Supramolecular Assemblies of DNA with Neutral Nucleoside Amphiphiles

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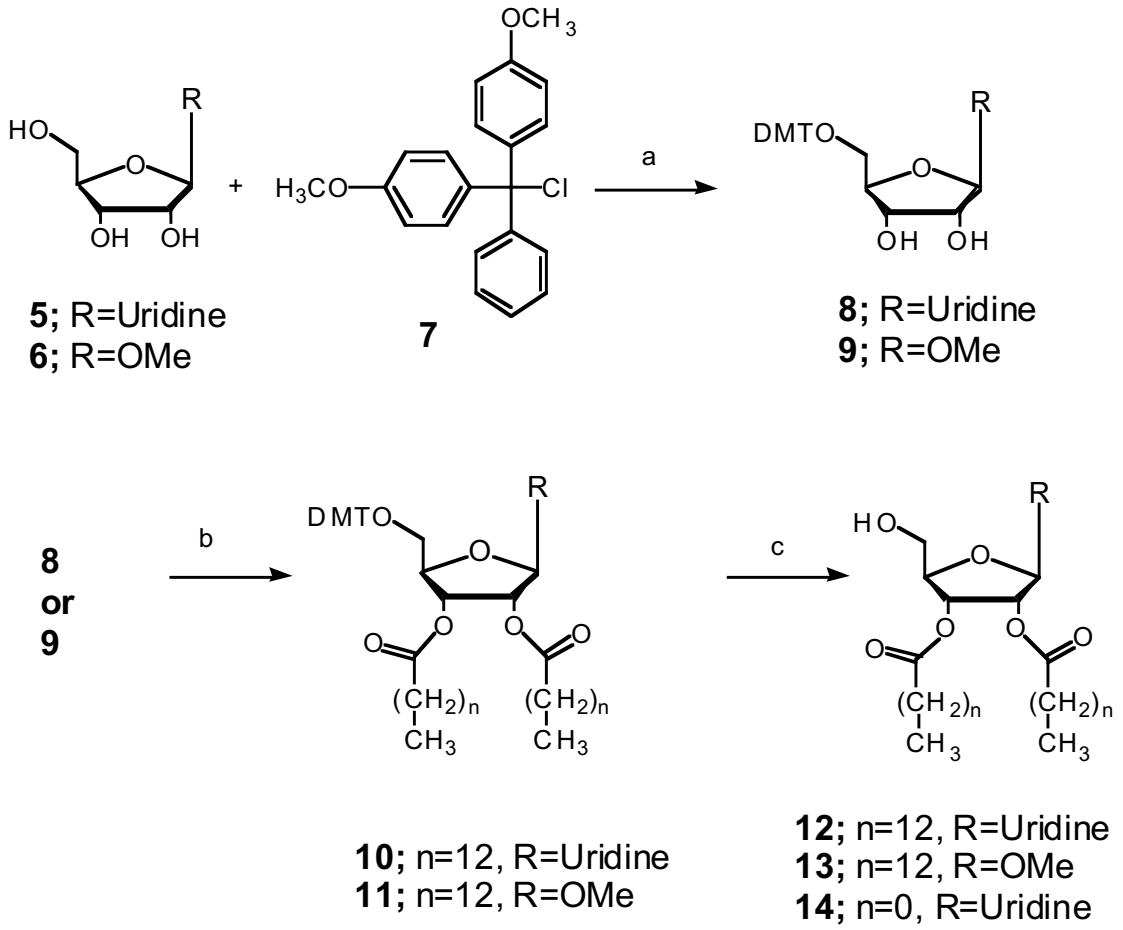
All solvents were dried and freshly distilled prior to use. All chemicals were purchased from Aldrich or Acros and used without further purification. All reactions were performed under nitrogen atmosphere. NMR spectra were recorded on a Varian INOVA spectrometer operating at 400 MHz (for ^{1}H). Plasmid DNA was purchased from Sigma. Fluorescence studies were carried out using a Hitachi F2500.

1-{5-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-3,4-dihydroxy-tetrahydro-furan-2-yl}-1H-pyrimidine-2,4-dione (8)

Dimethoxytritylchloride (2.0 g, 5.9 mmol), uridine (1.1 g, 4.5 mmol), and a catalytic amount of N,N-dimethyl aminopyridine were dissolved in 25 mL of pyridine. The reaction mixture was stirred for 24 hours at room temperature. Pyridine was removed under vacuum and the resulting crude material was purified on silica gel (DCM/MeOH 95/5) to yield 2.14 g of the expected product **8** (Yield: 87%). ^{1}H NMR ($CDCl_3$) δ in ppm: 3.48 (m, 2H, 2*H*-5'), 3.7 (s, 6H, 2 OCH_3), 4.17 (m, 1H, *H*-4'), 4.35 (m, 1H, *H*-3'), 4.42 (m, 1H, O*H*), 5.24 (s, 1H, *H*-2'), 5.31 (d, J=8.2Hz, 1H, C*H*), 6.00 (m, 1H, *H*-1'), 6.8 (d, J=8.9Hz 4H, *H*arom), 7.24 (m, 9H, *H*arom), 7.95 (d, J=8.2Hz, 1H, C*H*), 8.6(m, 1H, N*H*).^{13}C NMR ($CDCl_3$) δ in ppm: 55.25 (OCH_3), 61.80 (*C*5'), 69.57 (*C*3'), 72.90 (*C*4'), 83.46 (*C*2'), 86.90 (*C*1'), 90.14 (Ctrit), 102.18 (*C*H uridine), 113.21 (*C*arom), 140.43 (*C*H uridine), 123.82-149.41 (*C*arom), 151.17 (*C*O, uridine), 158.57 (OCH_3), 163.94 (*C*O, uridine). The data agreed with reported values (Smith et al.; *J. Amer. Chem. Soc.* ; 84; 1962; 430,436. Kawana, Masajiro; Kuzuhara, Hiroyoshi; *J. Chem. Soc. Perkin Trans.* 1; 4; 1992; 469-478.)



a/ Dimethoxytritylchloride, uridine, DMAP (cat) in pyridine, 24h. b/ RCO_2H, DCC, DMAP in DCM. c/ CCl_3CO_2H 3% in DCM

Scheme 1. Synthesis of compounds **8 - 14**.

2-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-5-methoxy-tetrahydro-furan-3,4-diol (9)

1-O-Methyl−β−D-ribofuranose **6** (0.82 g, 5.01 mmol) and dimethoxytritylchloride (1.63 g, 5.83 mmol) were dissolved in 100 mL of dry pyridine and heated to 120 °C for 3 hours. The solvent was evaporated under high vacuum and the resultant oil dissolved in chloroform. The chloroform was washed with water, 0.5 N HCl, and then again with water before being dried with Na_2SO_4. Silica gel column chromatography was performed (0-3% methanol in chloroform) yielding **2** in an 87% yield (1.77 g, 4.40 mmol). ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): 3.25 (m, 2H, *H*-5'); 3.32 (s 3H, OCH_3); 4.00 (d, 1H, J=4.4 Hz *H*-2'); 4.09 (m, 1H, *H*-4'); 4.23 (dd, 1H, J=6.4 Hz, 4.8 Hz, *H*-3'); 4.86 (s, 1H,

H-1'); 7.19-7.6 (m, 15 H, *Harom*). ^{1}H-NMR data agreed with literature values previously reported (Hird, G. S.; McIntosh, T. J.; Anthony, R. A.; Grinstaff, M. W., *J. Am. Chem. Soc.* **2002,** 124, 5983-5992. Kawana, M.; Kuzuhara, H.; Emoto, S. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1492-1504.)

Tetradecanoic acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (10)

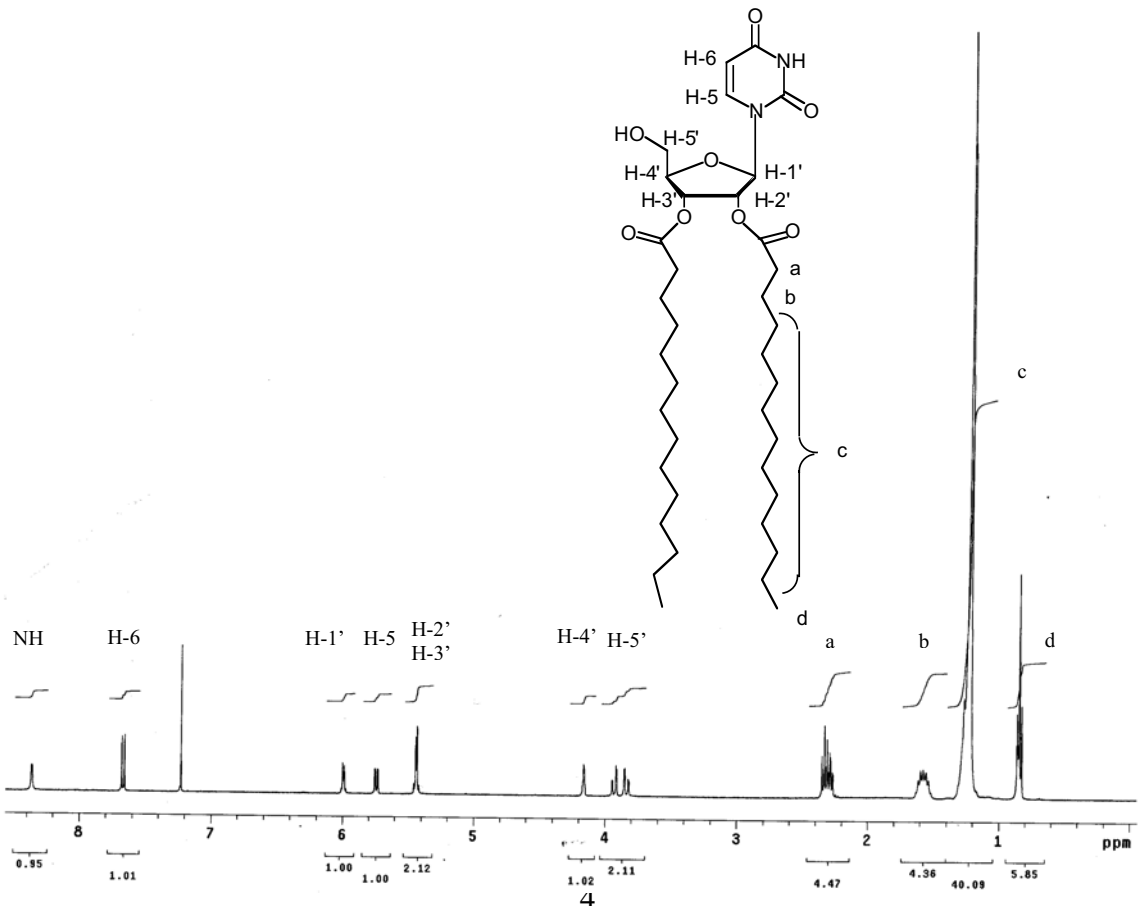
Compound **8** (0.50 g, 0.91 mmol), myristic acid (0.46 g, 2.01 mmol), dicyclohexylcarbodiimide (DCC, 0.41 g, 2.01 mmol) and N,N-dimethylaminopyridine (DMAP, 0.24 g, 2.01 mmol) were dissolved in 100 mL of freshly distilled methylene chloride. The mixture was stirred for 24 hours at room temperature under nitrogen. After filtration, the organic phase was successively washed with 20 mL of water 3 times and dried over sodium sulfate. Methylene chloride was removed under vacuum. The product (0.79 g) was obtained after chromatography (DCM/MeOH, 95/5) on silica gel (Yield: 90%). ^{1}H NMR ($CDCl_3$) δ in ppm: 0.8 (t, J=7Hz, 6 H, 2C*H*$_3$), 1.2 (m, 40H, 20C*H*$_2$), 2.3 (m, 4H, 2C*H*$_2$), 3.4 (m, 2H, 2*H*-5'), 3.8 (m, 6H, 2 OC*H*$_3$), 4.2 (s, 1H, *H*-4'), 5.25 (m, 2H, C*H* and *H*-2'), 5.6 (m, 1H, *H*-3'), 6.2 (m, 1H, *H*-1'), 6.8 (d, J=9Hz 4H, *H*arom), 7.24 (m, 9H, *H*arom), 7.7 (d, J=8Hz, 1H, C*H*), 8.2 (m, 1H, N*H*).

Tetradecanoic acid 2-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-5-methoxy-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (11)

Compound **9** (1.22 g, 7.46 mmol), myristic acid (5.45 g, 23.86 mmol), DMAP (2.92 g, 23.86 mmol) and DCC (4.93 g, 23.86 mmol) were stirred in 150 mL of DMF for 48 hours at 60 ^{o}C to afford **11**. The solution was filtered and the resultant mixture was purified on a silica gel column with the eluent of 9/1 Hex/EtOAc. The product was immediately converted to **13** as described below following the published procedure (Hird, G. S.; McIntosh, T. J.; Anthony, R. A.; Grinstaff, M. W., *J. Am. Chem. Soc.* **2002,** 124, 5983-5992.)

Tetradecanoic acid 5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-hydroxymethyl-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (12)

Compound **10** (0.788 g, 0.81 mmol) was dissolved in 50 mL of dried methylene chloride and an excess of a 3% tricloroethylacetic acid in methylene chloride was added. After thirty minutes, 3 mL of methanol was added to the mixture. The organic layer was then washed three times with 20 mL of water and dried over sodium sulfate. Crystallization in methylene chloride afforded 464 mg (0.70 mmol) of compound **12** (Yield: 86%). ^{1}H NMR ($CDCl_3$) δ in ppm: 0.84 (t, 6 H, 2C*H*$_3$), 1.22 (m, 40H, 20 C*H*$_2$), 1.55 (m, 4H, 2C*H*$_2$), 2.33 (m, 4H, 2C*H*$_2$), 3.84 (dd, 2H, *H*-5'), 4.17 (m, 1H, *H*-4'), 5.44 (m, 1H, *H*-2', *H*-3'), 5.74 (dd, 1H, *H*-5), 6.00 (m, 1H, *H*-1'), 7.66 (d, 1H, *H*-6), 8.15 (m, 1H, N*H*).^{13}C NMR ($CDCl_3$) δ in ppm: 14.07 (*C*H$_3$), 22.65 (*C*H$_2$), 24.67 (*C*H$_2$), 24.87 (*C*H$_2$), 29.04-29.62 (*C*H$_2$), 31.88 (*C*H$_2$), 33.83 (*C*H$_2$CO), 33.99 (*C*H$_2$CO), 61.80 (*C*5'), 71.04 (*C*3'), 72.90 (*C*4'), 83.66 (*C*2'), 87.61 (*C*1'), 103.15 (*C*H), 140.71 (*C*H), 150.40 (*C*O, uridine), 163.06 (*C*O, uridine), 172.46 (*C*O, ester), 172.84 (*C*O, ester). High Resolution MS: $[M+H]^+$, theoritical m/z=665.4663, observed m/z=665.4731.

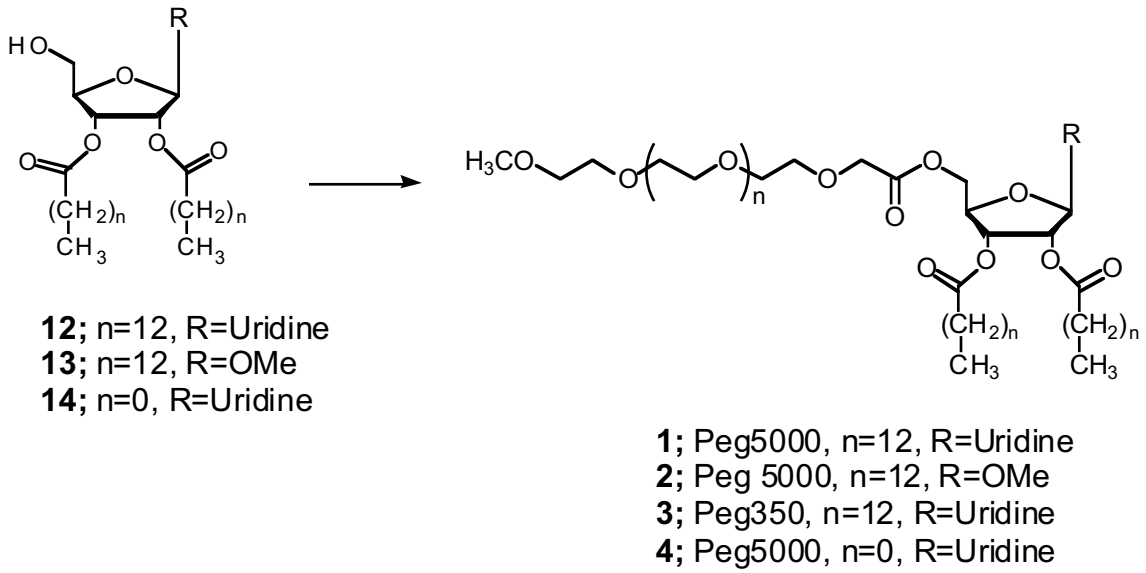


Tetradecanoic acid 2-hydroxymethyl-5-methoxy-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (13)

Next the solid, **11**, was immediately dissolved in aqueous acetic acid and stirred at 50 ^{o}C for 12 hours. The product, **13**, was subsequently purified by silica gel column chromotography (eluent 7/3 Hex /EtAc) with an overall yield of 49% for the last two steps (2.12 g, 3.63 mmol). ^{1}H-NMR ($CDCl_3$, 400 MHz, ppm): 0.863 (t, C*H3*), 1.260 (m, $(CH_2)_n$), 1.610 (m, C*H*$_2$), 2.331 (m, C*H*$_2$), 3.413 (3H, OC*H*$_3$), 3.863 (m, 2H, *H*-5'), 4.196 (m, 1H, *H*-4'), 4.888 (s, 1H, *H*-1'), 5.233 (d, 1H, *H*-2'), 5.339 (t, 1H, *H*-3'). ^{1}H-NMR data agreed with literature values previously reported (Hird, G. S.; McIntosh, T. J.; Anthony, R. A.; Grinstaff, M. W., *J. Am. Chem. Soc.* **2002,** 124, 5983-5992).

Acetic acid 4-acetoxy-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-hydroxymethyl-tetrahydro-furan-3-yl ester (14)

Compound **8** (1 g, 1.2 mmol) was dissolved in anhydride acetic/pyridine (1/1, 10 mL). The mixture was stirred overnight. After the solvent was removed under vacuum. The residual crude product was dried under high vacuum for one hour. The residue was dissolved in 50 mL of freshly distilled methylene chloride and an excess of a 3% tricloroethylacetic acid in methylene chloride was added under nitrogen to the solution. After 15 minutes, methanol (5 mL) was poured into the solution. The organic phase was washed 3 times with 20 mL of water and then dried over sodium sulfate. The solvent was removed under vacuum and 0.75 g of product **14** was obtained (Yield: 58%). ^{1}H NMR ($CDCl_3$) δ in ppm: 2.01 (s, 6 H, 2C*H*$_3$), 3.6 (m, 2H, $\underline{CH_2}$O*H*), (dd, 1H, *H*-5'), 4.42 (d, , J=2.2Hz, 1H, *H*-4'), 5.00 (s, 1H, *H*-2'), 5.54 (m, 1H, *H*-3'), 5.75 (d, J=8.2Hz, 1H, *H*-5), 6.40 (m, 1H, *H*-1'), 7.7 (d, J=8.2Hz, 1H, *H*-6), 8.5 (m, 1H, N*H*).^{13}C NMR ($CDCl_3$) δ in ppm: 17.01 (*C*H$_3$), 62.04 (*C*5'), 68.84 (*C*3'), 72.81 (*C*4'), 79.50 (*C*2'), 88.10 (*C*1'), 103.01 (*C*H), 140.29 (*C*H), 151.21 (*C*O, uridine), 162.85 (*C*O, uridine), 172.06 (*C*O, ester). MS: [M+H], m/z=329.



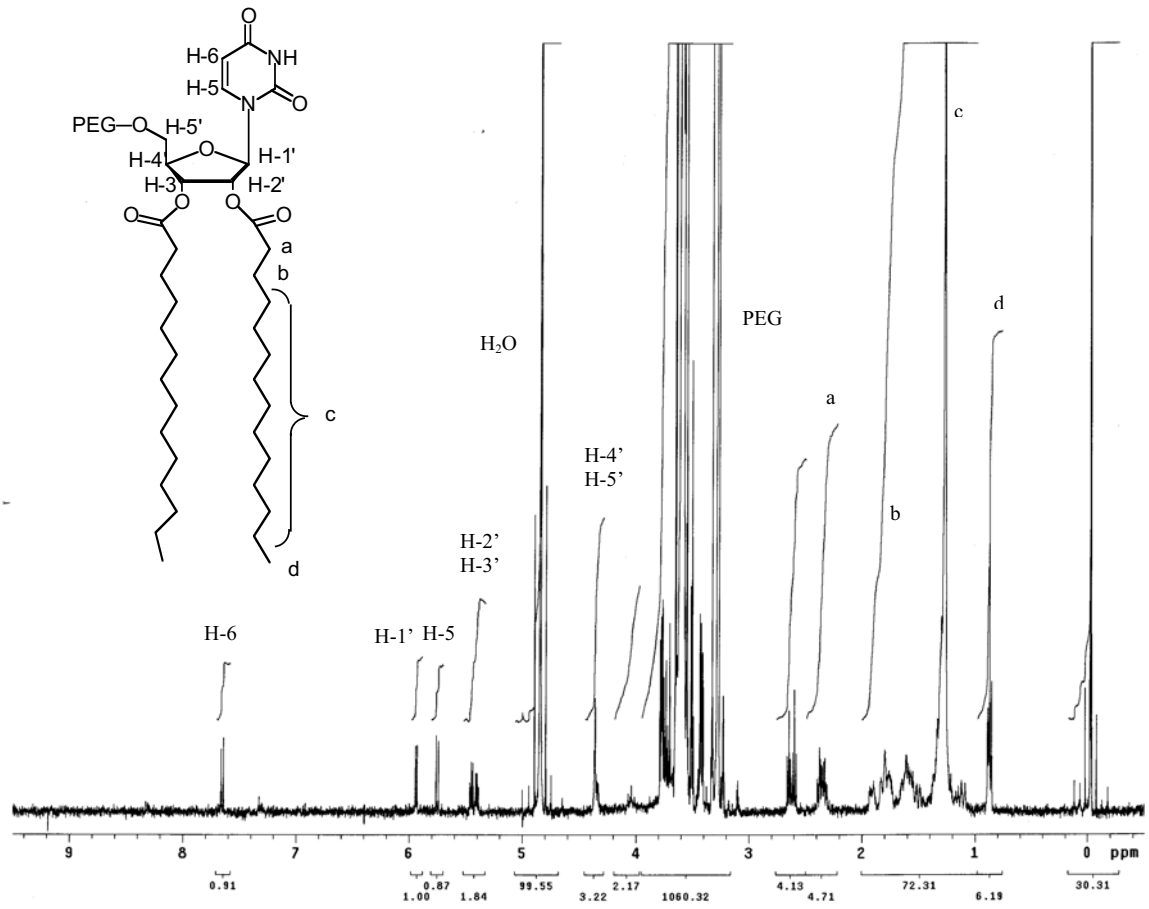
a/ $CH_3O(PEG5000)CH_2CO_2H$, DCC, DMAP in DCM, RT, 6 days.

Figure 2 Synthesis of the PEG derivatives **1** - **4**.

Tetradecanoic acid 5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-[3-(2-methoxy-PEG)-carboxymethyl]-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (1)

Compound **12** (10 mg, 0.015 mmol), methoxy-PEG5000-carboxymethyl (75 mg, 0.015 mmol), dicyclohexylcarbodiimide (10 mg, 0.05 mmol), and a catalytic amount of N,N-dimethylaminopyridine were dried for one hour under high vacuum. The starting materials were then dissolved under nitrogen in 5 mL of freshly distilled methylene chloride. The mixture was stirred for 6 days at room temperature under nitrogen. After filtration, the solvent was removed and the resulting crude product was purified with a LH20 size exclusion column in DCM/MeOH 50/50. The product (56 mg) was isolated after precipitation in methanol/ether (Yield: 64 %). ^{1}H NMR (CD_3OD) δ in ppm: (broad peaks were observed) 0.87 (t, 6 H, 2C*H*$_3$), 1.26 (m, C*H*$_2$), 1.60 (m, C*H*$_2$), 1.79 (m, C*H*$_2$), 2.35 (m, 4H, 2C*H*$_2$), 2.63 (m, 4H, 2C*H*$_2$), 3.22-3.78 (m, ~1000H, PEG), 4.35 (m, 3H, *H*-4', *H*-5'), 5.42 (m, 2H, *H*-2', *H*-3'), 5.75 (d, 1H, *H*-5), 5.94 (d, 1H, *H*-1'), 7.65 (d, 1H, *H*-5). MALDI MS: 1/ Starting material methoxy-PEG5000-carboxymethyl, Cal Mw=5359, found Mw=5484. Product **1,** Cal Mw=6023, found Mw=6025. Sephadex Exclusion Chromatography (based on PEG standards in THF using a Polymer Laboratories PL gel MIXED-E 3 μm column at a flow rate of 1mL/min) Mn = 6485,

Polydispersity index = 1.09, starting material methoxy-PEG5000-carboxymethyl in the same conditions, Mn = 5379, polydispersity index = 1.1.



Tetradecanoic acid 5-methoxy-2-[3-(2-methoxy-PEG)-carboxymethyl]-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (2)

A similar procedure was performed as for product **1**. Compound **13** (50 mg, 0.085 mmol), methoxy-PEG5000-carboxymethyl (428 mg, 0.085 mmol), dicyclohexylcarbodiimide (22 mg, 0.10 mmol), and a catalytic amount of N,N-dimethylaminopyridine were dissolved in methylene chloride. A white powder (261 mg) was isolated after precipitation in methanol/ether and purified by LH20 size exclusion

column in DCM/MeOH 50/50 (Yield: 55%). ^{1}H NMR ($CDCl_3$) δ in ppm: (broad peaks are observed) 0.8 (t, 6 H, 2C*H*$_3$), 1.25 (m, 40H, 20 C*H*$_2$), 2.3 (m, 4H, 2C*H*$_2$), 3.6 (m, ~440H, PEG). MALDI MS: Starting material methoxy-PEG5000-carboxymethyl, Cal Mw=5359, found Mw=5484. Product **2**, Cal Mw=5943, found Mw=5780. Sephadex Exclusion Chromatography (based on PEG standards in THF using a Polymer Laboratories PL gel MIXED-E 3 μm column at a flow rate of 1mL/min) Mn = 6052, Polydispersity index = 1.10.

Tetradecanoic acid 5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-2-[3-(2-methoxy-PEG)-carboxymethyl]-4-tetradecanoyloxy-tetrahydro-furan-3-yl ester (3)
Compound **12** (200 mg, 0.3mmol), methoxy PEG350-carboxymethyl (104 mg, 0.3 mmol), DCC (185 mg, 0.9 mmol) and a catalytic amount of N,N-dimethylaminopyridine were dried for 1 h under high vacuum. The compounds were then dissolved under nitrogen atmosphere in 20 mL of anhydrous methylene chloride. The reaction mixture was stirred at room temperature for 5 days. The DCU was filtered and the solvent was removed. The product was purified using a G-25 resin column in water. Lyophilization afforded the product (Yield: 65 %). ^{1}H NMR ($CDCl_3$) δ in ppm: (broad peaks are observed) 0.80 (t, J=7Hz, 6H, 2 C*H*$_3$), 1.25 (m, 40H, 20 C*H*$_2$), 2.30 (m, 4H, 2 C*H*$_2$), 3.30 (s, 3H, OC*H*$_3$), 3.60 (m, 36H, PEG), 4.30 (m, 5H, OC*H*$_2$CO; *H*-4', *H*-5'), 5.30 (m, 2H, *H*-2', *H*-3'), 5.74 (d, J=8Hz, 1H, *H*-5), 6.00 (d, 1H, *H*-1'), 7.50 (d, J=8Hz, 1H, *H*-6), 8.40 (m, 1H, N-H). FAB+ve Mass: Starting material methoxy PEG350-carboxymethyl m/z: 399.3. Product **3**, Cal 1046, found 1046.3.

3-(2-Methoxy-Peg)-carboxymethyl 3,4-diacetoxy-5-(2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-yl)-tetrahydro-furan-2-ylmethyl ester (4)
Compound **14** (10 mg, 0.03 mmol), methoxy-PEG5000-carboxymethyl (100 mg 0.015 mmol), dicyclohexylcarbodiimide (10 mg, 0.05 mmol), and a catalytic amount of N,N-dimethylaminopyridine were dried for 1 h under high vacuum. The compounds were then dissolved under nitrogen in 5 mL of freshly distilled methylene chloride. The mixture was stirred for 8 days at room temperature under nitrogen. After filtration, the solvent was removed and the product was isolated after precipitation in methanol/ether

(Yield: 77%). ^{1}H NMR (CD_3OD) δ in ppm: (broad peaks are observed) 0.8 (t, 6 H, 2C*H*$_3$), 1.25 (m, 40H, 20 C*H*$_2$), 2.3 (m, 4H, 2 C*H*$_2$), 3.6 (m, PEG), 4.33 (m, 3H, *H*-4', *H*-5'), 5.31 (m, 2H, *H*-2', *H*-3'), 5.74 (d, 1H, *H*-5), 6.00 (d, 1H, *H*-1'), 7.52 (d, 1H, *H*-6). MALDI MS: Starting material methoxy-PEG5000-carboxymethyl, Cal Mw=5359, found Mw=5484. Product **4**, Cal Mw=5794, found Mw=5780. Sephadex Exclusion Chromatography (based on PEG standards in THF using a Polymer Laboratories PL gel MIXED-E 3 μm column at a flow rate of 1mL/min) Mn = 5122, Polydispersity index = 1.11.

AFM Studies The AFM experimented were adapted from the publication Hansma (H. G. Hansma, I. Revenko, K. Kim and D. E. Laney, *Nucleic Acids Research* vol. 24, 4, pp723-720).

AFM imaging

The AFM images were collected in tapping mode using a Digital Instruments Nanoscope IIIa/Multimode Atomic Force Microscope. The silicon tapping mode AFM tips were purchased from Silicon/MDT (Model NSC-15) and used as received. Typical scanning and feedback parameters are as follows: oscillation frequency, 350 kHZ; integral gain, 0.2; proportional gain, 2.0; setpoint, 1.5 V; scan speed, 2 Hz.

Sample preparation

Preparation of DNA and amphiphiles-DNA solutions. Initially plasmid DNA was imaged in the absence of amphiphiles. 3 μL of the plasmid DNA, the initial solution (980 μg/mL) was diluted with 3 mL of the buffer (2 mM HEPES, 150 mM $MgCl_2$, 10 μm EDTA, pH 7.4). Amphiphiles-DNA condensates were prepared as following; 3 μL (plasmid DNA initial solution, 980 μg/mL) and varying amount of amphiphiles (0.5-5 mg dependent on the Amphiphile/DNA ratio required) were diluted to 1 mL with buffer (2 mM HEPES, 150 mM $MgCl_2$, 10 μm EDTA, pH 7.4). The solutions were mixed and incubated for 60 minutes at room temperature. Each solution was then diluted to 3 mL with buffer.

DNA and amphiphiles-DNA on mica. The resulting DNA-amphiphile solution was incubated on a freshly cleaved mica substrate for 10 minutes, rinsed twice with distilled water and dried in a desiccator under high vacuum for 1 h.

Fluorescence Measurements

For the measurements of critical micellar concentration, a stock solution of pyrene (0.5 mM) in ethanol was added to test tubes. The ethanol was then evaporated by a stream of nitrogen and thereafter under vacuum for 1 h. Various amounts of amphiphiles stock solution in buffer (2 mM HEPES, 150 mM $MgCl_2$, 10 μm EDTA, pH 7.4) were added to the tubes to give a final volume of 1 mL and a final pyrene concentration of 0.5 μM. The samples were incubated in the dark at 25 ºC for 24 h, with intermittent vigorous mixing. Fluorescence spectra were recorded on Hitachi F2500 using λ_{ex} = 320 nm and λ_{em} = 350-450 nm.