Supporting information

Mercury-Free Automated Synthesis of Guanidinium Backbone Oligonucleotides

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Materials

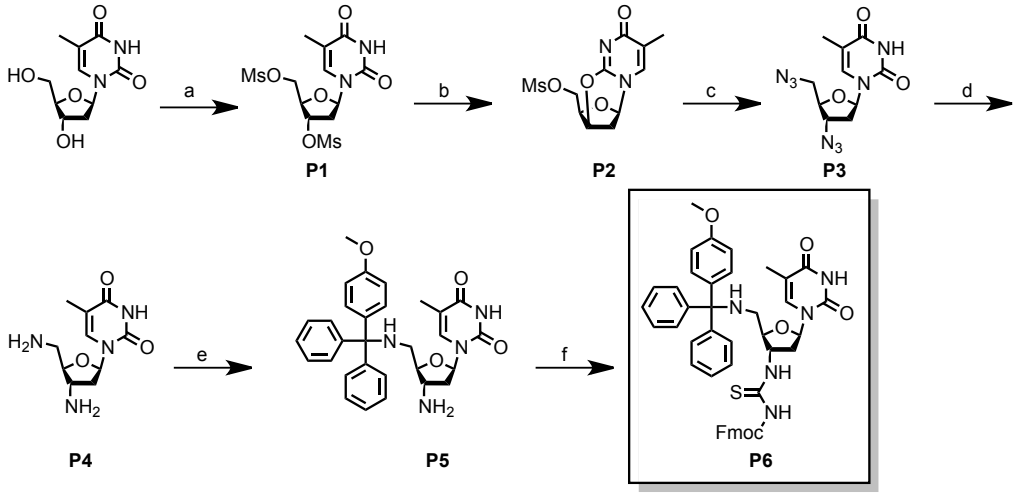
All commercial reagents were used without purification. Thymidine, all monomer synthesis reagents (unless otherwise noted), coupling reagents, nitrogenous bases for the solution phase screen, solvents, triethylammonium acetate (TEAA) pH 7 buffer for HPLC, 40% ammonium hydroxide solution, 30% methylamine solution, glacial acetic acid, and urea were purchased from Sigma-Aldrich or TCI. All DNA synthesis reagents and solid supports were acquired from Glen Research. Solid support controlled pore glass (CPG) beads were obtained from ChemGenes with Universal UnyLinker Support 1000 Å or Glen Research with a disulfide moiety (3-((3-dimethoxytrityl-O-propyl)disulfide)propyl succinyl-lcaa-CPG, thiol modifier C3 CPG). Pre-cast 4–15 % sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gels, non-denaturing Laemmli buffer (4×) and Precision Plus© protein ladder were obtained from Bio-Rad. 2′,6′-Dihydroxyacetophenone (DHAP) matrix, acrylamide, Tris-glycine-SDS buffer, 4% paraformaldehyde in phosphate-buffered saline (PBS), flow cytometry tubes and fluorenylmethyloxycarbonyl chloride (Fmoc)-isothiocyanate were acquired from Fisher Scientific. Cell culture reagents, Dulbecco's modified Eagle media (DMEM), 0.25% trypsin-EDTA, penicillin/streptomycin (P/S), fetal bovine serum (FBS), Hoechst 33342 staining solution, AlexaFluor 647 NHS ester, SimplyBlue stain and 8-well chambered coverglass were obtained from ThermoFisher Scientific.

Instrumentation

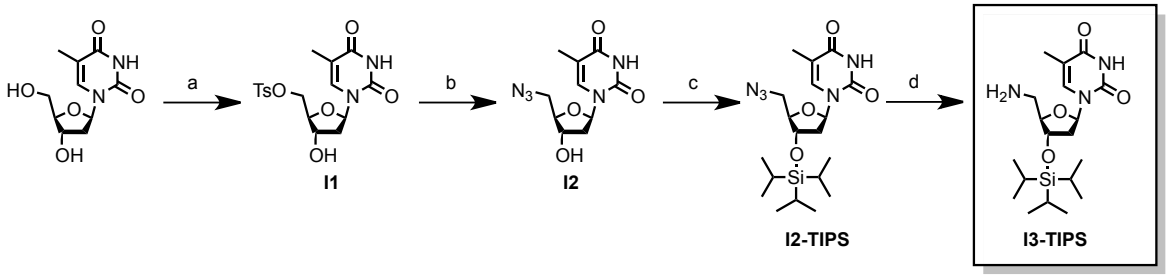
^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded using a Bruker Advance instrument (500 MHz) using the solvent as an internal standard. Electrospray ionization (ESI) mass spectrometry data was obtained through direct injection into a Bruker Amazon-SL. The solution phase screen was implemented on a Waters Acquity UPLC-MS (ESI quadrupole) instrument using a BEH C18 reverse-phase (RP) column with 1.7 um packing (Acquity, P.N. 18600350), kept at a constant temperature of 40 °C. All DNA synthesis was carried out on a MerMade 12 oligonucleotide synthesizer from BioAutomation using standard phosphoramidite chemistry protocols or conditions outlined in Figure 2C. HPLC purifications were performed on a Shimadzu Prominence instrument equipped with a C4 RP column running a linear gradient from 0-100% solvent B in 40 minutes with DNA detection at 254 nm where solvent A is 100 mM TEAA buffer at pH 7 with 3% acetonitrile and solvent B is acetonitrile. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) was performed on either a Bruker AutoFlex III or a Bruker Rapiflex. Gel electrophoresis was carried out on a mini-Protean setup from Bio-Rad or a Hoefer 600 setup from Fisher Scientific. Cell imaging was achieved using a Zeiss LSM 800 confocal laser scanning microscope using constant laser power (405, 488 and 633 nm lines) and PMT settings using a 63×/1.40 oil objective. Flow cytometry was conducted on a LSRFortessa cell analyzer from BD Biosciences, first identifying cell populations with forward and side scattering intensities, then reading APC fluorescence (670/30 nm filter) using a constant excitation from the 637 nm laser line. Fluorescence readouts from the Alamar Blue assay were obtained using a BioTek Cytation 5 imaging reader running Gen5 version 3.04 software, exciting at 560/10 nm and collecting fluorescence emission at 590/10 nm. Brightfield images were acquired on a Nikon Eclipse Ts2 microscope equipped with a 20×/0.40 objective running NIS-Elements Fluorescence software.

Synthetic routes to nucleoside monomers

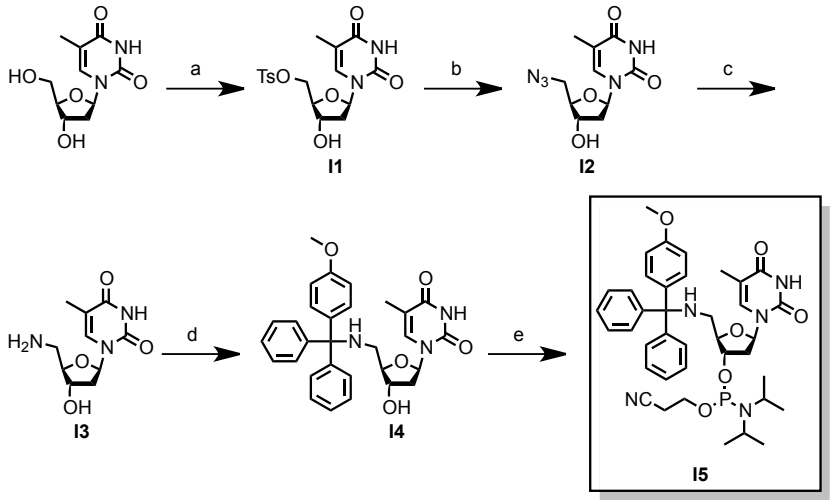
Thymidine initiator (**I5**), solution-phase coupling monomer (**I3-TIPS**), and propagator (**P6**) units were synthesized according to literature precedent with some minor changes as indicated below.[1-2] All monomer synthesis reactions were carried out with anhydrous solvents and under an inert gas atmosphere.



Scheme S1. Synthesis of the propagating unit **P6**. (a) Methanesulfonyl chloride (MsCl), pyridine (Pyr), 0 °C, 2 h, 91%; (b) Triethylamine (TEA), ethanol (EtOH), reflux, 24 h, 90%; (c) Sodium azide (NaN_3), *N*,*N*-dimethylformamide (DMF), 6 h, 130 °C, 58%; (d) 10% Palladium on carbon (Pd/C), hydrogen (H_2), 10% water in methanol (MeOH), room temperature (RT), 6 h, 90%; (e) Monomethoxytrityl chloride (MMTr), TEA, Pyr, 0 °C, 2 h, 25%; (f) Fmoc-isothiocyanate (Fmoc-NCS), dichloromethane (DCM), RT, 16 h, 84%.

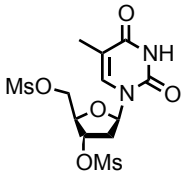


Scheme S2. Synthesis of solution phase coupling monomer **I3-TIPS**. (a) 4-Toluenesulfonyl chloride (Ts-Cl), Pyr, 0 °C, 2 h, 79%; (b) NaN_3, lithium chloride (LiCl), sodium iodide (NaI), DMF, 110 °C, 1 h, 62%; (c) Triisopropylsilyl chloride (TIPS-Cl), imidazole (Im), DMF, RT, 48 h, 76%; (d) Pd/C, H_2, 10% H_2O/MeOH, RT, 22 h, 82%.



Scheme S3. Synthesis of the initiator unit **I5**. (a) Ts-Cl, Pyr, 0 °C, 2 h, 79%; (b) NaN_3, LiCl, NaI, DMF, 110 °C, 1 h, 62%; (c) Pd/C, H_2, 10% H_2O/MeOH, RT, 24 h, 84%; (d) MMTr-Cl, TEA, Pyr, 0 °C, 24 h, 72%; (e) 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite, diisopropylethylamine (DIPEA), DCM, RT, 2 h, 30%.

Synthesis of propagator nucleoside P6

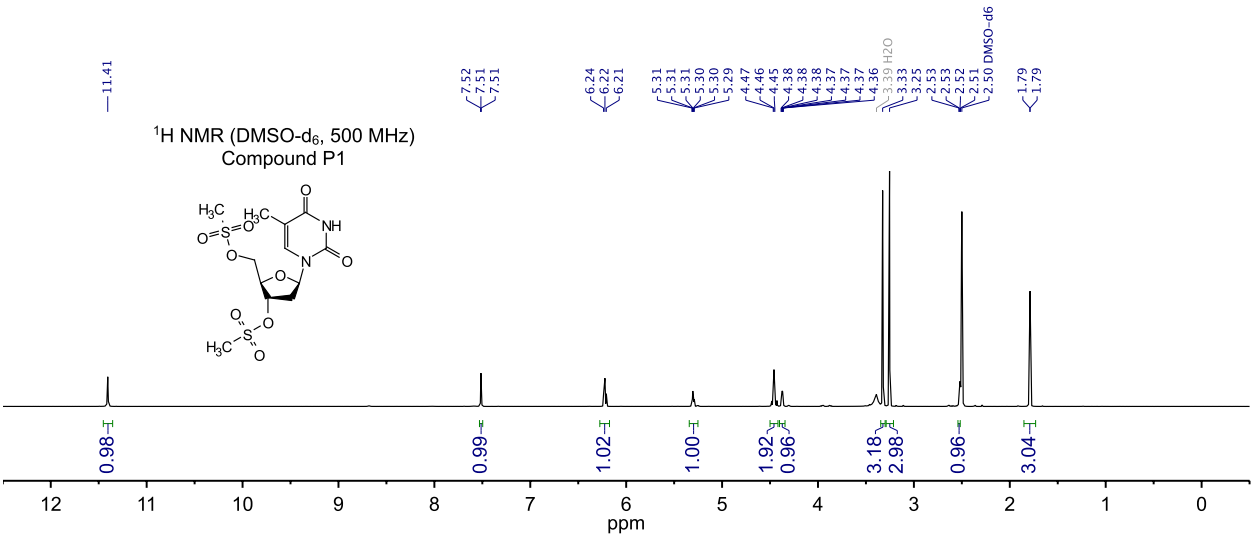


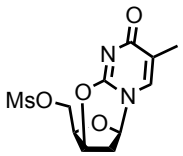
3′-O-mesyl-5′-O-mesyl-thymidine (P1)

Dissolved thymidine (20 g, 83 mmol, 1 equiv.) and triethylamine (23 mL, 165 mmol, 2 equiv.) in pyridine (120 mL) and cooled to 0 °C in an ice bath. Added methanesulfonyl chloride (19.2 mL, 248 mmol, 3 equiv.) to the solution dropwise. Let warm to room temperature and stir overnight. The solution was poured into 4 L of cold water to precipitate a tan solid, which was filtered and washed with a further 100 mL of water and lyophilized. (91% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.41 (s, 1H), 7.51 (d, J = 1.4 Hz, 1H), 6.22 (t, J = 7.1 Hz, 1H), 5.30 (td, J = 4.8, 3.1 Hz, 1H), 4.46 (t, J = 4.1 Hz, 2H), 4.37 (dt, J = 5.1, 3.6 Hz, 1H), 3.33 (s, 3H), 3.25 (s, 3H), 2.54 – 2.51 (m, 2H), 1.79 (d, J = 1.2 Hz, 3H).

Note: Multiplet signal at 2.54 -2.51 is partially obscured by the DMSO solvent peak, an integration of half the peak is given in the spectrum.

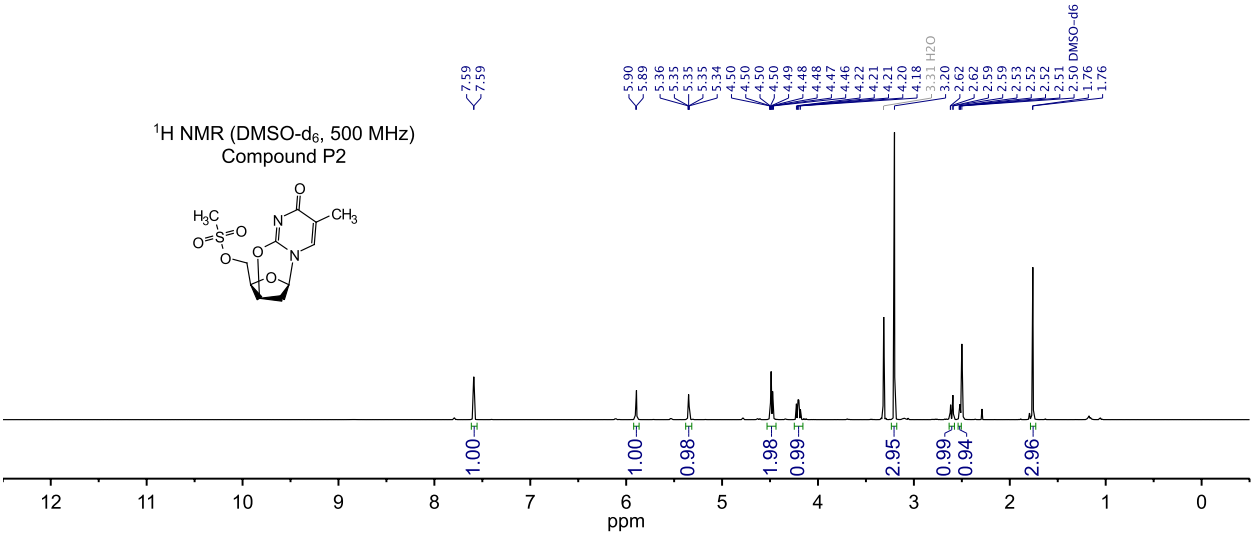


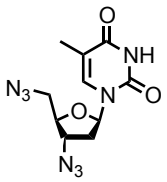


5′-O-mesyl-2′,3′-anhydro-thymidine (P2)

Suspended **P1** (26.7 g, 67 mmol) in ethanol (700 mL) and added triethylamine (70 mL). Heated at reflux overnight, then let cool to room temperature and filtered, washing with cold ethanol (ca. 100 mL) and lyophilized. Product is a light tan powder. (90% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.59 (d, J = 1.4 Hz, 1H), 5.90 (d, J = 3.8 Hz, 1H), 5.35 (dd, J = 2.9, 1.6 Hz, 1H), 4.54 – 4.44 (m, 2H), 4.24 – 4.17 (m, 1H), 3.20 (s, 3H), 2.61 (dd, J = 13.1, 1.5 Hz, 1H), 2.52 (dd, J = 4.1, 2.8 Hz, 1H), 1.76 (d, J = 1.2 Hz, 3H).

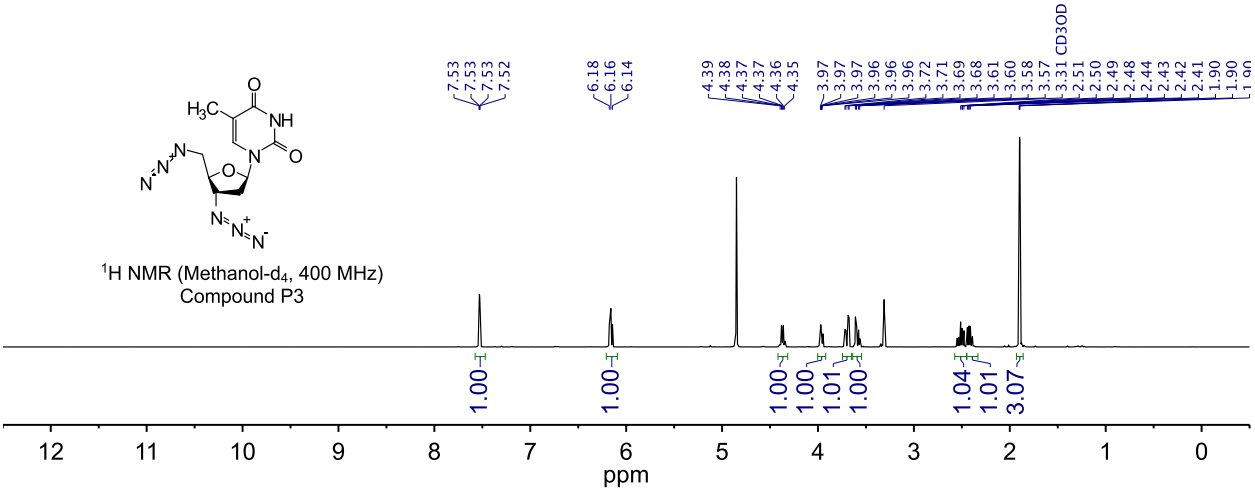


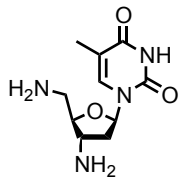


3′,5′-diazido-3′,5′-deoxythymidine (P3)

Suspended **P2** (9.1 g, 30.1 mmol, 1 equiv.) in DMF (100 mL), then added sodium azide (4.3 g, 66.2 mmol, 2.2 equiv., measured with a plastic spatula). The slurry was heated behind a blast shield while stirring to 130 °C, carefully controlling temperature. Reaction was monitored by TLC (ethyl acetate). After 6 hours the solution was cooled to room temperature prior to filtering through a pad of Celite, washing with DMF. The crude was then concentrated under reduced pressure. The product was purified on a silica gel column with a DCM to 30% methanol solvent gradient. Product was a viscous amber oil. (58% yield)

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.55 (q, J = 1.2 Hz, 1H), 6.18 (t, J = 6.6 Hz, 1H), 4.39 (dt, J = 7.7, 5.5 Hz, 1H), 3.98 (ddd, J = 5.5, 4.7, 3.7 Hz, 1H), 3.72 (dd, J = 13.3, 3.8 Hz, 1H), 3.61 (dd, J = 13.4, 4.7 Hz, 1H), 2.53 (ddd, J = 14.0, 7.8, 6.2 Hz, 1H), 2.43 (ddd, J = 14.0, 7.0, 5.4 Hz, 1H), 1.92 (d, J = 1.3 Hz, 3H).

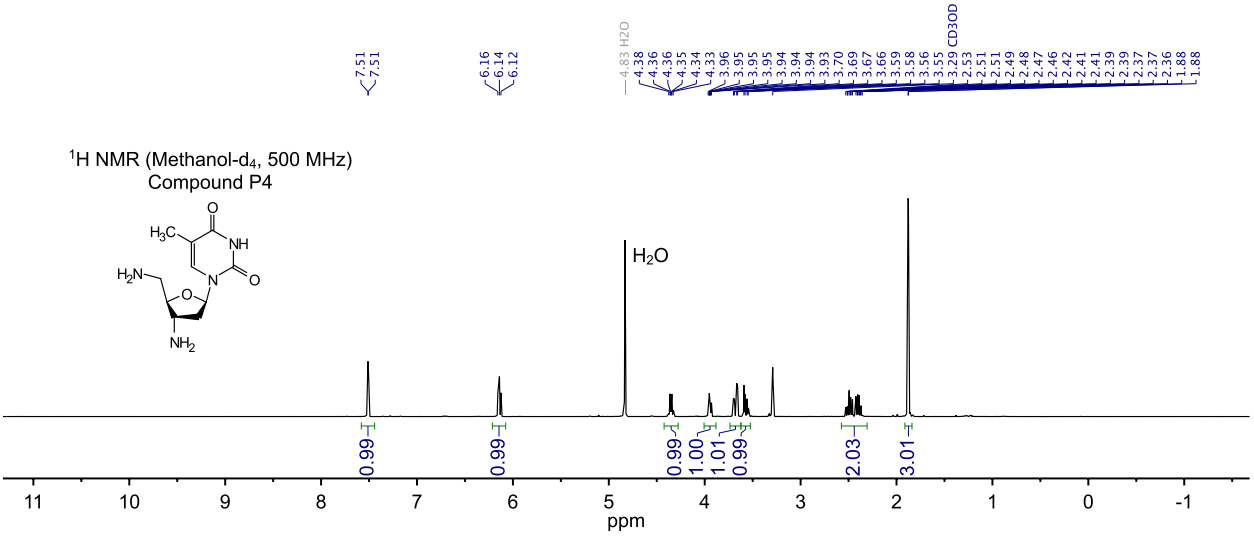


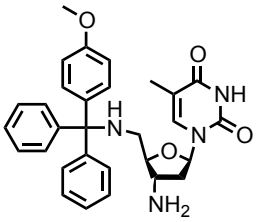


3′,5′-amino-3′,5′-deoxythymidine (P4)

Azide **P3** (7.86 g, 26.9 mmol) was dissolved in methanol (ca. 90 mL) and degassed by bubbling argon through the solution. 10% Palladium on carbon (1.18 g) was added to the reaction as a slurry in 10 mL of water. Hydrogen gas was bubbled through the reaction and the consumption of starting material monitored by TLC (60/40 ethyl acetate/hexanes). After ca. 6 h the reaction was complete and argon was bubbled through the solution. The solution was filtered through Celite, washing with copious amounts of methanol, then concentrated under reduced pressure. Product was a white foam. (90% yield)

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.51 (d, J = 1.2 Hz, 1H), 6.14 (t, J = 6.6 Hz, 1H), 4.35 (dt, J = 7.7, 5.5 Hz, 1H), 3.95 (ddd, J = 5.5, 4.7, 3.7 Hz, 1H), 3.68 (dd, J = 13.3, 3.8 Hz, 1H), 3.57 (dd, J = 13.4, 4.7 Hz, 1H), 2.49 (ddd, J = 14.0, 7.8, 6.2 Hz, 1H), 2.39 (ddd, J = 14.0, 7.0, 5.4 Hz, 1H), 1.88 (d, J = 1.3 Hz, 3H).

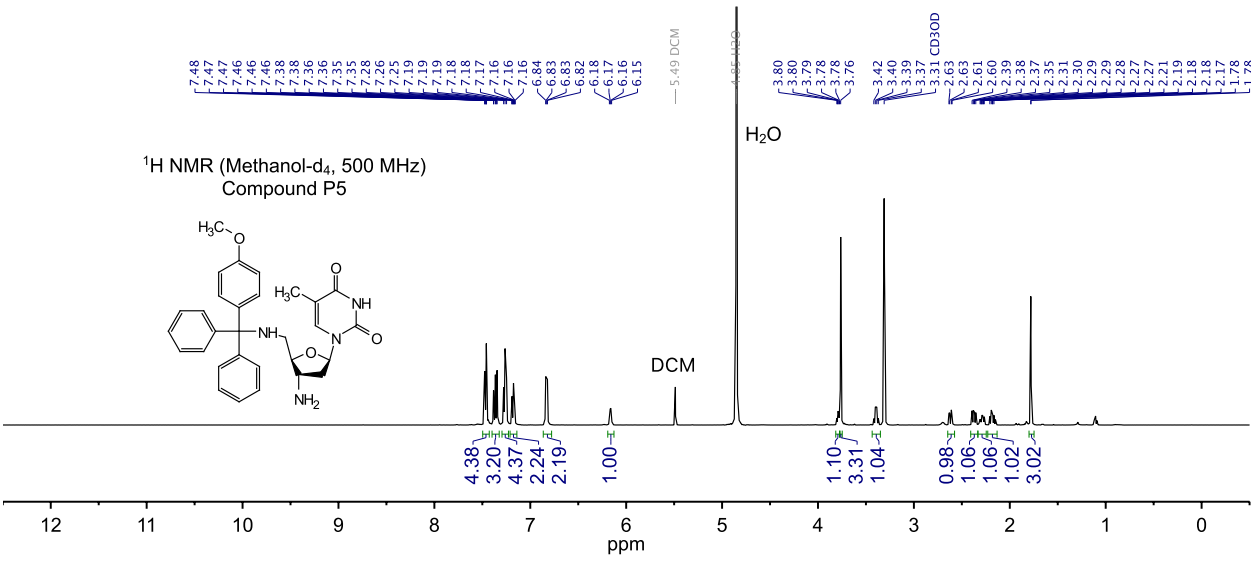


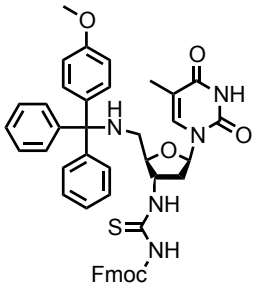


5′-N-monomethoxytrityl-3′-amino-3′,5′-deoxythymidine (P5)

Suspended **P4** (4.21 g, 17.5 mmol, 1 equiv.) in pyridine (350 ml) with 5% triethylamine at 0 °C, then added monomethoxytrityl chloride (5.42 g, 17.5 mmol, 1 equiv.) pre-dissolved in pyridine (25 ml) dropwise over 20 minutes. Allowed reaction to warm up to room temperature for 2 h, then removed solvent under reduced atmosphere. Purified on silica with 94.5% dichloromethane, 5% triethylamine and 0.5% methanol as eluent. Fractions corresponding to the product were dried, resuspended in dichloromethane and extracted with water (H_2O:DCM 1:10) 10× to remove excess triethylamine salts, yielding **P5**. (25% yield)

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.49 – 7.44 (m, 4H), 7.40 – 7.33 (m, 3H), 7.30 – 7.23 (m, 4H), 7.20 – 7.14 (m, 2H), 6.86 – 6.80 (m, 2H), 6.16 (dd, J = 7.4, 4.1 Hz, 1H), 3.82 – 3.76 (m, 1H), 3.76 (s, 3H), 3.39 (q, J = 7.8 Hz, 1H), 2.62 (dd, J = 12.6, 3.8 Hz, 1H), 2.37 (dd, J = 12.6, 6.6 Hz, 1H), 2.29 (ddd, J = 13.8, 7.9, 4.1 Hz, 1H), 2.18 (dt, J = 13.8, 7.7 Hz, 1H), 1.78 (d, J = 1.2 Hz, 3H).





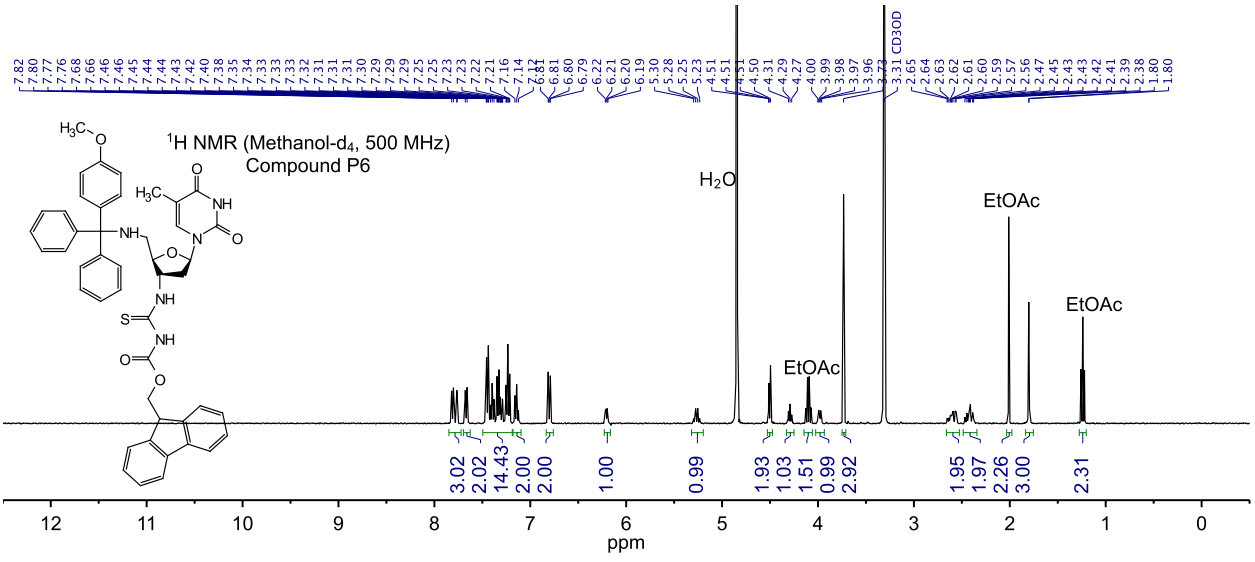
N-fluorenylmethoxycarbonyl-N′-[(3′,5′-deoxythymidin-3′-yl)-5′-N-monomethoxytrityl] thiourea (P6)

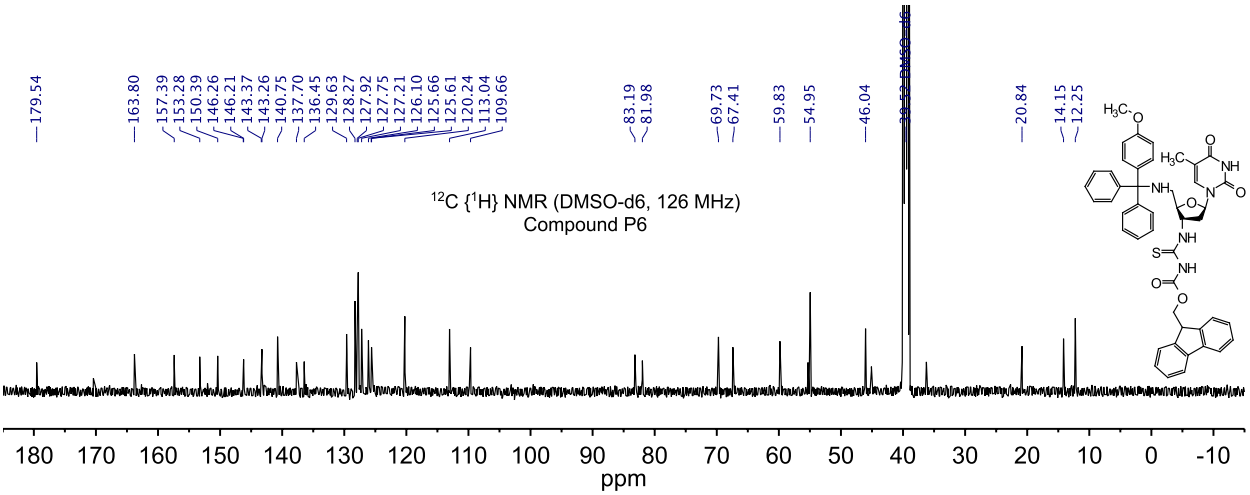
Dissolved **P5** (1.58 g, 2.0 mmol, 1 equiv.) in dichloromethane (40 ml) at room temperature, then added fluorenylmethoxycarbonyl isothiocyanate (0.67 g, 2.4 mmol, 1.2 equiv.). Allowed reaction to proceed for 16 h, then dried and purified on silica using a dichloromethane to ethyl acetate gradient. Fractions corresponding to the product were collected and dried yielding **P6** a pale-yellow foam. (84% yield)

HRMS–ESI (*m*/*z*): $[M + H]^+$ calcd for $C_{46}H_{44}N_5O_6S$, 794.3007; found 794.3016.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.86 – 7.73 (m, 3H), 7.67 (d, *J* = 7.5 Hz, 2H), 7.49 – 7.20 (m, 15H), 7.14 (t, *J* = 7.3 Hz, 2H), 6.85 – 6.73 (m, 2H), 6.20 (dd, *J* = 7.7, 3.7 Hz, 1H), 5.27 (q, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 6.9 Hz, 2H), 4.29 (t, *J* = 6.8 Hz, 1H), 3.98 (dt, *J* = 8.1, 4.0 Hz, 1H), 3.73 (s, 3H), 2.61 (ddd, *J* = 17.3, 13.5, 6.3 Hz, 2H), 2.50 – 2.34 (m, 2H), 1.80 (d, *J* = 1.1 Hz, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 179.54, 163.80, 157.39, 153.28, 150.39, 146.24 (d, *J* = 6.6 Hz), 143.31 (d, *J* = 13.5 Hz), 140.75, 137.70, 136.45, 129.63, 128.27, 127.92, 127.75, 127.21, 126.10, 125.63 (d, *J* = 6.5 Hz), 120.24, 113.04, 109.66, 83.19, 81.98, 69.73, 67.41, 59.83, 54.95, 46.04, 20.84, 14.15, 12.25.

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127.21
126.10
125.66
125.61
120.24
113.04
109.66
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59.83
54.95
46.04
20.84
14.15
12.25
^{12}C {^{1}H} NMR (DMSO-d6, 126 MHz)
Compound P6
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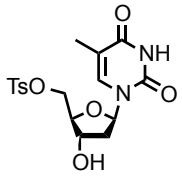
ppm

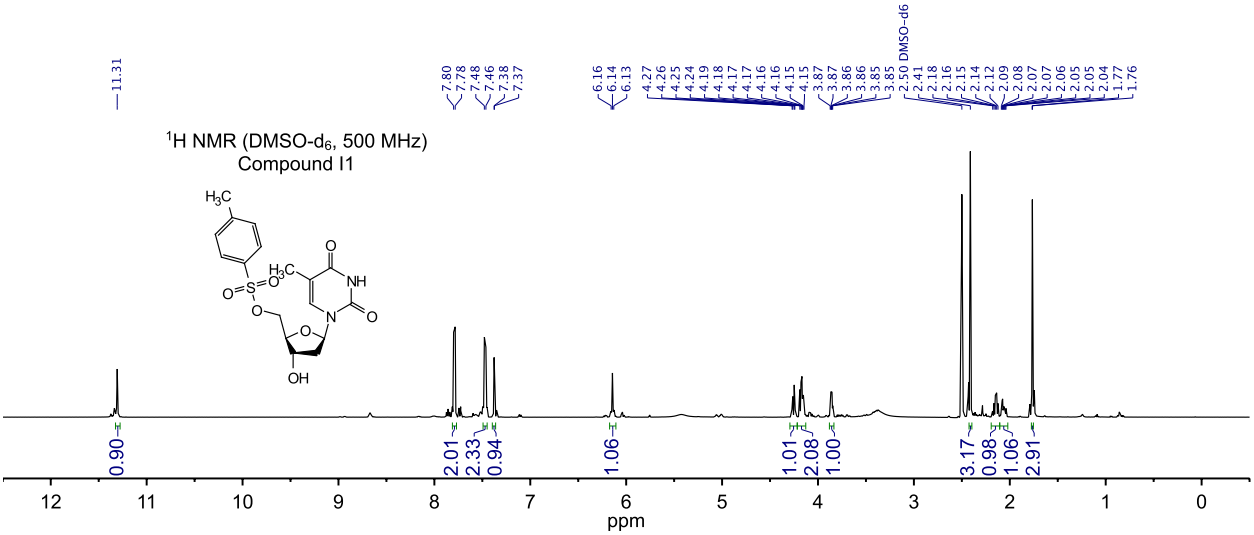
Synthesis of I4-TIPS nucleoside

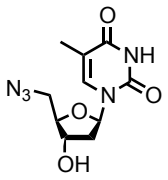


5′-O-tosyl-5′-deoxythymidine (I1)

Thymidine (5 g, 20.7 mmol, 1 equiv.) was dissolved in pyridine (35 mL) and cooled to 0 °C in an ice bath. P-toluenesulfonyl chloride (4.54 g, 23.7 mmol, 1.15 equiv.) was added as a solution in pyridine (5 mL) dropwise. The solution was allowed to warm to room temperature and monitored by TLC After the p-toluenesulfonyl chloride was consumed the solution was poured into ca. 200 mL of cold water to produce a white precipitate. Precipitate was filtered and washed with water, diethyl ether, and hexanes. The resulting white powder was used without further purification. (79% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.79 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 1.4 Hz, 1H), 6.14 (t, J = 6.9 Hz, 1H), 4.26 (dd, J = 10.9, 3.3 Hz, 1H), 4.21 – 4.13 (m, 2H), 3.86 (dt, J = 5.8, 3.5 Hz, 1H), 2.41 (s, 3H), 2.15 (dt, J = 13.8, 6.9 Hz, 1H), 2.06 (ddd, J = 13.6, 6.6, 3.9 Hz, 1H), 1.77 (d, J = 1.2 Hz, 3H).

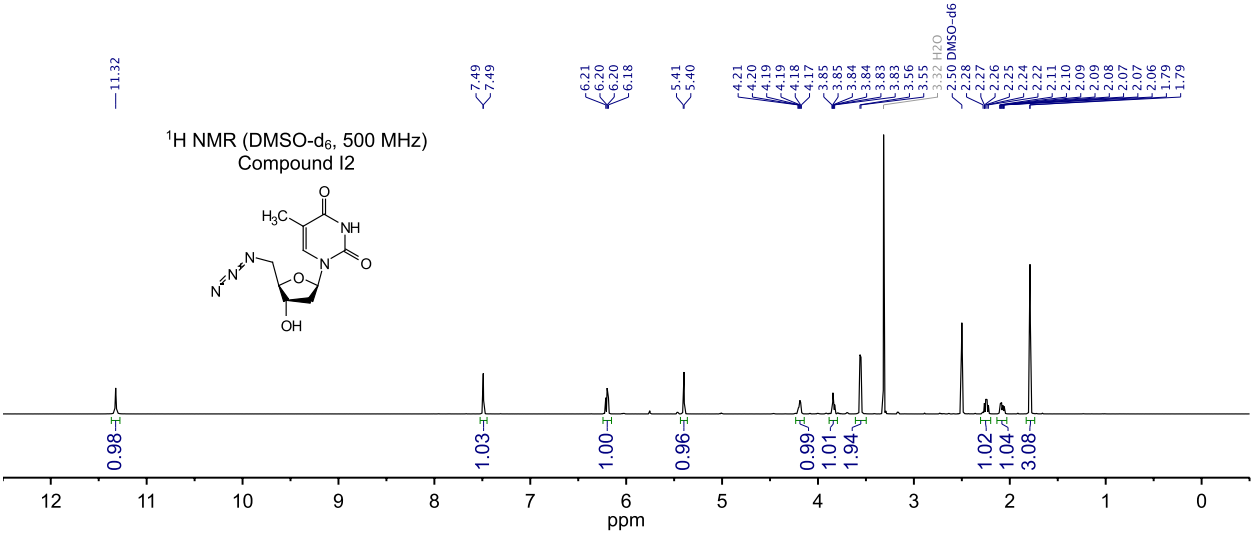


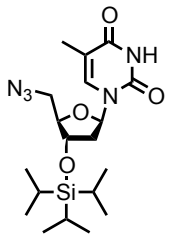


5′-Azido-5′-deoxythymidine (I2)

Combined **I1** (6.4 g, 16.1 mmol, 1 equiv.), sodium azide (1.17 g, 17.9 mmol, 1.1 equiv. weighed with a plastic spatula), and sodium iodide (0.24 g, 1.61 mmol, 0.1 equiv.) in DMF (30 mL). Heated to 110 °C for 1 h, carefully monitoring temperature behind a blast shield, then let cool to room temperature. Concentrated crude on a rotary evaporator and purified using silica chromatography with a gradient from DCM to methanol, fractions were analyzed with TLC using 10/90 MeOH/DCM solvent and UV light illumination. (62% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.32 (s, 1H), 7.49 (d, J = 1.3 Hz, 1H), 6.20 (dd, J = 7.6, 6.4 Hz, 1H), 5.40 (d, J = 4.4 Hz, 1H), 4.19 (dq, J = 7.5, 3.9 Hz, 1H), 3.84 (td, J = 5.2, 3.6 Hz, 1H), 3.56 (d, J = 5.3 Hz, 2H), 2.25 (dt, J = 13.9, 6.8 Hz, 1H), 2.08 (ddd, J = 13.5, 6.5, 3.7 Hz, 1H), 1.79 (d, J = 1.2 Hz, 3H).

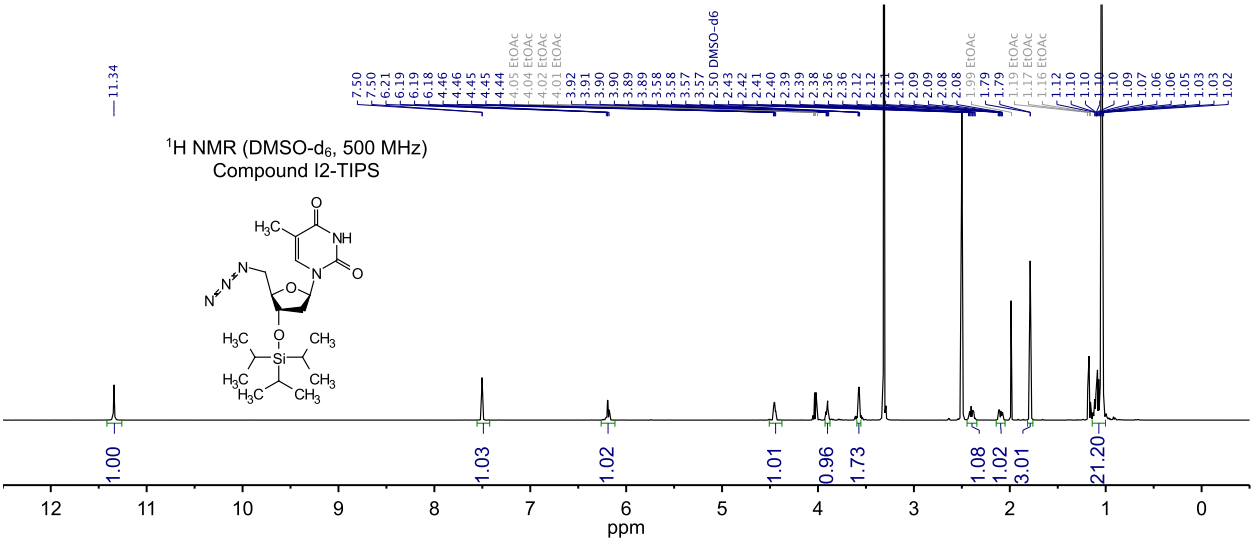


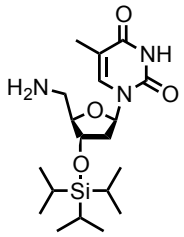


5′-azido-3′-O-triisopropylsilyl-5′-deoxythymidine (I2-TIPS)

Dissolved **I2** (1.0 g, 3.74 mmol, 1 equiv.) and imidazole (0.5 g, 7.49 mmol, 2 equiv.) in DMF (5 mL). Then added TIPS-Cl (0.94 mL, 4.4 mmol, 1.2 equiv.) and let stir at room temperature. Added more TIPS-Cl (0.78 mL, 3.74 mmol, 1 equiv.) after 24 h and let stir for another 24 h. Diluted with DCM and extracted with water, brine, and dried using magnesium sulfate. Concentrated under reduced pressure and purified with silica chromatography with a gradient from hexanes to ethyl acetate. Product was a white solid. (76% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (s, 1H), 7.50 (d, J = 1.4 Hz, 1H), 6.19 (dd, J = 8.2, 6.1 Hz, 1H), 4.45 (dt, J = 5.8, 2.8 Hz, 1H), 3.90 (td, J = 5.5, 2.7 Hz, 1H), 3.57 (dd, J = 5.5, 2.8 Hz, 2H), 2.40 (ddd, J = 13.9, 8.3, 6.0 Hz, 1H), 2.10 (ddd, J = 13.4, 6.1, 2.8 Hz, 1H), 1.79 (d, J = 1.2 Hz, 3H), 1.13 – 0.99 (m, 21H).





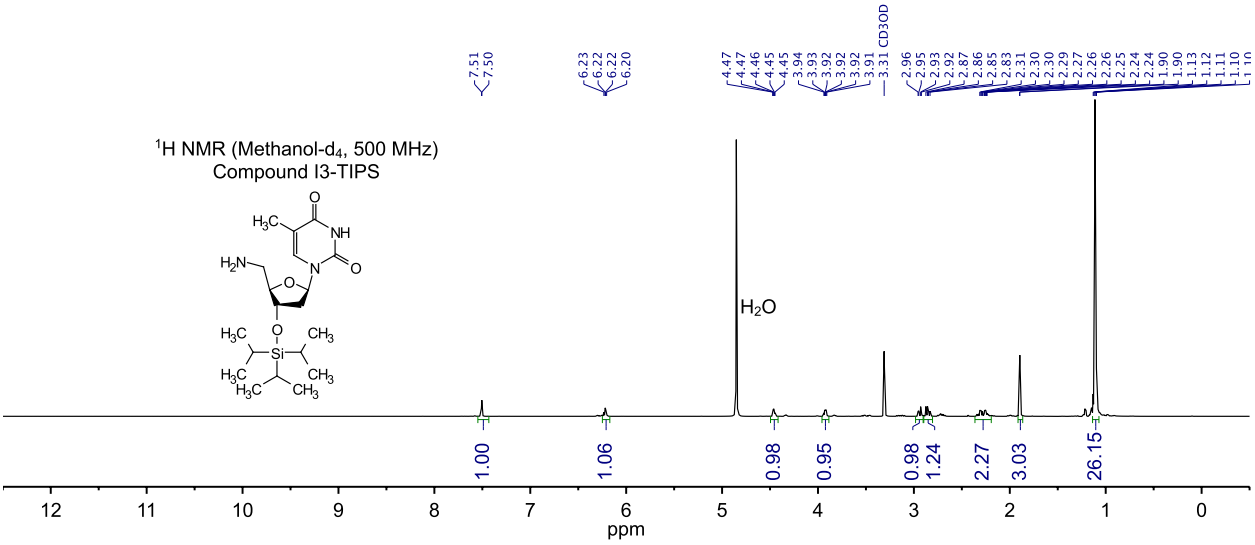
5′-amino-3′-O-triisopropylsilyl-5′-deoxythymidine (I3-TIPS)

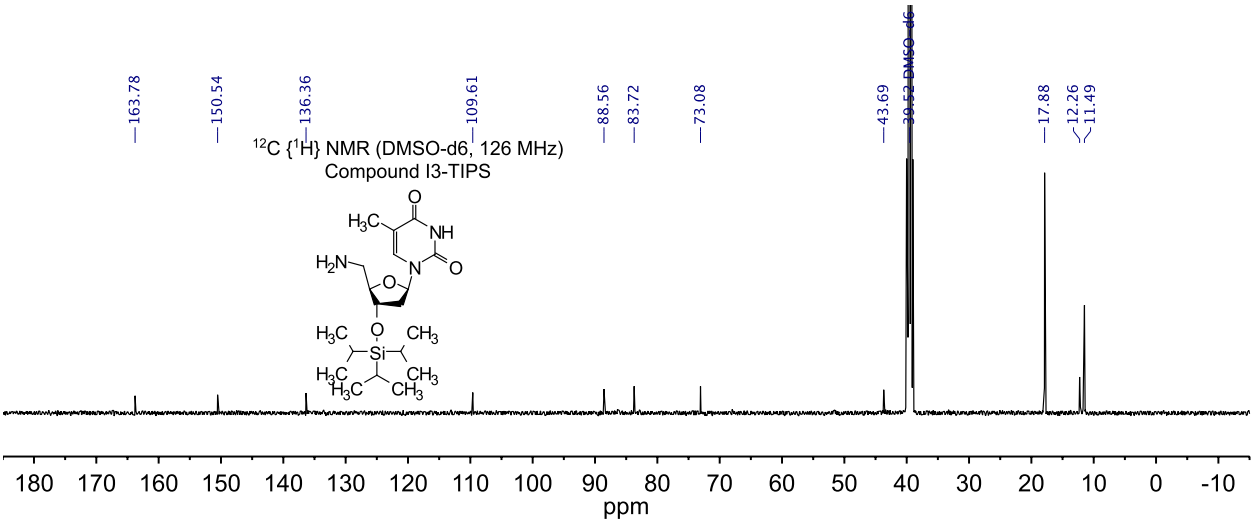
Dissolved **I2-TIPS** (1.30 g, 2.8 mmol) in methanol (30 mL) in a round bottom flask and degassed by bubbling argon. Then added 10% Pd on carbon (0.2 g) as a slurry in water. Replaced atmosphere by evacuating flask and refilling with hydrogen gas, cycled three times. Let stir vigorously at room temperature for 22 h. Bubbled argon through the reaction to remove hydrogen gas, then filtered through Celite washing with copious amounts of methanol. Removed methanol under reduced pressure and concentrated three times from benzene. A white foam remained. (82% yield)

HRMS–ESI (*m/z*): $[M + H]^+$ calcd for $C_{19}H_{36}N_3O_4Si$, 398.2470; found 398.2465.

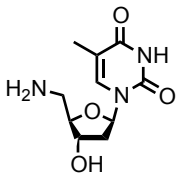
^{1}H NMR (500 MHz, Methanol-d_4) δ 7.51 (d, J = 1.4 Hz, 1H), 6.22 (dd, J = 8.0, 6.2 Hz, 1H), 4.46 (dt, J = 6.0, 3.0 Hz, 1H), 3.92 (dt, J = 8.2, 3.5 Hz, 1H), 2.94 (dd, J = 13.3, 3.9 Hz, 1H), 2.85 (dd, J = 13.3, 8.2 Hz, 1H), 2.35 – 2.22 (m, 2H), 1.90 (d, J = 1.2 Hz, 3H), 1.11 (d, J = 4.9 Hz, 21H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.78, 150.54, 136.36, 109.61, 88.56, 83.72, 73.08, 43.69, 17.88, 12.26, 11.49.




163.78
150.54
136.36
109.61
88.56
83.72
73.08
43.69
39.53 DMSO-d6
17.88
12.26
11.49
^{12}C {^{1}H} NMR (DMSO-d6, 126 MHz)
Compound I3-TIPS
O
H3C
NH
H2N
N
O
O
H3C
O
CH3
Si
H3C
CH3
H3C
CH3
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
ppm

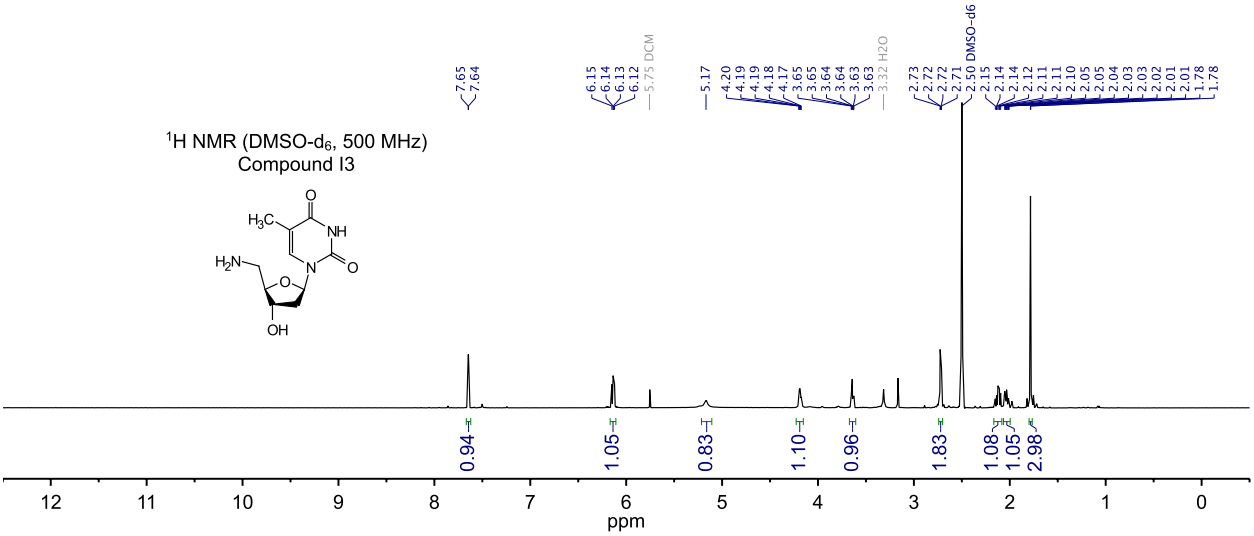
Synthesis of initiator nucleoside I5

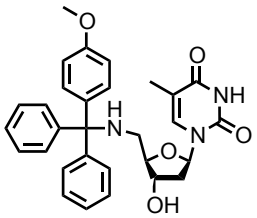


5′-Amino-5′-deoxythymidine (I3)

Dissolved **I2** (0.5 g, 1.87 mmol) in methanol (20 mL) previously degassed via bubbling argon. Added 10%$_m$ palladium on carbon (100 mg) as a slurry in water, then bubbled hydrogen gas through the solution while stirring vigorously and following reaction by TLC (15/85 MeOH/DCM, UV illumination). When all starting material was consumed, approximately 24 h, the solution was filtered through Celite with copious methanol washes. The solution was concentrated on a rotary evaporator and used without further purification. (84% yield)

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.65 (d, J = 1.4 Hz, 1H), 6.14 (dd, J = 7.7, 6.2 Hz, 1H), 5.17 (s, 1H), 4.19 (dt, J = 6.5, 3.4 Hz, 1H), 3.64 (td, J = 5.2, 3.3 Hz, 1H), 2.72 (dd, J = 5.2, 1.4 Hz, 2H), 2.12 (ddd, J = 13.9, 7.7, 6.4 Hz, 1H), 2.03 (ddd, J = 13.3, 6.3, 3.4 Hz, 1H), 1.78 (d, J = 1.2 Hz, 3H).

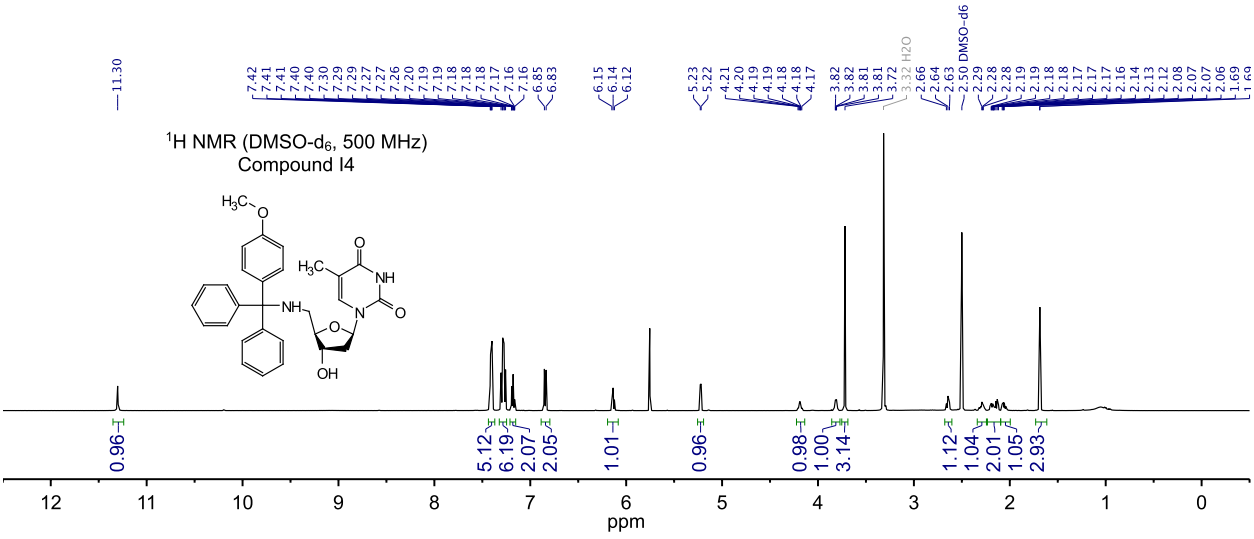


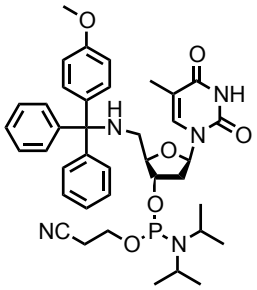


5′-N-monomethoxytrityl-5′-deoxythymidine (I4)

Dissolved **I3** (1.35 g, 5.6 mmol, 1 equiv.) and triethylamine (0.86 mL, 6.16 mmol, 1.1 equiv.) in pyridine (35 mL) under argon and cooled to 0 °C, then added monomethoxytrityl chloride dropwise (1.90 g, 6.16 mmol, 1.1 equiv.) as a solution in pyridine (8 mL). Let stir and warm to room temperature overnight. Concentrated on a rotary evaporator and purified with silica chromatography using methanol/DCM solvent system with 5% triethylamine. (72% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.30 (s, 1H), 7.44 – 7.38 (m, 5H), 7.33 – 7.23 (m, 6H), 7.21 – 7.15 (m, 2H), 6.84 (d, J = 8.9 Hz, 2H), 6.14 (t, J = 6.7 Hz, 1H), 5.22 (d, J = 4.7 Hz, 1H), 4.18 (dt, J = 6.8, 4.3 Hz, 1H), 3.81 (dt, J = 6.4, 4.3 Hz, 1H), 3.72 (s, 3H), 2.64 (t, J = 8.1 Hz, 1H), 2.33 – 2.25 (m, 1H), 2.22 – 2.10 (m, 2H), 2.09 – 2.01 (m, 1H), 1.69 (d, J = 1.2 Hz, 3H).





5′-N-monomethoxytrityl-5′-deoxythymidine, 3′-[(2-cyanoethyl)-(N,N-diisopropyl)]-phosphoramidite (I5)

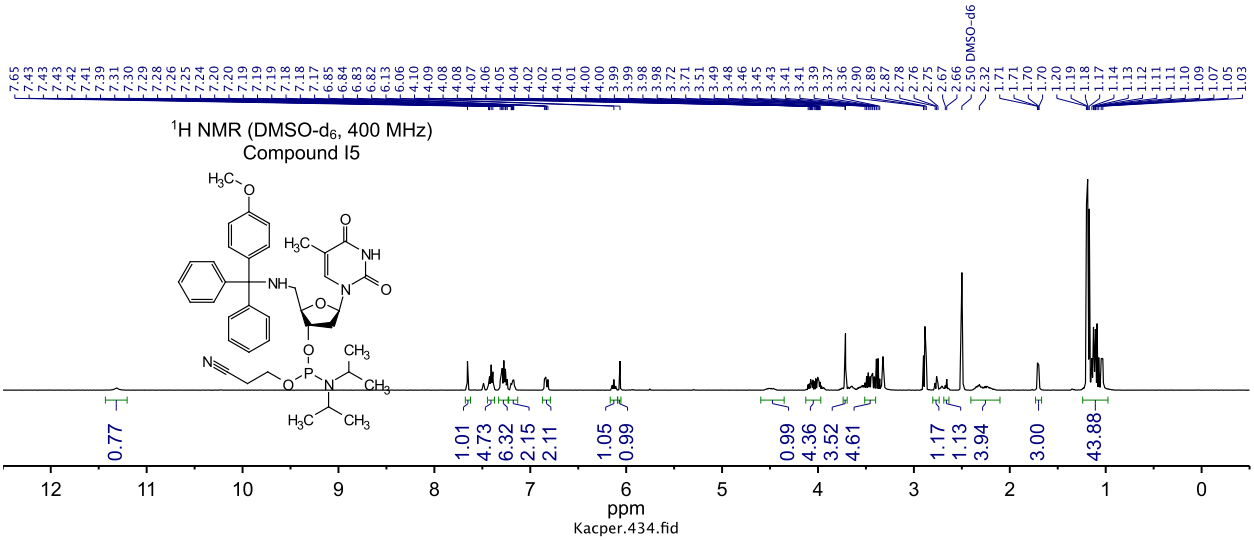
Under inert N_2 atmosphere, dissolved **I4** (0.80 mg, 1.6 mmol, 1 eq.) and DIPEA (1.0 g, 1.4 mL, 7.8 mmol, 5 eq) in DCM (16 ml). Added 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.1 g, 1.0 mL, 4.7 mmol, 3 eq) and allowed to stir at room temperature for 2 h. Concentrated under rotary evaporator and separated on silica using 60:40 Hex:DCM with 5% TEA under argon atmosphere using flash chromatography. Both diastereomers (*S*p/*R*p) were collected as a white foam. (30% yield).

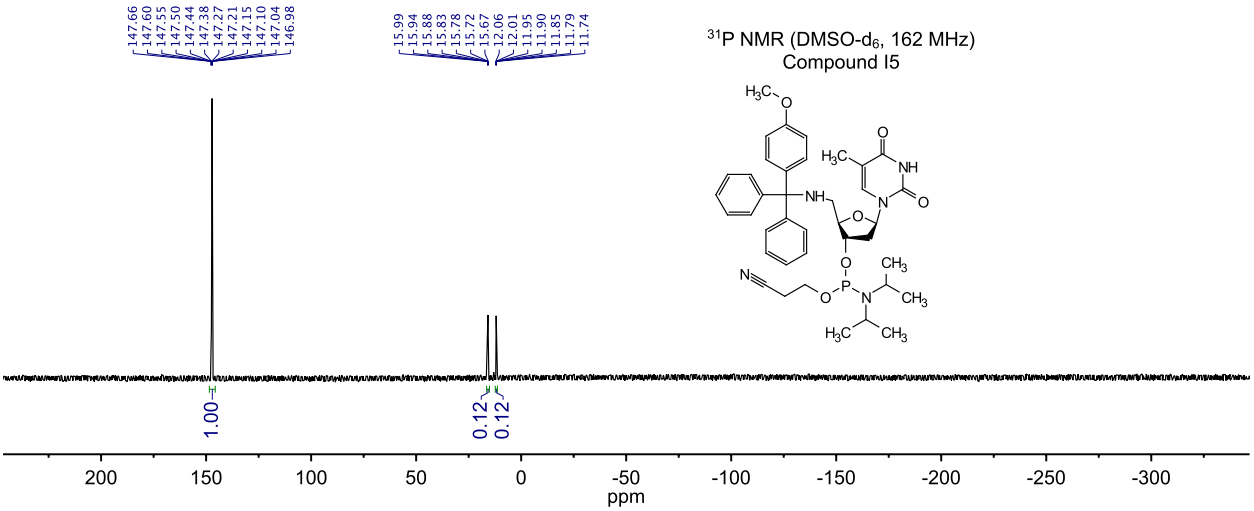
HRMS–ESI (*m*/*z*): [M + Na]$^+$ calcd for $C_{39}H_{48}N_5NaO_6P$, 736.3234; found 736.3239.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.31 (s, 1H), 7.65 (s, 1H), 7.45 – 7.36 (m, 5H), 7.28 (ddd, *J* = 14.2, 8.2, 6.1 Hz, 6H), 7.22 – 7.13 (m, 2H), 6.88 – 6.79 (m, 2H), 6.13 (s, 1H), 6.06 (s, 1H), 4.50 (ddd, *J* = 20.2, 10.7, 6.3 Hz, 1H), 4.04 (dddt, *J* = 30.2, 10.4, 7.6, 5.8 Hz, 3H), 3.72 (d, *J* = 1.9 Hz, 3H), 3.53 – 3.40 (m, 3H), 2.71 (dt, *J* = 42.5, 5.9 Hz, 2H), 2.39 – 2.13 (m, 2H), 1.70 (dd, *J* = 5.4, 1.1 Hz, 3H), 1.23 – 1.00 (m, 12H).

^{31}P NMR (162 MHz, DMSO-d_6) δ 147.52 (h, *J* = 9.6, 9.6, 9.6, 9.1, 9.1 Hz), 147.12 (h, *J* = 9.8, 9.8, 9.8, 9.5, 9.5 Hz), 16.25 – 15.38 (m), 12.18 – 11.51 (m).

The impurities visible in the ^{31}P NMR at 11.9 and 15.8 ppm are likely due to hydrolysis at the phosphorous center resulting in H-phosphonate degradation products.[3] This impurity was found to be difficult to eliminate and the product was used without further purification, the reported yield is corrected for the presence of this impurity.




147.66
147.60
147.55
147.50
147.44
147.38
147.27
147.21
147.15
147.10
147.04
146.98
15.99
15.94
15.88
15.83
15.78
15.72
15.67
12.06
12.01
11.95
11.90
11.85
11.79
11.74
31P NMR (DMSO-d6, 162 MHz)
Compound I5
1.00
0.12
0.12
200
150
100
50
0
-50
-100
-150
-200
-250
-300
ppm

Experimental

Solution-phase screen
96-well plates with aluminum foil covers or vials with small volume inserts were used for solution phase screening of reaction conditions. Reaction mixtures were injected directly into a UPLC-MS running a gradient from 95/5 to 5/95 of buffers A/B over 5.5 minutes, where buffer A consists of 0.1% formic acid in water or only water, and buffer B is made of 0.1% formic acid in acetonitrile or 10% THF in acetonitrile. Reactions were carried out by mixing a 5′-amino-dT derivative (0.20 mM, 1 equiv.), a 3′ thiourea-functionalized dT (1.00 mM, 5 equiv.), iodine (varied as indicated), and a base (varied as indicated), diluted to 200 μL with the indicated solvent.

Automated oligonucleotide synthesis
For coupling optimization, a $d(pT)_{19}$ strand was synthesized on CPG beads using standard phosphoramidite chemistry where the final base consisted of an initiator monomer **I5** with a 5′-MMTr-protected amine. A small amount of CPG was retained after **I5** addition to aid in measuring yield. Three DNG bases were subsequently synthesized atop the strands on the remaining CPGs. The full synthetic cycle of the MerMade 12 is given below. Briefly, the thiourea monomer was dissolved at a 25 mM concentration in a 1:1 mixture of acetonitrile (ACN) and dichloromethane (DCM), while the activator was a DCM solution of 50 mM iodine and 150 mM TMP. The activator was stored on the synthesizer wrapped in aluminum foil. Approximately 180 μL of thiourea and 60 μL of activator solution were added onto the beads per coupling step with a subsequent wait step of 10 minutes. The coupling step was repeated twice per base.

Strands were cleaved from the solid support and deprotected using AMA solution (1:1 mixture by volume of 30% aqueous ammonium hydroxide and 40% aqueous methylamine) at 55 °C for 30 minutes. Subsequently, Tris buffer (1 M, pH 8) was added to the solution to a final concentration of 0.1 M, and the liquid was dried under a stream of nitrogen gas at room temperature. The strands were then reconstituted with water to 2.2 mL, filtered with a 0.2 μm filter and injected onto a C4 reverse phase column using an HPLC system (Shimadzu). Collected fractions were lyophilized and resuspended in water.

Longer DNG oligonucleotides (10 and 20 bases in length, full DNG backbone) were synthesized similarly atop a CPG pre-coupled with initiator monomer **I5** using standard phosphoramidite chemistry. Additionally, the 20mer DNG strand was synthesized in two consecutive rounds of 9 and 10 bases to enable flushing of the activator line and minimize crystallization. Deprotection was carried out in AMA solution at 55 °C for 1 h prior to evaporation using a N_2 stream. CPGs were washed with water then acetonitrile prior to adding an aqueous 20% acetic acid solution, which was then filtered through 0.2 μm filter and lyophilized.

Polyacrylamide gel electrophoresis
T_{10} DNG oligonucleotide (630 picomoles) was mixed with non-denaturing Laemmli buffer and heated to 95 °C for 5 min prior to loading into a 4-15% SDS-PAGE gradient gel along with a Precision Plus protein ladder. The gel was run for 1 h at 100 V using Tris-Glycine-SDS buffer and stained with SimplyBlue, which is specific for cationic residues such as arginine, lysine and histidine.

Acetic Acid Urea Polyacrylamide gel electrophoresis

Longer DNG oligonucleotides were purified and analyzed using 24% acetic acid urea (AU) polyacrylamide gels as described.[4] This technique was adopted due to the structural similarities between histones and DNG, namely their low molecular weight and cationic charge due to the presence of guanidine groups. Briefly, 24% polyacrylamide gels with 6 M urea and 5% acetic acid were initiated using ammonium persulfate and tetramethylethylenediamine (TEMED), sonicated to remove bubbles and allowed to set. Samples were loaded in 5% acetic acid with 4 M urea, following heating to 95 °C for 5 min. The gels were then run for 1.5 h at 200 V (analytical gels) or 3.5 h at 250 V (purification gels) with reversed polarity, followed either by staining in SimplyBlue (analytical gels) or excision using a UV lamp shadow (purification gels). The excised bands were crushed, heat-shocked and soaked in 5% acetic acid for 24 h at 55 °C prior to desalting in 5% acetic acid. Final product was lyophilized, resuspended in water and its concentration was measured using UV-visible spectroscopy.

Cellular uptake experiments

Cellular uptake experiments were conducted using green fluorescent protein (GFP)-expressing mouse endothelial cells (C166) maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. For confocal microscopy experiments, cells were plated in 8-well chambered coverslips at a density of 100 cells/μl and treated the next day with 600 nM of T_{10} oligonucleotides (by label, DNA or DNG) in Opti-MEM for 2.5 h at 37 °C. Cells were then washed with PBS and fixed using 2% paraformaldehyde in PBS prior to imaging on a confocal fluorescence microscope using constant laser and PMT settings. For live cell imaging, the 2% paraformaldehyde was omitted, and PBS was added instead. Z-stacks were acquired in 1 μm slices from the bottom of the plate to the top of the cell using the signal from GFP in the cytoplasm as a marker in live cells.

Uptake quantification by flow cytometry was conducted with cells plated in a 24-well plate at a density of 100 cells/μl and treated the next day with 500 nM of T_{10} oligonucleotides (by label, DNA or DNG) in Opti-MEM for 2 h at 37 °C. Following treatment, cells were washed with PBS, treated with trypsin (100 μl) for 5 min at 37 °C and fixed using 2% paraformaldehyde in PBS prior to analysis by flow cytometry, where cells were gated using the forward and side scattering readouts and uptake was analyzed using the APC channel excited with the 637 nm laser. For live cell experiments, the paraformaldehyde addition was omitted and replaced by PBS, followed by pelleting (5 min, 1500 rpm, 4 °C), removal of the supernatant and resuspension in PBS for analysis.

Labeling strands with AlexaFluor 647 dye

CPG-bound 10-mer DNA or DNG strand were treated with 3% dichloroacetic acid in DCM to remove terminal trityl protecting groups and expose reactive amines at the 5′ terminus. A solution of approximately 4 mM AlexaFluor 647 N-hydroxysuccinimide ester dye (ThermoFisher) and 4 mM diisopropylethylamine in DMF was added to the CPG beads and allowed to react for 1 h at room temperature. The dye solution was removed, and beads washed with DMF and water. The strands were cleaved and deprotected with AMA solution at 55 °C for ~45 min then dried under a stream of nitrogen at 35 °C. DNA was resuspended in water, filtered through a 0.2 μm syringe filter and purified via RP-HPLC. The dry DNG film was washed with ~0.5 mL of water before reconstituting in 20% acetic acid and filtering through 0.2 μm syringe filter. Both oligonucleotide solutions were then lyophilized and reconstituted in water prior to use.

Alamar Blue cell viability assay

C166 mouse endothelial cells maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin were plated at a density of 100 cells/μL in a 96-well plate. Cells were treated the next day with T_{10} oligonucleotides (DNA or DNG), in triplicates, in a serial dilution 1:2 ranging from 5 uM to 78 nM of oligonucleotide in Opti-MEM at a final volume of 90 μL. Alamar Blue reagent (10 μL) was added and the cells were incubated at 37 °C for 6 h prior to measuring fluorescence at each well using an excitation wavelength of 560 nm and collecting emission 590 nm on a plate reader.

Crystal structure of iodine-TMP activator precipitate

Single crystals of TMP-iodine complex ($C_9H_{19}I_2N$) were crystalized from acetonitrile by making a 50 mM solution of iodine and adding TMP to a 150 mM concentration. Crystals began to precipitate from the solution within minutes. A suitable crystal was selected, and **the crystal was mounted on a MITIGEN holder with Paratone oil** on a **Bruker APEX-II CCD** diffractometer. The crystal was kept at 100.2 K during data collection. Using Olex2,[5] the structure was solved with the ShelXT[6] structure solution program using Intrinsic Phasing and refined with the XL[7] refinement package using least squares minimization. No special refinement was necessary.

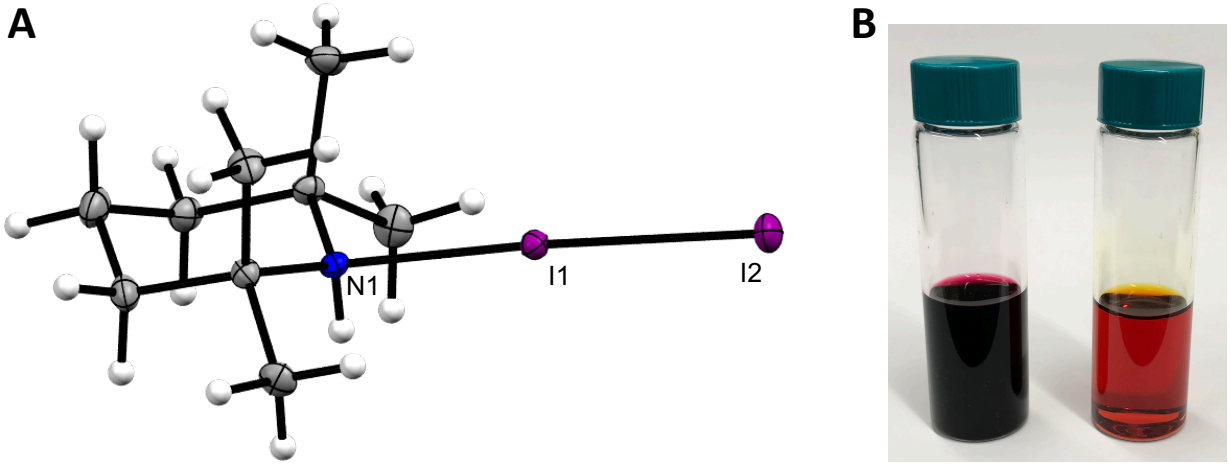


Figure S1. Iodine and TMP activator solution. (A) Crystal structure obtained from the precipitates of an iodine and TMP solution in acetonitrile, 50% probability ellipsoids shown. (B) Photograph of optimized activator solutions (50 mM iodine, 150 mM TMP in DCM) used for automated solid-phase DNG synthesis before (left) and after (right) addition of TMP base.

Crystal Data for $C_9H_{19}I_2N$ (M =395.05): monoclinic, space group $P2_1/c$ (no. 14), a = 8.0127(6) Å, b = 11.0751(8) Å, c = 14.6056(11) Å, β = 102.6380(14)°, V = 1264.72(16) Å^3, Z = 4, T = 100.2 K, μ(MoKα) = 4.932 mm^{-1}, *Dcalc* = 2.075 g/mm^3, 23884 reflections measured (4.658 ≤ 2Θ ≤ 72.676), 6116 unique (R_{int} = 0.0349, R_{sigma} = 0.0306) which were used in all calculations. The final R_1 was 0.0185 (I > 2σ(I)) and wR_2 was 0.0416 (all data).

The lengthened I1-I2 bond (2.85 Å, compared to 2.71 Å for molecular iodine[8]) indicates a weakened halogen bond. However, the relatively long N-I bond of 2.47 Å indicates a weaker complex with the nitrogen base compared to other electrophilic iodine sources (N-I bond lengths for dimethylaminopyridine complexes with N-iodosuccinimide and N-iodosaccharin are 2.15 and 2.29Å, respectively[9]). Complex formation is evidenced by immediate color change of the solution following addition of TMP to a solution of iodine in DCM, shown as in Figure S1B.

Various activator solutions over time

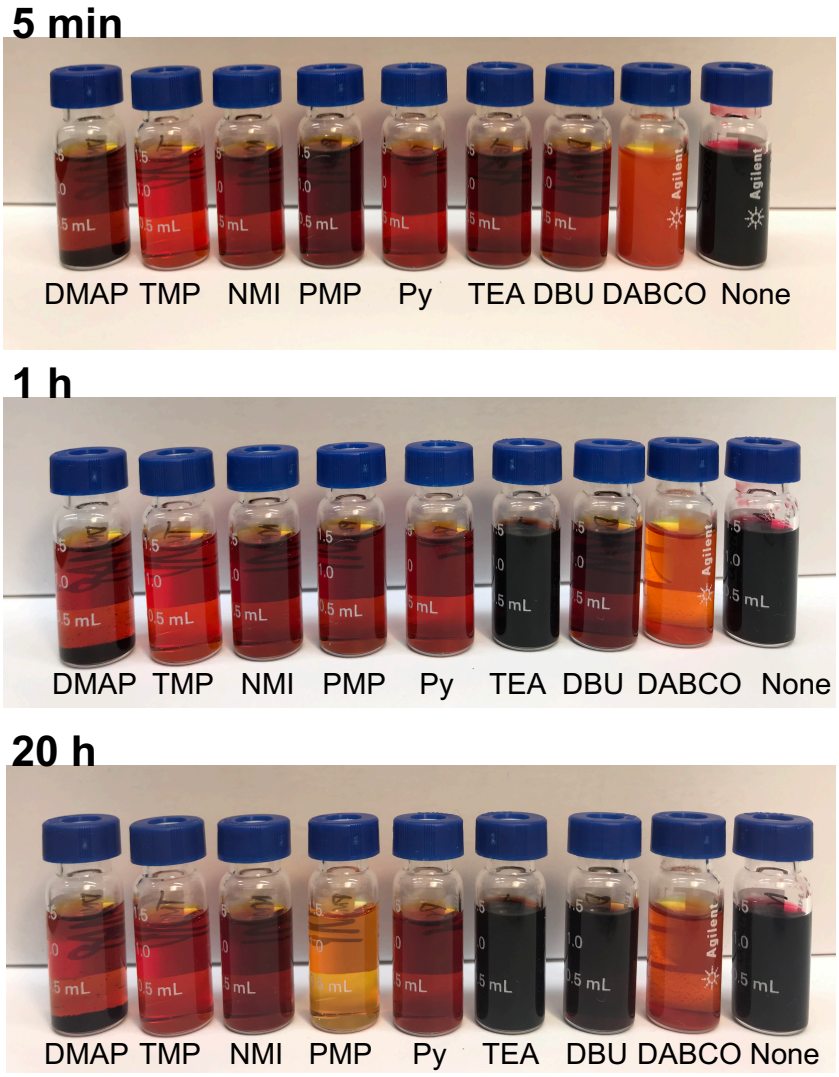


Figure S2. Activator solutions of iodine (50 mM) and various labeled bases (150 mM) in DCM after 5 min, 1 h, and 20 h. Solutions made by dissolving iodine in DCM first, then mixing with various neat bases. PMP, TEA, and DBU changed color over time, while DABCO and DMAP formed a precipitate.

Coupling rates as a function of base amount

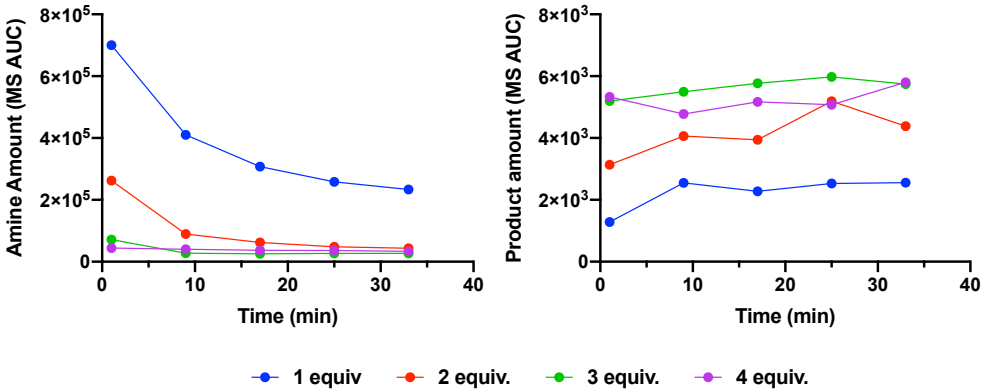


Figure S3. Product amount in reactions measured by the integration of the mass spectrum product peak while varying the number of equivalents of base in solution. Reaction conditions: thiourea **P6** (3 equiv.), amine **I3-TIPS** (1 equiv.), iodine (2 equiv.), and TMP (as specified) in 25% DCM in ACN.

Coupling yield as a function of solvent type

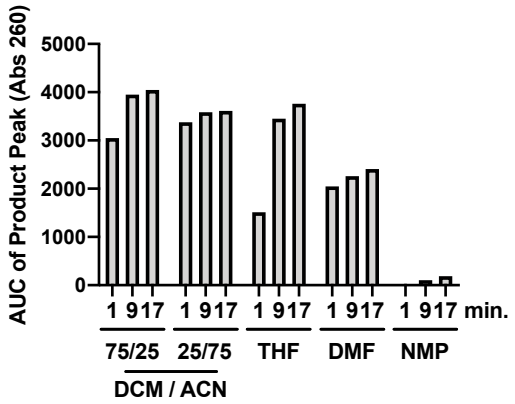


Figure S4. Coupling yield optimization using the iodine/base system. The impact of solvent choice on the coupling reaction with a thiourea/iodine/TMP ratio of 1.5/1/3.

DNG synthesis cycle on a MerMade 12

A standard 1 μmol scale synthesis cycle on the MerMade12 was adapted for DNG coupling. An alternative wash step was added by modifying the program files based on instructions from BioAutomation. An unused reagent bottle was designated as the alternative wash 1 position and filled with neat anhydrous DMF. To ensure that both standard phosphoramidite chemistry along with the iodine-mediated guanidinium synthesis could occur on the same machine, the activator line that corresponds to the thiourea monomer delivery tip was re-routed from the standard phosphoramidite activator manifold to an iodine-TMP activator stored in a separate bottle. This was accomplished simply by connecting the activator solenoid valve to a separate bottle and running tubing to join the atmosphere of the new activator bottle to the original phosphoramidite activator, which provides the pressure needed to drive the flow of reagents (Figure S5). The reagent flow was recalibrated to account for the solvent change.

Cycle commands:

1. START TRITYL
2. DEBLOCK
3. DEBLOCK
4. DEBLOCK
5. DEBLOCK
6. ACN WASH
7. ACN WASH
8. ACN WASH
9. END TRITYL
10. ALT WASH 1
11. ACN WASH
12. ACN WASH
13. ACN WASH
14. COUPLING
15. COUPLING
16. ACN WASH
17. CAPPING
18. ACN WASH
19. ACN WASH
20. ACN WASH

Step details:

DEBLOCK: 200 μL injection of deblock mix (3% dichloroacetic acid in DCM), 45 sec wait time with 3 pulses.

ACN WASH: 275 μL injection of acetonitrile, 6 sec wait time with 2 pulses.

ALT WASH 1: 275 μL injection of neat DMF, 6 sec wait time with 1 pulse.

COUPLING: 180 μL thiourea monomer at 25 mM in 1:1 ACN:DCM and 60 μL activator (I_2/TMP at 50/150 mM in DCM) co-injections followed by 5 sec equalize time and 10 min wait time with 2 vacuum pulses.

CAPPING: 125 μL injections of each cap A (THF/Pyr/Acetic anhydride) and cap B (16% 1-methylimidazole in THF) followed by 2 sec equalize time, 60 sec wait time with 31 pulses.

MerMade 12 DNG synthesis plumbing diagram

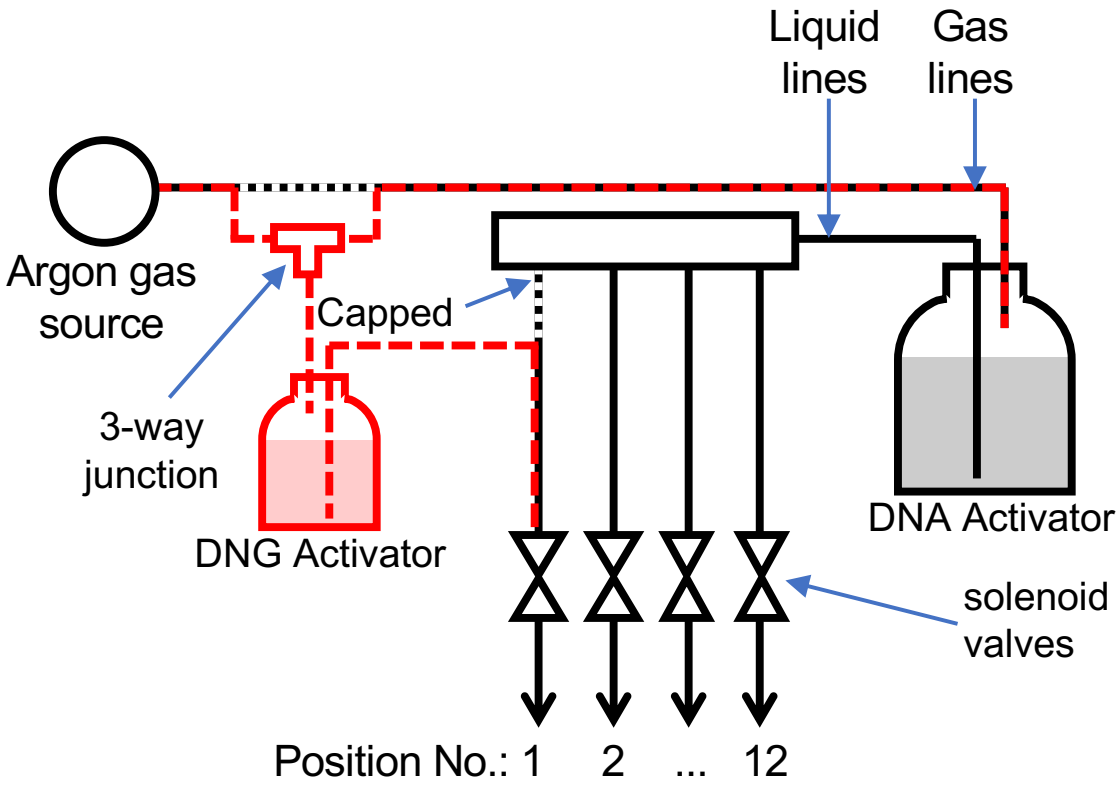


Figure S5. A diagram showing the gas and liquid line connections to transform a standard MerMade12 synthesizer to be able to synthesize DNG strands. Solid lines indicate unchanged components, dashed red lines indicate altered existing components, dashed black lines indicate removed components.

Coupling yield estimation using HPLC

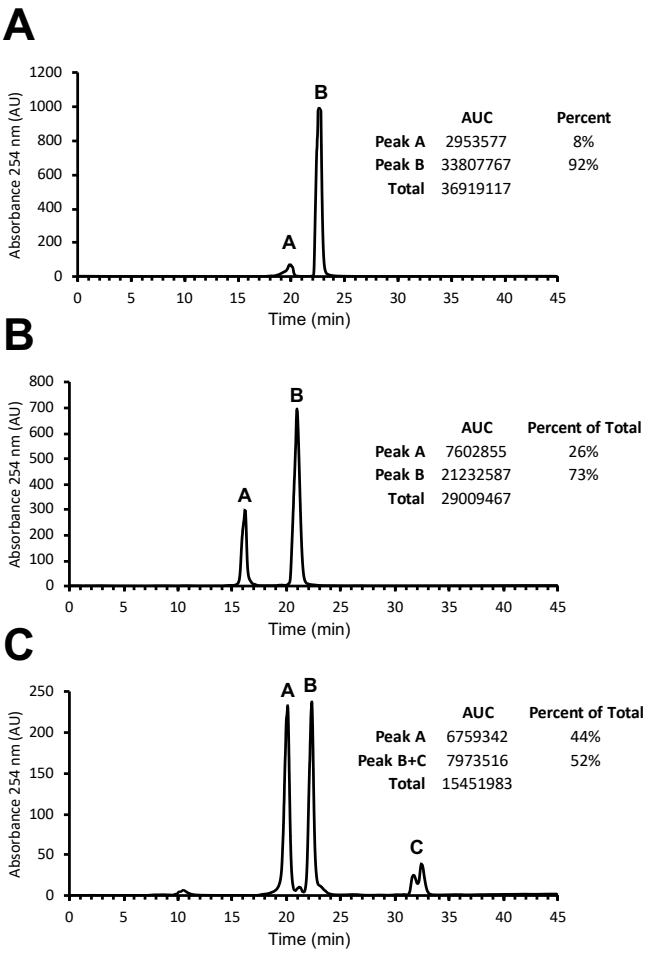


Figure S6. HPLC traces of DNG-DNA chimera oligonucleotides used for estimating coupling yields of DNGs. Small amounts of CPG were cleaved with ammonium hydroxide/methylamine solution and analyzed by HPLC at three different time points: (A) after synthesis of initial phosphate backbone $(pT)_{18}$ sequence, (B) after addition of initiator unit **I5** to make amine terminated $(pT)_{18}pT$, and (C) after addition of three DNG bases to make amine terminated $(pT)_{18}pT(gT)_3$. First peak to elute (Peak A) contains the failed strands that do not contain a trityl group, the second peak (Peak B) contains the full-length sequences with a trityl group, while the last peak (only observed in trace C) is hypothesized to consist of an electrostatic assembly of full-length strands.

Average per coupling DNG yields were estimated using the equation below where P_0 is the product (Peak B) percent of total AUC after addition of the initiator unit and P is the product (Peak B + C) after synthesis of the three DNG bases. We assumed that the failure strands from the initial 19-mer synthesis did not contribute significantly to the calculation and that the addition of the three thymidine bases did not significantly impact the extinction coefficient.

$$\left(\frac{P}{P_0}\right)^{1/3}$$

Retention of Fmoc Protecting Groups During Solid Phase Synthesis

To test the fate of Fmoc protecting groups during the synthesis we made a DNG 5-mer via four DNG couplings atop a C3 thiol CPG coupled with initiator **I5** through standard phosphoramidite chemistry. The CPG beads were then treated with a 0.1 M TCEP, 0.1 M phosphate buffered (pH 7.4) solution for 2 h, followed by addition of acetic acid to a final concentration of 20% (which substantially improves MALDI signal but removes the MMTr group from the 5′-amine). Zip Tips (C18, Millipore Sigma) were used to purify oligonucleotides in this solution away from the salts in preparation for MALDI-TOF MS. The MALDI spectrum shows retention of Fmoc and cyanoethyl protecting groups on the backbone of the oligonucleotide.

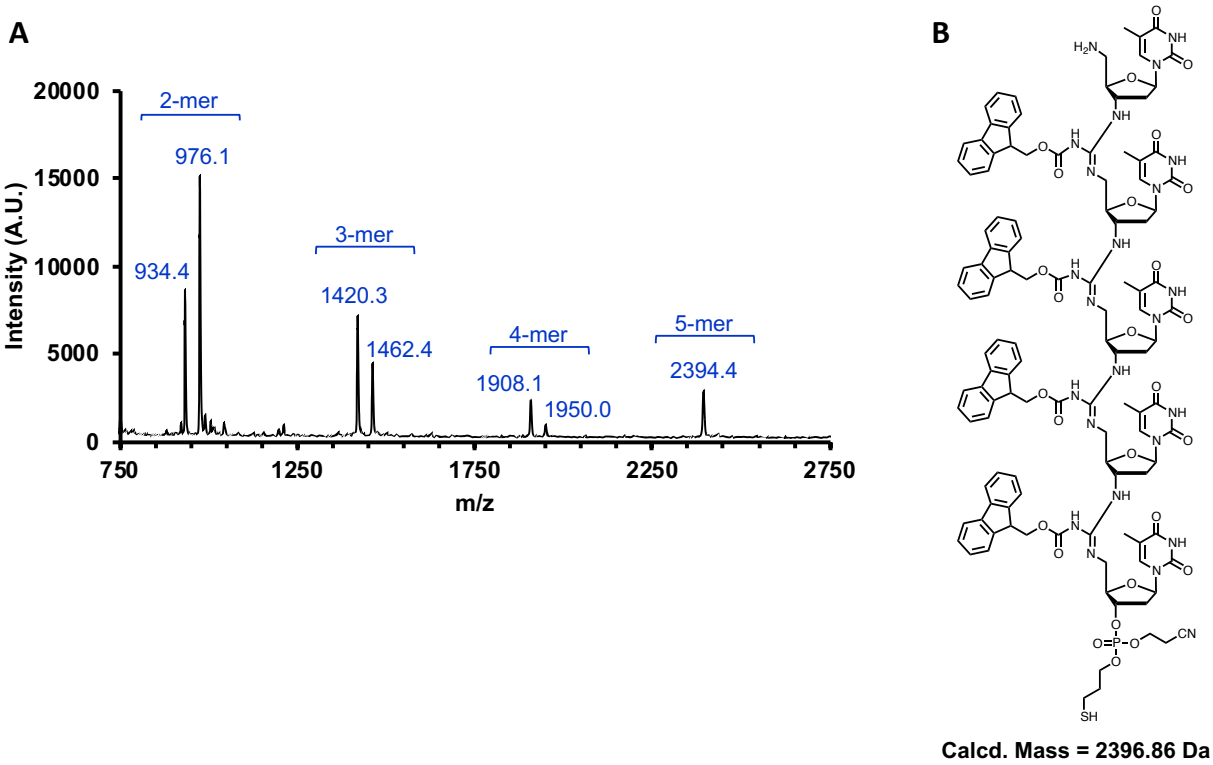
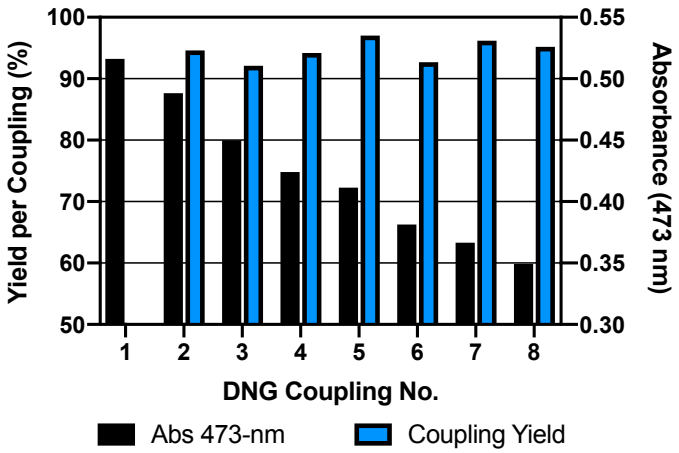


Figure S7. (A) MALDI-TOF MS spectrum of a DNG strand cleaved from CPG beads by reduction of the disulfide moiety. (B) Structure and mass of expected product with guanidine and phosphate protecting groups intact after reduction from CPGs.

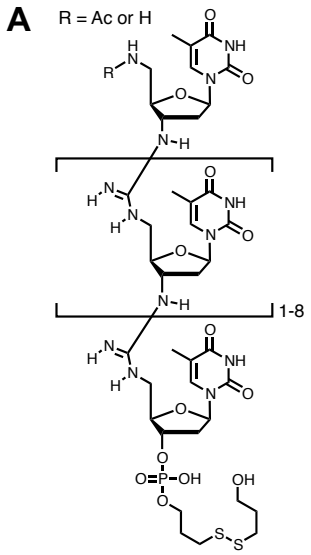
Coupling yield measured by trityl absorption



Coupling No.	*Abs. 473-nm*	*Coupling Yield (%)*
1	0.516	-
2	0.488	94.6%
3	0.450	92.1%
4	0.424	94.2%
5	0.411	97.0%
6	0.381	92.7%
7	0.367	96.2%
8	0.349	95.2%

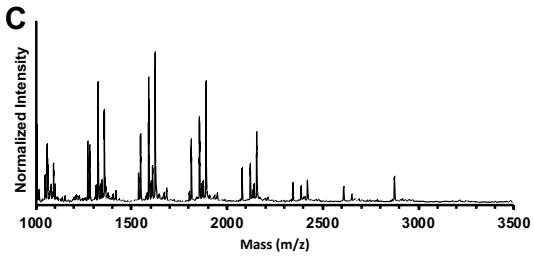
Figure S8. Coupling yield calculation based on trityl absorption. Synthesis was conducted on a MerMade 12 synthesizer with a dT_{10} synthesized previously on the beads using the same instrument and standard phosphoramidite chemistry followed by an initiator dT unit (**I5**) coupled in a glove box to give a dT_{11}. DNG bases were coupled using conditions in entry 5 in Figure 2C, and as outlined in the methods section. The column was removed from the synthesizer after each coupling cycle and the trityl group removed with 5 mL of standard deblocking solution (3% DCA in DCM). The resulting orange solution was diluted with 0.1 M TsOH•$1H_2O$ in ACN to 50 mL in a volumetric flask. UV-Vis absorption spectrum was measured and the value at the peak maximum (473 nm) was used to calculate the coupling yield, defined as the ratio between current absorption and the absorption after the last coupling.

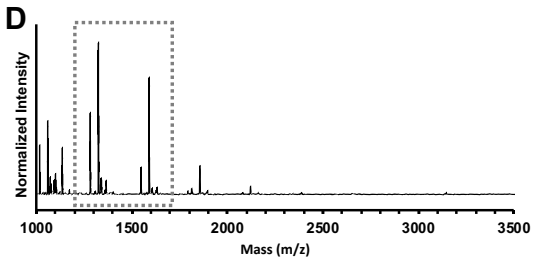
Additional characterization data for T_{10} and T_{20} DNG strands

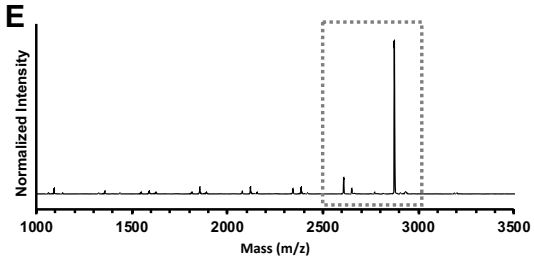


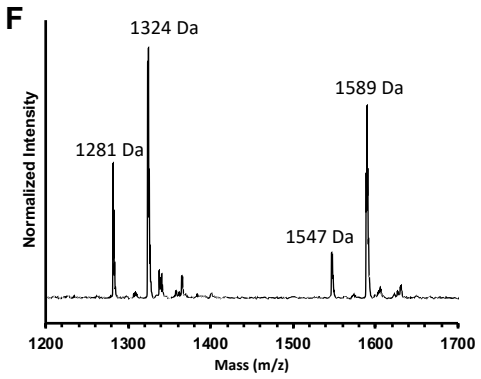
B

	Strand	Calcd. m/z [M+H⁺]	Observed m/z
Failure strands	4-mer (-NHAc)	1323	1324
	4-mer (-NH2)	1281	1281
	5-mer (-NHAc)	1589	1589
	5-mer (-NH2)	1547	1547
	9-mer (-NHAc)	2649	2651
	9-mer (-NH2)	2607	2608
Product	10-mer (-NH2)	2872	2873









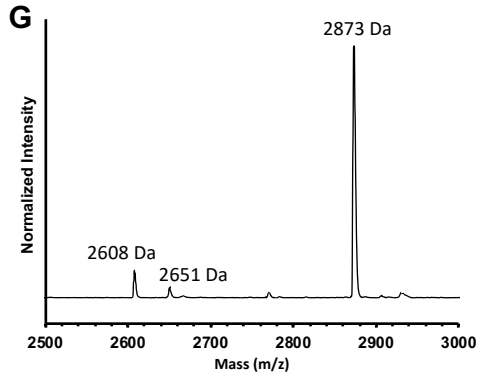


Figure S9. (A) Structure of DNG thymidine oligomers observed during the synthesis of the 10-mer DNG. (B) The calculated masses of selected failure and full-length peaks. MALDI-TOF MS spectra of (C) the crude DNG 10-mer product, (D) the initial water wash (TEAA buffer, pH 7), and (E) the 20% acetic acid wash. Expanded views of (F) the water wash and (G) the 20% acetic acid wash show the expected masses of failure strands with and without acetyl cap and the product peak.

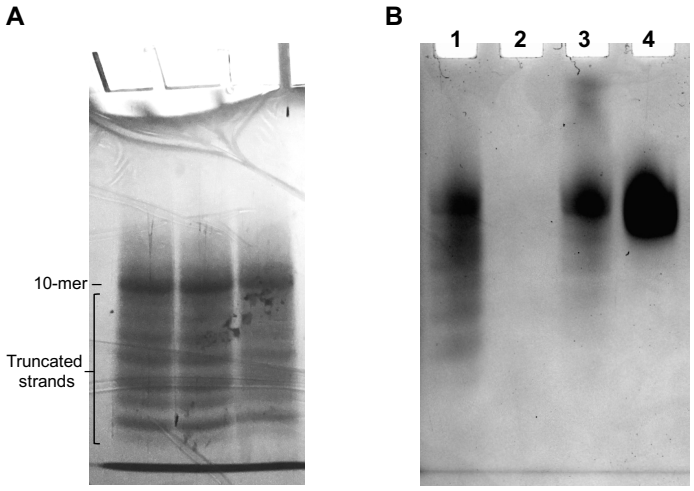


Figure S10. Acetic acid urea (AU) 24% poly-acrylamide gels. (A) Crude 10-mer DNG synthesis solubilized with 20% acetic acid following cleavage and deprotection with AMA without any purification. All three wells contain the same solution. Gel was imaged by hand-held UV lamp casting a shadow through the gel onto a UV-indicator TLC plate. (B) Gel of crude 10-mer (well 1), water wash (well 2), 20% acetic acid wash (well 3), and preparatory-scale AU gel-purified 10-mer DNG (well 4).

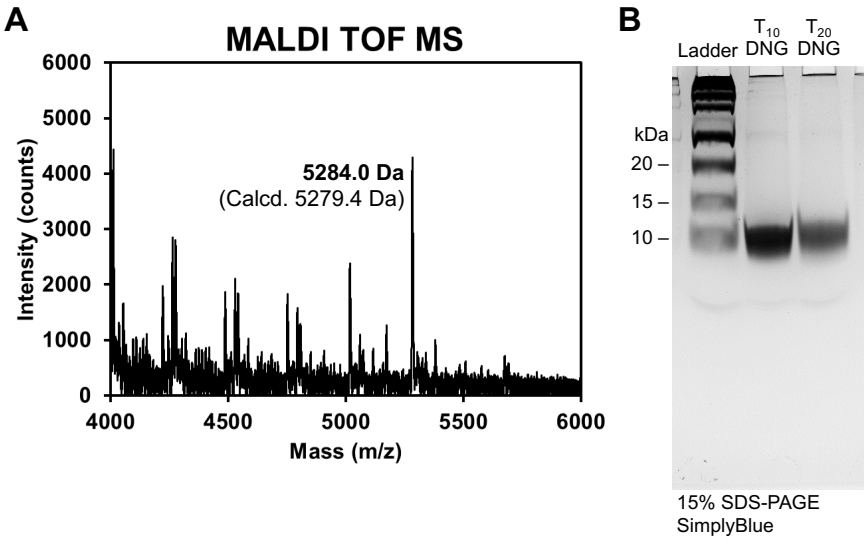


Figure S11. Additional characterization of the T_{10} and T_{20} DNG strands. (A) MALDI-TOF MS characterization of the crude T_{20} DNG strand showing the final product mass. (B) 15% SDS-PAGE gel stained with SimplyBlue, an anionic protein stain, showing a primary band for the T_{10} and T_{20} DNGs strands, the latter moving slightly slower through the gel.

Gating for cellular uptake by flow cytometry

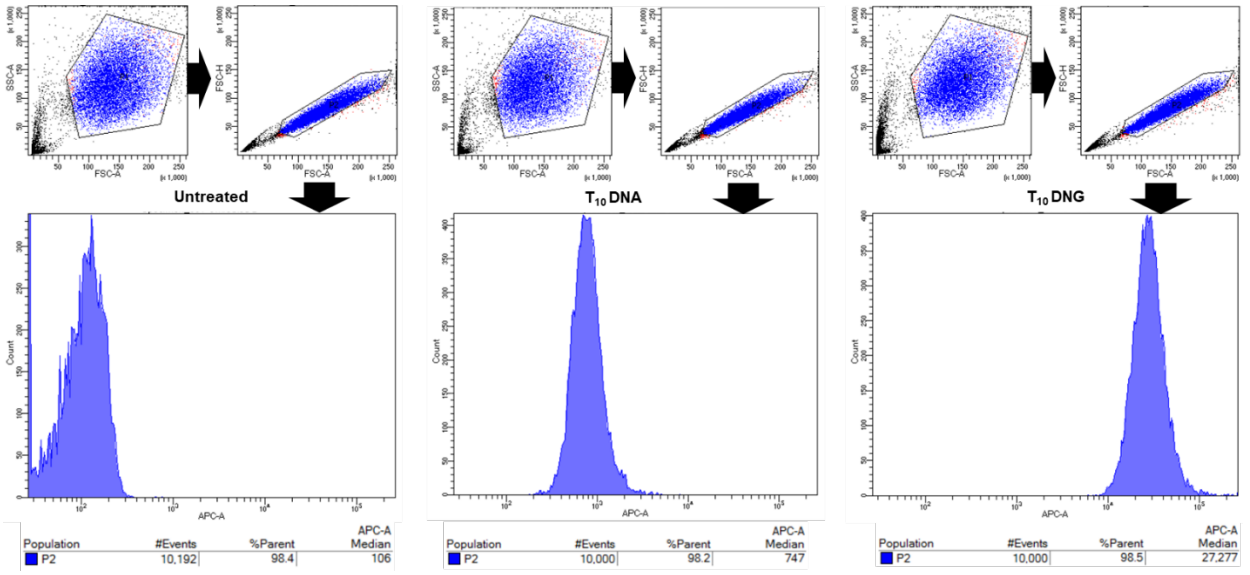


Figure S12. Gating of fixed C166 cells using forward and side scattering (top two dot plots) to identify cells and remove doublets, and fluorescence intensity in the APC channel (bottom histogram) for untreated, DNA, and DNG samples correlating with extent of cellular uptake.

Uptake in live cells by flow cytometry

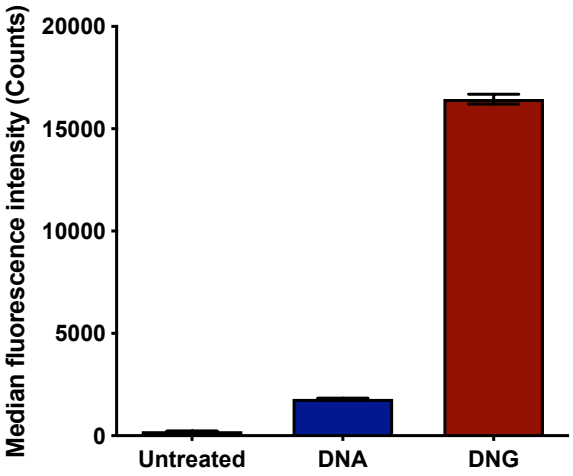


Figure S13. Uptake of AlexaFluor 647-labeled DNA and DNG strands into C166 cells as measured by flow cytometry on live cells.

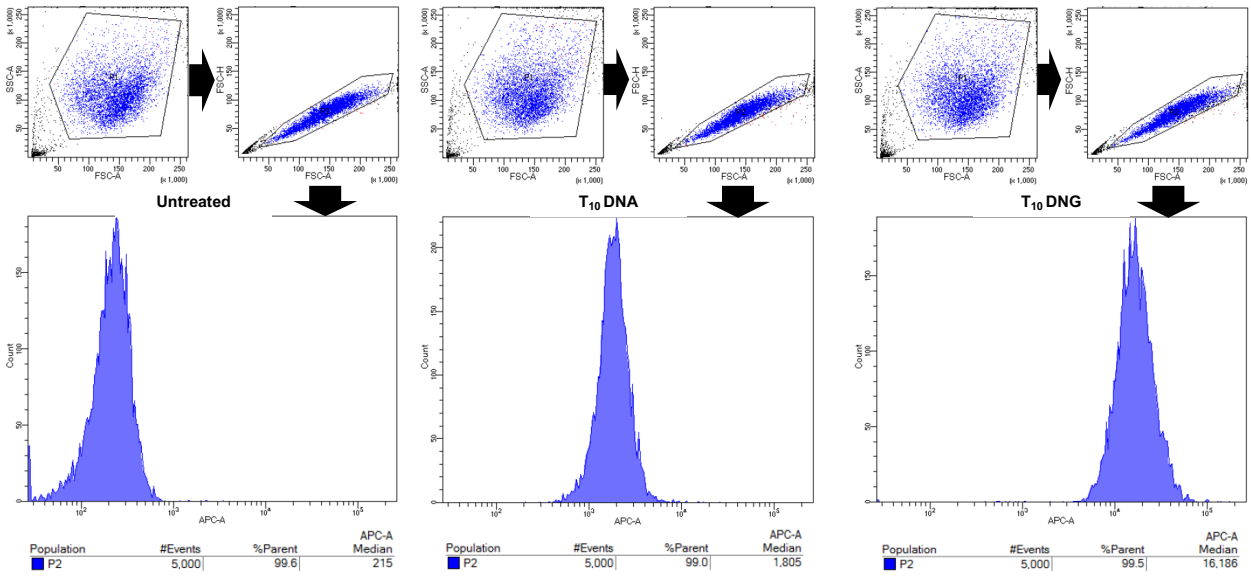
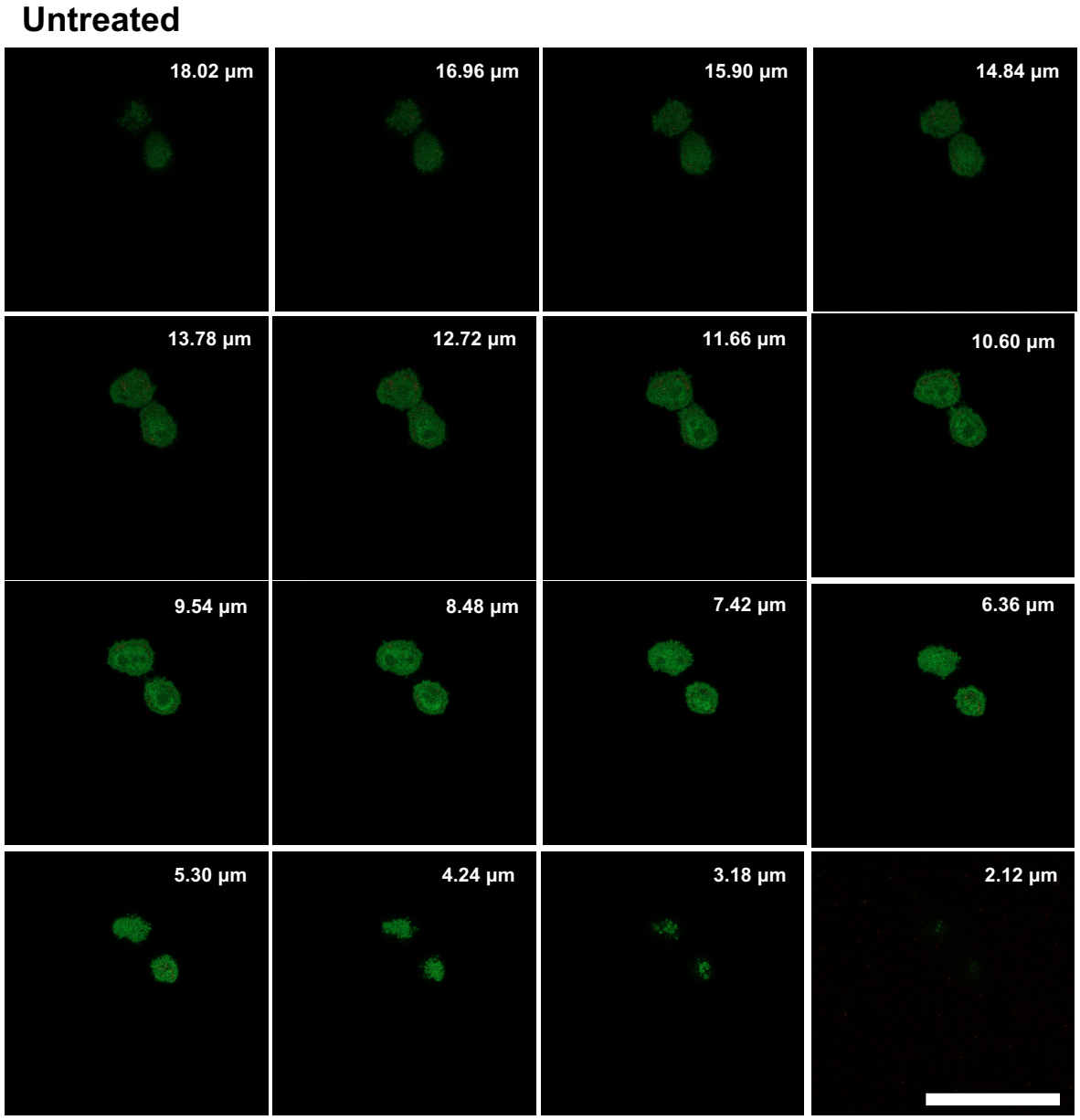
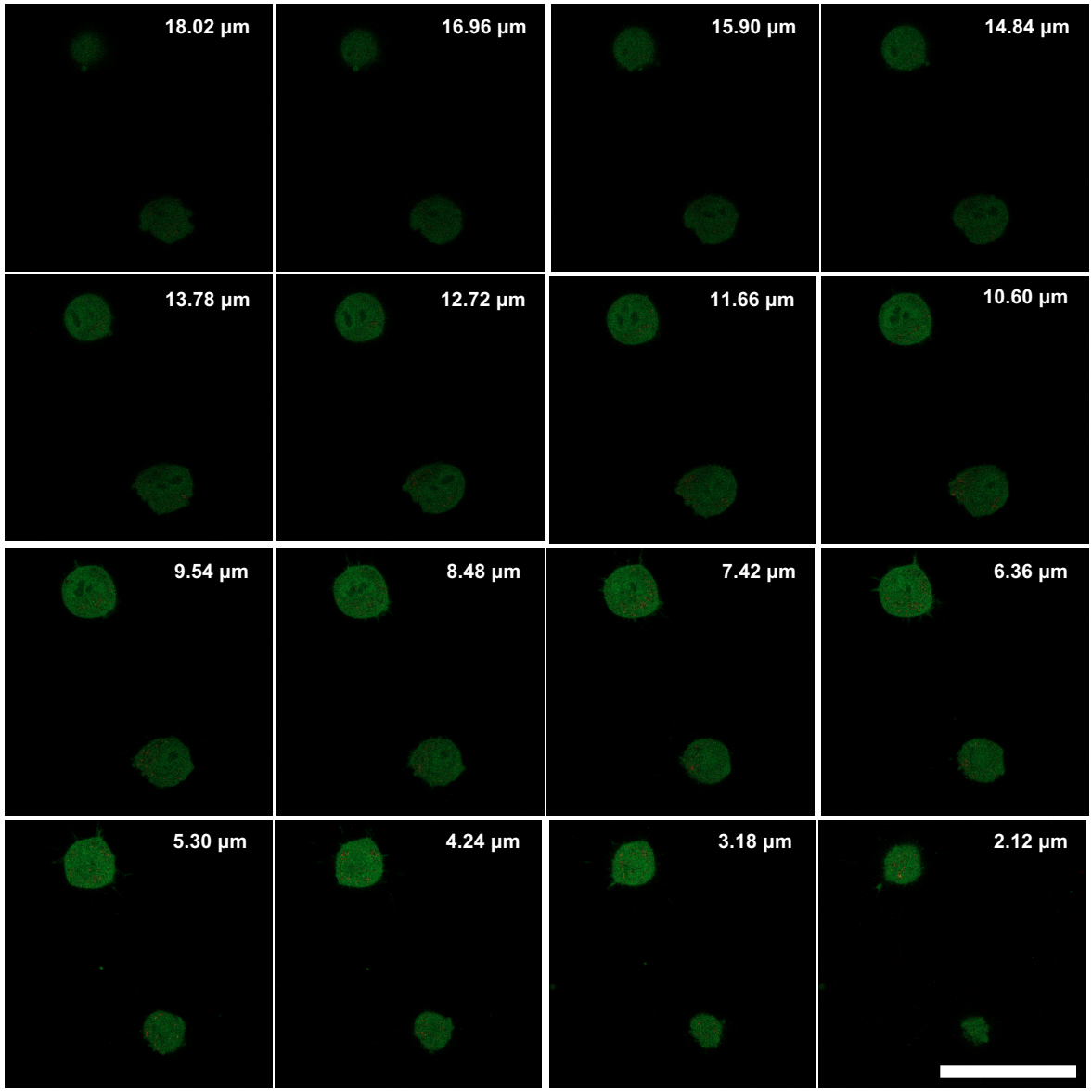


Figure S14. Gating of live C166 cells using forward and side scattering (top two dot plots) to identify cells and remove doublets, and fluorescence intensity in the APC channel (bottom histogram) for untreated, DNA and DNG samples correlating with extent of cellular uptake.

Cell internalization by confocal microscopy in live cells (Z-stacks)



T_{10} DNA


18.02 µm
16.96 µm
15.90 µm
14.84 µm
13.78 µm
12.72 µm
11.66 µm
10.60 µm
9.54 µm
8.48 µm
7.42 µm
6.36 µm
5.30 µm
4.24 µm
3.18 µm
2.12 µm

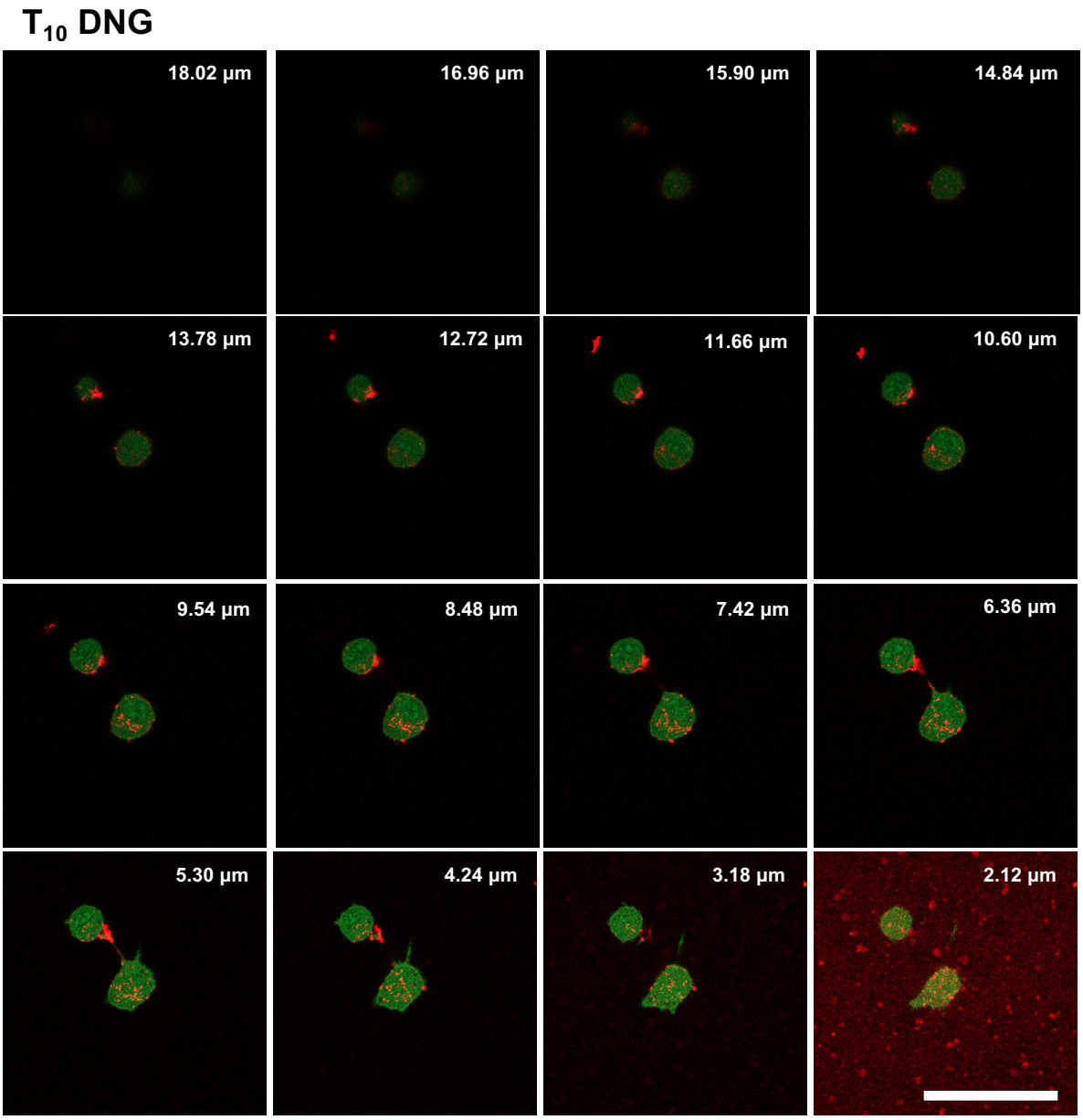


Figure S15. Confocal microscopy images of GFP-expressing C166 cells incubated with either T_{10} DNA or DNG across the z-axis (1 µm slices). DNG strand adhesion to the glass plate bottom is evident on the last image of the stack. Scale bars represent 50 µm. Height of stack is indicated on each image.

Brightfield images of cells treated with DNGs

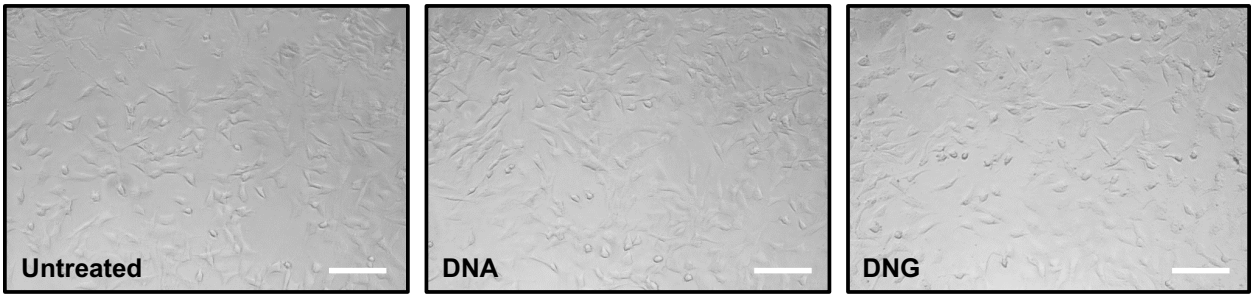


Figure S16. Brightfield images of C166 cells incubated with 5 uM of either T_{10} DNA or DNG showing no significant changes in morphology or density at the 6 h time point. Scale bars represent 50 μm.

References

1. Challa, H.; Bruice, T. C., Deoxynucleic guanidine: synthesis and incorporation of purine nucleosides into positively charged DNG oligonucleotides. *Biorg. Med. Chem.* **2004,** *12* (6), 1475-1481.
2. Lavandera, I.; Fernández, S.; Ferrero, M.; Gotor, V., First regioselective enzymatic acylation of amino groups applied to pyrimidine 3′,5′-diaminonucleoside derivatives. Improved synthesis of pyrimidine 3′,5′-diamino-2′,3′,5′-trideoxynucleosides. *J. Org. Chem.* **2001,** *66* (11), 4079-4082.
3. Krotz, A. H.; Rentel, C.; Gorman, D.; Olsen, P.; Gaus, H. J.; McArdle, J. V.; Scozzari, A. N., Solution stability and degradation pathway of deoxyribonucleoside phosphoramidites in acetonitrile. *Nucleosides Nucleotides & Nucleic Acids* **2004,** *23* (5), 767-775.
4. Shechter, D.; Dormann, H. L.; Allis, C. D.; Hake, S. B., Extraction, purification and analysis of histones. *Nat. Protoc.* **2007,** *2* (6), 1445-57.
5. Dolomanov, O. V.; Bourhis, L. J.; Gildea, R. J.; Howard, J. A. K.; Puschmann, H., OLEX2: a complete structure solution, refinement and analysis program. *J. Appl. Cryst.* **2009,** *42*, 339-341.
6. Sheldrick, G. M., Crystal structure refinement with SHELXL. *Acta Cryst. C* **2015,** *71*, 3-8.
7. Sheldrick, G. M., A short history of SHELX. *Acta Cryst. A* **2008,** *64*, 112-122.
8. Van Bolhuis, F.; Koster, P. B.; Migchelsen, T., Refinement of Crystal Structure of Iodine at 110°K. *Acta Cryst.* **1967,** *23*, 90-91.
9. Makhotkina, O.; Lieffrig, J.; Jeannin, O.; Fourmigué, M.; Aubert, E.; Espinosa, E., Cocrystal or Salt: Solid State-Controlled Iodine Shift in Crystalline Halogen-Bonded Systems. *Cryst. Growth Des.* **2015,** *15* (7), 3464-3473.