Enhanced mRNA delivery into lymphocytes enabled by lipid-varied libraries of Charge-Altering Releasable Transporters

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Supplementary Information

	1	2	3	4	5	6	7	8
1 (lipid only)	101	101	98	97	100	98	98	90
2 (dodecyl)	97	98	97	95	100	97	89	79
3 (oleyl)	96	94	90	92	82	94	87	73
4 (benzyl)	94	94	92	95	85	95	87	87
5 (ethyl)	101	94	89	96	89	93	92	81
6 (chol.)	98	95	94	98	95	99	95	93
7 (nonenyl)	89	87	87	91	85	96	89	80
8 (stearyl)	91	92	83	90	83	94	95	73

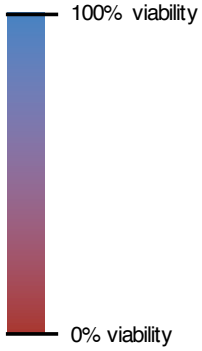


Fig. S1. MTT viability assay of Jurkat cells treated with mixtures of amphipathic CARTs for the delivery of luciferase (Fluc) mRNA.

CART	M_n (NMR)	M_n (GPC)	M_w (GPC)	$Đ$ (GPC)
D_{11} **1**	3716	2923	4585	1.56
D_{13}:A_{11} **2**	7002	6013	7516	1.25
O_{11}:A_9 **3**	6436	5080	7038	1.39
Bn_{10}:A_{11} **4**	4973	6458	8147	1.26
Et_{15}:A_{19} **5**	7013	8006	10100	1.26
$Chol_{13}$:A_{11} **6**	9185	3376	4287	1.27
N_{10}:A_{10} **7**	4964	4098	5424	1.32
S_{10}:A_9 **8**	6045	5683	7400	1.31
O_5-*b*-N_6:A_9 **9**	5671	4632	5992	1.29
N_5-*b*-O_6:A_{10} **10**	5998	4608	5931	1.28
O_4-*stat*-N_4:A_8 **11**	4492	5563	6676	1.20
BDK-D_{12}:A_{14} **12**	7471	4632	5992	1.29
BDK-O_7:N_7:A_{13} **13**	7782	6424	8497	1.32

Fig. S2. GPC characterization of new CARTs containing mixed lipid blocks. All GPC data is recorded in THF relative to polystyrene standards.

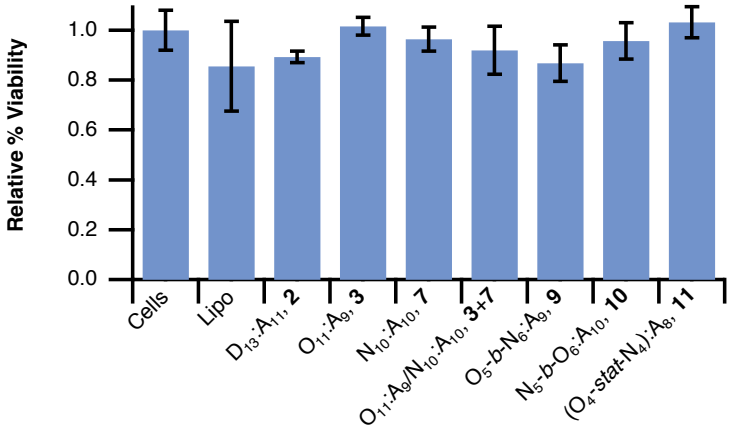


Fig. S3. MTT viability assay of Jurkat cells treated with single-lipid CART-mRNA complexes as well as covalent and non-covalent lipid-mixed CART-mRNA complexes. Each bar is the average of three separate experiments with error bar representing the standard deviation.

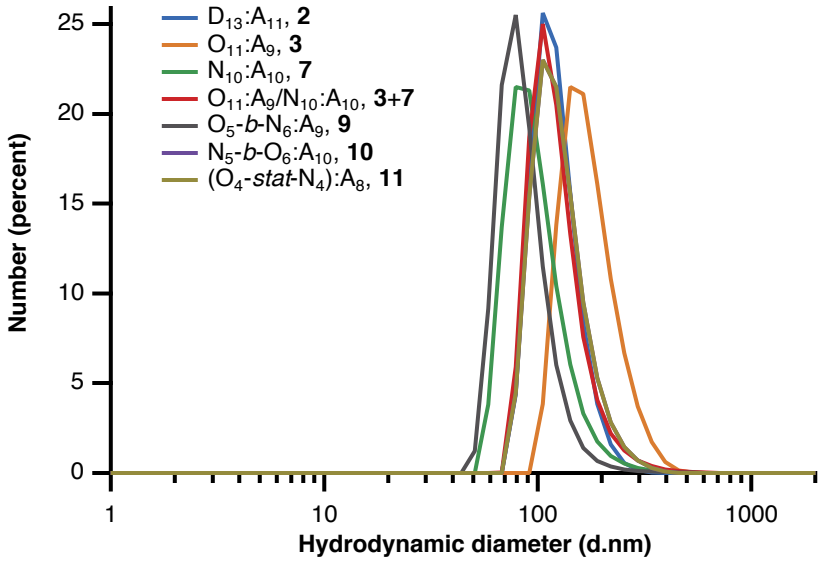


Fig. S4. DLS traces of single-lipid CARTs as well as covalent and non-covalent lipid-mixed CARTs complexed with luciferase (Fluc) mRNA.

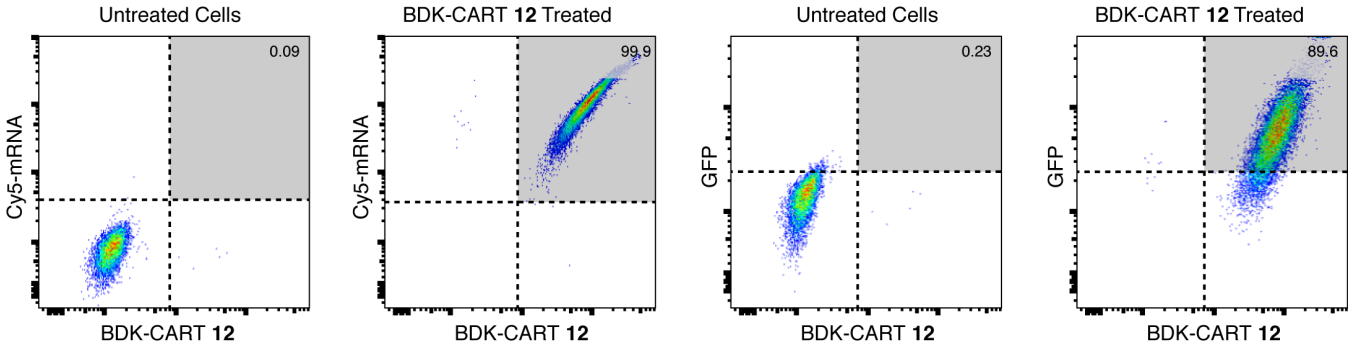


Fig. S5. Flow cytometry dot plots of HeLa cells treated with BDK-CART **12** complexed with Cy5-labeled eGFP mRNA, demonstrating a linear relationship between A) CART **12** fluorescence and Cy5 fluorescence and B) CART **12** fluorescence and resulting EGFP expression

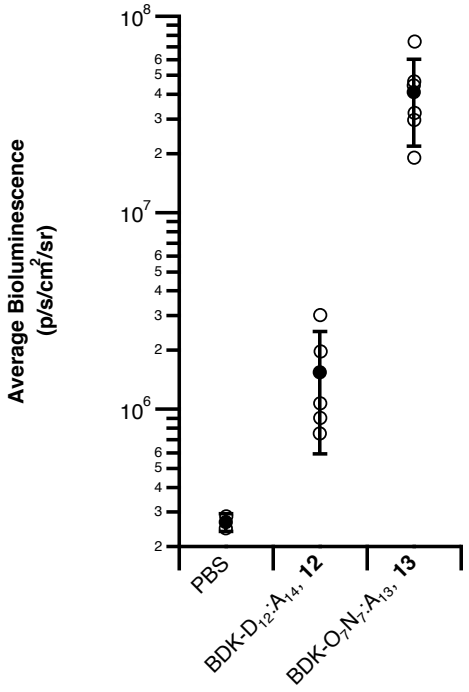


Fig. S6. Average bioluminescence intensity for female BALB/c mice injected with 3 ug Fluc mRNA complexed with either single-lipid CART BDK-D_{12}:A_{14} (**12**) or mixed-lipid CART BDK-O_7:N_7:A_{13} (**13**). Open markers represent individual measurements while filled markers are the average. Error bars represent standard deviation with n = 2 for PBS, n = 5 for CART **12**, and n = 6 for CART **13**.

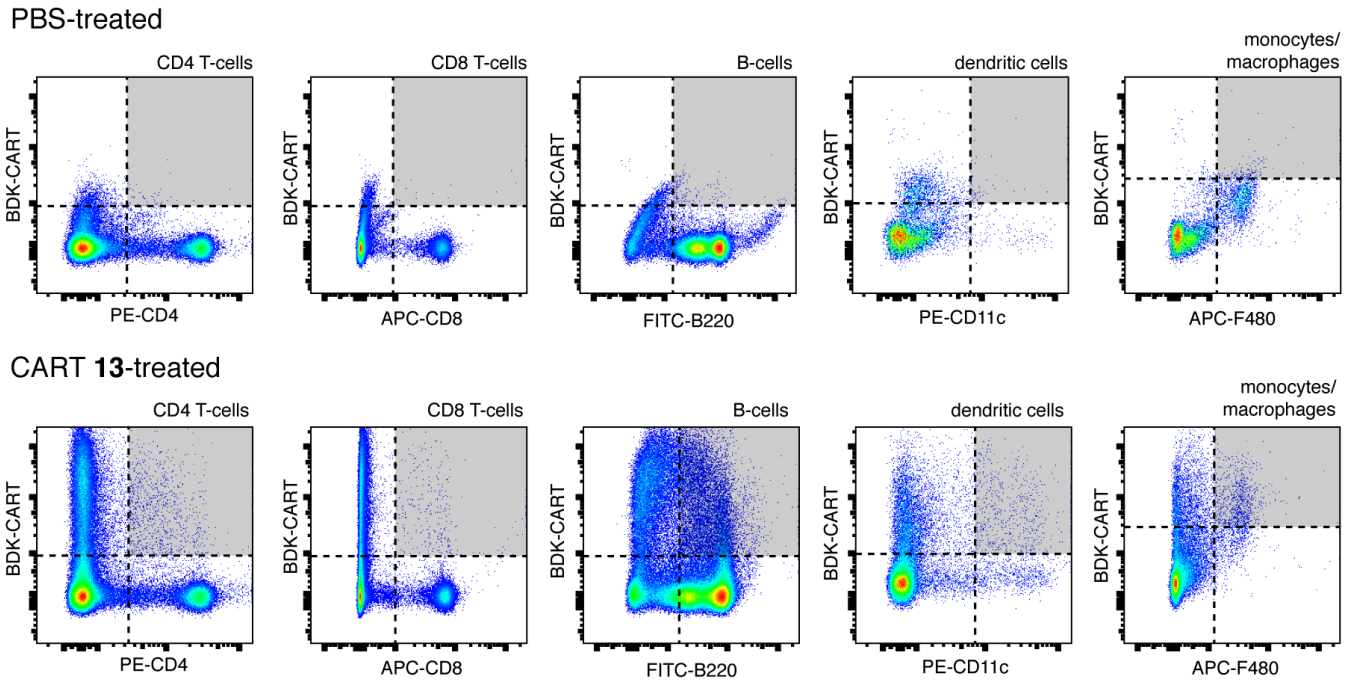
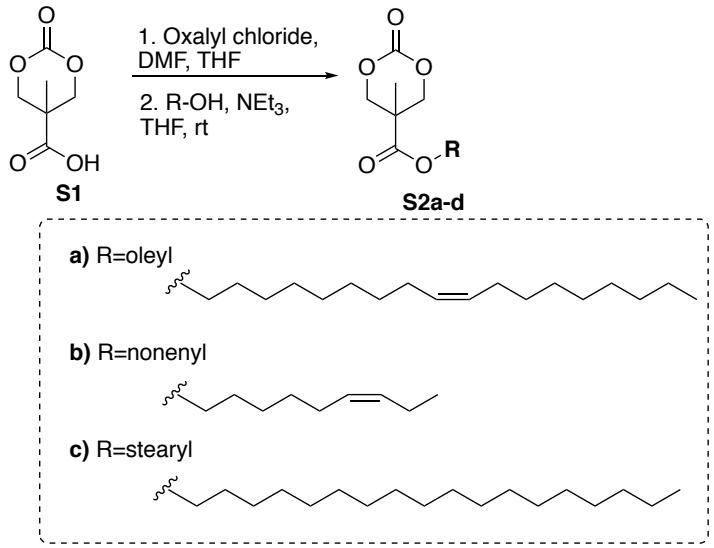


Fig. S7. Representative flow cytometry dot plots for *in vivo* transfection data for mice treated with PBS or CART BDK-O_7:N_7:A_{13} (**13**). Cells were first gated on scatter to isolate lymphocytes (for T-cells and B-cells) or monocytes (for DC's and macrophages) and then for singlets. Standard surface markers were used for cell-type determination as shown. Percentages for each transfected population are shown in Fig 8C.

Synthetic and Experimental Procedures



Synthesis of MTC monomers (S2a-c)

For the representative synthesis of MTC_{oleyl} **S2a**, to a flame-dried round bottom flask was added a solution of cyclic carbonate carboxylic acid **S1**[1] (1.56 mmol, 1 eq.), in dry THF (8 ml). To this was then added catalytic dry DMF (2 drops) followed by a solution of oxalyl chloride (1.59 mmol, 1.02 eq.) in 3 mL THF. The reaction stirred for 1 hour at room temperature before volatiles were removed to yield the acid chloride, which was used without further purification.

Triethylamine (1.71 mmol, 1.1 eq.) was added to a solution of oleyl alcohol (1.56 mmol, 1 eq.) in THF (2 mL). This solution was then added to the crude acid chloride prepared above in THF (3 mL). The reaction stirred at room temperature overnight under nitrogen. The reaction was then filtered and concentrated *in vacuo*. Purification of the resulting material by silica gel column chromatography (30→40% EtOAc/pentane) yielded the desired product.

MTC dodecyl, benzyl, ethyl, and cholesterol monomers were prepared as previously reported.[1,2]

MTC_{oleyl} (S2a) *(46 % yield)*

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.41-5.28 (m, 2H), 4.69 (d, 2H, J = 10.8 Hz), 4.24-4.13 (m, 4H), 2.07-1.91 (m, 4H), 1.66 (*app* p, 2H, J = 6.7 Hz), 1.39-1.18 (m, 25H), 0.88 (t, 3H, J = 6.6 Hz) ppm.

^{13}C NMR (300 MHz, $CDCl_3$) δ 171.2, 147.6, 130.1, 129.9, 73.1, 66.6, 40.3, 32.8 32.1, 29.9, 29.8, 29.7, 29.5, 29.5, 29.3, 29.3, 28.5, 27.4, 27.3, 25.9, 22.8, 17.8, 14.3 ppm.

$MTC_{nonenyl}$ (S2b) *(43 % yield)*

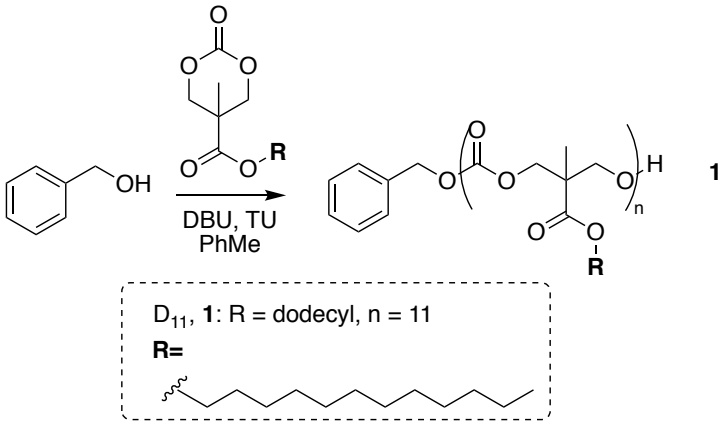
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.44-5.22 (m, 2H), 4.68 (d, 2H, J = 10.9 Hz), 4.24-4.13 (m, 4H), 2.02 (*app* p, 4H, J = 7.1 Hz), 1.73-1.60 (m, 2H), 1.43-1.29 (m, 4H), 1.33 (s, 3H), 0.95 (t, 3H, J = 7.5 Hz) ppm.

^{13}C NMR (300 MHz, $CDCl_3$) δ 171.2, 147.6, 132.1, 128.7, 73.1, 66.5, 40.3, 29.3, 28.4, 27.0, 25.4, 20.6, 17.8, 14.5 ppm.

$MTC_{stearyl}$ (S2c) *(52% yield)*

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.68 (d, 2H, *J* = 10.9 Hz), 4.23-4.14 (m, 4H), 1.70-1.58 (m, 2H), 1.37-1.17 (m, 33H), 0.92-0.82 (m, 3H) ppm.

^{13}C NMR (500 MHz, $CDCl_3$) δ 171.3, 147.7, 73.6, 66.7, 40.4, 32.2, 30.0 (broad), 29,8, 29.7, 29.6, 29.4, 28.9, 28.6, 28.4, 25.9, 23.0, 18.0, 17.8, 14.3 ppm.

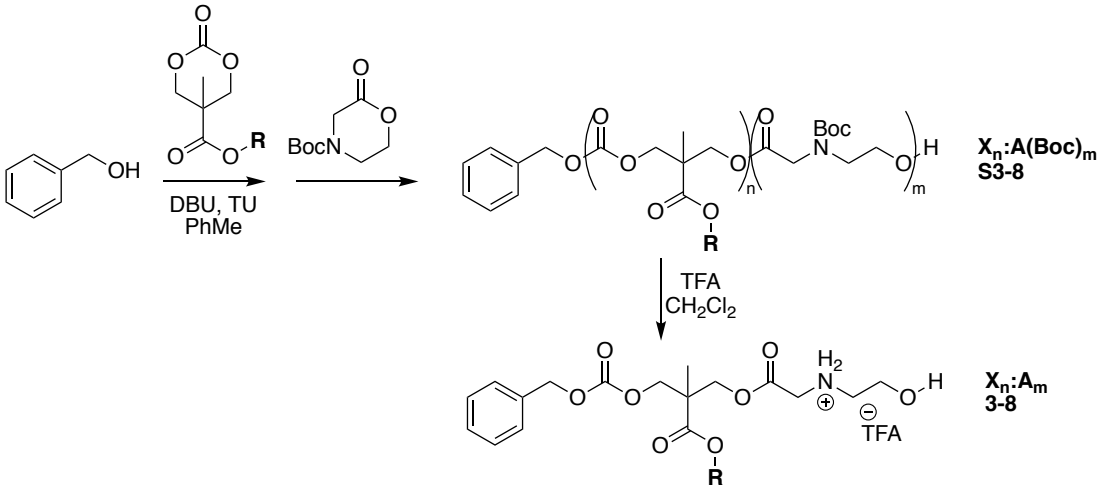


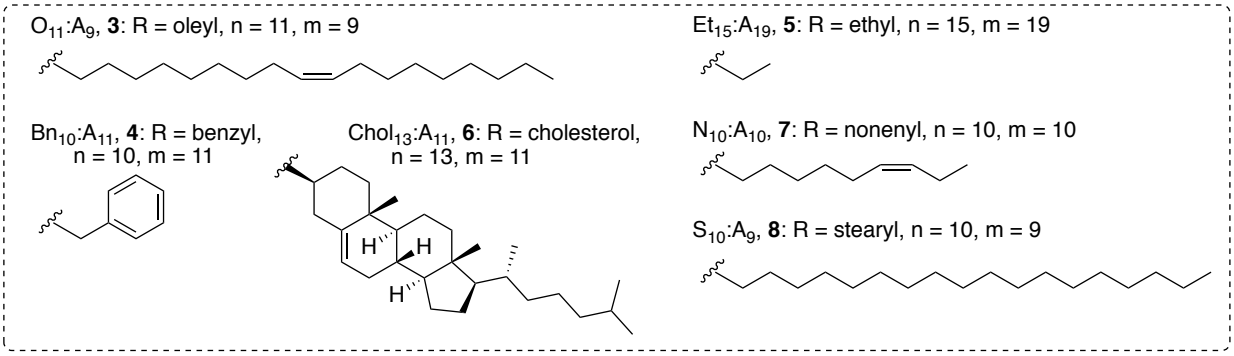
Synthesis of dodecyl homopolymer D_{11} (1)

A flame-dried vial was charged with $MTC_{dodecyl}$ monomer (0.1 mmol), benzyl alcohol (0.013 mmol), and 50 µL toluene under N_2 atmosphere in a glove box. DBU (0.005mmol) and TU (0.005 mmol) in 50 µL toluene were added to the reaction vial and allowed to stir. After 90 minutes, the reaction was quenched with benzoic acid then concentrated under reduced pressure. The crude material was dialyzed in CH_2Cl_2 against MeOH (1.0 kDa dialysis bag).

D_{11} (1)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.36 (d, *J* = 4.3 Hz, 5H), 5.14 (s, 2H), 4.47 – 4.18 (m, 44H), 4.10 (t, *J* = 6.6 Hz, 23H), 1.61 (p, *J* = 6.8 Hz, 22H), 1.39 – 1.11 (m, 248H), 0.91 – 0.81 (m, 34H).





General procedure for synthesis of single-lipid CARTs X_n:A_m (3-8)

For the representative synthesis of Bn_{10}:A_{11} **4**, a flame-dried vial was charged with MTC_{benzyl} monomer (0.22 mmol), benzyl alcohol (0.0185 mmol), and 460 μL toluene under N_2 atmosphere in a glove box. DBU (0.02mmol) and TU (0.02 mmol) were added to the reaction vial and allowed to stir. After 2 h, M_{BOC} monomer (0.277 mmol) was added to the vial as a solid and the reaction was allowed to stir for 3 h. After a total of 5 h, the reaction was quenched with benzoic acid then concentrated under reduced pressure. The crude material was dialyzed in CH_2Cl_2 against MeOH (1.0 kDa dialysis bag). End group analysis by ^{1}H NMR shows DP 10:11.

Dodecyl-based CART **2** was prepared as previously reported.[3]

Boc protected O_{11}:A_9 (S3)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.55-5.27 (m, 23H), 5.17-5.12 (s, 2H), 4.60-4.16 (m, 66H), 4.15-4.05 (m, 22H), 4.02-3.80 (m, 16H), 3.6-3.32 (m, 18H), 2.14-1.88 (m, 39H), 1.69-1.52 (m, 21H), 1.52-1.37 (m, 83H), 1.37-1.01 (m, 296H), 0.95-0.79 (m, 34H) ppm.

O_{11}:A_9 (3)

^{1}H NMR (500 MHz, CD_3OD) δ 7.48-7.20 (m, 5H), 5.49-5.27 (m, 20H), 5.17 (s, 2H), 4.68-4.51 (m, 14H), 4.53-4.24 (m, 44H), 4.24-4.01 (m, 33H), 3.59-3.40 (m, 18H), 2.15-1.85 (m, 39H), 1.80-1.51 (m, 26H), 1.51-1.03 (m, 289H), 1.03-0.72 (m, 37H) ppm.

Boc protected Bn_{10}:A_{11} (S4)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 – 7.20 (m, 55H), 5.13 (d, *J* = 6.8 Hz, 22H), 4.23 (dq, *J* = 33.9, 14.8, 10.1 Hz, 67H), 3.91 (dd, *J* = 28.3, 21.8 Hz, 25H), 3.81 – 3.60 (m, 1H), 3.59 – 3.29 (m, 27H), 1.53 – 1.31 (m, 108H), 1.31 – 1.14 (m, 34H).

Bn_{10}:A_{11} (4)
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.45 – 7.15 (m, 55H), 5.15 (d, *J* = 9.0 Hz, 18H), 4.64 – 4.48 (m, 19H), 4.48 – 4.19 (m, 48H), 4.19 – 4.06 (m, 17H), 4.06 – 3.92 (m, 12H), 3.44 (ddd, *J* = 27.7, 21.9, 5.8 Hz, 26H).

Boc protected Et_{15}:A_{19} (S5)
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (q, *J* = 4.3, 3.4 Hz, 4H), 5.17 – 5.08 (m, 2H), 4.44 – 4.07 (m, 125H), 3.94 (dd, *J* = 21.0, 6.0 Hz, 38H), 3.50 (dp, *J* = 18.5, 6.1, 5.5 Hz, 38H), 1.49 – 1.31 (m, 174H), 1.23 (q, *J* = 4.7, 3.7 Hz, 94H).

Et_{15}:A_{19} (5)
^{1}H NMR (400 MHz, Methanol-d_4) δ 7.45 – 7.29 (m, 5H), 4.52 – 4.38 (m, 34H), 4.38 – 4.24 (m, 31H), 4.24 – 4.02 (m, 73H), 3.57 – 3.37 (m, 43H), 1.40 – 1.12 (m, 113H).

Boc protected $Chol_{13}$:A_{11} (S6)
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45-7.30 (m, 5H), 5.43-5.21 (m, 13H), 5.17-5.11 (s, 2H), 4.69-4.57 (m, 13H), 4.45-4.12 (m, 68H), 4.11—3.90 (m, 22H), 3.59-3.47 (m, 22H), 2.35-2.19 (m, 26H), 2.05-1.90 (m, 29H), 1.89-1.75 (m, 43H), 1.63-1.28 (m, 242H), 1.28-0.89 (m, 263H), 0.88-0.81 (m,76H), 0.71 0.64 (m, 38H) ppm.

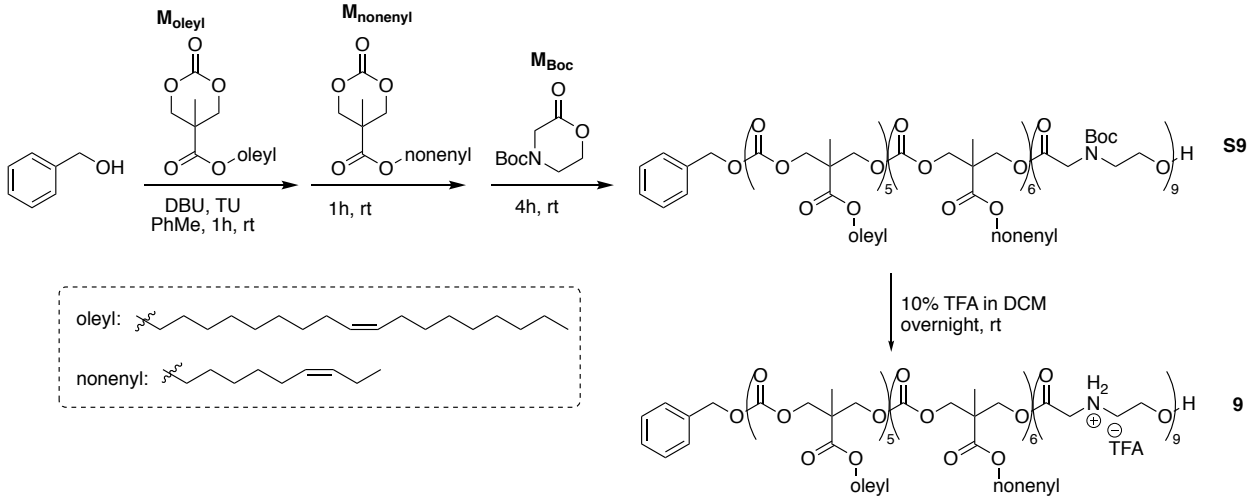
$Chol_{13}$:A_{11} (6)
^{1}H NMR (500 MHz, CD_3OD) δ 7.48-7.20 (m, 5H), 5.53-5.38 (m, 11H), 5.15 (s, 2H), 4.72-4.51 (m, 26H), 4.50-4.22 (m, 44H), 4.22-3.99 (m, 26H), 3.60-3.36 (m, 26H), 2.49-2.19 (m, 20H), 2.19-1.72 (m, 58H), 1.71-1.24 (m, 177H), 1.21-0.91 (m, 168H), 0.91-0.81 (m, 72H), 0.79-0.67 (m, 33H) ppm.

Boc protected N_{10}:A_{10} (S7)
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39-7.30 (m, 5H), 5.41-5.23 (m, 21H), 5.17-5.12 (s, 2H), 4.46-4.16 (m, 58H), 4.15-4.05 (m, 20H), 4.02-3.80 (m, 20H), 3.6-3.32 (m, 19H), 2.09-1.92 (m, 40H), 1.79-1.55 (m, 24H) 1.50-1.28 (m, 130H), 1.25-1.18 (m, 30H), 1.02-0.82 (m, 27H) ppm.

N_{10}:A_{10} (7)
^{1}H NMR (500 MHz, CD_3OD) δ 7.48-7.27 (m, 5H), 5.44-5.26 (m, 20H), 5.17-5.12 (s, 2H), 4.62-4.52 (m, 16.5H), 4.51-4.23 (m, 47H), 4.19-4.05 (m, 45H), 3.56-3.41 (m, 20H), 2.14-1.98 (m, 43H), 1.72-1.57 (m, 24H), 1.46-1.08 (m, 81H), 0.99-0.93 (m, 32H) ppm.

Boc protected S_{10}:A_9 (S8)
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40-7.30 (m, 5H), 5.17-5.12 (s, 2H), 4.46-4.16 (m, 56H), 4.15-4.05 (m, 18H), 4.02-3.80 (m, 18H), 3.6-3.32 (m, 18H), 1.68-1.53 (m, 20H) 1.51-1.35 (m, 89H), 1.34-1.08 (m, 310H), 0.93-0.80 (m, 27H) ppm.

S_{10}:A_9 (8)
^{1}H NMR (500 MHz, CD_3OD) δ 7.48-7.27 (m, 5H), 5.17 (s, 2H), 4.68-4.51 (m, 20H), 4.53-4.01 (m, 68H), 3.56-3.40 (m, 26H), 1.80-1.59 (m, 17H), 1.45-1.18 (m, 236H), 1.01-0.79 (m, 23H) ppm.



Synthesis of triblock CARTs O_5-*b*-N_6:A_9 (9) and N_5-*b*-O_6:A_{10} (10)

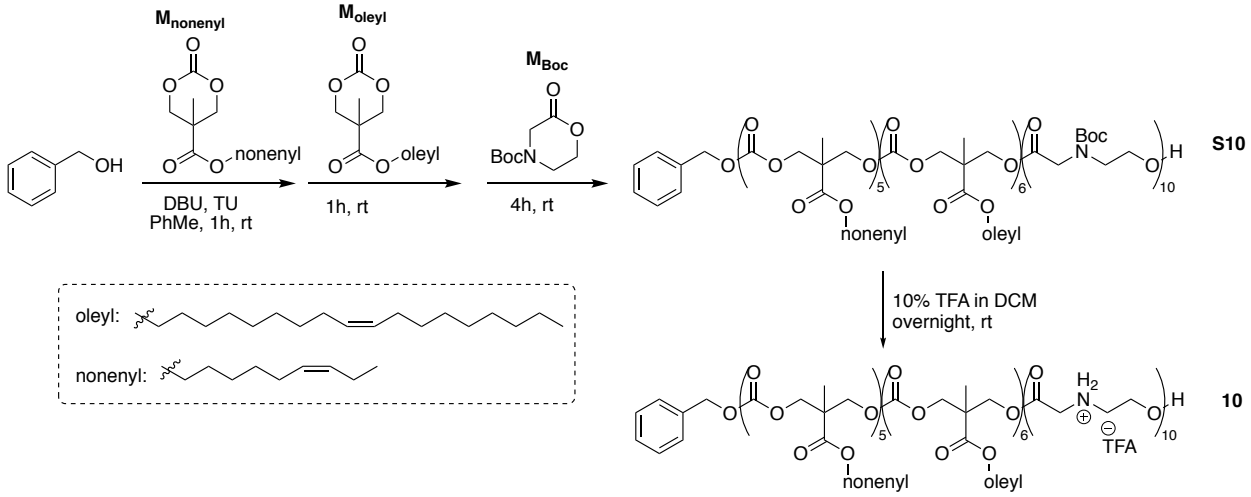
Followed general procedure for CART synthesis above with the addition of a second lipid block. Briefly, to an oven dried 1 dram vial equipped with stir bar under N_2 atmosphere in a glove box was added MTC_{oleyl} (6 eq., 0.077 mmol, 31.1 mg) and TU (0.05eq., 0.0038 mmol, 1.4 mg). To this was added benzyl alcohol as a 1M solution in toluene (1 eq., 0.0128 mmol, 12.8 µL) followed by an additional 134 µL of toluene (1M w.r.t. M_{BOC} monomer). Catalytic 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (1 drop) was then added to the solution and the reaction was allowed to stir at rt for 1 hour in the glove box. At this time $MTC_{nonenyl}$ (6 eq., 0.077 mmol, 21.7 mg) was added to the reaction. After stirring for another hour, M_{BOC} (12.66 eq., 0.123 mmol, 27 mg) was added to the reaction and stirred for an additional 4 hours before being quenched with benzoic acid. The reaction was dialyzed in methanol (1.0 kDa dialysis bag) overnight. Concentration yielded protected co-oligomer **S9** as a clear oil. Degree of polymerization was determined by ^{1}H NMR end group analysis and dispersity by GPC in THF. Subsequent deprotection in 10% TFA in DCM overnight at room temperature under N_2 afforded CART O_5-*b*-N_6:A_9 **(9)**. Complete protecting group removal was confirmed by ^{1}H NMR (CD_3OD).

Boc protected O_5-*b*-N_6:A_9 (S9)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 – 7.28 (m, 5H), 5.46 – 5.18 (m, 20H), 5.13 (s, 2H), 4.47 – 4.16 (m, 57H), 4.10 (td, *J* = 6.4, 2.0 Hz, 21H), 3.95 (dt, *J* = 24.4, 4.9 Hz, 19H), 3.51 (dt, *J* = 18.6, 6.1 Hz, 18H), 2.13 – 1.89 (m, 40H), 1.60 (hept, *J* = 6.1, 5.6 Hz, 22H), 1.52 – 1.37 (m, 83H), 1.37 – 1.07 (m, 165H), 0.94 (t, *J* = 7.5 Hz, 18H), 0.86 (t, *J* = 6.7 Hz, 14H).

O_5-*b*-N_6:A_9 (9)

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.47 – 7.23 (m, 5H), 5.33 (qd, *J* = 10.4, 9.9, 5.0 Hz, 20H), 5.15 (s, 2H), 4.56 (q, *J* = 6.0, 5.6 Hz, 13H), 4.51 – 4.39 (m, 9H), 4.39 – 4.20 (m, 27H), 4.20 – 4.00 (m, 38H), 3.48 (dt, *J* = 13.9, 5.0 Hz, 17H), 2.05 (p, *J* = 8.2, 7.4 Hz, 39H), 1.80 – 1.54 (m, 25H), 1.30 (qt, *J* = 22.2, 12.2 Hz, 177H), 0.93 (dt, *J* = 22.2, 7.3 Hz, 34H).



Synthesis of CART N_5-*b*-O_6:A_{10} (10)

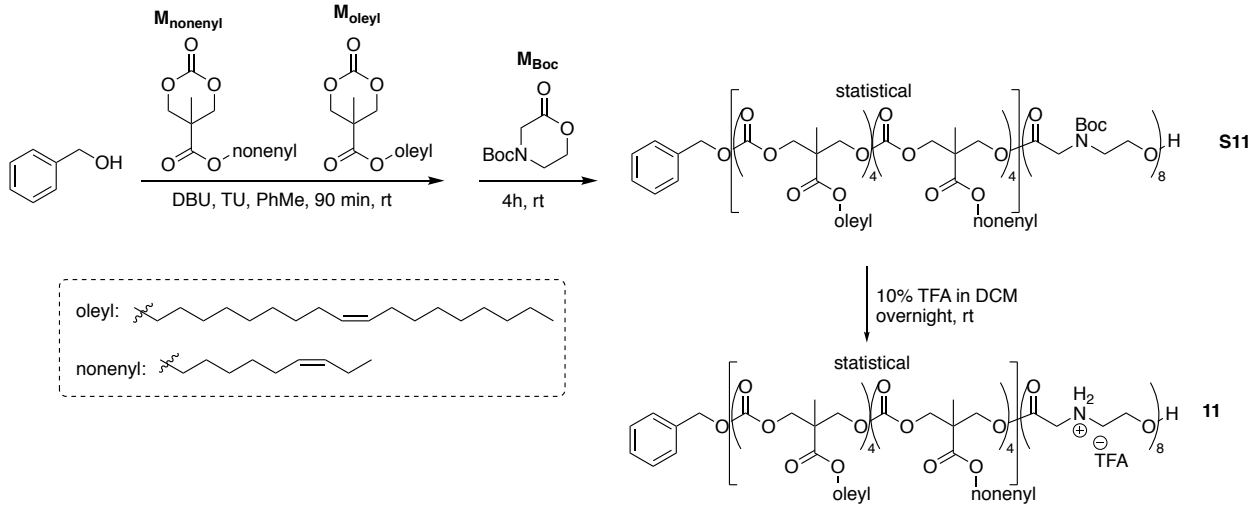
Followed general procedure for CART synthesis above with the addition of a second lipid block. Briefly, to an oven dried 1 dram vial equipped with stir bar under N_2 atmosphere in a glove box was added $MTC_{nonenyl}$ (6 eq., 0.063 mmol, 18 mg) and TU (0.05eq., .0033 mmol, 1.2 mg).To this was added benzyl alcohol as a 1M solution in toluene (1 eq., 0.0106 mmol, 10.6 µL)followed by an additional 127 mg of toluene (1M w.r.t. M_{BOC} monomer). Catalytic 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (1 drop) was then added to the solution and the reaction was allowed to stir at rt for 1 hour in the glove box. At this time MTC_{oleyl} (6 eq., 0.064 mmol, 26.2 mg) was added to the reaction. After stirring for another hour, M_{BOC} (12.66 eq., 0.123 mmol, 27 mg) was added to the reaction and stirred for an additional 4 hours before being quenched with benzoic acid. The reaction was dialyzed in methanol (1.0 kDa dialysis bag) overnight. Concentration yielded protected co-oligomer **S10** as a clear oil. Degree of polymerization was determined by ^{1}H NMR end group analysis and dispersity by GPC in THF. Subsequent deprotection in 10% TFA in DCM overnight at room temperature under N_2 afforded CART N_5-*b*-O_6:A_{10} **(10)**. Complete protecting group removal was confirmed by ^{1}H NMR (CD_3OD).

Boc protected N_5-*b*-O_6:A_{10} (S10)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.49 – 7.29 (m, 5H), 5.32 (qd, *J* = 10.9, 5.2 Hz, 21H), 5.12 (d, *J* = 11.9 Hz, 2H), 4.60 – 4.16 (m, 60H), 4.10 (t, *J* = 6.5 Hz, 21H), 4.03 – 3.81 (m, 22H), 3.51 (dt, *J* = 20.6, 7.3 Hz, 22H), 2.00 (tq, *J* = 13.0, 6.5, 5.7 Hz, 43H), 1.62 (p, *J* = 6.5 Hz, 25H), 1.53 – 1.06 (m, 300H), 0.94 (t, *J* = 7.5 Hz, 11H), 0.87 (t, *J* = 6.6 Hz, 17H).

N_5-*b*-O_6:A_{10} (10)

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36 (t, *J* = 6.4 Hz, 6H), 5.34 (t, *J* = 5.6 Hz, 15H), 5.15 (s, 2H), 4.56 (t, *J* = 5.0 Hz, 16H), 4.48 – 4.20 (m, 44H), 4.20 – 3.98 (m, 41H), 3.47 (q, *J* = 6.8, 5.8 Hz, 19H), 2.05 (t, *J* = 7.3 Hz, 42H), 1.64 (s, 25H), 1.53 – 1.06 (m, 187H), 0.93 (dt, *J* = 21.5, 7.3 Hz, 35H).



Synthesis of CART O_4-*stat*-N_4:A_8 (11)

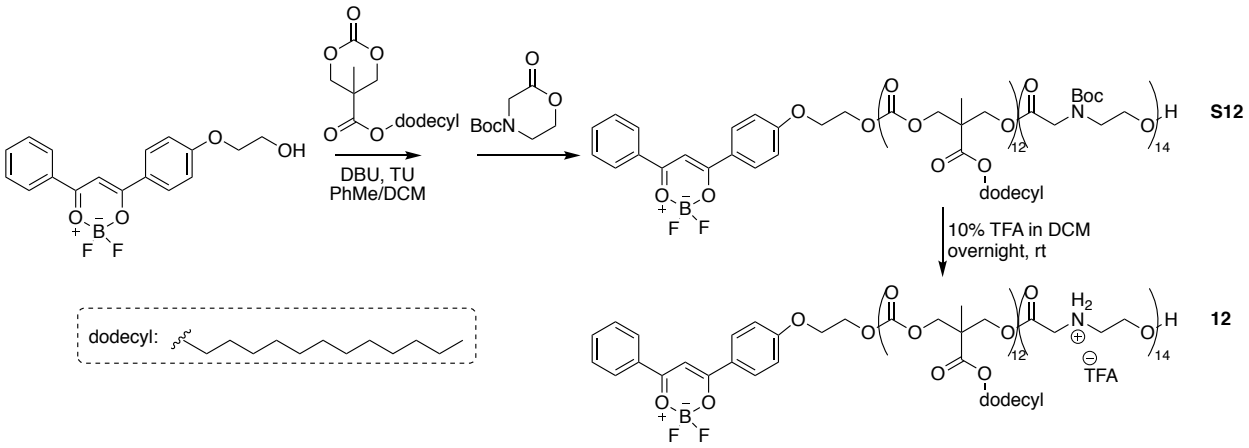
Followed general procedure for CART synthesis above with the modification that both lipid monomers were added simultaneously to produce a statistical block containing both oleyl and monomers, followed by the Boc-protected amino ester block.

Boc protected O_4-*stat*-N_4:A_8 (S11)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 – 7.28 (m, 5H), 5.46 – 5.20 (m, 13H), 5.14 (s, 2H), 4.25 (d, *J* = 17.0 Hz, 39H), 4.10 (qd, *J* = 6.6, 6.2, 3.5 Hz, 14H), 4.03 – 3.83 (m, 14H), 3.52 (dt, *J* = 18.3, 6.0 Hz, 14H), 2.00 (dtd, *J* = 14.7, 10.0, 8.7, 5.1 Hz, 27H), 1.62 (p, *J* = 6.5 Hz, 15H), 1.52 – 1.37 (m, 62H), 1.37 – 1.13 (m, 101H), 0.94 (t, *J* = 7.5 Hz, 11H), 0.87 (t, *J* = 6.8 Hz, 8H).

O_4-*stat*-N_4:A_8 (11)

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.46 – 7.25 (m, 5H), 5.33 (h, *J* = 8.2, 6.6 Hz, 17H), 5.15 (s, 2H), 4.57 (q, *J* = 5.2 Hz, 14H), 4.50 – 4.21 (m, 35H), 4.13 – 4.02 (m, 16H), 3.59 – 3.38 (m, 15H), 2.16 – 1.89 (m, 33H), 1.64 (s, 21H), 1.53 – 1.12 (m, 151H), 0.92 (dt, *J* = 22.1, 7.3 Hz, 28H).



Synthesis of BDK-initiated CARTs (12-13)

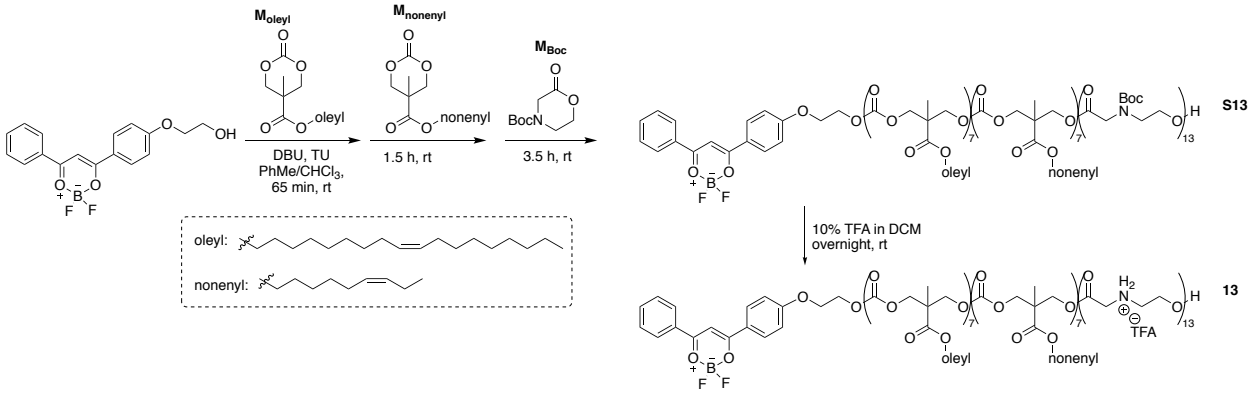
Followed general procedure for CART synthesis above using BDK-alcohol as the initiator (synthesized as previously described)[4] instead of benzyl alcohol. Synthesis, purification, and deprotection were carried out shielded from light.

Boc protected BDK-D_{12}:A_{14} (S12)

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 – 8.06 (m, 2H), 8.03 – 7.90 (m, 2H), 7.76 – 7.41 (m, 3H), 7.17 – 6.72 (m, 3H), 4.42 – 4.16 (m, 73H), 4.10 (t, *J* = 6.8 Hz, 24H), 4.02 – 3.84 (m, 29H), 3.62 – 3.31 (m, 31H), 1.62 (td, *J* = 14.7, 13.9, 8.0 Hz, 31H), 1.51 – 1.35 (m, 128H), 1.35 – 1.15 (m, 249H), 0.87 (t, *J* = 6.7 Hz, 34H).

BDK-D_{12}:A_{14} (12)

^{1}H NMR (400 MHz, Methanol-d_4) δ 8.30 (dd, *J* = 22.8, 8.2 Hz, 1H), 8.07 (t, *J* = 9.2 Hz, 2H), 7.66 – 7.43 (m, 2H), 7.22 – 6.96 (m, 2H), 4.57 (q, *J* = 6.1, 5.5 Hz, 21H), 4.50 – 4.20 (m, 44H), 4.20 – 4.00 (m, 49H), 3.49 (dt, *J* = 10.5, 4.8 Hz, 26H), 1.63 (s, 25H), 1.50 – 1.06 (m, 234H), 0.90 (t, *J* = 6.7 Hz, 36H).



Boc protected O_7-*b*-N_7:A_{13} (S13)

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.24-7.94 (4H, m), 7.72-7.36 (4H, m), 7.19-6.80 (2H, m), 5.52-5.15 (28H, b), 4.42-4.20 (84H, b), 4.20-4.06 (29H, b), 4.06-3.88 (26H, b), 3.63-3.46 (26H, b), 2.15-1.90 (55H, b), 1.74-1.54 (35H, b), 1.53-1.41 (124H, b), 1.40-1.20 (225H, b), 1.00-0.94 (22H, t), 0.94-0.84 (21H, t) ppm.

O_7-*b*-N_7:A_{13} (13)

^{1}H NMR (300 MHz, CD_3OD) δ 8.35-7.89 (4H, m), 7.70, 7.31 (4H, m), 7.16-6.99 (2H, m) 5.51-5.13 (23H, b), 4.72-4.51 (23H, b), 4.51-4.22 (58H, b), 4.22-4.01 (51H, b), 3.36-3.38 (27H, b), 2.20-1.91 (45H, b), 1.74-1.55 (31H, b), 1.50-1.15 (198H, b), 1.03-0.80 (44H, m) ppm.

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