Supplementary Information (SI) for RSC Advances.

Ethylene oxide graft copolymers reduce the immunogenicity of lipid nanoparticles

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1. Materials

Dulbecco's Modified Eagle Medium (DMEM) was purchased from Thermo Scientific (MA, USA). Fetal bovine serum (FBS), accutase cell detachment solution and Dulbecco's modified phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (MO, USA). Cholesterol was purchased from Chem Scene (NJ, USA). L-Glutamic acid was purchased from Sigma-Aldrich (MO, USA). Extra dry Dichloromethane, extra dry Tetrahydrofuran, and 50 mM citric acid buffer stock solution (pH 4) were purchased from Thermo Scientific (MA, USA). DLin-MC3-DMA (MC3), 1, 2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1, 2-dimyristoyl-sn-glycerolmethoxypolyethylene glycol 2000 (DMG-PEG2000) were purchased from A.V.T. (Shanghai, China). Triphosgene was purchased from TGI (OR, USA). Firefly luciferase-encoded mRNA (mFLuc) and enhanced green fluorescent protein-encoded mRNA (eGFP) were purchased from APExBIO (TX, USA) and TriLink BioTechnologies (CA, USA), respectively. Silica gel and thin layer chromatography (TLC) plate were purched from Sigma-Aldrich (MO, USA). Dimethyl sulfoxide-d_6 (DMSO-d_6), Chloroform-*d* ($CDCl_3$), and Deuterium Oxide (D_2O) were purchased from Sigma-Aldrich (MO, USA). Size exclusion chormatography (SEC) results were determined by Polymer Laboratories-gel permeation chromatography (GPC) 50 instrument (Polymer Laboratories, USA). The ^1H and ^{13}C NMR spectra were acquired using either a Bruker AVQ 400 or a Bruker NEO 500 spectrometer at the NMR Facility of the College of Chemistry, UC Berkeley. ^1H NMR spectra data are reported as follows: chemical shift δ (ppm) referenced to either $CDCl_3$ (7.26 ppm), DMSO-d_6 (2.50 ppm) or D_2O (4.79 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets), coupling constant *J* (Hz), and integration.

2. Methods

2.1 Gel permeation chromatography (GPC) characterization for PGEs

Preparation of samples

1. The $PGE_{2\text{-}5}$ and standard polystyrene were dissolved separately in DMF at a concentration of 0.1 mg/mL.
2. The solutions were filtered using a 0.45 μm filter to remove any particulates.

GPC system setup

1. The column (PL aquagel-OH, 8 μm, 300 × 7.5 mm, Agilent, USA) temperature was set to 35°C.
2. Used DMF(0.01 M LiBr) as the mobile phase.
3. Set the flow rate of the eluent to 1.0 mL/min.

Sample injection

1. 100 μL of the prepared polymer solution (0.1 mg/mL) was loaded into the syringe.
2. 100 μL of polymer sample (0.1 mg/mL) or 100 μL of standard polystyrene (0.1 mg/mL) was injected into the PL-GPC 50 system.

Data Analysis

GPC results were processed with software (Cirrus GPC Version 3.4, Agilent, USA) by using narrow standards..

2.2 General procedure for LNP formulation

Preparation of stock solution

DLin-MC3-DMA, DOPE, cholesterol, and either $PGE_{2\text{-}5}$ or DMG-PEG2000 were separately dissolved in 100% ethanol to form a 10 mg/mL solution each. All stock solutions were stored at -20 °C.

Preparation of LNP

1. Organic phase preparation
    a) Lipid stock solutions were allowed to warm to room temperature or heated up to 37 °C to ensure complete dissolution, and were vortexed whenever necessary.
    b) All lipids components were mixed in ethanol, maintaining the specific molar ratio of DLin-MC3-DMA (10 mg/mL), DOPE (10 mg/mL), cholesterol (10 mg/mL), and either $PGE_{2\text{-}5}$ polymer (10 mg/mL or 20 mg/mL) or DMG-PEG2000 (10 mg/mL).
2. Aqueous phase preparation

mRNA solution (1 mg/mL) was mixed with 10 mM citrate buffer (pH 4) at a 1:2 (V/V) ratio to creat a 0.33 mg/mL aqueous solution.

3. Mixing phases

The organic phase and aqueous phase were mixed at a 1:3 (V/V) or 2:3 (V/V) ratio in a 1.5 mL Eppendorf tube. The mixture was pipetted repeatedly and it became cloudy. The LNP/mRNA solutions were incubated at room temperature for 15 minutes.The resulting formulation was flushed with nitrogen for 5 minutes to remove ethanol prior to utilization in cell and mouse experiments.

A representative protocol: preparation of PGE_3-LNPs made with 0.84 mole% of PGE_3

4 µL of DLin-MC3-DMA (10 mg/mL), 3 µL of DOPE (10 mg/mL), 2.5 µL of cholesterol (10 mg/mL) and 3.33 µL of PGE_3 (10 mg/mL) were mixed to make the mixed lipid solution. The molar ratio of DLin-MC3-DMA, DOPE, cholesterol and PGE_3 in the lipid solution was 62.30:40.32:64.67:1.41. 1 µL or 2 µL of the mixed lipid solution was mixed with 3 µL of the mRNA solution (0.33 mg/mL) in a 1.5 mL Eppendorf tube to achieve final concentrations of 0.5 mg/mL or 0.6 mg/mL.The resulting mixtures were mixed by repeated pipetting, becoming cloudy after pipetting. The LNP solutions were incubated at room temperature for 15 min. The resulting formulation was flushed with nitrogen for 5 minutes to remove the ethanol prior to utilization in cell and mouse experiments.

2.3 Dynamic light scattering (DLS)

LNP samples were prepared as described above at a concentration of 0.6 mg/mL mRNA in 10 mM citrate buffer (pH = 4). The LNP solutions were diluted to a concentration of 0.01 mg/mL mRNA using phosphate-buffered saline (PBS, pH 7.4).

The diluted LNPs were filtered through a sterile 0.22 μm syringe filter, transferred into a sterile conical tube for collection, and stored at 4 °C.

The hydrodynamic size and polydispersity index (PDI) of the LNPs were measured via dynamic light scattering (DLS) using a Malvern Instruments particle sizer (Zetasizer Nano SZ, Malvern Instruments, UK). A 0.1 mL sample solution was placed in a ultra-Micro cuvette (polystyrene, center height 8.5 mm, BrandTech), and measurements were performed at 25 °C. The instrument parameters and measurement times were automatically determined by the instrument.

2.4 Zeta potential measurement

Zeta potential measurements were conducted using a Zetasizer Nano ZS instrument (Malvern Instruments). The zeta potential was determined utilizing the Smoluchowski equation. For each measurement, a 1 mL sample solution was prepared as described in Section **2.2** and placed in a folded capillary cell (DTS 1070; Malvern Instruments). To enhance the accuracy of the zeta potential measurements and data collection, a two-minute equilibration period was implemented prior to each measurement.

2.5 Ribogreen assay for determining mRNA encapsulation

The mRNA encapsulation efficiency of the PGE-LNPs and PEG-LNPs was determined using the RiboGreen RNA assay (Thermo Fisher), following literature procedures.[1] Briefly, to determine mRNA encapsulation efficiency, standard calibration curves were initially generated with pure mRNA. LNP formulations were diluted to a concentration of 25 ng/mL mRNA in a buffer that contained the RiboGreen reagent, starting from a 100 ng/mL mRNA. This was achieved by adding 175 μL of LNP formulations containing 100 ng/mL RNA to 350 μL of 2,000-fold diluted RiboGreen reagent, and 175 μL of 1× TE buffer (Tris-EDTA), the dilutions were made in a 1.5 mL RNase-free Eppendorf tube. The contents were mixed well. The RNA standard curve samples and the LNP dilutions (in triplicate) were added to a black, flat-bottom 96-well plate, with a final volume of 200 μL per well. The plate was mixed well and incubated for 5 minutes at room temperature, protected from light. Subsequently, the sample fluorescence was measured using a Tecan Infinite M200 plate reader (excitation ~485 nm, emission ~535 nm, Männedorf, Switzerland). The concentration of unencapsulated mRNA in the sample solution (C, ng/mL) was calculated according to the standard curve. The encapsulation efficiency of mRNA in each formulation was then determined using the formula: $(1 - C/25) \times 100$ (%).

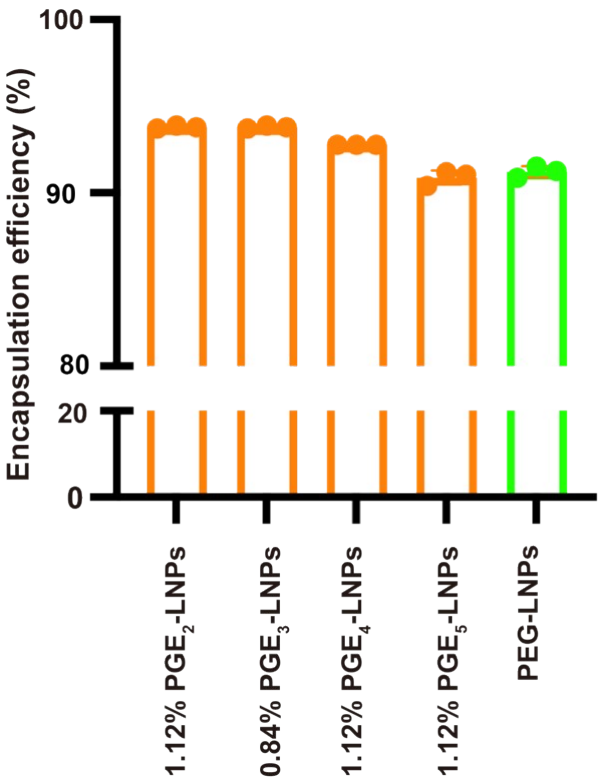


Figure S1. mRNA encapsulation efficiency of PGE_2-LNPs and PEG-LNPs. $PGE_{2\text{-}5}$-LNPs have similar encapsulation efficiency as PEG-LNPs. Data are represented as mean ± standard deviation, n = 3.

2.6 Small angel X-ray scattering (SAXS) of PGE_3-LNPs and PEG-LNPs

SAXS experiments were performed at SLAC SSRL Beamline BL4-2, California, USA, with a detector distance of 2.5 meters and X-ray wavelength of l = 1.127 Å, covering a Q range of 0.005 $Å^{-1}$ to 0.35 $Å^{-1}$. All LNPs were prepared as described in **2.1**. For example, a representative procedure is described for LNPs made with 0.84 mole% of PGE_3. 10 µL of mRNA at 1 mg/mL (in the original buffer from TriLink) was diluted with 20 µL of 10 mM citrate buffer (pH = 4), and then mixed the 20 µL of the lipid solution to yield a final volume of 50 µL of LNP solution (0.2 mg/mL mRNA). 30 µL samples were inserted into the flow-through cell using an autosampler setup. A consecutive series of 12 exposures of 1s were collected for samples and buffer blanks. During data collection, the solutions oscillated in a stationary quartz capillary cell to maximize the exposed volume and reduce the radiation dose per exposed volume. The collected data were radially integrated, screened for the effects of radiation damage to the sample, and scaled according to the transmitted beam intensity. The corresponding values for the buffer were subtracted using RAW data package. The data set was calibrated to an absolute intensity scale using water as a primary standard. The peak positions were analyzed using the integrated peak analysis tools in OriginPro 2022.

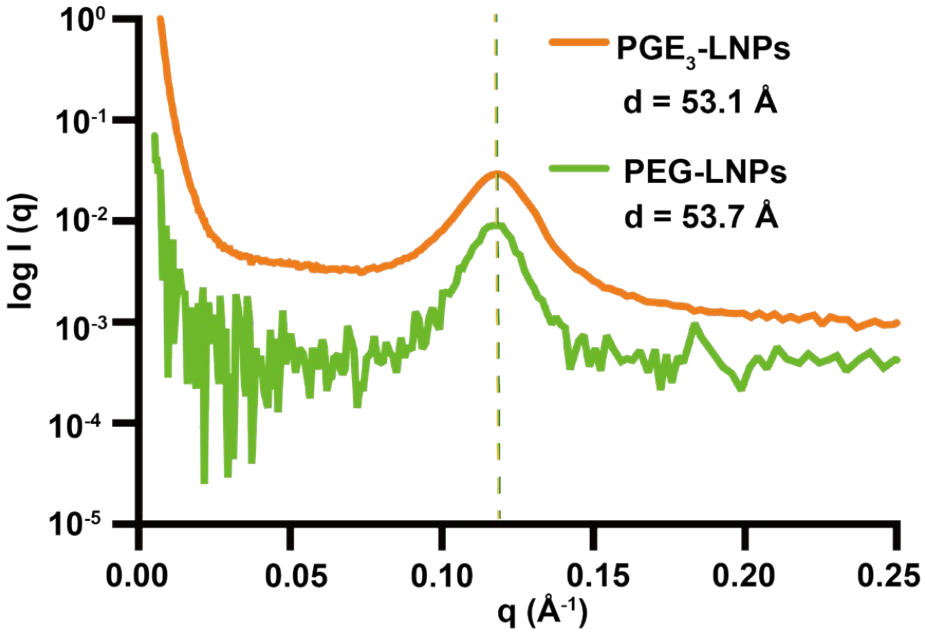


Figure S2. SAXS analysis of PGE_3-LNPs made with 0.84 mole% of PGE_3 and PEG-LNPs. The data show that PGE_3-LNPs made with 0.84 mole% of PGE_3 exhibit an internal structure comparable to that of the PEG-LNPs.

2.7 Delivery of eGFP mRNA to HEK293Tcells with $PGE_{2\text{-}5}$-LNPs and PEG-LNPs

HEK 293T cells were seeded in 96-well plates at a density of 2 × 10^4 cells per well and incubated at 37°C for 12 hours (DMEM 10% FBS). LNPs were formed as described in **2.2** and 4 µL or 5 µL of LNP solution were added to 300 µL Opti media to generate a 300 ng/100 µL concentration of mRNA. The LNP solution in Opti media was added to cells in a 100 µL volume and incubated with the cells for 12 hours. The cells were washed with PBS pH 7.4 and detached with Accutase® solution for 10 min at 37 °C. The transfection efficiency of the cells was determined using an Attune flow cytometer (Thermo Fisher, MA, USA). Data analysis was performed using FlowJo v10 10.7.1 software (FlowJo, LLC, Ashland OR). A blank group and PGE_3-LNPs containing 0.84 mole% of PGE_3 were used as examples for flow cytometry plots and gating (Figure S**3**).

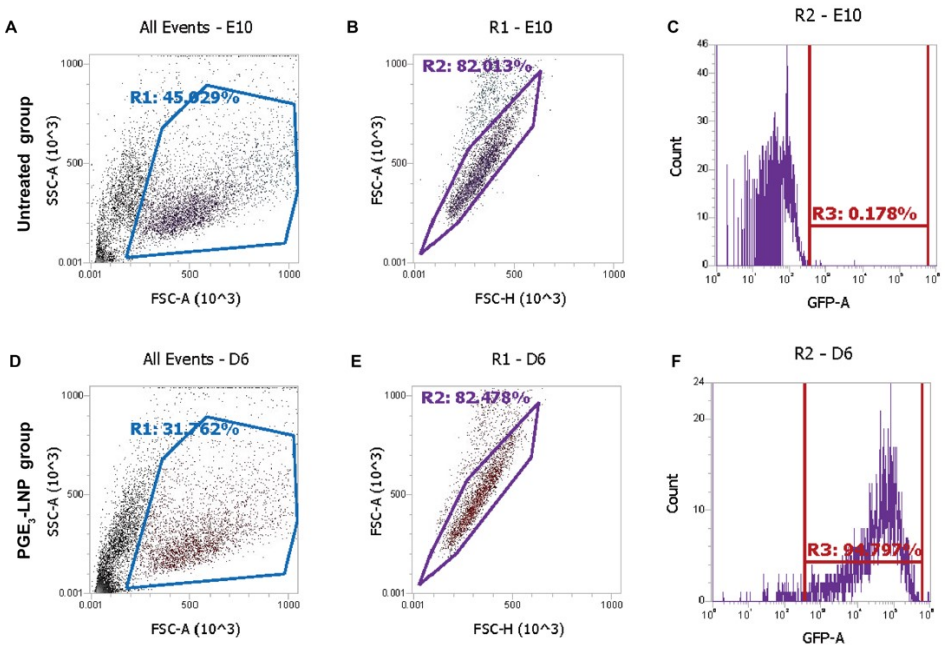


Figure S3. FACS gating strategy for PGE_3-LNPs deliverying eGFP mRNA to HEK 293T cells. (A)-(C) for untreated group. (A) Forward scatter versus side scatter plot. (B) Single cell gating. (C) eGFP expression. (D)-(F) for PGE_3-LNPs containing 0.84 mole% of PGE_3. (D) Forward scatter versus side scatter plot. (E) Single cell gating. (F) eGFP expression.

2.8 In vivo mRNA delivery

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Berkeley (UCB). All facilities used during the study were accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC). Wild-type BALB/cJ mice (female, Jackson Lab, 000651), aged 10-16 weeks and weighing 18-20 g, were used for firefly luciferase mRNA and human erythropoietin (hEPO) mRNA delivery experiments.

Delivery of luciferase mRNA in PGE-LNPs and PEG-LNPs

Wild-type BALB/cJ female mice weighing between 18-20 g were used for firefly luciferase mRNA delivery experiments. The mRNA encoding firefly luciferase (mFluc) was encapsulated within LNPs. Prior to injection, LNP/mRNA complexes (20 μL) were mixed with PBS (80 μL), resulting in a final injection volume of 100 μL per mouse (0.25 mg/kg mRNA). The LNP/mRNA complexes were injected into mice through either intravenous (*i.v.*) injection or intramuscular (*i.m.*) administration. At 4 h posttreatment, VivoGlo™ Luciferin (Promega) was intraperitoneally injected into mice at a dose of 150 mg/kg. Seven minutes after the luciferin injection, mice were anesthetized with isoflurane (2.5% (vol/vol) in 0.2 L/min O_2 flow) and imaged in a small animal imaging system (IVIS, PerkinElmer). Euthanize the mice in a chamber with a CO_2 fill rate of 30–70% of the chamber volume per minute. Dissect the mouse and collect the major

organs (heart, lungs, liver, spleen and kidneys). Image and quantify the luciferase expression of the organs by the IVIS system.

Delivery of human erythropoietin mRNA in PGE-LNPs and PEG-LNPs

Human erythropoietin (hEPO) mRNA (0.05 mg/kg) encapsulated in PGE_3-LNPs or PEG-LNPs in Dulbecco's PBS was administered intravenously (*i.v.*) into mice using 3/10-cc insulin syringes (BD Biosciences), with an injection volume of 100 µL. The mice were injected once a week for three consecutive weeks via intravenous (*i.v.*) injection. The mice were anesthetized with isoflurane (2.5% vol/vol in 0.2 L/min O_2 flow), the eyeballs were then removed, and blood was withdrawn from the eye sockets.

2.9 hEPO quantitation

Mouse blood samples were collected 24 hours post-injection, followed by centrifugation at 1500 × g for 10 minutes at 4°C to isolate serum. hEPO levels were measured using the EPO Human ELISA Kit (no. BMS2035; Thermo Fisher Scientific) following the manufacturer's recommendations.

2.10 Quantification of Anti-PEG IgG in mouse serum

A 96-well plate (Corning, 23321004) was coated with PGE_3 polymer at a coating density of 100 ng/well overnight at 4 °C. The plate was washed 3 times with 1 × PBS, then blocked with 5% BSA in 1 × PBS for 4 h at room temperature, followed by 3 times washing with 1 × PBS. Then, 50 × diluted plasma was added to each well, and incubated at 37 °C for 2 h. The plate was washed with 1 × PBS + 0.05% Tween-20 three times, then incubated with Rabbit Anti-Mouse IgG H&L (HRP) (Abcam, ab6728) for 1 h at 37 °C. The plate was washed with 1 × PBS + 0.05% Tween-20 three times, then freshly prepared OPT substrate was added (Thomas Scientific, C753Y67). The reaction was stopped with 3 M Sulfuric Acid and read at 450 nm on a plate reader.

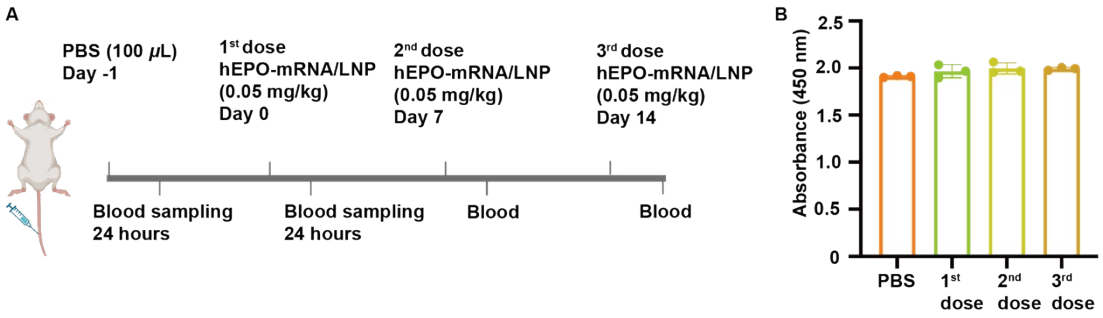


Figure S4. PGE_3-LNPs do not induce anti-PEG IgG. (A) Experiment schedule for chronic LNP dosing experiment with hEPO mRNA. Mice were intravenously injected with PBS (100 µL) on Day -1 and PGE_3-LNPs containing hEPO mRNA (0.05 mg/kg) on days 0, 7 and 14. Serum samples were collected 24 hours post-injection (n = 3 mice). (B) Quantitation of anti-PEG IgG in the blood of mice treated with PBS or PGE_3-LNPs. ELISA results indicated that the anti-PEG IgG levels in the serum of the mice treated with PGE_3 formulated LNPs did not significantly increase compared to the control group treated with PBS. This suggests that chronic dosing with PGE_3

formulated LNPs does not induce the production of anti-PEG antibodies in mice (n = 3 mice).

2.11 Statistics

GraphPad Prism version 10 (GraphPad Software) was used for all statistical analysis and for generating the plots. Data are reported as mean ± SD. Statistical analyses were performed with a Student's t test with $^{*}P < 0.05$.

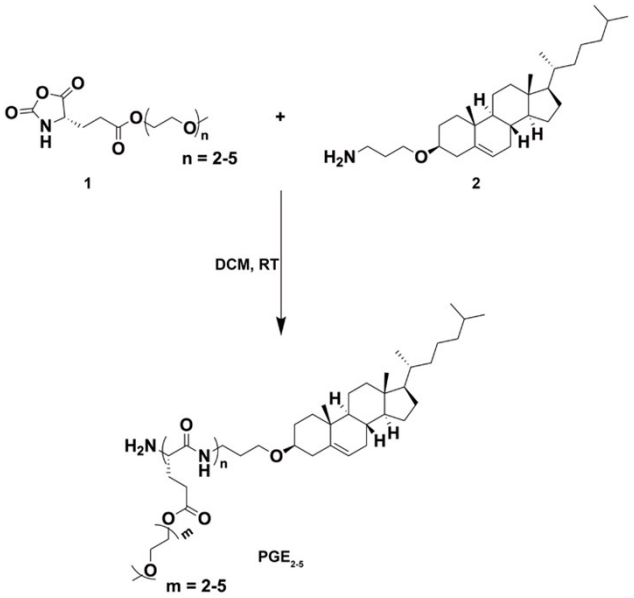
3. Synthetic procedures and characterization data

3.1 Synthesis of the *N*-carboxyanhydride monomers needed to make PGE_{2-5}

The monomers (**1**) were prepared from oligoethylene glycol glutamate ester and triphosgene accoding to previous literature procedures.[2]

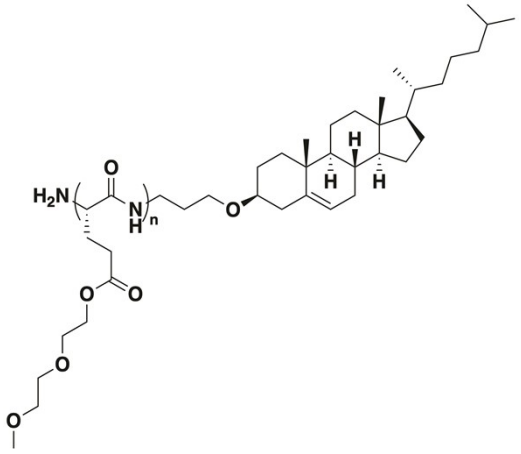
3.2 Synthesis of cholesteryloxypropan-1-amine

Cholesteryloxypropan-1-amine (**2**) was synthesized according to previous literature procedures.[3]



Scheme S1. Synthesis of PGE_{2-5}. PGE_{2-5} were synthesized via ring opening polymerization of the *N*-carboxyanhydride monomer **1**. The polymerization was initiated with the amine containing cholesterol derivative **2**. All polymerization reactions were performed in dry vials under N_2.

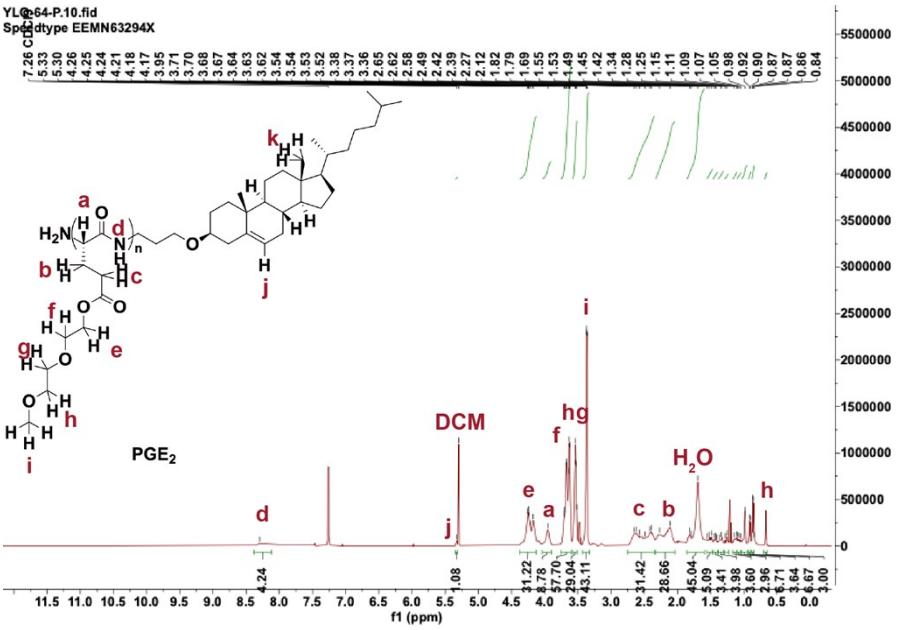
3.3 Synthesis of PGE_{2-5}



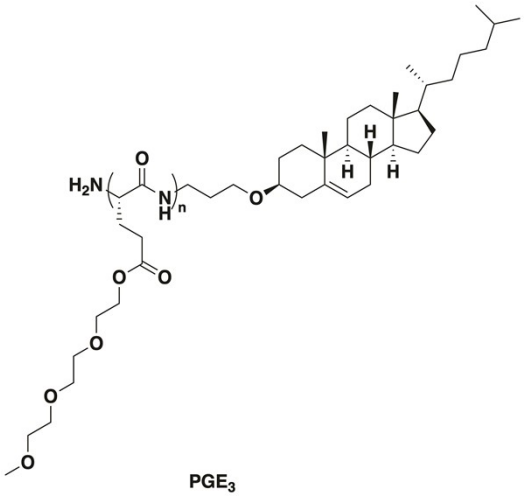
PGE_2

Synthesis of PGE_2

2-(2-methoxyethoxy)ethyl (*S*)-3-(2,5-dioxooxazolidin-4-yl)propanoate (275 mg, 1 mmol) was dissolved in dry DCM (500 μL), and added to cholesteryloxypropan-1-amine (8.8 mg, 0.020 mmol) in dry DCM (500 μL) under stirring in an N_2 atmosphere. The reaction was carried out in a 100 mL round-bottom flask equipped with a magnetic stir bar. After stirring vigorously at room temperature for 12 h, the mixture was precipitated by adding cold diethyl ether (15 mL), and the white product was collected by centrifugation at 4500 rpm for 5 minutes. This step was repeated three times. The resulting precipitate was dried in vacuo to give **PGE_2** as a colorless oil (178.01 mg, yield 62.75 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.29 (s, 7 H), 5.33 (s, 1 H), 4.38 – 4.13 (m, 31 H), 3.95 (s, 9 H), 3.76 – 3.60 (m, 58 H), 3.53 (q, *J* = 4.6, 29 H), 3.43 – 3.33 (m, 43 H), 2.75 – 2.35 (m, 31 H), 2.19 (d, *J* = 76.6, 29 H), 1.69 (s, 45 H), 1.56 – 1.47 (m, 5 H), 1.46 – 1.39 (m, 3 H), 1.35 (d, *J* = 13.4, 4 H), 1.27 (d, *J* = 15.3, 3 H), 1.13 (d, *J* = 19.7, 4 H), 1.10 – 1.04 (m, 3 H), 0.98 (s, 7 H), 0.91 (d, *J* = 6.4, 4 H), 0.88 – 0.84 (m, 7 H), 0.67 (s, 3 H).



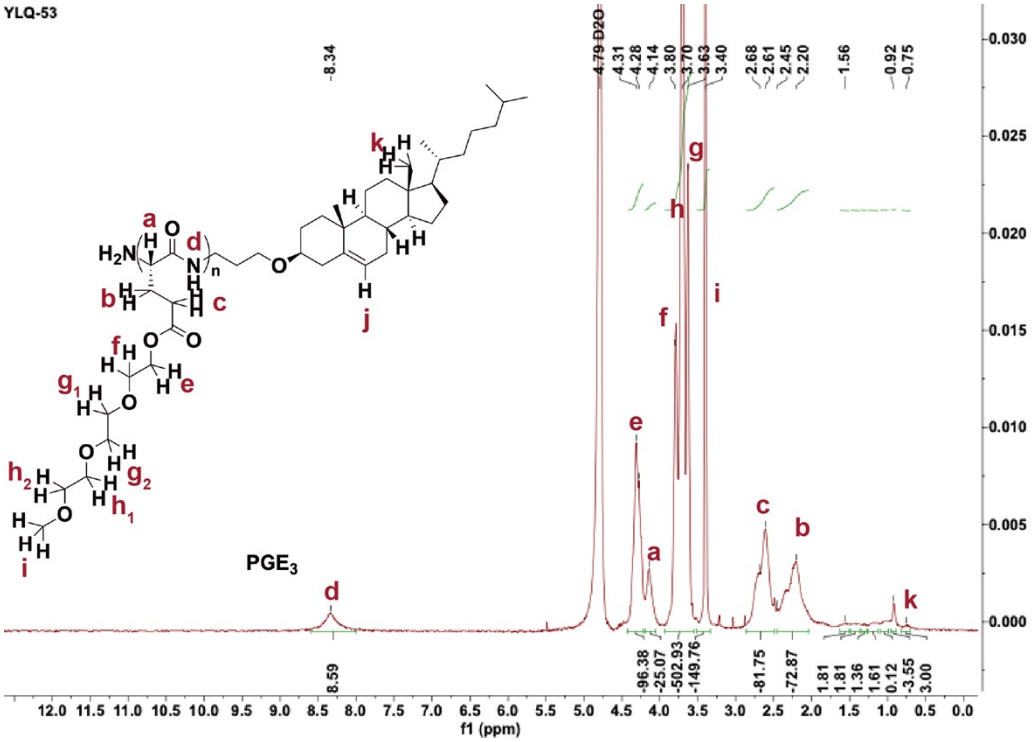
^{1}H NMR spectra of **PGE_2** in $CDCl_3$ (2 mg/mL).



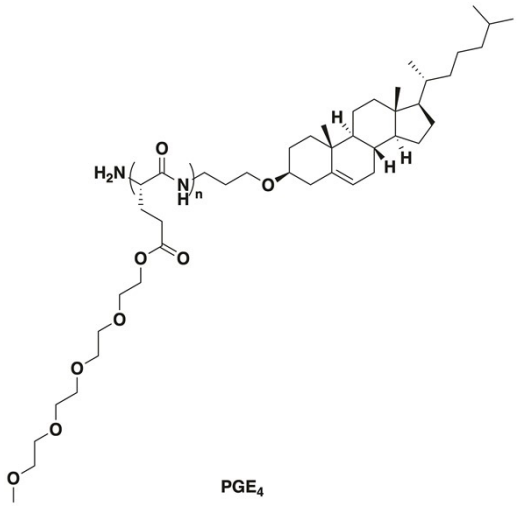
PGE$_3$

Synthesis of PGE$_3$

2-(2-(2-methoxyethoxy)ethoxy)ethyl (*S*)-3-(2,5-dioxooxazolidin-4-yl)propanoate (250 mg, 0.79 mmol) was dissolved in dry DCM (500 μL), and added to cholesteryloxypropan-1-amine (6.96 mg, 0.016 mmol) in dry DCM (500 μL) under stirring in an N_2 atmosphere. The reaction was carried out in a 100 mL round-bottom flask equipped with a magnetic stir bar. After stirring vigorously at room temperature for 12 h, the mixture was precipitated by adding cold diethyl ether (15 mL), and the white product was collected by centrifugation at 4500 rpm for 5 minutes. This step was repeated three times. The resulting precipitate was dried in vacuo to give PGE$_3$ as a colorless oil (187.90 mg, yield 85.37 %). ^{1}H NMR (400 MHz, D_2O) δ = 8.34 (s, 9 H), 4.29 (d, *J* = 13.9, 96 H), 4.14 (s, 25 H), 3.94 – 3.55 (m, 503 H), 3.40 (s, 150 H), 2.65 (d, *J* = 29.1, 82 H), 2.20 (s, 73 H), 1.56 (s, 2 H), 0.92 (s, 4 H), 0.75 (s, 3 H).

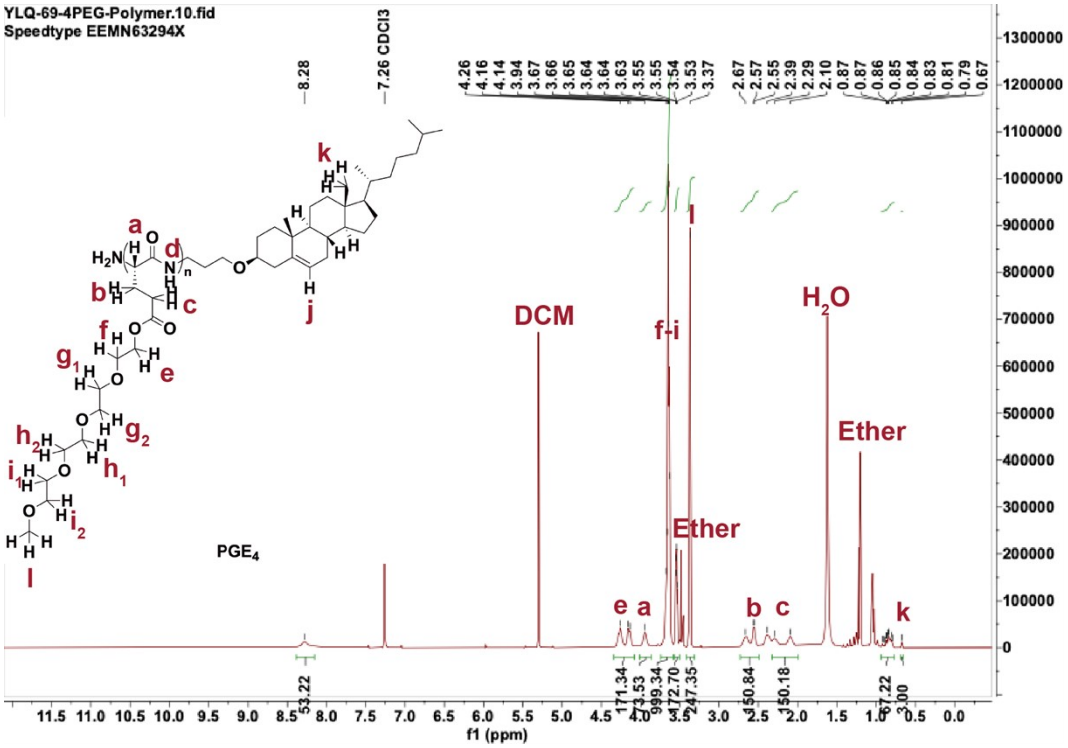


^{1}H NMR spectra of PGE$_3$ in D_2O (1 mg/mL).

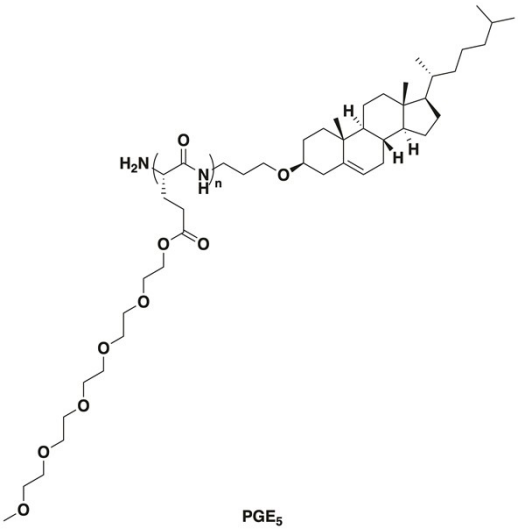


Synthesis of PGE_4

2,5,8,11-tetraoxatridecan-13-yl (S)-3-(2,5-dioxooxazolidin-4-yl)propanoate (250 mg, 0.69 mmol) was dissolved in dry DCM (500 µL), and added to cholesteryloxypropan-1-amine (6.2 mg, 0.014 mmol) in dry DCM (500 µL) under stirring in an N_2 atmosphere. The reaction was carried out in a 100 mL round-bottom flask equipped with a magnetic stir bar. After stirring vigorously at room temperature for 12 h, the mixture was precipitated by adding cold diethyl ether (15 mL), and the white product was collected by centrifugation at 4500 rpm for 5 minutes. This step was repeated three times. The resulting precipitate was dried in vacuo to give PGE_4 as a colorless oil (192.10 mg, yield 74.33 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.28 (s, 53 H), 4.34 – 4.08 (m, 171 H), 3.94 (s, 74 H), 3.74 – 3.59 (m, 999 H), 3.57 – 3.51 (m, 173 H), 3.37 (s, 247 H), 2.73 – 2.49 (m, 151 H), 2.19 (d, *J* = 98.9, 150 H), 0.94 – 0.77 (m, 67 H), 0.67 (s, 3 H).

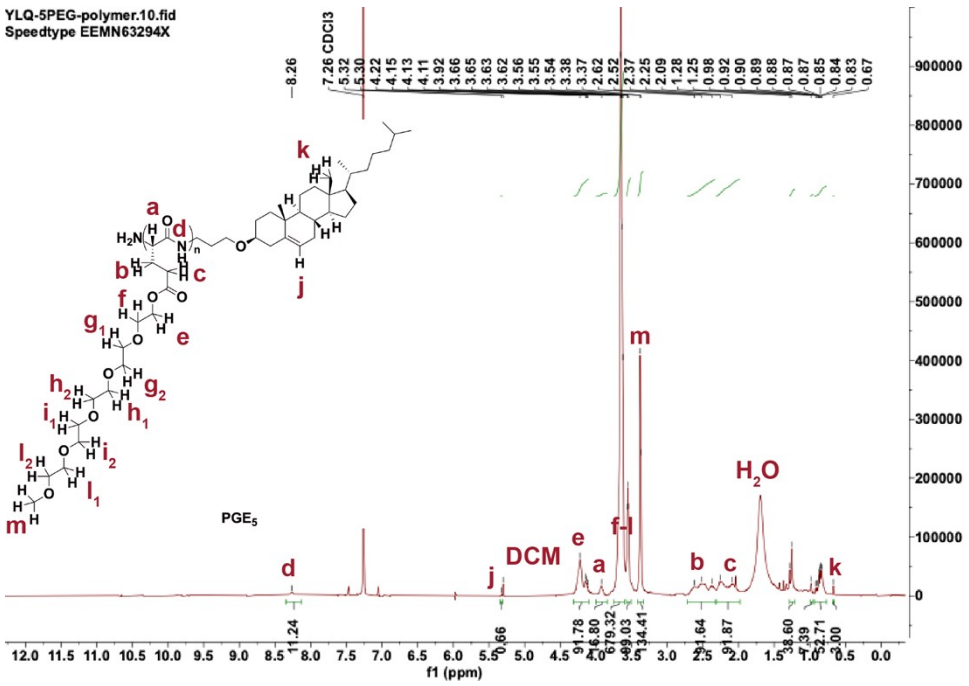


^{1}H NMR spectra of PGE_4 in $CDCl_3$ (2 mg/mL).



Synthesis of PGE_5

2,5,8,11,14-pentaoxahexadecan-16-yl (*S*)-3-(2,5-dioxooxazolidin-4-yl)propanoate (250 mg, 0.61 mmol) was dissolved in dry DCM (500 μL), and added to cholesteryloxypropan-1-amine (5.4 mg, 0.012 mmol) in dry DCM (500 μL) under stirring in an N_2 atmosphere. The reaction was carried out in a 100 mL round-bottom flask equipped with a magnetic stir bar. After stirring vigorously at room temperature for 12 h, the mixture was precipitated by adding cold diethyl ether (15 mL), and the white product was collected by centrifugation at 4500 rpm for 5 minutes. This step was repeated three times. The resulting precipitate was dried in vacuo to give **PGE_5** as a colorless oil (13.98 mg, yield 5.48 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.26 (s, 11 H), 5.32 (s, 1 H), 4.19 (d, *J* = 36.3, 92 H), 3.92 (s, 17 H), 3.75 – 3.60 (m, 679 H), 3.55 (t, *J* = 4.3, 99 H), 3.37 (d, *J* = 5.2, 134 H), 2.72 – 2.33 (m, 92 H), 2.17 (d, *J* = 82.7, 92 H), 1.27 (d, *J* = 15.6, 39 H), 0.98 (s, 7 H), 0.94 – 0.77 (m, 53 H), 0.67 (s, 3 H).



^{1}H NMR spectra of **PGE_5** in $CDCl_3$ (2 mg/mL).

4. Tables detailing the components of $PGE_{2\text{-}5}$-LNPs

Table S1 Components of PGE_2-LNPs

Molar ratio/Molar percentages (%)			
PGE_2	DLin-MC3-DMA	DOPE	Cholesterol
0.12/0.07%	62.29/37.22	40.32/24.09	64.67/38.63
0.24/0.14%	62.30/37.19	40.32/24.07	64.67/38.60
0.47/0.28%	62.30/37.14	40.32/24.03	64.67/38.55
0.94/0.56%	62.30/37.03	40.32/23.97	64.67/38.44
1.42/0.84%	62.30/36.93	40.32/23.90	64.67/38.33
1.90/1.12%	62.30/36.82	40.32/23.83	64.67/38.22
3.83/2.24%	62.30/36.41	40.32/23.56	64.67/37.79
7.84/4.48%	62.30/35.57	40.32/23.02	64.67/36.93

Table S2 Components of PGE_3-LNPs

Molar ratio/Molar percentages (%)			
PGE_3	DLin-MC3-DMA	DOPE	Cholesterol
0.12/0.07%	62.30/37.21	40.32/24.09	64.67/38.63
0.24/0.14%	62.30/37.19	40.32/24.07	64.67/38.60
0.47/0.28%	62.30/37.14	40.32/24.04	64.67/38.55
0.95/0.56%	62.30/37.03	40.32/23.97	64.67/38.44
1.41/0.84%	62.30/36.93	40.32/23.90	64.67/38.34
1.90/1.12%	62.30/36.82	40.32/23.83	64.67/38.22
3.84/2.24%	62.30/36.40	40.32/23.56	64.67/37.79
7.85/4.48%	62.30/35.57	40.32/23.02	64.67/36.93

Table S3 Components of PGE_4-LNPs

Molar ratio/Molar percentages (%)			
PGE_4	DLin-MC3-DMA	DOPE	Cholesterol
0.12/0.07%	62.30/37.21	40.32/24.09	64.67/38.63
0.24/0.14%	62.30/37.18	40.32/24.07	64.67/38.60
0.47/0.28%	62.30/37.14	40.32/24.04	64.67/38.55
0.95/0.56%	62.30/37.03	40.32/23.97	64.67/38.44
1.41/0.84%	62.30/36.93	40.32/23.90	64.67/38.33
1.90/1.12%	62.30/36.82	40.32/23.83	64.67/38.22
3.84/2.24%	62.30/36.41	40.32/23.56	64.67/37.79
7.85/4.48%	62.30/35.57	40.32/23.02	64.67/36.92

Table S4 Components of PGE_5-LNPs

Molar ratio/Molar percentages (%)			
PGE_5	DLin-MC3-DMA	DOPE	Cholesterol
0.12/0.07%	62.30/37.21	40.32/24.09	64.67/38.63
0.23/0.14%	62.30/37.19	40.32/24.07	64.67/38.60
0.47/0.28%	62.30/37.14	40.32/24.04	64.67/38.55
0.94/0.56%	62.30/37.03	40.32/23.97	64.67/38.44
1.41/0.84%	62.30/36.93	40.32/23.90	64.67/38.33
1.90/1.12%	62.30/36.82	40.32/23.83	64.67/38.22
3.83/2.24%	62.30/36.41	40.32/23.56	64.67/37.79
7.85/4.48%	62.30/35.57	40.32/23.02	64.67/36.92

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