Site-specific labeling of RNA at internal ribose hydroxyl groups: terbium-assisted deoxyribozymes at work

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General Information

All chemicals and solvents were purchased from commercial suppliers and used without further purification. Silica gel plates precoated with fluorescent indicator were used for thin layer chromatography (TLC) and the plates were visualized with UV light. Silica gel 60, 0.032-0.063 mm (230-450 mesh) was used for column chromatography. ^{1}H NMR and ^{13}C NMR were recorded on a 400 MHz instrument. The chemical shifts (in ppm) are reported downfield from TMS (0 ppm) or $CDCl_3$ (7.26 ppm for ^{1}H and 77.16 ppm for ^{13}C). Spin multiplicities in ^{1}H NMR are reported as singlet (s), doublet (d), triplet (t), triplet of triplet (tt) or multiplet (m).

Unmodified DNA oligonucleotides, including deoxyribozymes were purchased from Sigma Aldrich and purified by denaturing PAGE (10-15% polyacrylamide). RNAs were prepared by solid-phase synthesis using 5'-*O*-DMT-2'-*O*-TOM- protected 3'-β-cyanoethyl phosphoramidites of N^6-acetyladenosine, N^4-acetylcytidine, N^2-acetylguanosine, and uridine, and 5'-*O*-DMT-2'-*O*-TBDMS- protected 3'-β-cyanoethyl phosphoramidite of N^2-(*N*,*N*-dimethylaminoformamidine)-2-aminopurineriboside purchased from ChemGenes. Benzylthiotetrazole (BTT) was from Carbosynth.

Ribonucleotide triphosphates (NTPs) for in vitro transcription reactions were purchased from Jena Bioscience, α-^{32}P-ATP (3000 Ci/mmol) and γ-^{32}P-ATP (3000 Ci/mmol) were from Perkin Elmer. T4 DNA ligase and T4 polynucleotide kinase (PNK) were from Fermentas. *Taq* DNA polymerase was purchased from New England BioLabs.

F545-alkyne (Acetylene-Fluor 545) and modified GTP analogs EDA-GTP-biotin, EDA-GTP, MANT-GTP, 8-Oxo-GTP, 6-Thio-GTP, 8-[(6-Amino)hexyl]-amino-GTP and EDA-GTP-Cy3 were purchased from Jena Bioscience. C488- (Chromeo 488-Alkyne), Cy3- (Chromeo 546-Alkyne) and Cy5-alkyne (Chromeo 642-Alkyne) were purchased from baseclick. 2'-NH_2-GTP and 2'-N_3-GTP were purchased from TriLink Biotechnologies.

Anion exchange HPLC experiments were performed on an ÄKTApurifier 10 from GE Healthcare using a Dionex DNAPac PA200 (4 x 250 mm) column at 80°C. Eluent A: 25 mM Tris HCl, pH 8.0, 6 M urea; Eluent B: 25 mM Tris HCl, pH 8.0, 0.5 M $NaClO_4$, 6 M urea.

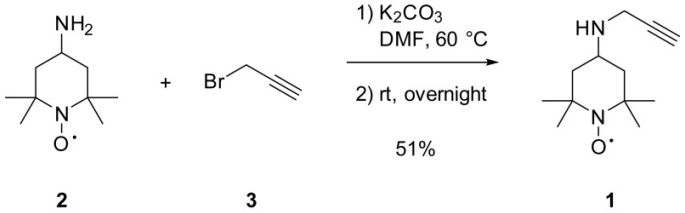
Reversed phase HPLC experiments were performed on a Shimadzu HPLC system with a diode array detector using a Nucleosil 100-5 C18 HD (4 x 250 mm) column at 40 °C. Eluent A: 100 mM TEAA in H_2O; Eluent B: 20 mM TEAA in H_2O:MeCN 1:4.

UV-vis spectra were recorded on a Cary 100 spectrometer (Varian) using 1 cm quartz cuvettes. Fluorescence spectra were recorded on Cary Eclipse fluorescence spectrometer (Varian) using 0.4 x 1 cm quartz cuvettes. Sample holders of both instruments were equipped with a Peltier temperature controller.

Denaturing polyacrylamide gels (0.5 or 0.7 mm thick, 20x30 or 20x40 cm, 8-20% acrylamide, 7 M urea, 1x TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) were run in 1x TBE buffer at 35 W. Bromophenol blue and xylene cyanol were used as dye markers.
On preparative gels, oligonucleotides were visualized by UV shadowing on a TLC plate. Analytical gels of unlabeled samples were stained with Toluidine blue O (0.001% (w/v) in H_2O, 15-20 min, r.t.) and photographed on a white light transilluminator with a DIAS-II Imaging and Analysis System from SERVA Electrophoresis. Gels of radioactive samples were exposed to phosphor storage screens and

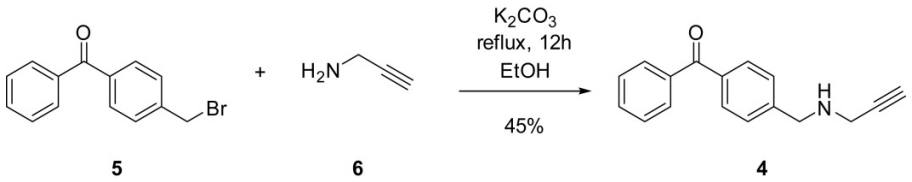
visualized with a Storm 820 PhosphorImager, GE Healthcare. Fluorescence gel images were recorded with a Biorad ChemiDoc MP with epi illumination using blue, green and red LEDs (emission filters 530/28 (blue), 605/50 (green) and 695/55 (red)), or with a Typhoon Trio$^+$ Imager from GE Healthcare equipped with a blue (488 nm), green (532 nm) and red (633 nm) laser.

Chemical synthesis of alkynes



Scheme S1. Synthesis of TEMPO-alkyne **TA** (**1**).

4-(Prop-2-yn-1-ylamino)-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO-alkyne, TA) (1).
4-Amino-2,2,6,6-tetramethylpiperidine-1-oxyl (100 mg, 0.6 mmol, 1.0 eq) (**2**) was dissolved in 3 mL dry DMF and mixed with K_2CO_3 (242 mg, 1.8 mmol, 3.0 eq) under argon atmosphere. The reaction mixture was heated at 60 °C for 30 min and allowed to cool to room temperature. Propargylbromide (83.4 mg (80% solution w/w in toluene), 0.7 mmol, 1.2 eq) (**3**) was added and the mixture was stirred at room temperature overnight (12h). The organic phase was washed with brine (20 mL) and the aqueous phase extracted twice with EtOAc (40 mL in total). The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude mixture was purified by column chromatography (silica gel, 2% NEt_3 in EtOAc). The desired product **1** was isolated as orange needles (62.3 mg, 0.3 mmol, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (s, 12H), 1.64-2.05 (m, 4H), 2.28 (d, *J* = 4.3 Hz, 1H), 3.10-3.35 (m, 1H), 3.53 (s, 2H). ESI-MS: calcd. for $C_{12}H_{21}N_2O$: 209.3; found: 210.2 $[M+H]^+$.



Scheme S2. Synthesis of UV-inducible crosslinker alkyne BPA (**4**).

4-[(*N*-prop-2-yn-1-yl)-aminomethyl]benzophenone (BP-alkyne, BPA) (4).
4-(Bromomethyl)benzophenone (138mg, 0.5 mmol, 1.0 eq) (**5**) and propargylamine (27.5 mg, 0.5 mmol, 1.0 eq) (**6**) were dissolved in 7.5 mL ethanol and K_2CO_3 (138 mg, 1.0 mmol, 2.0 eq) was added. The reaction mixture was heated at reflux for 12 h and allowed to cool to room temperature. The precipitated KBr was removed by filtration, the solvent was evaporated and the residue purified by column chromatography (silica gel, CH_2Cl_2/MeOH 95:5). The desired product **4** was isolated as yellow oil (56.1 mg, 0.2 mmol, 45%). Lit.:[1]. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.28 (t, *J* = 2.4 Hz, 1H), 3.46 (d, *J* = 2.4 Hz, 2H), 3.98 (s, 2H), 7.46-7.51 (m, 4H), 7.59 (tt, *J* = 7.4 Hz, 1.3 Hz, 1H), 7.76–7.82 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 37.4, 51.8, 71.8, 81.8, 128.1, 128.2, 130.0, 130.3, 132.3, 136.5, 144.3, 196.42. ESI-MS: calcd. for $C_{17}H_{15}NO$: 249.3; found: 250.1 $[M+H]^+$.

Synthesis of labeled GTPs by copper-catalyzed 1,3-dipolar cycloaddition

Synthesis of TEMPO-GTP

To a solution of 50 nmol 2'-N_3-GTP (0.5 µL of 100 mM in H_2O; 1.0 eq) in 17.25µL H_2O were added 0.75 µL of TA (**1**) (100 mM in CH_2Cl_2; 1.5 eq). 1.0 µL of a freshly prepared solution of CuBr (44 mM in DMSO/*t*BuOH 3:1; 0.8 eq) was mixed with 1.0 µL TBTA (44 mM in DMSO/tBuOH 3:1; 0.8 eq), and added to the aqueous solution, to give a final volume of 20 µL (in total 14% of organic solvent). The mixture was incubated at room temperature for 4 h, then diluted with water up to 50 µL and purified by size exclusion chromatography (Sephadex G 10, 3 × 5 mL HiTrap desalting columns, GE Healthcare). The product was eluted with water (monitored by UV-detection at 280 nm), lyophilized and redissolved in water to a final concentration of 2 mM.

Synthesis of BP-GTP and F545-, Cy3- and Cy5-GTP

To a solution of 100 nmol 2'-N_3-GTP (1.0 µL of 100 mM in H_2O; 1.0 eq) in 23 µL H_2O and 10 µL DMSO/*t*BuOH (3:1) were added 3.0 µL BPA (**4**) (100 mM in DMF; 3.0 eq). 1.0 µL of a freshly prepared solution of CuBr (88 mM in DMSO/^{t}BuOH 3:1; 0.9 eq) was mixed with 2.0 µL TBTA (88 mM in DMSO/tBuOH 3:1; 1.7 eq), and added to the solution, to give a final volume of 40 µL (in total 40% of organic solvent). The mixture was incubated at room temperature for 4h and precipitated by addition of 30 times the volume of icecold 2% $NaClO_4$ in acetone (centrifuged at 4 °C, 13200 rpm for 30 min). The crude sample was dissolved in water and purified by reversed phase HPLC on a Nucleosil 100-5 C18 HD column, 4 x 250 mm, 0 – 70% B in 40 min at 40 °C (25 nmol crude product per injection; buffer A: 100 mM TEAA in H_2O, buffer B: 20 mM TEAA in H_2O:MeCN 1:4). The isolated product was lyophilized and redissolved in 50 µL of water.

F545-, Cy3- and Cy5-GTP were synthesized following a similar procedure as for BP-GTP using F545-alkyne (**5**) (3.0 µL of 100 mM in H_2O), Cy3-alkyne (**6**) (3.0 µL of 100 mM in DMF) or Cy5-alkyne (**7**) (3.0 µL of 100 mM in DMF), respectively.

Synthesis of C488-GTP

To a solution of 100 nmol 2'-N_3-GTP (1.0 µL of 100 mM in H_2O; 1.0 eq) in 11 µL H_2O and 11 µL DMSO/*t*BuOH (3:1) were added 15.0 µL C488-alkyne (20 mM in H_2O/DMF 4:1). 1.0 µL of a freshly prepared solution of CuBr (88 mM in DMSO/*t*BuOH 3:1; 0.9 eq) was mixed with 2.0 µL TBTA (88 mM in DMSO/*t*BuOH 3:1; 0.8 eq), and added to the nucleotide-containing solution, to give a final volume of 40 µL (in total 40% of organic solvent). The mixture was incubated at room temperature for 4h and precipitated by addition of 30 times the volume of icecold 2% $NaClO_4$ in acetone (centrifuge at 4 °C, 13200 rpm for 30 min). The crude sample was dissolved in water and purified by reversed phase HPLC on a Nucleosil 100-5 C18 HD column, 4 x 250 mm, 0 – 70% B in 40 min at 40 °C (25 nmol crude product per run; buffer A: 100 mM TEAA in H_2O, buffer B: 20 mM TEAA in H_2O:MeCN 1:4). The isolated product was lyophilized and redissolved in 50 µL of water.

Table S1. Characterization of click-labeled GTP analogs. (HPLC traces of pure products in Figure S9).

clicked GTP-analog	scale nmol	yield (%)	mol. wt. calcd.	mol. wt. found
TEMPO-GTP	50	76	757.5	756.2
BP-GTP	100	64	797.5	796.0
C488-GTP	100	51[a]	1102.8	1101.1
F545-GTP	100	60[b]	1191.9	1190.1
Cy3-GTP	100	45	1192.0	1190.2
Cy5-GTP	100	45	1190.0	1188.1

[a] combined yield of both isomers (product ratio is 34:17 nmol);
[b] combined yield of both isomers (product ratio is 20:40 nmol)

Sequences of RNA and DNA oligonucleotides

Target RNAs (labeling sites are marked in bold)

#	name	5′-sequence-3′
R1	GAC	GGAUAAUACG**A**CUCAC
R2	GAG	GGAUAAUACG**A**GUCAC
R3	GAA	GGAUAAUACG**A**AUCAC
R4	GAU	GGAUAAUACG**A**UUCAC
R5	AAG	GGAUAAUACA**A**GUCAC
R6	AAA	GGAUAAUACA**A**AUCAC
R7	AAC	GGAUAAUACA**A**CUCAC
R8	AAU	GGAUAAUACA**A**UUCAC
R9	CAG	GGAUAAUACC**A**GUCAC
R10	CAA	GGAUAAUACC**A**AUCAC
R11	CAC	GGAUAAUACC**A**CUCAC
R12	CAU	GGAUAAUACC**A**UUCAC
R13	UAG	GGAUAAUACU**A**GUCAC
R14	UAA	GGAUAAUACU**A**AUCAC
R15	UAC	GGAUAAUACU**A**CUCAC
R16	UAU	GGAUAAUACU**A**UUCAC
R17	26mer	GAUGCCUUGUAACCGA**A**AGGGGGAAU
R18	SAMII-1	UCGCGCUG**A**UUU**A**ACCGUA
R19	SAMII-2	UUGCAAGCGCGUGAUAAAUGU**A**GCUAAAAAGGG
R20	SAMII	UCGCGCUG**A**UUU**A**ACCGUAUUGCAAGCGCGUGAUAAAUGU**A**GCUAAAAAGGG
R21	SAMII-1-2AP14	UCGCGCUGAUUUA(2AP)CCGUA
R22	SAMII-2-2AP41	UUGCAAGCGCGUGAUAAAUGU(2AP)GCUAAAAAGGG
R23	46mer-A	GGACUAGUCUAGGCG**A**ACGUUUA**A**GGCGAUCUCUGUUUACAACUCC
R24	46mer-B	GGAGUUGUA**A**ACAGAGAUCGCCUUAAACGUUCGCCUAGACUAGUCC
R25	U6 snRNA (S.cerevisiae) (121nt)	GGUUCGCGAAGUAACCCUUCGUGGACAUUUGGUCAAUUUG**AAA**C**A**AUACAG**A**GAUG**A**UCAGC**A**GUU CCCCUGCAUA**A**GGAUGAACCGUUUU**A**CA**A**AGAGAUUUAUUUCGUUUUAAAGGAUC
R26	ydaO riboswitch (B.subtilis) (156nt)	ggGAAAACAAAUCGCUUAAUCUGAAAUCAGAGCGGGGGACCCAAUAG**A**ACGGCUUUUUGCCGUUGG GGUGAAUCCUUUUUAGGUAGGGCUAACUCUCAUAUGCCCGAAUCCGUCAGCUAACCUCGU**A**AGCGU UCGUGAGAGGAGAUGAAUGAAACC
R27	R_Δ	GAAGAGAUGGCGACGG
R28	compR1	GUGAGUCGUAUUAUCC

RNA-labeling deoxyribozymes

All 10DM24-derived deoxyribozyme sequences are preceded by the constant binding site for R_Δ and loop A (5′-CCGTCGCCATCTCCCGTAGGTGAAGGGC), followed by the target binding arms (underlined) which flank loop B.

#	target RNA	deoxyribozyme: 5′-CCGTCGCCATCTCCCGTAGGTGAAGGGC...
D1	R1	...GTGAGGGTTCCATTCCCGTATTATCC-3′
D2	R2	...GTGACGGTTCCATTCCCGTATTATCC
D3	R3	...GTGATGGTTCCATTCCCGTATTATCC
D4	R4	...GTGAAGGTTCCATTCCCGTATTATCC
D5	R5	...GTGACGGTTCCATTCCTGTATTATCC
D6	R6	...GTGATGGTTCCATTCCTGTATTATCC

D7	R7	...GTGAGGGTTCCATTCCTGTATTATCC
D8	R8	...GTGAAGGTTCCATTCCTGTATTATCC
D9	R9	...GTGACGGTTCCATTCCGGTATTATCC
D10	R10	...GTGATGGTTCCATTCCGGTATTATCC
D11	R11	...GTGAGGGTTCCATTCCGGTATTATCC
D12	R12	...GTGAAGGTTCCATTCCGGTATTATCC
D13	R13	...GTGACGGTTCCATTCCAGTATTATCC
D14	R14	...GTGATGGTTCCATTCCAGTATTATCC
D15	R15	...GTGAGGGTTCCATTCCAGTATTATCC
D16	R16	...GTGAAGGTTCCATTCCAGTATTATCC
D17	26mer	...CCCCTGGTTCCATTCCTCGGTTACAAGG
D18	A-A16	...AACGTGGTTCCATTCCCGCCTAGACT
D19	A-A24	...TCGCCGGTTCCATTCCTAAACGTTCGCC
D20	B-A10′	...TCTGTGGTTCCATTCCTACAACTCC
D21	SAMII-A9	...TTAAAGGTTCCATTCCCAGCGCGA
D22	SAMII-A13	...ACGGTGGTTCCATTCCAAATCAGCGCGAAACAACAACAAC
D23	SAMII-A41	...TTAGCGGTTCCATTCCACATTTATCACGCGCT
D24	U6-A40	...TTGTTGGTTCCATTCCCAAATTGACC
D25	U6-A41	...ATTGTGGTTCCATTCCTCAAATTGACCAAATG
D26	U6-A42	...TATTGGGTTCCATTCCTTCAAATTGACCAAAT
D27	U6-A43	...TGTATGGTTCCATTCCGTTCAAATTGACCAAA
D28	U6-A51	...TCATCGGTTCCATTCCCTGTATTGTTTC
D29	U6-A56	...GCTGAGGTTCCATTCCCATCTCTGTATTG
D30	U6-A62	...GGAACGGTTCCATTCCGCTGATCATCTC
D31	U6-A76	...CATCCGGTTCCATTCCTATGCAGGGGAAC
D32	U6-A91	...CTTTGGGTTCCATTCCAAAACGGTTCATCC
D33	U6-A94	...TCTCTGGTTCCATTCCTGTAAAACGGTTCA
D37	ydaO-A46	...GCCGTGGTTCCATTCCCTATTGGGTCCCCC
D38	ydaO-A125	...ACGCTGGTTCCATTCCACGAGGTTAGCT

RNA-cleaving deoxyribozymes for analysis of cleavage efficiency

The binding arms are underlined, the deoxyribozyme family is indicated in parentheses.

#	target	5′-sequence-3′	
D39	U6-A35	TATTGTTTCAAAGGCTAGCTACAACGATGACCAAATGTCCACACACACACACACAC	(10-23)
D40	U6-A51	GCTGATCATTCCGAGCCGGACGACTGTATTGTTTCAAATTGACACACACACACACA	(8-17)
D41	U6-A62	TCATCCTTATGCAGGGGAATCCGAGCCGGACGAGCTGATCATCTCACACACACACA	(8-17)
D42	U6-A97	AAAACGAAATAAATTCCGAGCCGGACGACTTTGTAAAACGGACACACACACACACA	(8-17)
D43	ydaO-A17	CTGATTTCAGAGGCTAGCTACAACGATAAGCGATTTG	(10-23)
D44	ydaO-G60	TCACCCCAAGGCTAGCTACAACGAGGCAAAAAGC	(10-23)
D45	ydaO-A96	ATTCGGGCATAGGCTAGCTACAACGAGAGAGTTAGC	(10-23)
D46	ydaO-A143	GGTTTCATTCAGGCTAGCTACAACGACTCCTCTCAC	(10-23)

Other DNA oligonucleotides

#	description	5′-sequence-3′
D47	T7 promoter	CTGTAATACGACTCACTATA
D48	temp-R23(A)	GGAGTTGTAAACAGAGATCGCCTTAAACGTTCGCCTAGACTAGTCCTATAGTGAGTCGTATTACAG
D49	temp-R24(B)	GGACTAGTCTAGGCGAACGTTTAAGGCGATCTCTGTTTACAACTCCTATAGTGAGTCGTATTACAG
D50	SAMII-splint	CACGCGCTTGCAATACGGTTAAATCAGC
D58	SAMII-c25-48	TTTTAGCTACATTTATCACGCGCT

#	description	5′-sequence-3′
D51	revP-U6	GTCCTTTAAAACGAAATAAATCTC
D52	U6-c66-94	TTGTAAAACGGTTCATCCTTATGCAGGGG
D53	U6-c104-119	GTCCTTTAAAACGAAA
D54	fwd-ydaO	CTGTAATACGACTCACTATAGGGAAAACAAATCGCTTAATCTGAAATCAGAGCGGGGGACCCAATA GAACGGCTTTTTGCCGTTGGGGTGGAATC
D55	rev-ydaO	GGTTTCATTCATCTCCTCTCACGAACGCTTACGAGGTTAGCTGACGGATTCGGGCATATGAGAGTT AGCCCTACCTAAAAAGGATTCCACCCCAACG
D56	revP-ydaO	GGTTTCATTCATCTCCTC
D57	ydaO-c57-89	TAGCCCTACCTAAAAAGGATTCACCCCAACGGC

General methods for RNA synthesis

Solid-phase RNA synthesis (R1-R22)
As described previously, [2] all RNA sequences were prepared by solid-phase synthesis on custom primer supports at 0.7 µmol scale in conventional 3' → 5' direction using standard 5'-DMT-2'-TOM or 2'-TBDMS protected 3'-β-cyanoethyl RNA phosphoramidites. Deprotection was performed under standard conditions in two steps using methyl amine in H_2O/EtOH, and TBAF in THF. All oligonucleotides were purified by PAGE and analyzed by anion exchange HPLC on Dionex DNAPac PA200 column.

In vitro transcription of 5'-triphosphorylated RNA (R23-R26)
In vitro transcription reactions were performed with T7 RNA polymerase with 0.5-1 µM DNA template* (and 0.5-1 µM promotor D47 for single-stranded templates) in 40 mM Tris-HCl, pH 8.0, 30 mM $MgCl_2$, 10 mM DTT, 4 mM each NTP and 2 mM spermidine at 37°C for 5 h. The transcription products were purified by denaturing PAGE (10-15% polyacrylamide, 7 M urea, 0.7 mm, 20x30 cm, 35W, 1.5-2h). 100 µL transcription reactions yielded 2.1 nmol R23, 3.0 nmol R24, 0.9 nmol R25 and 1.5 nmol R26. For analytical purpose, R25 and R26 were also internally ^{32}P-labeled by including 1.0 µL of α-^{32}P-ATP in the transcription reaction.

**Template preparation for ydaO RNA.* The DNA template for the ydaO riboswitch R26 was generated by overlapping primer extension, followed by PCR amplification. 5 pmol of D54, 5 pmol of D55 (D54 and D55 share an overlapping region of 15nt), 100 pmol of D47 and 100 pmol of the reverse primer D56 were mixed in the presence of 200 µM each dATP, dCTP, dGTP and dTTP and 1x Taq DNA polymerase buffer (10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$) in a final volume of 49.5 µL. After addition of 0.5 µL of Taq DNA polymerase (5 u/µL), the mixture was subjected to a 30 cycle PCR (4 min 94 °C, 30x [30 sec 94 °C, 30 sec 45 °C, 1 min 72 °C], 7 min 72 °C, 4 °C) using an Eppendorf Mastercycler ep gradient S. The PCR solution was diluted with water to 100 µL, extracted first with 100 µL PCI (phenol-chloroform-isoamyl alcohol, 25:24:1) and afterwards 100 µL CI (chloroform-isoamyl alcohol, 24:1) followed by ethanol precipitation (10 µL 3 M NaCl, 300 µL EtOH).

**Template preparation for U6 snRNA*. An aliquot of a pUC18 plasmid containing the U6 snRNA sequence form Saccharomyces cerevisiae was kindly provided by Dr. Penghui Bao/Dr. Olex Dybkov, Department Prof. Reinhard Lührmann, MPIbpc. In a 50 µL PCR reaction, 0.5 µg plasmid were used with 100 pmol D47 and 100 pmol D51. Other reagents and conditions as described above, annealing temperature was 47 °C. The quality and proper length of the ds DNA templates was checked on 2% agarose gels, 1x TAE (40 mM Tris acetate, 1 mM EDTA, pH 8.0) run at 100V, using a 100-bp DNA ladder as size standard.

DNA-catalyzed labeling reactions

The target RNA (0.5-4 nmol), deoxyribozyme and R_Δ were mixed in a 1:1:1.5 ratio and annealed in 25 mM HEPES pH 7.5, 15 mM NaCl, and 0.1 mM EDTA by heating to 95 °C for 2 min and slow cooling to room temperature over 15 min. Four equivalents of GTP-analog with respect to target RNA was added and the reaction performed at room temperature or at 37 °C for 3-5 h in 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM KCl, 100 µM $TbCl_3$, and 20 or 80 mM $MgCl_2$. The final concentration of target RNA was 5, 10 or 20 µM, and the final concentration of labeled GTP was therefore 20, 40 or 80 µM. Nucleic acids were precipitated by adding 10% (v/v) of 3 M NaCl solution and 3 times the volume of ice-cold 100% ethanol. The sample was kept on dry ice for 15 min. After centrifugation at 4 °C, 13200 rpm for 30 min, the sample was washed with 70 µL 75% ethanol (centrifugation at 4 °C, 13200 rpm for 5 min), dried and the pellet dissolved in 10 µL water. The labeled RNA was purified by denaturing PAGE (10-20% polyacrylamide, 0.7 mm, 20x30 cm, 35W). Isolated yields ranged from 25-62%.
Additional information for individual RNAs:
46 nt RNAs for FRET ruler: The crude mixture was subjected to DNase I treatment (conditions: 67 µM DNA, 0.13 u/µL DNase I, 10 mM Tris HCl pH 7.6, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 37 °C, 10 min; final volume 15 µL) prior to PAGE purification.
SAM II RNA for FRET: Position A13 was first labeled with C488-G and D22 in the presence of two equivalents of disruptor DNA (D58). The labeled RNA was purified by PAGE prior to the second labeling reaction at position A41 with F545-G and D23.
U6 RNA and ydaO RNA: Labeling reactions were performed in the presence of 1.5-2.0 equivalents of complementary disruptor DNAs (D52 or D53 for U6, D57 for ydaO). See page S6-S7 for sequence information of all DNA oligonucleotides and deoxyribozymes used.

Labeling of 2'-N_3-G-functionalized RNAs with alkynes by CuAAC

2'-N_3-G modified RNA (2.0 nmol) and 5.0 equivalents of respective alkyne (10 nmol) were mixed in 16 µL H_2O. 2.0 µL of a freshly prepared solution of CuBr (4 mM in DMSO/*t*BuOH 3:1) were mixed with 2.0 µL TBTA (8 mM in DMSO/*t*BuOH 3:1), and added to the aqueous solution, to give a final volume of 20 µL (final RNA concentration: 100 µM). The mixture was incubated at 37 °C for 4h and purified by PAGE (15% polyacrylamide, 0.4 mm, 35 W, 1 h 30 min). The isolated yields ranged from 33-55%.

Enzymatic ligation of 2AP-containing and GTP-labeled SAM II fragments by T4 DNA Ligase

Equimolar amounts of acceptor RNA (0.5-1.0 nmol), 5′-phosphorylated donor RNA and DNA splint (ratio 1:1:1) were annealed by heating to 95 °C for 2 min and slow cooling to room temperature (final RNA concentration was 15 µM of each strand). T4 DNA ligase buffer (final concentration 40 mM Tris HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP) and T4 DNA ligase (final concentration 0.5 u/µL) were added, and the reaction mixture was incubated at 37 °C for 5-6 h and analyzed by PAGE (15% polyacrylamide, 0.4 mm, 35 W, 1 h 30 min). The isolated yields ranged from 32-50%.

Monitoring SAM binding to G-labeled SAM II RNA by 2-aminopurine fluorescence

SAM titration experiments were performed essentially as previously described.[3] A 0.5 µM solution of 2-AP RNA was prepared in a total volume of 120 µL containing 50 mM KMOPS pH 7.5, 100 mM KCl and 2 mM $MgCl_2$. The $MgCl_2$ was added after the samples were heated to 95 °C for 2 min and slowly cooled to room temperature over 15 min. The solution was transferred to a quartz cuvette and held at 20 °C in the peltier controlled sample holder. SAM was pipetted manually in a way not to exceed a volume increase of 2% and the solution was mixed with a pipette after each titration step. The spectra were recorded from 320 to 500 nm (excitation wavelength 308 nm, data interval 1 nm, scan rate 120 nm/min, slit widths 10 nm).

To determine the apparent binding constants $K_{D,app}$ the increase in fluorescence after each titration step was followed via integration of the area between 325 and 500 nm (all integrated spectra were background and dilution corrected). The values for changes in fluorescence (F_x-F_{min}) were compared to the initial value in the absence of SAM (F_{min}). The relative fluorescence Y was plotted against the concentration of SAM (Figure S14) and the data were fit using the following equation.

$$Y = \frac{F_x - F_{min}}{F_{min}} = \frac{Y_{max} \cdot [SAM]}{(K_{D,app} + [SAM])}$$

F_x corresponds to the fluorescence value at a particular [SAM]; F_{min} corresponds to the initial fluorescence value in the absence of SAM; [SAM] corresponds to the total SAM concentration at a particular titration step.

Monitoring RNA folding of SAM-II RNA via FRET

A 0.1 µM solution of the C488-G and F545-G labeled RNA was prepared in a total annealing volume of 120 µL (118.8 µL annealing volume, if titration experiment is performed with SAM ligand) containing 50 mM KMOPS pH 7.5 and 100 mM KCl. The samples were heated to 95 °C for 2 min and slowly allowed to cool to room temperature over 15 min. For all measurements with SAM ligand, 1.2 µL of a 1 mM SAM solution (final concentration SAM 10 µM) was added and the mixture kept at room temperature for additional 15 min for equilibration. The solution was transferred to a quartz cuvette and held at 20 °C in a temperature-controlled sample holder. $MgCl_2$ was titrated manually in a way not to exceed a volume increase of 2% per titration step. The spectra were recorded from 506 to 700 nm (excitation wavelength 496 nm, data interval 1 nm, scan rate 600 nm/min, slit widths 10 nm) and 575 to 700 nm (excitation wavelength 565 nm, data interval 1 nm, scan rate 600 nm/min, slit widths 10 nm) for the double fluorophore-labeled SAM II. The mono-labeled RNAs were treated in the same way for recording titration curves with only donor-labeled RNA and only acceptor-labeled RNA.

The FRET efficiency was calculated by the following equation:[4]

$$E = 1 - \frac{I_{DA}}{I_D}$$

I_{DA} corresponds to the intensity of the double-labeled SAM II at 520 nm (donor emission); I_D corresponds to the intensity of the donor-only labeled SAM II at 520 nm.
The data were fit by using the following equation:[5]

$$E_{obs} = \left(E_{low} + E_{high} \cdot K \cdot [Mg^{2+}]^n\right)/(1 + K \cdot [Mg^{2+}]^n)$$

E_{obs} is the observed FRET as a function of $[Mg^{2+}]$; E_{low} and E_{high} correspond to the minimal and maximal E values; *K* is the equilibrium constant that relates the concentration of unfolded and folded states, *n* is the Mg^{2+} Hill coefficient.[6]
$[Mg^{2+}]_{1/2}$ was calculated with the following equation:

$$[Mg^{2+}]_{1/2} = K^{-1/n}$$

UV melting curve analysis

Complementary RNA strands were mixed in a 1:1 ratio to give a final concentration 0.4 µM (R23 and R24 for samples in Figure 3c) or 2.0 µM (R1 and R28 for samples in Figure S11) of each strand in the presence of 10 mM KH_2PO_4 and 150 mM NaCl at pH 7.0. The solutions were transferred to quartz cuvettes (path length 1 cm) and the solution was covered with a layer of silicon oil. Absorbance versus temperature profiles were recorded at 250 nm, 260 nm, 270 nm and 280 nm on a Cary-100 spectrophotometer equipped with a multiple cell holder and a peltier-controlled temperature device. For each sample two full heating and cooling cycles (15-95 °C, 4 ramps) were collected at a heating/cooling rate of 0.7 °C/min. Data points were recorded every 0.5 °C. The % hyperchromicity was calculated as 100*(A(T)-A(min))/A(min) and plotted versus temperature. The T_m values are given as the maximum of the first derivative of the hyperchromicity. All calculations and analyses were performed with Kaleidagraph (Synergy Software).

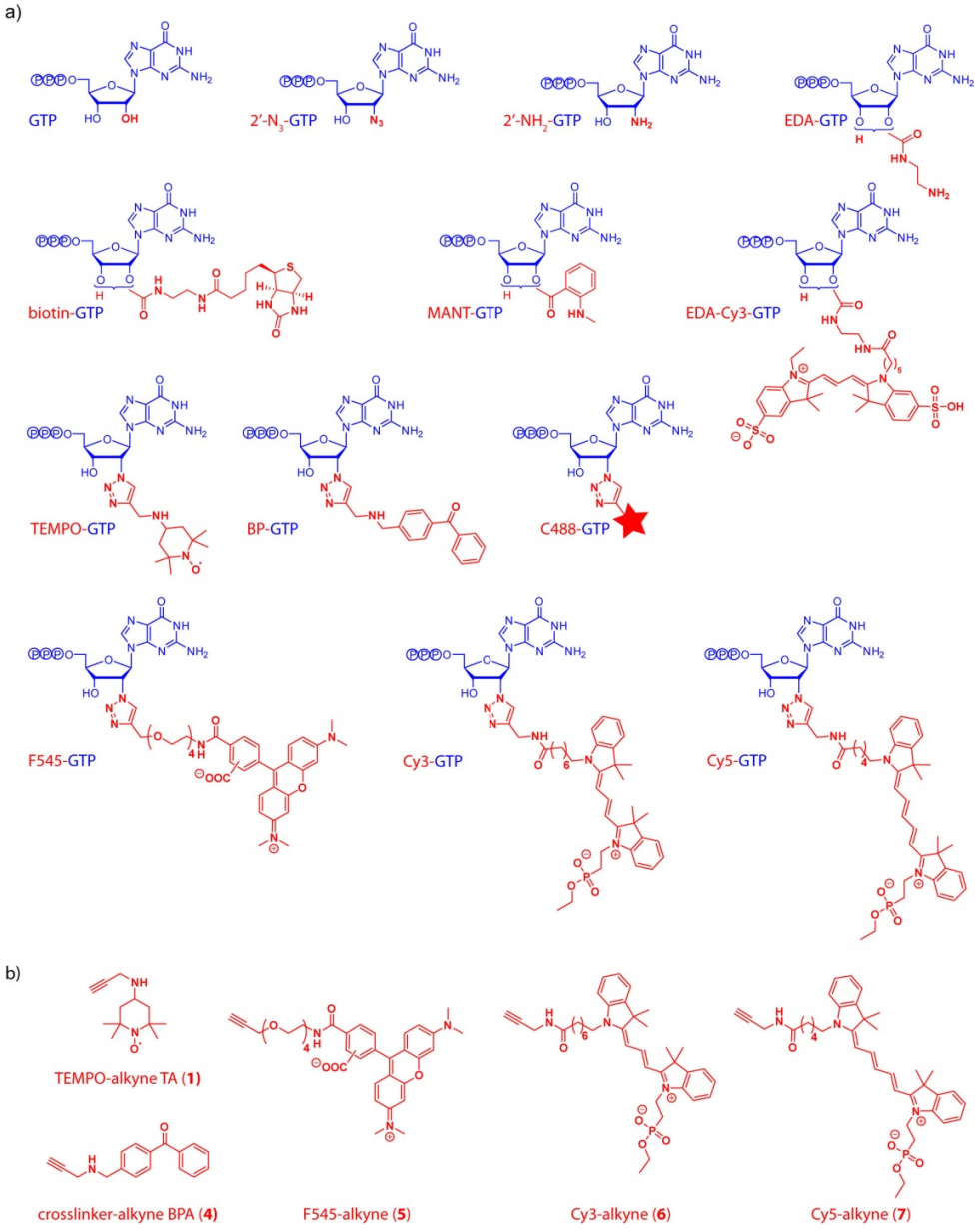


Figure S1. a) Structures of commercially available GTP-analogs: GTP, 2'-N_3-GTP, 2'-NH_2-GTP, biotin-GTP, MANT-GTP, Cy3-EDA-GTP, and GTP-analogs synthesized in this work via CuAAC: TEMPO-GTP, BP-GTP, C488-GTP, F545-GTP, Cy3-GTP and Cy5-GTP; b) Structures of the synthesized TEMPO-alkyne TA (**1**) and crosslinker-alkyne BPA (**4**) as well as commercially available alkyne-derivatives of fluorophore F545 (**5**), Cy3 (**6**) and Cy5 (**7**). The chemical structure of C488 has not been released.

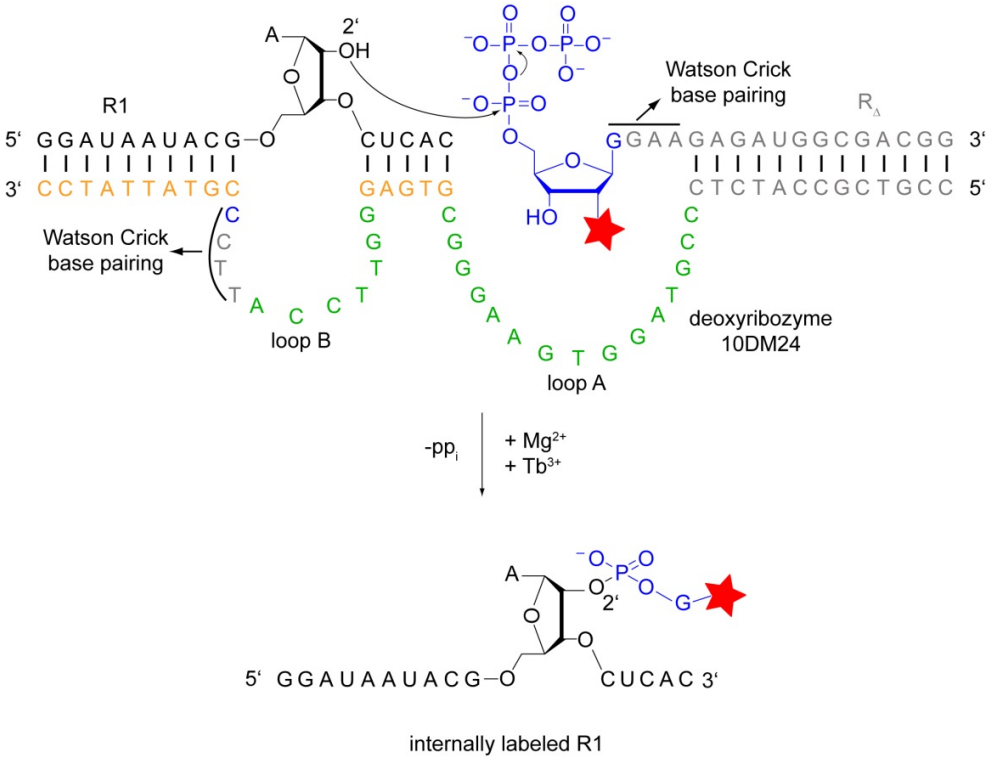


Figure S2. 10DM24-catalyzed attachment of 2'-labeled GTP to RNA R1 (shown in black). The deoxyribozyme 10DM24 contains two catalytic loops (depicted in green). The binding arms of 10DM24 that hybridize with the target RNA R1 and thus specify the reaction center are marked in orange. The crucial recognition nucleotide is depicted in blue, next to the three grey nucleotides that hybridize with the three 5'-terminal nucleotides of R_Δ to form the three-helix junction structure and the binding site for the labeled GTP.[7]

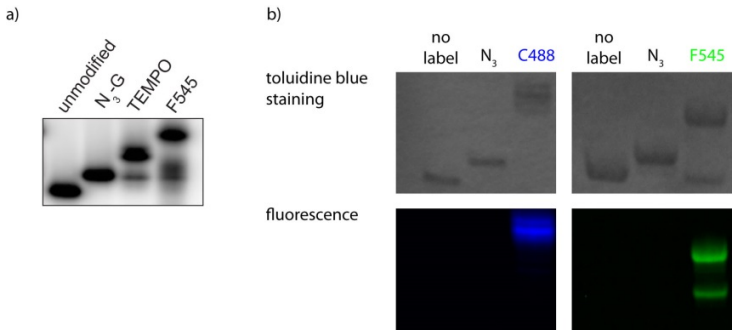


Figure S3. Labeling of 2'-N_3-G-modified RNA with alkynes by CuAAC. a) Analysis of analytical scale reactions for R18. Conditions: 1.0 µM 2'-N_3-G-R18, 5 µM TEMPO-alkyne or F545-alkyne, 0.4 mM CuBr/TBTA (final volume 10 µL) in H_2O/DMSO/*t*BuOH. b) Synthesis of fluorophore-modified short fragments of SAM-II RNA. Left: PAGE analysis after CuAAC of C488-alkyne to 2'-N_3-G-R18; right: PAGE analysis after CuAAC of F545-alkyne to 2'-N_3-G-R19.

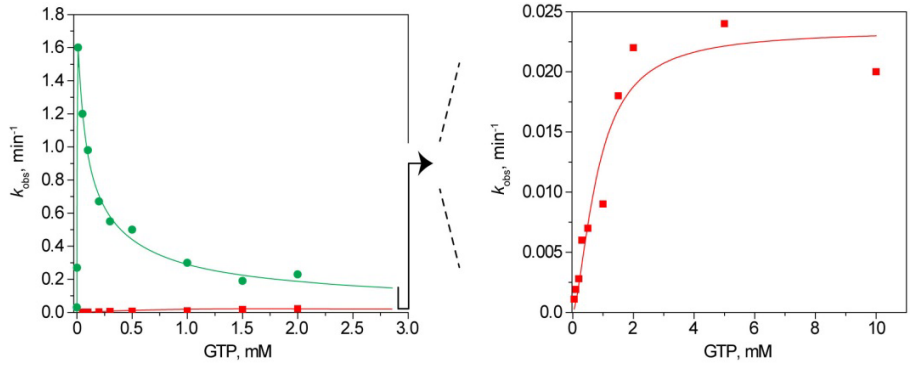


Figure S4. GTP concentration-dependence of labeling kinetics for R1 in absence (red) and presence (green) of Tb^{3+}. Conditions: RNA: DNA: R_Δ (1:5:10), 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM KCl, 80 mM Mg^{2+}, with or without 100 µM Tb^{3+}, 37 °C. The right panel is the zoomed view of the left panel for the k_{obs} of the reactions in the absence of Tb^{3+}.

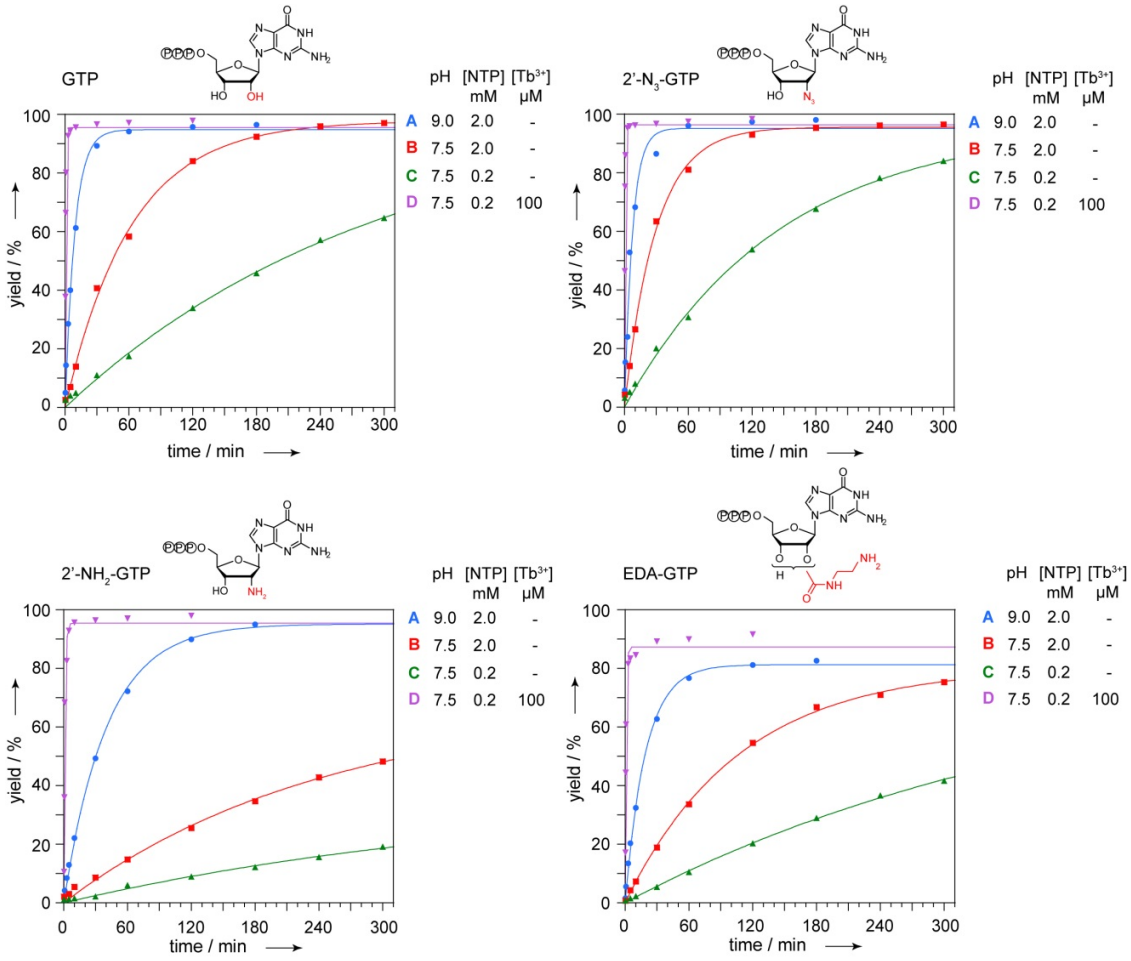


Figure S5. Kinetic plots for the determination of pH and concentration dependence of k_{obs} for the DNA-catalyzed ligation of 2'-modified GTP to RNA R1**.** The k_{obs} values are given in Table 1 in the manuscript. Reactions under condition D are too fast for accurate determination of k_{obs}, but are in the range of 1-2 min^{-1}.

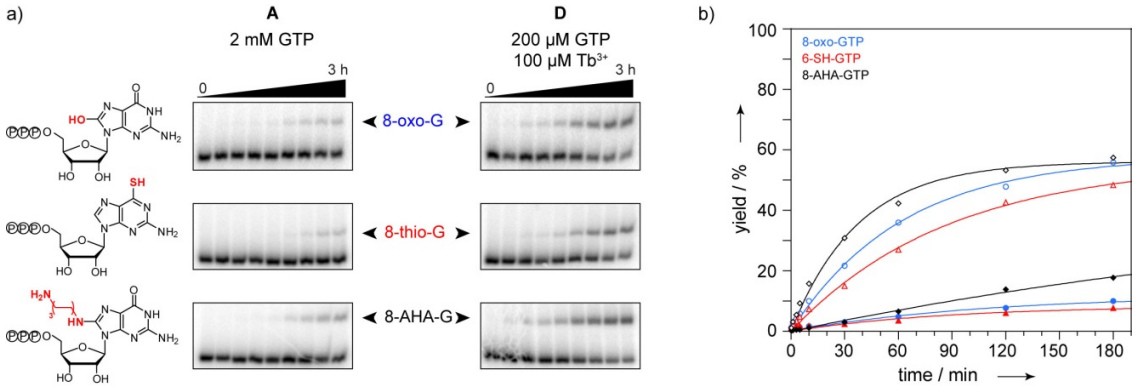


Figure S6. Reactivity of nucleobase-modified GTP analogs in presence and absence of Tb^{3+}. a) PAGE analysis of nucleobase-modified 8-oxo-GTP, 6-thio-GTP and 8-[(6-Amino)hexyl]-amino-GTP (8-AHA-GTP) attachment to RNA R1 (condition A and D are the same as described in Figure S5). b) Kinetic plots of reactions shown in a), closed symbols for condition A, open symbols for condition D.

Table S2. Examination of sequence context at labeling sites for GTP attachment under three different conditions of GTP and Mg^{2+} concentrations.

		3 mM GTP 100 mM Mg^{2+}			200 µM GTP 80 mM Mg^{2+}			200 µM GTP 80 mM Mg^{2+} 100 µM Tb^{3+}		
			yield (%)[b]			yield (%)[b]			yield (%)[b]	
RNA	Sequence context[a]	k_{obs} (min^{-1})	10min	5h	k_{obs} (min^{-1})	2h	5h	k_{obs} (min^{-1})	10min	2h
R1	**GAC**	0.10	59	96	0.004	25	46	0.67	72	95
R2	**GA**G	0.75	93	99	0.013	66	85	~2	95	99
R3	**GA**A	0.62	89	96	0.014	69	80	~2	92	99
R4	**GA**U	0.11	64	99	0.004	22	33	1-2	69	77
R5	A**AG**	0.05	36	86	0.003	13	24	~2	85	91
R6	A**AA**	0.03	24	83	0.003	11	25	~2	82	86
R7	A**AC**	0.007	8	76	~0.0001	4	5	0.02	15	74
R8	A**AU**	0.0004	5	17*	<0.0001	1	2	0.03	23	61
R9	C**AG**	0.04	27	73	0.002	11	25	1-2	77	87
R10	C**AA**	0.02	17	62	0.0005	7	14	1-2	62	73
R11	C**AC**	0.01	11	67	~0.0001	4	8	0.17	51	74
R12	C**AU**	0.0006	3	19*	~0.0001	5	5	0.03	23	65
R13	U**AG**	0.20	75	93	0.006	31	59	~2	95	99
R14	U**AA**	0.19	62	84	0.006	34	61	~2	82	87
R15	U**AC**	0.03	23	75	~0.0001	5	10	0.08	34	70
R16	U**AU**	0.001	5	25*	~0.0001	5	9	0.03	13	40

[a] The original sequence context is highlighted in orange and mutated nucleobases are marked in blue. [b] Yield indicates the fraction ligated in % at the given time point. * yields increased to >70% after 24 h (see Figure S7b). For yields < 20% in 5 h, the k_{obs} values were obtained from a linear fit. For all k_{obs} values given as ~2 min^{-1} the conversion was > 60% at the first time point (20 sec).

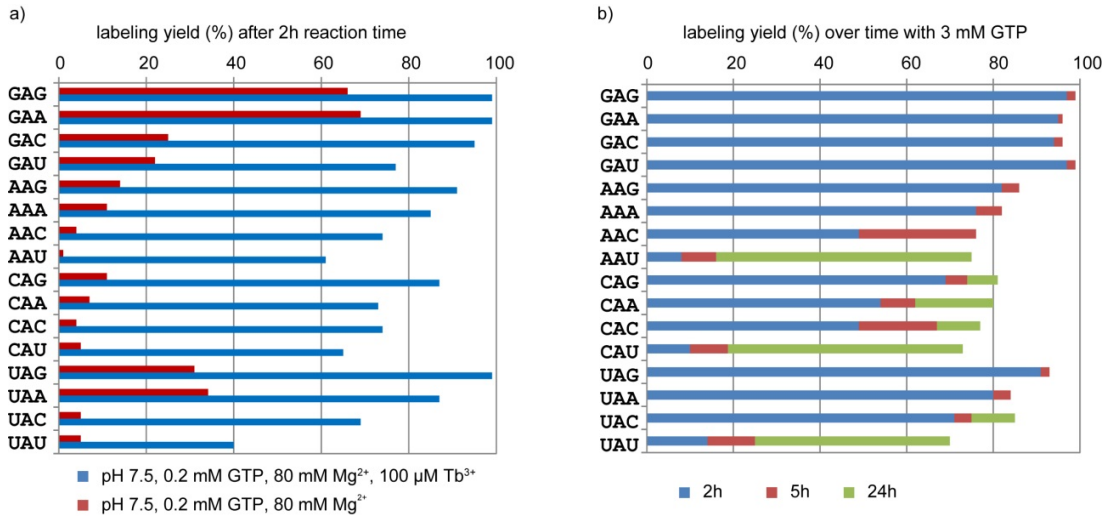


Figure S7. Labeling efficiencies of GTP in different sequence contexts of R1-R16. a) Comparison of labeling yield in the presence and absence of 100 µM Tb^{3+} with 200 µM GTP after 2h reaction time. The yield is significantly higher in the presence of Tb^{3+}; 13 out of 16 RNAs are >70% labeled. b) Time course of GTP labeling efficiency at 3 mM GTP, pH 9.0 and 100 mM $MgCl_2$ after 2, 5 and 24 h. See data in Table S2 and compare Figure 1e in the manuscript.

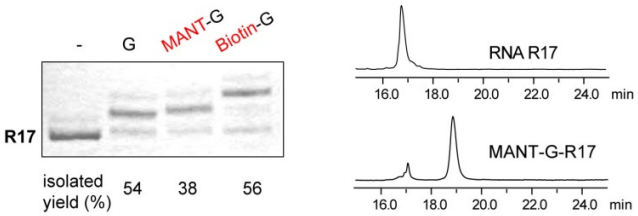


Figure S8. DNA-catalyzed labeling of R17 with ribose-labeled GTPs using commercial carbamoyl-linked MANT- and Biotin-GTP in comparison to unmodified GTP. The HPLC traces show analysis of unmodified and MANT-G labeled R17; Anion exchange HPLC Dionex DNA Pac PA200, detection at 260 nm.

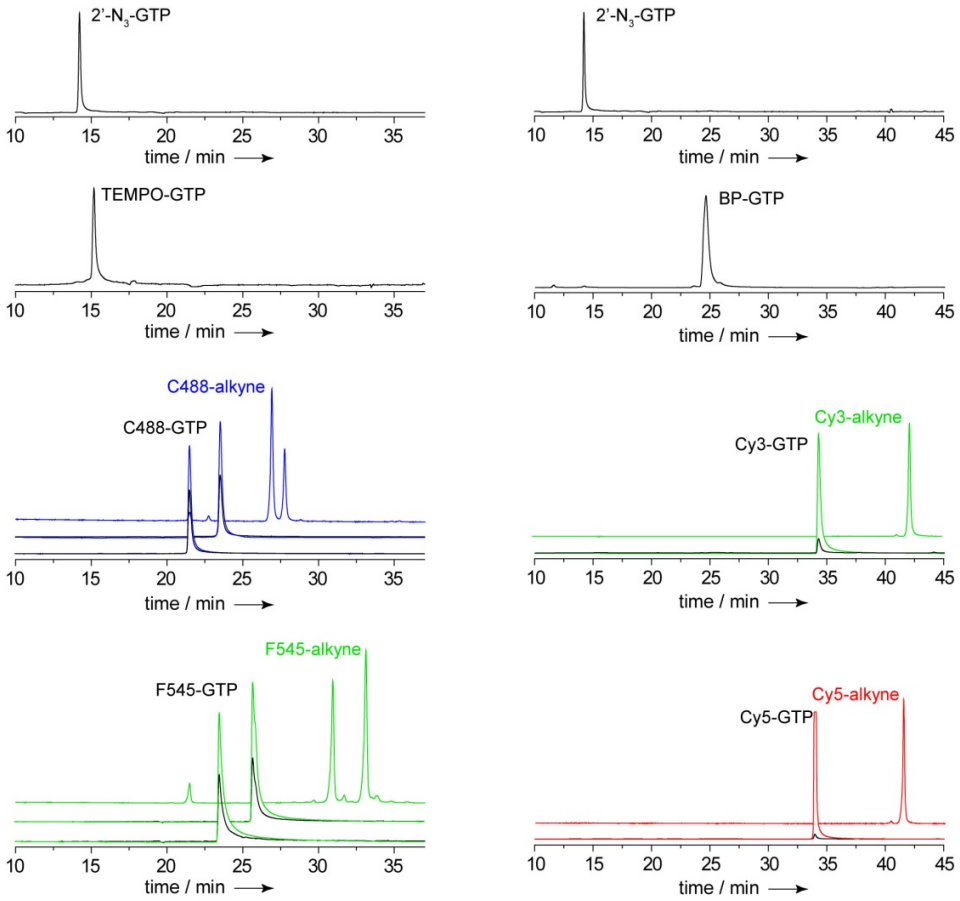


Figure S9. RP-HPLC analysis of clicked GTP analogs. Nucleosil 100-5 C18 HD column, 4 x 250 mm, 0 – 70% B in 40 min, 40 °C; Eluent A: 100 mM TEAA in H_2O; Eluent B: 20 mM TEAA in H_2O:MeCN 1:4; detection at 260 nm, 488 nm (blue), 546 nm (green), 642 nm (red). The two isomers of C488-GTP and F545-GTP were isolated separately and tested in kinetic labeling reactions. For C488-GTP and F545-GTP, the isomers showed similar labeling efficiencies and reaction rates. For further labeling reactions with C488-GTP, the fraction with a retention time of 21 min was used. In case of F545-GTP the fraction with 24 min retention time was chosen for further use.

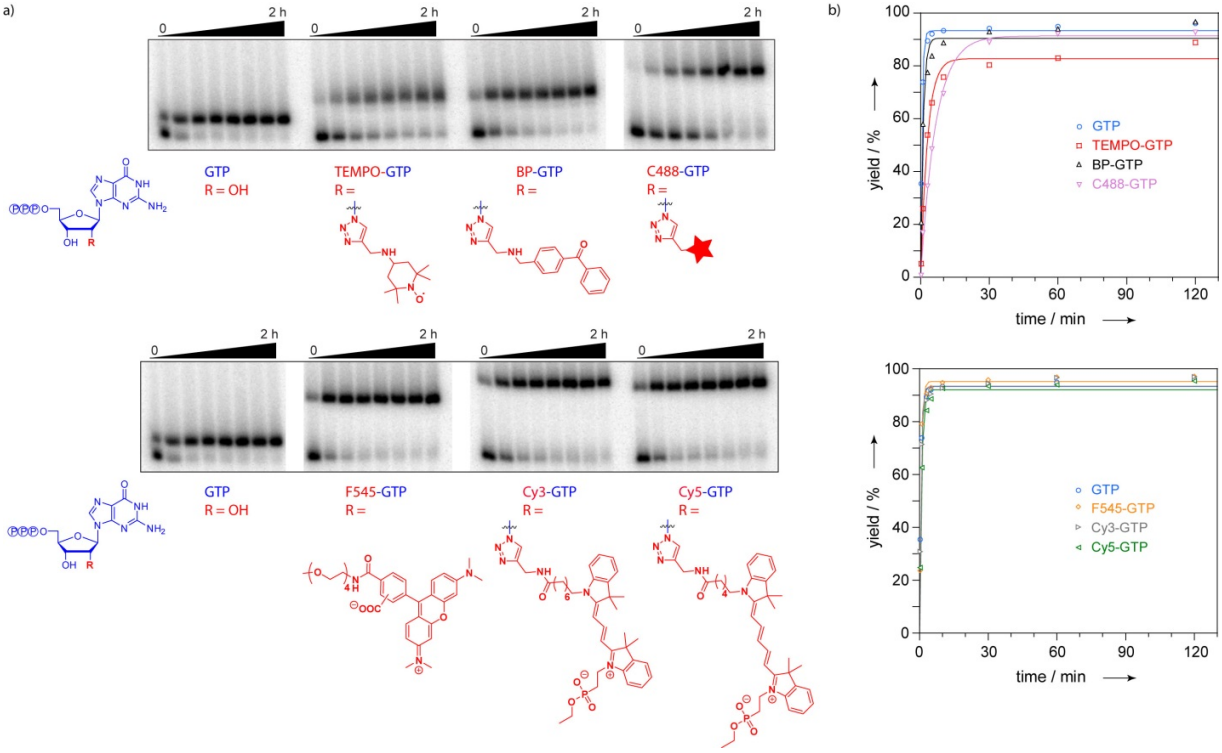


Figure S10. Labeling of RNA R1 with "click-labeled" GTP analogs. a) gel images and b) kinetic plots of reactions at following conditions: RNA: DNA: R_{Δ} 1:5:10; final concentration RNA: 0.2 µM, 100 µM GTP-analog, 100 µM Tb^{3+}, 80 mM Mg^{2+}, 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM KCl, 37 °C. For most analogs shown here, the k_{obs} values were not determined accurately due to very fast reactions (> 1 min^{-1}) and >50% conversion at first timepoint.

Table S3. Mg^{2+} dependence of DNA-catalyzed GTP attachment to RNA R1.[a]

Mg^{2+} mM	k_{obs} min^{-1}	$t_{1/2}$ min
80	0.7	1
40	0.4	2
20	0.2	4
10	0.03	23
7	0.008	87
3	0.0006	19 h

[a] Conditions: 50 mM HEPES pH 7.5, 150 mM NaCl, 2 mM KCl, 200 µM GTP, 100 µM Tb^{3+}. $t_{1/2}= \ln(2)/k_{obs}$

Table S4. ESI-MS of RNA R1 and labeled derivatives.[a]

RNA	mol. wt. calcd.	mol. wt. found [ESI-MS]
R1	5088.1	5088.6
R1-G	5433.4	5433.6
R1-N_3-G	5458.4	5458.6
R1-TEMPO-G	5667.7	5668.2
R1-Cy3-EDA-G	6132.2	6132.6

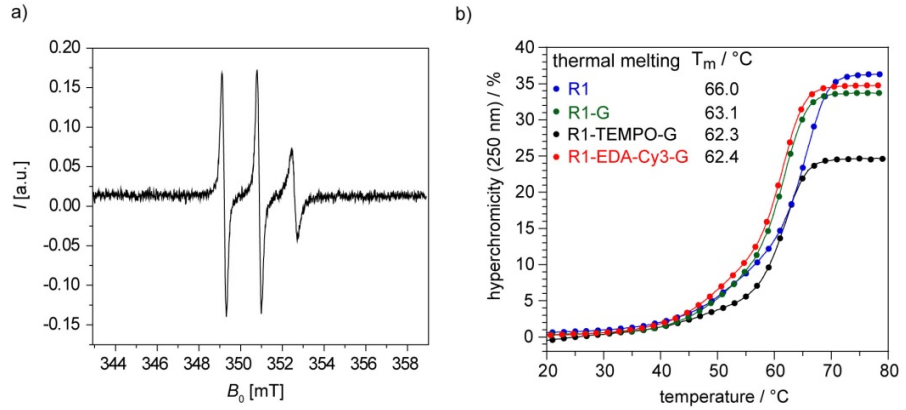


Figure S11. a) X-band EPR spectrum of 25 µM R18-TEMPO-G, in 10 mM KH_2PO_4 and 150 mM NaCl (in 20 µL). A Bruker ELEXsys Spectrometer with an ELEXSYS super high sensitivity probehead was used; the incident microwave power was 2 mW, the field modulation amplitude was 1.0 G at a 100 kHz frequency. Sample temperature was set at 25 °C; 64 averaged scans. b) UV melting curves of labeled duplexes containing R1and R28 with indicated guanosine labels attached at 2'-A11 of R1. Conditions: 2.0 µM duplex in the presence of 10 mM KH_2PO_4 pH 7.0 and 150 mM NaCl.

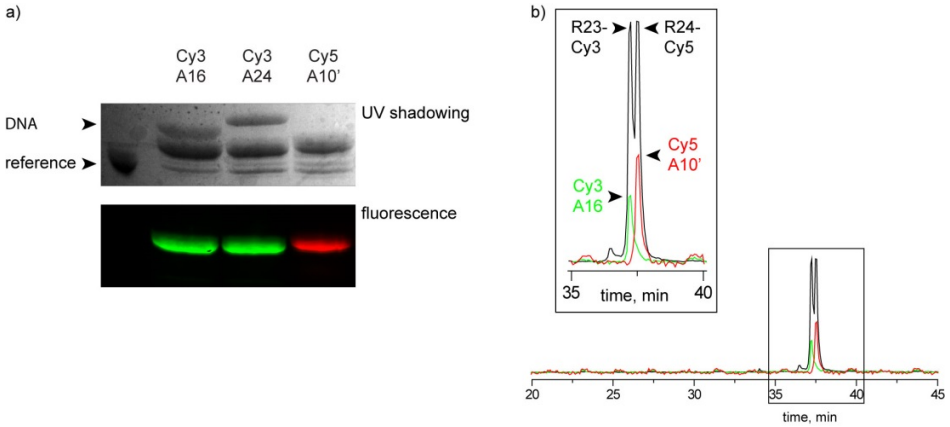


Figure S12. a) PAGE analysis of Cy3-G labeling of RNA A (R23) and Cy5-G labeling of RNA B (R24) for FRET ruler example. b) HPLC quality control of the labeled FRET ruler duplex. The absorbance at 260nm (black) shows that the labeled RNA strands were mixed in the desired 1:1 ratio. To confirm the successful labeling of the FRET ruler sequences with Cy3- and Cy5-G the absorbance was also measured at 550 nm (green) and 646 nm (red). The area ratio of Cy3 and Cy5 corresponds to the ratio of extinction coefficients at the given wavelengths.

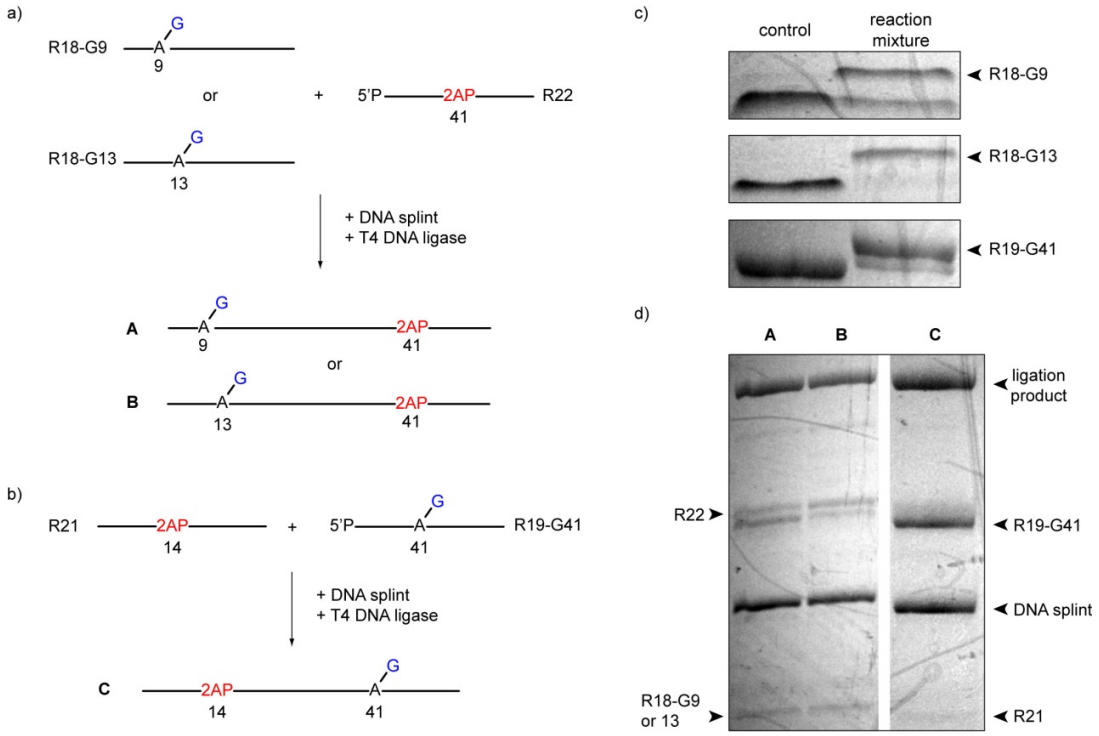


Figure S13. a) and b) show a schematic depiction for the preparation of 2AP- and G-labeled SAM-II RNAs. c) PAGE purification of the synthesis of G-labeled starting material R18-G9, R18-G13 and R19-G41 (15% polyacrylamide, 0.4 mm, 35 W, 1 h 30 min). d) PAGE purification of enzymatic ligation of 2AP- and GTP-labeled SAM II fragments (15% polyacrylamide, 0.4 mm, 35 W, 1 h 30 min). **A**: ligation of R18-G9 and R22; **B**: ligation of R18-G13 and R22; **C**: ligation of R21 and R19-G41.

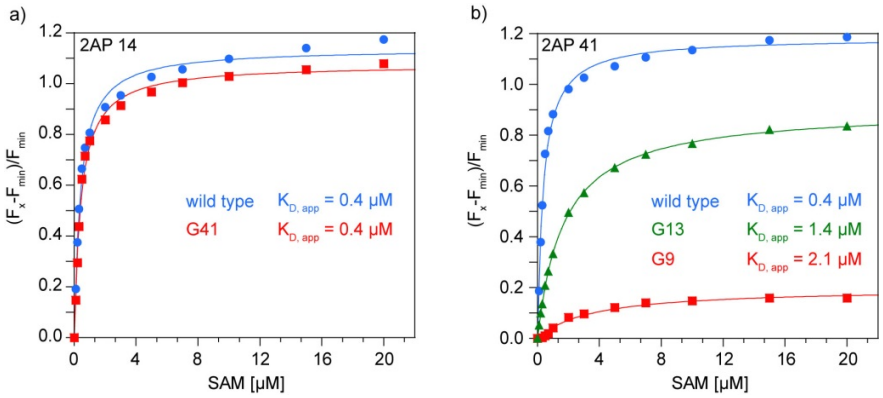


Figure S14. SAM binding to G-labeled SAM II RNA monitored by changes in 2-aminopurine (2AP) fluorescence. G = guanosine label. a) 2AP at position14: Comparison of SAM binding of wild type SAM II and SAM II with G-label on position A41. b) 2AP at position 41:Comparison of SAM binding of wild type SAM II and SAM II with G-label on position A13 or A9. The reduced amplitudes for G13 and G9 can be rationalized by the close proximity of the flexible G and 2AP in the folded structure, resulting in partial quenching of fluorescence. Conditions: 0.5 µM RNA, 2 mM Mg^{2+}, 50 mM KMOPS pH 7.5, 100 mM KCl, 20 °C.

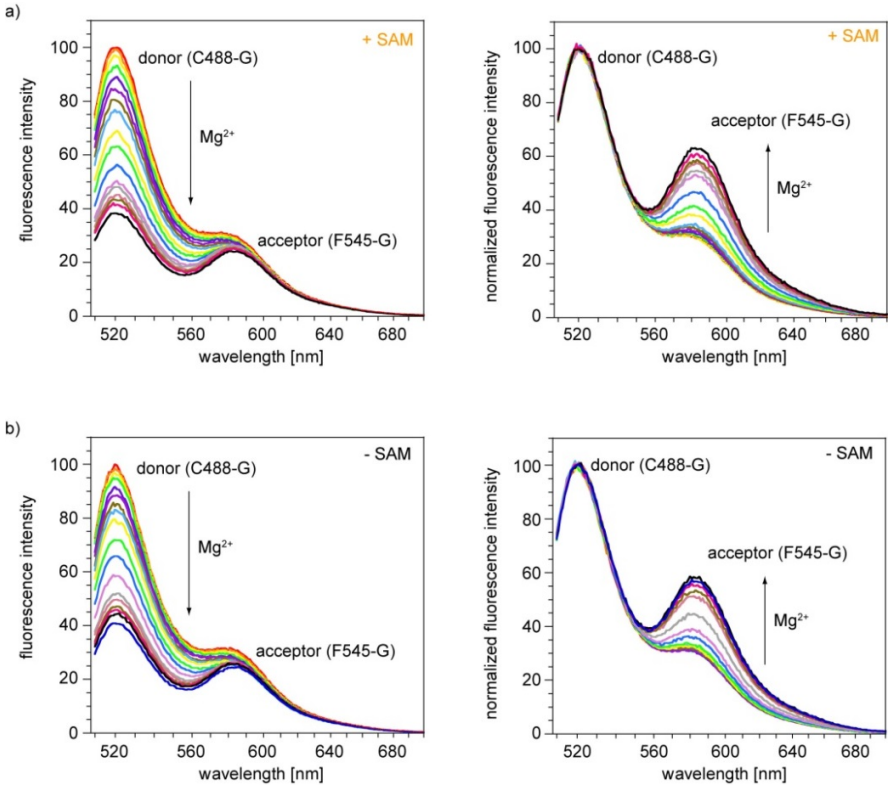


Figure S15. Monitoring of RNA folding by FRET. Fluorescence spectra for double-labeled SAM II-RNA with C488-G at A13 and F545-G at A41. a) Spectra were recorded in the presence of 10 µM SAM. b) Spectra were recorded without SAM. For a) and b): 0.1 µM double-labeled R20, 50 mM KMOPS pH 7.5, 100 mM KCl, 20 °C. left: original spectra after dilution correction. The donor emission peak is decreasing with increasing Mg^{2+} concentration; right: fluorescence emission spectra normalized at 519 nm (donor emission). The acceptor emission is increasing with increasing Mg^{2+} concentration due to the FRET effect. Mg^{2+}-concentrations ranged from 1 µM to 40 mM.

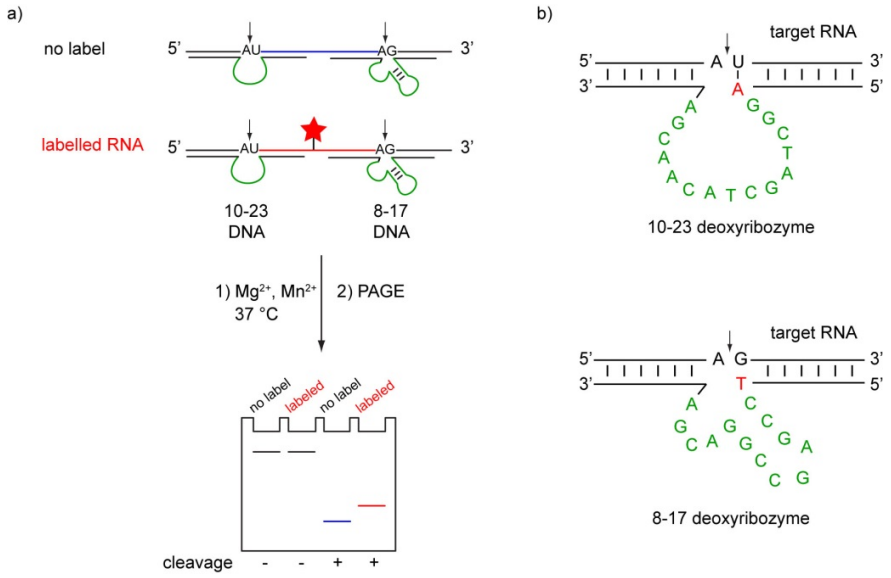


Figure S16. a) Schematic depiction of a 10-23 and 8-17 deoxyribozyme-catalyzed RNA cleavage reaction to determine the labeling efficiency when labeled and unlabeled RNAs cannot be separated due to their large size. The schematic PAGE analysis shows that the cleaved fragment of the labeled RNA (red) migrates slower than the fragment of non-labeled RNA (blue). b) Structures of the 10-23 and 8-17 deoxyribozyme (catalytic core marked in green). For both enzymes the sequence of the binding arms can be chosen according to the target RNA, as long as Watson-Crick base pairing is maintained. 10-23 can generally cleave R↓Y (R = purine, Y = pyrimidine), whereas 8-17 can best cleave A↓G.[8,9]

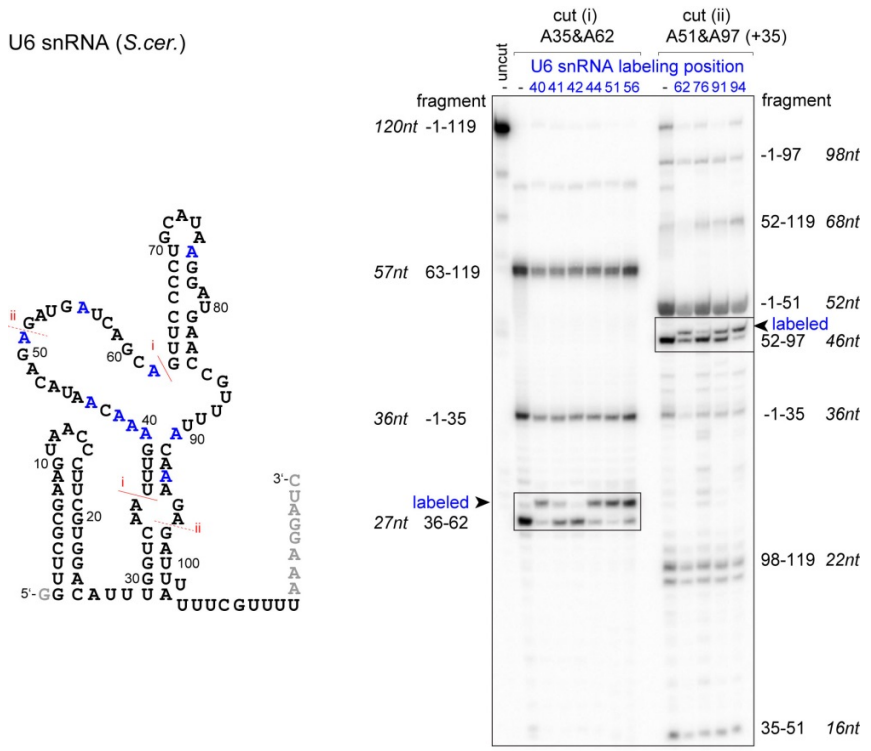


Figure S17. Analysis of labeling efficiency on U6 snRNA with MANT-GTP. In the sequence of U6 snRNA (*S.cerevisiae*) addressed labeling positions are indicated in blue, cleavage sites are marked in red. The labeled products and the control were cut at position A35 and A62 (cut i) or at position A51 and A97 (cut ii), respectively. The boxed areas of the gel image are shown in Figures 5d,e.

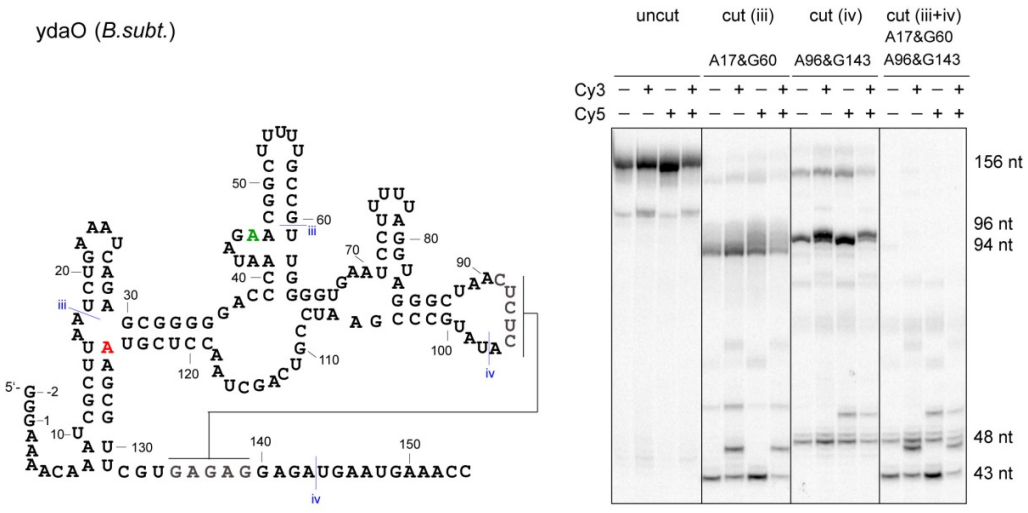


Figure S18. Sequence of ydaO RNA (*B.subtilis*) with labeling sites marked in green for Cy3 (position A46) and red for Cy5 (position A125). Autoradiograph of PAGE analysis of uncut and cut RNA samples. The RNA samples were either cut at A17 and G60 (cut iii) or at A96 and A143 (cut iv, cleavage sites are marked in blue). The efficiency of Cy3-G labeling at A46 was 60%, and that of Cy5-G labeling at A125 was 38%.

References for supporting information

[1] L.-W. Guo, A. R. Hajipour, K. Karaoglu, T. A. Mavlyutov, A. E. Ruoho, *ChemBioChem* **2012**, *13*, 2277-2289.
[2] B. Samanta, C. Höbartner, *Angew. Chem. Int. Ed.***2013**, 52, 2995-2999.
[3] A. Haller, U. Rieder, M. Aigner, S.C. Blanchard, R. Micura, *Nat. Chem. Biol*. **2011**, *7*, 393-400.
[4] D. Klostermeier, C. Hammann. *RNA Structure and Folding;* Walter de Gruyter: Berlin/Boston, **2013**.
[5] D. A Baum, S. K. Silverman, *Angew. Chem. Int. Ed.* **2007**, *46*, 3502-3504.
[6] S. K. Silverman, T. R. Cech, *Biochemistry* **1999**, *38*, 14224-14237.
[7] C. Höbartner and S. K. Silverman, *Angew. Chem. Int. Ed.* **2007**, *46*, 7420–7424.
[8] S. K. Silverman, D. A. Baum, *Methods Enzymol.* **2009**, *469*, 95-117.
[9] M. J. Cairns, A. King, L.-Q. Sun, *Nucl. Acids. Res.* **2003**, *31*, 2883-2889.