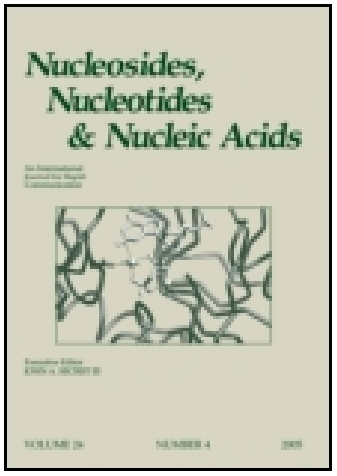


Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn20

Synthesis of 2′-O-Photocaged Ribonucleoside Phosphoramidites

Jun Lu[a], Selene C. Koo[a], Nan-Sheng Li[a] & Joseph A. Piccirilli[a]

[a] Department of Biochemistry & Molecular Biology and Department of Chemistry, University of Chicago, Chicago, Illinois, United States

Published online: 26 Jan 2015.

To cite this article: Jun Lu, Selene C. Koo, Nan-Sheng Li & Joseph A. Piccirilli (2015) Synthesis of 2′-O-Photocaged Ribonucleoside Phosphoramidites, Nucleosides, Nucleotides and Nucleic Acids, 34:2, 114-129, DOI: 10.1080/15257770.2014.965256

To link to this article: http://dx.doi.org/10.1080/15257770.2014.965256

Nucleosides, Nucleotides and Nucleic Acids, 34:114–129, 2015

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2014.965256

SYNTHESIS OF 2′-*O*-PHOTOCAGED RIBONUCLEOSIDE PHOSPHORAMIDITES

Jun Lu, Selene C. Koo, Nan-Sheng Li, and Joseph A. Piccirilli
Department of Biochemistry & Molecular Biology and Department of Chemistry, University of Chicago, Chicago, Illinois, United States

□ *The chemical synthesis and incorporation of the phosphoramidite derivatives of 2′-O-photocaged ribonucleosides (A, C, G and U) with o-nitrobenzyl, α-methyl-o-nitrobenzyl or 4,5-dimethoxy-2-nitrobenzyl group into oligoribonucleotides are described. The efficiency of UV irradiated uncaging of these 2′-O-photocaged oligoribonucleotides was found in the order of α-methyl-o-nitrobenzyl < 4,5-dimethoxy-2-nitrobenzyl < 2′-O-o-nitrobenzyl.*

Keywords 2′-*O*-photocaged ribonucleosides; 2′-*O*-photocaged phosphoramdites; 2′-*O*-photocaged oligomers; 2′-*O*-*o*-nitrobenzyl; 2′-*O*-α-methyl-*o*-nitrobenzyl; 2′-*O*-4,5-dimethoxy-2-nitrobenzyl.

INTRODUCTION

Photolabile protecting groups have been extensively investigated over the years to protect hydroxyl or amino groups in organic synthesis.[1,2] Photocaging provides a well-established strategy to cage functions of biologically important molecules until released by UV irradiation.[3–5] 2′-*O*-Photocaged oligonucleotides including the 3′-*S*-oligonucleotide and 5′-*S*-oligonucleotide phosphorothiolates as useful RNA analogues have been successfully utilized to investigate the mechanism of RNA catalyzed reactions[6–10] and pre-mRNA splicing.[11,12] Although a number of photolabile groups have been introduced into photocaged molecules for biological studies, to the best of our knowledge, only *o*-nitrobenzyl group has been introduced to 2′-*O*-caged RNAs.[6,10,13,14] For example, the 2′-*O*-*o*-nitrobenzyl derivatives of adenosine,[15] cytidine,[15] guanosine,[16] and uridine[17] were synthesized by Ohtsuka *et al.* and applied to the solution synthesis of 2′-*O*-caged dinucleotides and trinucleotides.[15,17,18] The first

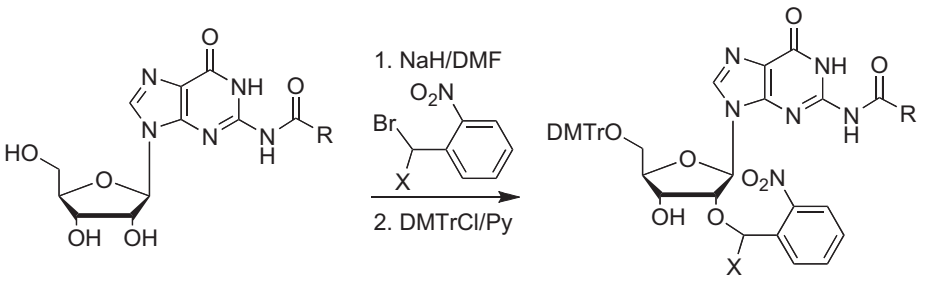
Received 30 July 2014; accepted 10 September 2014.
Address correspondence to Nan-Sheng Li or Joseph A. Piccirilli, Department of Biochemistry & Molecular Biology and Department of Chemistry, University of Chicago, Chicago, Illinois, United States. E-mail: nli@uchicago.edu or jpicciri@uchicago.edu

solid-phase synthesis of 2′-*O*-photocaged oligoribonucleotides was achieved by using 2′-*O*-*o*-nitrobenzyl-3′-*H*-phosphonate chemistry.[13] Later, the phosphoramidite derivatives of 2′-*O*-*o*-nitrobenzyl-N^6-benzoyladenosine[6] and 2′-*O*-*o*-nitrobenzyl-N^4-benzoylcytidine[10] were prepared and applied to synthesize the 2′-*O*-caged RNAs via phosphoramidite chemistry. These 2′-*O*-*o*-nitrobenzyl RNAs have proven useful as tools to probe RNA reactivity and dynamics via a photochemically-controlled manner.[6–12] The 2′-*O*-photolabile group and ultramild *N*-phenoxyacetyl group protected phosphoramidites are useful for the synthesis of oligoribonucleotides containing a 5′-*S*-phosphorothiolate linkage since these modified oligoribonucleotides are more susceptible to alkaline cleavage compared to the wild type RNAs.[7,19] Only three 2′-*O*-photolabile phosphoramidite derivatives (with 2′-*O*-*o*-nitrobenzyl and *N*-benzoyl or *N*-isobutyryl protection) have been synthesized and reported in the literature.[10,14,20] 2′-*O*-*o*-Nitrobenzyl caged RNAs synthesized by either solid phase synthesis or a two-step ligation method have been successfully applied to investigate the mechanisms of ribozyme catalyzed reactions.[7–9,19] However, the relatively long UV irradiation time (4 to 6 minutes)[9,19] for the full removal of *o*-nitrobenzyl group from 2′-*O*-photocaged RNAs may limit its applications in the case of unstable RNAs (or ribozymes) with UV exposure for a long time. To overcome this limitation, here we report the general synthetic methods to obtain 2′-*O*-photocaged phosphoramidites containing a number of more efficient 2′-*O*-photolabile and *N*-phenoxyacetyl protected ribonucleoside phosphoramidites.

RESULTS AND DISCUSSION

The 2′-*O*-photolabile 5′-*O*-DMTr-N^2-butyrylguanosine derivatives (**2a** and **2b**) were prepared in 21% and 18% yields, respectively, according to our previously improved procedures by the reactions of N^2-butyrylguanosine (**1a**) with *o*-nitrobenzyl bromide or α-methyl-*o*-nitrobenzyl bromide, followed by the 5′-*O*-DMTr protection.[16,19] The corresponding 2′-*O*-photolabile 5′-*O*-DMTr-N^2-phenoxyacetylguanosine derivatives (**2c** and **2d**) were prepared from N^2-phenoxyacetylguanosine (**1b**) in 20% and 5% yields, respectively (Scheme 1). It is worthy to note the free guanosine could not be selectively 2′-*O*-benzylated by the reaction with *o*-nitrobenzyl bromide due to the side reaction at N1 of the guanine ring.

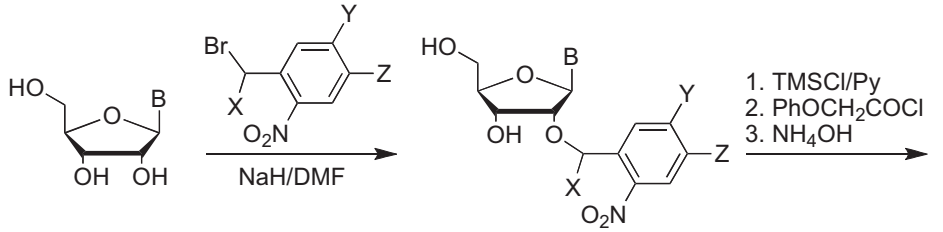
The 2′-*O*-photolabile 5′-*O*-DMTr-*N*-phenoxyacetyl adenosine and cytidine derivatives (**6a–6d**) could be prepared from their respective free ribonucleoside precursors (i.e. adenosine and cytidine) in three steps (Scheme 2). Without any protection both adenosine and cytidine could be selectively 2′-*O*-benzylated with 4,5-dimethoxy-2-nitrobenzyl bromide or α-methyl-*o*-nitrobenzyl bromide to give **4a-4d**. The amino group of nucleobases of



1a: R = $CH(CH_3)_2$
1b: R = CH_2OPh

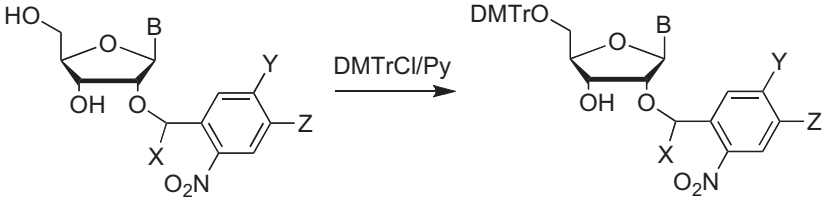
2a: R = $CH(CH_3)_2$, X = H, 21% yield
2b: R = $CH(CH_3)_2$, X = Me, 18% yield
2c: R = CH_2OPh, X = H, 20% yield
2d: R = CH_2OPh, X = Me, 5% yield

SCHEME 1



3a: B = A
3b: B = C

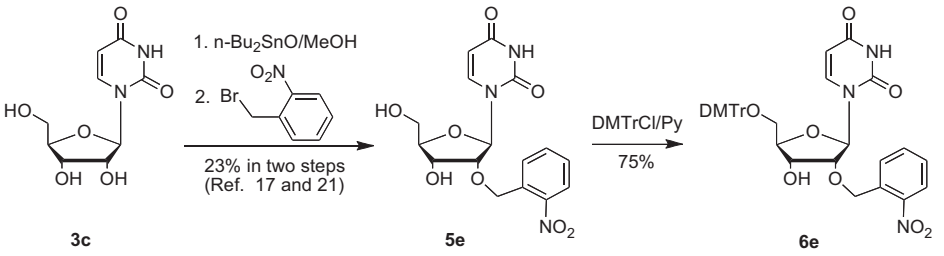
4a: X = H, Y = Z = OMe, B = A, 54% yield
4b: X = Me,Y = Z = H, B = A
4c: X = Y = Z = H, B = C, 18% yield (Ref. 15)
4d: X = Me,Y = Z = H, B = C



5a: X = H, Y = Z = OMe, B = A^{PhOAc}, 91% yield
5b: X = Me,Y = Z = H, B = A^{PhOAc}, 65% yield from **3a**
5c: X = Y = Z = H, B = C^{PhOAc}, 71% yield
5d: X = Me,Y = Z = H, B = C^{PhOAc}

6a: X = H, Y = Z = OMe, B = A^{PhOAc}, 72% yield
6b: X = Me,Y = Z = H, B = A^{PhOAc}, 67% yield
6c: X = Y = Z = H, B = C^{PhOAc}, 86% yield
6d: X = Me, Y = Z = H, B = C^{PhOAc}, 14% yield from **3b**

SCHEME 2

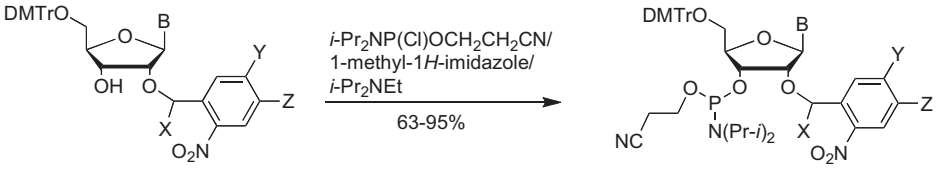


SCHEME 3

4a–4d was then selectively protected by a phenoxyacetyl group to give compounds **5a–5d**. 5′-*O*-DMTr protection of **5a–5d** gave the corresponding phophoramidite precursors **6a–6d** in three steps from free nucleosides **3a** and **3b** with 11–44% overall yields.

Ohtsuka and Ikehara reported the synthesis of 2′-*O*-*o*-nitrobenzyluridine (**5e**) in 24% yield by the reaction of 2′,3′-*O*-(dibutylstannyl)uridine[21] with *o*-nitrobenzyl bromide in DMF at 110°C for 4 hours (Scheme 3).[17] DMTr protection of **5e** then yielded the corresponding 2′-*O*-photocaged uridine phosphoramidite precursor **6e** in 75% yield. Unfortunately, we found this method cannot be extended for the preparation of 2′-*O*-α-methyl-*o*-nitrobenzyluridine by reacting of 2′,3′-*O*-(dibutylstannyl)uridine with α-methyl-*o*-nitrobenzyl bromide. No desired product was formed after this reaction, even at 110°C for 24 hours. According to the procedure for the synthesis of **2a**, direct deprotonation of uridine with NaH in DMF, followed by reaction with *o*-nitrobenzyl bromide generated only O^4-*o*-nitrobenzyluridine.

Phosphitylation of **2a–3d** and **6a–6e** with 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite yielded the corresponding 2′-*O*-photocaged nucleoside phosphoramidites **7a–7i** including the 2′-*O*-photolabile *N*-phenoxyacetylribonucleoside phosphoramidites **7c–7h** in good yields (Scheme 4 & Table 1).



SCHEME 4

To compare the efficiency of the UV irradiated decaging of RNAs containing various photolabile groups, the known phosphoramidite derivative of 2′-*O*-*o*-nitrobenzyl-N^6-benzoyladenosine **7j** was also prepared

TABLE 1 Phosphoramidites **7a-7j** prepared by the phosphitylation of **2a-2d** and **6a-6f**

Product	Precursor	X	Y	Z	B	Yield (%)
7a	**2a**	H	H	H	$G^{i\text{-}Bu}$	95
7b	**2b**	Me	H	H	$G^{i\text{-}Bu}$	86
7c	**2c**	H	H	H	G^{PhOAc}	77
7d	**2d**	Me	H	H	G^{PhOAc}	63
7e	**6a**	H	OMe	OMe	A^{PhOAc}	88
7f	**6b**	Me	H	H	A^{PhOAc}	92
7g	**6c**	H	H	H	C^{PhOAc}	92
7h	**6d**	Me	H	H	C^{PhOAc}	91
7i	**6e**	H	H	H	U	86
7j	**6f**	H	H	H	A^{Bz}	82

(Scheme 4).[6,14] The adenosine phosphoramidites **7e** (with 3,4-dimethoxy-2-nitrobenzyl group), **7f** (with α-methyl-*o*-nitrobenzyl group), and **7j** (with *o*-nitrobenzyl group) were then incorporated into a 30-mer RNA sequence- (an oligomer fragment for the construction of glms ribozyme):[22] 5′-GGU AAA UUA UAG AXG CGC CAG AAC UAC ACC-3′, where X represented the coupling of 2′-*O*-photocaged phosphoramidites (**7e**, **7f** or **7h**) at the ribozyme cleavage site. The solid-phase synthesis was carried out on Expedite 8900 Nucleic Acid Synthesizer with a modified RNA 1 μmole protocol. The standard RNA 1 μmole protocol for X was modified to double coupling before capping and oxidation. The trityl yields for the coupling of **7e**, **7f** or **7h** were comparable to the commercially available phosphoramidites. After deprotection with ammonium hydroxide, and desilylation with 1-methyl-2-pyrrolidinone (NMP)/Et_3N/Et_3N-3HF,[23] these RNAs were 5′-radiolabeled and purified by dPAGE gel as usual.[19] The progress of UV irradiated decaging of these RNAs was analyzed after alkaline hydrolysis and quantified by phosphorimager according to our reported method.[9,19] As expected, no alkaline hydrolysis was observed at the caged nucleotide site until UV irradiation (Figure 1). These data were then fitted into a first-order kinetics equation ($y = y_0 - A^*e^{-kt}$); and the resulting kinetics rates are shown in Table 2. From Table 2, we conclude that 2′-*O*-caged RNA containing an α-methyl-*o*-nitrobenzyl group has the most prominent photolabile properties. An RNA oligomer with α-methyl-*o*-nitrobenzyl group is uncaged by UV at 365 nm about 10 times faster compared to an RNA oligomer with *o*-nitrobenzyl group, whereas an RNA strand with 3,4-dimethoxy-2-nitrobenzyl group is uncaged by UV about 5 times faster than an RNA oligomer with *o*-nitrobenzyl group at 365 nm. The electronic donating groups (α-methyl and 3,4-dimethoxy) may have stabilized the resonance isomer (intermediate) formation thus facilitating the UV irradiated deprotection.[24]

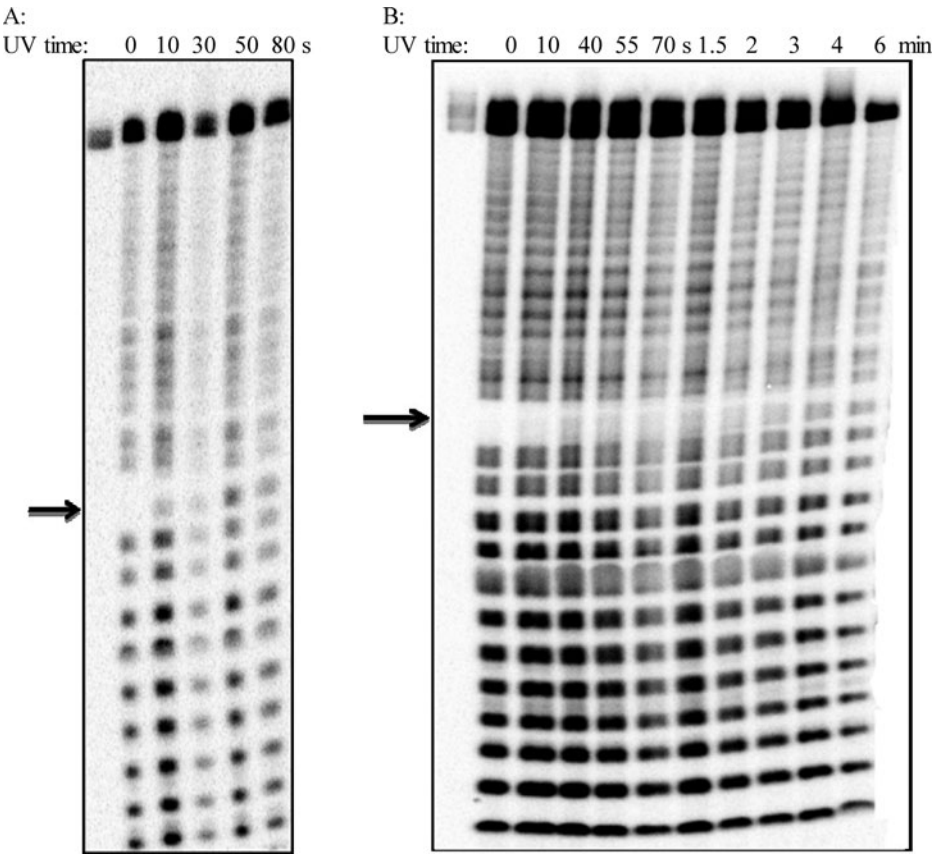


FIGURE 1 UV initiated deprotection and alkaline hydrolysis of 2′-*O*-caged RNAs containing 2′-*O*-photolabile groups. A: Gel image corresponds to the RNA containing α-methyl-*o*-nitrobenzyl protecting group. B: Gel image corresponds to the RNA containing *o*-nitrobenzyl protecting group. Method: A 5′-radiolabeled 30 mer RNA fragment in water (20 μL) was irradiated at 365 nm and 0.5-μL aliquots were taken and diluted in water (3 μL). Alkaline hydrolysis ladders were performed by adding sodium bicarbonate, pH 9, and incubating at 90 °C for 15 minutes. The resulting ladder of RNA fragments was separated by denaturing PAGE, and deprotection was quantitated by measuring the intensity of the band corresponding to cleavage by the deprotected nucleophile. The first lane in the depicted gel corresponds to deprotected substrate prior to alkaline hydrolysis.

In summary, we have described the synthesis of the phosphoramidite derivatives of adenosine, cytidine and guanosine with more 2′-*O*-photolabile and *N*-phenoxyacetyl protections. We also prepared the phosphoramidite derivative of 2′-*O*-*o*-nitrobenzyluridine. Solid phase synthesis using these phosphoramidites together with commercially available 2′-*O*-TBS-*N*-phenoxyacetylribonucleoside phosphoramidites would provide a general method to synthesize 2′-*O*-caged RNA oligonucleotides featured with ultramild deprotection conditions (NH_4OH/rt, 2 hours). Application of this new class of ribonucleoside phosphoramidites in the synthesis of

TABLE 2 Efficiency of the UV (365 nm) irradiated uncaging of 2′-*O*-caged RNAs containing various 2′-*O*-photolabile groups

2′−*O*-Caged RNAs	k (min^{-1})[a]	$t_{1/2}$ (min)[b]	Time (min)[c]
2′-*O*-α-methyl-*o*-nitrobenzyl-RNA	3.0	0.23	0.83
2′-*O*-2-nitro-3,4-$(MeO)_2C_6H_3CH_2$-RNA	1.6	0.43	1.5–3
2′-*o*-nitrobenzyl-RNA	0.27	2.6	4–6

[a] k is obtained from alkaline hydrolysis data of UV initiated uncaging of RNAs by fitting to the equation: $y = y_0 - A^*e^{-kt}$.

[b] $t_{1/2}$ is the time when 50% RNAs are uncaged.

[c] Time estimated for full uncaging.

RNA oligomers containing a 2′-*O*-photocaged-5′-*S*-phosphorothiolate linkage are currently being further evaluated and the syntheses are expected to be reported elsewhere later. Overall, 2′-*O*-photocaged RNAs containing an α-methyl-*o*-nitrobenzyl group seems to be the most photolabile amongst the three photocaged groups studied.

EXPERIMENTAL SECTION

5′-*O*-(Dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(*o*-nitrobenzyl)guanosine (2a).[19] Compound **2a** was prepared from N^2-isobutyrylguanosine (**1a)** in two steps (21% yield). Under argon N^2-Isobutyrylguanosine (**1a)** (1.717 g, 4.86 mmol) was treated with NaH (292 mg, 12.2 mmol) in dry DMF (40 mL) at 0°C. After hydrogen gas generation ceased (about 40 minutes), *o*-nitrobenzyl bromide (1.575 g, 7.29 mmol) was added and the mixture was stirred for 5 h at 0°C. The reaction was quenched with EtOH and neutralized with 1 N HCl. The mixture was evaporated and the crude product was purified by silica gel chromatography, eluting with 4–6% methanol in dichloromethane, to give N^2-isobutyryl-2′-*O*-(*o*-nitrobenzyl)guanosine[16,19] as a yellow foam: 0.76 g (32% yield). To a solution of N^2-isobutyryl-2′-*O*-(*o*-nitrobenzyl)guanosine (192 mg, 0.393 mmol) in dry pyridine (4.0 mL) under dry argon, DMTrCl (400 mg, 1.18 mmol) was added. After the mixture was stirred at rt overnight, methanol (2.0 mL) was added to quench the reaction. The solvent was removed by rotary evaporation, and the crude product was purified by silica gel chromatography, eluting with 2% MeOH in CH_2Cl_2 containing 0.5% Et_3N to give compound **2a**[19] as a yellow foam (206 mg, 66% yield). ^{1}H NMR ($CDCl_3$) δ 12.02 (br s, 1H), 8.61 (br s, 1H), 8.01 (d, $J = 8$ Hz, 1H), 7.87 (s, 1H), 7.67–7.18 (m, 12H), 6.8 (m, 4H), 6.01 (d, $J = 3.5$ Hz, 1H), 5.37 (d, $J = 15$ Hz, 1H), 5.22 (d, $J = 15$ Hz, 1H), 4.62 (t, $J = 4.5$ Hz, 1H), 4.55 (m, 1H), 4.27 (m, 1H), 3.77 (s, 3H), 3.767 (s, 3H), 3.55 (d, $J = 10.5$ Hz, 3H), 3.35 (m, 1H), 3.21 (br s, 1H), 2.29 (m, 1H), 1.12 (d, $J = 7$ Hz, 1H), 1.01 (d, $J = 7$ Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 231.0, 178.8, 158.6, 155.5, 147.5, 147.4, 146.9, 144.6, 137.5, 135.8, 135.6, 134.1,

133.8, 130.0, 129.9, 128.6, 128.5, 128.0, 127.9, 127.0, 124.7, 121.9, 113.2, 113.1, 87.0, 86.4, 83.6, 82.3, 69.4, 69.3, 63.1, 55.2, 36.2, 18.7, 18.6; HRMS calcd for $C_{42}H_{43}N_6O_{10}$ [MH^+] 791.3035, found 791.3041.

5′-*O*-(Dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(α-methyl-*o*-nitrobenzyl)guanosine (2b).[19] According to the procedure described for the synthesis of **2a**, N^2-isobutyryl-2′-*O*-(α-methyl-*o*-nitrobenzyl)guanosine[19] (636 mg, 26% yield) as a yellow foam was prepared from **1a** (1.717 g, 4.86 mmol), NaH (292 mg, 12.2 mmol) and α-methyl-*o*-nitrobenzyl bromide (1.677 g, 7.29 mmol). Compound **2b** was then prepared by the reaction of N^2-isobutyryl-2′-*O*-(α-methyl-*o*-nitrobenzyl)guanosine (307 mg, 0.61 mmol) with DMTrCl (621 mg, 1.83 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1–2% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **2b**[19] (345 mg, 70% yield) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 12.04 (s, 1H), 8.57 (s, 1H), 7.92–7.70 (m, 3H), 7.47–7.19 (m, 10H), 6.82–6.79 (m, 4H), 6.01 (d, J = 1.2 Hz, 1H), 5.77 (m, 1H), 4.33–4.26 (m, 2H), 3.91 (m, 1H), 3.770 (s, 3H), 3.767 (s, 3H), 3.57–3.45 (m, 2H), 2.72 (d, J = 8.3 Hz, 1H), 2.60 (m, 1H), 1.66 (d, J = 6.3 Hz, 3H), 1.27 (d, J = 6.9 Hz, 3H), 1.21 (d, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 178.8, 158.5, 155.3, 147.8, 147.6, 147.0, 144.4, 139.0, 136.6, 135.6, 135.5, 134.7, 130.0, 128.8, 128.0, 127.9, 127.0, 124.6, 121.9, 113.2, 87.6, 86.6, 82.7, 80.4, 72.4, 69.1, 62.5, 55.2, 36.5, 24.1, 19,2, 18.4; HRMS calcd for $C_{43}H_{45}N_6O_{10}$ [MH^+] 805.3192, found 805.3209.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (2c). According to the procedure described for the synthesis of **2a**, 2′-*O*-(*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (0.81 g, 30% yield) as a yellow foam was prepared from N^2-phenoxyacetylguanosine (**1b**)[25] (2.027 g, 4.86 mmol), NaH (292 mg, 12.2 mmol) and *o*-nitrobenzyl bromide (1.575 g, 7.29 mmol). Compound **2c** was then prepared by the reaction of 2′-*O*-(*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (308 mg, 0.56 mmol) with DMTrCl (565 mg, 1.68 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1–2% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **2c** (317 mg, 67% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 8.11 (s, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.73–7.69 (m, 2H), 7.55 (t, J = 6.6 Hz, 1H), 7.35–7.20 (m, 11H), 7.00–6.96 (m, 3H), 6.85–6.81 (m, 4H), 6.06 (d, J = 5.2 Hz, 1H), 5.47 (d, J = 2.6 Hz, 1H, disappeared with D_2O), 5.06 (d, J = 14.7 Hz, 1H), 4.97 (d, J = 14.6 Hz, 1H), 4.85 (d, J = 2.5 Hz, 2H), 4.59 (t, J = 5.1 Hz, 1H), 4.42 (m, 1H), 4.12 (m, 1H), 3.72 (s, 6H), 3.28–3.18 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 169.7, 158.6, 156.6, 147.7, 147.4, 144.3, 136.8, 135.5, 135.4, 133.8, 133.1, 130.0, 129.8, 128.9, 128.7, 128.1, 127.9, 127.0, 124.7, 122.6, 122.0, 114.8, 113.2, 86.7, 86.3, 83.6, 82.7, 69.8, 69.3, 67.0, 63.0, 55.2; HRMS calcd for $C_{46}H_{43}N_6O_{11}$ [MH^+] 855.2984, found 855.2996.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (2d). According to the procedure described for the synthesis

of **2a**, a crude 2′-*O*-(α-methyl-*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (530 mg, 19% yield) with some impurity as a yellow foam was prepared from **1b** (2.09 g, 5.01 mmol), NaH (301 mg, 12.54 mmol) and α-methyl-*o*-nitrobenzyl bromide (1.729 g, 7.52 mmol). Compound **2d** was then prepared by the reaction of 2′-*O*-(α-methyl-*o*-nitrobenzyl)-N^2-phenoxyacetylguanosine (107 mg, 0.19 mmol) with DMTrCl (192 mg, 0.57 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **2d** (47 mg, 28% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 7.76 (d, $J = 7.8$ Hz, 1H), 7.70 (s, 1H), 7.45–7.17 (m, 15H), 7.07–6.74 (m, 7H), 5.80 (d, $J = 4.7$ Hz, 1H), 5.43 (m, 1H), 4.63 (s, 2H), 4.53 (t, $J = 4.8$ Hz, 1H), 4.40 (t, $J = 4.8$ Hz, 1H), 4.21 (m, 1H), 3.75 (s, 6H), 3.42 (dd, $J = 2.4$, 10.7 Hz, 1H), 3.35 (dd, $J = 2.4$, 10.7 Hz, 1H), 1.59 (d, $J = 6.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.8, 158.5, 156.7, 147.4, 144.3, 138.7, 137.4, 135.5, 133.8, 129.9, 129.9, 129.7, 128.6, 128.0, 127.8, 127.4, 126.9, 123.8, 122.5, 114.7, 113.1, 86.5, 85.9, 84.1, 81.1, 73.9, 69.4, 67.0, 63.3, 55.1, 23.7; HRMS calcd for $C_{47}H_{45}N_6O_{11}$ [MH^+] 869.3141, found 869.3158.

2′-*O*-(4,5-Dimethoxy-2-nitrobenzyl)adenosine (**4a**). According to the procedure described for the synthesis of **2a**, compound **4a** was prepared from adenosine (1.51 g, 5.43 mmol), NaH (196 mg, 8.17 mmol) and 4,5-dimethoxy-2-nitrobenzyl bromide (2.248 g, 8.14 mmol). Silica gel chromatography (0–8% methanol in dichloromethane) of the crude product yielded **4a** (1.41 g, 54% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H), 8.06 (s, 1H), 7.56 (s, 1H), 7.35 (brs, 2H), 7.20 (s, 1H), 6.13 (d, $J =$ 5.0 Hz, 1H), 5.50 (d, $J = 5.0$ Hz, 1H), 5.42 (m, 1H), 5.07 (d, $J = 15.0$ Hz, 1H), 4.87 (d, $J = 15.0$ Hz, 1H), 4.56 (t, $J = 5.0$ Hz, 1H), 4.40 (m, 1H), 4.05 (m, 1H), 3.82 (s, 3H), 3.71 (s, 3H), 3.70 (m, 1H), 3.59 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 156.1, 153.2, 152.4, 148.9, 147.3, 139.5, 139.0, 129.4, 119.3, 110.1, 107.8, 86.3, 86.1, 81.3, 68.9, 67.9, 61.3, 56.0, 55.8; HRMS calcd for $C_{19}H_{23}N_6O_8$ [MH^+] 463.1572, found 463.1560.

2′-*O*-(4,5-Dimethoxy-2-nitrobenzyl)-N^6-phenoxyacetyladenosine (**5a**). Compound **4a** (0.68 g, 1.47 mmol) was dried by co-evaporation with dry pyridine (3 × 50 mL) under vacuum, and resuspended in dry pyridine (30 mL) under Ar. Chlorotrimethylsilane (1.5 mL) was added to the suspension at 0°C. After the reaction mixture was stirred at rt for 45 minutes, a solution of phenoxyacetyl chloride (310 μL, 2.21 mmol) and 1,2,4-triazole (155 mg, 2.26 mmol) in dry pyridine/acetonitrile (20 mL, 1:1) was slowly added. After the mixture was stirred at 55°C overnight, it was cooled to rt, and the reaction was quenched by addition of H_2O (1.5 mL). After stirring for 5 minutes, concentrated aqueous NH_4OH (1 mL) was added at 0°C, and the mixture was stirred for 30 minutes. The solvent was removed, the crude product was purified by silica gel chromatography, eluting with 3–5% MeOH in CH_2Cl_2 to give **5a** (797 mg, 91% yield) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.67 (br s, 1H), 8.71 (s, 1H), 8.17 (s,

1H), 7.44 (s, 1H), 7.37–7.33 (m, 2H), 7.07–7.04 (m, 3H), 6.76 (s, 1H), 6.07 (d, J = 7.3 Hz, 1H), 5.03 (d, J = 12.8 Hz, 1H), 4.96–4.93 (m, 3H), 4.71–4.65 (m, 2H), 4.40 (s, 1H), 3.99 (dd, J = 1.5, 13.0 Hz, 1H), 3.87 (s, 3H), 3.82 (s, 3H), 3.81 (dd, J = 1.5, 13.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 167.3, 157.1, 153.1, 152.0, 150.4, 149.1, 148.5, 143.7, 140.2, 129.9, 129.7, 126.7, 123.8, 115.0, 114.6, 111.4, 108.1, 89.1, 88.1, 81.2, 70.7, 70.1, 68.3, 65.1, 63.1, 56.5, 56.4; HRMS calcd for $C_{27}H_{29}N_6O_{10}$ [MH^+] 597.1940, found 597.1937.

2′-*O*-(*o*-Nitrobenzyl)-N^4-phenoxyacetylcytidine (5c).[19] 2′-*O*-(*o*-Nitrobenzyl)cytidine (4c)[15] (3.58 g, 9.46 mmol) was dried by co-evaporation with dry pyridine (3 × 100 mL) under vacuum and resuspended in dry pyridine (180 mL) under argon. Chlorotrimethylsilane (9.0 mL) was added to the suspension at 0°C. After the mixture was stirred at rt for 45 minutes, a solution of phenoxyacetyl chloride (2.00 mL, 14.4 mmol) and 1,2,4-triazole (0.980 g, 14.3 mmol) in pyridine-acetonitrile (120 mL, 1:1) was slowly added. After the mixture was stirred at 55°C overnight, it was cooled to rt, and the reaction was quenched by addition of H_2O (10 mL). After stirring for 5 minutes, concentrated aqueous NH_4OH (6.5 mL) was added at 0°C, and the mixture was stirred for 30 minutes. The solvent was removed, and the crude product was purified by silica gel chromatography, eluting with 3–5% MeOH in CH_2Cl_2 to give 5c[19] (3.44 g, 71% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 11.04 (s, 1H), 8.51 (d, 1H, J = 7.5 Hz), 8.08 (dd, 1H, J = 1.3, 8.2 Hz), 7.94 (d, 1H, J = 7.7 Hz), 7.77 (m, 1H), 7.57 (m, 1H), 7.35–7.25 (m, 2H), 7.11 (d, 1H, J = 7.5 Hz), 6.96 (m, 1H), 6.92 (m, 2H), 5.94 (d, 1H, J = 2.4 Hz), 5.34 (d, 1H, J = 6.7 Hz), 5.25 (t, 1H, J = 5.0 Hz), 5.15 (s, 2H), 4.83 (s, 2H), 4.12 (m, 1H), 4.15 (s, 1H), 4.03–3.95 (m, 3H), 3.79 (m, 1H), 3.64 (m, 1H); HRMS calcd for $C_{24}H_{25}N_4O_9$ [MH^+] 513.1622, found 513.1621.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(4,5-dimethoxy-2-nitrobenzyl)-N^6-phenoxyacetyladenosine (6a). According to the procedure described for the synthesis of **2a**, compound **6a** was prepared by the reaction of **5a** (797 mg, 1.34 mmol) with DMTrCl (1.25 g, 4.02 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **6a** (864 mg, 72% yield) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.47 (brs, 1H), 8.66 (s, 1H), 8.26 (s, 1H), 7.59 (s, 1H), 7.44–6.81 (m, 19H), 6.30 (d, J = 4.1 Hz, 1H), 5.24 (d, J = 14.0 Hz, 1H), 5.08 (d, J = 14.0 Hz, 1H), 4.86 (s, 2H), 4.74 (t, J = 4.6 Hz, 1H), 4.57 (t, J = 4.9 Hz, 1H), 4.32 (m, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 3.78 (s, 6H), 3.57 (dd, J = 3.2, 10.8 Hz, 1H), 3.46 (dd, J = 4.1, 10.7 Hz, 1H), 2.92 (b, 1H); ^{13}C NMR ($CDCl_3$) δ 158.7, 157.1, 153.6, 152.6, 151.4, 148.4, 148.3, 144.5, 141.9, 140.1, 135.62, 135.60, 130.2, 130.0, 128.2, 128.0, 127.8, 127.1, 123.2, 122.5, 115.0, 113.3, 110.6, 108.2, 87.1, 86.9, 84.3, 81.9, 70.1, 69.8,

68.2, 62.9, 56.5, 56.4, 55.3; HRMS calcd for $C_{48}H_{47}N_6O_{12}$ [MH^+] 899.3246, found 899.3244.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-N^6-phenoxyacetyl-adenosine (6b). According to the procedure described for the synthesis of **2a**, a crude 2′-*O*-(α-methyl-*o*-nitrobenzyl)adenosine (**4b**) (1.42 g) as a yellow foam was prepared from adenosine (2.00 g, 7.48 mmol), NaH (269 mg, 11.2 mmol) and α-methyl-*o*-nitrobenzyl bromide (2.51 g, 10.9 mmol). Compound **5b** was then prepared from the crude **4b** (1.42 g, 3.41 mmol), TMSCl (3.5 mL), and phenoxyacetyl chloride (0.71 mL, 5.12 mmol) as described for the synthesis of **5a**. Silica gel chromatography (5% methanol in dichloromethane) of the crude product yielded **5b** (1.20 g, 65% yield, two steps from adenosine) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.71 (s, 1H), 8.13 (s, 1H), 7.72–7.06 (m, 9H), 6.01 (d, $J=$ 7.0 Hz, 1H), 5.42 (m, 1H), 5.11 (m, 1H), 4.93 (s, 2H), 4.85 (m, 1H), 4.40 (d, $J=$ 4.6 Hz, 1H), 4.32 (d, $J=$ 1.3 Hz, 1H), 3.90 (m, 1H), 3.76 (m, 1H), 2.69 (s, 1H), 1.33 (d, $J=$ 6.4 Hz, 3H). Compound **6b** was then prepared by the reaction of **5b** (295 mg, 0.538 mmol) with DMTrCl (546 mg, 1.61 mmol) according to the procedure described for synthesis of **2a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **6b** (305 mg, 67% yield, or 44% from **3a**) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.36 (brs, 1H), 8.68 (s, 1H), 8.63–8.61 (m, 3H), 8.25 (s, 1H), 7.94–6.81 (m, 18H), 6.25 (d, J = 2.1 Hz, 1H), 5.68 (m, 1H), 4.86 (s, 2H), 4.34 (m, 1H), 4.29–4.25 (m, 2H), 3.79 (s, 6H), 3.55 (dd, J = 2.7, 10.8 Hz, 1H), 3.44 (dd, J = 4.0, 10.8 Hz, 1H), 2.56 (d, J = 8.0 Hz, 1H), 1.65 (d, J = 6.3 Hz, 3H); HRMS calcd for: $C_{47}H_{45}N_6O_{10}$, [MH^+]: 853.3197, found 853.3200.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-*N*-phenoxyacetylcytidine (6c).[19] According to the procedure described for the synthesis of **2a**, compound **6c** was prepared by the reaction of **5c** (1.24 g, 2.42 mmol) with DMTrCl (2.46 g, 7.26 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **6c**[19] (1.67 g, 86% yield) as a yellow foam. ^{1}H NMR (DMSO-d_6) δ 11.03 (s, 1H), 8.34 (d, J = 7.5 Hz, 1H), 8.08 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.76 (m, 1H), 7.57 (m, 1H), 7.40–6.85 (m, 19H), 5.94 (s, 1H), 5.4 (d, J = 7.3 Hz, 1H), 5.21 (dd, J = 15.2, 25.4 Hz, 2H), 4.82 (s, 2H), 4.35 (m, 1H), 4.15 (s, 1H), 4.01 (d, J = 4.8 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.42–3.36 (m, 2H); HRMS calcd for $C_{45}H_{43}N_4O_{11}$ [MH^+] 815.2928, found 815.2930.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-*N*-phenoxyacetyl-cytidine (6d). According to the procedure described for the synthesis of **2a**, a crude 2′-*O*-(α-methyl-*o*-nitrobenzyl)cytidine (**4d**) (~1.42 g) containing impurities as a yellow foam was prepared from cytidine (0.97 g, 4.0 mmol), NaH (192 mg, 8.00 mmol) and α-methyl-*o*-nitrobenzyl bromide (1.43 g,

6.22 mmol). The molecular weight of **4d** was confirmed by MS (ES-API), calcd for $C_{17}H_{21}N_4O_7$ [MH^+] 393.1, found 393.2. According to the procedure described for the synthesis of **5c** and **6c**, intermediate **4d** (∼1.42 g, 3.60 mmol) was first protected with phenoxyacetyl group to give a crude 2′-*O*-(*α*-methyl-*o*-nitrobenzyl)-N^4-phenoxyacetylcytidine (**5d**) (1.35 g). The molecular weight of **5d** was also confirmed by MS (ES-API), calcd for $C_{25}H_{27}N_4O_9$ [MH^+] 527.2, found 527.2. Compound **6d** was then prepared by the reaction of crude **5d** (1.35 g) with DMTrCl (2.57 g, 7.58 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **6d** (459 mg, three steps 14% yield from cytidine) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 9.78 (brs, 1H), 8.36 (d, J = 7.5 Hz, 1H), 7.86 (d, J = 8.5 Hz, 1H), 7.72 (d, J = 8.0 Hz, 1H), 7.59 (m, 1H), 7.40–7.20 (m, 14H), 7.09 (d, J = 7.5 Hz, 1H), 7.01 (t, J = 7.5 Hz, 1H), 6.86 (d, J = 8.5 Hz, 4H), 5.61 (q, J = 6.5 Hz, 1H), 5.56 (s, 1H), 4.65 (s, 2H), 4.35 (m, 1H), 4.11 (m, 1H), 3.92 (m, 1H), 3.81 (s, 6H), 3.62–3.52 (m, 2H), 1.64 (d, J = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 168.5, 161.8, 158.8, 156.9, 154.8, 148.0, 145.0, 144.2, 139.4, 135.6, 135.3, 133.3, 130.18, 130.15, 129.8, 128.3, 128.1, 127.2, 124.4, 122.4, 114.6, 113.4, 96.4, 88.5, 87.2, 83.4, 82.2, 75.0, 68.2, 67.6, 61.2, 55.3, 23.4; HRMS calcd for $C_{46}H_{44}N_4O_{11}Na$ [MNa^+] 851.2904, found 851.2874.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)uridine (6e). According to the procedure described for the synthesis of **2a**, compound **6e** was prepared by the reaction of 2′-*O*-(*o*-nitrobenzyl)uridine (**5e**)[17] (540 mg, 1.42 mmol) with DMTrCl (1.083 g, 3.20 mmol) in dry pyridine at rt overnight. Silica gel chromatography (1.5–2% methanol in dichloromethane) of the crude product yielded **6e** (727 mg, 75% yield) as a yellow foam. ^{1}H NMR ($CDCl_3$) δ 10.05 (brs, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.5 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.56 (m, 1H), 7.44–7.35 (m, 3H), 7.33–7.25 (m, 7H), 6.83 (d, 4H, J = 8.5 Hz), 6.05 (d, J = 1.8 Hz, 1H), 5.31 (d, J = 8.5 Hz, 1H), 5.28 (d, J = 13.5 Hz, 1H), 5.15 (d, J = 13.5 Hz, 1H), 4.53 (dd, J = 7.5, 5.5 Hz, 1H, 1H), 4.13–4.08 (m, 2H), 3.77 (s, 6H), 3.58 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 163.8, 158.7, 150.5, 147.9, 144.4, 139.9, 135.3, 135.1, 133.6, 133.0, 130.2, 130.1, 129.8, 128.8, 128.2, 128.0, 127.2, 124.8, 113.3, 102.2, 87.4, 87.1, 83.2, 82.6, 69.4, 68.7, 61.3, 55.3; HRMS calcd for $C_{37}H_{35}N_3O_{10}Na$ [MNa^+] 704.2220, found 704.2200.

5′-*O*-(Dimethoxytrityl)-N^2-isobutyryl-2′-*O*-(*o*-nitrobenzyl)guanosine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7a).[20] To a solution of compound **2a**[19] (47 mg, 0.059 mmol) in dry CH_2Cl_2 (5.0 mL) under Ar, *N,N*-diisopropylethylamine (52 μL, 0.30 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (42 mg, 0.18 mmol) and 1-methylimidazole (5.0 μL, 0.059 mmol) were added. The mixture was stirred at rt until all starting material was consumed (1 h). The reaction was quenched with MeOH (1 mL) and stirred for 5 minutes. After the solvent

was removed, the crude product was purified by silica gel chromatography, eluting with 1% MeOH in CH_2Cl_2 containing 0.5% Et_3N to give phosphoramidite **7a**[20] (56 mg, 95% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.8, 152.6; HRMS calcd for $C_{51}H_{60}N_8O_{11}P$ [MH^+]: 991.4119, found: 991.4110.

5′-*O*-(Dimethoxytrityl)-*N*^{2}-isobutyryl-2′-*O*-(α-methyl-*o*-nitrobenzyl)guanosine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7b). Phosphoramidite **7b** was prepared from 5′-*O*-(dimethoxytrityl)-*N*^{2}-isobutyryl-2′-*O*-(α-methyl-*o*-nitrobenzyl)guanosine **(2b)**[19] (255 mg, 0.317 mmol), *N,N*-diisopropylethylamine (277 μL, 1.59 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (224 mg, 0.95 mmol), and 1-methylimidazole (27 μL, 0.32 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7b** (275 mg, 86% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.7, 152.4; HRMS calcd for $C_{52}H_{62}N_8O_{11}P$ [MH^+]: 1005.4276, found: 1005.4276.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-*N*^{2}-phenoxyacetylguanosine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7c). Phosphoramidite **7c** was prepared from **2c** (284 mg, 0.332 mmol), *N,N*-diisopropylethylamine (290 μL, 1.66 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (234 mg, 0.996 mmol), and 1-methylimidazole (28 μL, 0.33 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7c** (270 mg, 77% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 153.0, 152.7; HRMS calcd for $C_{55}H_{60}N_8O_{12}P$ [MH^+]: 1055.4069, found: 1055. 4075.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-*N*^{2}-phenoxyacetylguanosine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7d). Phosphoramidite **7d** was prepared from **2d** (156 mg, 0.18 mmol), *N,N*-diisopropylethylamine (157 μL, 0.90 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (127 mg, 0.54 mmol), and 1-methylimidazole (15 μL, 0.18 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7d** (121 mg, 63% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.6, 152.5; HRMS calcd for $C_{56}H_{62}N_8O_{12}P$ [MH^+]: 1069.4225, found: 1069.4235.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(4,5-dimethoxy-2-nitrobenzyl)-*N*^{6}-phenoxyacetyladenosine 3′-*N,N*-diisopropyl (cyanoethyl)phosphoramidite (7e). Phosphoramidite **7e** was prepared from **6a** (210 mg, 0.234 mmol), *N,N*-diisopropylethylamine (194 μL, 1.11 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (156 mg, 0.66 mmol), and 1-methylimidazole (18 μL, 0.21 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7e** (225 mg, 88% yield) as a yellow

foam. ^{31}P NMR (CD_3CN) δ 152.8, 152.7; HRMS calcd for $C_{57}H_{64}N_8O_{13}P$ [MH^+] 1099.4325, found 1099.4309.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-N^6-phenoxyacetyl-adenosine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7f). Phosphoramidite **7f** was prepared from **6b** (284 mg, 0.33 mmol), *N,N*-diisopropylethylamine (276 μL, 1.58 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (221 mg, 0.94 mmol), and 1-methylimidazole (26 μL, 0.30 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7f** (322 mg, 92% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.6, 152.0; HRMS calcd for $C_{56}H_{62}N_8O_{11}P$ [MH^+]: 1053.4276, found: 1053.4277.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-*N*-phenoxyacetylcytidine 3′-*N,N*-Diisopropyl-(cyanoethyl)phosphoramidite (7g). Phosphoramidite **7g** was prepared from 5′-*O*-(dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-*N*-phenoxyacetylcytidine **(6c)**[19] (185 mg, 0.23 mmol), *N,N*-diisopropylethylamine (192 μL, 1.10 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (154 mg, 0.66 mmol), and 1-methylimidazole (18 μL, 0.21 mmol) as described for **7a**. Silica gel chromatography (0–4% acetone in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7g** (215 mg, 92% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.4, 151.6; HRMS calcd for $C_{54}H_{60}N_6O_{12}P$, [MH^+]: 1015.4007, found: 1015.4011.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(α-methyl-*o*-nitrobenzyl)-*N*-phenoxyacetyl-cytidine 3′-*N,N*-Diisopropyl(cyanoethyl)phosphoramidite (7h). Phosphoramidite **7h** was prepared from **6d** (150 mg, 0.18 mmol), *N,N*-diisopropylethylamine (158 μL, 0.91 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (129 mg, 0.54 mmol), and 1-methylimidazole (15 μL, 0.18 mmol) as described for **7a**. Silica gel chromatography (0–1% acetone in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7h** (169 mg, 91% yield) as a white foam. ^{31}P NMR (CD_3CN) δ 151.2, 149.4; HRMS calcd for $C_{55}H_{62}N_6O_{12}P$, [MH^+]: 1029.4163, found: 1029.4143.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)uridine 3′-*N,N*-Diisopropyl (cyanoethyl)-phosphoramidite (7i). Phosphoramidite **7i** was prepared from **6e** (121 mg, 0.18 mmol), *N,N*-diisopropylethylamine (158 μL, 0.91 mmol), 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (129 mg, 0.54 mmol), and 1-methylimidazole (15 μL, 0.18 mmol) as described for **7a**. Silica gel chromatography (0–2% acetone in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7i** (135 mg, 86% yield) as a white foam. ^{31}P NMR (CD_3CN) δ 150.9, 150.1; HRMS calcd for $C_{46}H_{53}N_5O_{11}P$, [MH^+]: 882.3479, found: 882.3469.

5′-*O*-(Dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-N^6-benzoyladenosine 3′-*N,N*-Diisopropyl-(cyanoethyl)phosphoramidite (7j).[6,14] Phosphoramidite

7j was prepared from 5′-*O*-(dimethoxytrityl)-2′-*O*-(*o*-nitrobenzyl)-N^6-benzoyladenosine (**6f**)[6,14,19] (99 mg, 0.12 mmol), *N*,*N*-diisopropylethylamine (106 μL, 0.61 mmol), 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (221 mg, 0.36 mmol), and 1-methylimidazole (10 μL, 0.12 mmol) as described for **7a**. Silica gel chromatography (1% methanol in dichloromethane containing 0.5% triethylamine) of the crude product yielded **7j**[6,14] (101 mg, 82% yield) as a yellow foam. ^{31}P NMR (CD_3CN) δ 152.9, 152.7.

ACKNOWLEDGMENT

We thank Dr. Qing Dai, Mr. Saurja Dasgupta, Dr. Armando Hernandez, Mr. Hao Huang and Dr. Sandip Shelke for helpful discussions and critical comments on the manuscript.

FUNDING

This work was supported by an N.I.H. grant to J.A.P. (1R01-AI081987).

REFERENCES

1. Pillai, V.N.R. Photoremovable protecting groups in organic synthesis. *Synthesis* **1980**, 1–26.
2. Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 3rd ed., John Wiley & Sons Inc., New York, 1999.
3. Adams, S.R.; Tsien, R.Y. Controlling cell chemistry with caged compounds. *Annu. Rev. Physiol.* **1993**, 55, 755–784.
4. Tang, X.; Dmochowski, I.J. Regulating gene expression with light-activated oligonucleotides. *Mol. Biosyst.* **2007**, 3, 100–110.
5. Casey, J.P.; Blidner, R.A.; Monroe, W.T. Caged siRNAs for spatiotemporal control of gene silencing. *Mol. Pharm.* **2009**, 6, 669–685.
6. Chaulk, S.G.; MacMillan, A.M. Caged RNA: photo-control of a ribozyme reaction. *Nucleic Acids Res.* **1998**, 26, 3173–3178.
7. Das, S.R.; Piccirilli, J.A. General acid catalysis by the hepatitis delta virus ribozyme. *Nat. Chem. Biol.* **2005**, 1, 45–52.
8. Kath-Schorr, S.; Wilson, T.J.; Li, N.-S.; Lu, J.; Piccirilli, J.A.; Lilley, D.M. General acid-base catalysis mediated by nucleobases in the hairpin ribozyme. *J. Am. Chem. Soc.* **2012**, 134, 16717–16724.
9. Wilson, T.J.; Li, N.-S.; Lu, J.; Frederiksen, J.K.; Piccirilli, J.A.; Lilley, D.M. Nucleobase-mediated general acid-base catalysis in the Varkud satellite ribozyme. *Proc. Natl. Acad. Sci. USA* **2010**, 107, 11751–11756.
10. Ohtsuka, E.; Ohta, T.; Koizumi, M. Sequence-specific cleavage of RNA by designed ribozymes. *Supramol. Chem.* **1993**, 2, 197–200.
11. Chaulk, S.G.; MacMillan, A.M. Separation of spliceosome assembly from catalysis with caged pre-mRNA Substrates. *Angew. Chem. Int. Ed.* **2001**, 40, 2149–2152.
12. Fica, S.M.; Tuttle, N.; Novak, T.; Li, N.-S.; Lu, J.; Koodathingal, P.; Dai, Q.; Staley, J.P.; Piccirilli, J.A. RNA catalyzes nuclear pre-mRNA splicing. *Nature* **2013**, 503, 229–234.
13. Tanaka, T.; Tamatsukuri, S.; Ikehara, M. Solid phase synthesis of oligoribonucleotides using the o-nitrobenzyl group for 2′-hydroxyl protection and H-phosphonate chemistry. *Nucleic Acids Res.* **1987**, 15, 7235–7248.
14. Chaulk, S.G.; MacMillan, A.M. Synthesis of oligo-RNAs with photocaged adenosine 2′-hydroxyls. *Nat. Protoc.* **2007**, 2, 1052–1058.

15. Ohtsuka, E.; Tanaka, S.; Ikehara, M. Studies on transfer ribonucleic acids and related compounds. XVI. Synthesis of ribooligonucleotides using a photoseneitive o-nitrobenzyl protection for the 2′-hydroxyl group. *Chem. Pharm. Bull.* **1977**, 25, 949–959.
16. Ohtsuka, E.; Tanaka, S.; Ikehara, M. Studies of transfer ribonucleic-acids and related compounds. XVIII. Photolabile 2′-ether of guanosine as an intermediate for oligonucleotide synthesis. *Synthesis* **1977**, 453–454.
17. Ohtsuka, E.; Tanaka, S.; Ikehara, M. Studies on transfer ribonucleic acids and related compounds. IX. Ribooligonucleotide synthesis using a photosensitive o-nitrobenzyl protection at the 2′-hydroxyl group. *Nucleic Acids Res.* **1974**, 1, 1351–1357.
18. Ohtsuka, E.; Tanaka, T.; Tanaka, S.; Ikehara, M. Studies on transfer ribonucleic acids and related compounds. 20. A new versatile ribooligonucleotide block with 2′-(o-nitrobenzyl) and 3′-phosphorodianilidate groups suitable for elongation of chains in the 3′ and 5′ directions. *J. Am. Chem. Soc.* **1978**, 100, 4580–4584.
19. Li, N.-S.; Frederiksen, J.K.; Koo, S.C.; Lu, J.; Wilson, T.J.; Lilley, D.M.; Piccirilli, J.A. A general and efficient approach for the construction of RNA oligonucleotides containing a 5′-phosphorothiolate linkage. *Nucleic Acids Res.* **2011**, 39, e31 1–16.
20. Li, N.-S.; Tuttle, N.; Staley, J.P.; Piccirilli, J.A. Synthesis and incorporation of the phosphoramidite derivative of 2′-o-photocaged 3′-s-thioguanosine into oligoribonucleotides: substrate for probing the mechanism of RNA catalysis. *J. Org. Chem.* **2014**, 79, 3647–3652.
21. Wagner, D.; Verheyden J.P.H.; Moffatt, J.G. Preparation and synthetic utility of some organotin derivatives of nucleosides. *J. Org. Chem.* **1974**, 39, 24–30.
22. Viladoms, J.; Scott, L.G.; Fedor, M.J. An active-site guanine participates in glmS ribozyme catalysis in its protonated state. *J. Am. Chem. Soc.* **2011**, 133, 18388–18396.
23. Wincott, F.; DiRenzo, A.; Shaffer, C.; Grimm, S.; Tracz, D.; Workman, C.; Sweedler, D.; Gonzalez, C.; Scaringe, S.; Usman, N. Synthesis, deprotection, analysis and purification of RNA and ribozymes. *Nucleic Acids Res.* **1995**, 23, 2677–2684.
24. Pelliccioli, A.P.; Wirz, J. Photoremovable protecting groups: reaction mechanisms and applications. *Photochem. Photobiol. Sci.* **2002**, 1, 441–458.
25. Singh, K.K.; Nahar, P. An improved method for the synthesis of N-phenoxyacetylribonucleosides. *Synth. Commun.* **1995**, 25, 1997–2003.