Supporting Information

Synthetic Biology Parts for the Storage of Increased Genetic Information in Cells

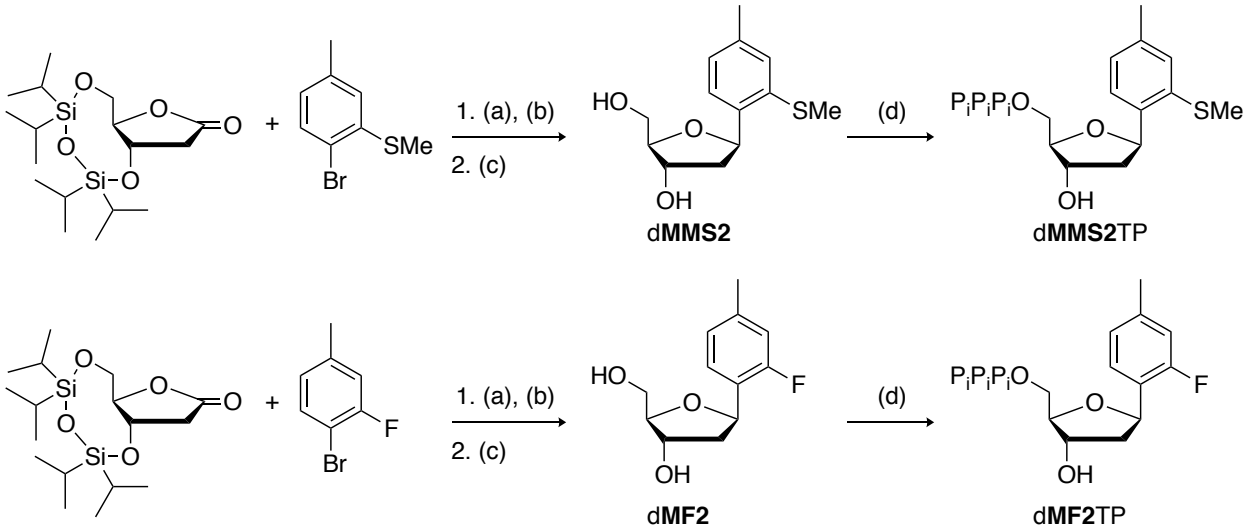
Sydney E. Morris, Aaron W. Feldman, and Floyd E. Romesberg
Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, United States

Supporting Methods

Nucleoside Triphosphate Synthesis

For synthetic procedures all reactions were carried out in oven-dried glassware under an inert atmosphere. Solvents were distilled and/or dried over 4 Å molecular sieves. Unless otherwise noted, all other chemical reagents were used without further purification. ^{1}H, ^{13}C, and ^{31}P spectra were taken on a Bruker NMR spectrometer (DRX-500 or DPX-400). The ^{1}H and ^{13}C chemical shifts are referenced to TMS, and the ^{31}P chemical shifts are referenced relative to 85% phosphoric acid in D_2O. Mass spectroscopic data were obtained from the core facilities at The Scripps Research Institute.

General Procedure For Nucleoside Triphosphate Synthesis



Scheme S1. (a) *n*-BuLi, THF, -78 °C; (b) Et_3SiH, $BF_3{\cdot}Et_2O$, THF, 3 h; (c) TBAF, THF, 1 h; (d) proton sponge, $POCl_3$, $(MeO)_3PO$, -15 °C, 3 h, then Bu_3N, $Bu_3N{\cdot}H_4P_2O_7$, DMF, -15 °C – 0 °C, 30 m, then 0.5 M aq. TEAB, rt, 15 m.

Synthesis of Nucleosides dMMS2 and dMF2

Under nitrogen, aryl bromide (1.0 mmol) was dissolved in dry THF (10 mL) and cooled to -78 °C. A solution of *n*-butyl lithium (1.6 M in THF, 0.67 mL) was added dropwise, and the reaction was stirred at -78 °C for 30 min. Disiloxane-protected 2-deoxyribonolactone (0.63 mmol) was dissolved in dry THF (5 mL) and added dropwise to the reaction. The resulting mixture was stirred at -78 °C for an additional hour before quenching with saturated NH_4Cl. THF was removed *in vacuo*, and the resulting residue was extracted with EtOAc and H_2O. The organic layer was dried over Na_2SO_4 and concentrated. Upon thorough concentration on high vacuum, the crude product was dissolved in dry DCM (10 mL) and cooled to -78 °C. Triethylsilane (1.88

mmol) and boron trifluoride diethyl etherate (48% in Et_2O, 1.88 mmol) were added dropwise, and the mixture was stirred at -78 °C for 3 h. The reaction was quenched with saturated $NaHCO_3$, warmed to room temperature, and extracted with DCM and H_2O. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The resulting crude product was purified by silica gel flash column chromatography (0 – 5% EtOAc/hexanes). The purified product was dissolved in THF (2 mL) and stirred with tetra-*n*-butylammonium fluoride (1.0 M in THF, 1.5 mL) at room temperature for 1 h. Volatiles were removed *in vacuo* and the crude residue was purified by silica gel flash column chromatography (0 – 5% MeOH/DCM), providing the desired nucleoside.

Synthesis of Nucleoside Triphosphates dMMS2TP and dMF2TP

Under nitrogen, proton sponge (0.13 mmol) and free nucleoside (0.1 mmol) were dissolved in dry trimethyl phosphate (4.0 mmol) and cooled to -15 °C in a salt ice bath. Freshly distilled phosphorous oxychloride (0.13 mmol) was added dropwise, and the reaction was stirred at -15 °C for 3 h. Dry tributylamine (0.6 mmol) and a solution of tributylammonium pyrophosphate (0.5 mmol) in dry DMF (1.0 mL) were added dropwise to the reaction, and the resulting mixture was warmed to 0 °C over 30 min. The reaction was then quenched by addition of aqueous triethylammonium bicarbonate (0.5 M TEAB, pH 7.5, 1.5 mL), and stirred at room temperature for 15 min. The crude reaction mixture was purified by anion exchange chromatography (DEAE Sephadex A-25) with an elution gradient of 0 to 1.2 M TEAB. The concentrated product was further purified by reverse-phase (C18) HPLC (0 – 35% CH_3CN in 0.1 M TEAB, pH 7.5) and concentrated by SpeedVac, affording the pure ß anomer of the desired nucleoside triphosphate.

dMMS2. Prepared from 4-bromo-3-(methylthio)toluene using the general synthesis of nucleosides procedure described above (21% yield, 3 steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (d, *J* = 7.8 Hz, 1H), 7.06 (d, *J* = 1.7 Hz, 1H), 7.00 – 6.97 (m, 1H), 5.49 (dd, *J* = 9.9, 5.9 Hz, 1H), 4.42 (ddd, *J* = 6.2, 3.4, 2.4 Hz, 1H), 4.03 – 3.98 (m, 1H), 3.86 (dd, *J* = 11.7, 4.2 Hz, 1H), 3.79 (dd, *J* = 11.6, 5.0 Hz, 1H), 2.46 (s, 3H), 2.37 (ddd, *J* = 13.3, 5.9, 2.3 Hz, 1H), 2.33 (s, 3H), 1.95 (ddd, *J* = 13.3, 9.9, 6.6 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 137.85, 136.60, 135.58, 127.31, 126.40, 125.33, 86.80, 77.31, 73.71, 63.38, 50.83, 42.70, 21.18, 16.58.
HRMS (ESI-TOF$^+$ high-acc) calcd for $C_{13}H_{18}O_3S$ $[MH]^+$ 255.1049; found 255.1050.

dMF2. Prepared from 4-bromo-3-fluorotoluene using the general synthesis of nucleosides procedure described above (25% yield, 3 steps).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (t, *J* = 7.8 Hz, 1H), 6.96 (dd, *J* = 7.9, 1.5 Hz, 1H), 6.88 (dd, *J* = 11.7, 1.6 Hz, 1H), 5.36 (dd, *J* = 10.2, 5.8 Hz, 1H), 4.48 (dt, *J* = 6.4, 2.5 Hz, 1H), 4.04 (td, *J* = 4.3, 3.1 Hz, 1H), 3.86 (dd, *J* = 11.7, 4.0 Hz, 1H), 3.77 (dd, *J* = 11.7, 4.6 Hz, 1H), 2.36 (s, 3H), 2.32 (ddt, *J* = 13.4, 5.9, 1.7 Hz, 1H), 2.11 (ddd, *J* = 13.4, 10.2, 6.4 Hz, 1H), 1.28 (s, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 160.50, 158.87, 139.37, 126.77, 124.41, 115.56, 86.57, 73.36, 62.88, 42.34, 29.24, 20.55.
HRMS (ESI-TOF$^+$ high-acc) calcd for $C_{12}H_{15}FO_3$ $[MNa]^+$ 249.0897; found 249.0896.

dMMS2TP. Prepared from d**MMS2** using the general synthesis of nucleoside triphosphates procedure described above (21% yield).

^{31}P NMR (162 MHz, D_2O) δ -6.00 (d, J = 21.0 Hz), -10.62 (d, J = 19.7 Hz), -22.17 (t, J = 20.3 Hz).
MS (MALDI-TOF$^-$, matrix: 9-aminoacridine) (m/z): [M-H]- calcd for $C_{13}H_{20}O_{12}P_3S^-$, 493.0; found, 493.2.

dMF2TP. Prepared from d**MF2** using the general synthesis of nucleoside triphosphates procedure described above (15% yield).

^{31}P NMR (162 MHz, D_2O) δ -5.93 (d, J = 18.5 Hz), -10.57 (d, J = 17.1 Hz), -22.18 (t, J = 17.8 Hz).
MS (MALDI-TOF$^-$, matrix: 9-aminoacridine) (m/z): [M-H]- calcd for $C_{12}H_{17}FO_{12}P_3^-$, 465.0; found, 465.4.

Steady-State Kinetics Assay
Steady-state kinetics reactions were performed as described previously (see ref. 21 of main text) with the primer and template oligonucleotides shown in Tables S1 and S2 obtained from IDT (natural templates and primers) or gifted from Synthorx (La Jolla, CA) (d**NaM** and d**TPT3** containing templates). Briefly, primers were 5'-radiolabeled with [γ-^{32}P]-ATP (PerkinElmer) and T4 polynucleotide kinase (10,000 U/mL, New England BioLabs) by incubating at 37 °C for 1 h and then heating at 65 °C for 30 min. Unincorporated [γ-^{32}P]-ATP was removed by spin column purification (Qiagen QIAquick Nucleotide Removal Kit), and labeled primers were eluted in 5-fold diluted elution buffer. Primers were annealed to template oligonucleotides in 10× NEBuffer 2.1 reaction buffer (New England BioLabs) by heating to 95 °C for 5 min and slowly cooling to room temperature. Klenow fragment (Kf) of *E. coli* DNA Pol I (5000 U/mL, New England BioLabs) was freshly diluted in 10× NEBuffer 2.1 and added to cooled primer-template. Kinetic reactions were initiated at 37 °C by combining Kf-primer-template with triphosphate at various concentrations, and reactions were stopped with quenching/gel loading buffer (95% formamide, 20 mM EDTA, bromophenol blue, xylene cyanol). Reaction products were analyzed by denaturing polyacrylamide gel electrophoresis (15%, 8M urea, 80 W, 3.5 h) followed by overnight exposure to a phosphor screen (Kodak) and imaging (Typhoon 9410, GE Amersham Biosciences). Image Studio Lite 5.2.5 software was used to quantify primer and extended primer gel band intensities and determine the fraction of extended primer or velocity at each triphosphate concentration. GraphPad Prism 7 was used to plot measured velocities versus the eight triphosphate concentrations examined and fit the Michaelis-Menten equation to determine k_{cat} and K_M; values reported are the averages and standard deviations of three, independent experiments.

Table S1. Oligonucleotides used in Table 2 of this study. Primer nucleotides are underlined.

Sequence Context I

5′-GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAA-d**TPT3**-TGTGAGCGGATAACAATTTCACACAGGAAACAG-3′

5′-GGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAA-T-TGTGAGCGGATAACAATTTCACACAGGAAACAG-3′

Sequence Context II

5′-GATTCCATTCTTTTGTTTGTCTGCCGTGATCT-d**TPT3**-TACATTGTGTGAGTTAAAGTTGTACTCGAG-3′

5′-GATTCCATTCTTTTGTTTGTCTGCCGTGATCT-T-TACATTGTGTGAGTTAAAGTTGTACTCGAG-3′

Sequence Context III

5′-GATTCCATTCTTTTGTTTGTCTGCCGTGATC-d**TPT3**-GTACATTGTGTGAGTTAAAGTTGTACTCGAG-3′

5′-GATTCCATTCTTTTGTTTGTCTGCCGTGATC-T-GTACATTGTGTGAGTTAAAGTTGTACTCGAG-3′

Table S2. Oligonucleotides used in Table 1 of this study. Primer nucleotides are underlined.

Sequence Context I

5′-CTGTTTCCTGTGTGAAATTGTTATCCGCTCACA-d**NaM**-TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC-3′

5′-CTGTTTCCTGTGTGAAATTGTTATCCGCTCACA-T-TTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC-3′

Sequence Context II

5′-CTCGAGTACAACTTTAACTCACACAATGTA-d**NaM**-AGATCACGGCAGACAAACAAAAGAATGGAATC-3′

5′-CTCGAGTACAACTTTAACTCACACAATGTA-T-AGATCACGGCAGACAAACAAAAGAATGGAATC-3′

Sequence Context III

5′-CTCGAGTACAACTTTAACTCACACAATGTAC-d**NaM**-GATCACGGCAGACAAACAAAAGAATGGAATC-3′

5′-CTCGAGTACAACTTTAACTCACACAATGTAC-T-GATCACGGCAGACAAACAAAAGAATGGAATC-3′

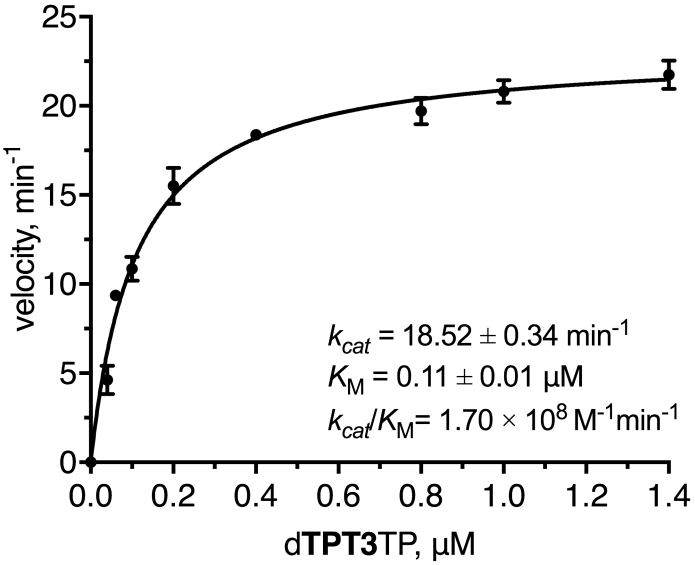


Figure S1. Representative Michaelis-Menten plot for d**TPT3**TP insertion against d**NaM** in sequence context III.

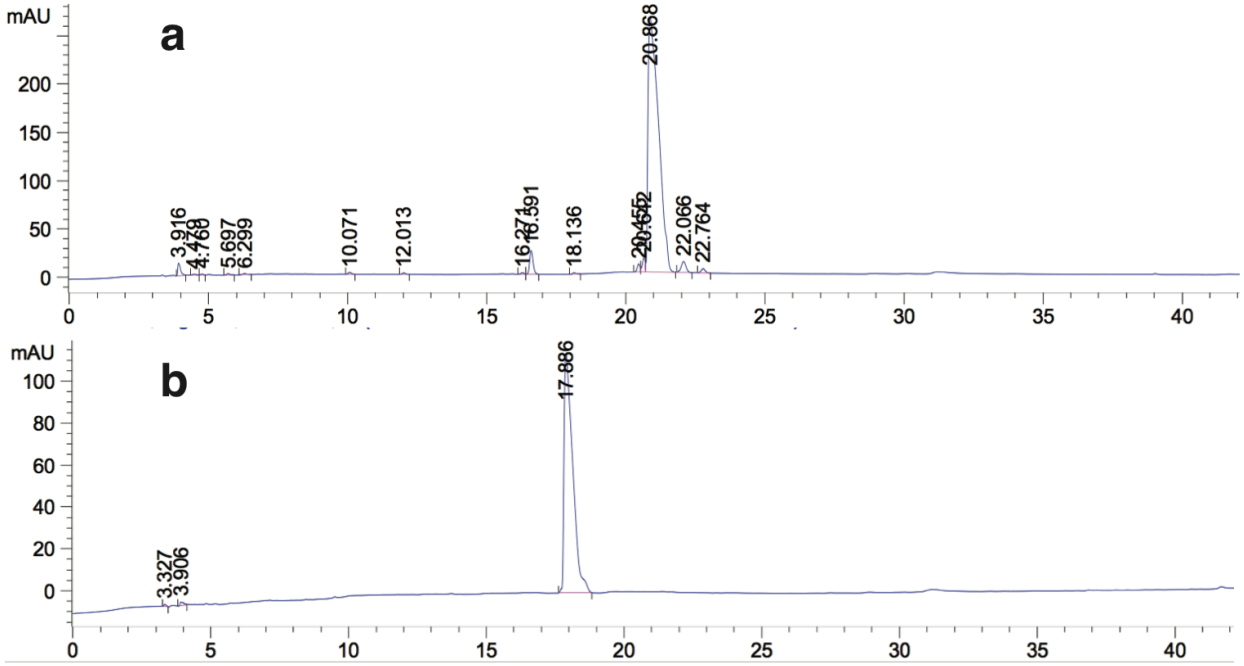


Figure S2. Representative HPLC traces of d**MMS2**TP (a) and d**MF2**TP (b) indicating purity of >90%.