Supporting Information

Developing a self-healing supramolecular nucleoside hydrogel

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S1: Synthetic procedure and analysis of N4-(3-hydroxytetradecanoyl)cytidine
S2: Synthetic procedures and analysis of 2’,3’-dideoxycytidine and 2’-deoxycytidine conjugates
S3: Gelation procedure for compounds containing ethanol
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S5: Vial inversion of N4-tetradecanoylcytidine (D), N4-tetradecanoyl-2’-deoxycytidine- (E) and 2’,3’-N4-tetradecanoyldideoxycytidine (J)
S6: Vial inversion of acylated 2’-deoxycytidine and 2’,3’-dideoxycytidine derivatives
S7: Oscillatory Rheology of N4-octanoyl-2’-deoxycytidine (H)
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$_2$Cl$_2$ (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$_2$Cl$_2$. 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, CH$_3$), 1.15-1.41 (m, 20H, CH$_3$-(CH$_2$)$_{10}$-CH(OH)), 2.25 (ddd, $J = 22.6$, 14.8, 6.5 Hz 1H, C=O-CH$_2$-CH(OH)), 2.45 (d, $J = 6.7$ Hz, 2H, C=O-CH$_2$), 3.55 - 3.78 (m, 2H, 5’-CH$_3$), 3.85 (m, 1H, 4’-CH), 3.95 (m, 1H, 3’-CH), 4.71 (t, $J = 5.3$ Hz, 1H, acyl-3-OH) 5.04 (t, $J = 5.4$ Hz, 1H, 3’-OH), 5.15 (d, $J = 5.2$ Hz, 1H, 5’-OH), 5.47 (d, $J = 4.7$ Hz, 1H, 2’-OH ), 5.77 (d, $J = 2.4$ Hz, 1H, 1’-CH), 7.21 (d, $J = 7.4$ Hz, 1H, 5-CH), 8.41 (d, $J = 7.4$ Hz, 1H, 6-CH), 10.73 (s, 1H, NH). $^{13}$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$_3$O$_7$ [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

To a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 1.1 mmol, 1 eqv, 193 mg) in anhydrous CH$_2$Cl$_2$ (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$_2$Cl$_2$.

**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, CH$_3$), 1.23 (s, 20H, CH$_2$-(CH$_2$)$_{10}$-CH$_3$), 1.54 (m, 2H, C=O-CH$_2$-CH$_2$), 1.98 - 2.32 (m, 2H, 2'-CH$_2$), 2.38 (t, $J = 7.3$ Hz, 2H, C=O-CH$_2$), 3.54 - 3.65 (m, 2H, 5'-CH$_2$), 3.87 (q, $J = 3.7$ Hz, 1H, 4'-CH), 4.20 - 4.24 (td, $J = 7.7$, 3.9 Hz, 1H, 3'-CH), 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$_3$), 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)**

![Chemical Structure](image)

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

\(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 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\(^+\)) \(\text{C}_{19}\text{H}_{32}\text{N}_3\text{O}_5\) [M+H]\(^+\) calculated 382.2336; found 381.7577

\(\text{m.p.}\): 133 – 134 °C

**N4-Hexanoyl-2’-deoxycytidine (I)**

![Chemical Structure](image)

Hexanoic acid (1.1 mmol, 1 eqv, 127 mg, 138 \(\mu\)L), 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-\(d_6\)) \(\delta\) 0.86 (t, \(J = 6.9\) Hz, 3H, \(\text{CH}_3\)), 1.22 – 1.31 (m, 4H, \(\text{CH}_2\)-(\(\text{CH}_2\)_2-\(\text{CH}_3\)), 1.50 – 1.58 (m, 2H, \(\text{C}=\text{O}-\text{CH}_2-\text{CH}_2\)), 1.98 - 2.31 (m, 2H, \(\text{HO}-\text{CH}-\text{CH}_2\)), 2.38 (t, \(J = 7.4\) Hz, 2H, \(\text{C}=\text{O}-\text{CH}_2\)), 3.55 - 3.63 (m, 2H, 5''-\(\text{CH}_2\)), 3.85 (q, \(J = 3.8\) Hz, 1H, 4''-\(\text{CH}\)), 4.19-4.23 (m, 1H, 3''-\(\text{CH}\)), 5.03 (t, \(J = 5.3\) Hz, 2H, \(\text{C}=\text{O}-\text{CH}_2\)), 5.25 (d, \(J = 4.1\) Hz, 1H, 3''-\(\text{OH}\)), 6.10 (t, \(J = 6.4\) Hz, 1H, 1''-\(\text{CH}\)), 7.21 (d, \(J = 7.4\) Hz, 1H, 6'-\(\text{CH}\)), 8.31 (d, \(J = 7.5\) Hz, 1H, 5-\(\text{CH}\)), 10.81 (s, 1H, \(\text{NH}\)).
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

$^{1}$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$_3$), 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$_3$O$_5$ [M+H]$^+$ calculated 422.3103; found 422.2637

m.p: 127 – 130 °C

$^{1}$H NMR (DMSO-$d_6$) of $\text{N4-Tetradecanoyl-2',3'-dideoxycytidine (J)}$

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

![Chemical structure of N4-dodecanoyl-2',3'-dideoxycytidine (K)](image)

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

**1H NMR (DMSO-d_6) δ** 0.85 (t, J = 6.8 Hz, 3H, CH_3), 1.24 (s, 16H, CH_2-(CH_2)_6-CH_3), 1.45 - 1.60 (m, 2H, C=O-CH_2-CH_2), 1.71 – 1.89 (m, 2H, 3’-CH_2), 1.91 – 2.01 (m, 1H, 2’-CH_2), 2.30-2.42 (m, 1H, 2’-CH_2), 2.37 (t, J = 7.3 Hz, 2H, C=O-CH_2), 3.54 - 3.80 (m, 2H, 5’-CH_2), 4.05 – 4.15 (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)

**13C 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_{3}O_{4} [M+H]^+ calculated 394.2700; found 394.5134

**m.p:** 114 – 118 °C

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

![Chemical structure of N4-Decanoyl-2',3'-dideoxycytidine (L)](image)

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

**1H NMR (DMSO-d_6) δ** 0.85 (t, J = 6.8 Hz, 3H, CH_3), 1.26 (s, 12H, CH_2-(CH_2)_6-CH_3), 1.45 - 1.60 (m, 2H, C=O-CH_2-CH_2), 1.69 – 1.89 (m, 2H, 3’-CH_2), 1.91 – 1.99 (ddt, J = 13.2, 7.6, 2.8 Hz, 1H, 2’-CH_2), 2.25-2.42 (m, 1H, 2’-CH_2), 2.36 (t, J = 7.1 Hz, 2H, C=O-CH_3), 3.56 - 3.77 (ddd, J = 68.0, 12.1, 3.7 Hz, 2H, 5’-CH_2), 4.07 – 4.13 (dq, J = 6.0, 3.4 Hz, 1H, 4’-CH), 5.10 (br-s, 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, 23.97, 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$_3$O$_4$ [M+H]$^+$ calculated 366.2387; found 366.2419

m.p: 110 - 111 °C

**N4-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, CH$_3$), 1.27 (s, 8H, CH$_2$-(CH$_2$)$_4$-CH$_3$), 1.50 - 1.57 (m, 2H, C=O-CH$_2$-CH$_3$), 1.69 - 1.91 (m, 2H, 3'-CH$_3$), 1.91 - 2.00 (m, 1H, 2'-CH$_2$), 2.25-2.45 (m, 1H, 2'-CH$_2$), 2.37 (t, J = 7.4 Hz, 2H, C=O-CH$_3$), 3.55 - 3.79 (ddd, J = 68.0, 12.1, 3.7 Hz, 2H, 5'-CH$_2$), 4.10 (dq, J = 6.1, 3.4 Hz, 1H, 4'-CH), 5.10 (br-s, 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 13.93, 22.04, 24.00, 24.48, 28.48, 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$_3$O$_4$ [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 $\Phi_{\text{EtOH}}$ 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 \textit{N4-tetradecanoylcytidine} (D), \textit{N4-tetradecanoyl-2'-deoxycytidine} (H) and \textit{N4-tetradecanoyl-2', 3'-dideoxycytidine} (J)

Table S1: \textit{N4-tetradecanoylcytidine} (D), \textit{N4-tetradecanoyl-2'-deoxycytidine} and \textit{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 $\Phi_{\text{SOL}}$ 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 (left to right). Representative image of n=3

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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 $\gamma = 0.05 - 100$, $\omega = 10$ rad/s, T = 37 °C b) Frequency sweep $\gamma = 5$, $\omega = 0.1 - 100$ rad/s, T = 37 °C c) Temperature sweep $\gamma = 5$, $\omega = 10$ rad/s, T = 37 - 70 °C