

**Novel Poly(amino ester)s Obtained from Michael Addition Polymerizations of
Trifunctional Amine Monomers with Diacrylates: Safe and Efficient DNA Carriers**

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Experimental Part

Materials and Reagents. 1-(2-aminoethyl)piperazine (AEPZ), 1,4-butanediol diacrylate (BDA) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) were used as received from Aldrich (Milwaukee, WI, USA). After being amplified in *E. coli* and purified according to the supplier's protocol (Qiagen, Hilden, Germany), Plasmid DNA (pCMV-Luc) (kindly donated by Yoshiharu Matsuura, National Institute of Infectious Diseases, Tokyo, Japan) was re-suspended in TE buffer and kept in aliquots at a concentration of 1 mg/ml for use. All other materials and solvents were used as received without further purification.

Characterization. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) were recorded on a Bruker DRX-400 spectrometer. ¹³C NMR was performed using an inverse-gated broadband decoupled

(INVGATE) program. Gel permeation chromatography (GPC) was carried out on a Waters 2690 apparatus with two columns in series (Waters Ultrahydrogel 250, 200) and a Waters 410 refractive index detector using 0.5 M acetic acid/0.5 M sodium acetate as the eluent at a flow rate of 0.75 ml/min. The molecular weights were calibrated against poly(ethylene oxide) standards.

Synthesis of polymer BDA-AEPZ. In a typical polymerization procedure, AEPZ (20 mmol) was dissolved in 15 ml chloroform under ambient temperature, then BDA (20 mmol) was added dropwise followed by washing with 5 ml chloroform into the solution. After being immersed in an oil bath at 45 °C for around 72 h, the solution was precipitated into 400 ml acetone containing 5 ml 10 M HCl. The obtained polymer, BDA-AEPZ, was purified by washing with fresh acetone and dried under vacuum.

The comparison of the ¹³C-NMR of BDA-AEPZ and a model compound, AEPZ, is depicted in Figure S1. The degradation of BDA-AEPZ in aqueous solution was monitored in situ by ¹H-NMR using deuterium water as the solvent and the results are shown in Figure S2. The results verified that the degradation of BDA-AEPZ was realized through the hydrolysis of ester bonds. After the hydrolysis, the peak attributed to the proton attached to the β-carbons in 1,4-butanediol shifted from around 1.50 ppm to 1.37 ppm. Therefore the hydrolysis degree could be monitored by the change in the ratio of the integral intensities of the two peaks as expressed as $I_{1.37}/(I_{1.50} + I_{1.37})$. The hydrolysis profile of BDA-AEPZ is illuminated in Figure S3.

The formation of DNA/polymer complexes and gel retardation analysis. Plasmid cDNA (pCMV-Luc) was diluted to a chosen concentration (usually 0.5-2 μg/μl) using 5% glucose under vortexing. Then an appropriate amount of 0.1 M solution of BDA-AEPZ in 5% glucose was added slowly into DNA solutions. The amount of polymer added was calculated based on a designed weight ratio of polymer/DNA. After the solution was incubated at ambient temperature for 30 min with gently vortexing, the formed polymer/DNA complexes were mixed with a

loading buffer and loaded onto 1% agarose gel containing ethidium bromide. Gel electrophoresis was run at room temperature in TEB buffer at 80 V for 60 min. DNA bands were visualized by a UV (254 nm) illuminator.

Cytotoxicity assay of poly(amino ester). 293 cells were cultured in DMEM supplemented with 10% FCS at 37 °C, 10% CO₂, and 95% relative humidity. For cell viability assay, polymer solutions were prepared in serum supplemented tissue culture medium. pH and osmolarity of the preparations were routinely measured and adjusted to pH 7.4 and 280-320 mosm/kg. The cells (10,000 cells/well) were seeded into 96-well microtiter plates (Nunc, Wiesbaden, Germany). After overnight incubation, the culture medium was replaced with 100 µl serial dilutions of the polymers, and the cells were incubated for another 3 h. 20 µl sterile filtered MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) stock solution in phosphate buffered saline (PBS) was added to each well to reach a final concentration of 0.5 mg MTT/ml. After 4 h, un-reacted dye was removed by aspiration. The formazan crystals were dissolved in 100 µl/well DMSO (BDH laboratory Supplies, England) and measured spectrophotometrically in an ELISA reader (Model 550, Bio-Rad) at a wavelength of 655 nm. The spectrophotometer was calibrated to 0 absorbance using culture medium without cells. The relative cell growth (%) related to control cells containing cell culture medium without polymer was calculated by $[A]_{\text{test}} / [A]_{\text{control}} \times 100$.

Assay of cell transfection efficiency of poly(amino ester). The in vitro transfection efficiency of poly(amino ester) was evaluated in 293 cells using the complexes with pCMV-Luc DNA. Cells were seeded 24 h prior to transfection into a 24-well plate (Becton-Dickinson, Lincoln Park, N.J) at a density of 5×10^4 per well with 0.5 ml of indicated medium. At the time of transfection, the medium in each well was replaced with 300 µl of Opti-MEM. The complexes of polymer/DNA

Supplementary Material (ESI) for Chemical Communications
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were incubated with the cells for 3 h at 37 °C. Then the medium was replaced with 0.5 ml of fresh complete medium and cells were further incubated for 24 h. After the incubation, cells were permeabilized with 100 µl of cell lysis buffer (Promega Co., Wis.) The luciferase activity in cell extracts was measured using a luciferase assay Kit (Promega.Co., Madison, Wis.) on a single-well luminometer (Berthold Lumat LB 9507, Germany) for 10 s. The light units (LU) were normalized against protein concentration in the cell extracts, which was measured using a protein assay kit (Bio-Rad Labs, Hercules, Calif.).

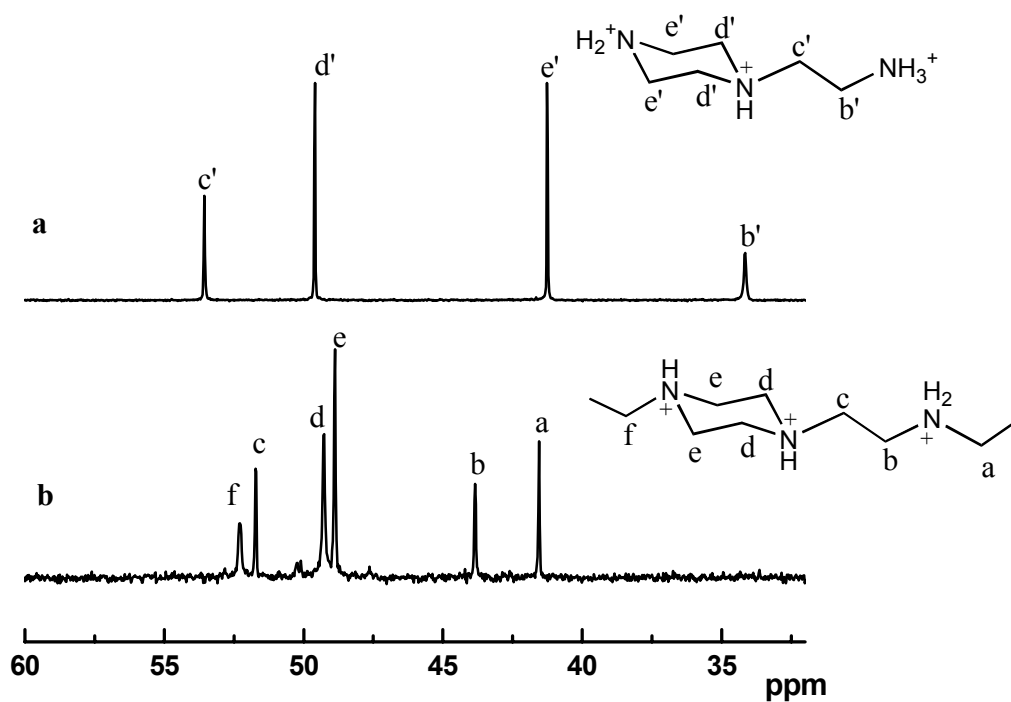


Figure S1. The comparison of the ^{13}C -NMR spectrum of a) model compound, AEPZ, and b) polymer, BDA-AEPZ.

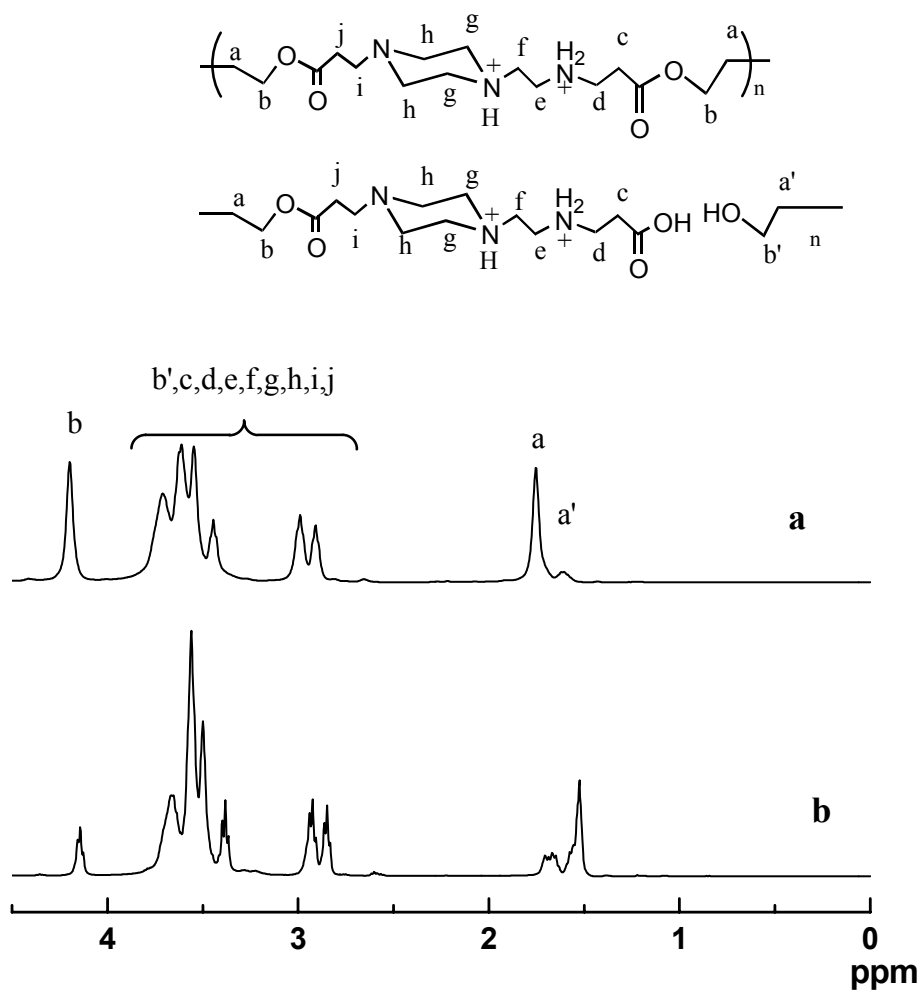


Figure S2. ¹H-NMR spectrum of BDA-AEPZ after being kept in water for a) 1 h and b) 26 days.

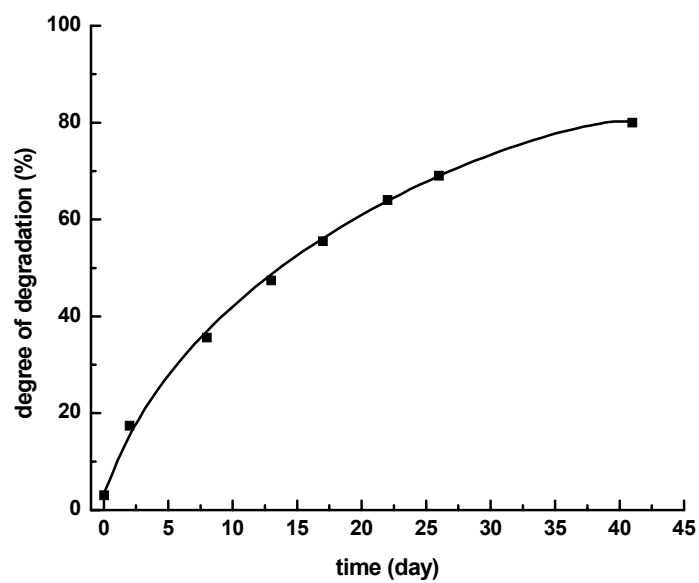


Figure S3. Hydrolysis profile of BDA-AEPZ in aqueous solution