Table S1. Selectivity of RNAP-inhibitory activity (related to Fig. 1B)

enzyme	IC50 (µM) (± SEM)	selectivity ratio
promoter-dependent transcription		
***E. coli* RNAP**		
6.25 µM UTP	0.1	[1]
50 µM UTP	2	[1]
250 µM UTP	8	[1]
human RNAP I		
6.25 µM UTP	>50	>500
50 µM UTP	ND	ND
250 µM UTP	ND	ND
human RNAP II		
6.25 µM UTP	1	10
50 µM UTP	7	4
250 µM UTP	>20	>3
human RNAP III		
6.25 µM UTP	9	90
50 µM UTP	ND	ND
250 µM UTP	ND	ND
promoter-independent transcription		
***E. coli* RNAP**		
1.56 µM UTP	0.9 (± 0.1)	[1]
25 µM UTP	4 (± 0.7)	[1]
400 µM UTP	10 (± 2)	[1]
HeLa nuclear extract (human RNAP I / II / III)		
1.56 µM UTP	4 (± 0.1)	4
25 µM UTP	15 (± 6)	4
400 µM UTP	51 (± 30)	5

Table S2. Antibacterial activity *in vivo* (mouse *S. pyogenes* peritonitis model; 7 day survival) (related to Fig. 1D)

intravenous (iv) administration, 10 min and 6 h post-infection		intravenous (iv) administration, 10 min post-infection		subcutaneous (sc) administration, 10 min post-infection	
PUM total dose (mg/kg)	**survivors / total**	**PUM total dose (mg/kg)**	**survivors / total**	**PUM total dose (mg/kg)**	**survivors / total**
50	7 / 8	40	5 / 8	40	6 / 8
20	6 / 8	16	4 / 8	16	2 / 8
8	5 / 8	6.4	0 / 8	6.4	1 / 8
3.2	0 / 8	2.56	2 / 8	2.56	1 / 8
0	0 / 8	1.024	0 / 8	1.024	0 / 8
		0	0 / 8	0	0 / 8

Table S3. Data collection and refinement statistics (related to Fig. 4)

	RPo-GpA-PUM (PDB: 5X21)	RPo-GpA-CMPcPP (PDB: 5X22)
data collection		
space group	C2	$P2_1$
cell dimensions		
a, *b*, *c* (Å)	186.8, 103.1, 296.2	186.4, 104.3, 297.3
α, β, γ (°)	90.0, 98.7, 90.0	90.0, 98.4, 90.0
resolution (Å)	40.00-3.30 (3.36-3.30)*	50.00-3.35 (3.41-3.35)*
R_{sym} or R_{merge}	0.138 (0.633)	0.197 (>1.0)
$I/\sigma(I)$	8.4 (1.7)	7.7 (1.8)
completeness (%)	0.920 (0.874)	0.956 (0.891)
redundancy	3.4 (3.4)	5.3 (5.3)
refinement		
resolution (Å)	50.00-3.32	50.00-3.33
number of reflections	70085	147976
R_{work} / R_{free}	0.232/0.280	0.208/0.250
number of atoms		
protein/DNA/RNA	28662	56787
ligand/ion	42	76
water	35	0
B factors		
protein	61.6	41.8
ligand/ion	31.8	21.0
Water	34.3	-
r.m.s deviations		
bond lengths (Å)	0.002	0.007
bond angles (°)	0.542	0.834

*Values in parentheses are for highest-resolution shell.

Table S4. Oligonucleotides (related to STAR Methods section)

name	sequence
recA promoter DNA fragment 1	5'-GGCGACCGTGATGCGGTGCGTCGTCAGGCTACTGCGTATGCATTGCAGACCTTGTGGCAAC AATTTCTACAAAACACTTGATACTGTATGAGCATACAGTATAATTGCTTCAGATCTCTAGAAGCT TTAATGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGGCACCGTGTATGAAATCTAAC AATGCGCTCATCGTCATCCTCGGCACCGTCACCCTGGATGCTCTAGGCATAGGCTTGGTTATG CCGGTACTGCCGGGCCTCTTGCGGGATATCGTCCATTCCGACAGCATCGCCAGTCACTATGG CGTGCTGCTAGCGCTATATGCGTTGATGCAATTTCTATGCGCACCCGTTCTCGGAGCACTGTC CGACCGCTTTGGCCGCCGCCCAGTCCTGCTCGCTTCGCTACTTGGAGCCACTATCGACTACG CGATCATGGCGACCACACCCGTCCTGT-3'
recA promoter DNA fragment 2	5'-GGCGACCGTGATGCGGTGCGTCGTCAGGCTACTGCGTATGCATTGCAGACCTTGTGGCAAC AATTTCTACAAAACACTTGATACTGTATGAGCATACAGTATAATTGCTTCAACAGAACCAGGGAC AAGTTAGTTCGTTCAGCGACACGCGGCAACAAG-3'
recA promoter DNA fragment 3	5'-GGCGACCGTGATGCGGTGCGTCGTCAGGCTACTGCGTATGCATTGCAGACCTTGTGGCAAC AATTTCTACAAAACACTTGATACTGTATGAGCATACAGTATAATTGCTTCAACAGAACCAGGGAC AAGGAGACCAACGCAGCGACACGCGGCAACAAG-3'
bacteriophage T4 N25 promoter DNA fragment	5'-TTGCTTTCAGGAAAATTTTTCTGTATAATAGATTCATAAATTTGAGAGAGGAGTTTAAATATGG CTGGTTCTCGCGAGAATTCCGAATAGCCATCCCAATCGAACAGGCCTGCTGGTAATCGCAGGC TTTTTATTT-3'
Cy5-labelled *lacUV5* promoter DNA fragment	5'-Cy5-AGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA-3'
S. pyogenes rpoB forward primer	5'-GGGCAAATGATAACTTAGTTGCGATTTGCTG-3'
S. pyogenes rpoB reverse primer	5'-CCTTTCTGCCTTTGATGACTTTACCAGTTC-3'
S. pyogenes rpoC forward primer	5'-GCTCAAGAAACTCAAGAAGTTTCTGAAACAACTGAC-3'
S. pyogenes rpoC reverse primer	5'-GTCAATGCTTTTTACTGCCAACAAACTCAGAC-3'

Table S5. Nucleic-acid scaffolds for single-nucleotide-addition reactions (related to STAR Methods section)

incoming NTP	RNA 3' end	nucleic-acid scaffold[1]
UTP	G	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGCATGCGGTCTGTCCC-5' 5'-*CGGCGCGCG-3'
GTP	G	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGCCTGCGGTCTGTCCC-5' 5'-*CGGCGCGCG-3'
ATP	G	5'-tCGCCAGACAGGG-3' 3'-GCCGCGCGCTaGCGGTCTGTCCC-5' 5'-*CGGCGCGCG-3'
CTP	G	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGCGTGCGGTCTGTCCC-5' 5'-*CGGCGCGCG-3'
UTP	A	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGTATGCGGTCTGTCCC-5' 5'-*CGGCGCGCA-3'
GTP	A	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGTCTGCGGTCTGTCCC-5' 5'-*CGGCGCGCA-3'
ATP	A	5'-tCGCCAGACAGGG-3' 3'-GCCGCGCGTTaGCGGTCTGTCCC-5' 5'-*CGGCGCGCA-3'
CTP	A	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGTGTGCGGTCTGTCCC-5' 5'-*CGGCGCGCA-3'
UTP	U	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGAATGCGGTCTGTCCC-5' 5'-*CGGCGCGCU-3'
GTP	U	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGACTGCGGTCTGTCCC-5' 5'-*CGGCGCGCU-3'
ATP	U	5'-tCGCCAGACAGGG-3' 3'-GCCGCGCGATaGCGGTCTGTCCC-5' 5'-*CGGCGCGCU-3'
CTP	U	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGAGTGCGGTCTGTCCC-5' 5'-*CGGCGCGCU-3'
UTP	C	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGGATGCGGTCTGTCCC-5' 5'-*CGGCGCGCC-3'
GTP	C	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGGCTGCGGTCTGTCCC-5' 5'-*CGGCGCGCC-3'
ATP	C	5'-tCGCCAGACAGGG-3' 3'-GCCGCGCGGTaGCGGTCTGTCCC-5' 5'-*CGGCGCGCC-3'
CTP	C	5'-ACGCCAGACAGGG-3' 3'-GCCGCGCGGGTGCGGTCTGTCCC-5' 5'-*CGGCGCGCC-3'

[1]black, DNA; red, RNA, asterisk, ^{32}P.