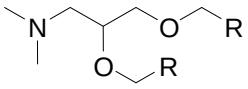
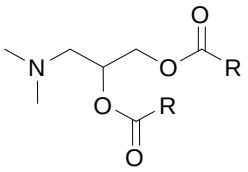
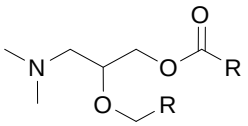
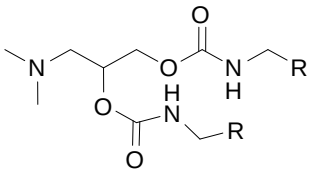
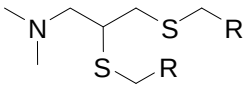
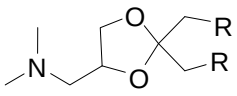
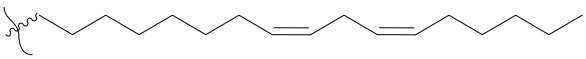
Rational design of novel cationic lipids for use in next-generation siRNA delivery systems

Supplementary Table 1. Linker modifications to DLinDMA

Abbreviated Name	Chemical Name	Structure	Mean Particle Size (nm)[a]	ED_{50} (mg/kg)
DLinDMA (Benchmark)	1,2-Dilinoleyloxy-3-dimethylaminopropane		71 ± 24	~1
DLinDAP	1,2-Dilinoleoyl-3-dimethylaminopropane		65 ± 21	40-50
DLin-2-DMAP	1-Linoleoyl-2-linoeyloxy-3-dimethylaminopropane		79 ± 26	5-12
DLin-C-DAP	1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane		73 ± 25	12-25
DLin-S-DMA	1,2-Dilinoleylthio-3-dimethylaminopropane		69 ± 25	12-25
DLin-K-DMA	2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane		67 ± 22	~0.4
R = 				

[a] Mean particle size of the PFV formulation as tested for FVII activity and containing the indicated cationic lipid at 40 mol%.

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Supplementary Syntheses 1

Synthesis of 1,2-Dilinoleyloxy-3-dimethylaminopropane (DLinDMA)

DLinDMA was synthesized according to the method previously published by Heyes et. al[15].

Synthesis of 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP)

DLinDAP was synthesized according to the method previously published by Bailey and Cullis[17].

1-Linoleoyl-2-linoeyloxy-3-dimethylaminopropane (DLin-2-DMAP)

*Synthesis of 1-Triphenylmethyloxy-3-(N,N-dimethylamino)-2-propanol (**I**)*

A mixture of 3-(dimethylamino)-1,2-propanediol (3.0 g, 25 mmol) and triphenylmethyl chloride (7.75 g, 27.8 mmol) in dry pyridine (100 mL) was refluxed for 30 minutes. Upon cooling, most of the solvent was evaporated in vacuo, and the resulting residual was re-dissolved in 400 mL of dichloromethane. The organic phase was washed with water (3 x 200 mL), then brine (150 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 6.3 g of yellow oil as a crude product. The crude product was purified by column chromatography on silica gel (230-400 mesh, 500 mL) eluted with 0-10% methanol gradient in dichloromethane. This afforded 4.0 g of **I** as yellow oil.

*Synthesis of 1-Triphenylmethyloxy-2-linoleyloxy-3-N,N-dimethylaminopropane (**II**)*

NaH (60%, 2.17 g, 54 mmol) was washed with hexanes (3 x 40 mL) under nitrogen and then suspended in anhydrous benzene (60 mL). To the suspension was added **I** (4.0 g, 11 mmol) dropwise in 20 mL of anhydrous benzene. The resulting mixture was stirred at room temperature for 20 minutes, then a solution of linoleyl methanesulfonate (4.5 g, 13 mmol) in 40 mL of anhydrous benzene was added dropwise under nitrogen. The mixture was stirred at room temperature for 30 minutes and then refluxed overnight. Upon cooling to room temperature, 30 mL of 1:1 (V:V) ethanol-benzene solution were added dropwise under nitrogen followed by 100 mL of benzene and 100 mL of water. Upon shaking, the aqueous phase was separated. The organic phase was washed with brine (2 x 100 mL) and dried over anhydrous sodium sulfate. Evaporation of the solvent afforded 6.8 g of yellowish oil. The crude product was

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chromatographed on a silica gel column (230-400 mesh, 400 mL) eluted with 0-3% methanol gradient in chloroform. 5.8 g (84%) of **II** were obtained as yellowish oil.

*Synthesis of 2-Linoleyloxy-3-(N,N-dimethylamino)-1-propanol (**III**)*

II (5.8 g, 9.2 mmoL) was refluxed in 80% HOAc (25 mL) under nitrogen for 10 minutes. Upon cooling to room temperature, the mixture was diluted with water (100 mL). The resulting aqueous solution was neutralized to about pH 6 with 0.5% NaOH solution. The aqueous phase was then extracted with dichloromethane (4 x 100 mL). The combined organic phase was washed with 0.1% NaOH solution (100 mL), water (100 mL), then brine (100 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 5.6 g of a mixture of product and starting material as yellowish oil. The mixture was chromatographed on a silica gel column (230-400 mesh, 400 mL) eluted with 0-10% methanol gradient in chloroform. 2.2 g (62%) of **III** were afforded as yellowish oil.

*Synthesis of 2-Linoleyoloxyl-3-linoleyloxyl-1-N,N-dimethylaminopropane (**DLin-2-DMAP**)*

To a solution of linoleic acid (2.36 g, 8.4 mmol) in anhydrous benzene (50 mL) was added dropwise oxalyl chloride (1.45 g, 11.4 mmol) under nitrogen. The resulting mixture was stirred at room temperature for 4 hours. Solvent and excess of oxalyl chloride was removed in vacuo to give linoleoyl chloride as light yellowish oil. This was re-dissolved in anhydrous benzene (85 mL). To the resulting solution was added dropwise a solution of **III** (2.9 g, 7.5 mmol) and dry pyridine (1 mL) in 15 mL of anhydrous benzene. The mixture was then stirred at room temperature under nitrogen for 2 days, resulting in a suspension. The mixture was diluted with benzene (100 mL). The organic phase was washed with a solution of 3:5 (V:V) ethanol-water (320 mL), then brine (2 x 75 mL), and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo affording 5.2 g of oil. The crude product was purified by column chromatography on silica gel (230-400 mesh, 450 mL) eluted with 0-4% methanol gradient in chloroform. This afforded 3.9 g (80%) of **DLin-2-DMAP** as yellowish oil. ^{1}H NMR (400 MHz, CDCl3) δ: 5.25 (8H, m, 4 x CH=CH), 4.17 (1H, dd, J = 11.6 and 4 Hz, OCH), 3.96 (1H, dd, J = 11.6 and 5.2 Hz, OCH), 3.53-3.64 (1H, m, OCH), 3.35-3.53 (2H, m, OCH2), 2.68 (4H, t, =CH-CH_2-CH=), 2.41 (2H, m, CH_2), 2.25 (6H, s, 2 x NCH_3), 2.21 (2H, m, CH_2), 1.96 (8H, q, allylic 4 x CH_2), 1.4-1.6 (4H, m, 2 x CH_2), 1.21 (30H, s, 15 x CH_2), 0.80 (6H, t, 2 x CH_3) ppm.

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1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP)

Preparation of Linoleyl Phthalimide

A mixture of potassium phthalimide (11.2 g, 59.5 mmol) and linoleyl methanesulfonate (9.3 g, 27 mmol) in 250 mL of anhydrous DMF was stirred at 70ºC under nitrogen overnight. The resulting suspension was poured into 500 mL of cold water. The aqueous phase was extracted with EtOAc (3 x 200 mL). The combined extract was washed with water (200 mL), then brine (200 mL), and dried over anhydrous Na_2SO_4. Solvent was evaporated to give a mixture of solid and oily materials. To the mixture was added 300 mL of hexanes. The solid was filtered and washed with hexanes (2 x 25 mL). The filtrate and washes were combined, and the solvent was evaporated to result in 11 g of Linoleyl Phthalimide, which was used in the next step without further purification.

Preparation of Linoleylamine

The above crude linoleyl phthalimide (11 g, ca. 27 mmol) and hydrazine (10 mL) were refluxed in 350 mL of ethanol under nitrogen overnight. The resulting white solid was filtered upon cooling the mixture to about 40 to 50ºC and the solid was washed with warm EtOH (2 x 30 mL). The filtrate and washes were combined and solvent evaporated. To the residual was added 400 mL of chloroform which resulted in precipitation of white solid. The solid was filtered again. The organic phase of the resulting filtrate was washed with water (2 x 100 mL), then brine (100 mL), and dried over anhydrous Na_2SO_4. Solvent was removed in vacuo to afford 7.3 g of yellow oil as a crude product. Pure linoleylamine was obtained by column chromatography on silica gel eluted with 0-20% methanol gradient in chloroform.

Preparation of Linoleyl Isocyanate

Anhydrous sodium carbonate (11g g) was suspended in a solution of linoleylamine (7.3 g, ca. 27 mmol) in anhydrous CH_2Cl_2 (200 mL) under good stirring and nitrogen. The suspension was cooled to 0 to 5ºC with an ice bath. To the suspension was added diphosgene (8.2 g, 41 mmol) in 10 mL of anhydrous CH_2Cl_2 under vigorous stirring. Upon addition, the resulting suspension was stirred at 0 to 5ºC under nitrogen for 60 minutes and then at room temperature for 2 hours. Upon completion of the reaction, 100 mL of water was added to the mixture and the mixture was stirred at room temperature for 30 minutes. The organic layer was separated, and washed with

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water (100 mL) then brine (100 mL). After drying with anhydrous Na_2SO_4, the solvent was evaporated to give 7.6 g of yellow oil as a crude product, which was used in the following step without further purification.

1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP)

To a solution of crude linoleyl isocyanate (7.6 g, ca. 25 mmol) in 150 mL of anhydrous benzene under nitrogen was added dropwise a solution of 3-(dimethylamino)-1,2-propanediol (0.99 g, 8.3 mmol) in 20 mL of anhydrous benzene. The resulting mixture was stirred at room temperature for 60 minutes and then refluxed for 4 hours, followed by stirring at room temperature overnight. Upon dilution of the mixture with 150 mL benzene, the organic phase was washed with water (3 x100 mL), then brine (100 mL), and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave 8.4 g of yellow oil. Column purification of the oily material (500 mL silica gel, 230-400 mesh, eluted with 0-3% methanol gradient in chloroform) afforded 2.2 g (38%) of yellowish oil as the product **DLin-C-DAP**. ^{1}H NMR (400 MHz, CDCl3) δ: 5.37 (8H, m, 4 x CH=CH), 5.06 (1H, br. CONH), 4.91 (1H, br. CONH), 4.79 (1H, m, OCH), 4.28 (1H, br. d, J = 11 Hz, OCH), 4.16 (1H, dd, J = 12 and 6 Hz, OCH), 3.16 (4H, m, 2 x NCH_2), 2.77 (4H, t, J = 6.4 Hz, =CH-CH_2-CH=), 2.4-2.7 (2H, m, NCH_2), 2.33 (6H, s, 2 x NCH_3), 2.05 (8H, m, allylic 4 x CH_2), 1.4-1.55 (4H, m, 2 x CH_2), 1.29 (40H, s, 20 x CH_2), 0.89 (6H, t, 2 x CH_3) ppm.

1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA)

Synthesis of Linoleylthio Acetate

First linoleyl mercaptane was synthesized. To a solution of triphenylphosphine (18.0g, 68.2 mmol) in 250 mL of anhydrous THF under nitrogen at 0-5ºC was added dropwise diisopropyl azodicarboxylate (DIAD, 14.7 mL, 68 mmol). Upon addition, the resulting mixture was stirred at 0 to 5ºC for 45 minutes. A yellow suspension was resulted. A solution of linoleyl alcohol (9.1g, 34 mmol) and thiolacetic acid (5.1 mL, 68 mmol) was then added at 0 to 5ºC dropwise over 30 minutes to the yellow suspension under nitrogen. The resulting mixture was stirred at 0 to 5ºC for 1 hour and then allowed to warm up to room temperature. After stirring at room temperature for 60 minutes, a brown solution was obtained. After solvent evaporation the residual was re-dissolved in 600 mL of ether. The ether phase was washed with water (2 x 250 mL), then brine (250 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated to afford 31g of brown

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