Supporting Information

Biodegradable Amino-ester Nanomaterials for Cas9 mRNA Delivery in Vitro and in Vivo

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Materials.

ψ-modified Cas9 mRNA and 5meC- ψ-modified Firefly Luciferase mRNA were purchased from TriLink Biotechnologies, Inc. (San Diego, CA). 1, 2-dioleoyl-sn-glycero-3-

phosphoethanolamine (DOPE) was purchased from Avanti Polar Lipids (Alabaster, Alabama), Inc. Other chemicals were purchased from sigma-Aldrich and used without further purification.

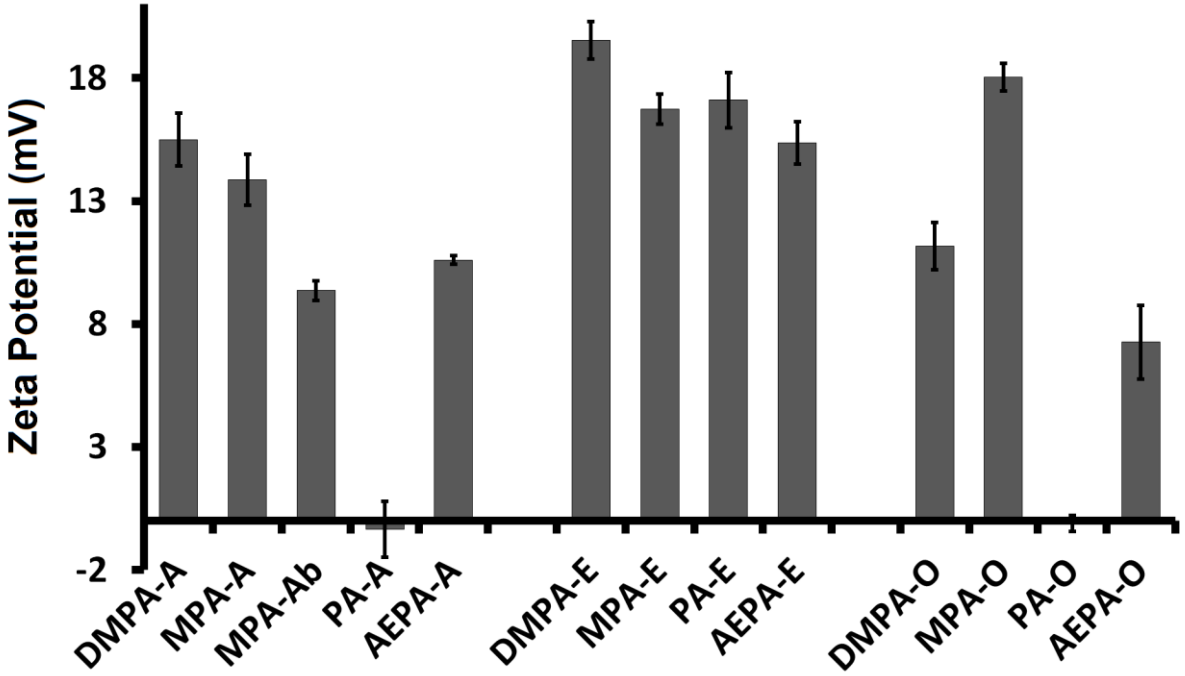

Figure S1. Zeta potential of amino-ester LLNs.

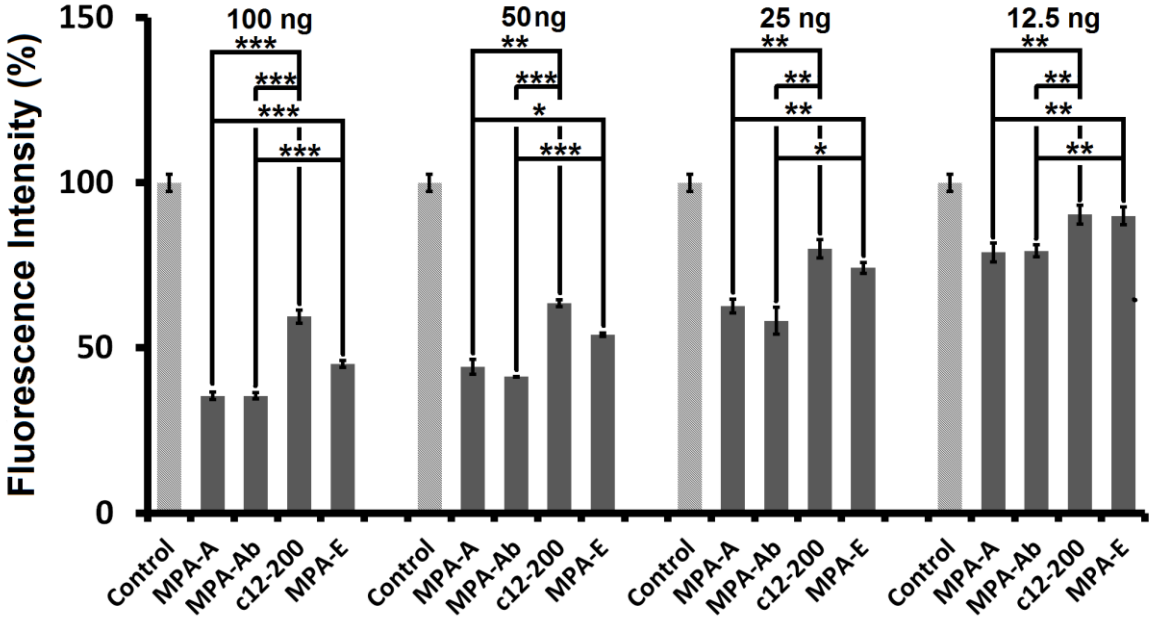

Figure S2. Dose-dependent assay. Delivery of Cas9 mRNA using **MPA-A**, **MPA-Ab**, **C12-200** and **MPA-E** LLNs *in vitro* at mRNA doses of 100, 50, 25 and 12.5 ng. Control: PBS. (triplicate; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; *t* test, double-tailed).

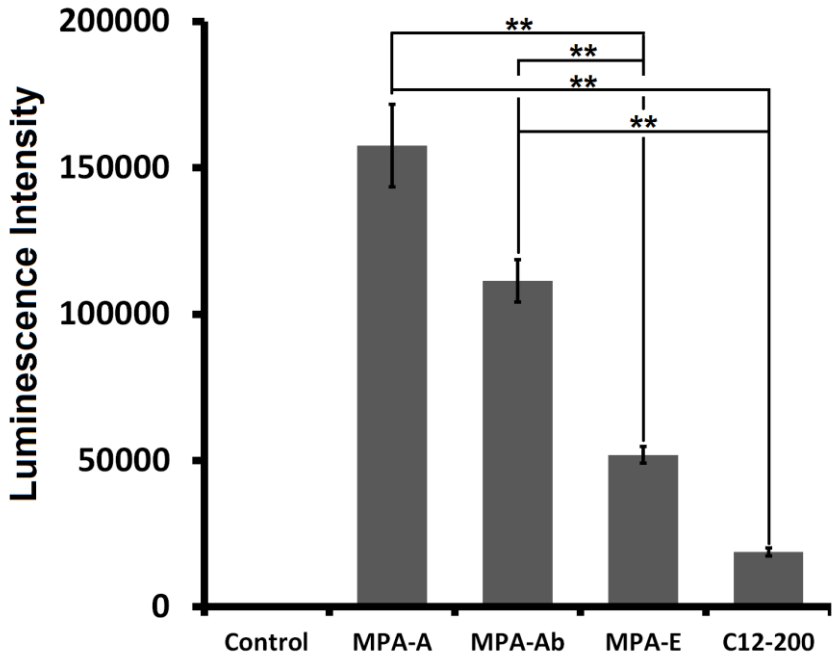

Figure S3. Delivery of mRNA encoding Firefly luciferase in Hep3B cells. Control: PBS. (triplicate; **, $P < 0.01$; *t* test, double-tailed).

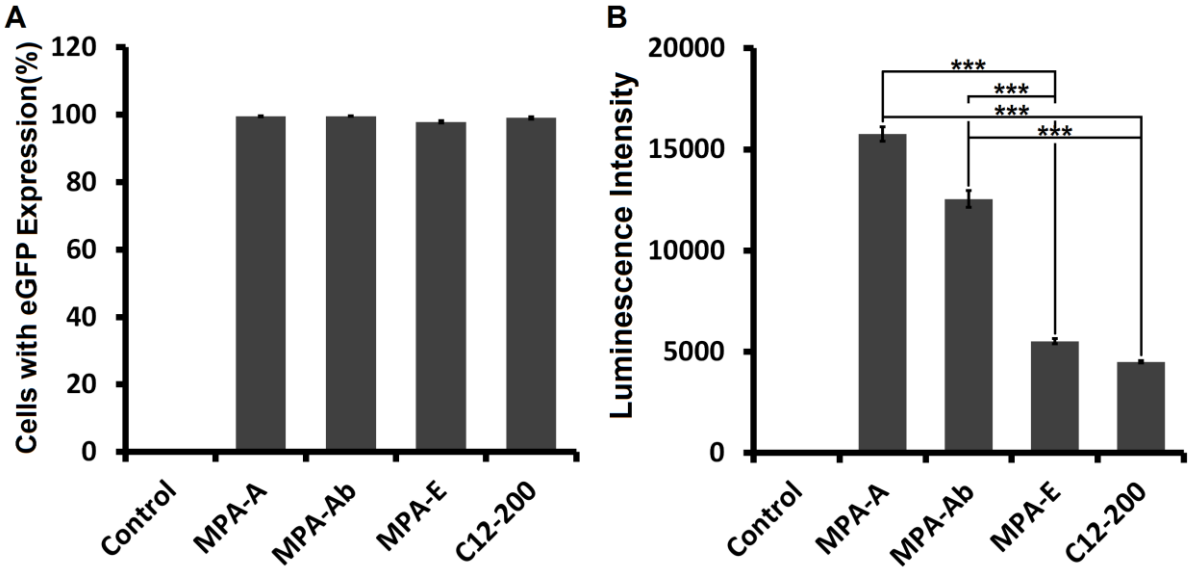

Figure S4. (A) Percentage of cells in population with eGFP expression. (B) Delivery of mRNA encoding eGFP in 293T cells. Control: PBS. (triplicate; ***, $P < 0.001$; *t* test, double-tailed).

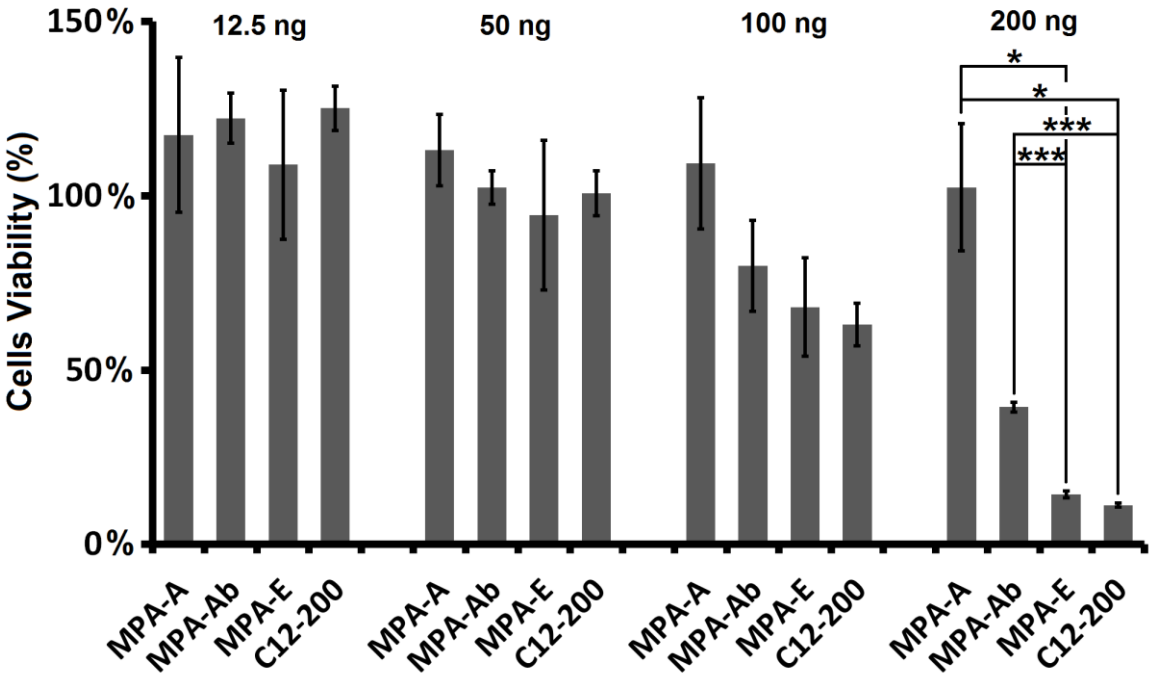

Figure S5. Cells viability through MTT assay on Hep3B cell line at mRNA dose of 12.5, 50 100 and 200ng. (triplicate; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; *t* test, double-tailed).

Synthesis.

9-oxononanoic acid, 9-Oxononanoic acid (*z*)-non-2-en-yl ester (**A**) and 9-Oxononanoic acid 2-ethyl-hexane-1-yl ester (**Ab**) were synthesized according to the method reported previously.[S1, S2]

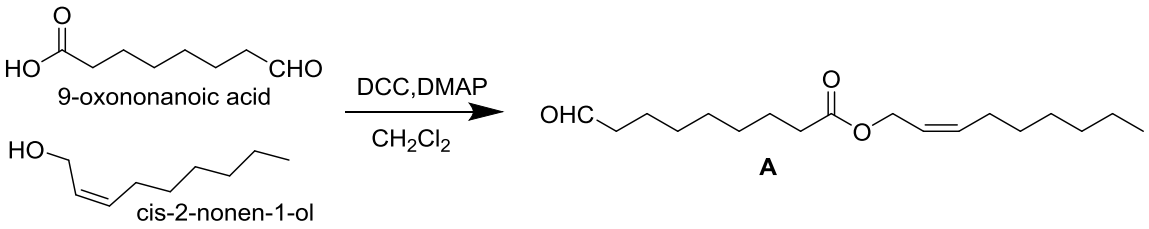

Figure S6. Synthesis route of compound **A**.

A: 9-oxononanoic acid (500 mg, 3.16 mmol) and DCC (700 mg, 3.40 mmol) were dissolved in 15 mL CH_2Cl_2 and cooled to 0 ^oC. After adding DMAP (10 mg, 0.08 mmol), the reaction mixture was stirred for 30 min at 0 ^oC. Cis-2-nonen-1-ol (670 mg, 4.72 mmol) was added and stirred for 2 h at 0 ^oC. The reaction mixture was warmed to RT and stirred overnight. The reacting mixture was then washed with water twice. The organic phase was dried with sodium sulfate and CH_2Cl_2 was evaporated. The crude product was purified by column chromatography using a Combiflash Rf system with hexanes/ethyl acetate, (95/5 by volume) to give the product in a colorless oil (650 mg, yield 69.4%).

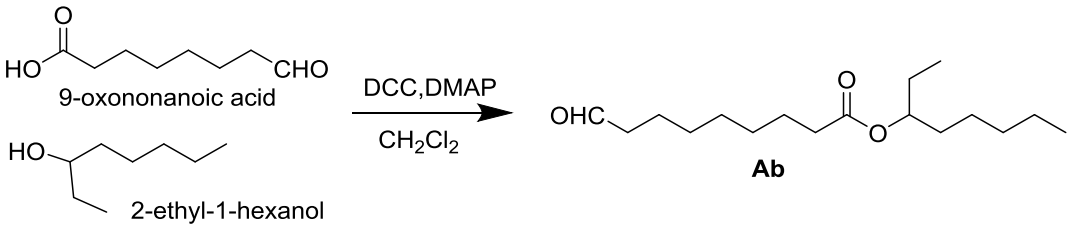

Figure S7. Synthesis route of compound **Ab**.

Ab: Following the same procedure as **A**.

MPA-A: yield 42.2%, ^1H NMR (400 MHz, $CDCl_3$) δ = 5.65 (3H, m), 5.56 (3H, m), 4.65-4.63 (6H, d, *J* = 8), 2.44 (6H, m), 2.34-2.30 (8H, t, *J* = 8), 2.24 (3H, s), 2.14-2.09 (6H, m), 1.65-

1.62 (9H, m), 1.46 (6H, m), 1.40-1.30 (50H, m), 0.92-0.88 (9H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{58}H_{109}N_2O_6$, 929.8286; found, 929.8266.

DMPA-A: yield 77.0%, ^1H NMR (400 MHz, $CDCl_3$) δ = 5.65 (2H, m), 5.55 (2H, m), 4.65-4.63 (4H, d, *J* = 8), 2.42-2.39 (8H, m), 2.37-2.32 (4H, t, *J* = 8), 2.26 (6H, s), 2.14-2.09 (4H, m), 1.73-1.62 (6H, m), 1.49 (4H, m), 1.40-1.31 (32H, m), 0.92-0.89 (6H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{41}H_{79}N_2O_4$, 663.6040; found, 663.6014.

PA-A: yield 61.9%, ^1H NMR (400 MHz, $CDCl_3$) δ = 5.64 (4H, m), 5.55 (4H, m), 4.64-4.63 (8H, d, *J* = 8), 2.42 (10H, m), 2.34-2.30 (8H, t, *J* = 8), 2.14-2.09 (8H, m), 1.65-1.62 (10H, m), 1.44-1.30 (74H, m), 0.92-0.88 (12H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{75}H_{139}N_2O_8$, 1196.0531; found, 1196.0490.

AEPA-A: yield 69.4%, ^1H NMR (400 MHz, $CDCl_3$) δ = 5.64 (5H, m), 5.55 (5H, m), 4.65-4.63 (10H, d, *J* = 8), 2.53 (5H, s), 2.43 (11H, s), 2.34-2.30 (10H, t, *J* = 8), 2.14-2.09 (10H, m), 1.66-1.62 (12H, m), 1.44-1.31 (92H, m), 0.92-0.88 (15H, t, *J* = 8). MS (*m/z*): $[M + H]^+$ calcd. for $C_{95}H_{176}N_3O_{10}$, 1519.3356; found,1519.3313.

MAP-Ab: yield 74.0%, ^1H NMR (400 MHz, $CDCl_3$) δ = 4.85-4.80 (3H, m), 2.47 (6H, m), 2.46-2.28 (10H, m), 2.25 (3H, s), 1.65-1.45 (26H, m), 1.31 (42H, m), 0.91-0.87 (18H, m). MS (*m/z*): $[M + H]^+$ calcd. for $C_{55}H_{109}N_2O_6$, 893.8286; found,893.8275.

^{1}H NMR spectra

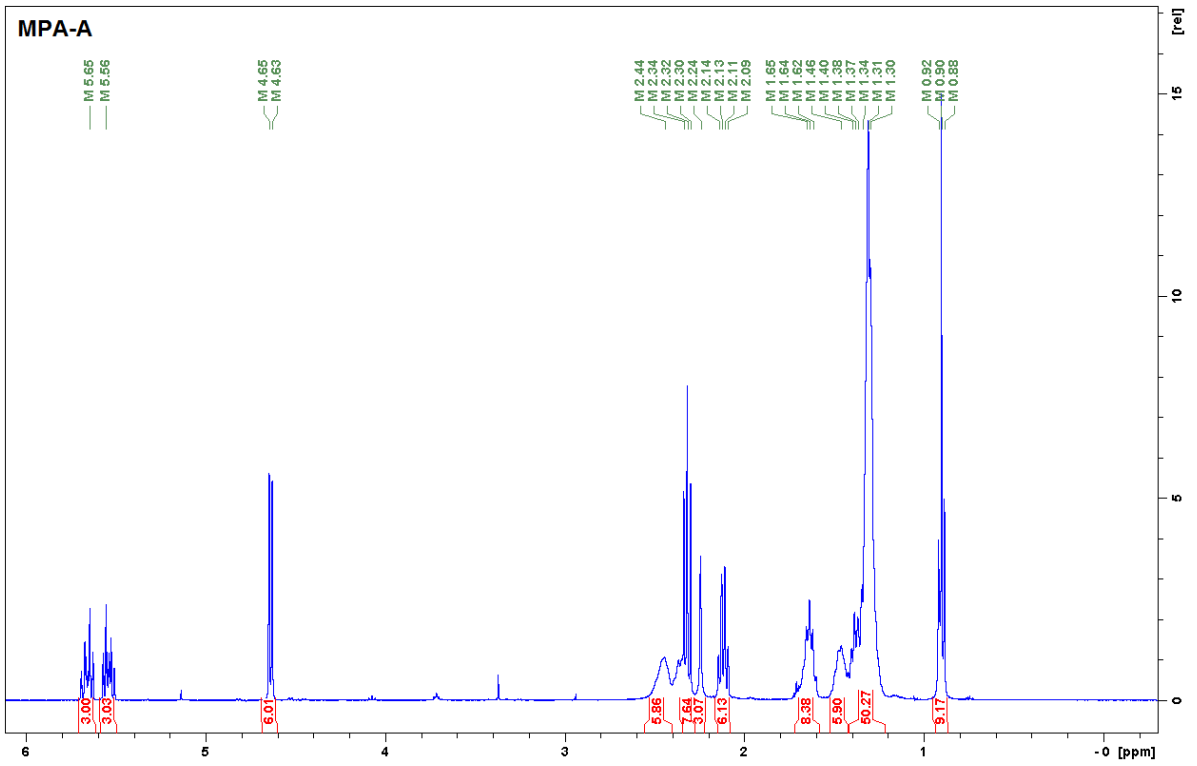

Figure S8. ^{1}H NMR of compound **MPA-A**.

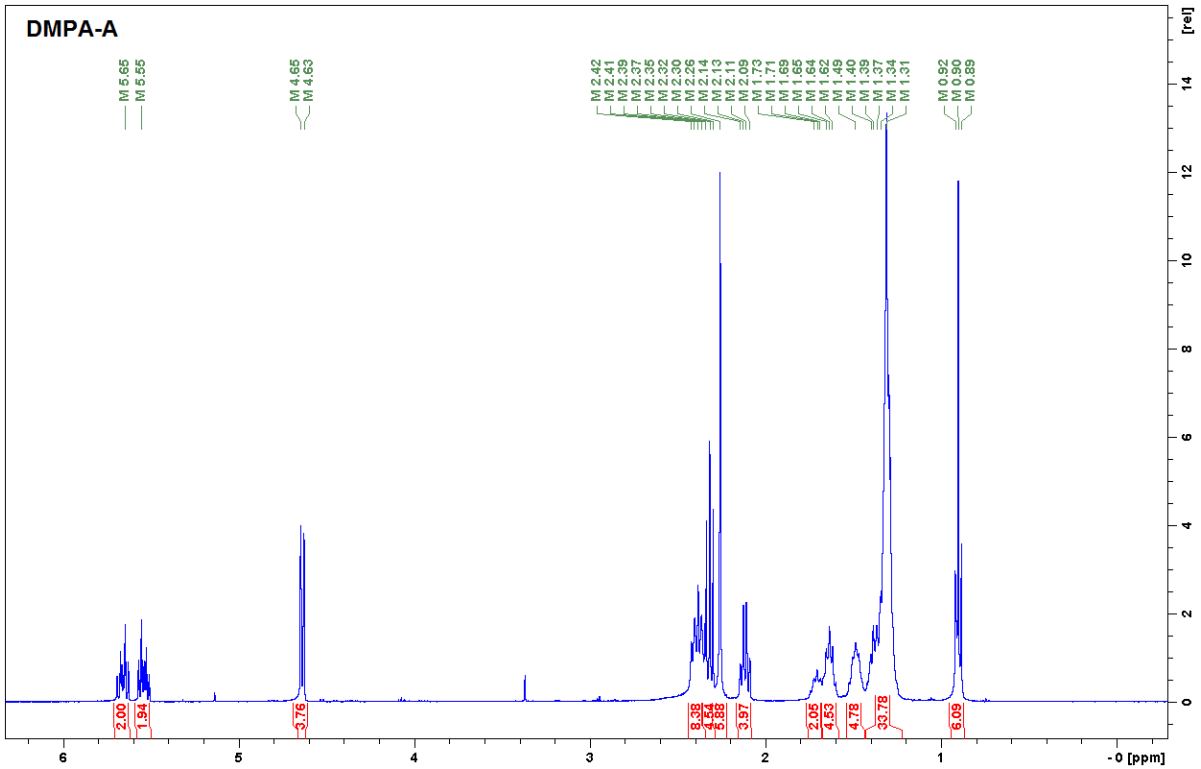

Figure S9. ^{1}H NMR of compound **DMPA-A**.

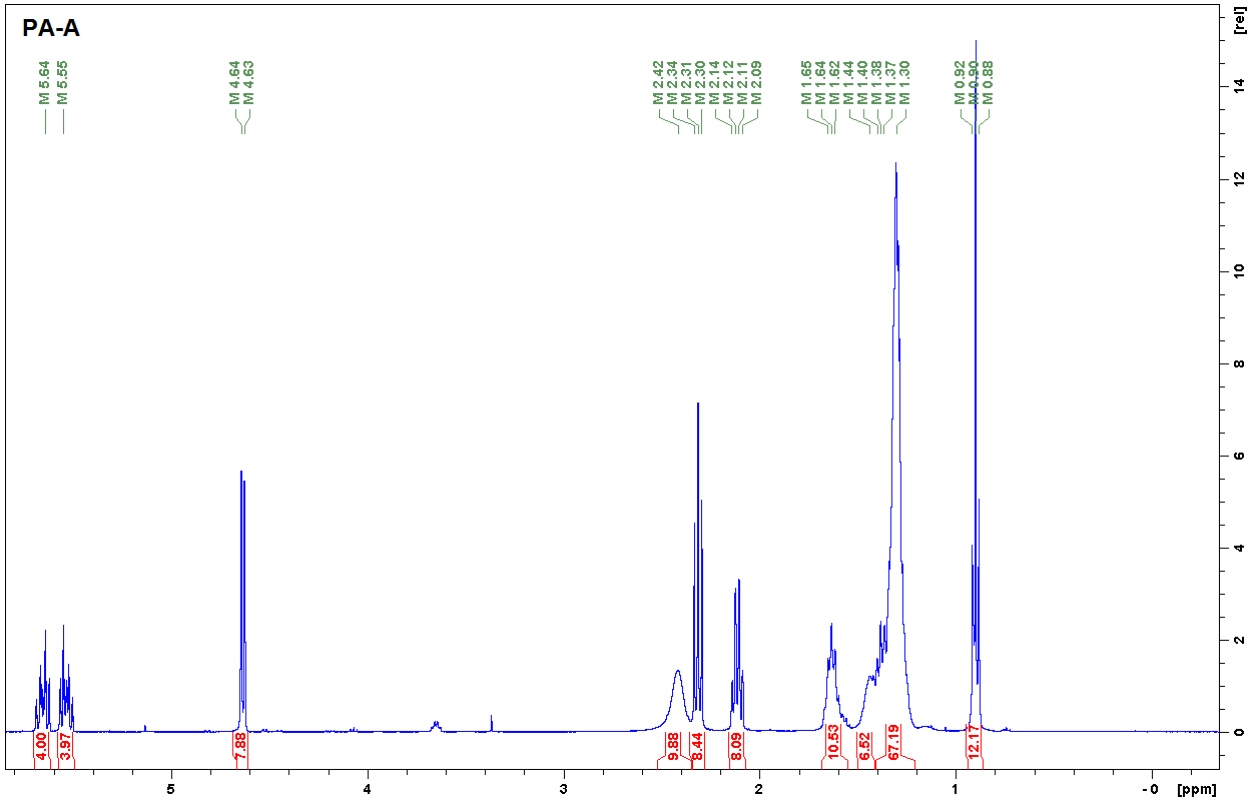

Figure S10. ^{1}H NMR of compound **PA-A**.

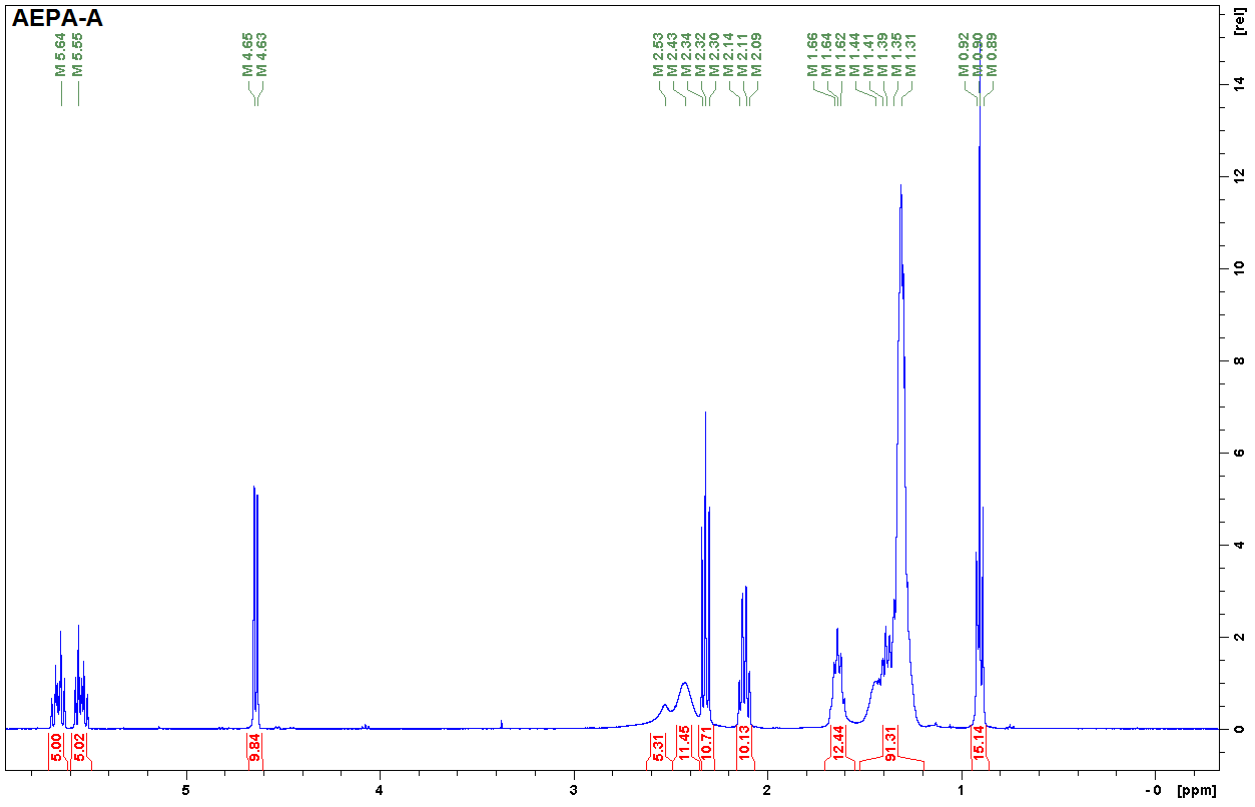

Figure S11. ^{1}H NMR of compound **AEPA-A**.

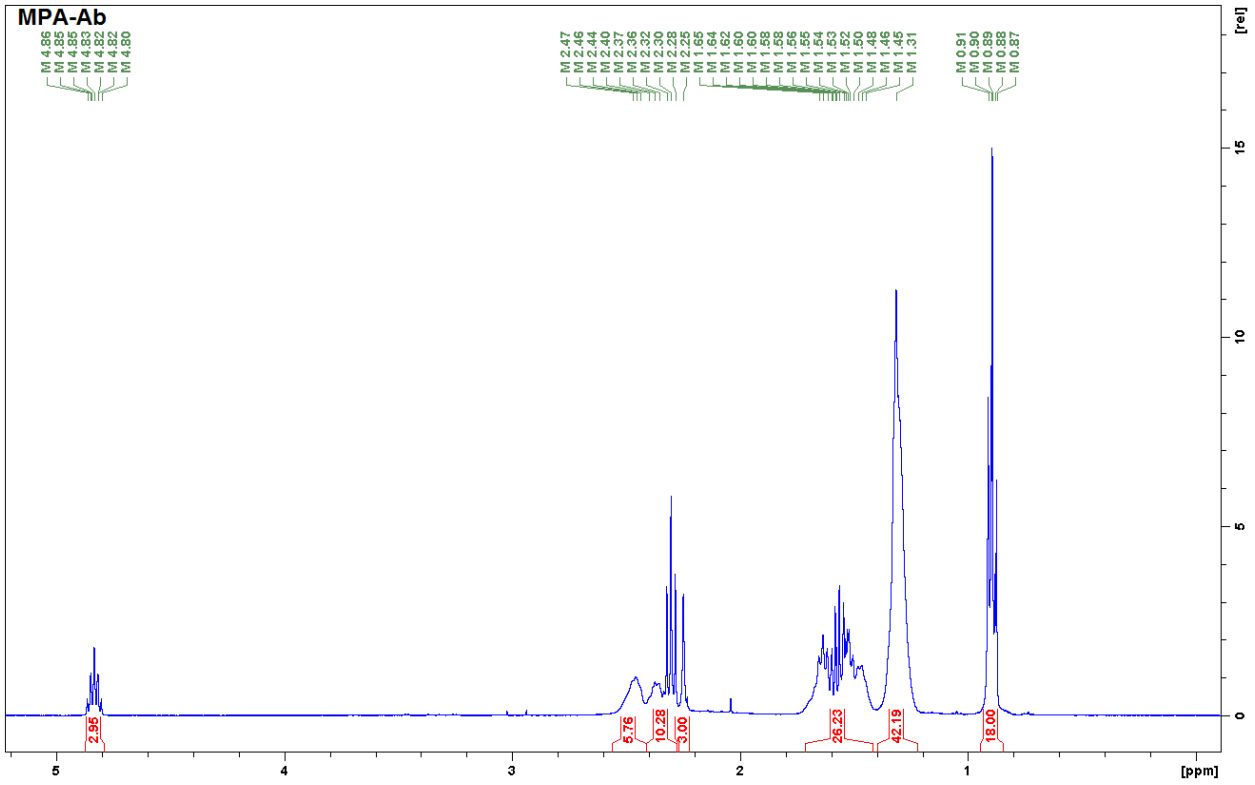

Figure S12. ^{1}H NMR of compound **MPA-Ab**.

Reference

(S1) Rajabi, M.; Lanfranchi, M.; Campo, F.; Panza, L. Synthesis of a Series of Hydroxycarboxylic Acids as Standards for Oxidation of Nonanoic Acid *Synth. Commun.* **2014**, *44*, 1149-1154.

(S2) Woodcock, S. R.; Marwitz, A. J. V.; Bruno, P.; Branchaud, B. P. Synthesis of Nitrolipids. All Four Possible Diastereomers of Nitrooleic Acids: (E)- and (Z)-, 9- and 10-Nitro-Octadec-9-Enoic Acids *Org. Lett.* **2006**, *8*, 3931-3934.