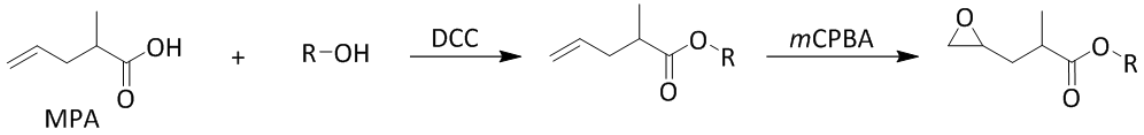
1 Experimental Section

1.1 Materials

The solvents acetone, dichloromethane (DCM), ethanol (EtOH), methanol (MeOH), paraformaldehyde (PFA), tetrahydrofuran (THF) and toluene (*Sigma-Aldrich*) as well as chloroform (*Carl Roth*) were analytical grade and used as received without further purification. Ethyl acetate (*Carl Roth*) was distilled prior to use. *Iso*-Hexane was technical grade and purchased from *Oelfabrik Schmidt*. All commercial reagents were obtained as analytical grade from *Sigma-Aldrich* and used as received. Materials for biological experiments were phosphate buffered saline without Mg/Ca (PBS), penicillin-streptomycin-amphotericin B mixture (PS) and trypsin were purchased from *PanBiotech*. Roswell Park Memorial Institute medium (RPMI) 1640 – Phenol Red was received from *Gibco* and VECTASHIELD® [HardSet antifade mounting medium with 4',6-Diamidin-2-phenylindol (DAPI)] from *Vector Laboratories*. Fetal bovine serum (FBS) was obtained from *Invitrogen*™.

1.2 Synthesis



R = Octadecanol / Cholesterol

Figure S1 Monomer synthesis strategy.

1.2.1 Esterifications

Synthesis of octadecyl 2-methylpent-4-enoate (OctMPA) was carried out by esterifying MPA (5.98 g, 5.00 mL, 41.5 mmol, 1.00 eq.) with octadecanol (11.8 g, 43.6 mmol, 1.05 eq.) using *N,N'*-dicyclohexylcarbodiimide (DCC) (8.50 g, 41.5 mmol, 1.00 eq.) as activation agent in DCM (250 mL) at 0 °C. The reaction was started by adding 4-(dimethylamino)pyridine (DMAP) (0.30 g, 2.49 mmol, 6 mol%) and stirred at room temperature (RT) overnight (Figure S1). The precipitate was filtered off and the filtrate was stirred with a saturated aqueous solution of $NaHCO_3$ (200 mL) for 1 h. The aqueous layers were extracted with DCM (8 × 25 mL). The combined organic layers were washed with saturated

aqueous solution of NaCl (1 × 50 mL) and were dried over Na_2SO_4 (1 × 50 mL). After removal of the solvent under reduced pressure OctMPA (15.2 g, 41.0 mmol, 99 %) was obtained as yellow oil.

Cholesteryl 2-methylpent-4-enoate (ChMPA), a white solid, was synthesized under identical conditions using cholesterol (16.8 g, 43.6 mmol, 1.05 eq.) as the alcohol component with a yield of 82 % (16.4 g, 34.0 mmol) after column chromatography (8.0 × 20 cm, chloroform).

1.2.2 Epoxidations

Synthesis of octadecyl 2-methyl-3-(oxiran-2-yl)propanoate (OctMOP) was carried out by dissolving OctMPA (10.0 g, 27.3 mmol, 1.0 eq.) in DCM (300 mL) and the subsequent addition of 3-chloroperbenzoic acid (*m*CPBA) (7.40 g, 30.1 mmol, 77 wt%, 1.10 eq.) in portions over the course of 45 min at 0 °C (Figure S1). The reaction mixture was stirred overnight at 0 °C and terminated by adding ice-cold aqueous NaOH (1M, 100 mL) solution. The precipitate was filtered off and the organic and aqueous layers were separated after which the aqueous layer was extracted with DCM (3 × 100 mL). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (1 × 300 mL), saturated aqueous NaCl (1 × 300 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure. Column chromatography (8.0 × 20 cm, 9:1 *iso*-Hexane:ethyl acetate) afforded OctMOP (4.0 g, 10.5 mmol, 38 %) as a colorless oil.

5α,6α-epoxycholesteryl 2-methyl-3-(oxiran-2-yl)propanoate (ChMOP) was synthesized under identical conditions using *m*CPBA (12.9 g, 56.0 mmol, 77 wt%, 2.05 eq.) as the epoxidation agent and ChMPA (13.2 g, 27.3 mmol, 1.0 eq.) as starting material. The purified product was obtained after column chromatography (8.0 × 20 cm, 8.5:1.5 *iso*-Hexane:ethyl acetate) as white solid substance with a yield of 40 % (11.5 g, 22.4 mmol).

1.2.3 Copolymers

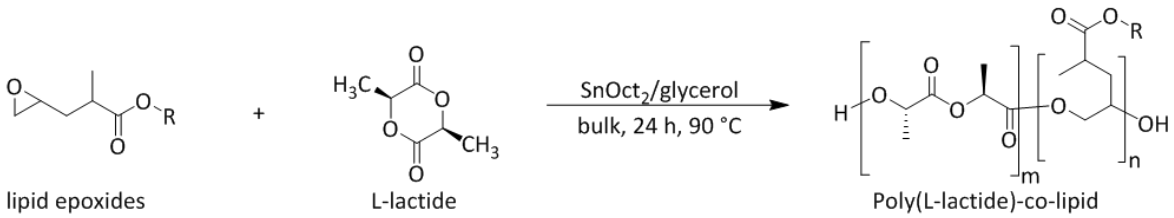


Figure S2 Copolymerization scheme.

Table S1 Initial weights of copolymerization agents.

comonomer	feed ratio	L-lactide	glycerol	$SnOct_2$
OctMOP	10 mol%	1.68 g, 11.7 mmol	23.9 mg, 0.26 mmol	158 mg, 0.39 mmol
0.50 g, 1.30 mmol	20 mol%	0.75 g, 5.20 mmol	12.0 mg, 0.13 mmol	79.0 mg, 0.20 mmol
	30 mol%	0.44 g, 3.03 mmol	8.00 mg, 0.09 mmol	52.6 mg, 0.13 mmol
ChMOP	10 mol%	1.26 g, 8.73 mmol	17.9 mg, 0.19 mmol	118 mg, 0.29 mmol
0.50 g, 0.97 mmol	20 mol%	0.56 g, 3.88 mmol	8.93 mg, 0.10 mmol	58.9 mg, 0.15 mmol
	30 mol%	0.33 g, 2.26 mmol	5.95 mg, 0.06 mmol	39.2 mg, 0.10 mmol

L-lactide and OctMOP or ChMOP (Table S1) were dissolved separately in DCM (10 mL) and transferred into a glass vial followed by stirring at 90 °C. Glycerol (0.02 mol%) and tin(II) 2-ethylhexanoate ($SnOct_2$) (0.03 mol%) were added and stirring was continued for 24 h (Figure S2). The polymerization was terminated by adding a small amount of EtOH, followed by dissolving the reaction mixture in DCM (2 mL) and subsequent precipitation in ice-cold *iso*-Hexane and EtOH (100 mL), respectively. After drying under reduced pressure to constant weight poly(L-lactide-co-octadecyl 2-methyl-3-(oxiran-2-yl)propanoate) (PLA-co-C_{18}) and poly(L-lactide-co-cholesteryl 2-methyl-3-(oxiran-2 yl)propanoate) (PLA-co Ch) were obtained with yields between 30 and 40 wt%.

1.2.4 Nanoparticles

The NPs were synthesized using the nanoprecipitation method.[4] PLA NPs served as a control. Copolymers (0.5 mg) were dissolved in a mixture of THF/acetone (1:1, 1.0 mL) and then Milli-Q water (1.0 mL) was added rapidly using a graduated micropipette. The resulting colloidal suspensions were dialyzed against deionized water for at least 3h. Dynamic light scattering (DLS) was used to determine the size distribution profile of nanoparticles in a colloidal suspension at RT. Particle size and zeta

potential were analyzed with a Beckman Coulter *Delsa^{TM} Nano Submicron* device measuring every sample three times using 70 data points for size and 50 data points for zeta potential determination.

1.3 Cellular Uptake of the Nanoparticles

1.3.1 Dye Labeling for NP Uptake Studies

To investigate the cellular uptake of the synthesized NPs (0.5 mg mL^{-1}) with HeLa cells they were labeled with the lipophilic dye Rhodamine B octadecyl ester perchlorate (RhC_{18}). RhC_{18} was non-covalently incorporated into the particles during the NP preparation by dissolving it in the organic solvent used in NP-preparation (0.5 wt% rel. to PLA-copolymer).

1.3.2 Cell Culture and NP Uptake Studies

HeLa cells were cultured in a humidified incubator at 5 % CO_2, 37 °C using RPMI medium supplemented with 10 % FBS and 1 % PS. Passages from number 3 to 6 were used. To study the cellular uptake of NP via fluorescence microscopy HeLa cells were seeded in 8 well chamber slides at a density of 20,000 cells per well and incubated overnight. Media was aspirated and the cells were washed with PBS and incubated with RhC_{18} labeled NP (10 µg mL^{-1}) in RPMI. After 5h of incubation, the medium was aspirated and the cells were washed three times with PBS for subsequent microscopy analysis. For Flow Cytometry analysis HeLa cells were seeded in 24 chamber slides at a density of 100,000 cells per well and grown overnight. Media was aspirated, subsequently washed with PBS and the cells were incubated with dye-labeled NPs (10 µg mL^{-1}) for 5h.

1.3.3 Fluorescence Microscopy

Following the incubation with dye-labeled NP the cells were washed three times with PBS, fixed (PBS, 4 vol% PFA) and washed again thoroughly three times with PBS. The 8-well chamber slide was mounted onto a cover slip using DAPI mounting medium for staining the cell nucleus and the images were acquired using *Zeiss Cell Observer Z1* fluorescent microscope using Zeiss filter set 43. Images processed and analyzed with *Axio Vision Rel. 4.8* software.

1.3.4 Flow Cytometry

After 5h incubation cells were washed three times with PBS, trypsinized and resuspended in a buffer (PBS, 2 vol% FBS, 1 mL). Flow cytometry was measured using a *LSRFortessa* analyzer from *Becton Dickinson* (Core Facility – Department of Medicine I, Hematology, Oncology and Stem Cell Transplantation) and analyzed with *Flowing Software 2.5.1* by Perttu Terho. 10000 events were recorded per sample and samples were prepared in triplicates.

1.4 Methods

1.4.1 Attenuated Total Reflection Infrared Spectroscopy (ATR-IR)

The spectra were measured using a *Vector 22 FT-IR* spectrometer (*Bruker*) equipped with a *Specac's Golden Gate™* ATR accessory, including a heated top-plate *MkII* and analyzed with *Opus 5.5* software from *Bruker*. Films of NP were prepared by overnight evaporation of NP suspensions on square glass cover slips which were cleaned prior to use by immersing in toluene, acetone and EtOH for 10 min each under ultrasonification and then dried for 24 h at 60 °C. The ensuing film was pulled off the glass surface and then placed on the diamond top plate through an area of 4 mm^{-2}. Using ATR-IR spectroscopy an approximate penetration depth of 100 nm is expected.

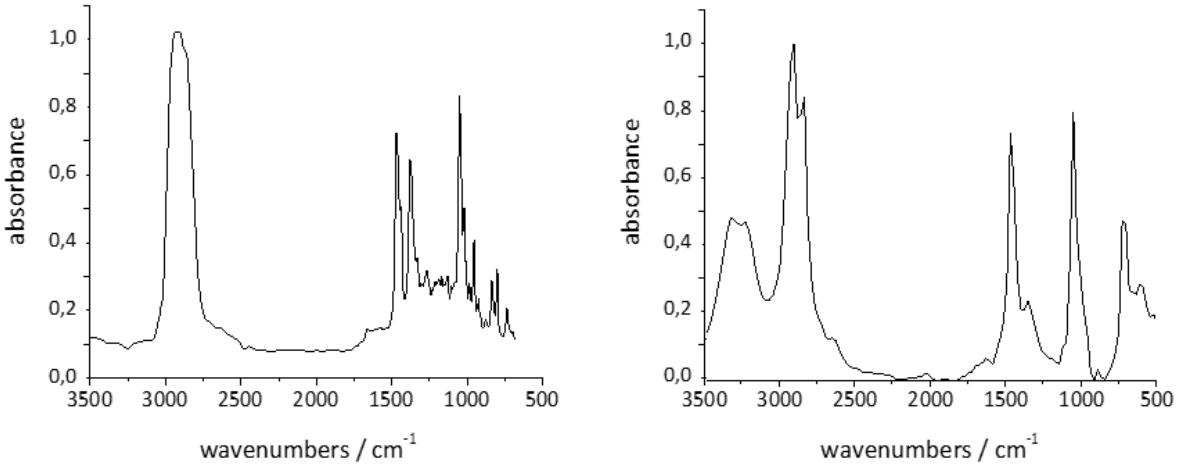


Figure S3 ATR-IR spectra of cholesterol (left) and octadecanol (right).

1.4.2 Static Contact Angle Measurement (CAM)

Static water contact angles were measured with a *KSV-CAM100* device and the droplet shape was analyzed using the corresponding *CAM200* software. The copolymer (1.0 wt%) was dissolved in DCM and spin coated onto cleaned cover glass (cleaning method described above). The integrity of the films were established via visual inspection and then a droplet of 30 µL of distilled deionized water was placed on the surface using a microsyringe and the contact angle was measured immediately. A minimum of three independent samples were analyzed at three random locations per sample. The values are presented as average ± SD.

1.4.3 Differential Scanning Calorimetry (DSC)

Polymer samples (10 – 15 mg) were sealed in aluminum pans, the thermal transitions were recorded under nitrogen on a *Perkin Elmer Pyris 1 DSC* and analyzed with *Pyris Manager Version 8.0.0.0172*. The samples were heated up from -50 °C to 150 °C with rates of 10 K min^{-1}.

1.4.4 Elemental Analysis (EA)

EA was carried out on a *VarioEL* device by Elementaranalysensysteme GmbH (Institute for Organic Chemistry, University of Freiburg).

OctMPA (Found: C, 77.39; H, 12.91; N, 0.69[a]. $C_{24}H_{46}O_2$ requires C, 78.63; H, 12.65%)

OctMOP (Found: C, 74.3; H, 12.11. $C_{24}H_{46}O_3$ requires C, 75.34; H, 12.12%)

ChMPA (Found: C, 81.55; H, 10.98. $C_{33}H_{54}O_2$ requires C, 82.1; H, 11.27%)

ChMOP (Found: C, 76.54; H, 10.72. $C_{33}H_{54}O_4$ requires C, 77.0; H, 10.57%)

1.4.5 Gel Permeation Chromatography (GPC)

A *Knaur A22* GPC solvent/sample module, using a column combination A32 [(PSS-SDVB)-column] and polystyrene [molecular weight (MW) 10^3-10^6 g mol^{-1}] standards, was used to determine relative MW and polydispersity of the synthesized polymers. Samples were prepared in chloroform at a concentration of 4 mg mL^{-1} and filtered through a 0.45 µm filter prior to injection and eluted using chloroform at a flow rate of 1 ml min^{-1} at a temperature of 30 °C. The elugram was analyzed using *PSS WinGPC Unity, Build 5403*.

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[a]Nitrogen was detected because of incomplete removal of 1,3-dicyclohexylurea.

1.4.6 Nuclear Magnetic Resonance (NMR)

^{1}H-NMR spectra were recorded with a *Bruker ARX 300* spectrometer at RT using d-chloroform as the solvent. The chemical shifts in ppm are indicated relative to chloroform (δH = 7.26 ppm). The spectra were analyzed with the *Bruker Topspin 3.0* software.

OctMPA δH(300 MHz, $CDCl_3$, Me_4Si) 0.88 (3 H, br s, CH_3^i), 1.15 (3 H, d, CH_3^e), 1.26 (30H, br s, $(CH_2)_{15}^h$), 1.62 (2 H, br s, CH_2^g), 2.30 (2 H, dddd, CH_2^c), 2.50 (1 H, tq, CH^d), 4.06 (2 H, t, CH_2^f), 5.05 (2 H, br s, CH_2^a), 5.75 (1 H, ddt, CH^b).

OctMOP δH(300 MHz, $CDCl_3$, Me_4Si) 0.88 (3 H, br s, CH_3^i), 1.23 (3 H, d, CH_3^e), 1.26 (30 H, br s, $(CH_2)_{15}^h$), 1.60 (2 H, br s, CH_2^g), 1.95 (2 H, br s, CH_2^c), 2.47 (1 H, br s, CH^d), 2.65 (1 H, br s, CH^a), 2.75 (1 H, br s, CH^a), 2.96 (1 H, br s, CH_2^b), 4.06 (2 H, t, CH_2^f).

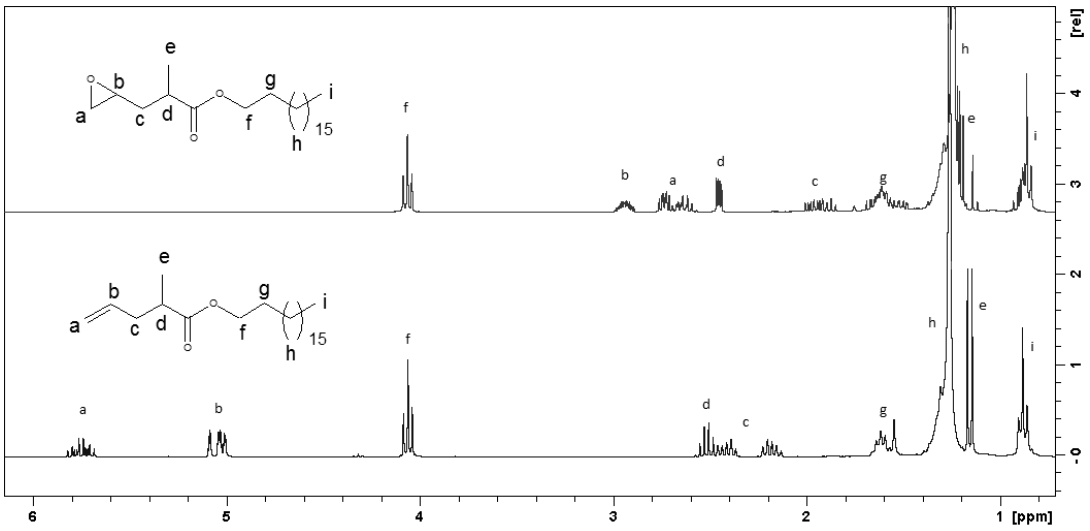

Figure S4 NMR-Data OctMPA (bottom) and OctMOP (top).

ChMPA δH(300 MHz, $CDCl_3$, Me_4Si) 0.68 (3 H, s, CH_3^i), 0.86-2.05 (25 H, br s, Steroid), 1.18 (3 H, d, CH_3^e), 2.17 (1 H, br s, CH_2^c), 2.30 (2 H, d, CH_2^g), 2.39 (1 H, br s, CH_2^c), 2.47 (1 H, br s, CH^d), 4.61 (1 H, br s, CH^f), 5.04 (2 H, br s, CH_2^a), 5.37 (1 H, br s, CH^h), 5.75 (1 H, br s, CH^b).

ChMOP δH(300 MHz, $CDCl_3$, Me_4Si) 0.62 (3 H, d, CH_3^i), 0.86-2.05 (25 H, br s, Steroid), 1.20 (3H, d, CH_3^e), 1.90-2.22 [(2 H, br s, CH_2^g), (2 H, br s, CH_2^c)], 2.47 (1 H, br s, CH^d), 2.60 (1 H, br s, CH^a), 2.75 (1 H, br s, CH^a), 2.89 (1 H, d, CH^{h1}), 2.93 (1 H, d, CH^b), 3.08 (1 H, d, CH^{h2}), 4.79 (1 H, d, CH^{f2}), 4.97 (1 H, br s, CH^{f1}).

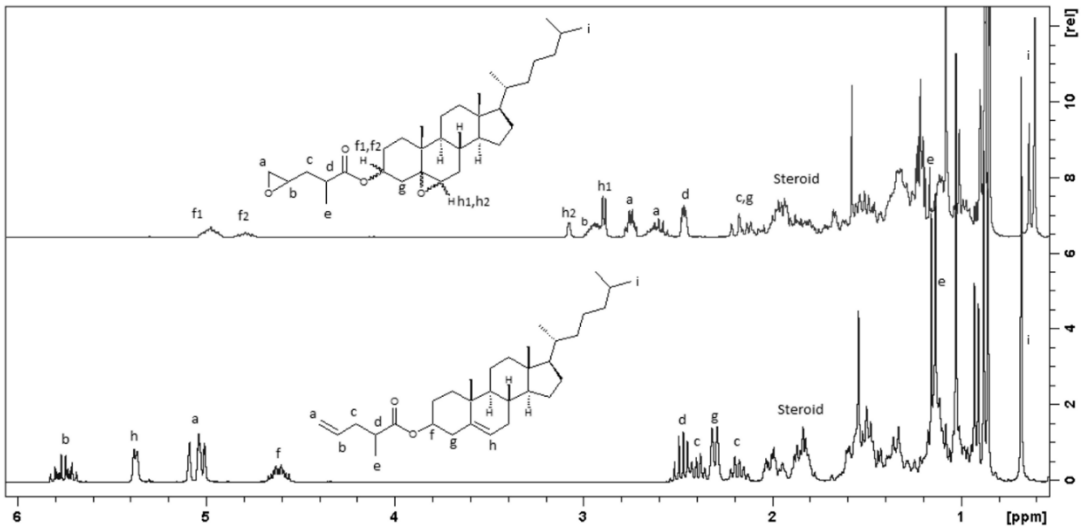

Figure S5 NMR-Data ChMPA (bottom) and ChMOP (top).

PLA-co-C_{18} δH(300 MHz, $CDCl_3$, Me_4Si) 0.87 (3 H, br s, CH_3^g), 1.25 (30 H, br s, $(CH_2)_{15}^f$), 1.49 (3 H, br s, CH_3^e), 1.57 (3 H, d, CH_3^a), 4.12 (1 H, br s, CH^d), 4.34 (2 H, br s, CH_2^c), 5.16 (1 H, q, CH^b).

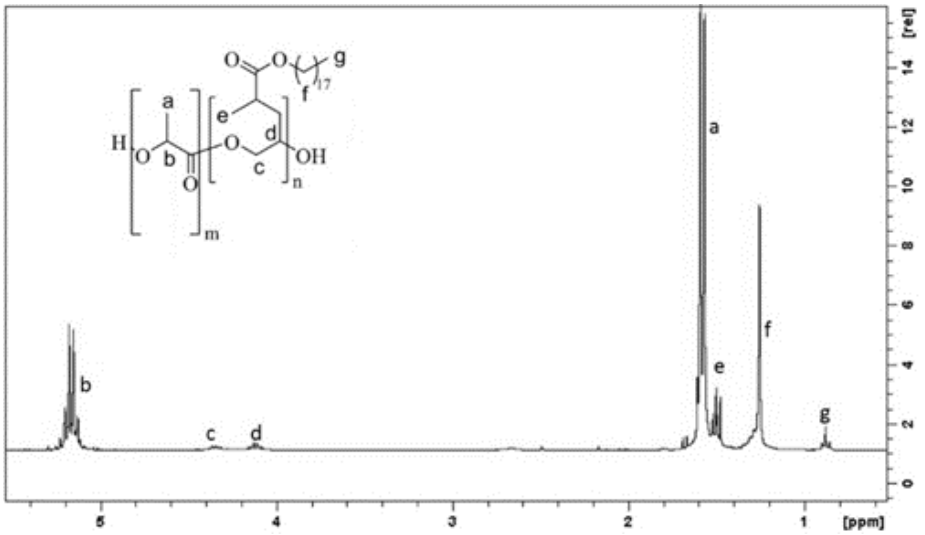

Figure S6 NMR-Data of PLA-co-C_{18}.

PLA-co-Ch δH(300 MHz, $CDCl_3$, Me_4Si) 0.61 (3 H, s, CH_3^g), 0.87-1.79 (25 H, br s, Steroid), 1.49 (3 H, d, CH_3^e), 1.57 (3 H, d, CH_3^a), 2.64 (1 H, d, CH^{f1}), 2.88 (1 H, d, CH^{f2}), 4.34 (3 H, br s, $CH_2^{c,d}$), 5.17 (1 H, q, CH^b).

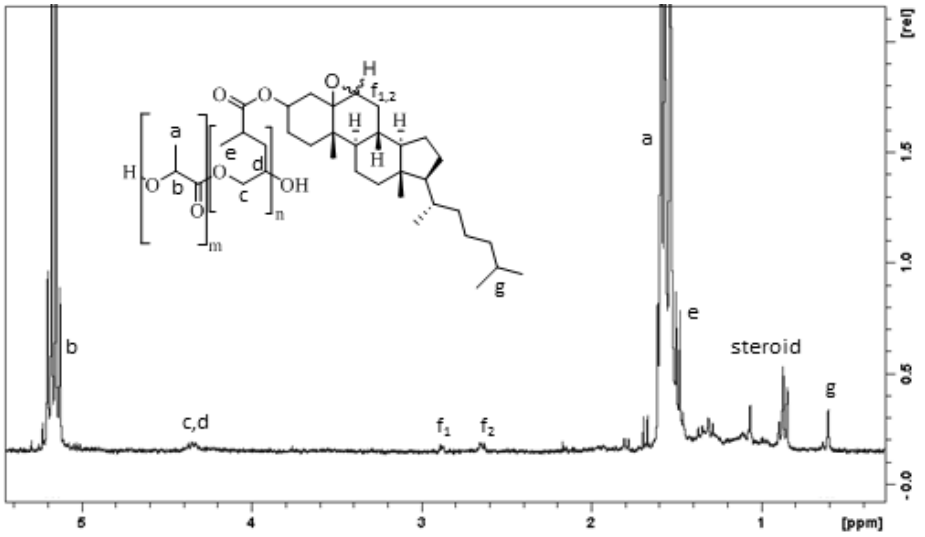

Figure S7 NMR-Data of PLA-co-Ch.