Supporting Information

Combinatorial Synthesis of a Lipidoid Library by Thiolactone Chemistry: *In Vitro* Screening and *In Vivo* Validation for siRNA Delivery

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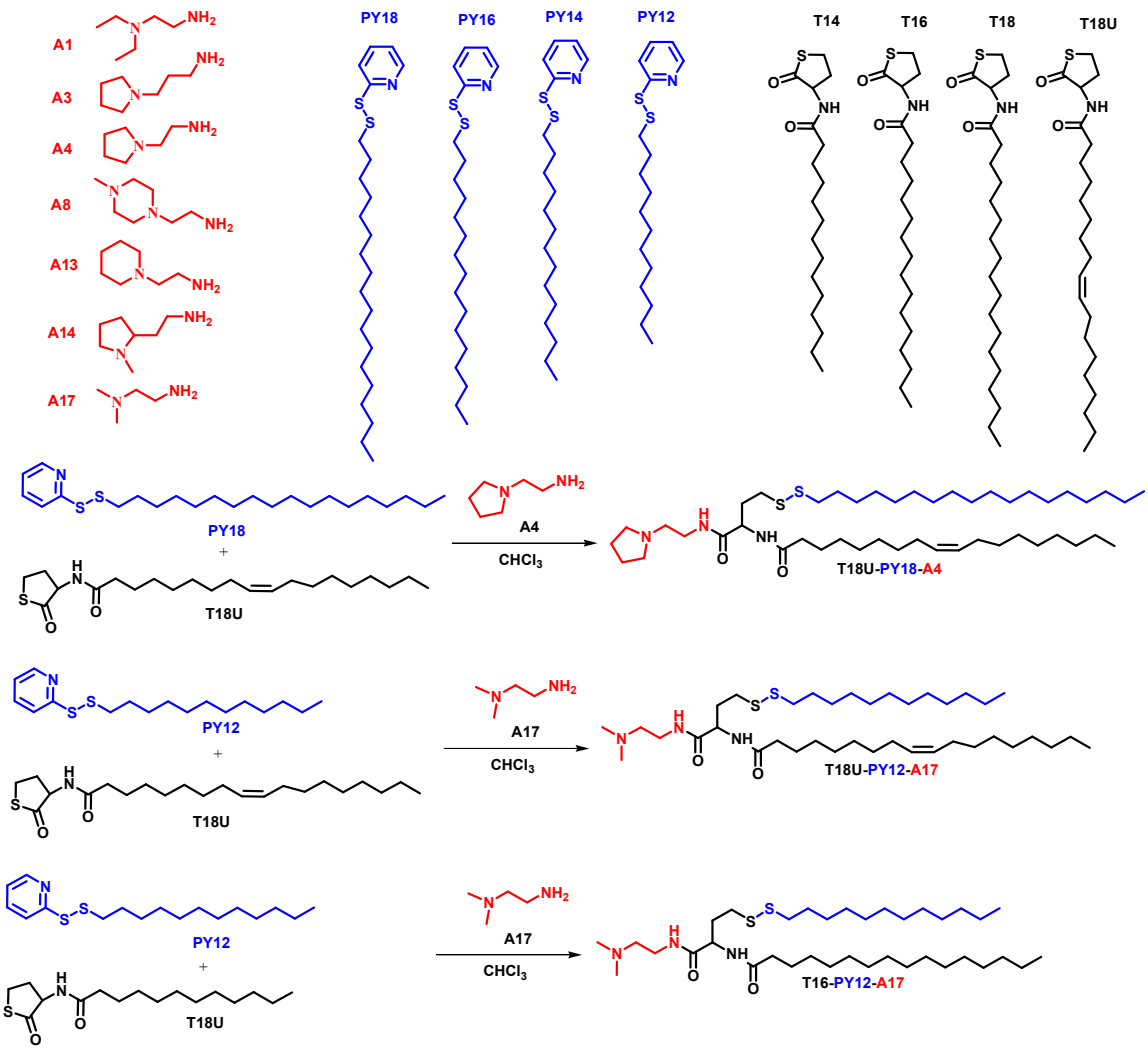
Synthesis and characterization of lipidoids

All the reagents were purchased from commercial source and used as such without further purification. ^{1}H NMR spectra were recorded on a DRX-500 MHz NMR spectrometer (Bruker GmbH, Germany) and all the spectra were calibrated against tetramethylsilane (Sigma-Aldrich Chemie GmbH, Germany). MALDI spectra were determined with a 4800 MALDI TOF/TOF Mass spectrometer (Applied Biosystems, CA, USA) in 'positive ion, reflector mode'.

Synthesis of Precursor: Synthetic protocol of all the precursors were taken from our previous report.[28]

Synthesis of Lipidoid: All the lipidoids are synthesized by same synthetic protocol. We have discussed here the synthesis of all top three lipidoids T18U-PY18-A4, T18U-PY12-A14 and T16-PY12-A17. The structure of precursor and top 3 lipidoids are given in the next page.



Scheme S1: Top: Structure of the amine (red), pyridyl disulfide (blue) and thiolactone precursor (black)

Synthesis of Lipidoid, T18U-PY18-A4: At first, amine **A4** (60µl, 0.469 mmol) was added to the solution of **T18U** (30mg, 0.0786 mmol) and **PY18** (32.5 mg, 0.0826 mmol) in $CHCl_3$. Then**,** the reaction mixture was stirred at room temperature for 6 hours. The reaction was stopped and $CHCl_3$ was evaporated to get a crude product as light green oil. Then 4 ml methanol was added to it and allowed to stand for 30 minutes. The methanol was decanted to get pure product as white solid.

MALDI-ToF MS Analysis: m/z calcd for $C_{46}H_{90}N_3O_2S2$ $[M+H]^+$: 780.6476; found: 780.5621

Synthesis of Lipidoid, T18U-PY12-A17: The amine **A17** (43µl, 0.471 mmol) was added to the solution of **T18U** (30mg, 0.0786 mmol) and **PY12** (25.8 mg, 0.0826 mmol) in $CHCl_3$. The reaction mixture was stirred at room temperature for 6 hours. The reaction was stopped and $CHCl_3$ was evaporated to get a crude product as greenish oil. Then 4 ml methanol was added to it and allowed to stand for 30 minutes. The methanol was decanted to get pure product as off-white solid.

MALDI-ToF MS Analysis: m/z calcd for $C_{38}H_{76}N_3O_2S2$ $[M+H]^+$: 670.5381; found: 670.5350

Synthesis of Lipidoid, T16-PY12-A17: The amine **A17** (43 µl, 0.471 mmol) was added to the solution of **T16** (30mg, 0.0782 mmol) and **PY12** (25.6 mg, 0.0821 mmol) in $CHCl_3$. The reaction mixture was stirred at room temperature for 6 hours. The reaction was stopped and $CHCl_3$ was evaporated to get a crude product as light greenish oil. Then 4 ml methanol was added to it and allowed to stand for 30 minutes. The methanol was decanted to get pure product as white solid.

MALDI-ToF MS Analysis: m/z calcd for $C_{36}H_{74}N_3O_2S2$ $[M+H]^+$: 644.5224; found: 644.5420

MALDI-TOF analysis for Lipidoid: The matrix solution and samples were spotted on Opti-TOF 384 well MALDI plate [123x81mm] (AB Sciex GmbH, Germany) made from stainless steel. The sample preparation for MALDI analysis is discussed below:

i) The compound 4-hydroxy cinnamic acid (α-CHCA) (10mg/ml) was used as a matrix and the solution was prepared in (acetonitrile:water) ACN: H_2O (1:1, v/v) and 0.1% TFA

(trifluoroacetic acid). Then 0.5 µl aliquot was pipetted on the required number of spots on a stainless steel MALDI plate. The matrix solution was then allowed to dry under nitrogen flow.

ii) 1 mg of sample was dissolved in 300 µl solvent to make a stock solution. Then 0.5 µl aliquot was spotted onto MALDI plate over previously spotted dried α-CHCA matrix. The second layer was allowed to dry under nitrogen flow.

iii) The MALDI plate was then used to analyze the MASS. MALDI spectra were measured with the MALDI TOF/TOF Mass spectrometer in 'positive ion, reflector mode' and a mass range of 600 to 1000 Da. The initial laser intensity was 3000 and later adjusted to get optimum spectra peaks. All the spectra were acquired using 4000 series Explorer ™ Software and data evaluation was carried out using Data Explorer Software 4.0 (Applied Biosystems). The experiments were performed in duplicates to be sure of the reproducibility of the data.

NMR Characterization: ^{1}H-NMR spectra were recorded at room temperature on the Bruker DRX-500 (500 MHz). Unless stated otherwise, all spectra were recorded in deuterated chloroform purchased from Sigma-Aldrich Chemie GmbH, Germany. All chemical shifts are given in ppm (δ) units relative to tetramethylsilane (singlet δH = 0.00). Calibration was achieved using the residual solvent signal of chloroform at δH = 7.27. ^{13}C NMR spectra were recorded as ^{1}H-decoupled spectra at room temperature on the Bruker DRX-500 (125 MHz). Spectra were calibrated to the signal of $CDCl_3$ (central line of triplet δC = 77.00).

Additional Figures:

Lipidoid	Hydrodynamic diameter of liposome (nm)	Surface charge of liposome (mV)	Hydrodynamic diameter of lipoplex (nm)	Surface charge of lipoplex (mV)	Knockdown Efficiency (%)
T18U-PY16-A1	110	+62	250	+15	38
T18U-PY18-A4	143	+45.5	113	+44	68
T18U-PY12-A17	130	+20	137	+63	66
T18U-PY12-A8	96	+63	98	+70	25
T18-PY12-A3	20	+71	52	+68	50
T14-PY12-A8	49	+68	56	+70	45
T14-PY16-A14	164	+44	136	+56	25
T14-PY16-A13	126	+56.5	64	+54	54
T14-PY16-A17	290	+43	734	+42	30
T14-PY14-A13	62	+56	69	+46	25
T14-PY14-A17	37	+60	34	+51	39
T16-PY12-A17	39	+45	37	+60	70
T16-PY12-A81	65	+70	51	+67	50

Table S1. Hydrodynamic diameter and surface charge of liposome and lipoplex. Knockdown efficiencies are displayed in the right-most column.

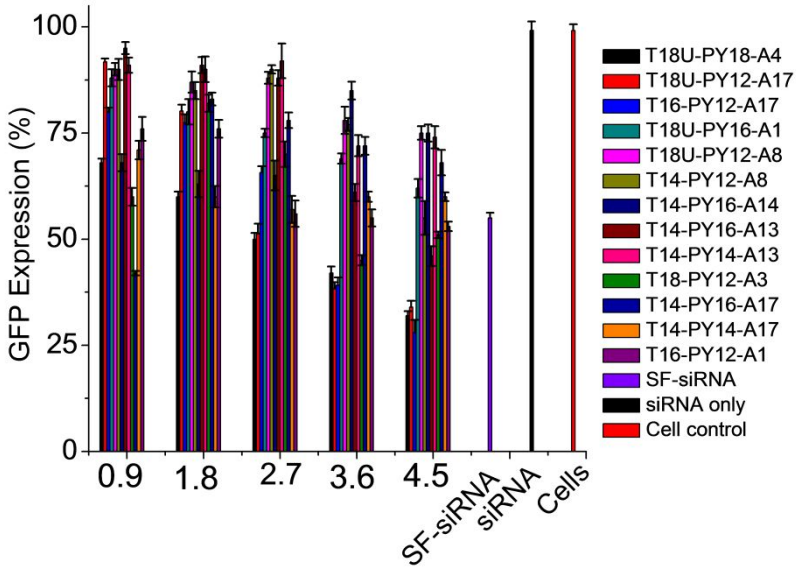


Figure S1. siGFP transfection to HeLa-GFP cells using 13 lipidoids with 5 different siGFP to lipidoid ratio. Data represent the mean±standard deviation (SD) from 3 independent experiments (n=3).

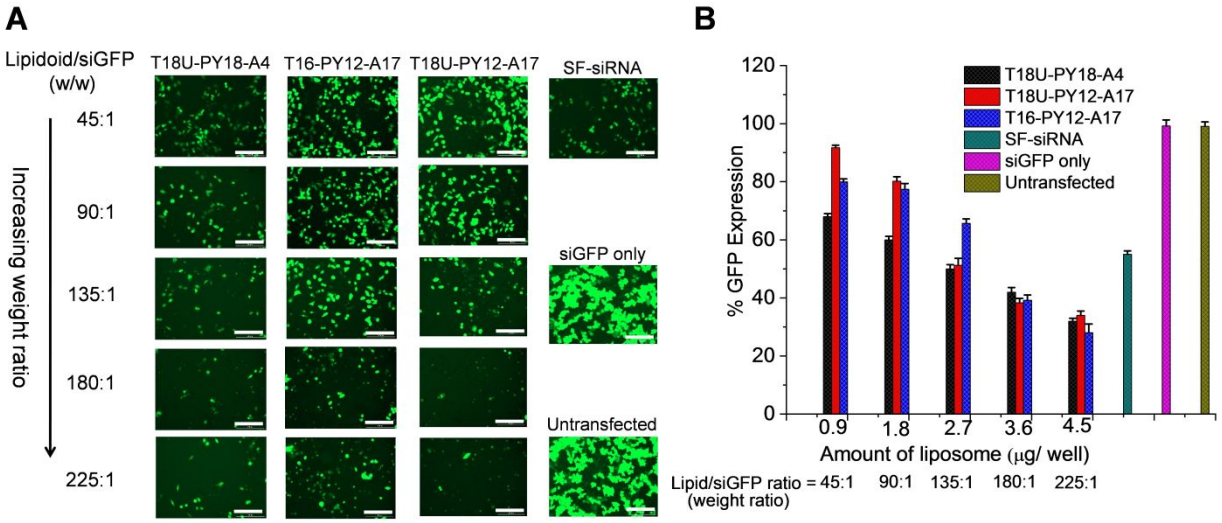


Figure S2. A) Fluorescence microscope images of siGFP transfection in HeLa-GFP cells with top three lipidoids in different lipidoid:siGFP ratios. Scale bar = 200 µm. B) Percentage of GFP expression post siGFP transfection in HeLa-GFP cells, as reflected from flow cytometry data. Data represent the mean±SD from 3 independent experiments (n=3).

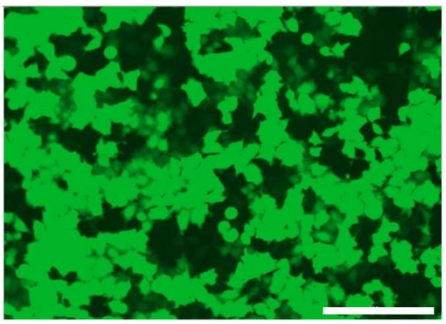


Figure S3. Fluorescence microscopy image of HeLa-GFP cells treated with lipoplex of T18U-PY18-A4 with scrambled siRNA. Scale bar = 200 µm.

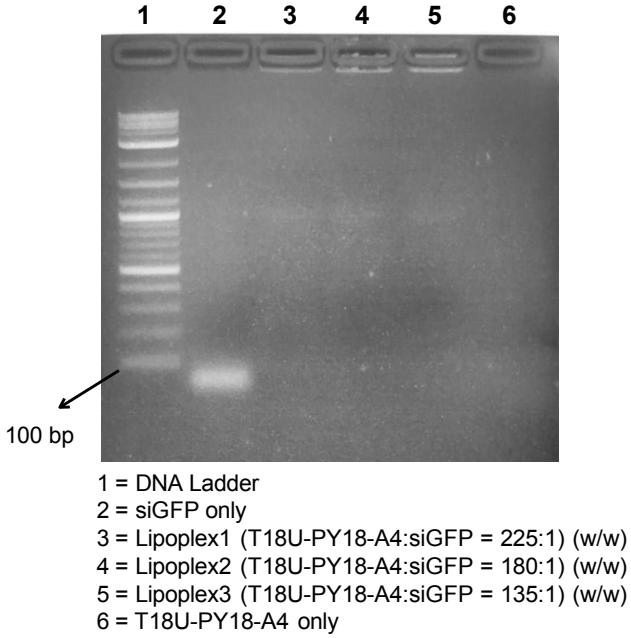


Figure S4. Establishment of siGFP complexation by T18U-PY18-A4 lipoplex, as evident by gel electrophoresis.

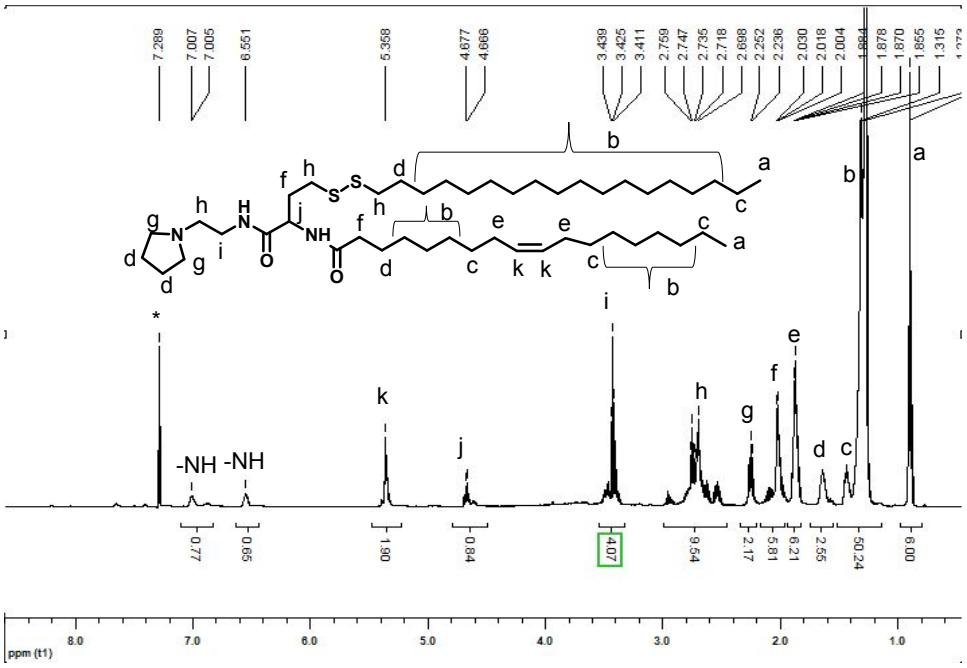


Figure S5. ^{1}H-NMR of T18U-PY18-A4

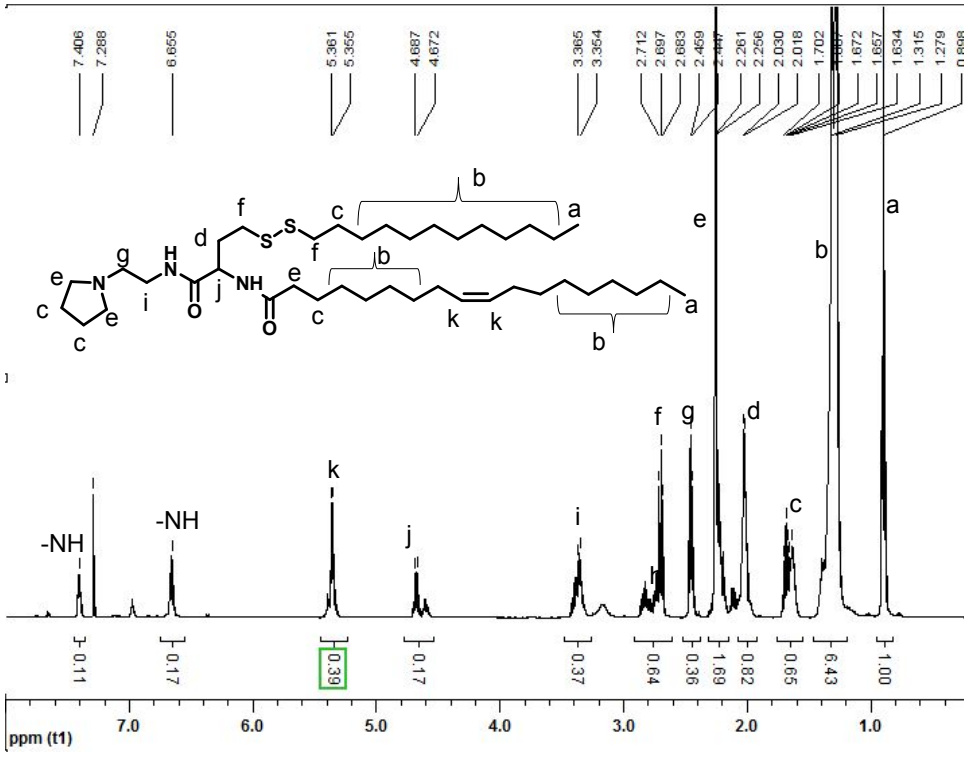


Figure S6. ^{1}H-NMR of T18U-PY12-A17

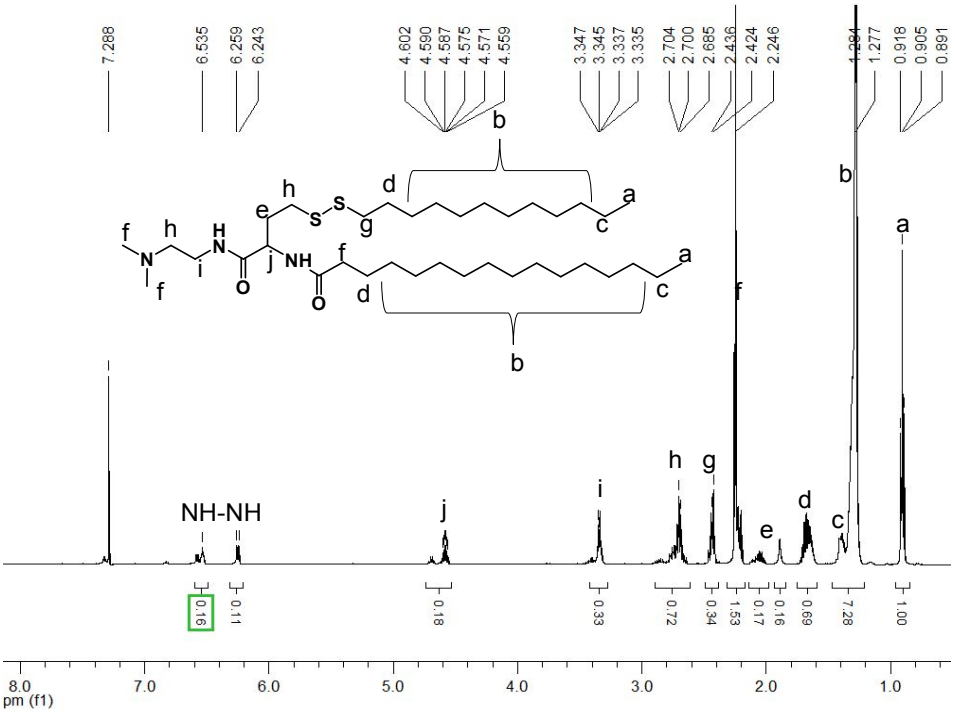


Figure S7. ^{1}H-NMR of T16-PY12-A17