

Electronic Supplementary Information

Thermally Stable and Photoreactive Polylactides by the Terminal Conjugation of Bio-based Caffeic Acid

Tran Hang Thi,^{ab} Michiya Matsusaki,^a and Mitsuru Akashi^{*a}

^a*Department of Applied Chemistry, Graduate School of Engineering, Osaka University,
2-1 Yamada-oka, Suita 565-0871, Japan.*

akashi@chem.eng.osaka-u.ac.jp

^b*Faculty of Technology of Organic Chemistry, College of Chemistry, Ministry of
Industry, Tien Kien, Lam Thao, Phu Tho, Viet Nam*

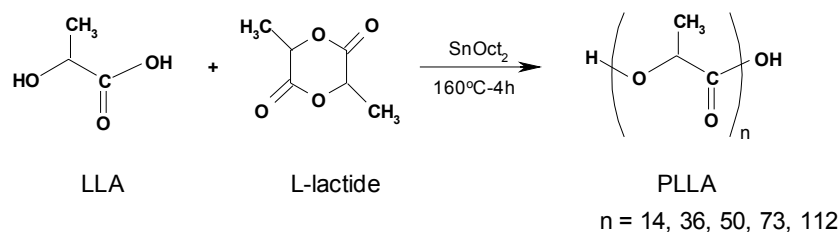
1. Materials
2. Synthesis of polyL-lactides (PLLAs)
 - 2-1. Synthetic method
 - 2-2. Analysis of chemical structure
3. Synthesis of 3,4-diacetoxycinnamic acid (DACA)
4. Synthesis of 3,4-diacetoxycinnamoyl chloride (DACC)
5. Synthesis of DACA-PLLAs
 - 5-1. Analysis of chemical structure
 - 5-2. Solubility of PLLAs and DACA-PLLAs
 - 5-3. Thermal properties of PLLAs and DACA-PLLAs
 - 5-3-1. Analysis of DSC
 - 5-3-2. Analysis of TGA
 - 5-4. Crystallinity of PLLAs and DACA-PLLAs
 - 5-5. Photoreactivity of PLLAs and DACA-PLLAs
 - 5-6. Molecular weight change of PLLAs and DACA-PLLAs by UV irradiation.
 - 5-7. Photo-conjugation of a fluorescent dye onto PLLAs and DACA-PLLA films
 - 5-7-1. Synthesis of DHCA conjugated Alexa fluor555
 - 5-7-2. Photo-conjugation method of the fluorescent dye onto the films

1. Materials

L-lactide (LLA; Tokyo Chemical Industry (TCI)) was recrystallized from ethyl acetate, and then dried *in vacuo* at room temperature for 24 h. L-lactic acid (Tokyo Chemical Industry (TCI)) was used as received. Thionyl chloride (SOCl₂), 3,4-dihydroxycinnamic acid (DHCA), acetic anhydride (Ac₂O) and Alexa fluor 555 cadaverine disodium salt (Wako Pure Chemical Industries, Ltd.) were used without purification.

2. Synthesis of polyL-lactides (PLLAs)

2-1. Synthetic method



Scheme S1. The synthesis of PLLA proceeded via a ring-opening polymerization (ROP) of L-lactide using L-lactic acid as an initiator and tin (II) ethyl hexanoate (SnOct₂) as a catalyst.

PLLA with L-lactide/L-lactic acid at a molar ratio of 10/1 mol/mol was prepared as follows. A total of 14.4 g of L-lactide (100 mmol), 0.9 g of L-lactic acid (10 mmol) and 43 mg of SnOct₂ (0.3 wt% of monomer) was dissolved in 0.43 ml of toluene, and was mixed and mechanically stirred *in vacuo* at room temperature for 1h to remove any air.

Table S1 Synthetic conditions and thermal properties of PLLAs^a

Sample ^b	L-lactide (mmol)	L-Lactic acid (mmol)	M _n ^c	M _w /M _n ^c	LLA unit ^c (Mn/72)	Yield ^d (wt%)	T _m ^e (°C)	T _c ^e (°C)	T ₁₀ ^f (°C)
PLLA14	100	50	1020	2.1	14	43	125	91	241
PLLA36	100	20	2620	2.4	36	74	148	91	231
PLLA50	100	10	3570	3	50	78	153	N	333
PLLA73	100	4	5290	2.8	73	85	156	N	248
PLLA112	100	2	8110	1.4	112	79	168	N	254

^a The polymerization of PLLAs was carried out in the presence of SnOct₂ as a catalyst (0.3 wt% of monomer) with mechanical stirring at 160 °C for 4 h.

^b The numbers in the sample name are the unit numbers of LLA.

^c The molecular weights and number of LLA units were estimated by GPC with polystyrene standards (eluent: THF).

^d The yields represent results after purification.

^e The T_m and T_c were measured by DSC upon a second heating (10 °C min⁻¹), the "T_c" means the crystallization temperature.

^f The 10% weight-loss temperatures, T₁₀, were measured by TGA under nitrogen (20 °C min⁻¹).

"N" means not detected.

Next, the mixture was reacted at 160 °C for 4 h in a nitrogen atmosphere. After the reaction, the product was dissolved in chloroform and purified by re-precipitation over methanol twice. The yield was 78 wt%. The other PLLAs were prepared by an analogous procedure (Table S1). The average molecular weight of the PLLAs was determined by a gel-permeation chromatograph (GPC) (HLC8220GPC system with a α -M and Ac900P columns) in tetrahydrofuran (THF), and then calibrated with polystyrene standards at a flow rate of 0.6 ml/min.

2-2. Analysis of chemical structure

The chemical structure of the PLLA was analyzed by FT-IR (Figure S1) and ^1H -NMR (Figure S2) measurements.

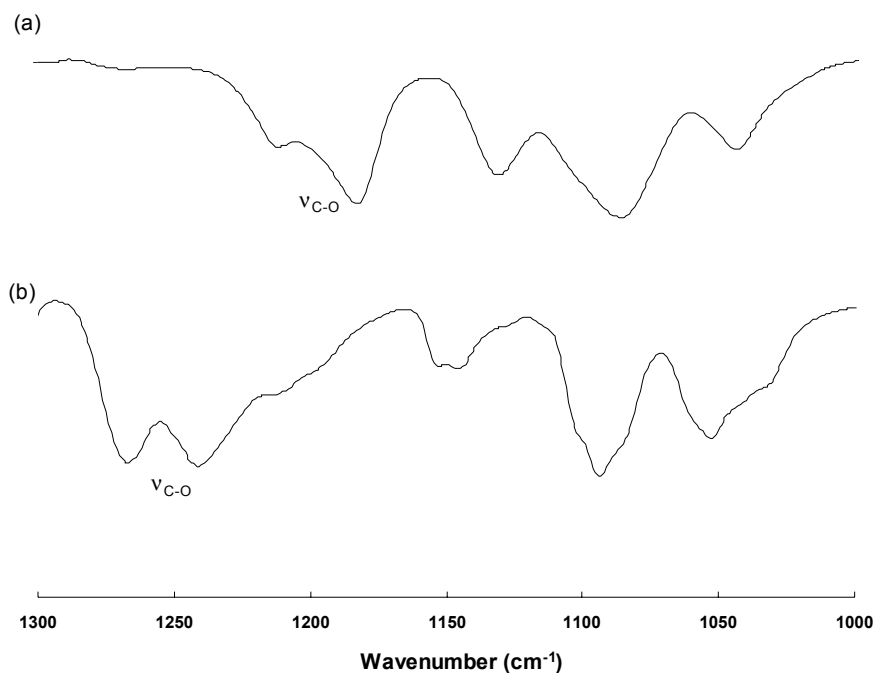


Figure S1. Representative FT-IR spectra of (a) PLLA50 and (b) L-lactide. The FT-IR spectra of the samples were measured at 25 °C on a Perkin Elmer Spectrum One spectropolarimeter between 4000-400 cm⁻¹. The stretching vibration peaks of the C-O groups in L-lactide were observed at 1267 and 1241 cm⁻¹, but were quenched in the FT-IR spectra of PLLAs and shifted to 1211 and 1182 cm⁻¹, thus indicating that the PLLAs were successfully polymerized.

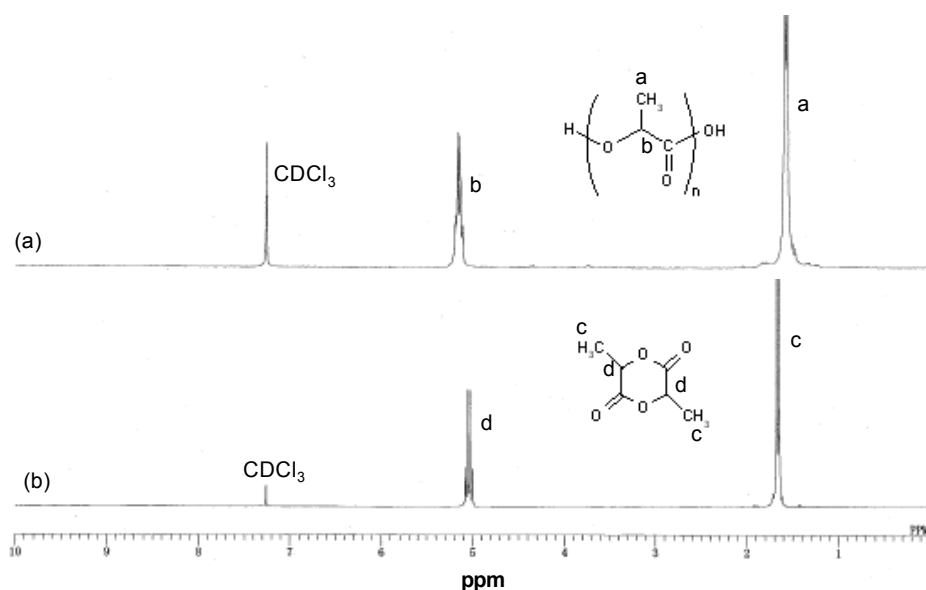
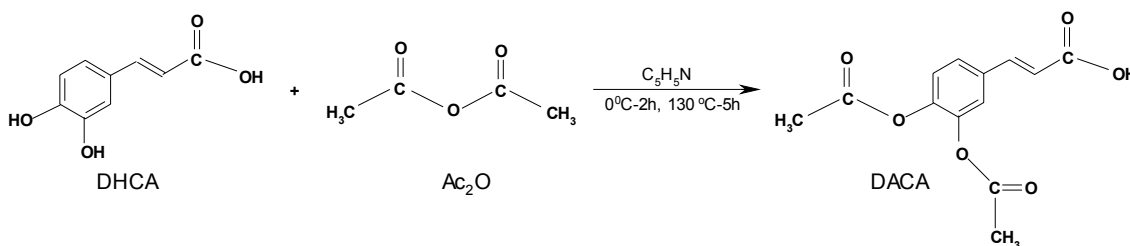


Figure S2. Representative ^1H -NMR spectra of (a) PLLA50 and (b) L-lactide. The ^1H -NMR spectra were measured with a NMR spectrometer (JEOL FX 400) at 400 MHz. The ^1H -NMR chemical shifts in parts per million (ppm) were recorded downfield from 7.26 ppm using CDCl_3 solvent as an internal reference. The protons of the methyl or C-H groups were shifted into a different position between L-lactide and PLLAs, and the peaks in the spectra of PLLAs were broader than in the spectrum of L-lactide, thus indicating that the PLLAs were polymerized.

3. Synthesis of 3,4-diacetoxycinnamic acid (DACA)



Scheme S2. Acetylation of DHCA.

A total of 25 g of DHCA (140 mmol) was dissolved in 50 ml of distilled pyridine at 0 °C for 30 min. Next, 75 ml of acetic anhydride was added and stirred at this temperature for 1.5 h. The mixture was then reacted at 130 °C under reflux for 5 h. After the reaction, the product was recrystallized in toluene, washed by 0.1N HCl twice, and dried at 50 °C *in vacuo* for 24 h. The yield was 83 wt%. The chemical structure of DACA was estimated by ^1H -NMR measurement (Figure S3).

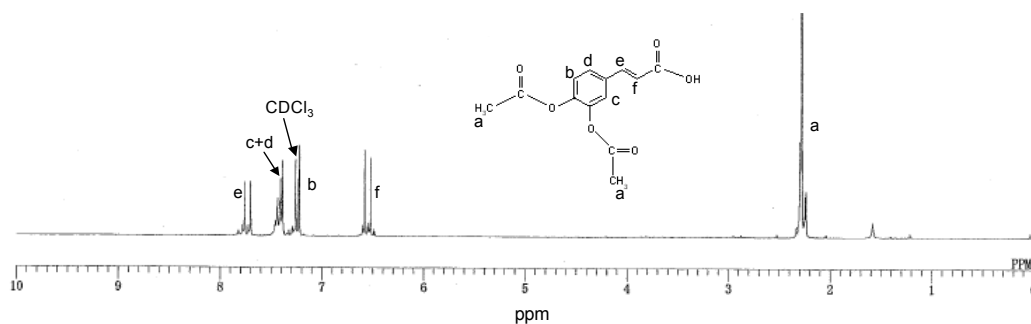
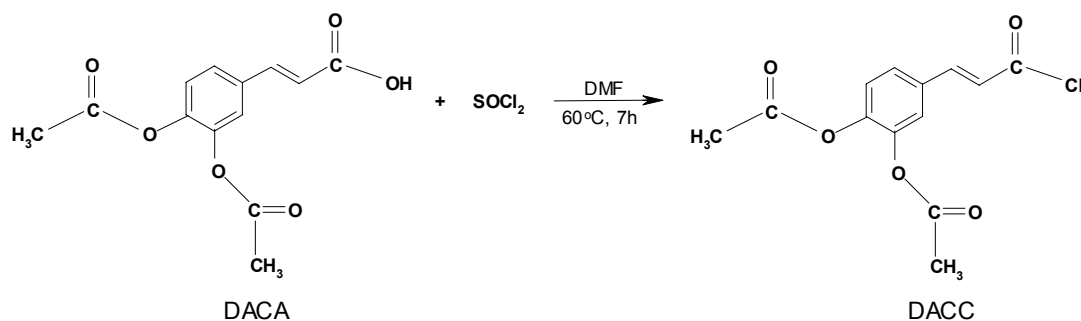


Figure S3. ^1H -NMR spectrum of DACA. The ^1H -NMR spectrum was measured with a NMR spectrometer (JEOL FX 400) at 400 MHz. The ^1H -NMR chemical shifts in parts per million (ppm) were recorded downfield from 7.26 ppm using CDCl_3 solvent as an internal reference. The ^1H -NMR signals showed the presence of methyl groups (2.3 ppm) in the DACA, and the integral value indicated that all OH groups of DHCA were esterified with acetic anhydride.

4. Synthesis of 3,4-diacetoxycinnamoyl chloride (DACC)



Scheme S3. Synthesis of DACC.

DACC was synthesized by 26.4 g of DACA (100 mmol) plus 50 ml of thionyl chloride (SOCl_2) (700 mmol) using 79 mg of DMF as a catalyst in 60 ml of distilled dichloromethane as a solvent at 60°C under reflux for 8 h. After this reaction, the residual SOCl_2 and solvent were removed *in vacuo*. The chemical structure of DACC was analyzed by FT-IR measurement (Figure S4).

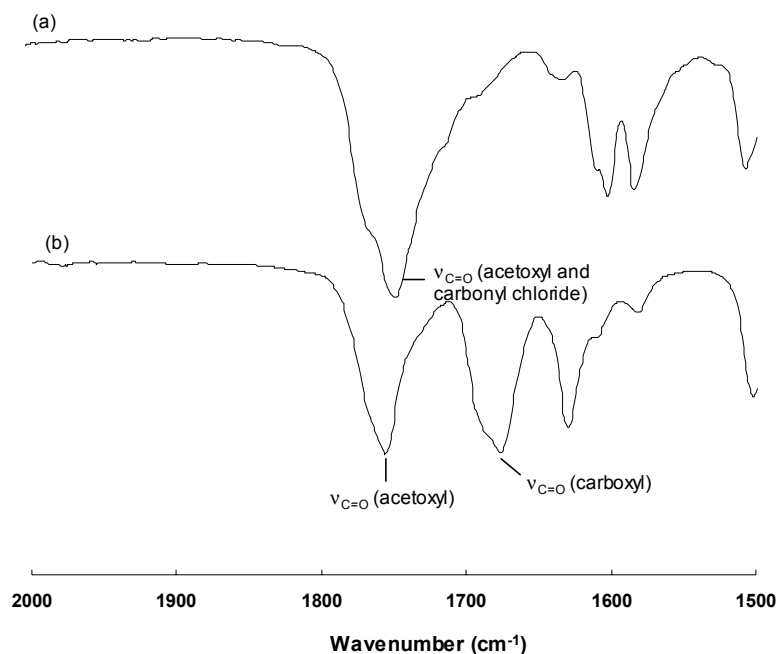


Figure S4. FT-IR spectra of (a) DACC and (b) DACA. The FT-IR spectra of the samples were measured at 25 °C on a Perkin Elmer Spectrum One spectropolarimeter between 4000-400 cm^{-1} . The C=O groups in the carbonyl chloride of DACC appeared at $\nu_{\text{C=O}}$ 1730 cm^{-1} , whereas the C=O groups in the carboxyl was not shifted at 1670 cm^{-1} , indicating that all carboxyl groups in DACA were converted to carbonyl chloride groups.

5. Synthesis of DACA-PLLAs

DACA-PLLA49 was prepared as follows. A total of 11.2 g of PLLA50 (3.7 mmol) was dissolved in 40 ml of distilled dichloromethane and 2 ml of distilled pyridine at 0 °C for 30 min, and 4 g of DACC (14.9 mmol; molar ratio of PLLA50: DACC = 1:4) was then added, stirred at this temperature for 1.5 h, and at room temperature for 24 h. After the reaction, the product was washed in pH 3 HCl solution, and re-precipitated into ethanol by dissolution in DCM twice. The yield was 94 wt%. The other DACA-PLLAs were prepared by an analogous procedure. The average molecular weight of DACA-PLLAs was determined in THF by a GPC (HLC8220GPC system with a α -M and Ac900P columns) calibrated with polystyrene standards at a flow rate of 0.6 ml min^{-1} .

5-1. Analysis of chemical structure

The chemical structures of DACA-PLLAs were analyzed by FT-IR (Figure S5) and $^1\text{H-NMR}$ (Figure S6) measurements.

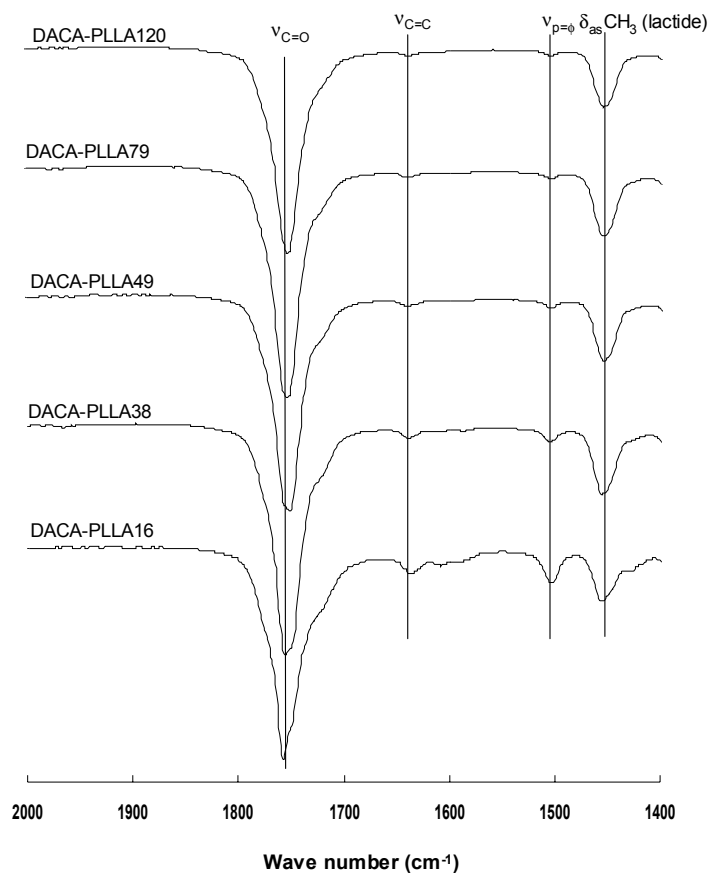


Figure S5. FT-IR spectra of various DACA-PLLAs. The FT-IR spectra of the samples were measured at 25 °C on a Perkin Elmer Spectrum One spectropolarimeter between 4000-400 cm^{-1} . The FT-IR spectra of the DACA-PLLAs confirmed the presence of the vinylene ($\nu_{\text{C}=\text{C}}$ 1640 cm^{-1}) and phenyl groups ($\nu_{\text{p}=\phi}$ 1504 cm^{-1}) of DACA, and the methyl group of PLLAs (δ_{as} 1455 cm^{-1}). In addition, the peak intensity of the vinyl or phenyl group in the DACA-PLLAs versus the peak intensity of the methyl group in PLLAs decreased with a decreasing composition of DACA. These results indicated that DACC was successfully conjugated to the terminal PLLAs.

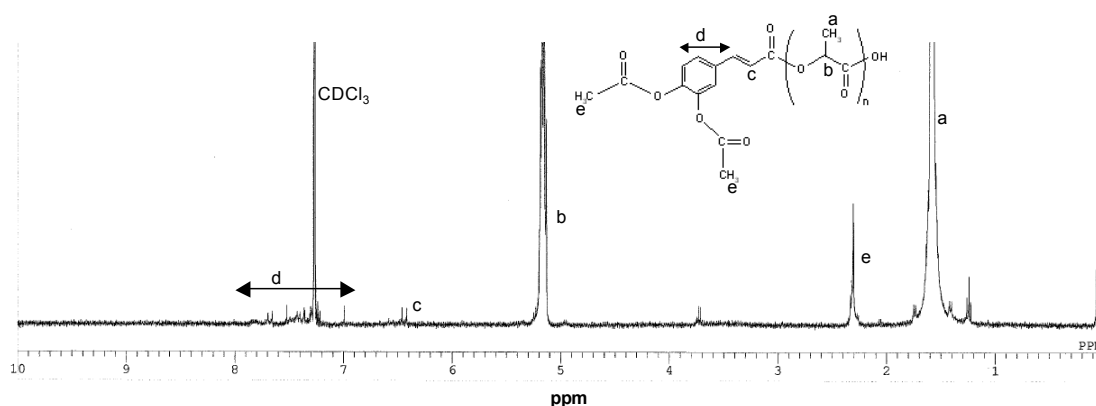


Figure S6. Representative ^1H -NMR spectrum of DACA-PLLA49. The ^1H -NMR spectra of various DACA-PLLAs were measured with a NMR spectrometer (JEOL FX 400) at 400 MHz. The ^1H -NMR chemical shifts in parts per million (ppm) were recorded downfield from 0.00 ppm using tetramethylsilane (TMS) as an internal reference. The solvent was CDCl_3 . The ^1H -NMR signals of DACA-PLLAs showed the presence of DACA and PLLA in the copolymers. The composition of PLLA or DACA in the DACA-PLLAs was calculated by the peak area ratio of the vinylene protons (c) in DACA and the C-H protons (b) in PLLAs.

5-2. Solubility of PLLAs and DACA-PLLAs

Solubility of PLLAs and obtained DACA-PLLAs was evaluated using various solvents. The samples were dissolved in various solvents with 1 mg/mL concentration

Table S2 Solubility of PLLAs and DACA-PLLAs

Solvents	LLA unit in PLLAs					LLA unit in DACA-PLLAs				
	14	36	50	73	112	16	38	49	79	120
Water	×	×	×	×	×	×	×	×	×	×
Methanol	×	×	×	×	×	×	×	×	×	×
Ethanol	×	×	×	×	×	×	×	×	×	×
Acetone	○	×	×	×	×	○	×	×	×	×
Acetonitrile	○	○	○	○	○	○	○	○	○	○
DMF	○	○	○	○	○	○	○	○	○	○
Pyridine	○	○	○	○	○	○	○	○	○	○
NMP	○	○	○	○	○	○	○	○	○	○
DMSO	○	○	○	○	○	○	○	○	○	○
Toluene	×	×	×	×	×	×	×	×	×	×
Hexane	×	×	×	×	×	×	×	×	×	×
DCM	○	○	○	○	○	○	○	○	○	○
THF	○	○	○	○	○	○	○	○	○	○
Chloroform	○	○	○	○	○	○	○	○	○	○

Marks "○" and "×" mean soluble and insoluble, respectively.

for a few hours. As shown in Table S2, solubility of DACA-PLLAs was identical to a PLLA of the same molecular weight.

5-3. Thermal properties of PLLAs and DACA-PLLAs

Thermal properties of PLLAs and DACA-PLLAs were analyzed by DSC measurement (EXSTAR6100, Seiko Instruments Inc.) and TGA measurement (Q500, TA Instrument-Dupont).

5-3-1. Analysis of DSC

The melting temperature (T_m) and crystallization temperature (T_c) of PLLAs and DACA-PLLAs were estimated at heating and cooling rates of $10\text{ }^{\circ}\text{C min}^{-1}$. One sample measurement was carried out over two cycles of heating or cooling. Figure S7 shows DSC curves of all DACA-PLLAs and PLLAs. The T_m of DACA-PLLA was higher than that of PLLA with the same molecular weight.

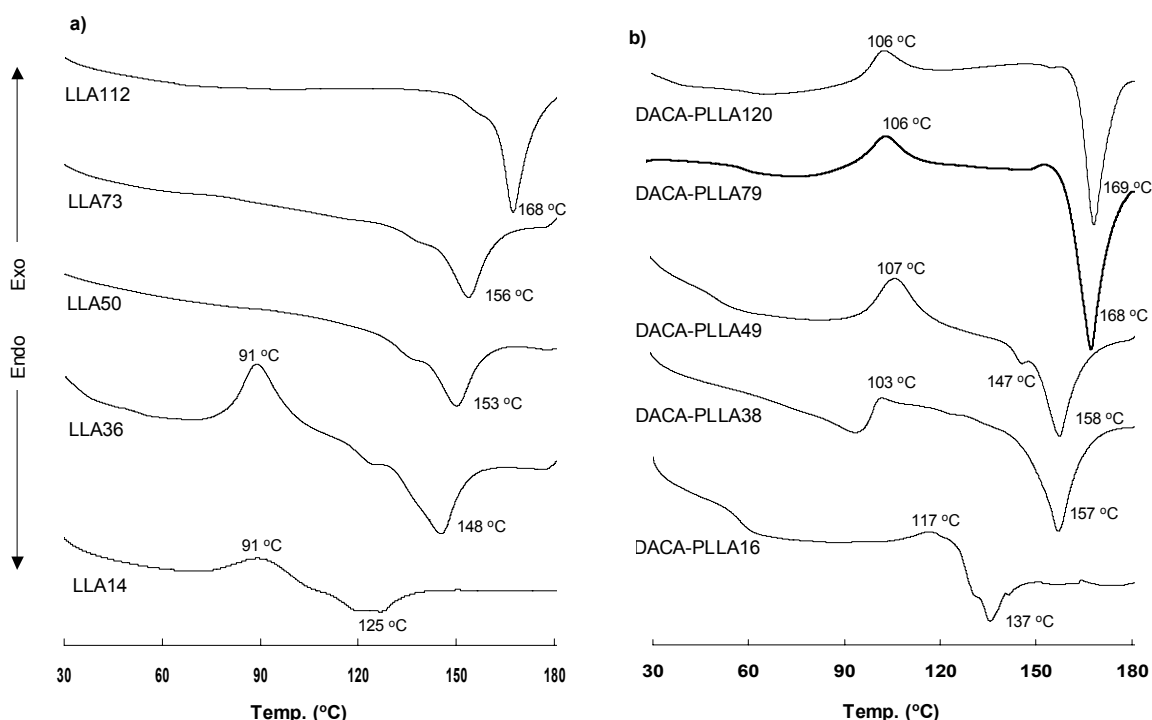


Figure S7. DSC curves upon a second heating of (a) PLLAs and (b) DACA-PLLAs.

5-3-2. Analysis of TGA

The thermal degradation behavior of the samples was observed from the TG curves by heating from 80 °C to 500 °C at a rate of 20 °C min⁻¹ under nitrogen atmosphere with flow rate of 200 ml min⁻¹.

5-4. Crystallinity of PLLAs and DACA-PLLAs

Samples at a concentration of 30 mg ml⁻¹ in chloroform were cast onto glass slides. They were then dried at room temperature for 24 h, and measured after annealing at 100 °C for 1h by WAXD measurement (X-ray diffractometer (RINT UltraX18), equipped with a scintillation counter) using CuK α radiation (40 kV, 200 mA; wavelength = 1.5418 Å). (Figure S8). The crystals of DACA-PLLAs were identical with PLLAs; i.e. DACA did not influence the crystal properties of PLLA.

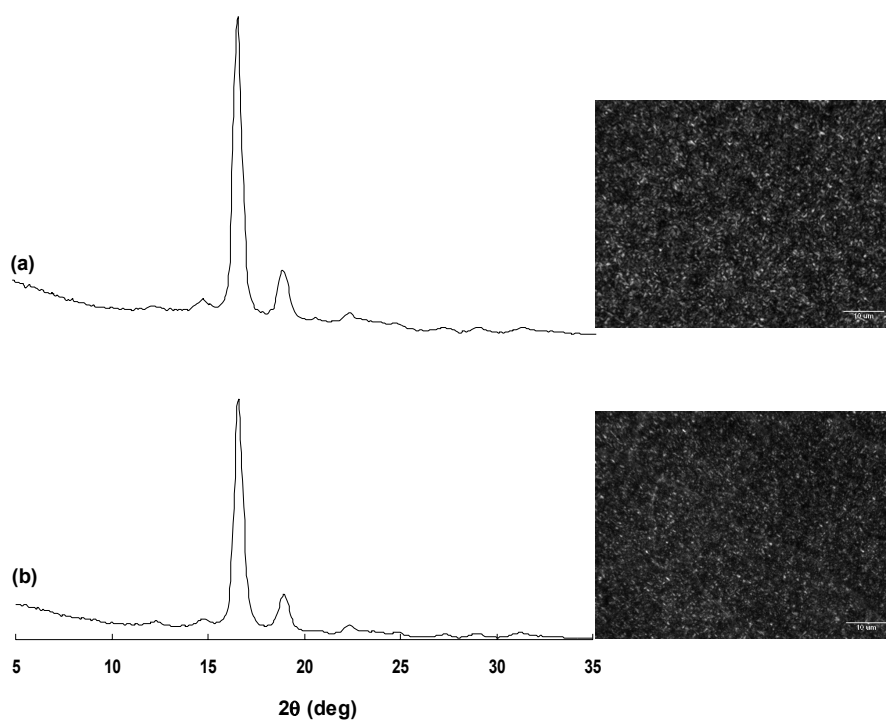


Figure S8. Representative WAXD patterns and differential interference microscopy (DIM) of (a) DACA-PLLA49 and (b) PLLA50.

5-5. Photoreactivity of PLLAs and DACA-PLLAs

DACA-PLLA16, DACA-PLLA38, DACA-PLLA49, DACA-PLLA79 and DACA-PLLA120 were dissolved in DMF to concentrations of 0.08 mg ml⁻¹, 0.18 mg ml⁻¹, 0.25 mg ml⁻¹, 0.4 mg ml⁻¹ and 1.8 mg ml⁻¹, respectively. A glass-filtered

high-pressure Hg Lamp ($\lambda > 280$ nm, 56 mW cm^{-2}) was irradiated. The time course of the photoreaction conversion was monitored by UV-visible absorption spectroscopy of the copolymer solutions using a HITACHI U3010 Spectrophotometer. The cyclobutane formation via UV-irradiation was detected by ^1H -NMR measurement (Figure S9). Small signals appeared in the chemical shift range of 4.0-4.2 ppm upon UV irradiation, supporting the formation of an aliphatic group (cyclobutane) substituted by the benzene ring or carbonyls.

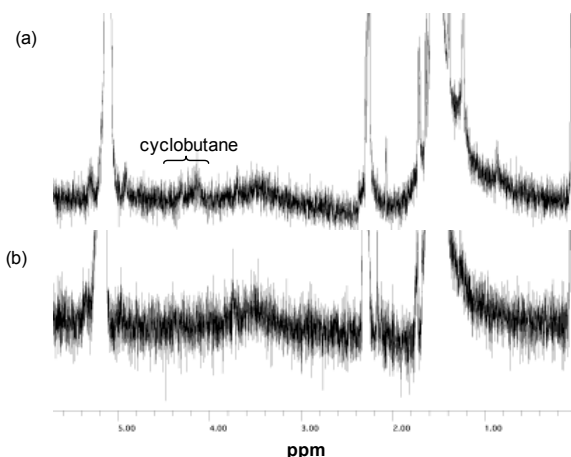


Figure S9. Representative ^1H -NMR spectra of DACA-PLLA49 (a) after UV irradiation for 90 min. and (b) before UV irradiation.

5-6. Molecular weight change of PLLAs and DACA-PLLAs by UV irradiation.

Molecular weight change of samples after UV irradiation at $\lambda > 280$ nm was measured by GPC analysis. Figure S10 shows GPC profiles of DACA-PLLA49 before and after UV irradiation. The elution time of the sample of after UV irradiation shifted to earlier than that before UV irradiation. In other words, a dimerization of the PLLA molecules had occurred upon the photoreaction of terminally-conjugated DACA.

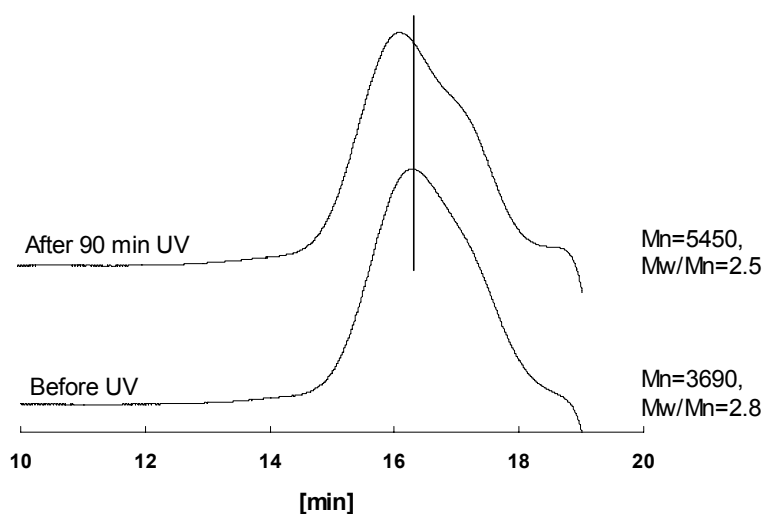
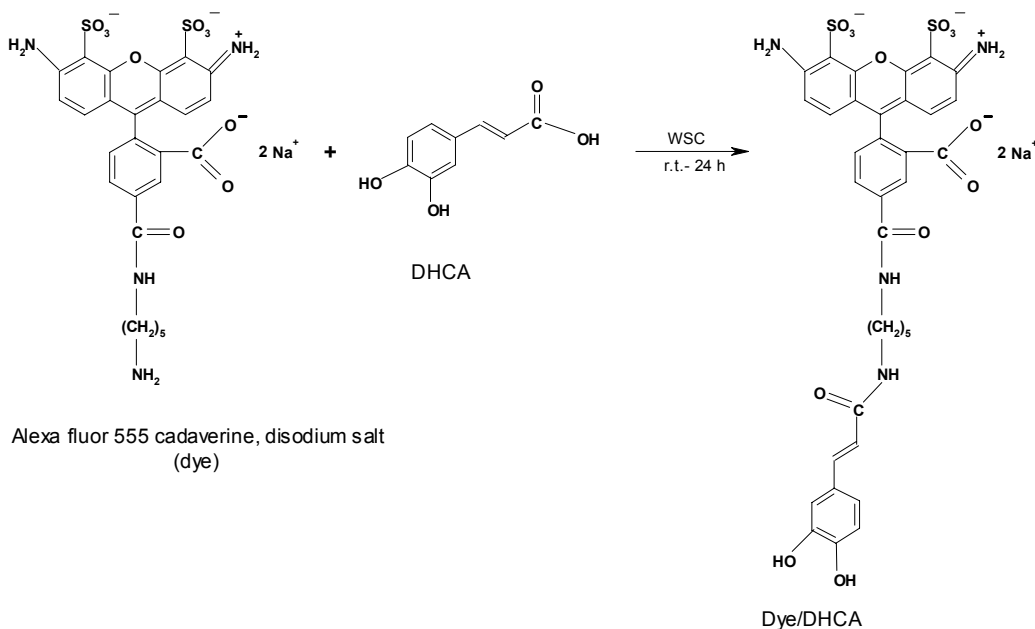


Figure S10. Representative GPC charts of DACA-PLLA49 before and after UV irradiation for 90 min. ($\lambda > 280$ nm, 56 mW cm^{-2}).

5-7. Photo-conjugation of a fluorescent dye onto PLLAs and DACA-PLLA films

5-7-1. Synthesis of DHCA conjugated Alexa fluor555



Scheme S4. Reaction of the dye and DHCA.

A total of 0.72 mg of DHCA (4 μmol) was dissolved in 0.42 ml of PBS buffer (pH = 7.4), and then 0.76 mg of WSC (4 μmol) was added and stirred at room temperature for 10 min. Next, 1 mg of Alexa fluor 555 cadaverine disodium salt (dye) was added, and

the reaction was carried out at room temperature for 24 h. After this reaction, the product was dialyzed in water for 3 days to purify it, and freeze-dried for 2 days.

5-7-2. Photo-conjugation method of the fluorescent dye onto the films

DACA-PLLA38 or PLLA36 films were prepared by casting 100 μl of the samples dissolved in DCM (10 mg ml^{-1}) onto glass slides, and then dried at room temperature for 2 days. Next, 0.5 ml of dye/DHCA dissolved in PBS buffer ($\text{pH} = 7.4$, 0.006 wt%) was dropped onto the films, and exposed to UV light at $\lambda > 280 \text{ nm}$ for 100 min. After the UV irradiation, the films were washed with pure water to remove any unreacted dye/DHCA, and observed by confocal fluorescence scanning microscopy.