Supporting Information

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SI Text

General Methods for Chemical Synthesis and Analysis. Reagents and anhydrous solvents were purchased from commercial sources and were used as received. Epoxide lipid synthesis was performed in capped glass vials under room air; other reactions were performed in oven-dried glassware under argon unless otherwise noted. Flash chromatography was performed using an automated purification system (Combiflash®, Teledyne Isco) over silica gel (40–63 μm particle size). NMR spectra were obtained on a 600 MHz (^{1}H) spectrometer; chemical shifts are reported in parts per million (ppm) on the δ scale, and were referenced to residual protonated solvent peaks. Spectra obtained in chloroform-*d* were referenced to $CHCl_3$ at δ_H 7.27 and $CDCl_3$ at δ_C 77.2.

Mass spectra were obtained on a Bruker Daltonics Omniflex MALDI-TOF mass spectrometer. 2,5-Dihydroxybenzoic acid (2,5-DHB) was used as matrix for the epoxide lipids (20–25 mg of 2,5-DHB into 1 mL of 60:40 MeOH/H_2O). Epoxide lipid stock solutions were prepared by dissolving 1–2 mg of the lipid in 1 mL of MeOH containing approximately 250 μL CH_2Cl_2; 12 μL of the stock solution was added to 100 μL of matrix solution. Mass spectra were calibrated using an external reference (ProteoMass™ Peptide MALDI-MS Calibration standards, Sigma-Aldrich) with α-cyano-4-hydroxycinnamic acid as matrix.

In Vitro Transfection Assay. 24 h prior to transfection, HeLa cells stably expressing firefly and Renilla luciferase were seeded in opaque white 96-well plates in growth medium. Cells were seeded at a density of 15,000 cells per well and incubated overnight at 37°C in 5% CO_2 to allow cell attachment. For transfection, working dilutions of lipidoids were prepared in 25 mM sodium acetate (pH 5) at concentrations necessary to yield weight ratios of 2.5:1, 5:1, 10:1, and 15:1, lipidoid:siRNA. To form lipidoid:siRNA complexes, 25 ul of lipidoid solution was mixed with 25 ul of 5ug/ml siRNA in a 96-well plate using a multichannel pipette. Mixtures were allowed 20 minutes incubation at room temperature for complex formation. After incubation, 30 ul of the complexes were then diluted in 200 ul growth media, of which 150 ul was transferred to cells after aspiration of spent media. Transfection with Lipofectamine 2000 was performed as described by the vendor and served as a control. Complexes were allowed 24 h incubation with cells prior to analysis for luciferase expression. Firefly and Renilla luciferase expression was analyzed using the Dual-Glo assay (Promega) as described by the vendor. Luminescence was measured using a Victor3 luminometer (Perkin Elmer). For data analysis, firefly expression was normalized by Renilla expression and treated groups were compared to untreated cells alone to determine degree of luciferase silencing.

In Vitro Non-Human Primate Experiments. All procedures using cynomolgus monkeys were conducted by a certified contract research organization using protocols consistent with local, state, and federal regulations, as applicable, and approved by the IACUC. Cynomolgus monkeys (*n* equals 3 per group) received either PBS or 0.03, 0.1, or 0.3 mg/kg siTTR formulated in C12-200 as 15-min intravenous infusions (5 mL/kg) via the cephalic vein. An ultrasound-guided liver biopsy was taken from each animal at 48 h post-administration and snap-frozen in liquid nitrogen. *TTR* mRNA levels, relative to *GAPDH* mRNA levels, were determined in ground liver samples using a branched DNA assay (QuantiGene Assay, Affymetrix, CA) (14).

Nucleic Acids. Luciferase GL3 siRNA (Dharmacon) was used for *in vitro* silencing experiments. For *in vivo* studies, siRNAs were produced by Alnylam. All siRNAs were tested negative for activation of the immune system (specifically, IFN-α and TNF-α) in primary human blood monocytes. Sequences can be found in *SI Text*. All siRNAs were synthesized by Alnylam and were characterized by electrospray mass spectrometry and anion exchange HPLC. The sequences for the sense and antisense strands of the TTR siRNA is as follows:

siLuc sense: 5'-CUUACGCUGAGUACUUCGATT-3', antisense: 5'-UCGAAGUACUCAGCGUAAGTT-3'

siFVII sense: 5'-GGAUfCfAUfCfUfCfAAGUfCfUfUfACfdTsdT-3', antisense: 5'-GUfAAGACfUfUfGAGAUfGAUfCfCfdTsdT-3'

siPCSK9 sense: 5'-GccuGGAGuuuAuucGGAAdTsdT-3', antisense: 5'-UUCCGAAuAAACUCcAGGCdTsdT-3'

siXBP-1 sense: 5'-cAcccuGAAuucAuuGucudTsdT-3', antisense: 5'-AGAcAAUGAAUUcAGGGUGdTsdT-3'

siApoB sense: 5'-GGAAUCuuAuAuuuGAUCcAsA-3', antisense: 5'-uuGGAUcAAAuAuAAGAuUCcscsU-3'

siSort1 sense: 5'-AuGuGAuuAAGuucuccAcdTsdT-3', antisense: 5'-GUGGAGAACUuAAUcAcAUdTsdT-3'

GFP-Alexa647 sense: 5'-AcAuGAAGcAGcACGACuUdTsdT-3', antisense:5'-AAGUCGUGCUGCUUCAUGUdTdT-3'-Alexa647

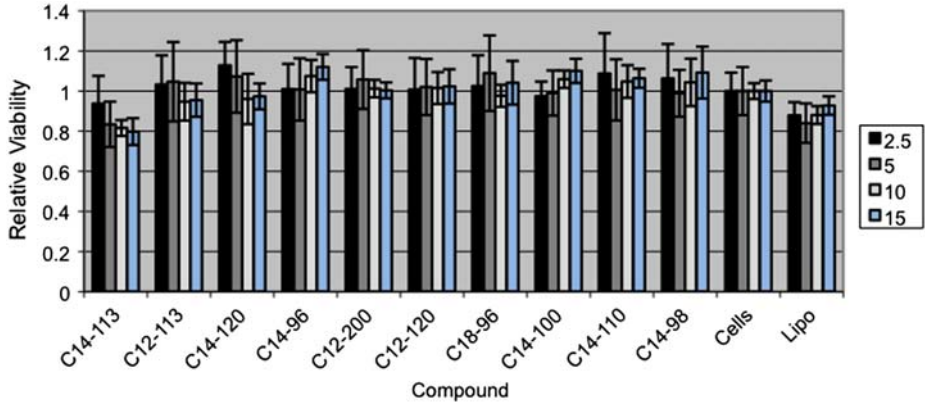


Fig. S1. *In vitro* cytotoxicity assay of top 10 performing lipidoids. Viability of HeLa cells after transfection with lipidoid:siRNA complexes at varying w:w ratios as measured by MTS assay. Relative to untreated cells, minimal toxicity is observed 24 h after transfection.

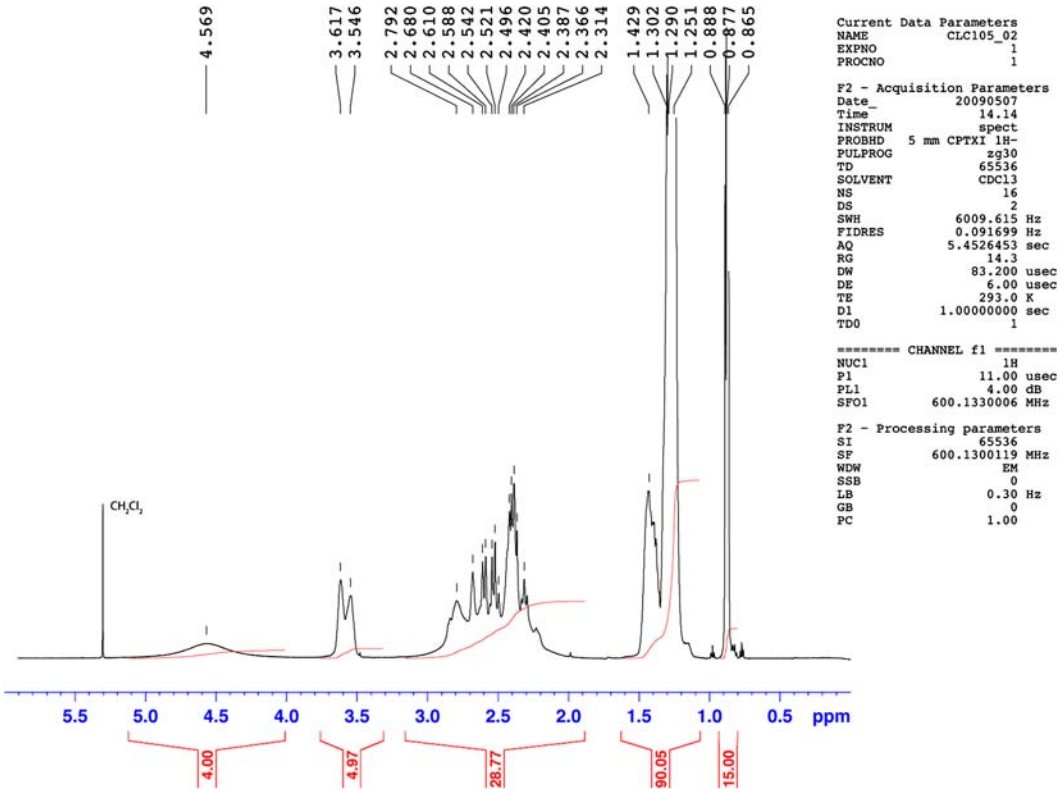
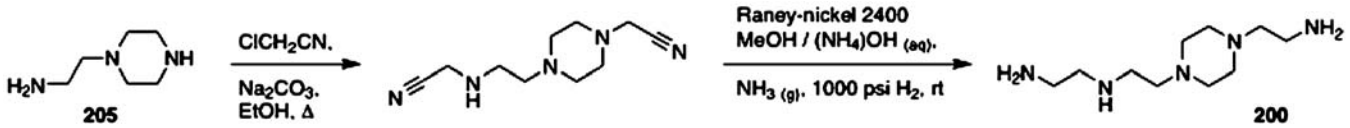
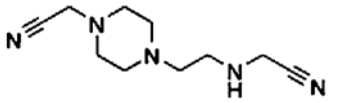


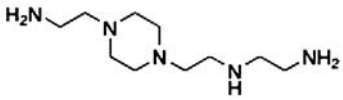
Fig. S2. Room temperature 600 MHz ^{1}H NMR spectrum of C12-200 in $CDCl_3$.



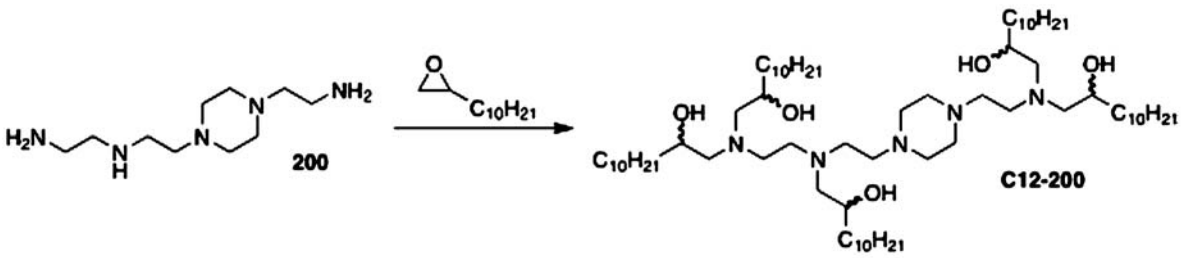
Preparation of amine 200 and C12-200. Scheme 1. Synthesis of amine 200.



2-(2-(4-(cyanomethyl)piperazin-1-yl)ethylamino)acetonitrile. Anhydrous ethanol (150 mL) was added to an oven dried 250 mL round bottom flask containing a magnetic stirbar. 1-(2-aminoethyl)piperazine (205, 4.00 mL, 30.4 mmol) was added to the ethanol using a syringe. Chloroacetonitrile (4.25 mL, 67.2 mmol, 2.2 equiv) was added to the stirred ethanolic solution slowly. Anhydrous sodium carbonate (12.9 g, 122 mmol, 4 equiv) was added to the mixture in one portion. The round bottom flask was fitted with a reflux condenser with drying tube and submersed in an oil bath; the solution was heated to reflux with vigorous stirring. After 7 h, the reflux condenser was removed from the flask, and the flask was removed from the oil bath. A portion of decolorizing charcoal was added cautiously to the reaction mixture. The hot suspension was stirred briefly and then gravity filtered through paper. The yellow filtrate was concentrated by rotary evaporation affording an orange oil that crystallized. The crude product was purified by chromatography on silica gel (gradient elution from CH_2Cl_2 to 9:1 CH_2Cl_2/MeOH). Fractions containing the desired product were pooled and concentrated by rotary evaporation; residual solvent was removed under reduced pressure affording the nitrile (3.7 g, 58%) as a pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.57 (s, 2H), 3.47 (s, 2H), 2.74 (t, $J = 5.4$ Hz, 2H), 2.56 (t, Hz, 4H), 2.48 (t, $J = 5.4$ Hz, br s, overlapped, 6H), 1.81 (br s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 118.2, 114.9, 57.0, 52.6, 51.9, 46.1, 45.1, and 37.6.



***N*^{1}-(2-(4-(2-aminoethyl)piperazin-1-yl)ethyl)ethane-1,2-diamine (200).** 2-(2-(4-(cyanomethyl)piperazin-1-yl)ethylamino)acetonitrile (500 mg, 2.41 mmol) was added to a 100 mL oven dried round bottom flask containing a magnetic stir bar. MeOH (25 mL) and concentrated aqueous NH_4OH (3 mL) were added to the flask. Raney®-Nickel-2400 catalyst (approximately 250 mg, in water) was transferred into a small pipette plugged with cotton. The catalyst was washed sequentially with water and MeOH (as the dried catalyst is flammable in air, pressurized Ar gas was used to force solvent through the pipette). The catalyst was transferred into the reaction solution, and the flask was placed into a stainless steel high pressure vessel (Parr apparatus); the pressure vessel was assembled. The vessel was charged with H_2 gas to 500 psi, then vented. This was repeated once. Stirring was activated, and the vessel was filled with H_2 to 1,000 psi; this pressure was maintained for 6 h by occasional addition of H_2. The pressure in the vessel was released slowly and the reaction mixture was filtered through paper. The filtrate was concentrated by rotary evaporation affording a bluish-green oil. This oil was dissolved in 50 mL EtOH; $HCl_{(g)}$ was bubbled into the solution causing the formation of an off-white precipitate. The precipitate (the HCl salt of amine 200) was filtered and dried under reduced pressure. This solid was dispersed in $CHCl_3$ by sonication. $NH_{3(g)}$ was bubbled into the $CHCl_3$ solution; the amine salt dissolved; this was accompanied by formation of solid NH_4Cl. The $CHCl_3$ solution was filtered; the filtrate was concentrated by rotary evaporation affording a pale yellow oil (300 mg). This material was used in the following reaction without further purification. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.82–2.77 (m, 4H), 2.74–2.67 (m, 4H), 2.50 (t, $J = 9.3$ Hz, overlapped with br s, 8H), 2.42 (t, $J = 9.3$ Hz, 4H), 1.32 (s, 5H).



Scheme 2. Synthesis of C12-200 from amine 200. C12-200. 1,2-epoxydodecane (2.10 g, 11.4 mmol, 7 equiv) was added to a 4 mL vial containing crude amine 200 (300 mg) and a magnetic stir bar. The vial was sealed and warmed to 80 °C. The reaction mixture was stirred for 2 days, whereupon analysis of the reaction mixture by MALDI-TOF spectroscopy suggested that the crude reaction mixture contained a significant amount of the desired product (MALDI-TOF-MS *m/z* calcd for $C_{70}H_{146}N_5O_5$ $[M + H^+]$ 1,137, found 1,137). The crude mixture was purified by chromatography on silica gel (gradient elution from CH_2Cl_2 to 87.5:11.0:1.5 CH_2Cl_2/MeOH/NH_4OH (aq.) affording C12-200 (668 mg, 42% over two steps) as a pale yellow viscous oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.00–4.00 (br s, 5H), 3.62 and 3.55 (apparent br s, 5H), 3.00–2.00 (m, 30H), 1.52–1.25 (m, 90H), 0.87 (t, $J = 7.2$ Hz, 15H).

Table S1. Summary of particle sizing data for formulated lipidoid particles used for in vivo screening as measured by dynamic light scattering

Lipidoid	Mean Particle Diameter	Polydispersity
C14-100	251.2–8.4	0.224–0.047
C14-96	126.1–0.5	0.243–0.017
C12-120	65.1–0.2	0.128–0.023
C12-113	67.7–0.4	0.147–0.021
C18-62	167.4–5.9	0.142–0.070
C14-98	250.6–16.1	0.227–0.045
C18-96	252.2–5.9	0.178–0.024
C14-113	135.7–1.0	0.184–0.016
C14-120	135.5–5.6	0.227–0.015
C14-110	236.2–1.1	0.162–0.080
C16-96	181–2.1	0.117–0.027
C12-200	140–1.9	0.242–0.012

Table S2. A summary of the calculated and observed *m/z* ratios in the MALDI-TOF spectra of a selection of crude reaction mixtures

			m/z: [*N*] tails		*m/z*: [*N* – 1] tails		*m/z*: [*N* – 2] tails		
Amine	Epox.	*N*[a]	calcd.	observed	calcd.	observed	calcd.	observed	other
114	C_{14}	5	1165.2	1165.6	953.0	953.4, 975.4(M + Na^+)	740.8	741.0	
113	C_{12}	4	854.9	855.2, 877.2 (M + Na^+)	670.7	670.9, 692.8(M + Na^+)	486.5	486.5, 508.5 (M + Na^+)	
120	C_{12}	4	885.9	886.2, 908.1 (M + Na^+)	701.7	701.9, 723.8(M + Na^+)	517.5	517.5	
98	C_{16}	6	1588.6	1590.3(trace)	1348.4	1349.0, 1371.0(M + Na^+)	1108.1	1108.6	868.3[*N* – 3]
103	C_6	2	349.3	349.1, 371.1(M + Na^+)	249.2	248.8, 270.8(M + Na^+)			
120	C_{10}	4	773.7	774.1, 796.0(M + Na^+)	617.6	617.8, 639.7(M + Na^+)	461.4	461.5(trace)	
103	C_{12}	2	517.5	517.6, 539.5(M + Na^+)	333.3	333.1, 355.1(M + Na^+)			
100	C_{14}	4	924.0	924.3	711.7	712.0	499.5	499.6	
63	C_{18}	2	639.7	639.8, 661.8(M + Na^+)	371.4	371.2			
109	C_{12}	3	657.7	657.8, 679.8(M + Na^+)	473.5	473.5, 495.4(M + Na^+)	289.3	289.0	

The *m/z* ratios are reported as ($M + H^+$) unless otherwise noted. Notes: (*A*) *N* equals maximum number of epoxide tails for the given amine ([number of 2° amines] plus [2 times number of 1° amines] in the polyamine starting material).

Table S3. A summary of the calculated and observed *m/z* ratios in the MALDI-TOF spectra of a selection of the epoxide lipids following column purification

				m/z: [*N*] tails		*m/z*: [*N* – 1] tails		*m/z*: [*N* – 2] tails		
Amine	Epox.	A/B	*N*	calcd.	observed	calcd.	observed	calcd.	observed	other
96	C_{14}	B	3	725.8		513.5	513.51	301.3		
120	C_{12}	A	4	885.9	886.14, 908.11 (M + Na^+)	701.7		517.5		
120	C_{12}	B	4	885.9		701.68	701.92, 723.91 (M + Na^+)	517.5		
96	C_{18}	B	3	893.9	894.26, 916.3 (M + Na^+)	625.66		357.4		
100	C_{14}	A	4	924.0	924.4, 946.3 (M + Na^+)	711.7		499.5		
100	C_{14}	B	4	924.0		711.7		499.5	499.5, 521.5 (M + Na^+)	
114	C_{16}	B	5	1305.3		1065.1	1065.5, 1087.5 (M + Na^+)	824.9	825.2, 847.2 (M + Na^+)	
114	C_{14}	B	5	1165.2		953.0	953.3, 975.3 (M + Na^+)	740.8	741.0, 763.0 (M + Na^+)	
98	C_{14}	A	6	1420.4		1208.2	1208.6, 1231.6 (M + Na^+)	996.0	996.3	
98	C_{14}	B	6	1420.44	1421.0, 1444.0 (M + Na^+)	1208.2	1208.7, 1230.7 (M + Na^+)	996.0		
98	C_{14}	B	6	1420.4		1208.23		996.02	996.4, 1018.5 (M + Na^+)	[*N* – 3]: 784.1
63	C_{18}	A	2	639.7	639.8, 661.7 (M + Na^+)	371.4				
63	C_{18}	B	2	639.7	639.8, 661.7 (M + Na^+)	371.4	371.2, 387.1 (M + Na^+)			
113	C_{14}	B	4	967.0	967.3	754.8	755.2, 777.1 (M + Na^+)	542.6		
113	C_{12}	B	4	854.8	855.3, 877.3 (M + Na^+)	670.7	671.1, 693.1 (M + Na^+)	486.5		
120	C_{14}	A	4	998.0	998.4, 1020.4 (M + Na^+)	785.8		573.6		
120	C_{14}	A	4	998.0		785.8	786.3, 808.2 (M + Na^+)	573.6	573.4(trace)	
110	C_{14}	B	6	1420.4		1208.2		996.0	996.6, 1018.5 (M + Na^+)	
110	C_{14}	B	6	1420.4	1420.7	1208.2	1208.7	996.0		
120	C_{16}	B	4	1110.1		869.9	870.4, 892.3 (M + Na^+)	629.6	629.9	

The *m/z* ratios are reported as ($M + H^+$) unless otherwise noted. The expected *m/z* ratios are shaded in the table. "A": fractions enriched in those less polar compounds expected to have [*N*] tails. "B": fractions enriched in more-polar compounds expected to have [*N* – 2] tails.