Synthesis of Oligoribonucleotides Containing a 2′-Amino-5′-S-phosphorothiolate Linkage

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ABSTRACT: Oligoribonucleotides containing a photocaged 2′-amino-5′-S-phosphorothiolate linkage have potential applications as therapeutic agents and biological probes to investigate the RNA structure and function. We envisioned that oligoribonucleotides containing a 2′-amino-5′-S-phosphorothiolate linkage could provide an approach to identify the general base within catalytic RNAs by chemogenetic suppression. To enable preliminary tests of this idea, we developed synthetic approaches to a dinucleotide, trinucleotide, and oligoribonucleotide containing a photocaged 2′-amino-5′-S-phosphorothiolate linkage. We incorporated the photocaged 2′-amino-5′-S-phosphorothiolate linkage into an oligoribonucleotide substrate for the hepatitis delta virus (HDV) ribozyme and investigated the pH dependence of its cleavage following UV irradiation both in the presence and absence of the oligoribonucleotide substrate for the hepatitis delta virus (HDV) ribozyme. The substrate exhibited a pH-rate profile characteristic of the modified linkage but reacted slower when bound to the ribozyme. Cleavage inhibition by the HDV ribozyme could reflect a non-productive ground-state interaction with the modified substrate’s nucleophilic 2′-NH2 or a poor fit of the modified transition state at the ribozyme’s active site.

INTRODUCTION

The synthesis of modified nucleosides, nucleotides, and oligonucleotides has been extensively investigated and motivated, in part, by creation of potential therapeutic agents (antisense, antiviral, and anticancer agents)1−6 and biological probes for the investigation of the relationship between the RNA structure and function. Chemical synthesis provides access to both naturally occurring and designed modified nucleotides and oligonucleotides, endowing biochemists and chemical biologists with tools to probe RNA chemistry and biology deeply and comprehensively.7,9 For example, the replacement of RNA’s 2′-OH with a 2′-NH2 (Figure 1A) maintains the hydrogen bonding capacity of 2′-OH but alters nucleophilicity, pKₐ, and metal-ion coordination properties.10 These defined changes in chemical properties form the basis of biochemical strategies to define the functional roles of RNA’s 2′-hydroxyl groups at specific locations. Owing to the weak nucleophilicity of the amino group toward the adjacent phosphodiester bond, 2′-amino substitution renders the ribose phosphate backbone inert to cleavage via internal transphosphorylation.11 Analogously, substitution of the 5′-bridging oxygen atom of the phosphodiester linkage with a sulfur atom (Figure 1B) alters hydrogen bonding, metal-ion coordination properties, and leaving group ability. However, in contrast to the 2′-amino group, a 5′-sulfur renders the phosphodiester backbone much more susceptible to transphosphorylation, owing to the greater leaving ability of sulfur relative to oxygen. This hyperactivation of the leaving group underpins a chemogenic strategy to identify groups that activate the 5′-oxygen leaving group within the active site of a biological catalyst.12−14 These 2′-NH2 and 5′-S-RNA modifications have been used independently in studies of RNA, including as mechanistic probes for ribozyme-catalyzed reactions.12−32 Many reports have described the synthesis and incorporation of 2′-amino-modified nucleosides or nucleotides into RNA, including as a photocaged precursor (Figure 1A).33 Nevertheless, 2′-NH2 substitution of the nucleophilic 2′-OH at the cleavage site of an endonucleolytic ribozyme has limited use as a mechanism probe on its own because the modification essentially abolishes cleavage.27,32 In contrast, the inherent instability of RNA containing a 5′-S-phosphorothiolate linkage makes working with this modification more challenging (Figure 1B). Protection of 2′-hydroxyl with a photolabile group such as an o-nitrobenzyl group, which could be removed by UV irradiation, has facilitated the use of this modification (Figure 1B).12−14 We previously developed a strategy to identify the general acid in an enzymatic reaction using sulfur substitution...
of the leaving group.\textsuperscript{12,13} The better leaving ability of the sulfur obviates the need for general acid catalysis. As a consequence, mutations to the general acid that adversely affect catalysis in the context of the natural oxygen leaving group become suppressed in the context of the sulfur leaving group, whereas mutations elsewhere remain deleterious.

We have been interested in an analogous strategy to identify a potential general base in catalysis. However, there appear to be no simple chemical modifications of the 2′-hydroxyl group that would suppress the need for a general base. A nucleotide analogue whose nucleophilic hydroxyl group ionizes fully within the pH range of the ribozyme reaction could provide a suitable probe, but this is not obviously accessed within the nucleotide framework. An amino group represents another possibility, but as noted above, oligonucleotides bearing 2′-amino groups do not undergo backbone cleavage via attack of the nitrogen at the adjacent phosphorus center.

Alternatively, Eckstein and co-workers have shown that cleavage of a dinucleotide with a 2′-amino group can occur readily when the adjacent phosphorus bears a 5′-sulfur leaving group (\(k \sim 10^{-4} \text{ s}^{-1}\) with half-life time \(\sim 2\) h).\textsuperscript{33} Moreover, the cleavage reaction occurs independently of pH at pH values \(>7\), indicating no susceptibility of the linkage to base catalysis. Accordingly, mutations that disable the ability of a ribozyme to deprotonate the nucleophile would be expected to affect cleavage of a substrate containing a 2′-amino group nucleophile and a 5′-S leaving group less adversely than a substrate containing only the 5′-S leaving group (Figure 2).

Testing this approach in a ribozyme reaction requires installation of 2′-NH\(_2\)/5′-S modifications beyond dinucleotides and into oligoribonucleotides. Here, we report the synthesis of oligoribonucleotides containing a photocaged 2′-amino-5′-S-phosphorothiolate linkage (Figure 1C\(^*\)) and determine its cleavage rate versus pH in the presence and absence of the hepatitis delta virus (HDV) ribozyme.

![Figure 1. Oligoribonucleotides containing 2′-aminonucleotide (A), 5′-S-phosphorothiolate linkage (B), and 2′-amino-5′-S-phosphorothiolate linkage (C).](https://pubs.acs.org/doi/10.1021/acs.joc.1c01059)

![Figure 2. Possible mechanism of ribozyme-catalyzed cleavage of the RNA substrate containing a 2′-NH\(_2\)/5′-S linkage at the cleavage site at pH > 7.](https://pubs.acs.org/doi/10.1021/acs.joc.1c01059)
RESULTS AND DISCUSSION

We have previously reported approaches to synthesize RNA containing 2′-O-photocaged 5′-S-phosphorothiolate linkages using either 5′-S-phosphoramidite chemistry or ligations to a synthetic dinucleotide containing the modified linkage: \((5′-\text{C}_2\text{-O-\text{NHar}}\text{Ps-G-3})\). We adapted these two strategies to enable the synthesis of RNAs containing 2′-amino-5′-S-phosphorothiolate linkages. We prepared the 2′-photocaged 2′-amino-5′-S-dinucleotide \((5′-\text{C}_2\text{-NHar-Ps-G-3})\), the trinucleotide derivative \((5′-\text{C}_2\text{-NHar-Ps-GG-3})\), and 2′-photocaged 2′-amino-3′-phosphoramidites.
Synthesis of 2′-Photocaged 2′-Aminocytidine Phosphoramidites and a Dinucleoside Containing a Photocaged 2′-Amino-5′-S-phosphorothiolate Linkage (5′-C2′-NHX-ps-G-3′). Photocaged 2′-aminocytidine 3′-phosphoramidites (4a, 4b) and the corresponding 3′-H phosphate (5) were synthesized as shown in Scheme 1. 5′-O-DMT-Tr-2′-aminocytidine 1 was prepared according to a literature procedure.16 The 2′-amino group of 1 could be selectively protected using a large photocaging group (4,5-dimethoxy-2-nitrobenzylchloroformate) followed by benzoyl protection of the amino group on the cytosine ring to afford compound 2. The nucleoside 2 was further converted to phosphoramidite 4a or the 3′-H-phosphate 5 in good yield. If excess 4,5-dimethoxy-2-nitrobenzyl chlorofomate was used in the reaction of 1, the exocyclic amine of the cytosine ring also became photocaged, giving derivative 3, which could be converted to the corresponding double-photocaged phosphoramidite 4b in 36% overall yield.

To prepare a 2′-photocaged 5′-S dinucleotide, various protected 5′-disulfanylguanosine derivatives (8a, 8b, 8c, and 8d) were synthesized from compounds 6a/6b,35 as shown in Scheme 2 and reacted with 3′-H-phosphate 5 (Scheme 3).

Scheme 3

However, only 8a and 8c containing the facile 5-nitro-2-pyridinyl leaving group reacted efficiently with 3′-H-phosphate 5 to afford the 2′-photocaged 5′-S-dinucleotide (5′-C2′-NHX-G-G-3′) (Scheme 3).

Attempts to prepare an oligonucleotide containing a photocaged 2′-amino-5′-S-phosphorothiolate linkage from the dinucleotide 5′-C2′-NHX-ps-G-G-3′ through enzymatic ligation were not successful (Figure 3). We were able to install the 5′-phosphate enzymatically onto the dinucleotide to obtain 5′-C2′-NHX-ps-G-3′ and successfully ligate it to RNA. However, the second ligation step failed to afford the full-length RNA, possibly because the large photocaged protecting group hinders the capacity of the dinucleotide to serve as an acceptor substrate in the enzymatic ligation reaction.36,37 We hypothesized that an oligonucleotide bearing the large photocaged group more distal to the acceptor site might serve as a better acceptor substrate for ligation. To test this idea, we set out to prepare the trinucleotide, that is, 5′-C2′-NHX-ps-G-G-G-3′, for incorporation into RNA via the two-step ligation approach.35

Figure 3. Construction of RNA-containing 5′-C2′-NHX-ps-G-3′ by a consecutive ligation approach.
Scheme 4

Scheme 5
containing a photocaged 2′-amino-5′-S-phosphorothiolate linkage (24a and 24b) (Scheme 8).

The corresponding RNAs containing a photocaged 2′-amino-5′-O-phosphonate linkage (26a and 26b) were prepared by the solid-phase synthesis with the first coupling to phosphoramidite 25 and then coupling to 4a (Scheme 9).

Characterization and pH-Dependent Cleavage of a Ribozyme Substrate Containing a 2′-Amino-5′-S-phosphorothiolate Linkage. All photocaged RNA oligonucleotides 24a, 24b, 26a, and 26b were analyzed by MALDI-TOF mass spectrometry, confirming their molecular weights. HPLC confirmed that under neutral conditions, 26a and 26b were photodeprotected to the corresponding 5′-C2′-NH2-GGGUCGGC-3′ (~25% conversion) and 5′-UUC2′-NH2-GGGUCGGC-3′ (~30% conversion) after UV irradiation (365 nm, 15–30 min). The UV deprotection rates were $k_{26a} = 0.27 \text{ min}^{-1}$ and $k_{26b} = 0.065 \text{ min}^{-1}$, respectively. The photodeprotection of the shorter oligonucleotide (26a, 9 mer) occurred about 4 times faster than the longer oligonucleotide (26b, 11 mer). After 3′-radiolabeling, the RNA oligonucleotides 24b and 26b were treated with Ag⁺ solution. As expected, 24b cleaves in the presence of Ag⁺ ion, confirming the

Scheme 7

Scheme 8
presence of the phosphorothiolate linkage (Figure 4, lane 10), but 26b, which contains no phosphorothiolate linkage, was unaffected in the presence of Ag\(^+\) ion (Figure 4, lane 9). Additionally, comparison of the alkaline hydrolysis of 24b and 26b before and after UV irradiation also confirmed the 2′-amino-5′-S-phosphorothiolate linkage in 24b (Figure 4, lanes 7 and 8).

We then studied the pH-dependent cleavage reaction of 5′-radiolabeled 24b in the presence and absence of the anti-genomic HDV ribozyme and 10 mM MgCl\(_2\) (Figure 5). As expected, the cleavage rate of 24b increases in a log-linear fashion at pH values below the pK\(_a\) of the 2′-amino group and becomes independent of pH at pH values above the pK\(_a\) (6.2).\(^{12}\) This pH rate profile resembles that for the cleavage of the corresponding U2-\(\text{NH}_2\)-ps-U dinucleotide.\(^{33}\) We found that in the presence of the HDV ribozyme, 24b underwent cleavage 3–9-fold slower than in the absence of the HDV ribozyme throughout the tested pH range. This result indicates that ribozyme binding to the substrate inhibits cleavage of the 2′-amino-5′-S-phosphorothiolate linkage. The inhibition may reflect a non-productive ground-state interaction involving 2′-OH in the natural reaction.\(^{13}−\)\(^{46}\) The possible non-productive ground-state interactions in the enzyme substrate complex most likely involve hydrogen bonding or metal coordination to the nucleophilic amino group. These interactions could diminish nucleophilicity through interaction with the amino group’s lone pair of electrons or disfavor acquisition of the in-line conformation required for reaction.\(^{46}\) Alternatively, the HDV ribozyme may not be able to accommodate the transition state for 2′-N-transphosphorylation of the 2′-amino-5′-S-phosphorothiolate linkage. We have shown previously using model systems that amine nucleophiles react at phosphodiester bonds via expanded transition states, with less bonding to both the nucleophile and the leaving group, relative to analogous reactions of phosphodiester bonds bearing oxygen leaving groups.\(^{47}\) Possibly, the expanded transition state does not fit well at the HDV-active site, resulting in slower cleavage relative to the corresponding reaction in the absence of ribozyme.

### CONCLUSIONS

We have prepared RNAs containing a C2-\(\text{NH}_2\)-psG linkage by solid-phase synthesis using 2′-photocaged S′-disulfanyl guanosine derivative 23 and 2′-aminophotocaged cytidine 3-H phosphonate 5. The structures of these modified RNAs were confirmed by MS and Ag\(^+\) treatment. In the context of a trans-acting HDV ribozyme substrate, the modified linkage exhibited the expected pH-dependent cleavage in the absence of ribozyme, verifying its integrity. Unexpectedly, instead of facilitating substrate cleavage, the HDV ribozyme inhibited cleavage of the modified substrate, possibly reflecting non-productive ground-state interactions or poor accommodation of the transition state within the RNA active site.

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**Figure 4.** Characterization of 3′-radiolabeled RNAs (11 mer) containing 2′-amino-5′-O- (26b) or 2′-amino-5′-S-linkage (24b). The figure was depicted from the right side of a large gel, so the oligo on lane 9 moved a little bit slower than the same oligo on lanes 1 and 5.
EXPERIMENTAL SECTION

2′-Amino-N4-benzoyl-2′-N-(4,5-dimethoxy-2-nitrobenzoyl carbonyl)-5′-O-DMTr-cytidine (2). Under argon to a solution of 2′-amino-5′-O-DMTr-cytidine (1) (160 mg, 0.27 mmol) in tetrahydrofuran (THF) (3 mL), disopropylethylamine (94 μL, 0.54 mmol) and 4,5-dimethoxy-2-nitrobenzyl chloroformate (91 mg, 0.33 mmol) were added. The reaction mixture was stirred at rt for 16 h. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in chloroform to afford 2′-amino-2′-N-(4,5-dimethoxy-2-nitrobenzoylcarbonyl)-5′-O-DMTr-cytidine: 185 mg as a light yellow foam. HRMS (ESI/APCI) m/z: [M + Na]+ calcd for C50H50N6O18Na, 1045.3079; found, 1045.3076.

1′-(4,5-dimethoxy-2-nitrobenzoyl)-5′-O-DMTr-cytidine (3). Under argon to a solution of 2′-amino-N4-dibenzoic acid-2′,5′-O-diisopropylphosphoramidite (4a). To a solution of 2′-amino-2′-N-(4,5-dimethoxy-2-nitrobenzoylcarbonyl)-5′-O-DMTr-cytidine (1) (162 mg, 0.30 mmol) in THF (10 mL), disopropylethylamine (314 μL, 1.80 mmol), DMAP (37 mg, 0.30 mmol), and 4,5-dimethoxy-2-nitrobenzyl chloroformate (492 mg, 1.80 mmol) were added. The reaction mixture was stirred at rt overnight. Thin-layer chromatography (TLC) showed that the reaction was complete, and the reaction was quenched with methanol (1.0 mL). The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 3% methanol in chloroform to afford 3 as a yellow foam: 119 mg (39% yield). 1H NMR (400 MHz, CDCl3/TMS): δ 8.01 (br s, 1H), 7.71 (s, 1H), 7.59 (s, 1H), 7.45–6.90 (m, 11H), 6.82 (d, 4H, J = 8.4 Hz), 6.38 (d, 1H, J = 7.8 Hz), 5.58 (s, 2H), 5.49 (d, 1H, J = 15.0 Hz), 5.36 (d, 1H, J = 15.0 Hz), 4.57 (m, 1H), 4.47 (br s, 2H), 4.29 (m, 1H), 4.00–3.70 (m, 18H), 3.43 (m, 2H); 13C NMR (101 MHz, CDCl3): δ 158.6, 156.1, 153.7, 153.6, 148.3, 147.8, 139.5, 135.2, 134.9, 130.0, 128.0, 127.9, 127.0, 113.2, 110.2, 108.5, 107.8, 87.0, 85.9, 71.7, 64.8, 63.8, 63.5, 60.6, 56.8, 56.4, 56.3, 56.1, 55.1; HRMS (ESI/APCI) m/z: [M + Na]+ calcld for C50H50N6O18Na, 1045.3079; found, 1045.3076.

2′-Amino-N4-benzoyl-2′-N-(4,5-dimethoxy-2-nitrobenzoyl carbonyl)-5′-O-DMTr-cytidine 3′-O,N,N-Diisopropyl(cyanoethyl)phosphoramidite (4b). To a solution of 2′-amino-2′-N,N′-diisopropylphosphoramidite (4b). To a solution of 2′-amino-2′-N,N′-diisopropylphosphoramidite (4b).
dimethoxy-2-nitrobenzoxycarbonyl)-3′-O-DMTr-cytidine (3) (103 mg, 0.10 mmol) and i-Pr$_2$NET (87 µL, 0.50 mmol) in anhydrous dichloromethane (5 mL) at 0 °C. CIP(NP$_3$)$_2$OCH$_2$CH$_2$CN (45 µL, 0.20 mmol) was added, followed by the addition of 1-methylimidazole (4.0 µL, 0.05 mmol). After stirring the reaction mixture at rt for 1 h, the reaction was quenched with methanol (1.0 mL). The solvent was removed, and the residue was purified by silica gel chromatography, eluting with 2% CH$_2$COOH in CH$_2$Cl$_2$ containing 0.5% Et$_3$N to afford 4b as a yellow foam: 113 mg (92% yield, >95% purity).

$^{31}$P[H] NMR (162 MHz, CDCl$_3$): δ 151.1, 150.3; HRMS (ESI/APCI) m/z: [M + Na]$^+$ calc for C$_{22}$H$_{24}$N$_3$O$_4$P$_2$: 542.1204; found, 542.1199.

2′-Amino-N$^4$-benzoyl-2′-N-(4,5-dimethoxy-2-nitrobenzoxycarbonyl)-3′-O-dimethoxytrityl-2′-deoxy-2′-O-phosphonate (5). To the solution of 2′-amino-N$^4$-benzoyl-2′-N-(4,5-dimethoxy-2-nitrobenzoxycarbonyl)-3′-O-dimethoxytrityl-2′-O-deoxytidine (2) (72 mg, 0.081 mmol) in pyridine (5 mL), diphenyl phosphate (77 µL, 0.41 mmol) was added. After 15 min, the reaction was quenched by addition of a mixture of water/triethylamine (1:1 v/v, 2 mL), and the resulting mixture was stirred for 15 min. The solvent was evaporated, and the residue was partitioned between dichloromethane (25 mL) and saturated aqueous NaHCO$_3$ (10 mL). The organic layer was washed for additional two times with aqueous NaHCO$_3$ (10 mL) and subsequently dried over MgSO$_4$. Following the removal of the solvent by evaporation under vacuum, the resulting residue was purified by silica gel chromatography, eluting with 3% methanol in dichloromethane containing 3% of triethylamine to afford compound 5 (71 mg, 81% yield) as a light yellow solid. $^1$H NMR (400 MHz, CDCl$_3$/TMS): δ 8.05 (d, 1H, J = 7.2 Hz), 7.88 (d, 2H, J = 7.6 Hz), 7.73 (s, 1H), 7.68 (s, 1H), 7.59 (s, 1H, J = 7.6 Hz), 7.49 (t, 2H, J = 7.6 Hz), 7.41 (d, 1H, J = 7.6 Hz), 7.35–7.27 (m, 6H), 7.23 (t, 1H, J = 7.6 Hz), 7.09 (m, 2H), 6.85 (d, 4H, J = 8.8 Hz), 6.44 (d, 4H, J = 8.4 Hz), 5.60–5.40 (m, 2H), 4.92 (m, 1H, J = 4.67 Hz), 4.48 (br s, 1H, J = 4.04) (s, 3H), 3.92 (s, 3H), 3.80 (s, 3H), 3.60–3.45 (m, 2H); $^{13}$C{$_1$H} NMR (101 MHz, CDCl$_3$): δ 162.1, 158.7, 156.0, 154.0, 147.9, 144.7, 141.1, 139.2, 135.2, 135.1, 133.1, 130.1, 129.3, 128.9, 128.1, 127.7, 127.2, 113.4, 109.8, 107.9, 87.3, 86.6, 84.8, 74.3, 73.3, 63.6, 58.6, 56.9, 56.3, 55.3; [$^{31}$P]$_{1}$NMR (162 MHz, CDCl$_3$): δ 7.67; HRMS (ESI/APCI) m/z: [M + Na]$^+$ calc for C$_{22}$H$_{24}$N$_3$O$_4$P$_2$: $^{59}$250.6250, found, 592.6253.

5′-Deoxy-2′, 3′-O-isopropylidene-5′-S′-pyridinylidinyl-2′-diﬂuoroglucosamine (7). From 5′-Benzythio-5′-deoxy-2′, 3′-O-isopropylidene sulfoxide (6a).$^{11}$ A solution of 6a (371 mg, 0.837 mmol) in THF (15 mL) and CH$_2$OH (15 mL) was saturated with ammonia at 0 °C for 30 min, and the mixture was kept at 4 °C for 24 h. After removing the solvent, the residue was dried under vacuum for 30 min. The residue was then dissolved into DMF (15 mL). To the resulting solution, 2,2′-dithiobis(5-nitropyridine) (521 mg, 1.68 mmol) was added. The reaction mixture was stirred at rt for 1.5 h. The resulting mixture was stirred at rt for 15 h. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in chloroform to afford 2′-acetylthio-5′-S′-O-isopropylidene sulfoxide (6b).$^{12}$ Disulfide was also prepared from 6b according to the procedure from 6a with a slight modification. A solution of 6b (100 µg, 0.26 mmol) in THF (5 mL) and CH$_2$OH (5 mL) was saturated with ammonia at 0 °C for 30 min, and the mixture was kept at 0 °C for additional 30 min (instead of at 4 °C for 24 h for 6a). The solvent was removed, and the residue was dissolved into DMF (5 mL) and then reacted with 2,2′-dithiobis(5-nitropyridine) (161 mg, 0.52 mmol) to afford 6 as a light yellow foam (71 mg, 55% yield). $^1$H NMR (400 MHz, DMF-d$_5$): δ 6.05 (br s, 1H), 9.13 (d, 1H, J = 2.8 Hz), 8.48 (dd, 1H, J = 2.8, 9.2 Hz), 8.23 (s, 1H), 7.88 (s, 1H), 6.77 (br s, 2H), 6.05 (s, 1H), 5.33 (d, 1H, J = 6.0 Hz), 5.15 (m, 1H), 4.32 (m, 1H), 4.18 (m, 1H, J = 6.0 Hz), 3.96 (s, 1H), 3.27 (s, 3H); $^{13}$C{$_1$H} NMR (101 MHz, DMF-d$_5$): δ 167.0, 155.7, 154.1, 149.7, 144.8, 144.2, 136.8, 132.4, 119.5, 114.6, 113.1, 89.8, 86.3, 83.8, 83.4, 40.5, 26.8, 25.2; HRMS (ESI/APCI) m/z: [M + H]$^+$ calc for C$_{32}$H$_{24}$N$_3$O$_5$S$_2$: 549.1207; found, 549.1201.
5'-Deoxy-2', 3'-O-di-(tert-butyldimethylsilyl)-N2'-[dimethylamino)methylene]-5'-[(pyridin-2-ylidene)sulfanyl]guanosine (8b). Under argon to a solution of 10a (108 mg, 0.170 mmol) in methanol (10 mL), N,N-dimethylformamide dimethyl acetal (0.226 mL, 1.70 mmol) was added. The mixture was stirred at rt overnight. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in dichloromethane to afford 8b as a light yellow foam: 61 mg (52% yield), eluting with 10% methanol in dichloromethane to recover 8a.

Synthesis of 5'-Deoxy-2', 3'-O-di-(tert-butyldimethylsilyl)-N2'-[dimethylamino)methylene]-5'-[(pyridin-2-ylidene)sulfanyl]guanosine (8c). From 10b: Under argon to a solution of 10b (105 mg, 0.120 mmol) in methanol (10 mL), N,N-dimethylformamide dimethyl acetal (0.294 mL, 2.20 mmol) was added. The mixture was stirred at rt overnight. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in chloroform to afford 8c as a light yellow foam: 81 mg (52% yield).

From 8b: To a solution of 8b (30 mg, 0.043 mmol) in CHCl3 (5 mL), DTT (17 mg, 0.11 mmol) was added, and the mixture was stirred at rt for 4 h. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in chloroform to afford 8c as a light yellow foam: 20 mg (68% yield). 1H NMR (400 MHz, CDCl3/THF): δ 9.53 (br s, 1H, 9.25 (d, 1H, J = 2.8 Hz)), 8.53 (s, 1H), 8.36 (dd, 1H, J = 2.8, 8.8 Hz), 7.90 (dd, 1H, J = 0.4, 8.8 Hz), 7.71 (s, 1H), 5.83 (d, 1H, J = 4.0 Hz), 4.57 (m, 1H), 4.25 (m, 1H), 4.11 (t, 1H, J = 4.4 Hz), 3.35–3.18 (m, 2H), 3.20 (3H, 3.15 (s, 3H), 1.89 (s, 2H), 0.81 (s, 3H), 0.86 (s, 3H), −0.02 (s, 3H), 1.1C (1H NMR) (101 MHz, CDCl3): δ 168.1, 158.2, 157.8, 156.9, 150.1, 145.2, 142.3, 136.9, 131.7, 121.2, 119.8, 88.9, 81.9, 75.3, 74.7, 42.3, 41.5, 35.4, 25.9, 25.8, 18.2, 18.0, −4.1, −4.5, −4.6; HRMS (ESI/APCI) m/z: [M + H]+ calc for C29H34N10O16PS, 671.3259; found, 671.3259.

5'-Deoxy-2', 3'-O-di-(tert-butyldimethylsilyl)-N2'-[dimethylamino)methylene]-5'-[(pyridin-2-ylidene)sulfanyl]guanosine (8d). To a solution of 8b (20 mg, 0.029 mmol) in CHCl3 (2 mL), 2-methyl-2-propanethiol (32 μmol, 1 M) was added. The mixture was stirred at rt overnight. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 5% methanol in chloroform to afford 8d as a colorless foam (20 mg, 76% yield).
Et,N−3HF (300 μL) (6:3:4, v/v/v) at 65 °C in a water bath for 25 min. The solvent was removed at rt under vacuum. The residue was extracted with water (1.0 mL) and washed with chloroform (3 × 0.3 mL). The aqueous phase was desalted by a C18 Sep-Pak column. The product was then purified by a reverse-phase HPLC column to afford the desired trinucleotide 13 as a colorless foam (50 mmol, 5% yield).

MALDI-TOF mass m/z: [M + H]⁺ calcld for C₂₃H₂₇N₆O₈S, 547.1606; found, 547.1595.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

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MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

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MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

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MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.

MALDI-TOF mass m/z: [M + Na]⁺ calcld for C₂₃H₂₇N₆O₈SNa, 569.1764; found, 569.1777.
190; HRMS (ESI/APCI) m/z: [M + H]+ calc'd for C34H52N20O11S, 589.1711; found, 589.1714.

5′-Deoxy-N2-isobutyryl-2′-O-(n-octo benzyl)-5′-(5-nitropyrindin-2-yl disulfanyl)guanosine (22). From 21a: Under argon, to the mixture of 21a (300 mg, 0.55 mmol) and 2,2′-dithiobis(5-nitropyridine) (341 mg, 1.10 mmol) in anhydrous dichloromethane (20 mL) at 0 °C, the solution of guanidine hydrochloride/guanidine (4:1) in methanol (10 mL) prepared from sodium methoxide (0.50 M solution in CH3OH, 1.2 mL, 0.60 mmol) and guanidine hydrochloride (268 mg, 2.80 mmol) in methanol (9.0 mL) was added. After stirring the reaction mixture at rt for 3 h, TLC showed that the reaction was not complete. Additional sodium methoxide (0.50 M solution in CH3OH, 1.2 mL, 0.60 mmol) was added, and the mixture was stirred at rt for an additional 5 h. The reaction mixture was neutralized with 1 N HCl. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 0–3% methanol in dichloromethane to afford 22 as a light yellow foam: 147 mg (41% yield).

From 21b: Under argon, to the mixture of 21b (187 mg, 0.32 mmol), 2,2′-dithiobis(5-nitropyridine) (199 mg, 0.64 mmol), and guanidine hydrochloride (366 mg, 3.83 mmol) in a mixed solvent of anhydrous dichloromethane/methanol (20 mL, v/v = 1:1) at 0 °C, sodium methoxide (0.50 M solution in CH3OH, 1.36 mL, 0.68 mmol) was added, and the mixture was stirred at rt for 3.5 h. The reaction mixture was neutralized with 1 N HCl. The solvent was removed, and the residue was isolated by silica gel chromatography, eluting with 0–3% methanol in dichloromethane to afford 22 as a light yellow foam: 133 mg (63% yield).

1H NMR (400 MHz, CDCl3/TMS): δ 12.33 (br s, 1H), 9.97 (br s, 1H), 9.15 (s, 1H), 8.52 (d, 1H, J = 7.6 Hz), 7.57 (t, 1H, J = 7.6 Hz), 7.39 (m, 1H), 5.95 (s, 1H), 5.24 (d, 1H, J = 14.8 Hz), 5.15 (d, 1H, J = 12.0 Hz), 4.82 (br s, 1H), 4.72 (br s, 1H), 4.50 (m, 1H), 4.31 (m, 1H), 3.40 (m, 1H), 3.27 (m, 1H), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz), 1.28 (d, 3H, J = 6.8 Hz). 13C NMR (101 MHz, CDCl3): δ 180.1, 168.5, 155.8, 148.2, 148.0, 147.1, 145.0, 143.2, 134.0, 134.3, 131.8, 129.1, 128.6, 128.4, 128.1, 119.7, 88.4, 82.7, 81.9, 72.3, 69.7, 41.9, 36.4, 19.1, 19.0; HRMS (ESI/APCI) m/z: [M + H]+ calc'd for C34H52N20O11S, 569.1337; found, 569.1331.

5′-Deoxy-N2-isobutyryl-2′-O-(n-octo benzyl)-5′-(5-nitrobenzyl)-2-disulfanyl)guanosine 3′-O-Phosphonate (23). 2-Chlorophenyl phosphorochloridate (140 mg, 0.58 mmol) was added to a magnetically stirred solution of 1,2,4-triazole (88 mg, 1.3 mmol) and dry Et3N (0.16 mL, 1.2 mmol) in dry THF (5.0 mL) after 15 min at rt; 22 (76.0 mg, 0.115 mmol) in THF (4.0 mL) and 1-methylimidazole (74 µL, 0.92 mmol) were added. After 60 min at rt, the resulting mixture was quenched by adding distilled water (29 µL) and Et3N (0.16 mL, 1.2 mmol). The solvent was removed, and the recovered crude yellow oil was partitioned between saturated aqueous NaHCO3 and dichloromethane. The organic layer was washed with brine and dried over MgSO4. The solution was evaporated, and the residue was purified by silica gel chromatography, eluting with 2% methanol in dichloromethane containing 2% EtN to afford 23 as a brown solid: 104 mg (95% yield). 1H NMR (500 MHz, CDCl3/TMS): δ 11.08 (br s, 1H), 9.16 (d, 1H, J = 2.5 Hz), 8.31 (dd, 1H, J = 9.0, 2.5 Hz), 7.95 (d, 1H, J = 9.0 Hz), 7.89 (d, 1H, J = 8.0 Hz), 7.76 (s, 1H), 7.69 (d, 1H, J = 7.5 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.51 (t, 1H, J = 7.5 Hz), 7.38 (t, 1H, J = 7.8 Hz), 7.22 (d, 1H, J = 7.5 Hz), 6.95 (m, 1H), 6.85 (m, 1H), 5.88 (m, 1H), 5.84 (d, 1H, J = 2.5 Hz), 5.16 (m, 1H), 5.06 (dd, 1H, J = 23, 14.5 Hz), 3.50–3.30 (m, 3H), 2.90 (m, 1H); 13C NMR (126 MHz, CDCl3): δ 180.1, 169.2, 155.9, 149.1, 148.0, 147.7, 147.3, 144.7, 141.9, 138.5, 134.1, 133.4, 131.7, 130.02, 129.99, 129.3, 128.3, 127.5, 124.8, 123.8, 121.8, 121.0, 119.4, 88.1, 80.5, 80.1, 76.2, 69.7, 42.1, 35.9, 19.02, 18.98; 31P NMR (202 MHz, CDCl3): δ = -6.42; HRMS (ESI/APCI) m/z: [M + H]+ calc’d for C37H49N3O10P(S)2 (378.1437); HRMS (ESI/APCI) m/z: [M + H]+ calc’d for C37H49N3O10P(S)2 (378.1437); HRMS (ESI/APCI) m/z: [M + H]+ calc’d for C37H49N3O10P(S)2 (378.1437); HRMS (ESI/APCI) m/z: [M + H]+ calc’d for C37H49N3O10P(S)2 (378.1437).
Ribozyme.

Following the previously described protocol for HDV and added to a quenching solution (8 solution was withdrawn, added to quenching solution (15 μL) was treated with NaHCO₃ (50 mM, pH 9, 2 μL) and run on a 20% dPAGE. (ii) Hydrolysis ladder: 2K cpm of the 3’-radiolabeled oligonucleotide 24b (2 μL) was treated with NaHCO₃ (50 mM, pH 9, 2 μL) in a total volume of 10 μL solution at 90 °C on a heating block for 15 min. The mixture was chilled on ice and added to a quenching solution (8 μL) and run on a 20% dPAGE. Quenching solution: 0.01% bb/xc in 90% formamide, 10 mM EDTA, 2 mM tris, pH 7.78, 100 mM MgCl₂, 100 mM DTT, 10 mM ATP.

Cleavage of 24b in the Presence and Absence of HDV Ribozyme. Following the previously described protocol for HDV ribozyme-catalyzed substrate cleavage, we investigated the cleavage reaction of 5'-radiolabeled 24b (~1 nM) in the presence and absence of anti-genomic HDV ribozyme (1 μM) and 10 mM MgCl₂. The yield of photodeprotection was about 30%, and the ribozyme kinetics were evaluated based on the reacted materials.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.1c01059.

1H NMR and 31P NMR of phosphoramidites 4a and 4b; dinucleotide 11; and a dinucleotide intermediate 18; MALDI-TOF MS of 13, 18, 24a, 24b, 26a, and 26b; and 31P NMR and 13C NMR spectra of all the other new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Khvorova, A.; Watts, J. K. Nat. Biotechnol. 2017, 35, 238–248.
(2) Uihlmann, E.; Peyman, A. Chem. Rev. 1990, 90, 543–584.
(3) Seley-Radtké, K. L.; Yates, M. K. Antiviral Res. 2018, 154, 66–86.
(4) Jordheim, L. P.; Durandel, D.; Zoulim, F. Nat. Rev. Drug Discovery 2013, 12, 447–464.
(5) Mahmoud, S.; Hasabelnaby, S.; Hammad, S.; Sakr, T. Adv. Pharmacol. 2018, 2, 73–88.
(6) Smith, R. A.; Sidwell, R. W.; Robins, R. K. Annu. Rev. Pharmacol. Toxicol. 1980, 20, 259–284.
(7) Li, N.-S.; Frederiksen, J. K.; Piccirilli, J. A. Acc. Chem. Res. 2011, 44, 1257–1269.
(8) Flamme, M.; McKenzie, L. K.; Sarac, I.; Hollenstein, M. Methods 2019, 161, 64–82.
(9) Phelps, K.; Morris, A.; Beal, P. A. ACS Chem. Biol. 2012, 7, 100–109.
(10) Shan, S.-O.; Herschlag, D. Biochemistry 1998, 39, 10958–10975.
(11) Hobb, J.; Sternbach, H.; Sprinzl, M.; Eckstein, F. Biochemistry 1973, 12, 5138–5145.
(12) Das, S. R.; Piccirilli, J. A. Nat. Chem. Biol. 2005, 1, 45–52.
(13) Wilson, T. J.; Li, N.-S.; Lu, J.; Frederiksen, J. K.; Piccirilli, J. A.; Lilley, D. M. J. Proc. Natl. Acad. Sci. U.S.A. 2010, 107, 11751–11756.
(14) Hiller, D. A.; Duncan, B. F.; Nallur, S.; Li, N.-S.; Piccirilli, J. A.; Strobel, S. A. Biochemistry 2020, 59, 1665–1671.
(15) Karpeisky, A.; Sweedler, J.; Haebeler, P.; Read, J.; Jarvis, K.; Beigelman, L. Bioorg. Med. Chem. Lett. 2002, 12, 3345–3347.
(16) McGee, D. P. C.; Vaughan-Suttle, A.; Vargese, C.; Zhou, Y. J. Org. Chem. 1996, 61, 781–785.
(17) Imazawa, M.; Eckstein, F. J. Org. Chem. 1979, 44, 2039–2041.
(18) Hobb, J. B.; Eckstein, F. J. Org. Chem. 1977, 42, 714–719.
(19) Lin, T. S.; Zhang, X. H.; Wang, Z. H.; Prusoff, W. H. J. Med. Chem. 1988, 31, 484–486.
(20) Bressi, J. C.; Verlinde, C. L. M. J.; Aronov, A. M.; Shaw, M. L.; Shin, S. S.; Nguyen, L. N.; Suresh, S.; Buckner, F. S.; Van Voorhis, W. C.; Kuntz, I. D.; Hol, W. G. J.; Gelb, M. H. J. Med. Chem. 2001, 44, 2080–2093.
(21) Ikehara, M.; Maruyama, T.; Miki, H. Tetrahedron Lett. 1976, 17, 4485–4488.
(22) Ranganathan, R. Tetrahedron Lett. 1977, 18, 1291–1294.
(23) Ikehara, M.; Maruyama, T.; Miki, H. Tetrahedron Lett. 1978, 34, 1133–1138.
(24) Robins, M. J.; Hawrelak, S. D.; Hernández, A. E.; Wnuk, S. F. Nucleosides Nucleotides 1992, 11, 821–834.
(25) McGee, D. P. C.; Vargese, C.; Zhai, Y.; Kirschheuter, G. P.; Settle, A.; Siedem, C. R.; Piekien, W. A. Nucleosides Nucleotides 1995, 14, 1329–1339.
(26) Morisawa, H.; Utagawa, T.; Yamanaka, S.; Yamazaki, A. Chem. Pharm. Bull. 1981, 29, 3191–3195.
(27) Beitelman, L.; Karpeisky, A.; Matulic-Adamic, J.; Haebeler, P.; Sweedler, D.; Usman, N. Nucleic Acids Res. 1995, 23, 4434–4442.
(28) Hendrix, C.; Devreece, B.; Rozenki, J.; Van Aerschot, A.; De Bruyn, A.; Beeumen, J. V.; Herewijn, P. Nucleic Acids Res. 1995, 23, 51–57.
(29) Greiner, B.; Pfleiderer, W. Helv. Chim. Acta 1998, 81, 1528–1544.
(30) Hwang, J.-T.; Greenberg, M. M. Org. Lett. 1999, 1, 2021–2024.
(31) Hwang, J.-T.; Greenberg, M. M. J. Org. Chem. 2001, 66, 363–369.
(32) Unpublished result. When using RNA substrate containing a 2′-aminocytidine at the cleavage site to study the HDV catalyzed reaction, we found the reaction was very slow (~1.7 × 10⁻⁶ min⁻¹) with only 4 times faster than hydrolysis of a substrate containing a 2′-deoxy cytidine at the cleavage site (~4 × 10⁻⁸ min⁻¹).
(33) Thomson, J. B.; Patel, B. K.; Jiménez, V.; Eckart, K.; Eckstein, F. J. Org. Chem. 1996, 61, 6273–6281.
(34) Li, N.-S.; Lu, J.; Piccirilli, J. A. J. Org. Chem. 2017, 82, 12003–12013.
(35) Li, N.-S.; Frederiksen, J. K.; Koo, S. C.; Lu, J.; Wilson, T. J.; Lilley, D. M. J.; Piccirilli, J. A. Nucleic Acids Res. 2011, 39, No. e31.
(36) Zhao, B.; Tong, Z.; Zhao, G.; Mu, R.; Shang, H.; Guan, Y. Acta Biochim. Biophys. Sin. 2014, 46, 727–737.
(37) Kestemont, D.; Renders, M.; Leonczak, P.; Abramov, M.; Schepers, G.; Pinheiro, V. B.; Rozenski, J.; Herdewijn, P. Chem.
Commun. 2018, 54, 6408−6411.
(38) S′-DMTr-N-PAC guanosine (14) was purchased from ChemGenes.
(39) Fauster, K.; Hartl, M.; Santner, T.; Aigner, M.; Kreutz, C.; Bister, K.; Ennifar, E.; Micura, R. 2′-Azido RNA. ACS Chem.
Biol. 2012, 7, 581−589.
(40) Lu, J.; Koo, S. C.; Li, N.-S.; Piccirilli, J. A. Nucleosides Nucleotides & Nucleic Acids 2015, 34, 114−129.
(41) Koo, S. C.; Lu, J.; Li, N.-S.; Leung, E.; Das, S. R.; Harris, M. E.; Piccirilli, J. A. J. Am. Chem. Soc. 2015, 137, 8973−8982.
(42) Aurup, H.; Tuschl, T.; Benseler, F.; Ludwig, J.; Eckstein, F. Nucleic Acids Res. 1994, 22, 20−24.
(43) Ganguly, A.; Weissman, B. P.; Piccirilli, J. A.; York, D. M. ACS Catal. 2019, 9, 10612−10617.
(44) Bingaman, J. L.; Zhang, S.; Stevens, D. R.; Yennawar, N. H.; Hammes-Schiffer, S.; Bevilacqua, P. C. Nat. Chem. Biol. 2017, 13, 439−445.
(45) Thaplyal, P.; Ganguly, A.; Hammes-Schiffer, S.; Bevilacqua, P. C. Biochemistry 2015, 54, 2160−2175.
(46) Shih, I.-h.; Been, M. D. Biochemistry 2000, 39, 9055−9066.
(47) Ye, J.-D.; Barth, C. D.; Anjaneyulu, P. S. R.; Tuschl, T.; Piccirilli, J. A. Org. Biomol. Chem. 2007, 5, 2491−2497.
(48) Independent experiments revealed that photolytic removal of the 2′-N-(4,5-dimethoxy-2-nitrobenzyloxy carbonyl) occurred more sluggishly and limited the overall yield. See: Burgess, K.; Jacutin, S. E.; Lim, D.; Shitangkoon, A. J. Org. Chem. 1997, 62, 5165−5168.
Amit, B.; Zehavi, U.; Patchornik, A. J. Org. Chem. 1974, 39, 192−196.