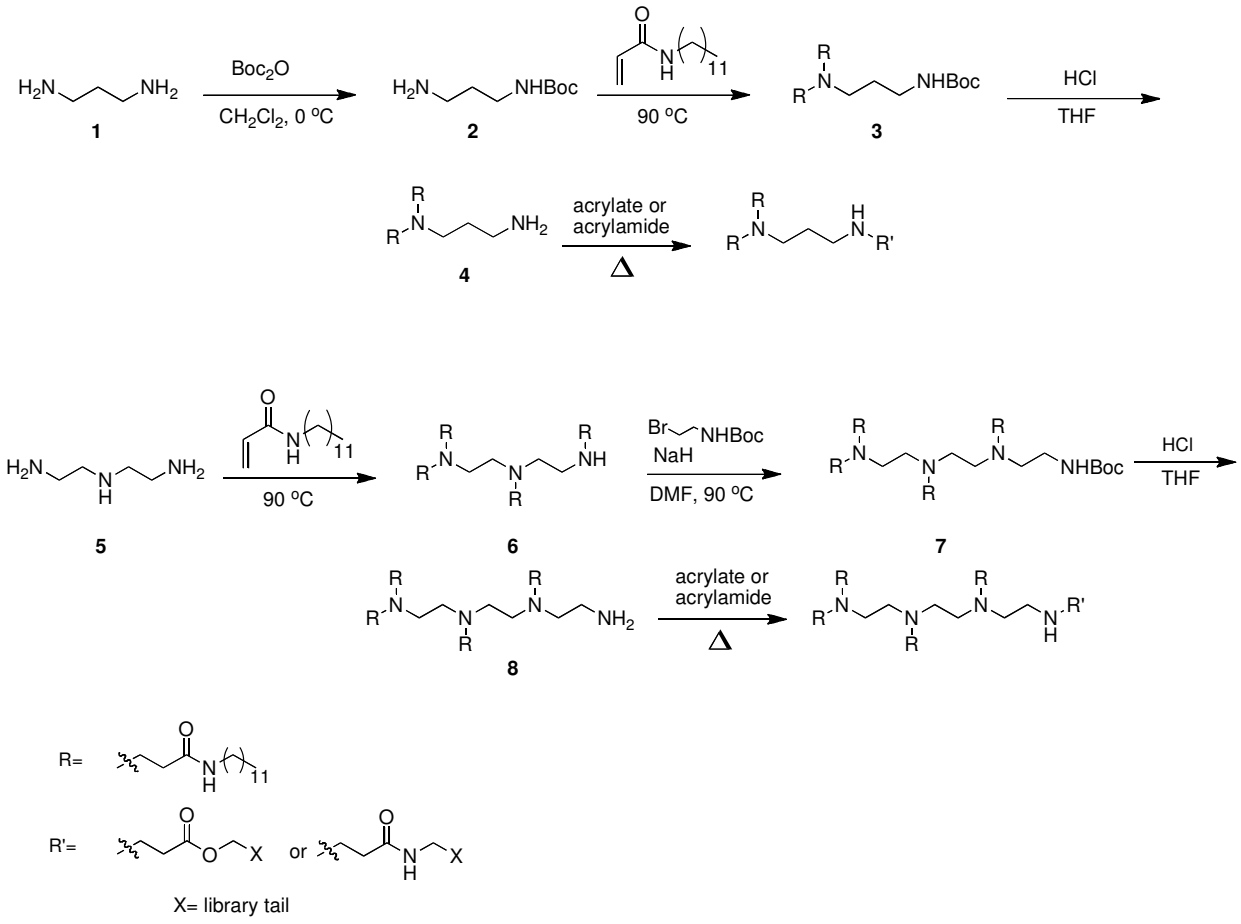
A combinatorial approach to determine functional group effects on lipidoid-mediated siRNA delivery

Kerry P. Mahon[†], Kevin T. Love[†], Kathryn A. Whitehead[†], June Qin[‡], Akin Akinc[‡], Elizaveta Leshchiner[†], Ignaty Leshchiner[†], Robert Langer[†], Daniel G. Anderson[*†]

[†]Department of Chemical Engineering and David H. Koch Institute of Integrative Cancer Research, Massachusetts Institute of Technology, 77 Massachusetts Ave., Cambridge, MA, 02139.[‡]Alnylam Pharmaceuticals, Inc., 300 Third Street, Cambridge, MA 02142

Supporting Information

Scheme 1 Synthesis of amine modified core lipidoids



*Synthesis of **2***

15g of **1** (1,3-diaminopropane, 74.12g/mol, 243mmol, 1.0 eq.) was added to 150ml of CH_2Cl_2 and the solution was cooled in an ice bath to 4^{O}C. In a separate vessel, 3.9g Di-*tert*-butyl dicarbonate (218.25g/mol, 24.3mmol, 0.1 eq.) was dissolved in 20ml CH_2Cl_2 and added to the amine solution dropwise over one hour. The reaction was allowed to gradually warm to room temperature while stirring overnight. After extraction 5 times with 20ml H_2O, the organic layer

was dried with $MgSO_4$ and concentrated to an oil. 2.6g (83%). MW calc. ($C_8H_{18}N_2O_2$)= 174.13 found 171.15.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 4.95 (s, 1H, N*H*), 3.25-3.13 (m, 2H, NCH_2), 2.80-2.73 (m, 2H, NCH_2), 1.55-1.55 (m, 2H, $CH_2CH_2CH_2$), 1.45 (s, CH,(OCH_3)$_3$).

*Synthesis of **3***

Added 6.05g dodecylacrylamide (239.4g/mol, 19.0mmol, 2.2 eq.) and 2.0g **2** (174.14g.mol, 8.6mmol, 1.0 eq.) to a 20ml scintillation vial equipped with a stirbar. Loosely capped the vial and placed at 90^{O}C for 5 days. Purified using silica gel chromatography with a solvent gradient of 5-30% Ultra (75:22:3 CH_2Cl_2: MeOH: NH_4OH) in CH_2Cl_2 over 120 min. 2.5g (33%).

MW calc.($C_{38}H_{76}N_4O_4$)= 652.58 found 652.57

^{1}H NMR (300 MHz, $CDCl_3$, δ):

6.95–6.85 (m, 2H, N*H*), 4.6 (s, 1H, N*H*), 3.25–3.1 (m, 6H, NCH_2), 2.70–2.60 (m, 4H, NCH_2), 2.36-2.31 (m, 2H, NCH_2)2.29-2.25 (m, 4H, C(O)CH_2), 1.71 (s, 2H, $NCH_2CH_2CH_2N$), 1.57-1.50 (m, 4H, $NHCH_2CH_2CH_2$), 1.47 (s, 9H, C(O)O(CH_3)$_3$), 1.26-1.23 (m, 36H, $CH_2CH_2CH_2$), 0.93-0.85 (m, 6H, CH_3).

*Synthesis of **4***

Dissolved 2.5g **3** (653.03g/mol, 8.1mmol, 1.0 eq.) in 3ml THF. In a separate vessel, added 3.2g 37% HCl (36.46g/mol, 81mmol, 10 eq.) to 1ml THF. Added the HCl solution dropwise to the solution of **2** over 5 min. Stirred rapidly overnight at room temperature. Diluted the reaction mixture in 75ml CH_2Cl_2 and extracted 2x 50 ml H_2O, 1x 50ml brine. Dried the CH_2Cl_2 solution

with $MgSO_4$ and concentrated under vacuum. Purified using silica gel chromatography with a solvent gradient of 20-50% Ultra in CH_2Cl_2 over 80 min. 1.6g (76%)

MW calc.($C_{33}H_{68}N_4O_2$)= 552.53 found 552.54

^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.80-6.75 (m, 2H, N*H*), 3.24-3.13 (m, 4H, NC*H*$_2$), 2.73-2.67 (m, 6H, NC*H*$_2$), 2.51-2.45 (m, 2H, NC*H*$_2$), 2.35-2.29 (m, 4H, C(O)C*H*$_2$), 1.65-1.52 (m, 2H, $NCH_2CH_2CH_2NH_2$), 1.51-1.40 (m, 4H, $NHCH_2CH_2CH_2$), 1.36-1.19 (m, 36H, $CH_2CH_2CH_2$), 0.92-0.83 (m, 6H, C*H*$_3$).

Conjugation to library

Added 120mg **4** (0.21mmol, 1.0 eq.) to a 2ml vial equipped with stir bar. Added 0.24 mmol, 1.1 eq. of the appropriate acrylate/ acrylamide. Placed in a 90^{O}C oven for 18 hrs. (acrylates) or 3 days (acrylamides).

*Synthesis of **6***

Added 515mg **5** (diethylenetriamine, 103.17g/mol, 5.0 mmol, 1.0 eq) and 5.0g dodecylacrylamide (239.4g/mol, 20.9mmol, 4.2eq.) to a 20ml scintillation vial equipped with a stirbar. Set up in duplicate. Loosely capped the vial and placed in an oven at 90^{O}C for 6 days. After cooling, dissolved the material in both vials in 5ml CH_2Cl_2. And purified using silica gel chromatography with a solvent gradient of 10-40% Ultra in CH_2Cl_2 over 40 min. Left with 2.26g (21%) of the desired product.

MW calc.($C_{64}H_{129}N_7O_4$)= 1060.01 found 1060.02

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.40–7.35 (m, 1H, N*H*), 7.15-7.10 (m, 1H, N*H*), 6.75-6.70 (m, 1H, N*H*), 3.25-3.1 (m, 8H, C(O)CH_2), 2.90-2.25 (m, 24H, NHCH_2, NCH_2), 1.55-1.40 (m, 8H, $NHCH_2CH_2$), 1.40-1.17 (m, 72H, $CH_2CH_2CH_2$), 0.93-0.86 (m, 12H, CH_3).

*Synthesis of **7***

Added 2.2g **6** (1060.1g/mol, 2.1mmol, 1.0 eq) and 1.63g N(Boc)-bromoethylamine (224 g/mol, 7.3mmol, 3.5eq) to a 20ml scintillation vial. Added 3ml DMF and stirbar. Added 292mg NaH.(60% dispersion, 24g/mol, 7.3mmol, 3.5 eq). Loosely capped the vial and placed in a 90^{O}C oven for 2.5 days. Diluted the reaction with 10ml $CHCl_3$ and filtered to remove the solids in the reaction. Further diluted with 100ml $CHCl_3$ and washed 2x 50ml H_2O and 1x50ml brine. Combine aqueous layers and extract with 50 ml $CHCl_3$. Combined the organic layers, dried the solution with $MgSO_4$ and concentrated under vacuum. Purified using silica gel chromatography with a solvent gradient of 10-40% Ultra in CH_2Cl_2 over 60 min. 1.47g (59%).

MW calc.($C_{71}H_{142}N_8O_6$)= 1203.10 found 1203.08

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.40-7.35 (m, 1H, N*H*), 7.20-7.05 (m, 2H, N*H*), 5.25-5.20 (m, 1H, N*H*), 3.27-3.10 (m, 8H, C(O)CH_2), 2.80-2.25 (m, 28 H, NCH_2CH_2N, NC$H_2$$CH_2$C(O), NHC$H_2$), 1.55-1.40 (m, 17H, $NHCH_2CH_2$, C(O)O(CH_3)$_3$), 1.37-1.20 (m, 72H, $CH_2CH_2CH_2$), 0.95-0.85 (m, 12H, CH_3).

*Synthesis of **8***

Dissolved 1.47g **7** (1203.94g/mol, 1.22mmol, 1.0 eq.) in 2ml THF. In a separate vessel, added 1.2g 37% HCl (36.46g/mol, 12.2mmol, 10 eq.) to 1ml THF. Added the HCl solution dropwise to the solution of **7** over 5 min. Stirred rapidly overnight at room temperature. Brought to pH 10

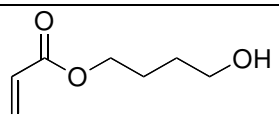
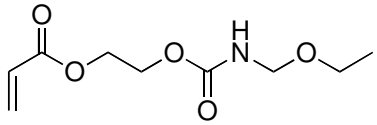
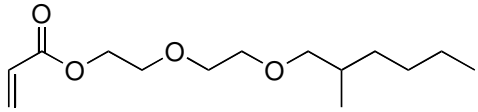
using NH_4OH. Diluted the reaction mixture in 100ml CH_2Cl_2 and extracted 3x 30 ml H_2O, 1x 50ml brine. Combined the aqueous washes and extracted with 60ml CH_2Cl_2. Combined the CH_2Cl_2 solutions, dried the CH_2Cl_2 solution with $MgSO_4$, filtered and concentrated under vacuum. Pruified using silica gel chromatography with a solvent gradient of 10-35% Ultra in CH_2Cl_2 over 40 min. 710mg (53%).

MW calc.($C_{66}H_{134}N_8O_4$)= 1103.05 found 1103.04

^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.60-7.55 (m, 1H, N*H*), 7.40-7.35 (m, 1H, N*H*), 7.25-7.17 (m, 2H, N*H*), 3.25-3.10 (m, 8H, NHCH_2), 2.80-2.60 (m, 10H, NCH_2), 2.55-2.40 (m, 10H, NCH_2), 2.38-2.25 (m, 8H, C(O)CH_2), 1.55-1.45 (m, 8H, $NHCH_2CH_2$), 1.39-1.20 (m, 72H, $CH_2CH_2CH_2$), 0.92-0.83 (m, 12H, CH_3)

Conjugation to library

Added 120mg **8** (0.11mmol, 1.0 eq.) to a 2ml vial equipped with stir bar. Added 0.12 mmol, 1.1 eq. of the appropriate acrylate/ acrylamide. Placed in a 90^{O}C oven for 18 hrs. (acrylates) or 3 days (acrylamides).

Compound	Library Tail	MALDI Analysis
1a		Calc.($C_{73}H_{146}N_8O_7$): 1247.99 Found: 1248.02
2a		Calc.($C_{76}H_{151}N_9O_8$): 1319.07 Found: 1319.12
6a		Calc.($C_{81}H_{162}N_8O_8$): 1376.2 Found: 1376.44

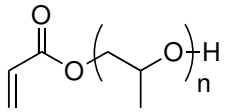
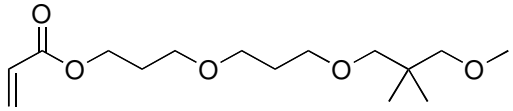
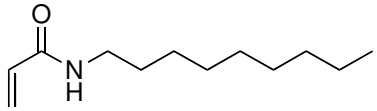
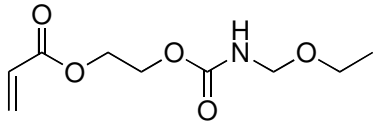
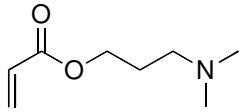
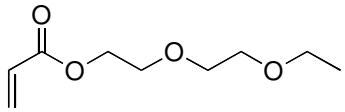
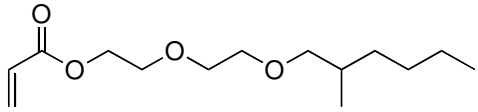
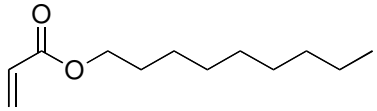
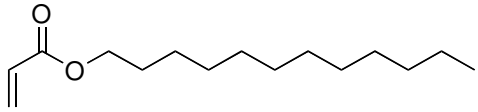
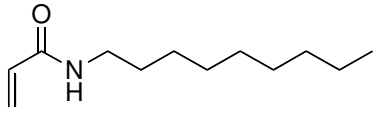
10a		Calc.($C_{90}H_{180}N_8O_{13}$): 1582.44 Found 1584.2
11a		Calc.($C_{81}H_{162}N_8O_9$): 1392.2 Found 1391.96
15a		Calc.($C_{78}H_{157}N_9O_5$): 1301.14 Found 1301.54
2b		Calc.($C_{43}H_{85}N_5O_6$): 768.16 Found: 768.53
3b		Calc.($C_{41}H_{83}N_5O_4$): 710.55 Found: 710.13
4b		Calc.($C_{42}H_{84}N_4O_6$): 741.14 Found: 741.85
6b		Calc.($C_{48}H_{96}N_4O_6$): 825.30 Found 825.65
12b		Calc.($C_{45}H_{90}N_4O_4$): 751.22 Found: 751.61
13b		Calc.($C_{48}H_{96}N_4O_4$): 792.74 Found 792.75
15b		Calc.($C_{45}H_{91}N_5O_3$): 750.24 Found: 750.64

Table S1 Formulation parameters for library testing *in vivo*

	Formulation Parameters				Characterization	
Compound	**Lipidoid**	**Cholesterol**	**PEG**	**Total Lipid:siRNA**	**Size (nm)**	**Entrapment (% siRNA content)**
1a	42	48	10	8	103.5	82
2a	42	48	10	8	48.2	95
6a	42	48	10	8	55.6	96
10a	42	48	10	8	114.1	61
11a	42	48	10	8	45.0	95
15a	42	48	10	8	62.1	95
2b	42	48	10	15	98.7	97
3b	42	48	10	15	138.5	98
4b	42	48	10	15	109.6	86
6b	42	48	10	15	55.4	92
12b	42	48	10	15	85.1	94
13b	42	48	10	15	92.3	91
15b	42	48	10	15	65.1	96

Table S2 Formulation parameters for *in vivo* dose response

	Formulation Parameters				Characterization	
Compound	**Lipidoid**	**Cholesterol**	**PEG**	**Total Lipid:siRNA**	**Size (nm)**	**Entrapment (% siRNA content)**
6a 15:1	42	48	10	15	111.6	95
10a 12:1	42	48	10	12	99.9	96
6a 15:1	42	48	10	15	167.7	95
10a12:1	42	48	10	12	113.7	84