Supporting Information

Tuning molecular interactions in Lipid-Oligonucleotides assemblies *via* locked nucleic acid (LNA)-based lipids

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I. Experimental Section

General Procedures and Materials

All the compounds were purchased from *Sigma-Aldrich*, *Fluka* and *Alfa Aesar* unless otherwise mentioned. Solvents for reactions were purchased from *Sigma-Aldrich* in the highest quality and from *VWR* for other uses. All the reactions were run under nitrogen atmosphere unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel F_{254} plates with fluorescent indicator from *Merck*. The detection of compounds was accomplished using a UV light (254 nm) and visualized on TLC plates by subsequent spraying with 10 % conc. H_2SO_4 solution in ethanol, followed by heating. Column chromatography was performed with flash silica gel (0.04-0.063 mm) from *Merck*. All the compounds were characterized using ^{1}H, ^{13}C and ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy. These NMR spectra were recorded (in $CDCl_3$ obtained from *Eurisotop*) on BRUKER Avance DPX-300 spectrometer (^{1}H at 300.13 MHz, ^{13}C at 75.46 MHz and ^{31}P at 121.49 MHz). The chemical shifts (δ) are given in parts per million (ppm) relatively to tetramethylsilane or residual solvent peaks ($CHCl_3$: ^{1}H: 7.26, ^{13}C: 77.0). The coupling constants *J* are given in Hertz (Hz); the peak multiplicity is reported as follows: s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet. High resolution electronspray ionization mass spectra (HR ESI-MS) were performed by the CESAMO (Bordeaux, France) on a QSsat Elite mass spectrometer (applied Biosystems). The instrument is equipped with an ESI source and spectra were recorded in negative mode. The electrospray needle was maintained at 4500 V and operated at room temperature. Samples were introduced by introduced by injection through a 10 µL sample loop into a 200 µL/min flow of methanol from the LC pump.

Isothermal Titration Calorimetry (ITC) experiments

Titration experiments were performed with iTC_{200} microcalorimeter from MicroCal Inc. The working cell (205.8 µL) was filled with desired nucleolipid (diC16-3'-LNA-T **1a**, diC16-3'-LNA-A **1b**, diC16-3'-dT **2a**, diC16-3'-dA **2b**) solution (1.25 mM) in HEPES buffer (50 mM, pH 7.2) and the reference cell with water. The injection syringe was filled nucleic acid (polyA or polyU) solution (14.6 mM) in HEPES buffer (50 mM, pH 7.2). The titration schedule consisted of 20 consecutive injections of 2 µL with an interval of 500 s between each injection. The corresponding reference blank experiments were also performed namely titration of nucleic acid-free HEPES buffer in the nucleolipid solution and titration of nucleic acid solution in the nucleolipid-free HEPES buffer. To avoid the presence of bubbles, all the samples were degassed for 10 min shortly before starting the measurements and centrifuge for 5 min. at 6000 rpm to avoid any insoluble particles, if any present. The syringe was constantly stirred at a rate of 500 rpm, and the measurements were performed at 25 °C. The first injection was carried out without taking into account the corresponding observed heat because the first injection was subject to large errors as a result of the diffusion of solutions across the syringe tip during the pre-titration equilibrium period. The data analyses were carried out with Origin 7.0 software (provided by MicroCal) using the 'one-set-of-sites' or 'two-set-of-sites' binding model. A complete list of binding data is given in Table SI1.

Differential Scanning Calorimetry (DSC)

DSC measurements of nucleolipid samples (2mg /ml, HEPES Buffer 50 mM) were performed using a micro DSC III calorimeter (SETARAM instrumentation, Lyon, France), in temperature intervals of 5 °C to 55 °C. Second heating run was selected for analysis. Baseline subtractions and enthalpy calculations were done, using the Calisto processing software (SETARAM Instrumentation, Lyon, France).

2D NMR spectral characterization

1D and 2D 1H-1H NOESY NMR experiments were acquired on a Bruker® Advance 700 MHz NMR spectrometer equipped with a TXI triple resonance $^{1}H/^{15}N/^{13}C/^{2}H$ with a z-axis gradient running under TopSpin (version 2.1, Bruker Biospin, Karlsruhe). Samples were tested at 288 K, 293K and 310 K, with mixing times of 50 ms and 300 ms. Usually a spectral width of ~11160 Hz on both dimension was used, with a time domain of 2048 point in F2 dimension and 128 point in the F1 dimension. A relaxation delay of 2 s was used and suppression of water was performed using Watergate W5 pulse sequence with gradients[1] or alternatively using excitation sculpting with gradients.[2] All the samples used for NMR study were prepared in 5% D_2O in H_2O. Following samples (combinations) were prepared in separate NMR tubes.

(a) diC16-3′-LNA-A + Poly U (2:1); (b) diC16-3′-dA + Poly U (2:1); (c) diC16-3′-LNA-A; (d) diC16-3′-dA; (e) Poly U

Particle size and zeta potential determination

Solution of nucleolipid (10 mg/mL in dichloromethane) were prepared at room temperature and stored at -20 °C. 100 µL of this solution was placed in glass tube. The solution was dried under dry N_2 and then desiccated under vacuum overnight. HEPES buffer (50 mM, pH 7.2) (for particle size measurement) or Milli-Q Water (for zeta potential measurement and TEM) was added to the dried nucleolipid to obtain dispersions (1 mg/mL, stock solution) after vortex agitation for 1 min. The resultant solution was extruded at room temperature through a 400 then 200 and finally 100 nm pore-size nucleopore polycarbonate filters.

Particle size and zeta potential were determined using Zitasizer 3000 HAS MALVERN. Samples were prepared as described above. Particle size measurement experiments were realized with 1 mg/mL of nucleolipid in HEPES buffer (50 mM, pH 7.2) and performed at 25

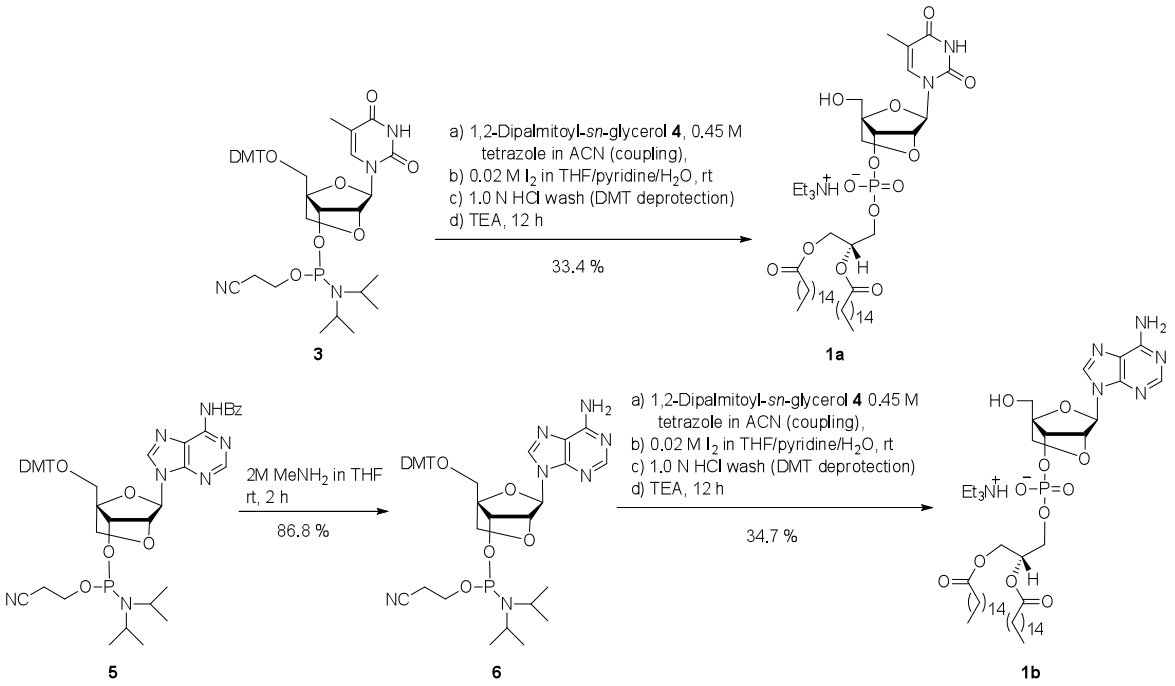
°C. Zeta potential measurement experiments were realized with 100 µL (1 mg/mL, stock solution) of nucleolipid diluted in 1 mL of Milli Q Water and performed at 25 °C.

Transmission Electron Microscopy (TEM)

TEM studies were performed on a HITACHI H7650 electron microscope in high resolution mode, at the BIC platform (Bordeaux Imaging Center). The software used for images acquisition was "Digital Micrograph (Gatan)". 10 µL of sample were dispensed on a carbon-Formvar–coated 200-mesh nickel grid and dried for 10 min and stained with uranyl acetate just prior to observation.

II. Synthesis

Scheme SI1. Synthesis of LNA-based amphiphiles



Synthesis of LNA-Thymidine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-T) 1a:

(1*R*,3*R*,4*R*,7*S*)-3-(thymine-1-yl)-1-(4,4′-dimethoxytrityloxy)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-2,5-dioxabicyclo[2.2.1]heptane **3** (150 mg, 1 equiv. 0.19 mmol), 1,2-dipalmitoyl-*sn*-glycerol **4** (144 mg, 1.3 equiv, 0.25

mmol/dissolved in 1.5 mL of THF) and a tetrazole solution in acetonitrile (0.45 M, 0.58 mL, 1.3 equiv, 0.25 mmol) were dissolved in 1.5 mL of dry acetonitrile under argon. The reaction mixture was stirred for 7 h at room temperature followed by oxidation with 17 mL of a solution of I_2 (0.02 M in THF/Pyridine/H_2O, 0..34 mmol, 1.75 equiv.). After 12 h at room tempereature, the solvent was evaporated under high vacuum to yield intermediate products. The contents of the reaction flask were dissolved in 20 mL of methylene chloride and then washed first with 3 X 20 mL of HCl (0.5 N) and second with 3 X 20 mL of saturated $Na_2S_2O_3$. The organic layer was evaporated and dissolved in 20% TEA in dichloromethane and stir over night (to ensure the complete deprotection of cyanoethyl chain). Product **1a** was isolated after purification on silica gel (DCM/MeOH/TEA from 97:2:1 to 74:25:1). Yield: 115 mg (65.7 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ in ppm 0.80 (t, 6H, J = 6.5 Hz, $2CH_3$ of palmitoyl chain), 1.18 (s, 48H, $24CH_2$ (palmitoyl chain)), 1.32 (t, J = 7.2, $3CH_3$ (triethylammonium)), 1.43-1.55 (m, 4H, $2CH_2$-CH_2-CO), 1.82 (s, 3H, CH_3-base), 2.17-2.22 (m, 4H, $2CH_2$-CO), 3.04 (q, J = 7.2, $3CH_2$ (triethylammonium)), 3.52-4.28 (m, 9H, $2CH_2$ (glycerol), -CH_2-O, 2H5′, 5'-OH), 4.37-4.42 (m, 2H, H2′,H3′), 5.07-5.16 (m, 1H, -CH-glycerol), 5.49 (s, 1H, H1′), 7.62 (s, 1H, H-6(base)), 8.93 (1H, -NH(base)), 11.72 (bs, -NH (triethylammonium)). ^{13}C NMR (75 MHz, $CDCl_3$): δ in ppm 8.47 (CH_3 triethylammonium), 12.5 (CH_3 base), 13.8 (CH_3 chain), 22.4 (CH_2-CH_3 chain), 24.8 (CH_2-CH_2-C=O), 28.9-29.4 (CH_2 chain), 31.6 (CH_2-CH_2-CH_3 chain), 33.8 (CH_2-C=O chain), 33.9 (CH_2-C=O chain), 45.8 (CH_2 triethylammonium), 56.6 (CH_2, C5'-sugar), 62.2 (CH_2-O-C=O glycerol), 63.71, 63.78 (CH_2-O-P=O glycerol, diastereomer), 69.96, 70.06 (CH glycerol, diastereomer), 71.1 (CH_2, C5"(C2'-C4' locked)), 72.00, 73.05 (CH, C3'-sugar, diastereomer), 78.47, 78.56 (CH, C1'-sugar, diastereomer), 86.48 (CH, C2'-sugar), 89.32 (C, C4'-sugar), 109.8 (C, C5-base), 134.6 (CH, C6-base), 149.6 (C=O(2) base), 163.5 (C=O(4) base), 172.7 (C=O chain), 173.1 (C=O

chain). ^{31}P NMR (121 MHz, $CDCl_3$): δ in ppm 2.99. High-resolution ESI MS $[M-H]^-$, theoretical *m/z* – 899.5404, observed *m/z* – 899.5399.

Synthesis of LNA-Adenosine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-A) 1b:

Step 1 (deprotection of benzoyl group): To a solution of 2M $MeNH_2$ in THF (4 mL) was added (1*R*,3*R*,4*R*,7*S*)-3-(6-*N*-Benzoyladenine-9-yl)-1-(4,4′-dimethoxytrityloxy)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-2,5-dioxabicyclo[2.2.1]heptane **5** (350 mg, 0.395 mmol) at room temperature. After being stirred for 2 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with 1% MeOH/DCM (v/v) containing 1% TEA and then 3% MeOH/DCM (v/v) containing 1% TEA to give the fractions containing the target (1*R*,3*R*,4*R*,7*S*)-3-(adenine-9-yl)-1-(4,4′-dimethoxytrityloxy)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-2,5-dioxabicyclo[2.2.1]heptane **6**. Yield: 295 mg (95.4 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ in ppm 0.84-1.15 (m, 12H, $4CH_3$), 2.33-2.52 (m 2H, $-CH_2CN$), 2.8 (bs, 2H, 2CH-isoproply), 3.44-3.59 (m, 4H, 2-CH_2O (cyanoethyl), 2H5′), 3.82 (s, 6H, 2-OCH_3), 3.94-4.16 (m, 2H, $-CH_2$-O-), 4.39-4.57 (m, 1H, H3′), 4.84-4.90 (m, 1H, H2′), 5.64 (s, 2H, $-NH_2$), 6.13 (s, 1H, H1′), 6.80-6.92 (m, 4H, DMT), 7.30-7.55 (m, 9H, DMT), 8.13-8.15 (m, 1H, H-2(base)), 8.36 (s, 1H, H-8(base)).

Step 2 (coupling with lipid): (1*R*,3*R*,4*R*,7*S*)-3-(adenine-9-yl)-1-(4,4′-dimethoxytrityloxy)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-2,5-dioxabicyclo[2.2.1]heptane **6** (product of step 1) (295 mg, 1 equiv. 0.38 mmol), 1,2-dipalmitoyl-*sn*-glycerol **4** (279 mg, 1.3 equiv, 0.49 mmol/dissolved in 3 mL of THF) and a tetrazole solution in acetonitrile (0.45 M, 1.09 mL, 1.3 equiv, 0.49 mmol) were dissolved in 3 mL of dry acetonitrile under argon. The reaction mixture was stirred for 7 h at room temperature followed by oxidation with 24.5 mL

of a solution of I_2 (0.02 M in THF/Pyridine/H_2O, 0.49 mmol, 1.3 equiv.). After 12 h at room tempereature, the solvent was evaporated under high vacuum to yield intermediate products. The contents of the reaction flask were dissolved in 20 mL of methylene chloride and then washed first with 3 X 20 mL of HCl (0.5 N) and second with 3 X 20 mL of saturated $Na_2S_2O_3$. The organic layer was evaporated and dissolved in 20% TEA in dichloromethane and stir over night (to ensure the complete deprotection of cyanoethyl chain). Product **1b** was isolated after purification on silica gel (DCM/MeOH/TEA from 97:2:1 to 74:25:1). Yield: 125 mg (34.7 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ in ppm 0.86 (t, 6H, J = 6.7 Hz, $2CH_3$ of palmitoyl chain), 1.24 (s, 48H, $24CH_2$ (palmitoyl chain) and $3CH_3$ (triethylammonium)), 1.57 (bs, 4H, $2CH_2$-CH_2-CO), 2.21-2.27 (m, 4H, $2CH_2$-CO), 3.01 (q, J = 7.25, $3CH_2$ (triethylammonium)), 3.77-4.34 (m, 8H, $2CH_2$ (glycerol), -CH_2-O, 2H5′), 4.78-4.89 (m, 2H, H2′,H3′), 5.14-5.24 (m, 1H, -CH- glycerol), 6.02 (s, 1H, H1′), 6.44 (bs, 2H, -NH_2), 8.21 (s, 1H, H-2(base)), 8.24 (s, 1H, H-8(base)). ^{13}C NMR (75 MHz, $CDCl_3$): δ in ppm 8.5 (CH_3, triethylammonium), 13.9 (CH_3 chain), 22.6 (CH_2-CH_3 chain), 24.8 (CH_2-CH_2-C=O), 29.1-29.6 (CH_2 chain), 31.8 (CH_2-CH_2-CH_3 chain), 33.9 (CH_2-C=O chain), 34.2 (CH_2-C=O chain), 45.8 (CH_2 triethylammonium), 56.9 (CH_2, C5'-sugar), 62.32, 62.37 (CH_2-O-C=O glycerol, diastereomer), 63.91, 63.98 (CH_2-O-P=O glycerol, diastereomer), 70.12, 70.22 (CH glycerol, diastereomer), 71.8 (CH_2, C5"(C2'-C4' locked)), 72.97, 73.03 (CH, C3'-sugar, diastereomer), 78.92, 78.98 (CH, C1'-sugar, diastereomer), 86.0 (CH, C2'-sugar), 88.77, 88.81 (C, C4'-sugar, diastereomer), 119.8 (C, C5-base), 138.2 (CH, C8-base), 148.8 (C, C6-base), 152.8 (CH, C2-base), 155.4 (C, C4-base), 172.8 (C=O chain), 173.2 (C=O chain). ^{31}P NMR (121 MHz, $CDCl_3$): δ in ppm 2.92. High-resolution ESI MS $[M-H]^-$, theoretical m/z – 908.5519, observed m/z – 908.5509.

III. NMR Spectra

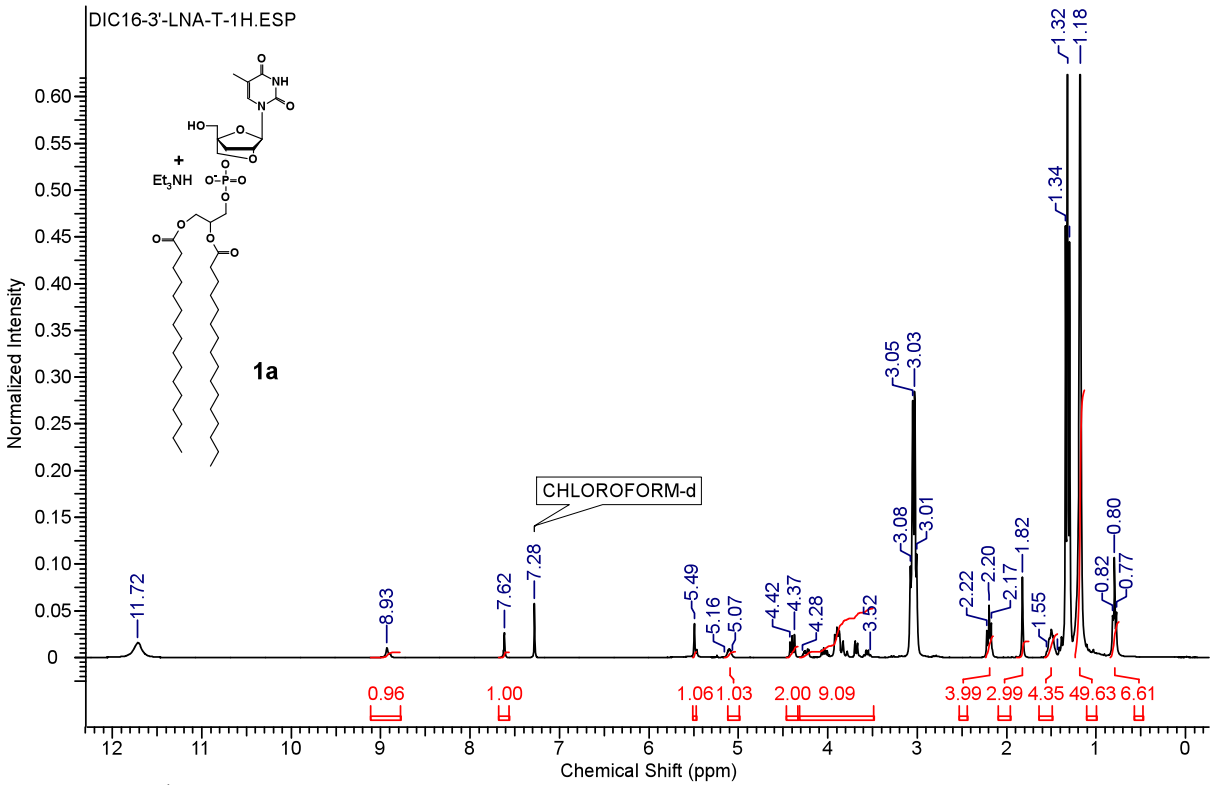


Figure SI1. ^{1}H NMR of LNA-Thymidine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-T) **1a**

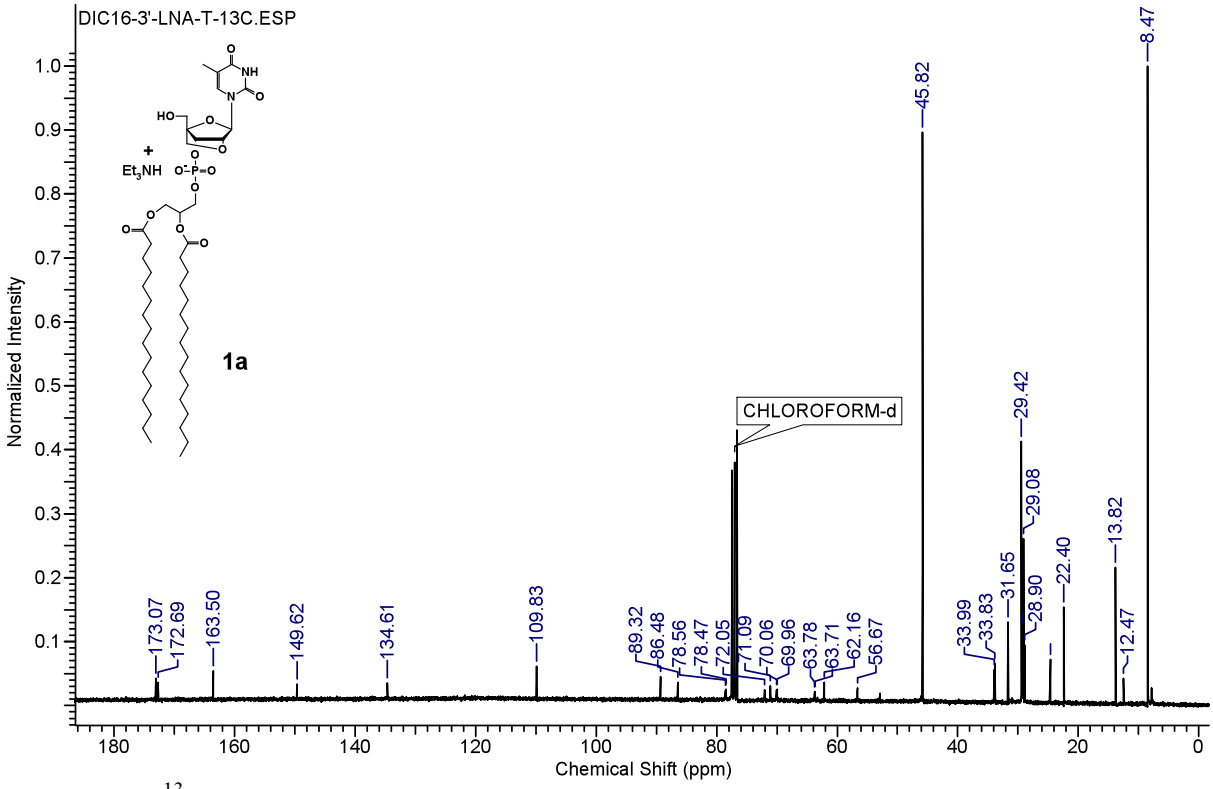


Figure SI2. ^{13}C NMR of LNA-Thymidine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-T) **1a**

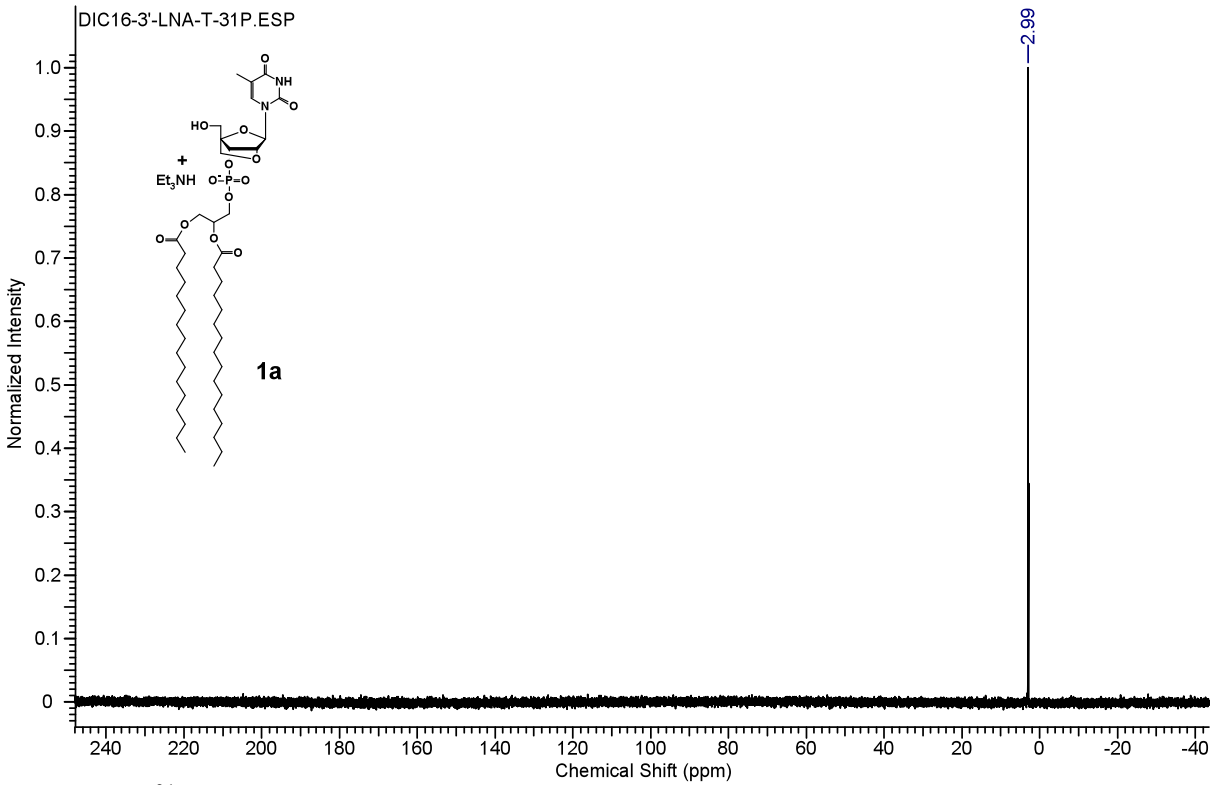


Figure SI3. ^{31}P NMR of LNA-Thymidine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-T) **1a**

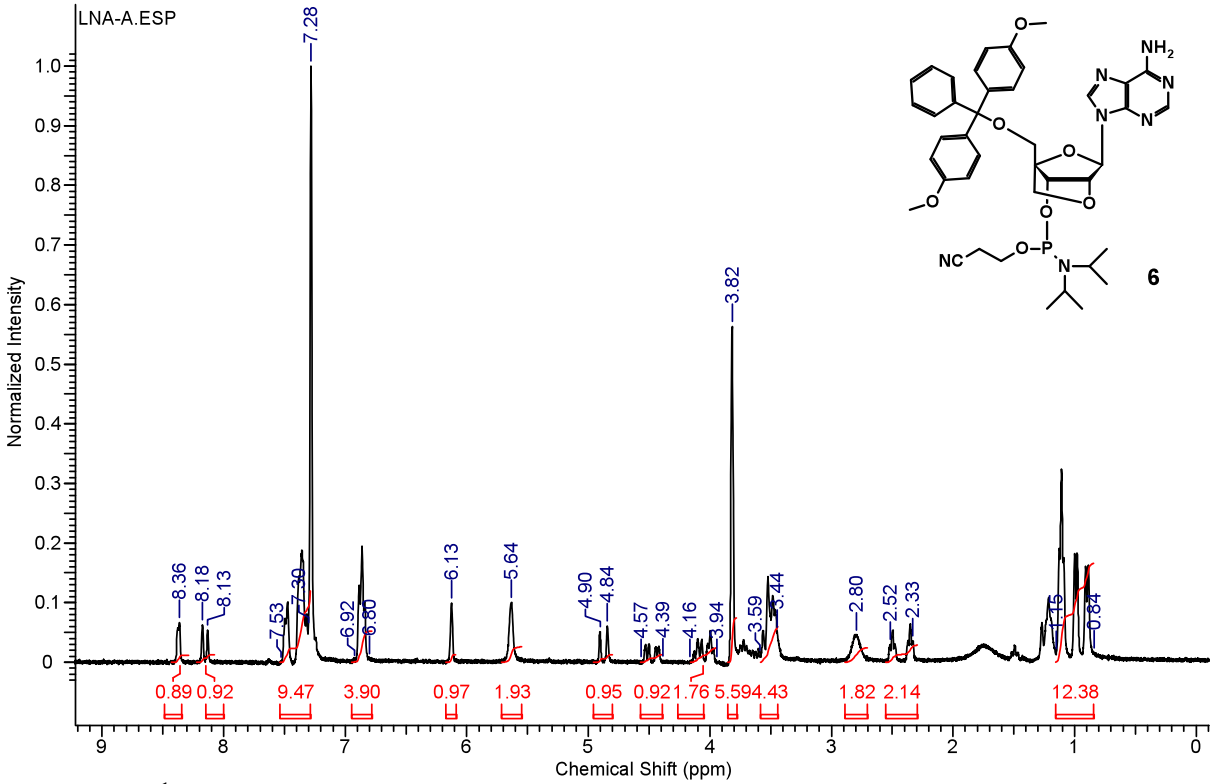


Figure SI4. ^{1}H NMR of (1*R*,3*R*,4*R*,7*S*)-3-(adenine-9-yl)-1-(4,4′-dimethoxytrityloxy)-7-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-2,5-dioxabicyclo[2.2.1]heptane **6**

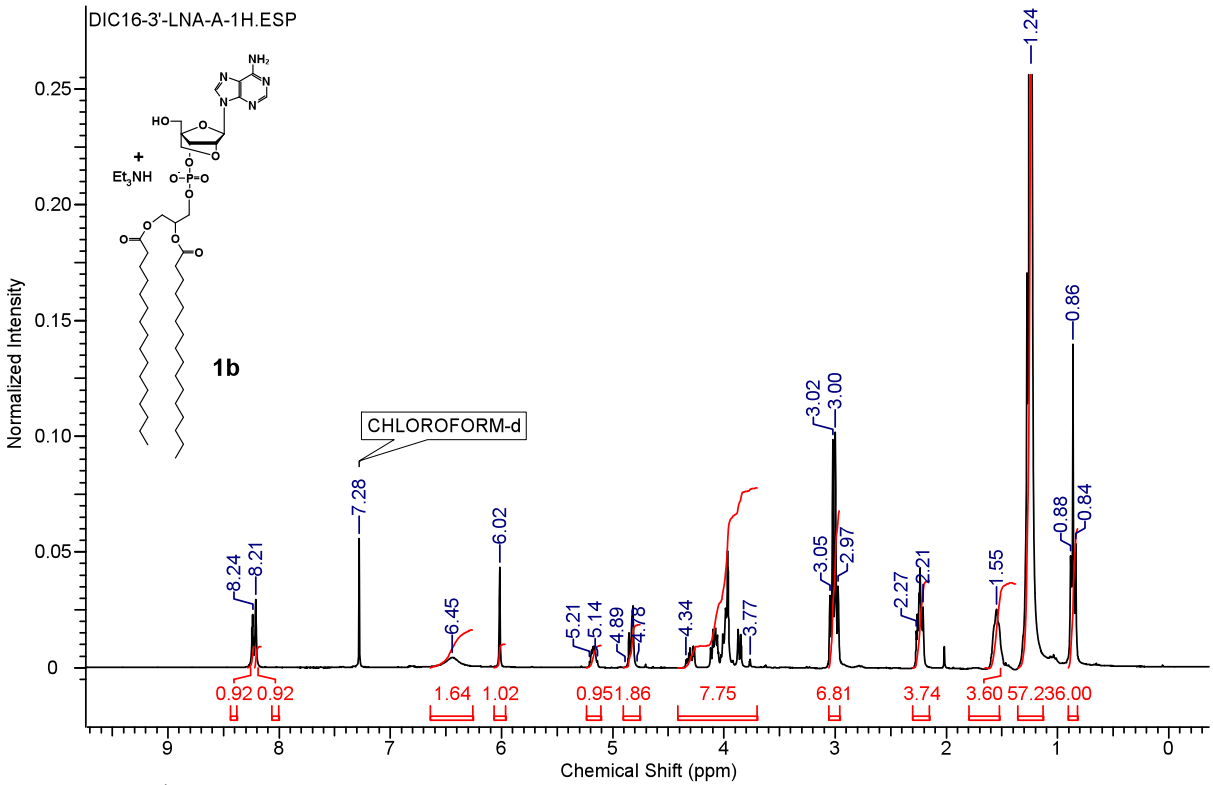


Figure SI5. [1]H NMR of LNA-Adenosine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-A) **1b**

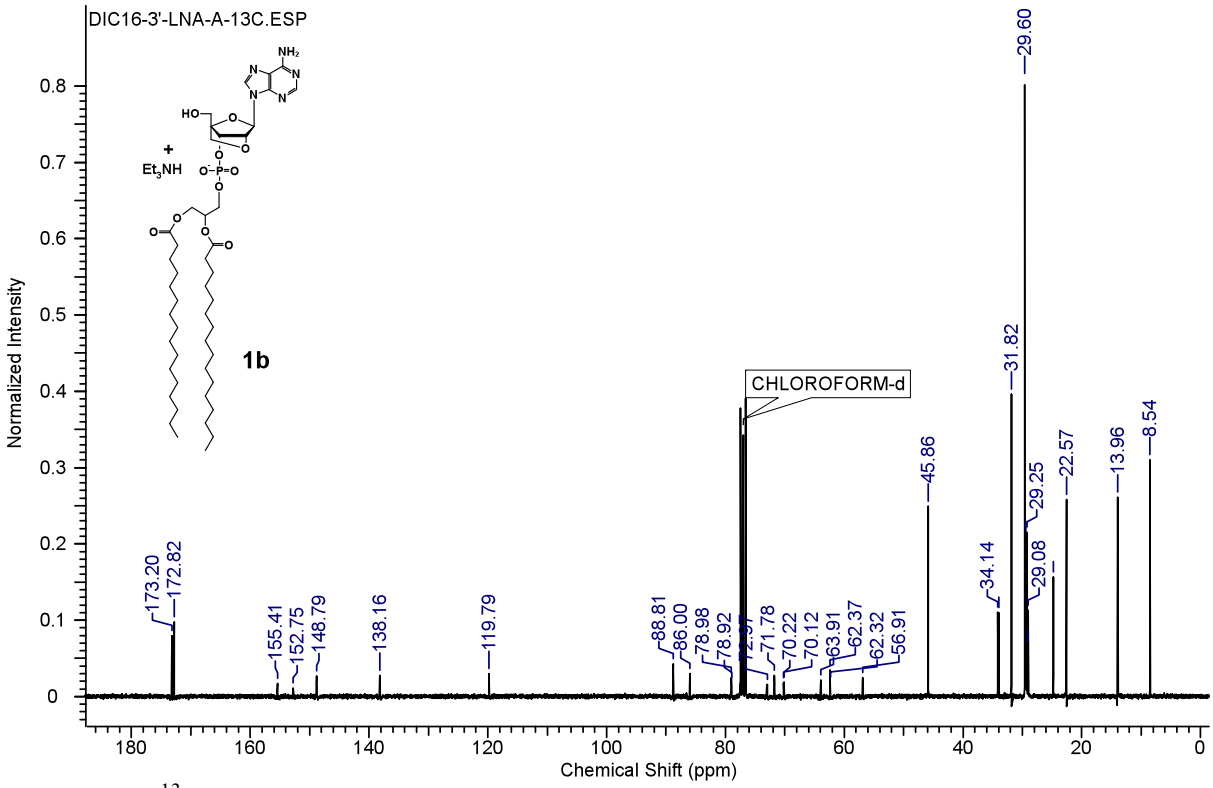


Figure SI6. [13]C NMR of LNA-Adenosine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-A) **1b**

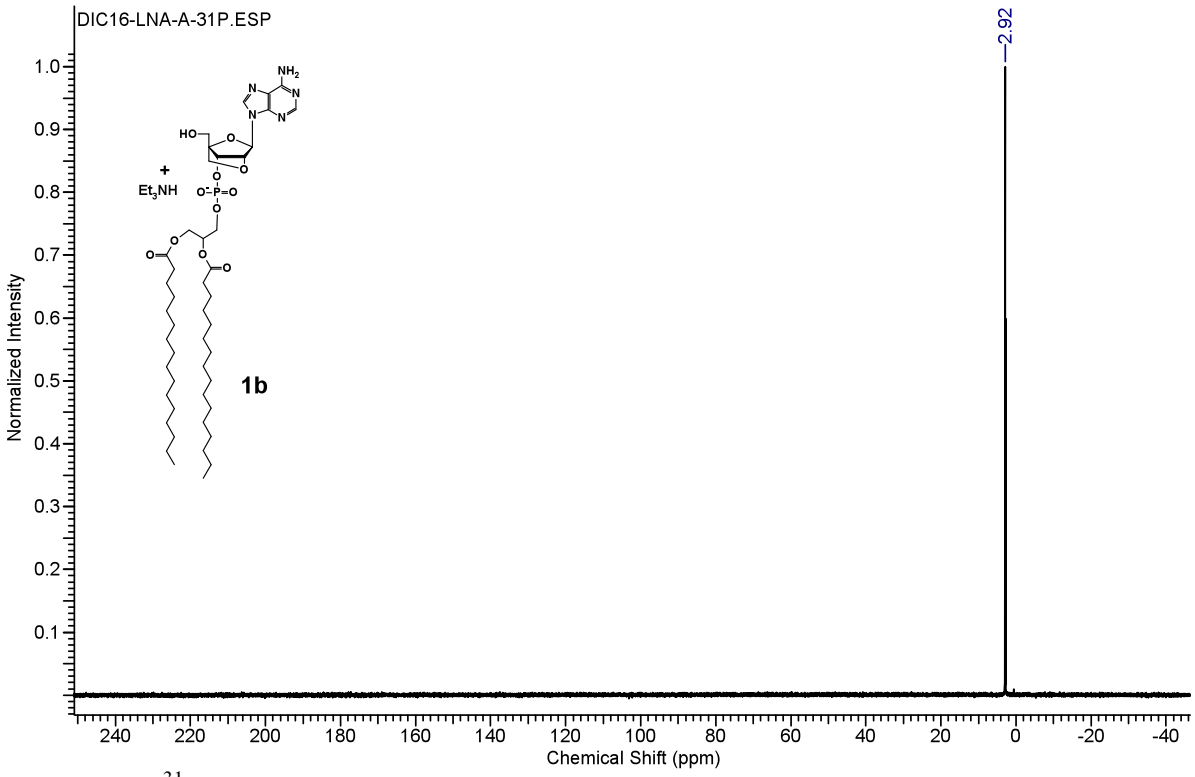


Figure SI7. ^{31}P NMR of LNA-Adenosine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-A) **1b**

IV. Mass Spectra (HR ESI-MS)

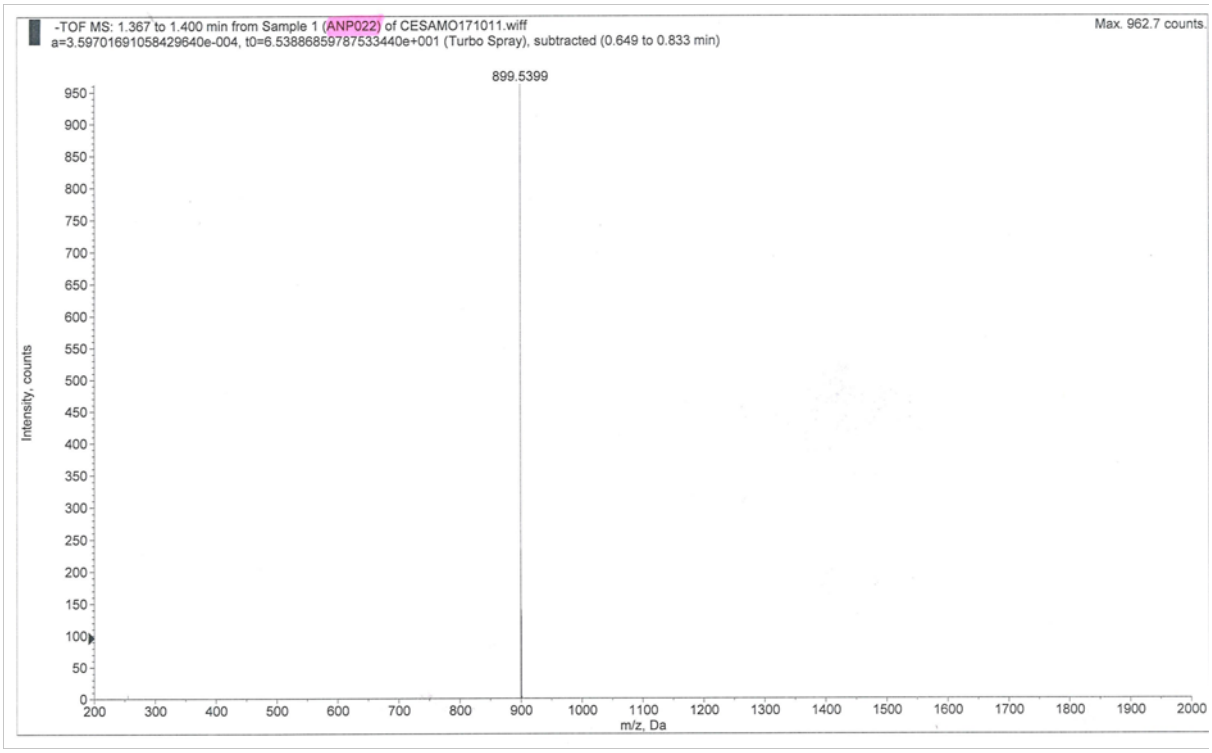


Figure SI8. Mass spectra of LNA-Thymidine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-T) **1a**

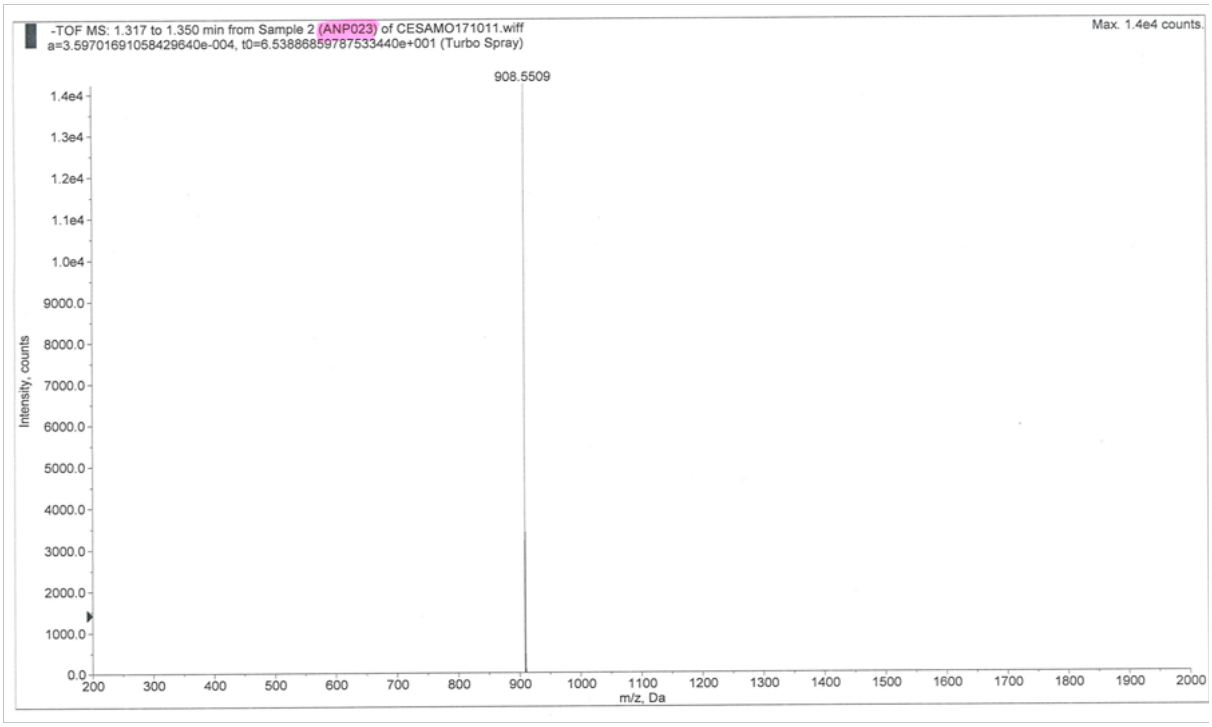


Figure SI9. Mass spectra of LNA-Adenosine-3′-(1,2-dipalmitoyl-*sn*-glycero-3-phosphate), (diC16-3′-LNA-A) **1b**

V. Additional data

Particle size and zeta potential determination

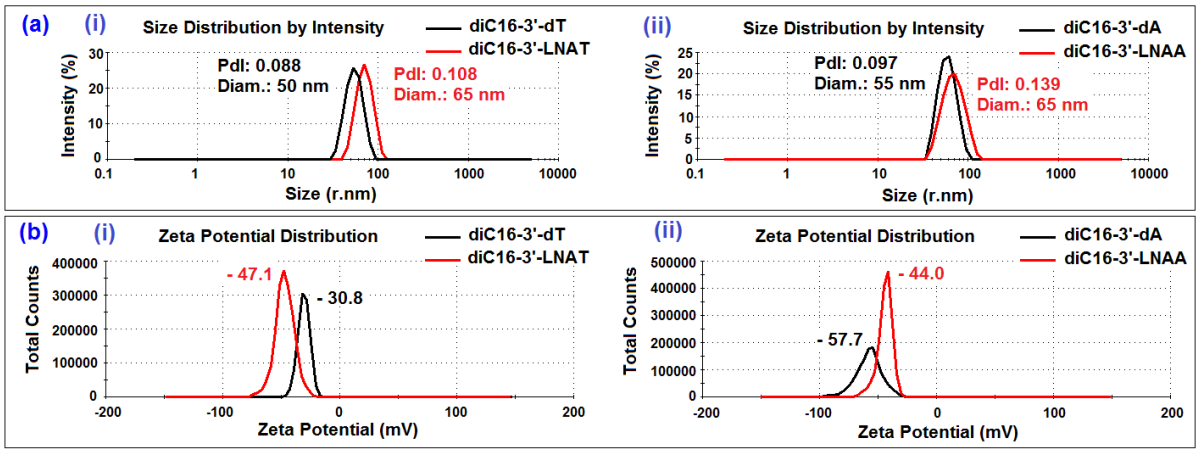


Figure SI10. (a) DLS profiles showing the intensity-averaged radius of (i) diC16-3′-LNA-T 1a and diC16-3′-dT 2a and (ii) diC16-3′-LNA-A 1b and diC16-3′-dA 2b, 0.1 mM in HEPES buffer (50 mM, pH 7.2) (b) zeta-potential profiles of (i) diC16-3′-LNA-T 1a and diC16-3′-dT 2a and (ii) diC16-3′-LNA-A 1b and diC16-3′-dA 2b, 0.01 mM in water.

2D NMR spectra

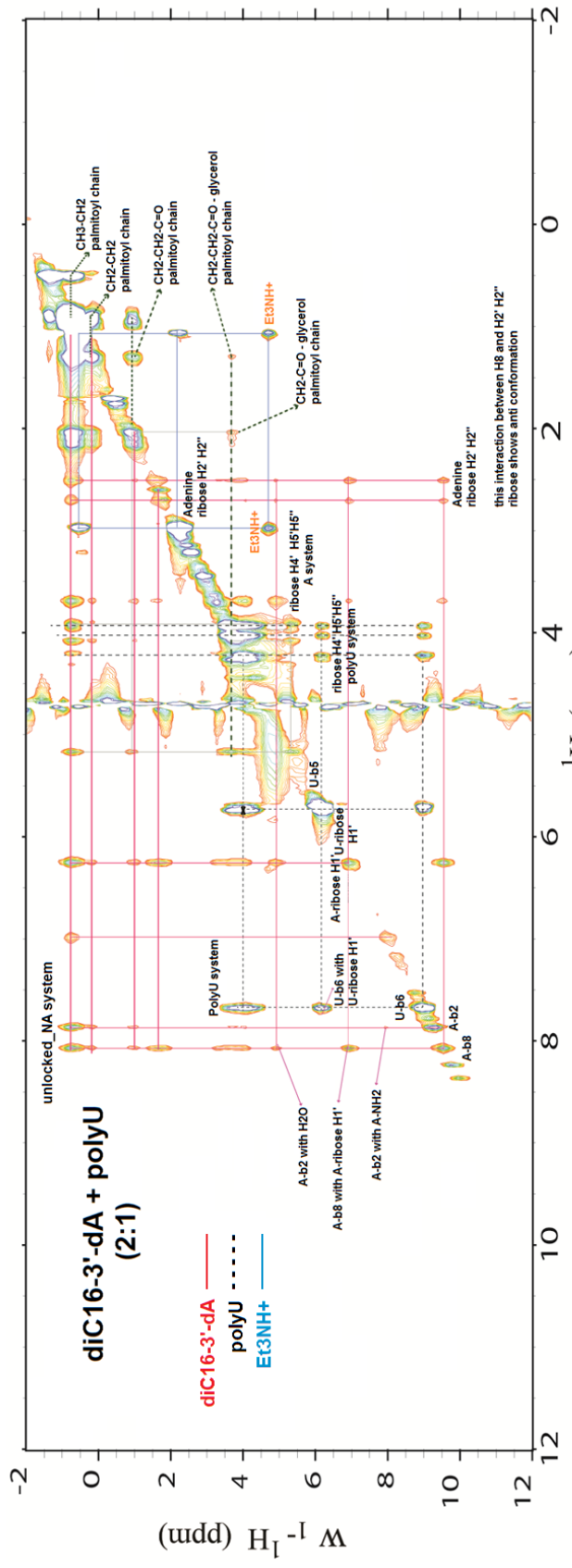


Figure SI11. 2D ^{1}H-^{1}H NOESY spectra of (A) diC16-3'-dA + polyU complex.

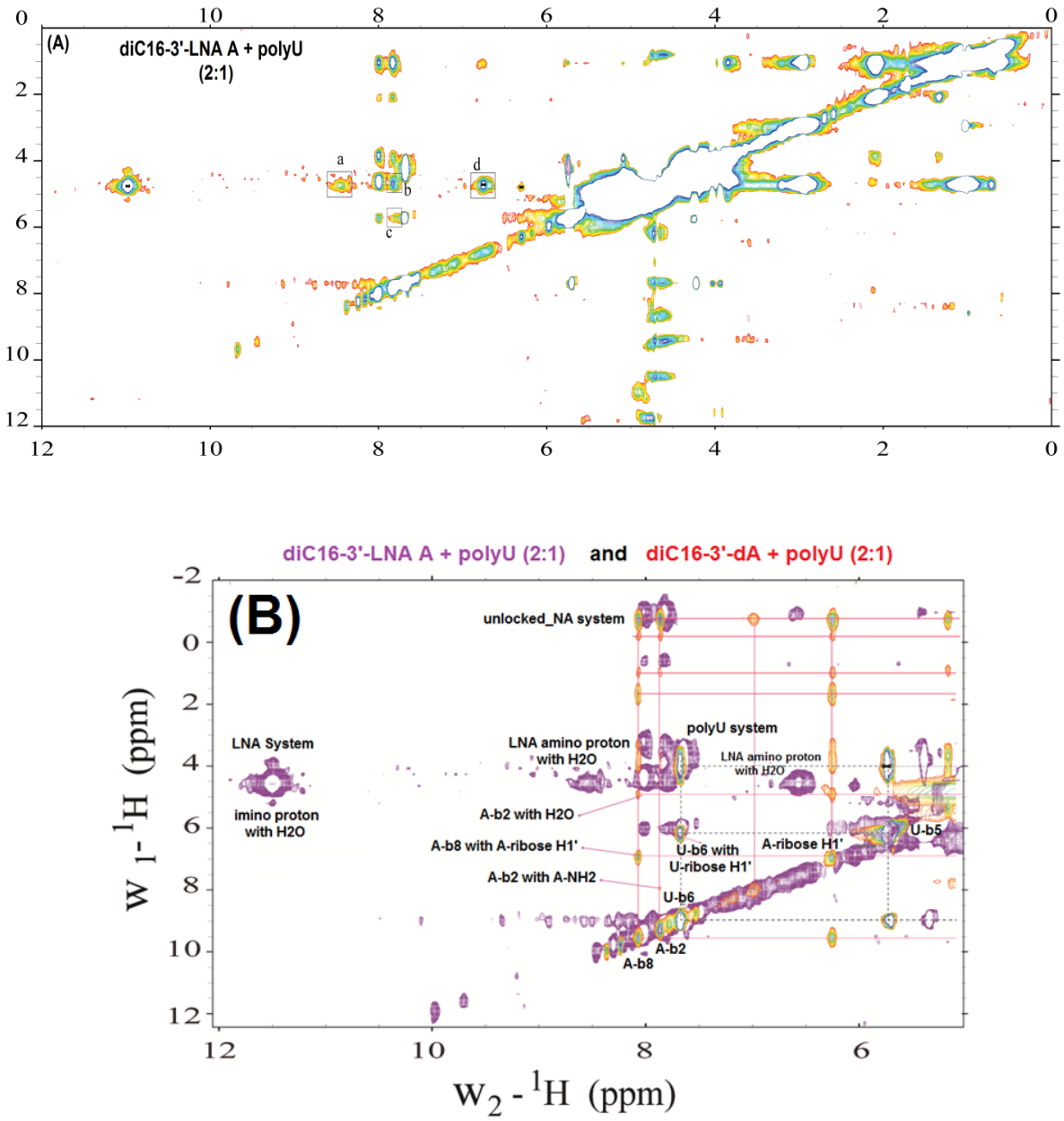


Figure S12. 2D ^{1}H-^{1}H NOESY spectra of (A) diC16-3'-LNA A + polyU complex (B) diC16-3'-dA + polyU (red-yellow-green gradient) overlay with diC16-3'-LNA A + polyU complex (purple)

ITC profiles

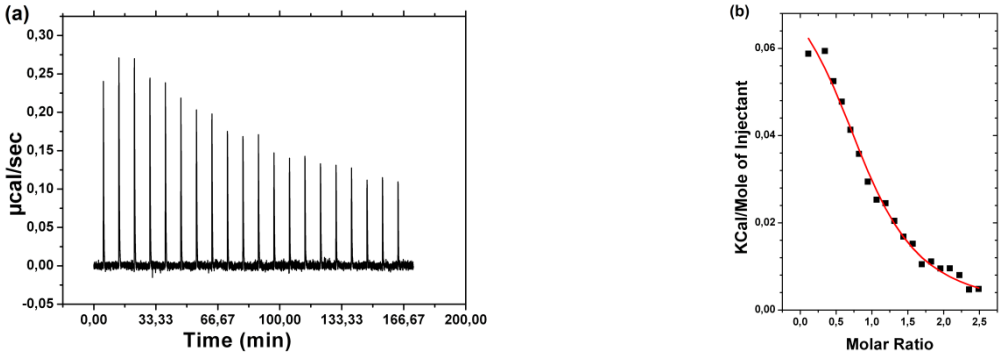


Figure SI13. ITC data for the titration of polyA to diC16-3′-LNA-T **1a** in HEPES buffer (a) power versus time (b) molar enthalpy versus the molar ratio of polyA/diC16-3′-LNA-T **1a**

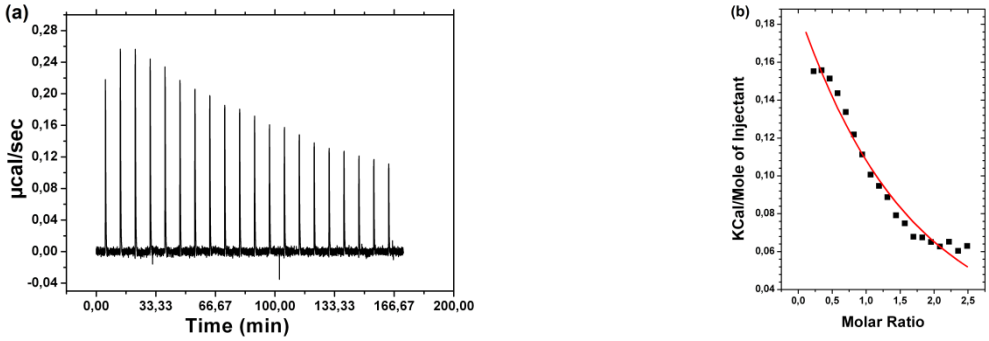


Figure SI14. ITC data for the titration of polyA to diC16-3′-dT **2a** in HEPES buffer (a) power versus time (b) molar enthalpy versus the molar ratio of polyA/diC16-3′-dT **2a**

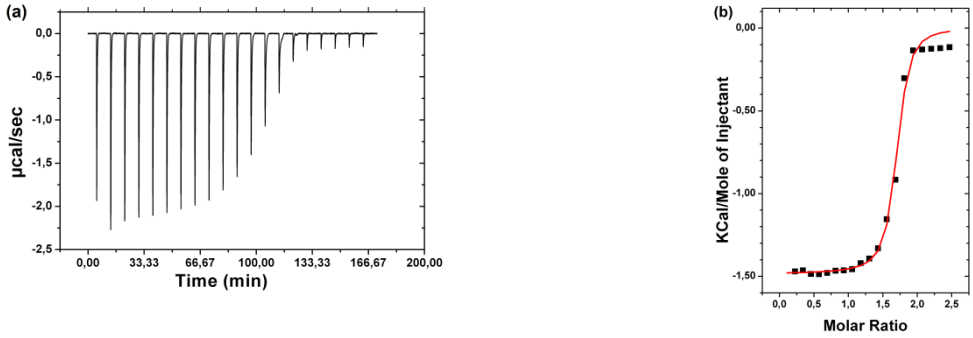


Figure SI15. ITC data for the titration of polyU to diC16-3′-dA **2b** in HEPES buffer (a) power versus time (b) molar enthalpy versus the molar ratio of polyU/diC16-3′-dA **2b**

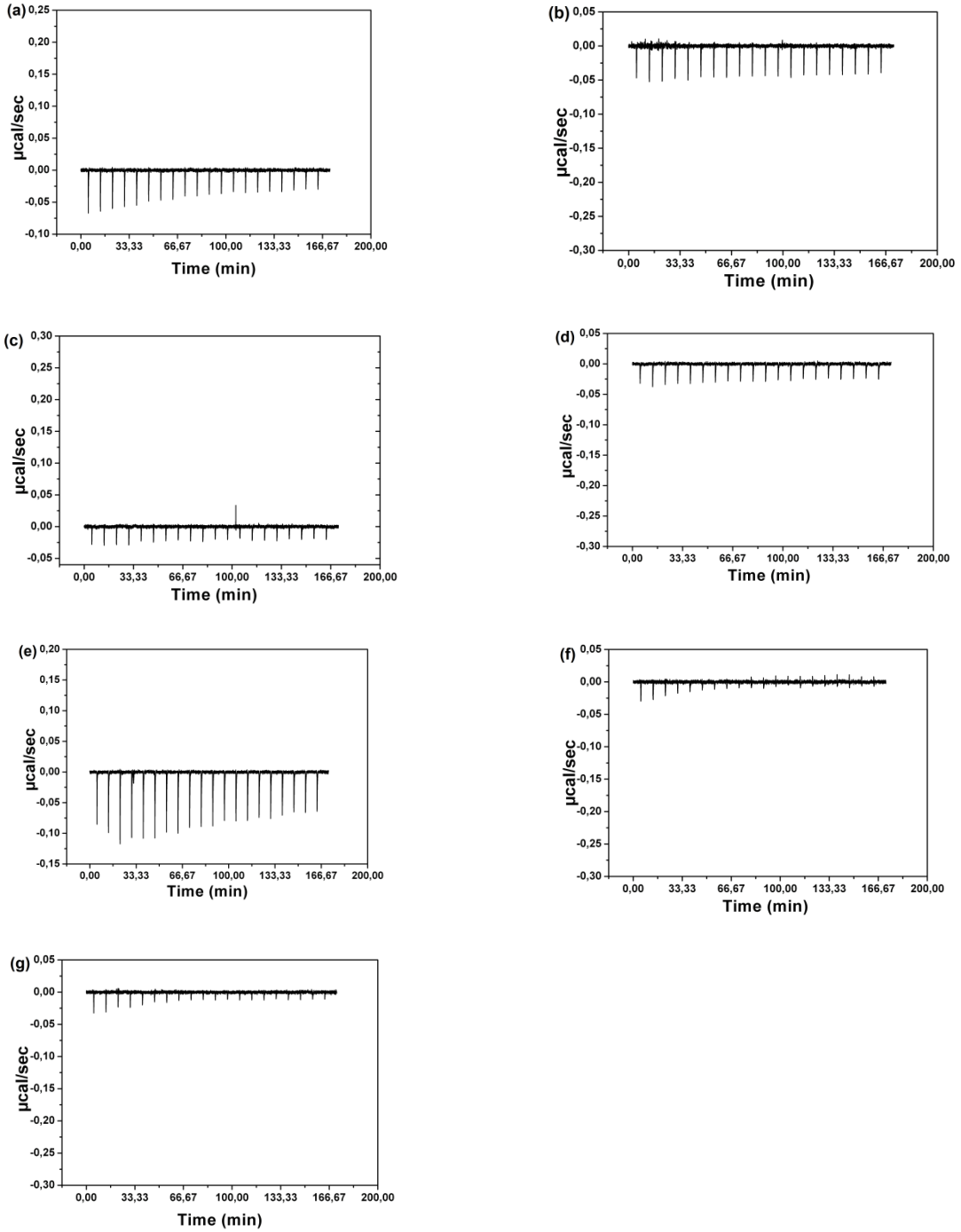


Figure SI16. ITC data for titration of (a) buffer[a] to diC16-3′-LNA-T **1a** (b) buffer to diC16-3′-LNA-A **1b** (c) buffer to diC16-3′-dT **2a** (d) buffer to diC16-3′-dA **2b** (e) polyA to buffer (f) polyU to buffer and (g) buffer to buffer. [a]HEPES buffer (50 mM, pH 7.2)

Table SI1. Thermodynamic data for ITC experiments

	Poly A (in the Syringe)[a] (14.6 mM)		Poly U (in the Syringe)[a] (14.6 mM)		
Nucleo-Lipid (in the Cell) ▸	diC16-3'-dT **2a**[a,b] (1.25 mM)	diC16-3'-LNA-T **1a**[a,b] (1.25 mM)	diC16-3'-dA **2b**[a,b] (1.25 mM)	diC16-3'-LNA-A **1b**[a,b] (1.25 mM)	
N^c	0.995	0.964	1.65	N_1	0.379
				N_2	1.47
K (M^{-1})	380 (K_3)	3.23 x10^3 (K_3^*)	1.73 x10^5 (K)	K_1^*	2.32 x 10^7
				K_2^*	6.11 x 10^4
ΔH (cal/mol)	565.3	80.1	-1484	ΔH_1	-156.8
				ΔH_2	-84.96
ΔS (cal/mol/deg)	13.7	16.3	19.0	ΔS_1	33.2
				ΔS_2	21.6
	Endothermic	Endothermic	Exothermic	Exothermic (two wave)	

[a]All the solutions were prepared in HEPES buffer (50 mM, pH 7.2) and HEPES buffer was prepared with degassed water.
[b]Lipids are in the form of its triethylammonium salts.
[c]Reaction stoichiometry-Molar ratio (N): syringe (here poly A or poly U)/ cell (nucleolipid **1a-b**,**2a-b**)

Transmission Electron Microscopy (TEM) images

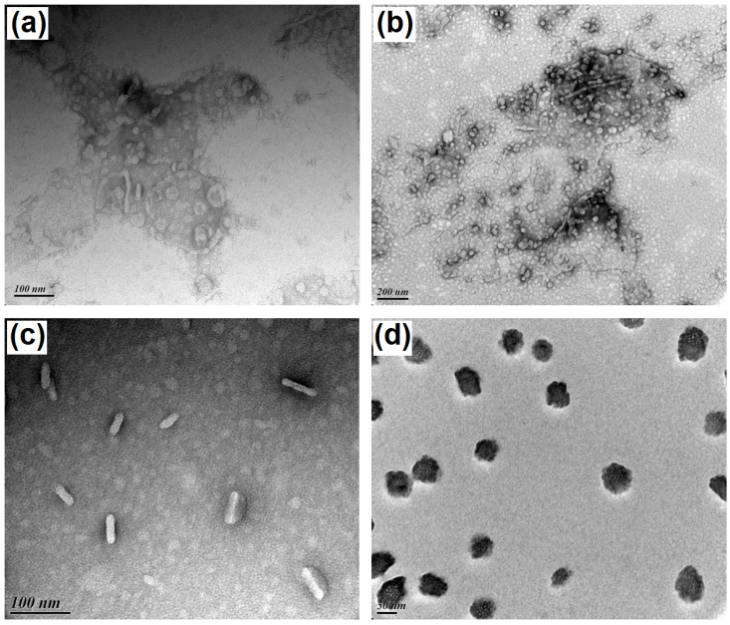


Figure SI17. TEM images of the supramolecular assemblies (a) diC16-3′-LNA-A, (b) diC16-3′-dA, (c) diC16-3′-LNA-A: poly U (2:1) and (d) diC16-3′-dA: poly U (2:1) in HEPES buffer (50 mM, pH 7.2). a, b, c are negative stain, d is positive stain.

References:

(1) Liu, M.; Mao, X.-A.; Ye, C.; Huang, H.; Nicholson, J. K.; Lindon, J. C. *J. Magn. Reson.* **1998**, *132*, 125–129.
(2) Thrippleton, M. J.; Keeler, J. *Angew. Chem. - Int. Ed.* **2003**, *42*, 3938–3941.