Synthesis and Incorporation of the Phosphoramidite Derivative of 2′-O-Photocaged 3′-S-Thioguanosine into Oligoribonucleotides: Substrate for Probing the Mechanism of RNA Catalysis

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Supporting Information

ABSTRACT: Oligoribonucleotides containing 3′-S-phosphorothiolate linkages possess properties that can reveal deep mechanistic insights into ribozyme-catalyzed reactions. "Photocaged" 3′-S-RNAs could provide a strategy to stall reactions at the chemical stage and release them after assembly steps have occurred. Toward this end, we describe here an approach for the synthesis of 2′-O-(o-nitrobenzyl)-3′-thioguanosine phosphoramidite starting from N2-isobutyrylguanosine in nine steps with 10.2% overall yield. Oligonucleotides containing the 2′-O-(o-nitrobenzyl)-3′-S-guanosine nucleotide were then constructed, characterized, and used in a nuclear pre-mRNA splicing reaction.

Oligonucleotides containing phosphorothiolate linkages, in which sulfur replaces the 3′- or 5′-bridging oxygen connected to the ribofuranose ring (Figure 1, II and III), serve as powerful biochemical probes to investigate fundamental features of enzyme catalysis.1−4 RNAs containing a 3′-S-phosphorothiolate linkage have been used in metal ion rescue experiments to elucidate the mechanism of metalloribozymes such as the group I intron, the group II intron, and the spliceosome.5−8 These studies rely on careful comparison of the 3′-O and 3′-S substrates and must include assays that monitor the chemical step. Despite broad utility of these substrates in the investigation of phosphoryl transfer reactions, complex assembly processes and conformational changes that accompany biological catalysis frequently mask analysis of the chemical step. Photocaging provides a well-established strategy to protect functional groups from chemical reactions until released by UV irradiation.9−11 In this respect, having access to "photocaged" 3′-S-modified RNAs could provide a strategy to stall reactions at the chemical stage and release them only after assembly steps have occurred. Moreover, photocaging provides the added advantage of increasing the stability of these phosphorothiolate-modified oligonucleotides by eliminating side reactions involving 2′-O-transphosphorylation.1

The 2′-O-(o-nitrobenzyl) derivatives of uridine,12 adenosine,13 cytidine,13 and guanosine14 have been synthesized and applied to the synthesis of oligoribonucleotides containing one or multiple 2′-O-photolabile groups (Figure 1, I†).12,13,15−18 Dinucleotides and trinucleotides containing a 2′-O-(o-nitrobenzyl) group were usually synthesized via a solution method involving condensation between the 3′-phosphate or 5′-phosphate of one nucleoside and the 5′-OH or 3′-OH group of another nucleoside.12,13,17 Longer oligoribonucleotides (>3-mer) containing 2′-O-(o-nitrobenzyl) groups could be

Figure 1. Structures of wild type, 3′-S-modified, and 5′-S-modified oligonucleotides.

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efficiently synthesized via solid-phase synthesis using 3′-phosphoramidite\textsuperscript{15,16} or 3′-H-phosphonates of 2′-O-(o-nitrobenzyl) nucleosides.\textsuperscript{18} After removal of the 2′-O-(o-nitrobenzyl) group by photolysis, these RNAs could initiate efficient and accurate ribozyme-catalyzed reactions.\textsuperscript{15,16} This RNA-caging approach has also been used to investigate spliceosome assembly during pre-mRNA splicing through introducing a 2′-O-(o-nitrobenzyl) group into the branch adenosine nucleotide, which blocks the first step of splicing, thereby allowing the study of the precatalytic stages during assembly.\textsuperscript{19} The synthesis of oligonucleotides containing a 2′-O-photocaged group and 3′-S-phosphorothiolate linkage via solid-phase synthesis.\textsuperscript{20} For the synthesis of 2′-O-(o-nitrobenzyl)-3′-thioguanosine phosphoramidite \textsuperscript{8} (Scheme 1), we adapted previous approaches for the synthesis of 2′-O-TBS-3′-thioguanosine phosphoramidite\textsuperscript{20} and 2′-O-methyl-3′-thioguanosine phosphoramidite.\textsuperscript{21} We chose the o-nitrobenzyl group in this study because the starting material o-nitrobenzyl bromide is commercially available and relatively inexpensive. Additionally, use of the o-nitrobenzyl group is well-established for the synthesis of 2′-O-photocaged RNAs. N°-Isobutryl-2′-O-(o-nitrobenzyl)guanosine (2) was prepared from N°-isobutyrylguanosine (1) in 47% yield as described previously, except that we quenched the reaction with dilute aqueous HCl.\textsuperscript{22} Reaction of 2 with (tert-butyl)diphenyldifluorosilane chloride gave the corresponding S°-silyl derivative in 94% yield. In the presence of 1.4 molar equiv of DMAP, the S°-silyl derivative reacted with 1.05 molar equiv of trifluoromethanesulfonyl chloride at 0 °C to give the corresponding 3′-triflate derivative 3 in 62% yield. When 2.0 molar equiv of DMAP and 1.5 molar equiv of trifluoromethanesulfonyl chloride were used, the reaction afforded 3′-triflate derivative 3 in 50% yield along with the guanosine 3′,O°-ditriflate derivative (~13% yield). Subsequent S°-2 substitution with 4 molar equiv of NaBr in refluxing acetone afforded 3′-bromo derivative 4 in 95% yield. Reaction of 4 with KSAc in DMF at 60 °C gave a mixture of the 3′-S-acetyl and 3′,4′-unsaturated derivatives in a 1.3/1 ratio. Desilylation of the mixture with TBAF+xH\textsubscript{2}O/AcOH in THF and purification by silica gel chromatography gave the desired pure 5′-deprotected derivative 5 in 54% yield over the two steps. Although the 3′-β-iodo derivative could be prepared by S°-2 substitution of 3 with NaI in 96% yield, the subsequent reaction with KSAc, followed by desilylation of the reaction mixture, produced predominantly the 3′,4′-unsaturated derivative and gave the desired 5′-deprotected derivative 5 only in 19% yield. Protection of 5 with DMTCl in pyridine for 24 h afforded the 5′-O-DMT ether 6 in 95% yield. The 3′-S-acetyl group of 6 was selectively removed by treatment with a 5:1 mixture of guanidine hydrochloride and guanidine\textsuperscript{23} to give 3′-SH derivative 7 in 90% yield. Phosphitylation of compound 7 with 2-cyanoethyl N°,N°-disopropylchlorophosphoramidite yielded the corresponding 2′-O-(o-nitrobenzyl)-3′-thioguanosine phosphoramidite 8 in 85% yield. Incorporation of phosphoramidite 8 into an 18-mer oligonucleotide was then accomplished by manual coupling using our previously described protocol.\textsuperscript{24} The modified oligonucleotide, 5′-UUU AG\textsubscript{3.S.2}-3′-(o-NBn) A GGU UGC UGC \scriptsize{3652} \footnotesize{dx.doi.org/10.1021/jo4028374 J. Org. Chem. 2014, 79, 3647–3652}
hydrolysis, silver ion cleavage, and RNase T1 cleavage (Figure 2). For comparison, we prepared the corresponding 2′-photocaged wild-type RNA, 5′-UUU AG₁₋₂-O-(sNBn) A GGU UGC UGC UUU-3′ and 2′-photocaged 3′-S-substrate (5′-UUU AG₁₋₂-O-(sNBn) A GGU UGC UGC UUU-3′): (A) 5′-pUUUAG₂-OH₃-Sp₃-3′, (B) 5′-pUUUAG₂-O₃-Sp₃-3′ (2′-O₃-S-cyclic phosphoryl 5-mer), (C) 5′-pUUUAG₂-O₃-Sp₃-3′ (3′-S-phosphoryl 5-mer), and (D) 5′-pUUUA-G₂₋₁-O-(sNBn)-Sp₃-3′. The numbers in the picture are assigned for the ladders of alkaline hydrolysis and T1 treatment of these two 18-mer RNAs.

Figure 2. Alkaline hydrolysis, silver ion cleavage, and RNase T1 cleavage of 2′-photocaged 3′-O-substrate (5′-UUU AG₁₋₂-O-(sNBn) A GGU UGC UGC UUU-3′) and 2′-photocaged 3′-S-substrate (5′-UUU AG₁₋₂-O-(sNBn) A GGU UGC UGC UUU-3′): (A) 5′-pUUUAG₂-OH₃-Sp₃-3′, (B) 5′-pUUUAG₂-O₃-Sp₃-3′ (2′-O₃-S-cyclic phosphoryl 5-mer), (C) 5′-pUUUAG₂-O₃-Sp₃-3′ (3′-S-phosphoryl 5-mer), and (D) 5′-pUUUA-G₂₋₁-O-(sNBn)-Sp₃-3′. The numbers in the picture are assigned for the ladders of alkaline hydrolysis and T1 treatment of these two 18-mer RNAs.

Figure 3. (a) Schematic for synthesis of ACT1 yeast splicing substrates. RNA is shown as black lines and boxes; the DNA splint is shown in gray. The boxes represent the exons, and the black line represents the intron. The red star indicates the position of the radiolabel. The modified 2′-O-photocaged residue is the last nucleotide of the intron. (b) Schematic of the two steps of splicing. (c) Exon ligation of the 3′-O-pc and 3′-S-pc substrates in an in vitro splicing assay (60 mM K₂PO₄ (pH 7), 3% PEG 8000, 2.5 mM MgCl₂, 2 mM ATP, 40% yeast extract in the initial incubation); “metal” indicates the addition of 0.5 mM MgCl₂, MnCl₂, or nothing as indicated in the main text.
position and shows that the photocage can be removed efficiently.

We next tested whether the 2'-O-photocaged 3'-S-oligonucleotide could be used to stage nuclear pre-mRNA splicing reactions at the chemical step. Nuclear pre-mRNA splicing is catalyzed by the spliceosome, a complex ribonucleoprotein particle that undergoes assembly steps and rearrangements en route to the chemical steps.23 We incorporated the photocaged 3'-S-oligonucleotide into a widely used ACTI yeast splicing substrate and used it in metal rescue experiments with the spliceosome (Figure 3a).28 The oligonucleotide was designed to contain the photocaged residue at the intron terminus, such that the phosphorothioate was in the leaving group position for the next step of splicing. To assemble the full-length substrate, we 25P-radioabeled the O- and S-containing oligonucleotides and then ligated them to two RNA oligonucleotides generated by in vitro transcription (Figure 3a). Next we incubated these RNA substrates in splicing-competent Saccharomyces cerevisiae extracts so that spliceosomes could assemble on them and catalyze branching (Figure 3b). The lariat intermediate product of the branching step runs aberrantly slowly and can be seen as the top band of the splicing gel (Figure 3c).

With the photocage in place, the spliceosomes stalled prior to exon ligation regardless of the identity of the atom (O or S) in the leaving group position (Figure 3c, lanes 1 and 7). To remove the photocage and permit exon ligation (Figure 3b), we irradiated spliceosomes for 5 min with 365 nm UV light (lanes 4 and 10–12). To initiate exon ligation, we then added additional MgCl2, MnCl2, or nothing and assayed for exon ligation. As expected, appearance of the exon ligation of the 3'-O-pc substrate proceeded efficiently in the UV-treated spliceosomes (lanes 4–6) but not without irradiation (lanes 1–3). Exon ligation of the 3'-S-pc substrate only proceeded efficiently with UV treatment and in the presence of MnCl2 (compare lane 12 with lanes 7–11). These results demonstrate that the photocage comprises an effective block to splicing chemistry and that, upon removal, splicing occurs in the presence of an added metal ion. The dependence of the 3'-S-pc substrate reaction on the “thiophilic” manganese- (II) ion reflects the role of the metal ion in leaving group stabilization.29

In summary, we have developed an efficient synthesis of the phosphoramidite derivative of 2'-O-(o-nitrobenzyl)-3'-thio-
guanosine starting from N2-isobuturylguanosine in nine steps. From this phosphoramidite, an 18-nucleotide RNA containing a 2'-O-photolabile group adjacent to a 3'-S-phosphorothiolate linkage was successfully synthesized by the solid-phase synthesis. Irradiation with UV light released the photocaging group without affecting the integrity of the 3'-S modification. This capability can enable effective use of the 3'-sulfur modification to perform metal rescue experiments even in systems that undergo complex assembly and conformational changes en route to the chemical step. As proof-of-principle, we have incorporated this modified RNA into a model yeast pre-
mRNA splicing substrate and used it to probe for catalytic metal ion interactions in the spliceosome.30

■ EXPERIMENTAL SECTION

N2-Isobuturyl-2'-O-(o-nitrobenzyl)guanosine (2). Under argon, N2-isobutyrylguanosine 1 (1.72 g, 4.86 mmol) was treated with NaH (307 mg, 95%, 12.15 mmol) in DMF (40 mL) at 0 °C. After hydrogen gas generation ceased (45 min), o-nitrobenzyl bromide (1.58 g, 7.30 mmol) was added, and the mixture was stirred at rt for 5 h. The reaction was neutralized with 1 N HCl. The mixture was evaporated, and the residue was purified by silica gel chromatography, eluting with 4–6% methanol in dichloromethane to give the product 2a,2b as a yellow foam (1.12 g, 47% yield).

5'-O-(tert-Butyldiphenylsilyl)-N2-isobuturyl-2'-O-(o-nitro-
benzyl)-3'-O-trifluoromethylsulfonylguanosine (3). To a stirred solution of N2-isobuturyl-2'-O-(o-nitrobenzyl)guanosine (2) (2.312 g, 4.73 mmol) in dry pyridine (20 mL) was added tert-butyldiphenylsilyl chloride (1.82 mL, 7.10 mmol) under argon. The mixture was stirred at rt for 24 h, then quenched with CH2OH (4.0 mL), and evaporated to a syrup. The residue was dissolved in CH2Cl2 and washed with H2O. The organic layer was dried over anhydrous MgSO4. After filtration and removal of solvent, the residue was isolated by silica gel chromatography, eluting with 2% CH2Cl2 to give 5'-O-(tert-butyldiphenylsilyl)-N2-isobuturyl-2'-O-(o-nitrobenzyl)guanosine 3 as a white foam: 3.24 g (94% yield); 1H NMR (CDC13/TMS) δ 12.20 (brs, 1H), 9.95 (brs, 1H), 7.97 (s, 1H), 7.96 (d, 1H, J = 8.8 Hz), 7.70–7.60 (m, 5H), 7.53 (m, 1H), 7.45–7.25 (m, 7H), 6.03 (d, 1H, J = 2.8 Hz), 5.28 (d, 1H, J = 15.0 Hz), 5.14 (d, 1H, J = 15.0 Hz), 4.61 (m, 1H), 4.28 (m, 1H), 4.22 (m, 1H), 4.05 (m, 1H), 3.88 (m, 1H), 2.84 (m, 1H), 1.26 (d, 3H, J = 4.8 Hz), 1.24 (d, 3H, J = 4.8 Hz), 1.02 (s, 9H); 13C NMR (CDC13) δ 179.6, 155.6, 147.9, 147.7, 146.9, 136.8, 135.5, 135.4, 134.0, 133.8, 132.7, 132.4, 129.4, 128.9, 128.0, 127.8, 127.7, 127.4, 124.1, 122.1, 86.9, 84.6, 83.3, 69.1, 68.5, 63.0, 36.1, 26.8, 19.0; HRMS calcd for C47H33N6O10Si: [MH]+ 727.2906, found 727.2903.

Under argon, to a solution of 5'-O-(tert-butyldiphenylsilyl)-N2-isobuturyl-2'-O-(o-nitrobenzyl)guanosine 3 (3.24 g, 4.46 mmol) and DMAP (763 mg, 6.25 mmol) in dry CH2Cl2 (30 mL) at 0 °C was added CF3SO2Cl (0.50 mL, 4.7 mmol). After the mixture was stirred at 0 °C for 3 h, the reaction was quenched with ice water (20 mL) and the mixture stirred for 15 min. The organic layer was separated, and the aqueous layer was extracted with CH2Cl2 (2 × 30 mL). The organic layers were combined, washed with brine, and subsequently dried over anhydrous MgSO4. After filtration and removal of solvent, the residue was purified by silica gel chromatography, eluting with 1.5–2% methanol in CH2Cl2, to give 5'-O-(tert-butyldiphenylsilyl)-N2-isobuturyl-2'-O-(o-nitrobenzyl)guanosine 4 as a white solid: 1.43 g (95% yield); 1H NMR (CDC13/TMS + a few drops of CD3OD) δ 8.00 (d, 1H, J = 8.4 Hz), 7.85 (s, 1H), 7.70–7.50 (m, 6H), 7.50–7.30 (m, 7H), 6.03 (d, 1H, J = 4.8 Hz), 5.71 (m, 1H), 5.30 (d, 1H, J = 14.8 Hz), 5.09 (d, 1H, J = 14.8 Hz), 4.89 (t, 1H, J = 4.8 Hz), 4.46 (m, 1H), 4.08 (dd, 1H, J = 3.4, 12.0 Hz), 3.86 (dd, 1H, J = 3.2, 12.0 Hz), 2.63 (m, 1H), 1.27 (d, 3H, J = 6.0 Hz), 1.26 (d, 3H, J = 6.0 Hz), 1.04 (s, 9H); 13C NMR (CDC13) δ 178.8, 155.4, 147.8, 147.7, 147.0, 137.1, 135.6, 135.5, 134.3, 133.3, 132.2, 132.0, 130.5, 130.4, 128.8, 128.6, 128.18, 125.8, 124.8, 122.8, 111.6, 86.4, 82.2, 81.9, 80.1, 69.2, 61.9, 36.6, 26.9, 19.1, 18.9; HRMS calcd for C46H34N6O7S2Si: [MH]+ 758.2399, found 589.2399.

5'-O-(2-Isobutyryl-2'-O-(o-nitrobenzyl)-3'-thio-
guanosine (5). Under argon to a solution of 4 (2.99 g, 3.78 mmol) in dry DMF (25 mL) was added potassium thioacetate (1.30 g, 11.4 mmol), and the mixture was stirred at 60 °C for 7 h. After the solvent was removed under reduced pressure, the residue was partitioned between a saturated aqueous NaHCO3 solution/brine (v/v, 1:1) and CH2Cl2.

Note
The the organic layer was dried over MgSO4. After filtration and removal of the solvent, the residue was purified by silica gel chromatography, eluting with 3% MeOH in CH2Cl2 to give 5 as a pale yellow foam: 1.28 g (95% yield); 1H NMR (CD3CN) δ 12.04 (brs, 1H), 9.18 (brs, 1H), 8.08 (d, 1H, J = 8.0 Hz), 7.98 (s, 1H), 7.82 (d, 1H, J = 7.6 Hz), 7.69 (m, 1H), 7.50−7.15 (m, 10H), 6.80 (d, 4H, J = 8.8 Hz), 6.09 (s, 1H), 5.51 (d, 1H, J = 15.6 Hz), 5.15 (d, 1H, J = 15.2 Hz), 4.84 (dd, 1H, J = 5.2, 11.2 Hz), 4.42 (d, 1H, J = 4.8 Hz), 4.35 (m, 1H), 3.77 (s, 6H), 3.51 (dd, 1H, J = 2.8, 11.2 Hz), 3.32 (m, 1H), 2.71 (m, 1H), 2.30 (s, 1H), 1.26 (d, 3H, J = 7.2 Hz), 1.24 (d, 3H, J = 6.9 Hz). 13C NMR (CDCl3) δ 196.0, 181.6, 157.4, 149.7, 149.1, 139.2, 134.7, 134.4, 132.45, 130.15, 130.06, 128.5, 128.4, 128.14, 128.08, 127.1, 124.8, 113.1, 88.7, 86.5, 84.8, 82.9, 69.5, 61.4, 55.3, 43.7, 36.5, 30.5, 19.0 18.8 ppm; HRMS calcd for C19H16N8O10S [M+H]+ 584.2912, found 584.2922.

5′-O-(Dimethoxymethyl)-N′-isobutyl-2′-O-(o-nitrobenzyl)-3′-thioguanosine (7). To a solution of guanidine hydrochloride (108 mg, 0.11 mmol) in dry pyridine (15.0 mL) under argon was added DMTMTI (1.23 mL, 21.7 mmol) and 1-methylimidazole (5.0 μL). The mixture was stirred at rt for 1 h. quenched with MeOH (1 mL) and stirred for 5 min. After solvent was removed, the residue product was purified by silica gel chromatography, eluting with 5% MeOH in CH2Cl2, containing 0.5% Et3N, to give the corresponding phosphoramidite as a yellow foam: 56 mg (95% yield); 1H NMR (CD3CN) δ 158.4, 155.5, 147.9, 147.1, 146.7, 144.8, 143.7, 137.0, 135.6, 134.5, 132.8, 128.5, 128.2, 127.9, 127.0, 124.8, 122.0, 113.1, 88.7, 86.5, 84.8, 82.9, 69.5, 61.4, 55.3, 43.7, 36.5, 30.5, 19.0 18.8 ppm; HRMS calcd for C19H16N8O10S [M+H]+ 584.2912, found 584.2922.

Oligonucleotide Characterization. Alkaline hydrolysis: 4 cpk of the 5′-radiolabeled oligonucleotide (1 μL) with or without UV deprotection (UVP-B1000, 365 nm, 4 min) was treated with NaHCO3 (pH 9, 50 mM, 2 μL) in a total volume of 10 μL solution at 90 °C for 10 min. Formamide loading dye (2×) and 10 μL was added, and the mixture was run on a 20% dPAGE gel.

Silver ion cleavage: 4 cpk of the 5′-radiolabeled oligonucleotides (2 μL) with or without UV deprotection (UVP-B1000, 365 nm, 4 min) was treated with AgNO3 (100 mM, 0.4 μL) in a total volume of 20 μL solution in the dark at rt for 60 min. DTT (100 mM, 0.6 μL) was then added, and the mixture was spun at full speed for 5 min. A 15 μL aliquot of solution was withdrawn, added to 2× formamide loading dye (15 μL), and run on a 20% dPAGE gel.

Synthesis of ACT1 Yeast Splicing Substrates. The ACT1-1-373 (nucleotides 1–373) was synthesized by in vitro transcription from a plasmid template linearized with HindIII restriction and containing ACT1-1-373 followed by an HDV ribozyme sequence. In cases where HDV cleavage was inefficient during transcription, the RNA was resuspended in Tris (10 mM, pH 7.5) and MgCl2 (20 mM).

Ribozyme cleavage was induced via 2–4 cycles of 90 °C for 1 min, rt for 15 min, and 37 °C for 15 min. The buffer conditions were then adjusted for T4 PNK treatment of the transcript to remove the 2′,3′-cyclic phosphate left by the ribozyme. The ACT1-392-590 (nucleotides 392–590) was synthesized by in vitro transcription using a PCR-derived template generated using plasmid bPS149. As the subsequent ligation requires a 5′-monophosphate group, a 4-fold excess of GMP over GTP was included in the transcription reaction.

ACT1 ligation reactions consisted of 500 pmol of ACT1-1-373, 50 pmol of oligonucleotide ACT1-1′-3′-O- or ACT1-3′-S, and 500 pmol of ACT1-392-590. The RNA was hybridized to 50 pmol of ACT1-3′-S in buffer TEN50 (10 mM Tris-HCl, pH 7.5; 1 μM EDTA; 50 mM NaCl) on a thermal cycle by heating to 90 °C for 2 min followed by reduction of the temperature by 1 °C for 1 min to 24 °C, then cooling to 4 °C for 5 min. T4 DNA ligase (~100 pmol, synthesized in-house) was added, and the mixture was incubated for 1 h at 37 °C.
or T4 RNA ligase 2 (2 units, New England Biolabs) was then added, and reactions were incubated at 37 °C for 4 h. The ligation reactions were DNase-treated (RNase-free DNase) for 15 min to remove splint, phenol–chloroform extracted, and ethanol precipitated before purification on 6% denaturing polyacrylamide gel. Bands containing full-length ACT1 pre-mRNA were excised and recovered by passive elution in TEN250 buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 250 mM NaCl) overnight at 4 °C. Yields typically ranged from 200 to 600 fmol, enough for 50–150 splicing reactions.

**Splicing Reactions.** Yeast splicing-competent extracts were prepared using the liquid nitrogen method, as described in the literature. In vitro splicing reactions consisted of 32P-labeled substrates (0.2–0.4 nM), 40% yeast extract that was pretreated with 1 mM EDTA, 3% PEG 8000, 60 mM K2PO4 (pH 7), 3.5 mM MgCl2, and 2 mM ATP. Reactions were incubated at 20 °C for 20 min, then on ice for 5 min for 365 nm UV light treatment. Metal ion concentrations were adjusted to 4 mM with either MgCl2 or MnCl2, and the reactions were incubated at 20 °C for another 20 min. The reactions were then quenched and analyzed by 6% dPAGE gel as described previously.

**ASSOCIATED CONTENT**

- Supporting Information
  - MALDI-TOF MS of 2′-photocaged 3′-S-RNA, 1H NMR and 13C NMR spectra of compounds 3–7, 1H NMR and 31P NMR of phosphoramidites 8 and 9. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**
The authors declare no competing financial interest.

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