}C under an argon atmosphere. After 5 days, the methanol and unreacted ethylenediamine were removed from the reaction mixture under vacuum. The obtained product was purified through a Sephadex LH-20 column using methanol as the eluent. The Boc-PAMAM dendron (generation 3.0) was thus obtained.

^{1}H NMR (D_2O): δ1.43 (s, (C**H**$_3$)$_3$OCO-), 2.44 (m, -C**H**$_2$CONH-), 2.64 (m, -C**H**$_2$N(CH$_2$)$_2$-), 2.75 (t, -C**H**$_2$NH$_2$), 2.84 (m, -C**H**$_2$CH$_2$CO-), 3.17 (t, -OCONHC**H**$_2$-), 3.26 (m, -CH$_2$CONHC**H**$_2$-)

The Boc-PAMAM dendron (generation 3.0) (3.04 g, 1.73×10^{-3} mol) dissolved in methanol (8 mL) was added to distilled methyl acrylate (300 mL, 3.35 mol). This mixture was stirred at 35 ^{o}C for 5 days under an argon atmosphere. The methanol and unreacted ethylenediamine were then removed under vacuum. The obtained product was purified through a Sephadex LH-20 column using methanol as the eluent. The Boc-PAMAM dendron (generation 3.5) was thus obtained.

^{1}H NMR ($CDCl_3$): δ1.42 (s, (C**H**$_3$)$_3$OCO-), 2.37 (m, -C**H**$_2$CONH-), 2.43 (t, -C**H**$_2$COO-), 2.54 (m, -C**H**$_2$N(CH$_2$)$_2$-), 2.79 (br, -C**H**$_2$CH$_2$CO-), 3.16 (br, -OCONHC**H**$_2$-), 3.28 (br, -CH$_2$CONHC**H**$_2$-), 3.67 (s, -OC**H**$_3$)

In order to remove the Boc group, the Boc-PAMAM dendron (generation 3.5) (1.10 g, 3.51×10^{-4} mol) was dissolved in trifluoroacetic acid (11.5 mL) and stirred for 3 hours after cooling with ice. The trifluoroacetic acid was removed under vacuum, and chloroform (41 mL) and triethylamine (14 mL, 0.01 mol) were added to the residue. Distilled water (20 mL) was added to the solution, and the chloroform phase was collected after stirring. The chloroform was then removed under vacuum, and the PAMAM dendron (generation 3.5) having a primary amino group at the root was thus obtained.

^{1}H NMR ($CDCl_3$): d2.37 (br, -C**H**$_2$CONH-), 2.44 (t, -C**H**$_2$COO-), 2.54 (m, -C**H**$_2$N(CH$_2$)$_2$-), 2.76 (br, -C**H**$_2$CH$_2$CO-), 3.29 (br, -CH$_2$CONHC**H**$_2$-), 3.67 (s, -OC**H**$_3$)

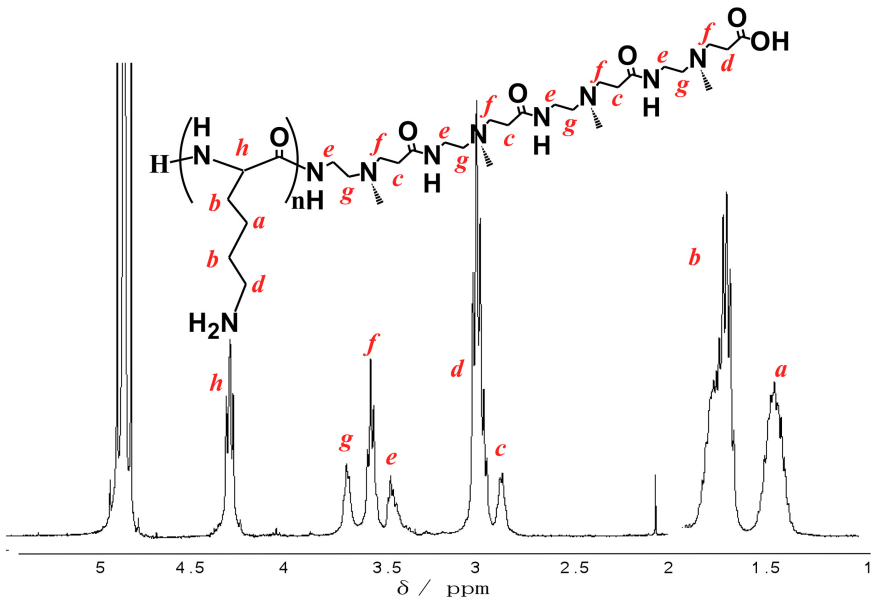
Polymerization of ε-benzyloxycarbonyl L-lysine N-carboxy anhydride [Lys(Z)-NCA] from PAMAM dendron.

The PAMAM dendron (generation 3.5) (1.03 g, 3.39×10^{-4} mol) and Lys(Z)-NCA (5.18 g, 1.69×10^{-2}

mol), which was synthesized by the Fuchs-Farthing method using triphosgene, were separately dissolved in distilled DMF (22 and 30 mL). Both solutions were mixed under an argon atmosphere and stirred at 40 °C. After 20 hours, the reaction mixture was added dropwise to diethyether cooled with ice, and the precipitant was dried under vacuum. The gel permeation chromatography measurement using DMF with 10 mM LiCl as the eluent and the combination column of TSK-gel G4000H_{HR} and G3000H_{HR} was carried out for the obtained product [PAMAM dendron-PLL(Z)]. A unimodal distribution was confirmed, and the polydispersity was determined to be 1.39 based on the calibration curve using PEG standards.

Removal of ε-benzyloxycarbonyl group from PLL(Z) block and methyl group from PAMAM dendron block.

In order to remove the ε-benzyloxycarbonyl group, the PAMAM dendron-PLL(Z) (0.507 g) was dissolved in trifluoroacetic acid (3 mL), and then 30% HBr / AcOH (21 mL) was added and stirred at ambient temperature. After 3 hours, the mixture was added dropwise to diethylether cooled with ice. The precipitant was dissolved in 0.5 M NaOH aq. (10 mL) in order to remove the methyl groups at the periphery of the dendron block. After 40 min of stirring, the reaction solution was neutralized using 1 M HCl aq., and dialyzed against distilled water. Finally, the PAMAM dendron-PLL was obtained by lyophilization of the reaction solution. The ^{1}H NMR spectra in D_2O including DCl of the obtained PAMAM dendron-PLL was as follows.



All peaks were assigned to the PAMAM dendron-PLL. Also, the polymerization degree of the PLL block

was calculated to be 70 from the ratio of the peak area of the PLL block against that of the PAMAM dendron.

Acid-base titration

150 mg of PAMAM dendron-PLL were dissolved in 45 mL of 0.01 M HCl and titrated with 0.01 M NaOH. The titrant was added in 0.1mL quantities, and the change in pH was monitored at 25 ^{o}C.

Dye exclusion assay

Polyplex solutions in Tris-HCl buffer (pH 7.4, 20 mM) prepared at various N/P ratios were adjusted to 10 μg of pDNA / mL with 2.5 μg of ethidium bromide (EtBr) / mL by adding 20 mM Tris-HCl buffer containing EtBr. The ratio of the residual molar concentration of EtBr to that of the base pair of pDNA was 0.033. The sample solutions were incubated overnight under dark conditions. Fluorescence measurements of the sample solutions were carried out using an FP-6500 spectrofluorometer (JASCO, Japan). The excitation and emission wavelengths were 510 and 590 nm, respectively. The relative fluorescence intensity was caluculated by setting the fluorescence intensity of EtBr with pDNA as 100 % and that without pDNA as the background (0 %).

Gel retardation assay

Polyplex solutions in Tris-HCl buffer (pH 7.4, 20 mM) were prepared at various N/P ratios (50 μg of pDNA / mL). The sample solutions were then electrophoresed at 100 V/cm for 30 min with 0.6 w/w% agarose gel in the buffer (40 mM Tris, 20 mM sodium acetate, and 2 mM EDTA buffer containing 1 μg/mL ethidium bromide). The migrated ethidium bromide-stained bands were visualized and the magnitude of the band intensity was analyzed using LAS-1000UVmini (FIJIFILM, Japan).

Tranfection to HaLa cells

The transfection of PLL and the PAMAM dendron-PLL polyplexes to HeLa cells was evaluated as follows:

The cells were seeded in 0.5 mL of DMEM supplemented with 10 % FCS in 24-well culture plates at 5×10^4

cells per well the day before the transfection. The cells were washed with PBS containing 0.36 mM $CaCl_2$ and 0.42 mM $MgCl_2$ [PBS(+)] and then covered with DMEM (1 mL). The polyplexes containing pDNA (1 μg), which were prepared at varying N/P ratios, were gently added to the cells and incubated at 37 ^{o}C for 4 hours. The cells were then rinsed with PBS(+), covered with DMEM containing 10 % FCS, and incubated at 37 ^{o}C. After 40 hours, the cells were lysed by adding 50 μL of the Luc-PGC-50 detergent. A 20 mL aliquot from each dish was used for one luciferase assay using a kit and a Lumat LB9507 luminometer. The protein content of the lysate was measured by Coomassie Protein Assay Reagent using bovine serum albumin as the standard.

Laser-doppler electrophoresis measurement

Laser-doppler electrophoresis measurement was carried out by using ELS-8000 (Otsuka Electronics Co., Ltd.). This instrument measures the particle velocity using a laser light scattering technique. Due to the Doppler effect, the frequency of the scattered laser light is different from that of the original laser beam. The relationship between the frequency shift and the electrophoretic mobility is expressed by the following equation;

$$u = (\nu_d \lambda) / [2 E n \sin(\theta/2)]$$

where ν_d is the Doppler frequency, u is the electrophoretic mobility, E is the electrical field strength, n is the refractive index, λ is the wavelength of the original laser beam, and θ is the scattered angle. From the determined electrophoretic mobility, the zeta-potential (ζ) was calculated by the Smoluchouski equation as follows;

$$\zeta = 4 \pi \eta u / \varepsilon$$

where η is the viscosity of the solution and ε is the dielectric constant of the solvent.