Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information

Developing a self-healing supramolecular nucleoside hydrogel

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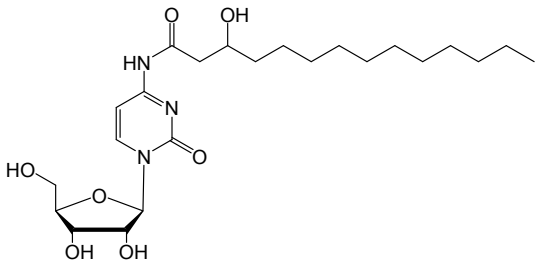
Supplementary 1:

Procedure for the synthesis of *N4*-(3-hydroxytetradecanoyl)cytidine To a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 0.9 mmol, 1 eqv, 158 mg) in anhydrous CH_2Cl_2 (3.5 mL) at 0 °C, was added *N*-methylmorpholine (NMM, 27.2 mmol, 1.36 eqv, 98 µL) with continuous stirring until a white suspension had formed. The mixture was then left to stir for 1 h. 3-hydroxy-tetradecanoic acid (0.9 mmol, 1 eqv, 207 mg) was added directly into the mixture as a solution in anhydrous DMF (2 mL) and stirred for a further 1 h. A solution of cytidine (A) (0.9 mmol, 1 eqv, 219 mg) in anhydrous DMF (2 mL) was made up at 0 °C. The cold triazine solution was added drop wise to the cooled cytidine solution over 30 mins, before heating to 50 °C and stirring for 12 h. The cooled solution was evaporated *in vacuo*. The product was purified using flash silica column chromatography, eluting at 5 - 7 % methanol in CH_2Cl_2. Product was a white powder. Purity was determined by NMR.



Yield: 21 %

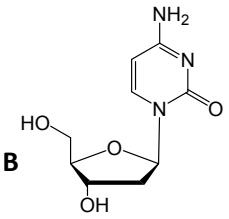
NMR purity: 98.2 %

^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.8 Hz, 3H, C<u>H</u>$_3$), 1.15-1.41 (m, 20H, CH_3-(C<u>H</u>$_2$)$_{10}$-CH(OH)), 2.25 (ddd, *J* = 22.6, 14.8, 6.5 Hz 1H, C=O-CH_2-C<u>H</u>(OH)), 2.45 (d, *J* = 6.7 Hz, 2H, C=O-C<u>H</u>$_2$), 3.55 - 3.78 (m, 2H, 5'-C<u>H</u>$_2$), 3.85 (m, 1H, 4'-C<u>H</u>), 3.95 (m, 1H, 3'-C<u>H</u>), 4.71 (t, *J* = 5.3 Hz, 1H, acyl-3-O<u>H</u>) 5.04 (t, *J* = 5.4 Hz, 1H, 3'-O<u>H</u>), 5.15 (d, *J* = 5.2 Hz, 1H, 5'-O<u>H</u>), 5.47 (d, *J* = 4.7 Hz, 1H, 2'-O<u>H</u>), 5.77 (d, *J* = 2.4 Hz, 1H, 1'-C<u>H</u>), 7.21 (d, *J* = 7.4 Hz, 1H, 5-C<u>H</u>), 8.41 (d, *J* = 7.4 Hz, 1H, 6-C<u>H</u>), 10.73 (s, 1H, N<u>H</u>). **^{13}C NMR (DMSO-d_6)** δ 22.56, 29.18, 29.49, 29.52, 31.76, 40.04, 40.25, 40.46, 40.67, 67.55, 69.12, 75.00, 84.66, 90.64, 65.73, 141.71, 145.82, 183.11

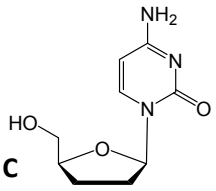
m/z: HRMS (TOF ES$^+$) $C_{23}H_{40}N_3O_7$ $[M+H]^+$ calculated 470.2861; found 470.1759

Supplementary 2:

General Procedure for the synthesis of substituted *N4*-acylated 2'-deoxycytidine (B) and 2',3'-dideoxycytidine (C) conjugates



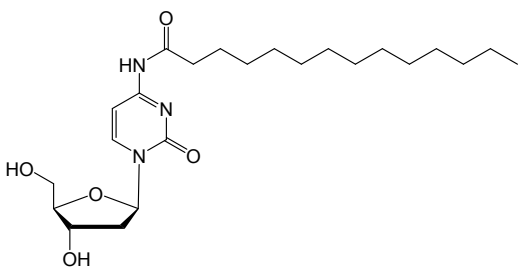
B



C

To a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 1.1 mmol, 1 eqv, 193 mg) in anhydrous CH_2Cl_2 (3.5 mL) at 0 °C, was added *N*-methylmorpholine (NMM, 1.50 mmol, 1.36 eqv, 171 µL) with continuous stirring until a white suspension had formed. The mixture was then left to stir for 1 h. Medium or short chain carboxylic acids (1.1 mmol, 1 eqv) were added directly into the mixture as a solution in anhydrous DMF (2 mL) and stirred for a further 1 h. A solution of either **B** or **C** (1.1 mmol, 1 eqv) in anhydrous DMF (2 mL) was made up at 0 °C. The cold triazine solution was added drop wise to the cooled cytidine solution over 30 mins, before heating to 50 °C and stirring for 7-8 h. The cooled solution was evaporated *in vacuo*. The product was purified using flash silica column chromatography, eluting at 5 - 8 % methanol in CH_2Cl_2.

***N4*-Tetradecanoyl-2'-deoxycytidine (E)**



Tetradecanoic acid (1.1 mmol, 1 eqv, 251 mg), **C** (1.1 mmol, 1 eqv, 250 mg)

Yield: 40.0 %

HPLC purity: 98.0 %

HPLC t_R: 24.1 min

^{1}H NMR (DMSO-d_6) δ 0.86 (t, *J* = 6.7 Hz, 3H, C$\underline{H}_3$), 1.23 (s, 20H, CH_2-($\underline{CH}_2)_{\underline{10}}$-$CH_3$), 1.54 (m, 2H, C=O-$CH_2$-C$\underline{H}_2$), 1.98 - 2.32 (m, 2H, 2'-C$\underline{H}_2$), 2.38 (t, *J* = 7.3 Hz, 2H, C=O-C$\underline{H}_2$), 3.54 - 3.65 (m, 2H, 5'-C$\underline{H}_2$), 3.87 (q, *J* = 3.7 Hz, 1H, 4'-C$\underline{H}$), 4.20 - 4.24 (td, *J* =7.7, 3.9 Hz, 1H, 3'-C$\underline{H}$), 5.03 (t, *J* = 5.3

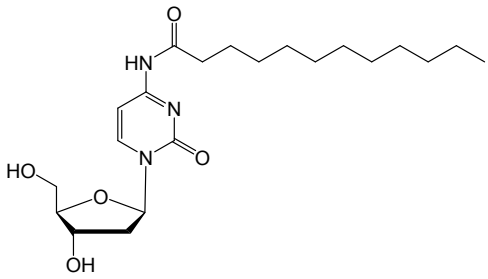
Hz, 1H, 5'-OH), 5.25 (d, *J* = 4.3 Hz, 1H, 3'-OH), 6.11 (t, *J* = 6.3 Hz, 1H, 1'-CH), 7.22 (d, *J* = 7.7 Hz, 1H, 6-CH), 8.32 (d, *J* = 7.5 Hz, 1H, 5-CH), 10.81 (s, 1H, NH).

^{13}C NMR (DMSO-d_6) δ 13.95, 22.09, 24.44, 28.43, 28.70, 28.85, 28.98, 29.00, 29.05, 31.29, 36.33, 40.89, 60.94, 69.93, 86.12, 87.89, 95.24, 144.94, 154.45, 162.27, 173.92

m/z: HRMS (TOF ES$^+$) $C_{23}H_{40}N_3O_5$ $[M+H\}^+$ calculated 438.2965; found 438. 4150

m.p: 141 - 142 °C

***N4*-Dodecanoyl-2'-deoxycytidine (F)**



Dodecanoic acid (1.1 mmol, 1 eqv, 242 mg), **C** (1.1 mmol, 1 eqv, 250 mg)

Yield: 59.0 %

HPLC purity: 98.7 %

HPLC t_R: 21.5 min

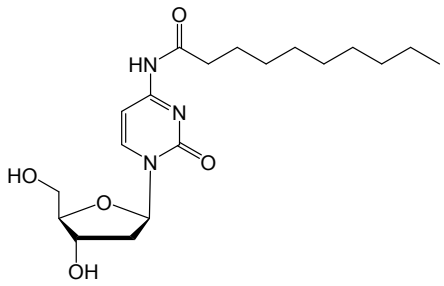
^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.9 Hz, 3H, CH_3), 1.25 (s, 16H, CH_2-$(CH_2)_8$-CH_3), 1.47-1.54 (m, 2H, C=O-CH_2-CH_2), 1.95-2.32 (m, 2H, HO-CH-CH_2), 2.37 (t, *J* = 7.3 Hz, 2H, C=O-CH_2), 3.54 - 3.5 (m, 2H, 5'-CH_2), 3.86 (q, *J* = 3.8 Hz, 1H, 4'-CH), 4.20 -4.24 (td, *J* = 7.6, 3.9, 1H, 3'-CH), 5.03 (t, *J* = 5.2 Hz, 1H, 5'-OH), 5.26 (d, *J* = 4.3 Hz, 1H, 3'-OH), 6.10 (t, *J* = 6.3 Hz, 1H, 1'-CH), 7.22 (d, *J* = 7.4 Hz, 1H, 6-CH), 8.32 (d, *J* = 7.4 Hz, 1H, 5-CH), 10.82 (s, 1H, NH).

^{13}C NMR (DMSO-d_6) δ 13.95, 22.08, 24.44, 28.42, 28.68, 28.70, 28.85, 28.97, 28.98, 31.28, 36.33, 40.88, 55.29, 60.94, 69.92, 86.11, 87.88, 95.24, 144.94, 154.45, 162.27, 173.92

m/z: HRMS (TOF ES$^+$) $C_{21}H_{36}N_3O_5$ $[M+H]^+$ calculated 410.2649; found 410.2086

m.p: 135 – 137 °C

***N4*-Decanoyl-2'-deoxycytidine (G)**



Decanoic acid (1.1 mmol, 1 eqv, 189 mg), **B** (1.1 mmol, 1 eqv, 250 mg)

Yield: 54.0 %

HPLC purity: 97.2 %

HPLC t_R: 19.0 min

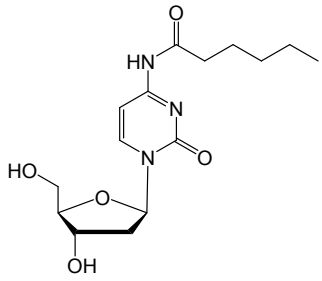
^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.6 Hz, 3H, C$\underline{H}_3$), 1.24 (s, 12H, CH_2-($\underline{CH}_2$)$_6$-CH_3), 1.53 (m, 2H, C=O-CH_2-C$\underline{H}_2$), 1.97-2.31 (m, 2H, HO-CH-C$\underline{H}_2$), 2.38 (t, *J* = 7.2 Hz, 2H, C=O-C$\underline{H}_2$), 3.52 - 3.66 (m, 2H, 5'-C$\underline{H}_2$), 3.85 (q, *J* = 3.7 Hz, 1H, 4'-C$\underline{H}$), 4.19-4.23 (m, 1H, 3'-C$\underline{H}$), 5.03 (t, *J* = 5.3 Hz, 1H, 5'-O$\underline{H}$), 5.25 (d, *J* = 4.1 Hz, 1H, 3'-O$\underline{H}$), 6.10 (t, *J* = 6.4 Hz, 1H, 1'-C$\underline{H}$), 7.21 (d, *J* = 7.4 Hz, 1H, 6-C$\underline{H}$), 8.31 (d, *J* = 7.5 Hz, 1H, 5-C$\underline{H}$), 10.81 (s, 1H, N$\underline{H}$).

^{13}C NMR (DMSO-d_6) δ 13.95, 22.09, 24.45, 28.44, 28.65, 28.71, 28.82, 31.26, 36.34, 40.88, 55.32, 60.94, 69.92, 86.12, 87.89, 95.25, 144.95, 154.45, 162.27, 173.93

m/z: HRMS (TOF ES$^+$) $C_{19}H_{32}N_3O_5$ $[M+H]^+$ calculated 382.2336; found 381.7577

m.p: 133 – 134 °C

***N*4-hexanoyl-2'-deoxycytidine (I)**



Hexanoic acid (1.1 mmol, 1 eqv, 127 mg, 138 µL) , **45** (1.1 mmol, 1 eqv, 250 mg)

Yield: 21.5 %

HPLC purity: 97.8 %

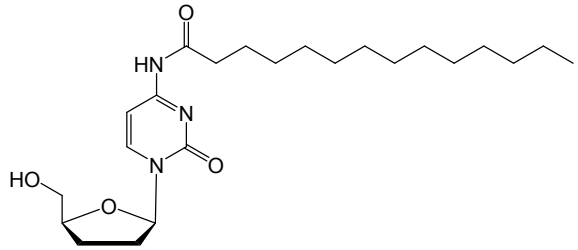
HPLC t_R: 13.2 min

^{1}H NMR (DMSO-d_6) δ 0.86 (t, *J* = 6.9 Hz, 3H, C$\underline{H}_3$), 1.22 – 1.31 (m, 4H, CH_2-($\underline{CH}_2$)$_2$-CH_3), 1.50 – 1.58 (m, 2H, C=O-CH_2-C$\underline{H}_2$), 1.98 - 2.31 (m, 2H, HO-CH-C$\underline{H}_2$), 2.38 (t, *J* = 7.4 Hz, 2H, C=O-C$\underline{H}_2$), 3.55 - 3.63 (m, 2H, 5'-C$\underline{H}_2$), 3.85 (q, *J* = 3.8 Hz, 1H, 4'-C$\underline{H}$), 4.19-4.23 (m, 1H, 3'-C$\underline{H}$), 5.03 (t, *J* =

4.2 Hz, 1H, 5'-OH), 5.25 (d, *J* = 3.8 Hz, 1H, 3'-OH), 6.10 (t, *J* = 6.3 Hz, 1H, 1'-CH), 7.22 (d, *J* = 7.5 Hz, 1H, 6-CH), 8.31 (d, *J* = 7.5 Hz, 1H, 5-CH), 10.82 (s, 1H, NH).

^{13}C NMR (DMSO-d_6) δ 13.81, 21.83, 24.14, 30.67, 36.30, 40.87, 60.94, 69.92, 86.12, 87.88, 95.24, 144.95, 154.45, 162.27, 173.92

***N4*-Tetradecanoyl-2',3'-dideoxycytidine (J)**



Tetradecanoic acid (1.18 mmol, 1 eqv, 269 mg), **C** (1.18 mmol, 1 eqv, 250 mg)

Yield: 56.9 %

HPLC purity: 98.9 %

HPLC t_R: 25.5 min

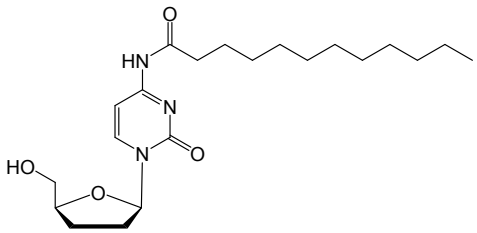
^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.7 Hz, 3H, CH_3), 1.23 (s, 20H, CH_2-$(CH_2)_{10}$-CH_3), 1.46 - 1.59 (m, 2H, C=O-CH_2-CH_2), 1.69 – 1.89 (m, 2H, 3'-CH_2), 1.90 – 2.00 (m, 1H, 2'-CH_2), 2.31-2.42 (m, 1H, 2'-CH_2), 2.37 (t, *J* = 7.3 Hz, 2H, C=O-CH_2), 3.56 - 3.78 (m, 2H, 5'-CH_2), 4.10 (m, 1H, 4'-CH), 5.10 (t, *J* = 5.3 Hz, 1H, 5'-OH), 5.92 (dd, *J* = 6.7, 2.5 Hz, 1H, 1'-CH), 7.19 (d, *J* = 7.4 Hz, 1H, 6-CH), 8.46 (d, *J* = 7.4 Hz, 1H, 5-CH), 10.78 (s, 1H, NH)

^{13}C NMR (DMSO-d_6) δ 13.95, 22.09, 23.96, 24.47, 28.43, 28.70, 28.85, 28.97, 29.00, 29.04, 31.28, 32.85, 36.32, 55.16, 61.54, 82.68, 86.86, 94.60, 145.03, 154.49, 162.22, 173.87

m/z: HRMS (TOF ES$^+$) $C_{23}H_{40}N_3O_5$ $[M+H]^+$ calculated 422.3103; found 422.2637

m.p: 127 – 130 °C

***N4*-dodecanoyl-2',3'-dideoxycytidine (K)**



Dodecanoic acid (1.18 mmol, 1 eqv, 236 mg), **C** (1.18 mmol, 1 eqv, 250 mg)

Yield: 70.7 %

HPLC purity: 96.9 %

HPLC t_R: 22.9 min

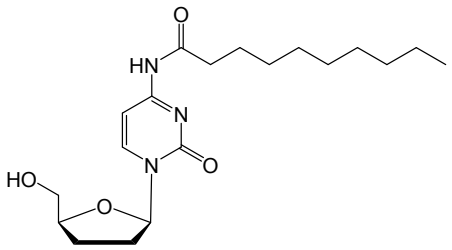
^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.8 Hz, 3H, C$\underline{H}_3$), 1.24 (s, 16H, CH_2-($\underline{CH}_2)_8$-CH_3), 1.45 - 1.60 (m, 2H, C=O-CH_2-C$\underline{H}_2$), 1.71 – 1.89 (m, 2H, 3'-C$\underline{H}_2$), 1.91 – 2.01 (m, 1H, 2'-C$\underline{H}_2$), 2.30-2.42 (m, 1H, 2'-C$\underline{H}_2$), 2.37 (t, *J* = 7.3 Hz, 2H, C=O-C$\underline{H}_2$), 3.54 - 3.80 (m, 2H, 5'-C$\underline{H}_2$), 4.05 – 4.15 (m, 1H, 4'-C$\underline{H}$), 5.10 (t, *J* = 5.3 Hz, 1H, 5'-O$\underline{H}$), 5.92 (dd, *J* = 6.7, 2.5 Hz, 1H 1'-C$\underline{H}$), 7.19 (d, *J* = 7.4 Hz, 1H, 6-C$\underline{H}$), 8.46 (d, *J* = 7.4 Hz, 1H, 5-C$\underline{H}$), 10.78 (s, 1H, N$\underline{H}$)

^{13}C NMR (DMSO-d_6) δ 13.95, 22.09, 23.97, 24.47, 28.43, 28.70, 28.86, 28.97, 31.29, 32.85, 36.33, 55.17, 61.54, 82.69, 86.87, 94.60, 145.04, 154.49, 162.23, 173.86

m/z: HRMS (TOF ES$^+$) $C_{21}H_{36}N_3O_4$ $[M+H]^+$ calculated 394.2700; found 394.5134

m.p: 114 – 118 °C

***N4*-Decanoyl-2',3'-dideoxycytidine (L)**



Decanoic acid (1.18 mmol, 1 eqv, 189 mg), **C** (1.18 mmol, 1 eqv, 250 mg)

Yield: 68.3 %

HPLC purity: 92.1 %

HPLC t_R: 20.2 min

^{1}H NMR (DMSO-d_6) δ 0.85 (t, *J* = 6.8 Hz, 3H, C$\underline{H}_3$), 1.26 (s, 12H, CH_2-($\underline{CH}_2)_6$-CH_3), 1.45 - 1.60 (m, 2H, C=O-CH_2-C$\underline{H}_2$), 1.69 – 1.89 (m, 2H, 3'-C$\underline{H}_2$), 1.91 – 1.99 (ddt, *J* = 13.2, 7.6, 2.8 Hz, 1H, 2'-C$\underline{H}_2$), 2.25-2.42 (m, 1H, 2'-C$\underline{H}_2$), 2.36 (t, *J* = 7.1 Hz, 2H, C=O-C$\underline{H}_2$), 3.56 - 3.77 (ddd, *J* = 68.0, 12.1, 3.7 Hz, 2H, 5'-C$\underline{H}_2$), 4.07 – 4.13 (dq, *J* = 6.0, 3.4 Hz, 1H, 4'-C$\underline{H}$), 5.10 (br-s, 1H, 5'-O$\underline{H}$), 5.92

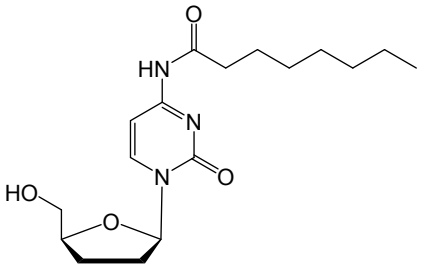
(dd, *J* = 6.7, 2.5 Hz, 1H 1'-C<u>H</u>), 7.19 (d, *J* = 7.4 Hz, 1H, 6-C<u>H</u>), 8.46 (d, *J* = 7.4 Hz, 1H, 5-C<u>H</u>), 10.78 (s, 1H, N<u>H</u>)

^{13}C NMR (DMSO-d_6) δ 13.95, 22.09, 23.97, 24.48, 28.44, 28.65, 28.71, 28.83, 31.26, 32.85, 36.33, 61.54, 82.69, 86.87, 94.61, 145.04, 154.50, 162.23, 173.88

m/z: HRMS (TOF ES$^+$) $C_{19}H_{32}N_3O_4$ $[M+H]^+$ calculated 366.2387; found 366.2419

m.p: 110 - 111 °C

***N*4-Octanoyl-2',3'-dideoxycytidine (M)**



Octanoic acid (1.18 mmol, 1 eqv, 159 mg, 174 µL), **C** (1.18 mmol, 1 eqv, 250 mg)

Yield: 58.9 %

HPLC purity: 90.1 %

HPLC t_R: 17.4 min

^{1}H NMR (DMSO-d_6) δ 0.87 (t, *J* = 6.9 Hz, 3H, C<u>H$_3$</u>), 1.27 (s, 8H, CH_2-(<u>CH_2</u>)$_4$-CH_3), 1.50 - 1.57 (m, 2H, C=O-CH_2-C<u>H$_2$</u>), 1.69 – 1.91 (m, 2H, 3'-C<u>H$_2$</u>), 1.91 – 2.00 (m, 1H, 2'-C<u>H$_2$</u>), 2.25-2.45 (m, 1H, 2'-C<u>H$_2$</u>), 2.37 (t, *J* = 7.4 Hz, 2H, C=O-C<u>H$_2$</u>), 3.55 - 3.79 (ddd, *J* = 68.0, 12.1, 3.7 Hz, 2H, 5'-C<u>H$_2$</u>), 4.10 (dq, *J* = 6.1, 3.4 Hz, 1H, 4'-C<u>H</u>), 5.10 (br-s, 1H, 5'-O<u>H</u>), 5.92 (dd, *J* = 6.7, 2.5 Hz, 1H 1'-C<u>H</u>), 7.19 (d, *J* = 7.4 Hz, 1H, 6-C<u>H</u>), 8.46 (d, *J* = 7.4 Hz, 1H, 5-C<u>H</u>), 10.78 (s, 1H, N<u>H</u>)

^{13}C NMR (DMSO-d_6) δ 13.93, 22.04, 24.00, 24.48, 28.83, 28.42, 31.10, 32.84, 36.33, 61.54, 82.69, 86.66, 94.60, 145.05, 154.49, 162.22, 173.87

m/z: HRMS (TOF ES$^+$) $C_{17}H_{28}N_3O_4$ $[M+H]^+$ calculated 338.2074; found 338.2345

m.p: 103 – 106 °C

Supplementary 3:

Gelation procedure for gels containing ethanol

Compounds were weighed using an A and D GR-202 semi micro-analytical balance into 1.5 mL sample vials so that a final compound concentration was 0.5 % (*w/v*) with a final sample volume was 500 µL. The compound was solubilized in ethanol and sonicated for 1-2 min. Solutions were heated to 60 °C, using a made to measure aluminium heating vessel, to solubilize the compound before adding pre-heated (60 °C) ultra-purified water. The samples were left to cool to room temperature for 18 h, prior to inversion.

Supplementary 4:

Vial inversion of *N4*-tetradecanoylcytidine and *N4*-(3-hydroxytetradecanoyl)cytidine and representation of intramolecular hydrogen bonding

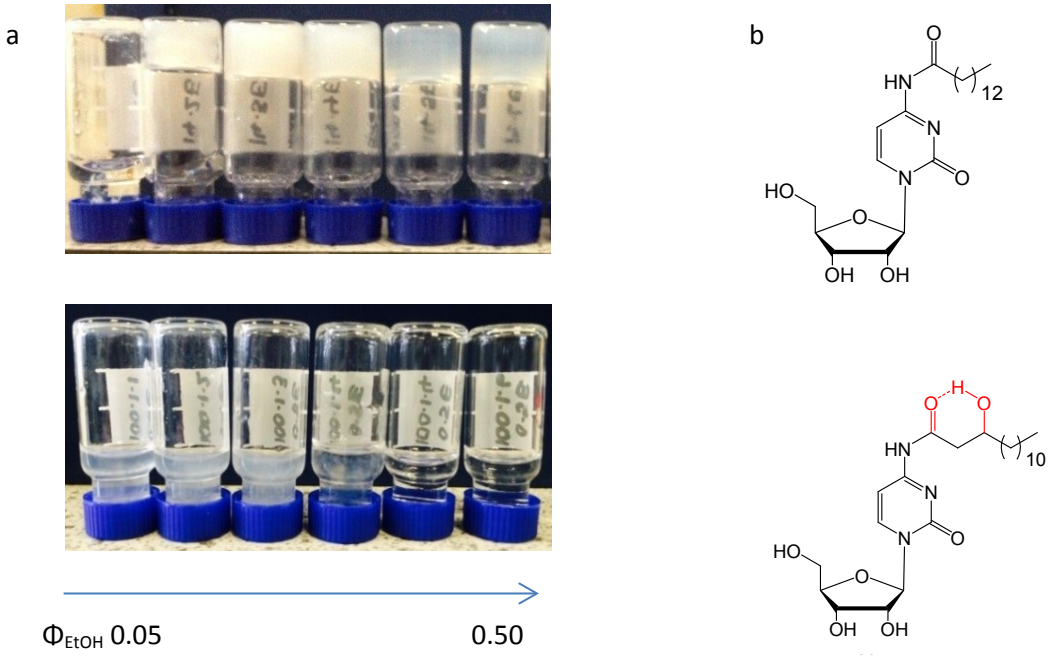
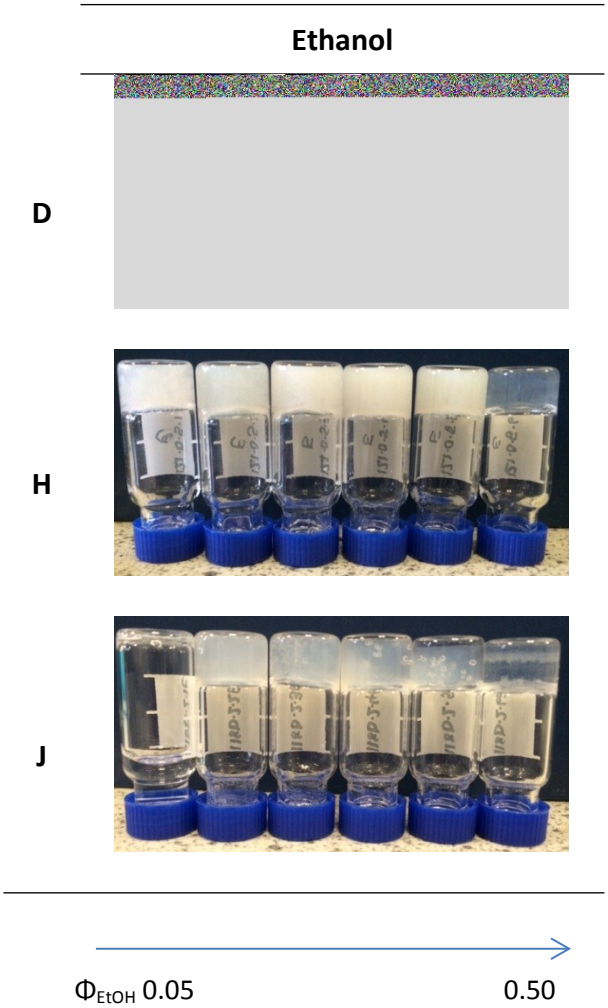


Figure S1: a) Vial inversion of *N4*-tetradecanoylcytidine (top) and *N4*-(3-hydroxytetradecanoyl)cytidine in ethanol. Image shows Φ_{EtOH} 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 (left to right). Representative image of n=4. b) Structure of *N4*-tetradecanoylcytidine (top) and *N4*-(3-hydroxytetradecanoyl)cytidine (bottom); *N4*-(3-hydroxytetradecanoyl)cytidine showing intra-molecular hydrogen bonding (red) between amide carbonyl and 3-hydroxyl.

Supplementary 5:

Vial Inversion of *N4*-tetradecanoylcytidine (D), *N4*-tetradecanoyl-2'-deoxycytidine (H) and *N4*-tetradecanoyl-2', 3'-dideoxycytidine (J)

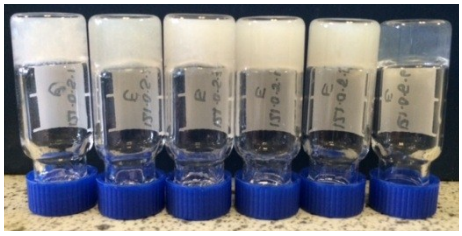
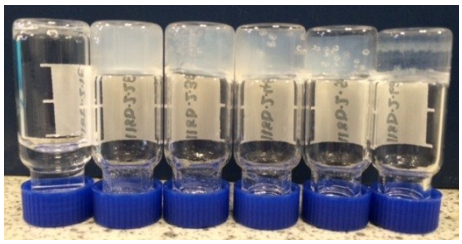
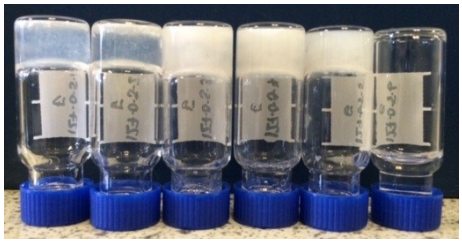
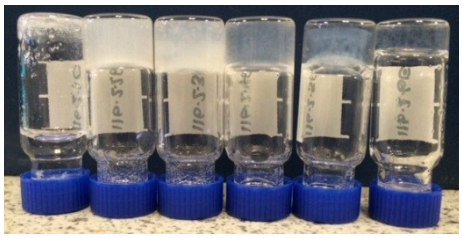
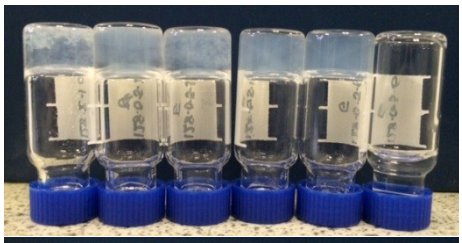
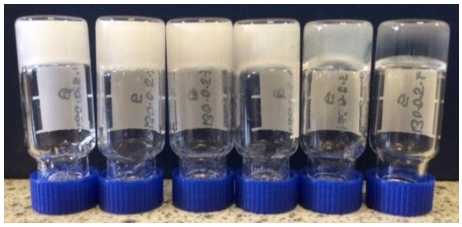
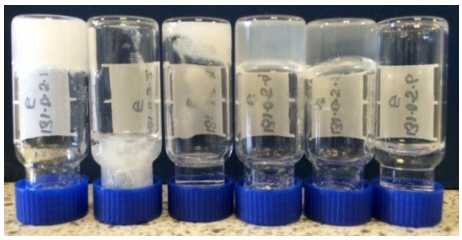
Table S1: *N4*-tetradecanoylcytidine (D), *N*4-tetradecanoyl-2'-deoxycytidine and *N4*-tetradecanoyl-2', 3'-dideoxycytidine (J) 0.5 % (*w/v)* in and ethanol/water. Images shown Φ_{SOL} 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 (left to right) are representative images of n=3



Supplementary 6:

Vial inversion of *N4*-acylated 2'-deoxycytidine and 2', 3'-dideoxycytidine derivatives

Table S2: Stability to inversion in ethanol/water , where n is the number of carbons in the acyl chain with final compound concentration of 0.5 % (*w/v*). Each image shows Φ_{SOL} 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 (left to right). Representative image of n=3

n		2'-deoxycytidine series		2', 3'-dideoxycytidine series
12	E		J	
10	F		K	
8	G		L	
6	H		M	
4	I			-

Supplementary 7:

Oscillatory rheology of *N4*-octanoyl-2'-deoxycytidine *(H)*

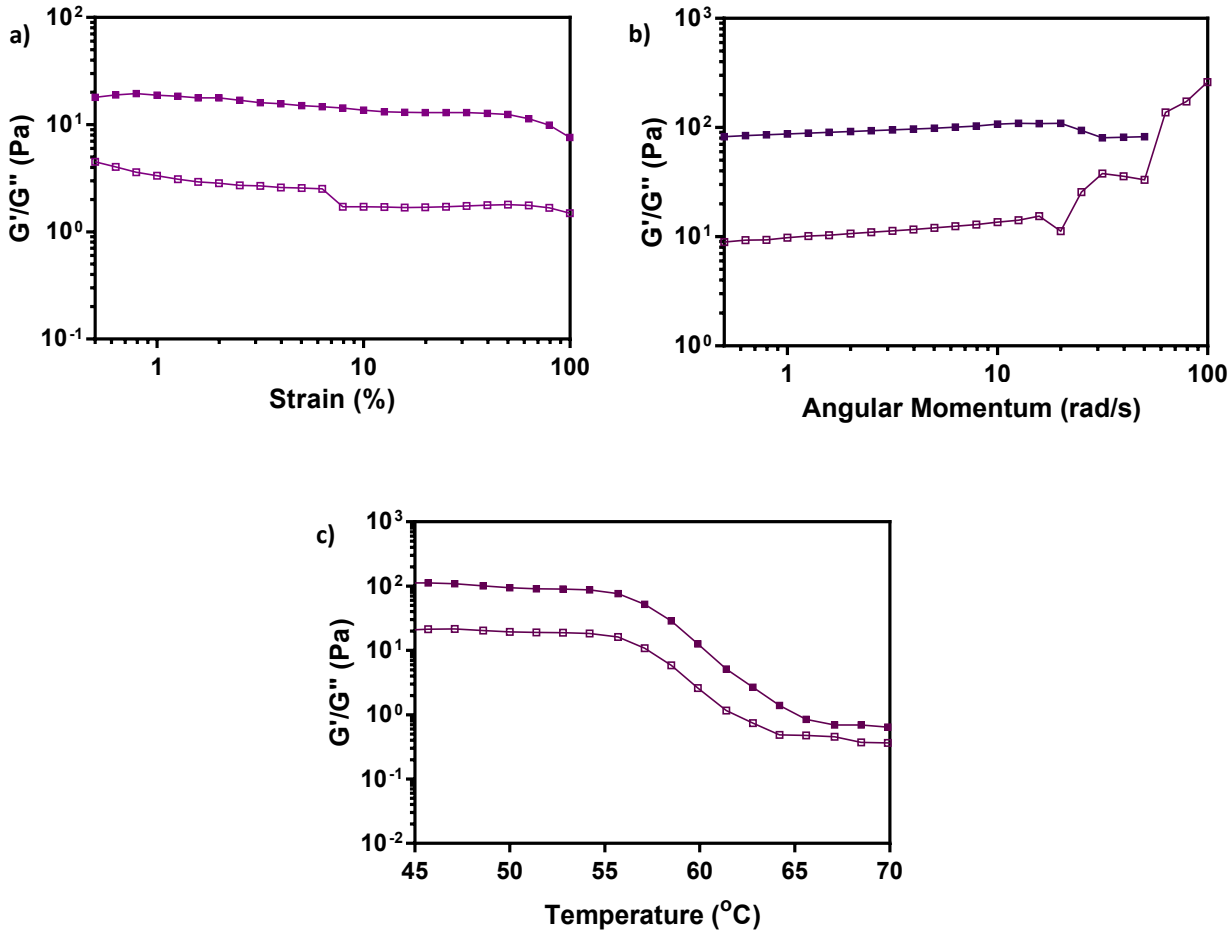


Figure S2**:** Representative plots from rheological studies of 0.00 0.5 % (*w/v*) where n = 4. a) Amplitude sweep γ = 0.05 – 100 %, ω = 10 rad/s, T = 37 °C b) Frequency sweep γ = 5 %, ω = 0.1 - 100 rad/s, T = 37 °C c) Temperature sweep γ = 5 %, ω = 10 rad/s, T = 37 - 70 °C