Electronic Supplementary Material (ESI) for Chemical Science

ESI for 'Chemical fidelity of an RNA polymerase ribozyme'

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Materials and Methods

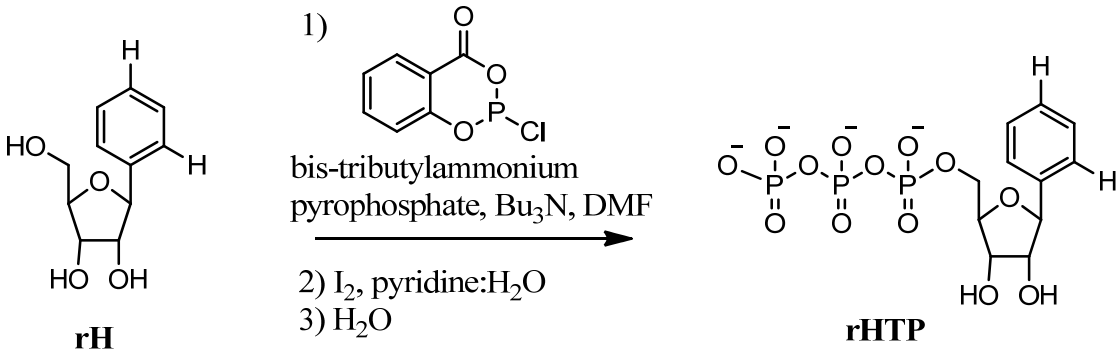
Nucleotides and buffers

Ribonucleoside triphosphates were obtained as sodium salts from Sigma (Sigma-Aldrich Co., Missouri, USA) and deoxyribonucleoside triphosphates from GE Illumina series (GE Life Sciences UK Ltd, UK). 2' -F, -NH_2, -OCH_3, and -N_3 ribonucleoside triphosphates were obtained from Trilink Biotechnologies (San Diego, California, USA), along with araU, araC and araA triphosphates and the ribonucleoside triphosphates of 2-thio-U, 4-thio-U, 5-iodo-U, thymine, ψ, D, and 5,6-DHU. Isoguanosine triphosphate was obtained from Chemgenes (Chemgenes Co., Massachusetts, USA). F-araU and F-araG triphosphates were obtained from Metkinen Chemistry (Kuusisto, Finland). Syntheses of homoTTP, glucoTTP, hexTTP, hexGTP, CeTTP, CeGTP, locked-TTP, locked-GTP, araGTP, PaTP, yTP, d-xyloTTP, unlocked-UTP and unlocked-GTP were performed according to established protocols[30, 54, 63-69].

The sea salt mixture was obtained from Sigma, and dissolved at 3.8 g/dm^3 to yield the unsupplemented brine. The mineral water derives from the Adobe springs (California, USA), and is non-carbonated with a pH of 8.65 with principal components 4.5 mM Mg^{2+}, 0.2 mM Ca^{2+}, 0.4 mM Na^+, 0.24 mM SO_4^{2-}, 0.13 mM Cl^-, and 7.2 mM bicarbonate (Noah's Water, California, USA).

Synthesis of ribonucleoside isostere triphosphates

The ribonucleosides **rH**, **rF**, **rL**, and **rB** were prepared as described previously[70].

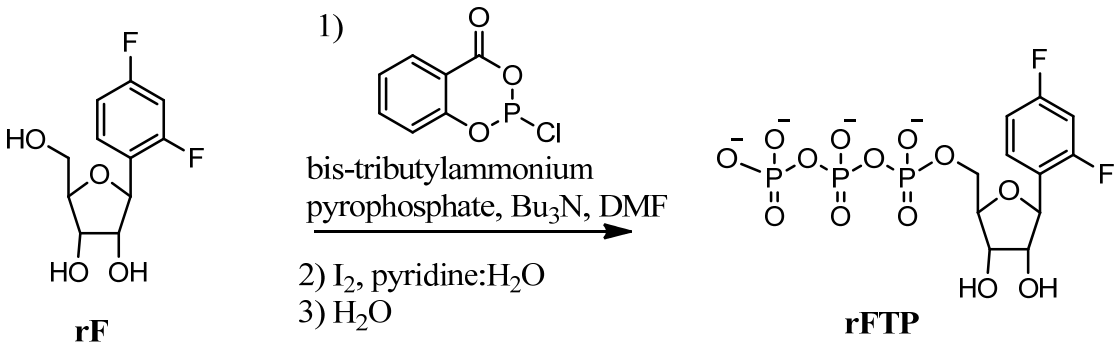


((2R,3S,4R,5S)-3,4-dihydroxy-5-phenyltetrahydrofuran-2-yl)methyl triphosphate (**rHTP**)

This reaction was performed as described previously[71]. Bis-tributylammonium pyrophosphate (0.16 mmol) was placed in a flask under Ar and treated with DMF (0.25 ml) and Bu_3N (0.3 ml). A solution of 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-on (0.16 mmol in 0.25 ml DMF) was added and the mixture was stirred for 30 min. This mixture was then added to an Ar-purged flask containing **rH** (0.08 mmol, 37 mg), and stirred for 90 min. A solution of I_2 (0.02 M in 9:1 pyridine:water) was added until a brown color persisted (about 1 ml). The mixture was stirred for 15 min followed by addition of water (2 ml), and followed by stirring for 90 min. This mixture was treated with 0.9 ml of a 3M aqueous solution of NaCl. EtOH (15 ml) was added and the mixture was transferred to a centrifuge tube and cooled at -80°C for 1 hour. The precipitate was collected by centrifugation and the supernatant was decanted. The solid was taken up in TEAA (20mM, pH 7.1) and purified by HPLC. HPLC conditions: Phenomenex Synergi semi-prep C18 column, 10 X 250mm, 5 ml/min flow rate, 5 to 35% MeCN in 20mM TEAA (pH 7.1) over 40 min. The product elutes at 7 min. The

product-containing fraction was lyophilized, dissolved in MeOH (0.4 ml), then treated with 0.15M $NaClO_4$ in acetone (1.2 ml). The precipitate was collected by centrifugation and the liquid decanted. The precipitate was washed with 2ml acetone and collected by centrifugation a total of 3 times. The solid was dried and Tris-HCl (5mM, pH 7.5) was added to make a 20 mM solution of **rHTP**, sodium salt, which was stored at -80°C. Yield: 1.5 µmol (2%). Yield was determined using the experimentally determined molar absorbtivity constant for **rH**: 156 M^{-1} cm^{-1}.

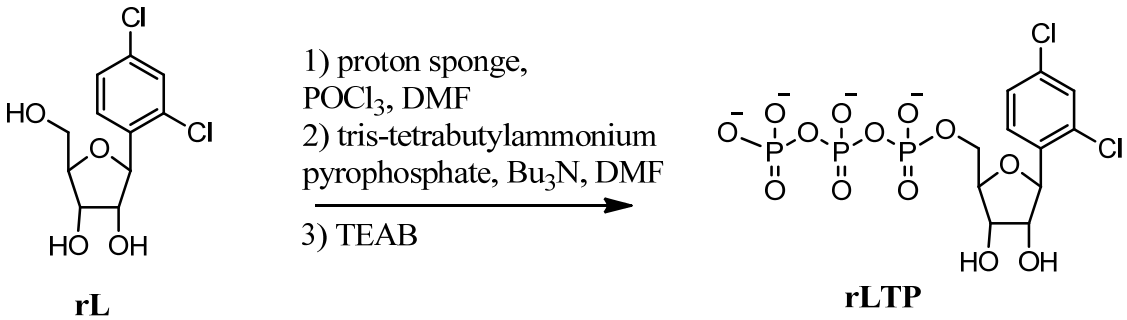
^{1}H NMR (D_2O, ppm) 7.36 (d, 2H, J=7.3 Hz), 7.30 (t, 2H, J=7.0 Hz), 7.27 (m, 1H), 4.62 (obscured by H_2O peak), 4.23 (m, 1H), 4.09 (m, 1H), 4.05 (m, 3H). ^{31}P NMR (D_2O, ppm): -9.92 (d), -10.27 (d), -22.32 (t). MS (ESI-) $[M-H]^-$ mass calculated: 449.0 mass found: 449.3.



((2R,3S,4R,5S)-5-(2,4-difluorophenyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl triphosphate (**rFTP**)

Synthesis of **rFTP** followed the same conditions as synthesis of **rHTP**. The crude product was purified by HPLC. HPLC conditions: Phenomenex Synergi semi-prep C18 column, 10 X 250mm, 5 ml/min flow rate, 5 to 35% MeCN in 20mM TEAA (pH 7.1) over 40 minutes. The product elutes at around 11 min. The product-containing fraction was lyophilized, dissolved in MeOH (0.4 ml), and treated with 0.15M $NaClO_4$ in acetone (1.2 ml). The precipitate was collected by centrifugation and the liquid decanted. The precipitate was washed with 2ml acetone and collected by centrifugation a total of 3 times. The solid was dried and Tris-HCl (5mM, pH 7.5) was added to make a 20 mM solution of **rFTP**, sodium salt, which was stored at -80°C. Yield: 2.0 µmol (3%). Yield was determined using the experimentally determined molar absorbtivity constant for **rF**: 844 M^{-1} cm^{-1}.

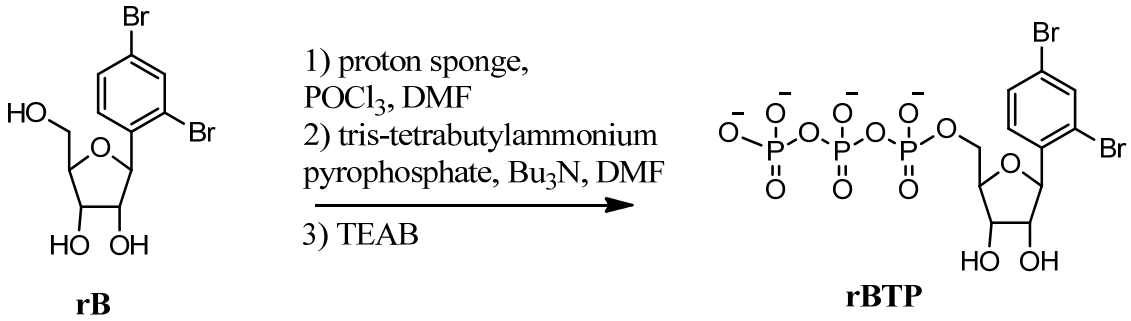
^{1}H NMR (D_2O, ppm) 7.28 (m, 1H), 6.88 (m, 2H), 5.03 (d, 1H, J=7.5 Hz), 4.44 (m, 1H), 4.21 (m, 1H), 4.02 (m, 3H). ^{31}P NMR (D_2O, ppm) -9.75 (d), -10.27 (d), -22.29 (t). MS (ESI-) $[M-H]^-$ mass calculated: 485.0, mass found: 485.2.



((2R,3S,4R,5S)-5-(2,4-dichlorophenyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl triphosphate (**rLTP**)

To an Ar-purged round bottom flask was added proton sponge (24 mg), **rL** (21 mg, 0.075 mmol) and $PO(OMe)_3$ (0.2 ml). This was taken to 0°C and treated with $POCl_3$ (10 µl) and stirred for 2 hours at 0°C. The mixture was treated with a solution of tris-tetrabutylamine pyrophosphate (0.375 mmol, 338 mg, in 0.11 ml Bu_3N and 1ml DMF). This was stirred for 20 min and treated with 8 ml of TEAB (0.5 M, pH 8.5). The mixture was stirred for 5 min, frozen, and dried by lyophilization. The product was purified by HPLC. HPLC conditions: Phenomenex Synergi semi-prep C18 column, 10 X 250mm, 7.5 ml/min flow rate, 5 to 35% MeCN in 50mM TEAA (pH 7.5) over 40 min. The product elutes as a broad peak at around 18 min. The product-containing fraction was lyophilized, dissolved in MeOH (0.4 ml), then treated with 0.15M $NaClO_4$ in acetone (1.2 ml). The precipitate was collected by centrifugation and the liquid decanted. The precipitate was washed with 2ml acetone and collected by centrifugation a total of 3 times. The solid was dried and Tris-HCl (5mM, pH 7.5) was added to make a 20 mM solution of **rLTP**, sodium salt, which was stored at -80°C. Yield: 8.8 µmol (12%). Yield was determined using the experimentally determined molar absorbtivity constant for **rL**: 207 M^{-1} cm^{-1}.

^{1}H NMR (D_2O, ppm) 7.55 (dd, 1H, J=8.4 Hz), 7.38 (s, 1H), 7.29 (dd, 1H, J=8.5 Hz), 5.15 (d, 1H, J=5.1 Hz), 4.21 (t, 1H, J=5.2 Hz), 4.10 (m, 4H). ^{31}P NMR (D_2O, ppm) -9.82 (d), -10.21 (d), -22.34 (t). MS (ESI-) $[M-H]^-$ mass calculated: 516.9, mass found: 517.2

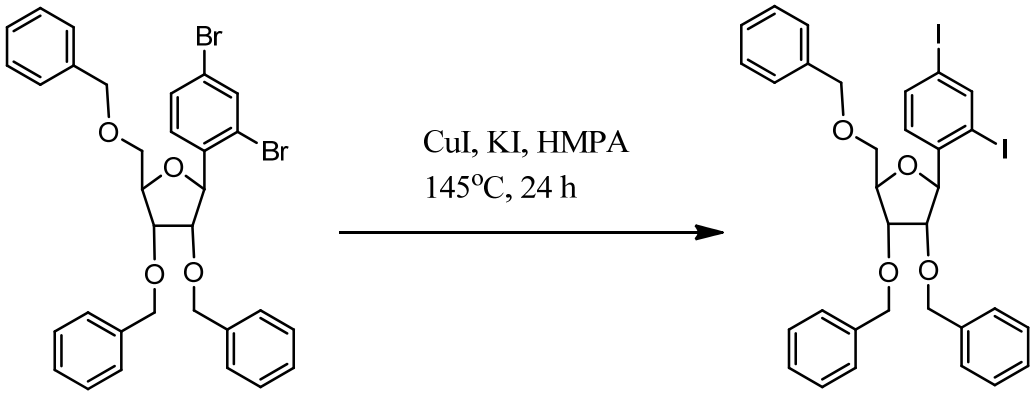


rB **rBTP**

((2R,3S,4R,5S)-5-(2,4-dibromophenyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl triphosphate (**rBTP**)

Synthesis of **rBTP** followed the same conditions as synthesis of **rLTP**. The crude product was purified by HPLC. HPLC conditions: Phenomenex Synergi semi-prep C18 column, 10 X 250mm, 5 ml/min flow rate, 5 to 35% MeCN in 100mM TEAA (pH 7.1) over 40 min. The product elutes as a broad peak at around 20 min. The product-containing fraction was lyophilized, dissolved in MeOH (0.4 ml), then treated with 0.15M $NaClO_4$ in acetone (1.2 ml). The precipitate was collected by centrifugation and the liquid decanted. The precipitate was washed with 2 ml acetone and collected by centrifugation a total of 3 times. The solid was dried and Tris-HCl (5mM, pH 7.5) was added to make a 20 mM solution of **rBTP**, sodium salt, which was stored at -80°C. Yield: 2.0 µmol (4%) of **rBTP**. Yield was determined using the experimentally determined molar absorbtivity constant for **rB**: 292 M^{-1} cm^{-1}.

^{1}H NMR (D_2O, ppm) 7.71 (s, 1H), 7.48 (m, 2H), 5.10 (d, 1H, J=5.1 Hz), 4.13 (t, 1H, J=5.03 Hz), 4.08 (m, 4H). ^{31}P NMR (D_2O, ppm) -9.95 (d), -10.28 (d), -22.33 (t). MS (ESI-) $[M-H]^-$ mass calculated: 607.0, mass found: 607.2

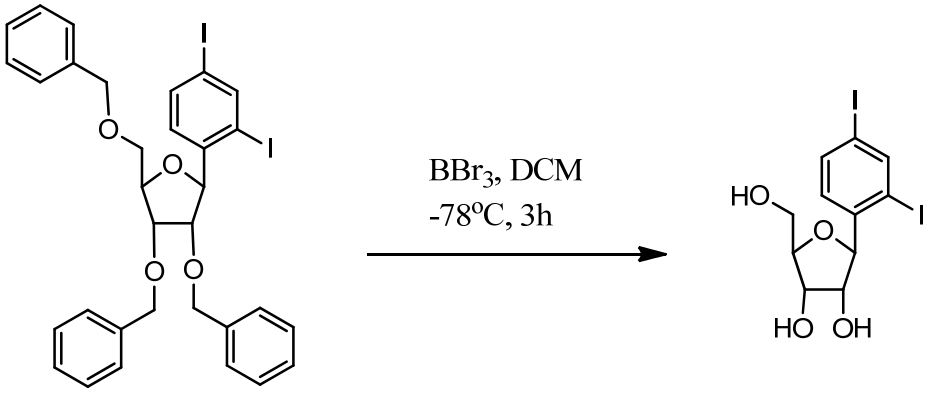


rB-3Bz **rI-3Bz**

(2R,3R,4S,5S)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-5-(2,4-diiodophenyl)tetrahydrofuran (**rB-3Bz**)

rB-3Bz (0.20g, 0.31 mmol), CuI (0.118g, 0.62 mmol), and KI (0.175g, 1.05 mmol) were placed in a sealed tube along with 0.35 ml HMPA and heated at 145°C for 24 hours, under Ar, with gentle stirring. The reaction was quenched with sat. aq. NH_4Cl (20 ml) and extracted with ether (3 x 20 ml). The combined organic fractions were washed with brine, dried (Na_2SO_4) and concentrated. Purification by column chromatography (0 to 5% EtOAc in hexane as eluent) gave the product, which was then further purified by recrystallization in MeOH. Yield: 103 mg.

^{1}H NMR ($CDCl_3$, ppm) 8.12 (s, 1H), 7.39 (s, 2H), 7.71-7.24 (m, 20H, overlaps with CDCl3 peak), 5.19 (d, 1H, 3.6 Hz), 4.71 (m, 2H), 4.63 (m, 2H), 4.45 (m, 2H), 4.31 (m, 1H), 4.07 (m, 1H), 3.85 (m, 2H), 3.65 (m, 1H). ^{13}C NMR ($CDCl_3$, ppm) 146.79, 142.71, 138.32, 138.25, 138.09, 137.63, 130.21, 128.64, 128.53, 128.12, 128.06, 128.02, 127.95, 127.90, 127.85, 98.47, 94.08, 87.23, 83.22, 80.90, 73.65, 73.31, 72.74, 69.37. MS (ESI+) $[M+Na]^+$ Mass calculated: 755.01 Found: 755.03. HRMS (ESI+) $[M+Na]^+$ Mass calculated for $[C_{32}H_{20}I_2NaO_4]^+$: 755.0126; Found: 755.0128.



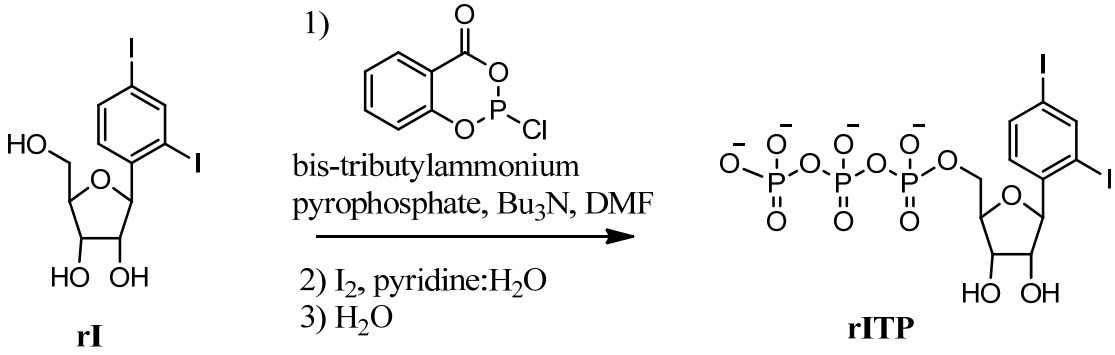
rI-3Bz **rI**

(2S,3R,4S,5R)-2-(2,4-diiodophenyl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol (**rI**)

rI-3Bz (0.25 g, 0.34 mmol) was placed in a round bottom flask, under Ar, along with DCM (3 ml), and cooled to -78°C. The mixture was treated with BBr_3 (1.36 ml of a 1M solution in DCM), and stirred for 3 hours at -78°C. The reaction was quenched with 20 ml of 1:1 MeOH:DCM and stirred at RT for 12h. The residue was concentrated and purified by column chromatography using a gradient of 0 to 10%

MeOH in DCM. The product-containing fractions were identified by TLC (CAN stain), pooled and concentrated. The product was recrystallized from CH_2Cl_2, giving 86 mg yield.

^{1}H NMR (MeOD, ppm) 8.17 (s, 1H), 7.68 (d, 1H, J = 8.4 Hz), 7.40 (d, 1H, J = 8.5 Hz), 4.98 (d, 1H, J = 4.0 Hz), 3.96 (m, 2H), 3.89 (m, 2H), 3.74 (m, 1H). ^{13}C NMR (MeOD, ppm) 146.69, 142.98, 137.34, 129.55, 98.08, 93.16, 87.88, 83.82, 77.72, 70.55, 61.66. MS (ESI+) $[M+Na]^+$ mass calculated: 484.87 mass found:484.87. HRMS (ESI+) Mass calculated for $[C_{11}H_{12}I_2NaO_4]^+$: 484.8717; Found: 484.8712



((2R,3S,4R,5S)-5-(2,4-diiodophenyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl triphosphate (**rITP**)

Synthesis of **rITP** followed the conditions for synthesis of **rHTP** exactly. The crude product was purified by HPLC. HPLC conditions: Phenomenex Synergi semi-prep C18 column, 10 X 250mm, 5 ml/min flow rate, 5 to 35% MeCN in 20mM TEAA (pH 7.1) over 40 min. The product elutes as a broad peak at around 20 min. The product-containing fraction was lyophilized, dissolved in MeOH (0.4 ml), then treated with 0.15M $NaClO_4$ in acetone (1.2 ml). The precipitate was collected by centrifugation and the liquid decanted. The precipitate was washed with 2ml acetone and collected by centrifugation a total of 3 times. The solid was dried and Tris-HCl (5mM, pH 7.5) was added to make a 20 mM solution of **rITP**, sodium salt, which was stored at -80^{o}C. Yield: 3.4 µmol (4%) of **rITP**. Yield was determined using the experimentally determined molar absorbtivity constant for **rI**: 1720 M^{-1} cm^{-1}.

^{1}H NMR (D_2O, ppm) 8.19 (s, 1H), 7.70 (dd, 1H, J=8.4 Hz), 7.25 (d, 1H, J=8.2 Hz), 4.96 (d, 1H, J=5.3 Hz), 4.16 (t, 1H, J=5.1 Hz), 4.10-4.01 (m, 4H). ^{31}P NMR (D_2O, ppm) -9.92 (d), -10.28 (d), -22.32 (t). MS (ESI-) $[M-H]^-$ mass calculated: 700.8, mass found: 701.2

Oligonucleotides and ribozymes

Sequences and provenance of oligonucleotides used are described in detail in ESI table S1. Ribozymes were transcribed using Ambion MegaShortScript kit from DNA comprising the ribozyme sequence downstream of a T7 promoter sequence. The Z ribozyme (Fig 1, 'tethered' format of Fig. S1 in ESI) and tC19Z sequences are as described previously[7] (3'-RTT = 3' run-through transcript: -GCGGCCGCAAAAAAAAAAGGCUUACC-3'). Ribozyme Z (for all three duplex arrangement formats) was purified using Qiagen RNeasy Mini Kit.

Ribozyme polymerase assays

To set up typical ribozyme polymerase assays, 10 pmol each of fluorescent RNA primer, RNA template and ribozyme were annealed together (80˚C for 2 min, 17˚C for 10 min) in 1 µl water. To start the reaction, 9 µl of chilled extension buffer was added (final concentrations 0.2 M $MgCl_2$, 50 mM Tris·HCl pH 8.3, 4 mM of any

NTPs, unless otherwise described), and reactions were incubated at 17˚C. Frozen reactions used 1 mM of any NTPs, unless otherwise described; to freeze, reactions were frozen at -25˚C for 10 minutes to induce ice crystal formation, before incubation in a Techne RB-5 refrigerated bath, maintained at -7˚C by a Techne Tempette TE-8D thermostat. Reactions were incubated for the indicated times before quenching with a 25% excess of Na-EDTA, and heating (94˚C, 5 min) in 6 M urea in the presence of a 20-fold excess over template of complementary competing RNA oligonucleotide compI; extension products were separated by denaturing PAGE. Fluorescence imaging was carried out using a Typhoon Trio.

The mineral water reactions in Fig. 2b were set up differently: 10 pmol each of ribozyme Z, primer BioA10 and template I were annealed together in 1 μl water in a 0.5 ml DNA LoBind microfuge tube (Eppendorf, Germany), before sufficient chilled mineral water (supplemented with the indicated concentrations of NTPs) was added to yield the indicated final RNA concentrations. Due to the large, variable volumes of these reactions, 'frozen' reactions were incubated at -7˚C to supercool, before freezing was effected by addition of an ice crystal. After incubation and EDTA quenching, the biotinylated primers were bound to aliquots of MyOne C1 streptavidin-coated paramagnetic beads, to allow elution in equal volumes of formamide-EDTA (94˚C 5 min) with compI before denaturing PAGE. A similar loading protocol was employed in Fig. 3e.

Extension quantification

Gel densitometry was carried out using ImageQuant. Each lane in a reaction was subdivided into bands corresponding to unextended primer and extension products. Band intensities were corrected for background with the help of a negative lane. Average extension per primer in a reaction was calculated as previously described[9]; equations for this and positional extension success ratios are described in Fig. S7 in ESI. Before calculating the latter, band intensities were further corrected to account for extension resulting from increased misincorporation of the three standard ribonucleotides present due to reduced U variant incorporation. A fraction of the observed extension band intensities beyond the template A in the lane lacking any U variant were subtracted from those in the lane with the U variant under investigation, with the fraction dependent on the degree of pausing before the A (base variant experiments) or the directly-observed error incorporation opposite A (for 2' variant experiments, whose altered gel migration permits this).

ESI Figures

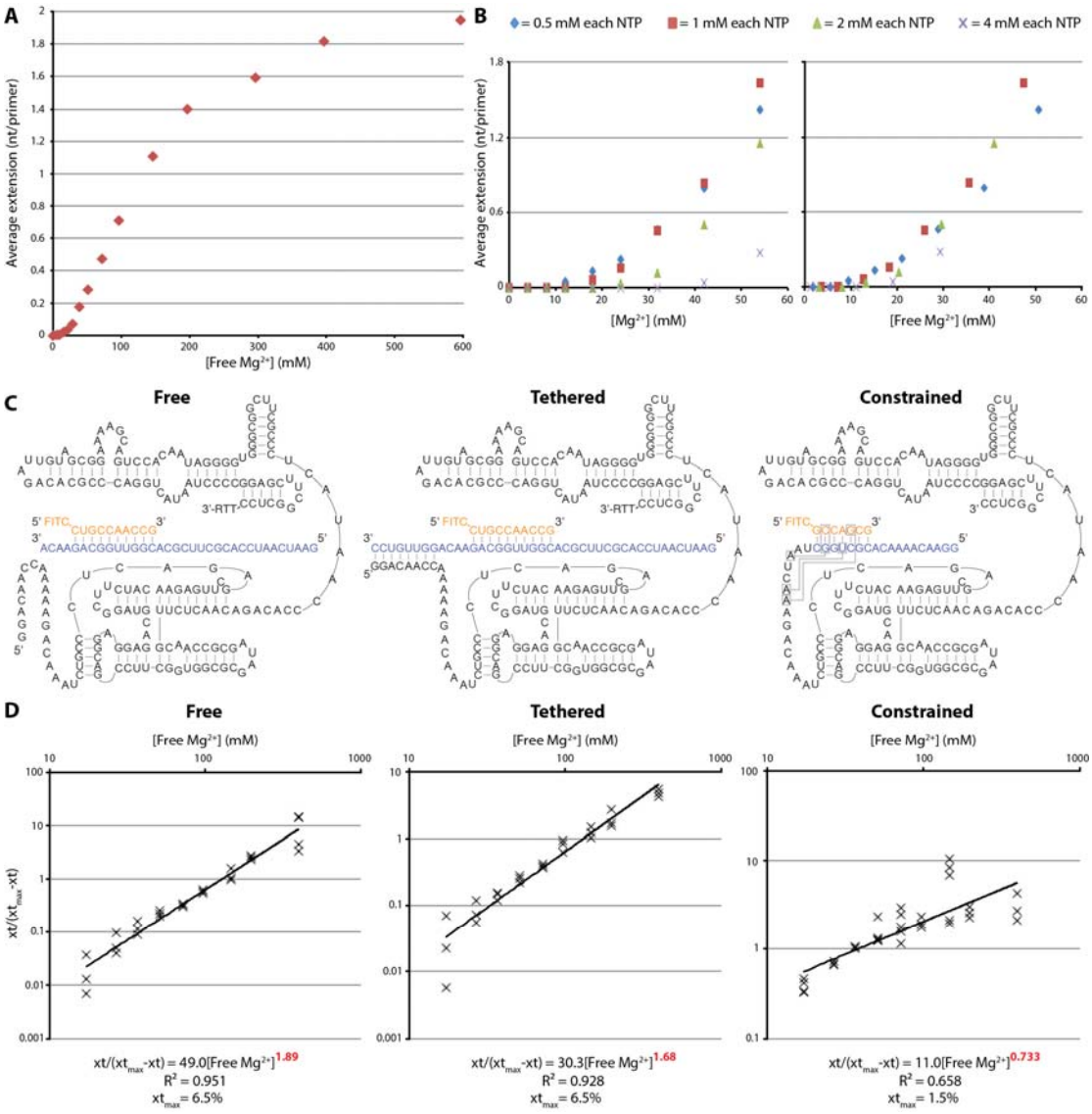


Fig. S1. (A) Quantification of average number of nucleotides added per primer in Fig. 1b, as a function of free magnesium concentration. (B) Quantification of observed extension by Z on duplex A10/I (17˚C, 7 days) at a range of NTP concentrations, plotted against total Mg^{2+} concentration ($[Mg^{2+}]$) in the reaction derived from added $MgCl_2$ (left), and available 'free' Mg^{2+} concentration after taking into account metal ion chelation by triphosphates (right, calculated using K_D values of 30 μM ($MgNTP^{2-} \rightarrow$ free Mg^{2+} and NTP^{4-}) and 25 mM ($Mg_2NTP \rightarrow$ free Mg^{2+} and $MgNTP^{2-}$))[72]. (C) Secondary structures of the polymerase ribozyme arranged with a primer (orange)/template (purple) duplex in three different formats: *Free* (Primer A10, template I-C, ribozyme Z), *Tethered* (Primer A10, template I, ribozyme Z) and *Constrained* (Primer L7, ribozyme LZ). The 5' region of the *Constrained* ribozyme

has a similar sequence to the equivalent region of the Class I ligase ribozyme with a known crystal structure, yielding a template in *cis*. Some of the tertiary interactions seen within this region in the crystal structure are highlighted[16]. (D) Hill analysis of the extension data in Fig. 1c for the three ribozyme/duplex arrangements, using percentage extension by one UTP as a proxy for fractional saturation of all ribozyme Mg^{2+} binding sites critical for activity. The extent of maximum extension (xt_{max}) used in each analysis was estimated from the extension plateaus in Fig. 1c and are indicated below each plot, along with the trendline formulae and R^2 values. The Hill coefficient for each plot is highlighted in red, where a value >1 indicates some degree of cooperative contributions to ribozyme activity over the [Mg^{2+}] range examined.

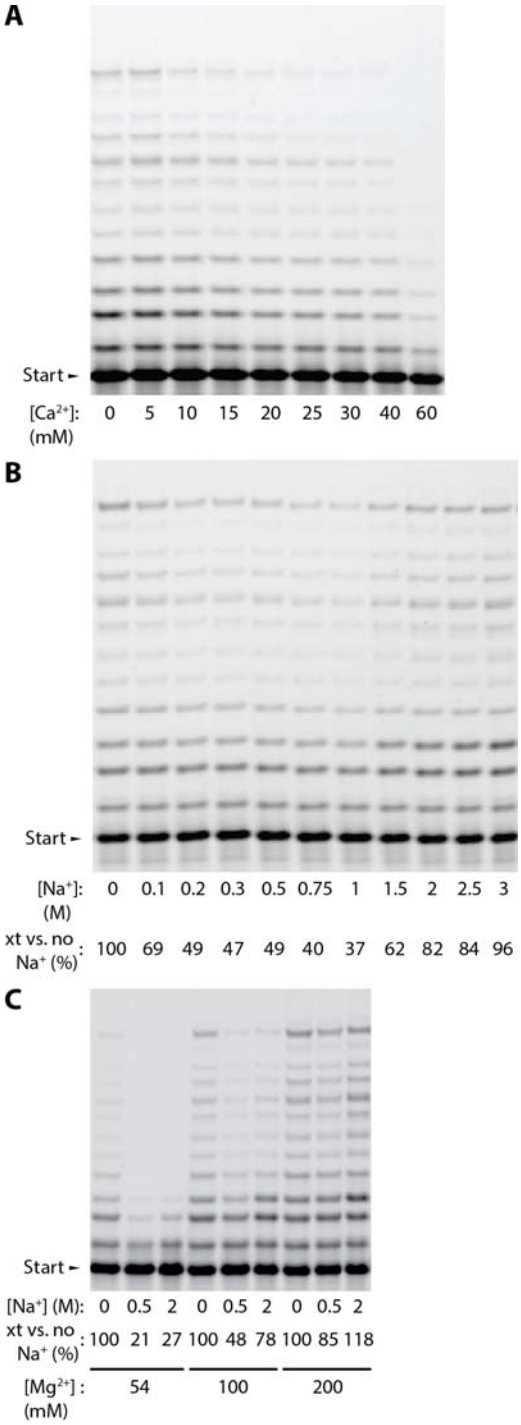


Fig. S2. Extension by Z upon the A10/I duplex using low (0.5 mM each) NTP concentrations under a range of chloride salt conditions. (A) Influence of Ca^{2+} upon extension at the 54 mM Mg^{2+} concentration found in brine (17˚C, 4 days). Concentrations of Ca^{2+} above 60 mM were not examined as they could trigger precipitation of NTPs. (B) Influence of Na^{+} upon extension at 100 mM Mg^{2+} (17˚C, 2 days). (C) Influence of Na^{+} upon extension in the context of a range of Mg^{2+} concentrations (17˚C, 1 day). The average extension per primer is shown as a percentage of the extension in the absence of Na^{+}.

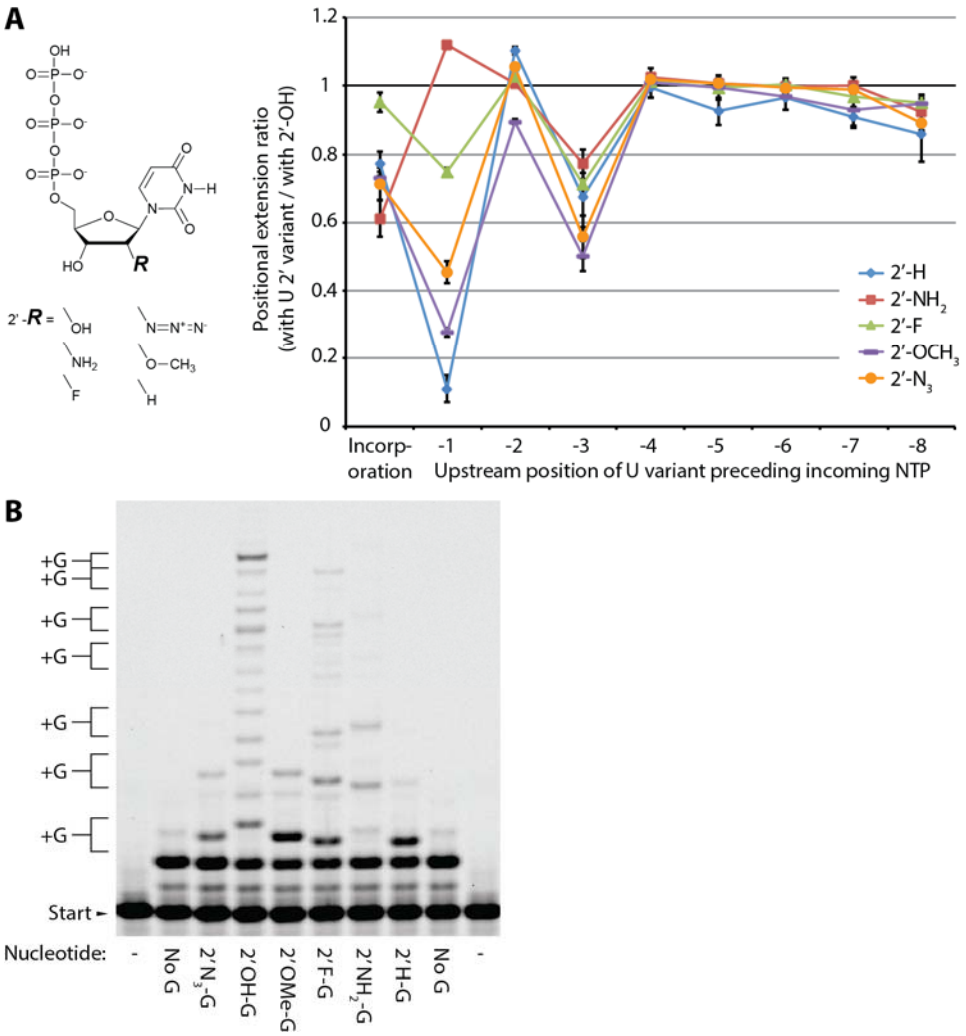


Fig. S3. (A) The positional extension ratios of each 2' variant of U/T relative to UTP/TTP incorporation, after extension as in Fig. 3b but in ice (-7˚C frozen, 5 days; error bars represent s.d., N = 3). (B) Extension upon the A7/I duplex (17˚C, 2 days) using UTP, ATP, CTP and a 2' variant of GTP (- = no NTPs). The positions in the ribonucleotide product ladder where the template encodes G incorporation are indicated.

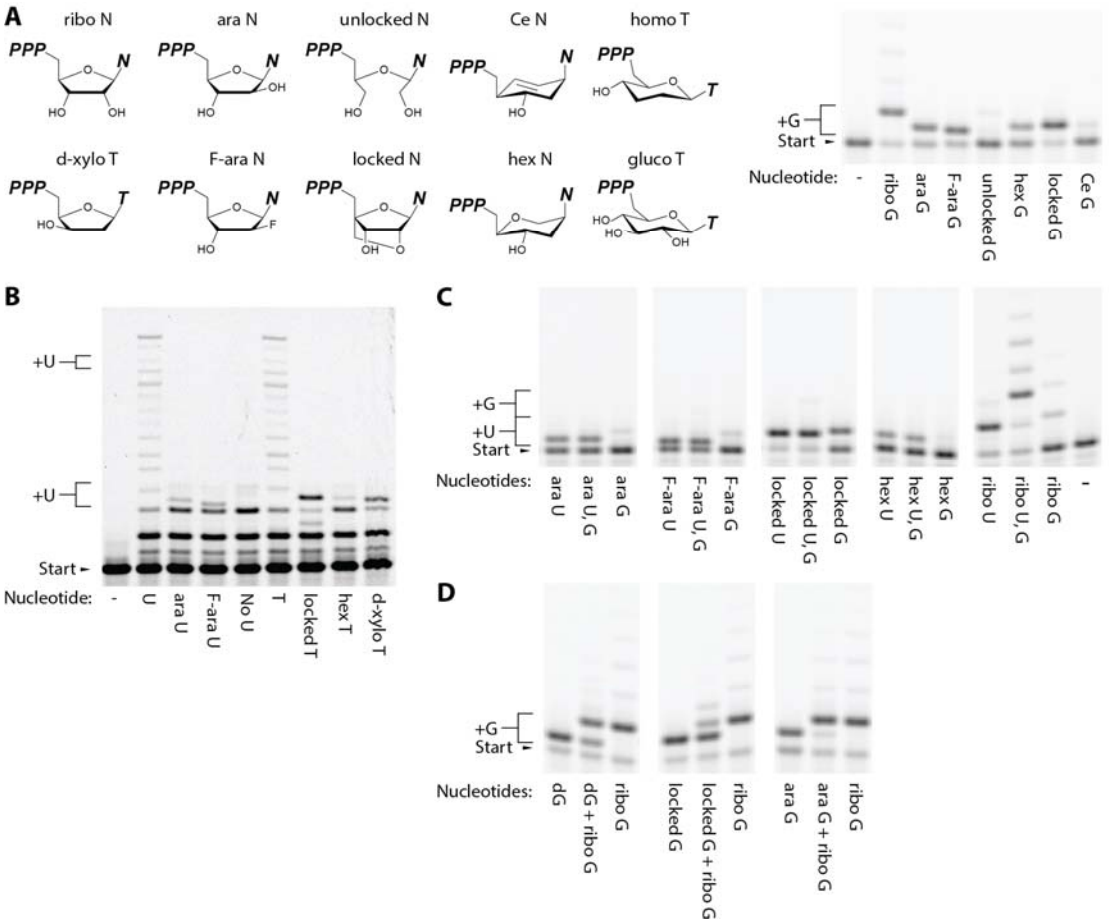


Fig. S4. (A) Structures of sugar variants/congeners, and incorporation of single G sugar-variant or congener nucleoside triphosphates opposite C in the A11/I duplex (17˚C, 10 days). (B) Extension upon the A7/I duplex (17˚C, 2 days) using GTP, ATP, CTP and a sugar variant or congener of UTP or TTP (- = no NTPs). The positions in the product ladder where the template encodes U incorporation are indicated. (C) Extension upon the A10/I duplex (17˚C, 10 days) testing consecutive incorporation of sugar-modified nucleotides. The template codes for addition of the indicated bases to the left of the gel. (D) Extension upon the A11/I duplex (17˚C, 2 days) testing competition of different sugar-variant or congener G nucleotides with the equivalent cognate GTP (riboG) nucleotide at 4 mM.

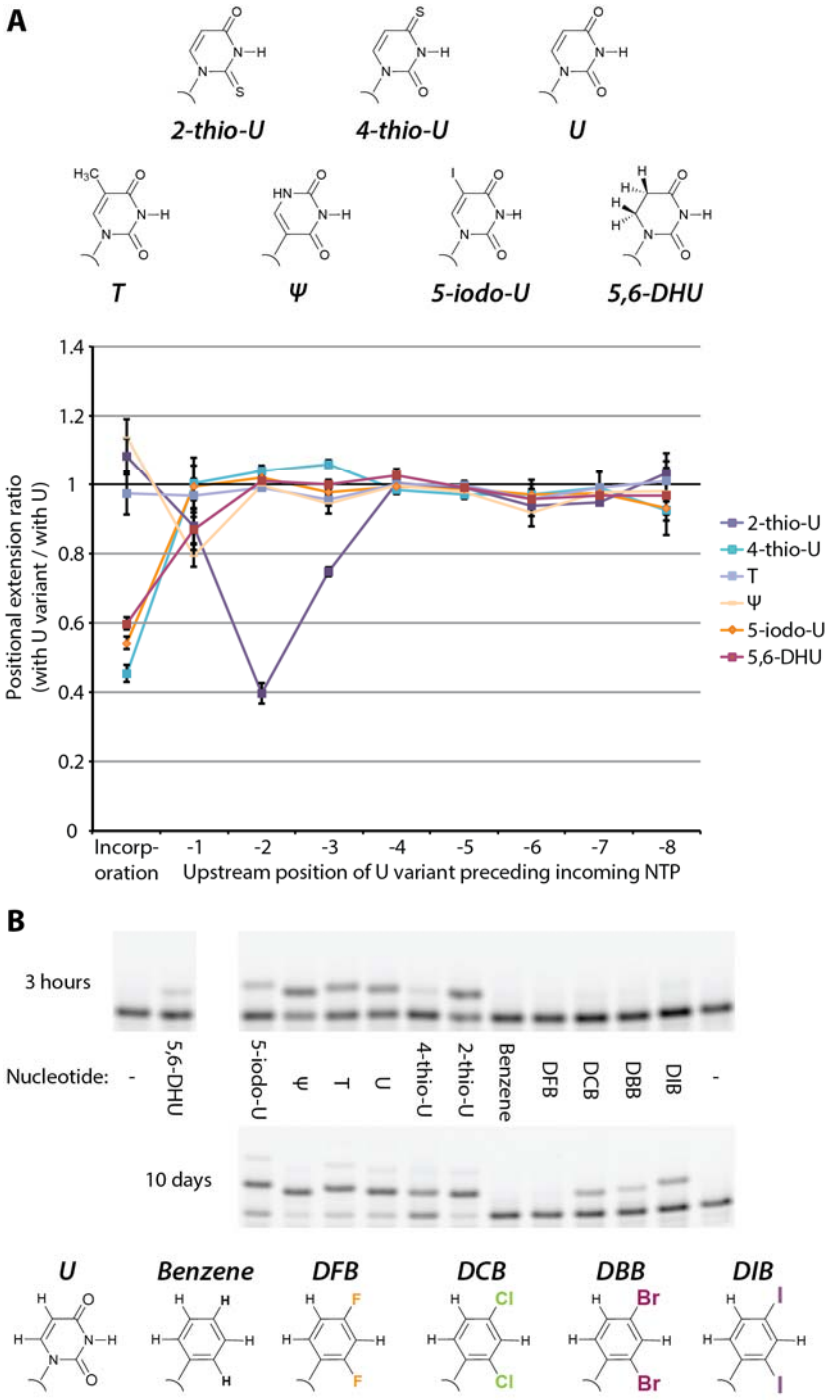


Fig. S5. (A) The positional extension success ratios of U base variants relative to UTP incorporation, after extension as in Fig. 4b but in ice (-7˚C, 5 days, error bars represent s.d.; N = 3). (B) Comparison of single incorporation by Z of U variant ribonucleotides along with benzene and a set of halogenated nucleotide isosteres (DFB, DCB, DBB, DIB) of U (structures shown below) on the A10/I duplex (17˚C for the indicated times).

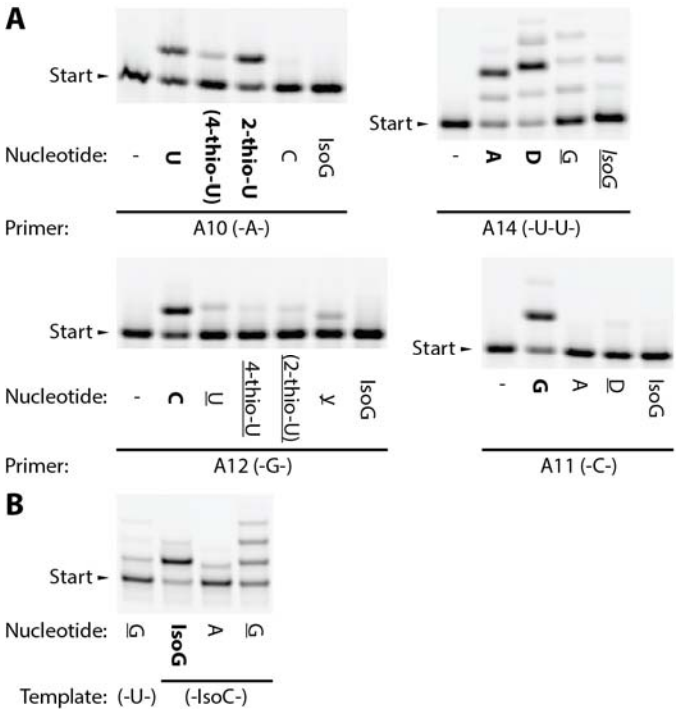


Fig. S6. (A) Extension by Z (17˚C, 3 hours) upon template I using primers of different lengths; the first template bases downstream of the primers are indicated. Single ribonucleotides were present at 4 mM concentration. Those able to Watson-Crick base pair to the template are highlighted in bold, and those able to wobble pair or reverse wobble pair are undelined. Pairing of nucleotides in parentheses relies on hydrogen bonding to sulphur; IsoG is italicized when its minor tautomer can Watson-Crick base pair to the template. (B) Extension of primer A18 by Z (-7˚C frozen, 7 days) upon template I-U and I-IsoC with the indicated base directly downstream of the primer; nucleotide notation as in (A).

A

$$\frac{\sum_{n=1}^{20} nx_n}{\sum_{n=1}^{20} x_n}$$

B

$$\frac{\sum_{n=p+1}^{20} x_{m,n} \cdot \sum_{n=p}^{20} x_{OH,n}}{\sum_{n=p}^{20} x_{m,n} \cdot \sum_{n=p+1}^{20} x_{OH,n}}$$

Fig. S7. (A) Equation used to calculate average extension in a lane, where x_n represents the intensity of the band corresponding to *n* nucleotide additions. (B) Equation used to calculate positional extension success ration beyond band p when comparing modified nucleotide m to UTP (where m=OH).

ESI table S1

All oligonucleotides except compI were gel-purified. *tx indicates preparation by transcription using established strategies[54].

Application	*Name*	*Source*	*Sequence (5´→3´)*
RNA primers	LA7	IDT	Fluorescein-GCCAGCG
	A7	Dharmacon	Fluorescein-CUGCCAA
	A10	Dharmacon	Fluorescein-CUGCCAACCG
	A11	IDT	Fluorescein-CUGCCAACCGU
	A12	IDT	Fluorescein-CUGCCAACCGUG
	A14	IDT	Fluorescein-CUGCCAACCGUGCG
	A18	IDT	Fluorescein-CUGCCAACCGUGCGAAGC
	BioA10	IDT	Fluorescein-biotin-CUGCCAACCG
RNA templates	I	Dharmacon	CAAUGAAUCCACGCUUCGCACGGUUGGCAGAACAGGUUGUCC
	I-U	IDT	CAAUGAAUCCAUGCUUCGCACGGUUGGCAGAACA
	I-IsoC	Chemgenes	CAAUGAAUCCA-IsoC-GCUUCGCACGGUUGGCAGAACA
	I-s	*tx	GGGUCAAUGAAUCCA-s-GCUUCGCACGGUUGGCAGAACA
	I-Ds	*tx	GGGUCAAUGAAUCCA-Ds-GCUUCGCACGGUUGGCAGAACA
	I-C	Dharmacon	CAAUGAAUCCACGCUUCGCACGGUUGGCAGAACA
Competing	CompI	Dharmacon	CUGCCAACCGUGCGAAGCGUGGAUUCAUUG

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