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Synthesis of Amine- and Thiol-Modified Nucleoside Phosphoramidites for Site-Specific Introduction of Biophysical Probes into RNA

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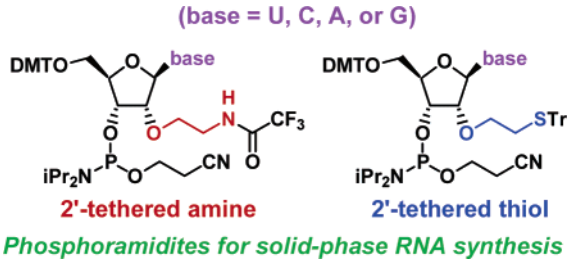
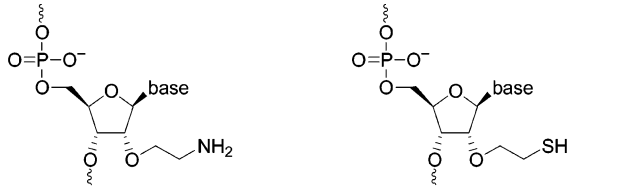


For studies of RNA structure, folding, and catalysis, site-specific modifications are typically introduced by solid-phase synthesis of RNA oligonucleotides using nucleoside phosphoramidites. Here, we report the preparation of two complete series of RNA nucleoside phosphoramidites; each has an appropriately protected amine or thiol functional group. The first series includes each of the four common RNA nucleotides, U, C, A, and G, with a 2′-(2-aminoethoxy)-2′-deoxy substitution (i.e., a primary amino group tethered to the 2′-oxygen by a two-carbon linker). The second series encompasses the four common RNA nucleotides, each with the analogous 2′-(2-mercaptoethoxy)-2′-deoxy substitution (i.e., a tethered 2′-thiol). The amines are useful for acylation and reductive amination reactions, and the thiols participate in displacement and oxidative cross-linking reactions, among other likely applications. The new phosphoramidites will be particularly valuable for enabling site-specific introduction of biophysical probes and constraints into RNA.

Introduction

Appending chromophores and other biophysical probes and constraints onto biological macromolecules enables us to develop a detailed understanding of their structure, folding, and catalysis.[1] For such purposes, an important prerequisite is the development of the chemical methods necessary to synthesize the modified biomolecules. Here, we describe the preparation of eight modified RNA nucleoside phosphoramidites. These reagents collectively allow the solid-phase synthesis of RNA oligonucleotides that incorporate a primary amine or thiol group tethered to the ribose 2′-oxygen of any specific nucleotide via a two-carbon tether (Chart 1). The ribose 2′-position is particularly useful for chemical modification because of its location in the minor groove of A-form duplex nucleic acid, where it points outward into solution.[2] The ribose 2′-position is also readily modified synthetically, as described in this work. Both established[3] and emerging[4]

CHART 1. RNAs with Site-Specific 2′-Tethered Amine or 2′-Tethered Thiol Groups



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† S.J. and C.V.M. are co-first authors of this paper.

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ligation techniques are used to construct RNAs larger than those that can be prepared by direct solid-phase synthesis (<100 nt),[5] as required for the study of many catalytically active RNAs.[6] In conjunction with such techniques, the modified nucleoside phosphoramidites reported here should be immediately useful in ongoing biochemical and biophysical studies of nucleic acids.

We chose to prepare phosphoramidites with relatively short two-carbon tethers rather than longer tethers for two specific reasons. First, for some purposes, such as certain fluorescence applications, a long tether can provide too little interaction between the RNA and a chromophore appended onto the tether. Because a spacer of variable length can typically be incorporated during the reaction with a chromophore reagent,[1h] the most generally useful tethers for such applications are relatively short. Second, for applications involving structural constraints, a long tether may introduce too much flexibility into the RNA, thereby precluding the goal of a structural constraint. In contrast, a short tether permits more structural control over the RNA conformation. For such applications, the two-carbon tethers in Chart 1 are the shortest chemically stable tethers that retain the 2′-oxygen atom (thereby avoiding certain concerns over destabilizing RNA structure with unnatural functional groups[7]) and also provide reactive amine or thiol functional groups for subsequent derivatization. Our synthetic routes to the nucleoside phosphoramidites that are required to incorporate these tethers into RNA are designed to be maximally convergent. Experimental protocols for preparing sufficient quantities of each phosphoramidite (at least 100 mg) for conventional solid-phase RNA oligonucleotide synthesis are described.

Results and Discussion

Pyrimidine Nucleoside Phosphoramidites with 2′-Tethered Amines. A small number of pyrimidine nucleosides with a primary amino group tethered via the ribose 2′-oxygen have been reported previously. For tethers longer than two carbon atoms, the only reported approach has been the reaction of a pyrimidine nucleoside 2′,3′-stannylene derivative[8] with an alkyl halide. The reaction proceeds with a modest chemical yield and little, if any, selectivity between 2′- and 3′-alkylation,[9] in those cases for which the yield and selectivity were reported.[10] We chose to avoid such alkylation routes to prevent separating mixtures of 2′- and 3′-isomers. For the two-carbon tethers, others have reported nucleophilic attack on a 2,2′-anhydrothymidine derivative, either by a boron derivative of 2-hydroxyethylphthalimide[11] or by the latter reagent itself in the presence of $Ti(OiPr)_4$.[12] The former procedure requires a sealed stainless steel bomb heated to 150 °C and provides only a 21% yield of 2′-modified product after silica gel column chromatography; the yield is 18% for a three-carbon tether and anhydrouridine.[13] The latter procedure with $Ti(OiPr)_4$ is reported to produce a 32% yield, but no experimental protocol was provided. An alternative approach to the 2′-modified pyrimidine nucleosides first protects the 3′- and 5′-hydroxyl groups as a TIPDS silyl derivative (see below); this step is followed by the alkylation of the 2′-hydroxyl group with an α-bromo ester.[14,15] Then, reduction of the ester produces the 2′-OCH_2CH_2OH substitutent for further manipulation. In one case, the tethered hydroxyl group was activated with TsCl and displaced with NaN_3 to introduce the nitrogen, but the communication provided no experimental protocols.[14] In another case, phthalimide was used in a Mitsunobu reaction to introduce the nitrogen, and again no experimental protocol was provided.[15]

On the basis of these limited precedents, we decided to protect the pyrimidine 3′- and 5′-hydroxyl groups at the outset and to employ a different 2′-alkylation strategy. Our route began with the simultaneous TIPDS (tetraisopropyldisiloxane-1,3-diyl) protection[16] of the 3′- and 5′-hydroxyl groups of uridine **U1** (Scheme 1).[17] The $TIPDSCl_2$ silylating reagent is commercially available, albeit expensive. As an alternative, $TIPDSCl_2$ may conveniently be generated from the corresponding bissilane and CCl_4 using substoichiometric $PdCl_2$.[18] The crude TIPDS-uridine was immediately protected as its

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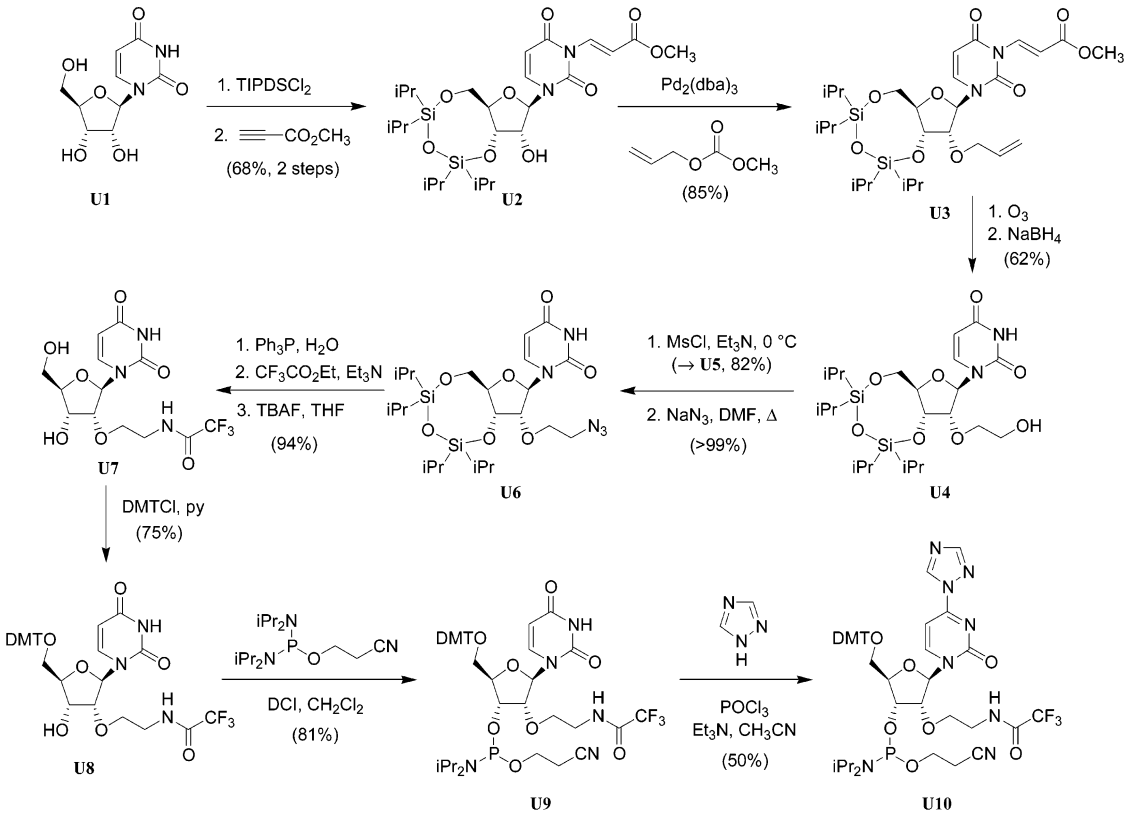
SCHEME 1



N^3-methoxycarbonylvinyl (mocvinyl) derivative,[19] **U2**;[20] this protection was necessary to avoid undesired derivatization of N^3 in the subsequent alkylation step. Palladium-catalyzed allylation[21] of the free 2′-hydroxyl group of **U2** readily produced **U3** in high yield. This 2′-*O*-allyl nucleoside was ozonolyzed and treated with $NaBH_4$ to provide 2′-(2-hydroxyethyl) nucleoside **U4**. The ozonolysis conditions also removed the mocvinyl group, presumably by the oxidative cleavage of the C=C double bond and the subsequent breakdown of the hemiaminal. TIPDS-protected nucleoside **U4** has been accessed before by different routes,[22−24] but at most partial experimental protocols were provided; in all cases, our route is one to five steps shorter.

The tethered hydroxyl group of **U4** was exchanged for an azido moiety by mesylation to **U5**[23] and displacement with sodium azide in the presence of 18-crown-6, forming **U6**. In a one-pot procedure, the azide of **U6** was reduced via the Staudinger reaction (PPh_3, H_2O) to the amino group, which was protected as the trifluoromethyl amide, and the TIPDS 3′,5′-protecting group was removed with TBAF, producing **U7**.[14] The Staudinger reaction was used instead of H_2 reduction over Pd/C because, in our hands, the latter method led to ∼15% hydrogenation of the uracil C^5=C^6 double bond.[25] Trifluoromethyl amide protection was chosen because the trifluoromethyl group is readily removed by transamidation during the standard NH_3 deprotection of RNA oligonucleotides following solid-phase synthesis.[26]

Selective reprotection of the 5′-hydroxyl group of **U7** as its 4,4′-dimethoxytrityl (DMT) derivative led to **U8**, which was phosphitylated with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite using 4,5-dicyanoimidazole (DCI) as the activator[27] to provide uridine-tethered amine nucleoside phosphoramidite **U9**. Starting with uridine **U1**, the overall route provides phosphoramidite **U9** in a 20% overall yield in 9 steps with 7 chromatographic purifications; 440 mg of **U9** was obtained. This route to the 2′-(2-aminoethoxy)-substituted pyrimidine phosphoramidite represents a substantial improvement in yield and convenience for moderate-scale laboratory synthesis when compared to previous reports for similar compounds, as cited above.

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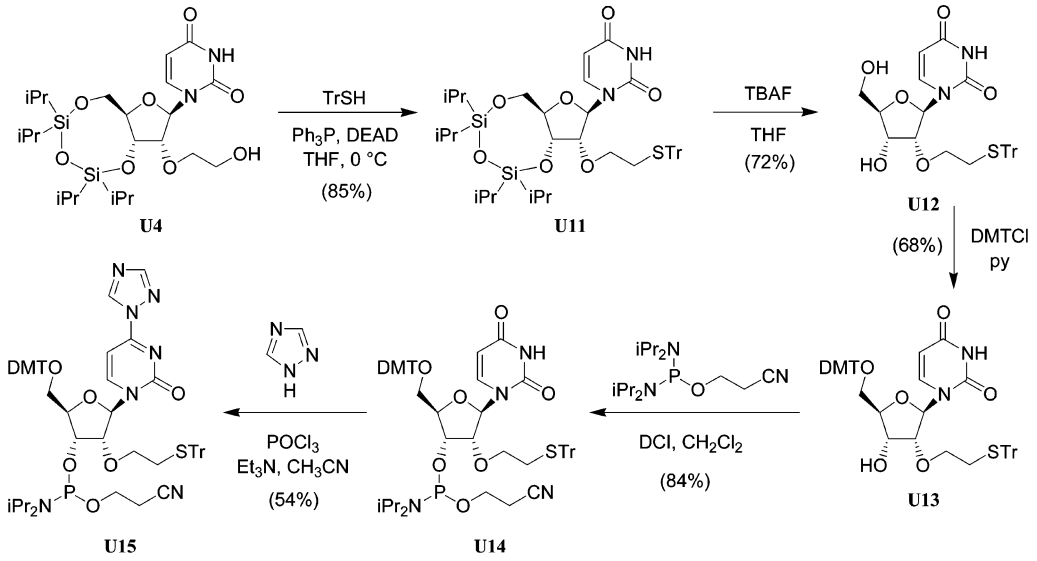
SCHEME 2



U9 was converted to its triazolide derivative **U10** to obtain the cytidine phosphoramidite analogue (Scheme 1). This reagent can be incorporated as usual during solid-phase RNA synthesis and upon standard oligonucleotide deprotection with NH_3 leads to cytidine at the desired nucleotide position.[28] This highly convergent route is greatly preferred over the independent synthesis of the cytidine nucleoside phosphoramidite starting from cytidine.[29] Such an independent route would entail nearly twice the number of reaction steps as synthesis of the uridine nucleoside phosphoramidite **U9** plus its one-step conversion to the triazolide **U10**. Starting from uridine **U1**, phosphoramidite **U10** was obtained in a 10% overall yield in 10 steps with 8 chromatographic purifications; 100 mg of **U10** was prepared.

Pyrimidine Nucleoside Phosphoramidites with 2′-Tethered Thiols. The route to the pyrimidine nucleosides with 2′-tethered thiols started with intermediate **U4** from the pyrimidine-tethered amines strategy (Scheme 2). Displacement of the hydroxyl group with trityl mercaptan under Mitsunobu conditions produced tritylprotected thiol **U11**.[22] The conversion of **U4** to **U11** appears to be the first reported use of trityl mercaptan as the nucleophile in a Mitsunobu reaction. Intermediate **U11** was treated with TBAF to remove the 3′,5′-protecting group, providing diol **U12**. As was done for the amine route, the 5′-hydroxyl was reprotected as its DMT derivative to produce **U13**, which was phosphitylated to provide uridine-tethered thiol nucleoside phosphoramidite **U14**. The overall route from uridine **U1** to **U14** is 8 steps with 7 chromatographic purifications and proceeds in a 13% overall yield; 340 mg of **U14** was produced. Compounds **U11**−**U14** were previously accessed by a different route with little experimental information provided;[22] our route to **U11** is three steps shorter and has a higher yield starting from uridine (30% vs 16−20%). The cytidine phosphoramidite analogue was obtained from **U14** as the triazolide derivative **U15**, in a manner analogous to the tethered amine route. A cytidine-tethered thiol was prepared previously by a completely different route that did not involve the uridine triazolide.[29] The route from **U1** to **U15** is 9 steps with 8 chromatographic purifications in 7% overall yield; 112 mg of **U15** was prepared.

The trityl group has been used previously for thiol protection of nucleosides.[30,31] For the 2′-tethered thiol, we chose trityl rather than *tert*-butyl disulfide protection[23] because the *S*-trityl deprotection procedure (treatment with $AgNO_3$[31]) avoids the use of reducing agents such as DTT. Application of such reducing agents would complicate the subsequent use of oligonucleotides with *tert*-butyl disulfide protecting groups in RNA ligation reactions catalyzed by protein enzymes, which are normally used in DTT-containing buffers.[3]

Adenosine Nucleoside Phosphoramidite with 2′-Tethered Amine. Our preparation of the adenosine nucleoside phosphoramidite with a 2′-tethered amine followed a route related to that used for the pyrimidines (Scheme 3). Adenosine **A1** was alkylated preferentially at the 2′-position[10,32] with NaH and methyl bromoacetate to provide **A2** in a 47% yield.[33] Although ethyl bromoacetate had a slightly higher yield of 58%, greater than 50% of the ethyl ester analogue of **A2** underwent transesterification during silica gel chromatography with $MeOH/CH_2Cl_2$ as the eluent (for separation purposes, methanol proved to be superior to ethanol as the chromatography cosolvent). Therefore, although the yield was

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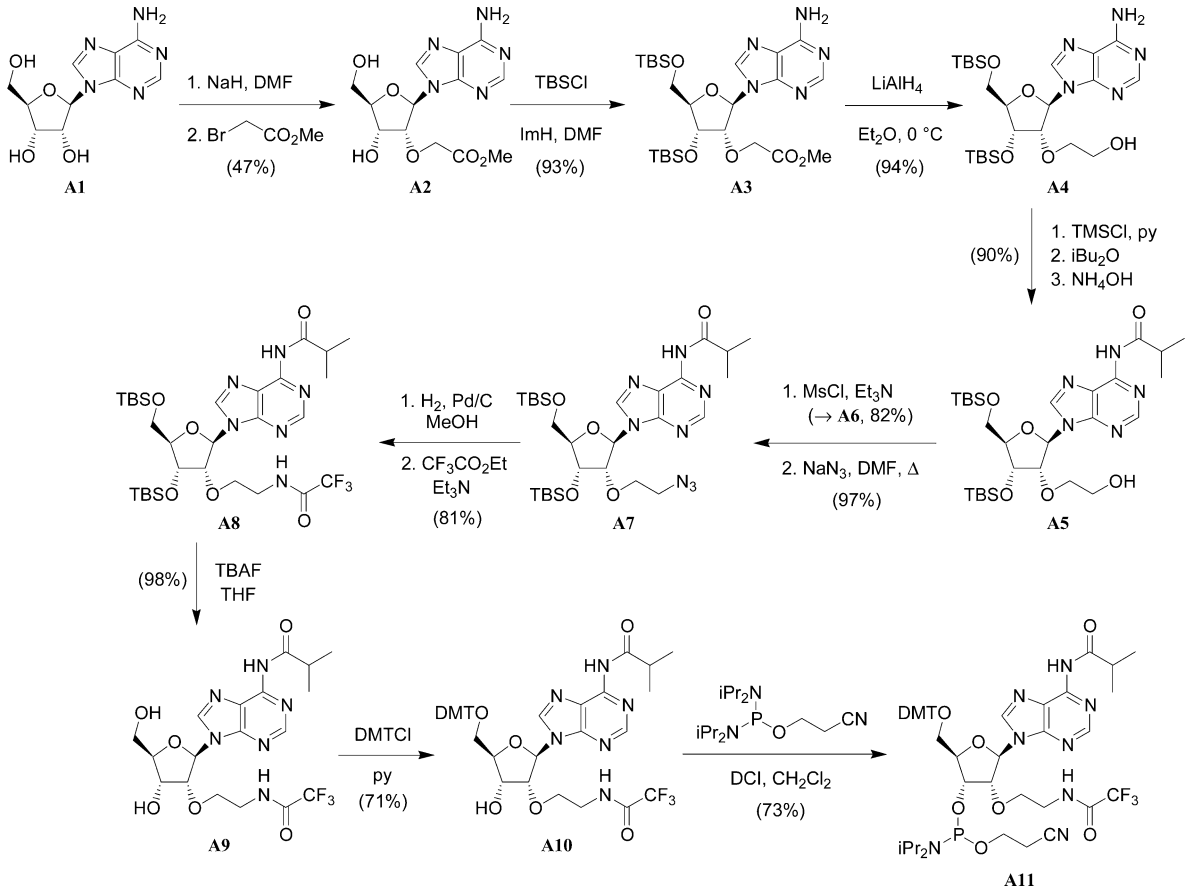
SCHEME 3



slightly lower, we chose to use methyl bromoacetate in the alkylation reaction so that the product, **A2**, was homogeneous and fully characterizable by 2D NMR spectroscopy. In the 2D HMBC spectrum of **A2**, both of the long-range (four-bond) *H*−C−O−*C* couplings between $H^{2'}$ and 2′-OCH_2 and between 2′-OCH_2 and $C^{2'}$ were clearly observed, thereby confirming the identity of **A2** as the 2′-alkylated isomer.

Nucleoside **A2** was doubly protected with *tert*-butyldimethylsilyl (TBS) at its 3′- and 5′-positions to produce **A3**. We used double TBS protection rather than 3′,5′-TIPDS protection because the site-selectivity of $TIPDSCl_2$ for the 3′- and 5′-positions was not required (the 2′-hydroxyl was already substituted) and TBSCl is less expensive than $TIPDSCl_2$. Nucleoside **A3** was reduced with $LiAlH_4$ to provide the 2′-(2-hydroxyethyl) nucleoside **A4**. In our hands, $NaBH_4$ was ineffective for reductions of this type.[33]

Our overall strategy to the 2′-(2-hydroxyethyl)adenosine nucleoside is an alternative to the previously used 2′-*O*-allylation of adenosine followed by ozonolysis and reduction of the tethered aldehyde group, which was performed with a different combination of hydroxyl and nucleobase protecting groups.[34] Here, we favored adenosine 2′-alkylation with the α-bromo ester because we found that the 2′-isomer, **A2**, and its 3′-isomer could easily be separated by silica gel chromatography, which was not the case for the 2′-*O*-allyl derivatives.[34] We also found that direct 2′-alkylation of adenosine with functionalized alkyl halides such as *N*-(2-bromoethyl)phthalimide, which was at least moderately successful with longer-chain substituted alkyl halides,[9,10,32] was not preparatively useful for the two-carbon tether (R. L. Coppins and S. K. Silverman, data not shown).

Protection of nucleobase N^6 of **A4** as its isobutyryl amide was achieved with transient TMS protection[35] of the 2′-tethered hydroxyl group, leading to **A5**. The 2′-tethered hydroxyl group was exchanged for an azido group by mesylation to **A6**, which was followed by azide displacement yielding **A7**. The amine was obtained by reduction with H_2 over Pd/C and then protected as trifluoromethyl amide **A8**. Removal of the 3′- and 5′-silyl groups with TBAF, yielding **A9**, was followed by 5′-DMT protection to produce **A10** and phosphitylation to yield adenosine tethered amine nucleoside phosphoramidite **A11**. The route from adenosine **A1** to **A11** takes 10 steps with 8 chromatographic purifications in a 12% overall yield; 198 mg of phosphoramidite **A11** was prepared.

Adenosine Nucleoside Phosphoramidite with 2′-Tethered Thiol. Adenosine nucleoside derivatives with 2′-OCH_2CH_2SR substituents appear to be unreported. The adenosine-tethered thiol phosphoramidite was prepared from intermediate **A5** by a route analogous to that used for the pyrimidine thiol phosphoramidites (Scheme 4). Mitsunobu displacement with trityl mercaptan produced **A12** from **A5**; **A12** was 3′,5′-deprotected to form

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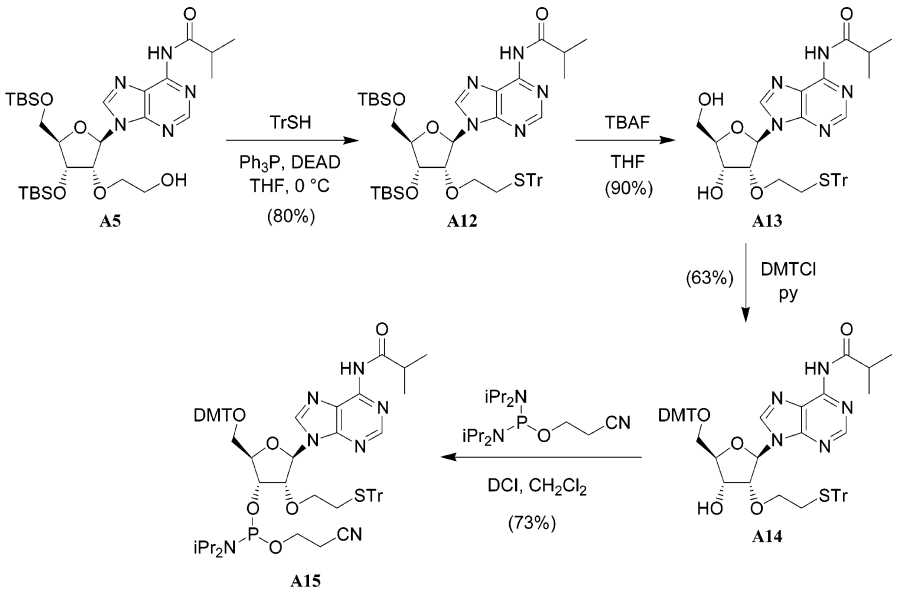
SCHEME 4



A13, and then **A13** was 5′-DMT-protected to produce **A14**. Finally, phosphitylation yielded the adenosine-tethered thiol nucleoside phosphoramidite **A15**. Phosphoramidite **A15** was obtained in 8 steps with 7 chromatographic purifications from adenosine **A1** in a 12% overall yield; 102 mg of **A15** was synthesized.

Guanosine Nucleoside Phosphoramidite with 2′-Tethered Amine. Direct 2′-alkylation of unprotected guanosine is impractical because of the unwanted reactivity of the nucleobase.[36,37] Therefore, our route to the guanosine derivatives began with commercially available 2,6-diaminopurine ribonucleoside **G1** (Scheme 5). Nucleoside **G1** was preferentially 2′-alkylated with NaH and benzyl 2-bromoethyl ether to provide **G2**, which was obtained free of the 3′-isomer after silica gel chromatography.[38] Although the yield of **G2** was modest (33%), the reaction and separation were readily performed on a large scale, and for expediency we judged this route preferable to multistep options involving protecting groups (e.g., initial 3′,5′-TIPDS protection of **G1**, then 2′-alkylation and 3′,5′-deprotection). The enzymatic conversion of the 2,6-diaminopurine derivative **G2** to the guanosine analogue with adenosine deaminase (ADA) provided 2′-functionalized guanosine nucleoside **G3**.[38,39] Protection of the 3′- and 5′-hydroxyl groups using 5 equiv of TBSCl was accompanied by guanine O^6-silylation to form **G4**. This compound appears to be stable to silica gel chromatography, which is somewhat unexpected on the basis of literature reports for similar compounds.[37] Isobutyryl protection of the guanosine N^2 was achieved along with removal of the O^6-TBS group, producing **G5**. The 2′-tethered benzyl group was hydrogenolyzed to provide **G6**, which was converted via the mesylate **G7** to the azide **G8**. Reduction with H_2 over Pd/C to the amine, protection as the trifluoromethyl amide, and removal of the 3′- and 5′-silyl groups with TBAF produced **G9**. The 5′-hydroxyl was protected as its DMT derivative, yielding **G10**. To complete the route, the nucleoside was phosphitylated to produce the guanosine-tethered amine nucleoside phosphoramidite **G11**. The 10-step route from 2,6-diaminopurine ribonucleoside **G1** to **G11** was achieved with 8 chromatographic purifications in a 6% overall yield. This includes the modest 33% yield for the first-step conversion of **G1** into the 2′-alkylated **G2**. A total of 160 mg of **G11** was synthesized.

Guanosine Nucleoside Phosphoramidite with 2′-Tethered Thiol. The last of the eight phosphoramidites, the guanosine-tethered thiol, was prepared starting from intermediate **G6** (Scheme 6). Exchange of the 2′-tethered hydroxyl group for the trityl-protected thiol group was achieved under Mitsunobu conditions, and the 3′- and 5′-silyl groups were removed with TBAF to produce **G12**. 5′-DMT protection led to **G13**, and phosphitylation provided the guanosine-tethered thiol nucleoside phosphoramidite **G14**. The overall 8-step route from **G1** to **G14** proceeded with 7 chromatographic purifications in a 7% overall yield; 109 mg of **G14** was prepared.

Solid-Phase RNA Synthesis Using the Modified Phosphoramidites. The new nucleoside phosphoramidites were successfully incorporated into RNA oligonucleotides by solid-phase synthesis. For the tethered amine series, each of the four nucleoside phosphoramidites, **U14**, **U15**, **A11**, and **G11**, was incorporated into a separate oligonucleotide, as detailed in the Experimental Section. This demonstrates that all nucleobase deprotections and the U-triazolide-to-C conversion were successful in the context of oligonucleotides. For the tethered thiol series, adenosine phosphoramidite **A15** was used for solid-phase synthesis, thereby establishing that the thiol modification is successfully incorporated. Subsequently, in a larger RNA to which the tethered thiol oligonucleotide derived from **A15** was ligated,[40] a standard $AgNO_3$ deprotection procedure[31] was employed, which demon-

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(38) Gundlach, C. W., IV; Ryder, T. R.; Glick, G. D. *Tetrahedron Lett.* **1997**, *38*, 4039−4042.

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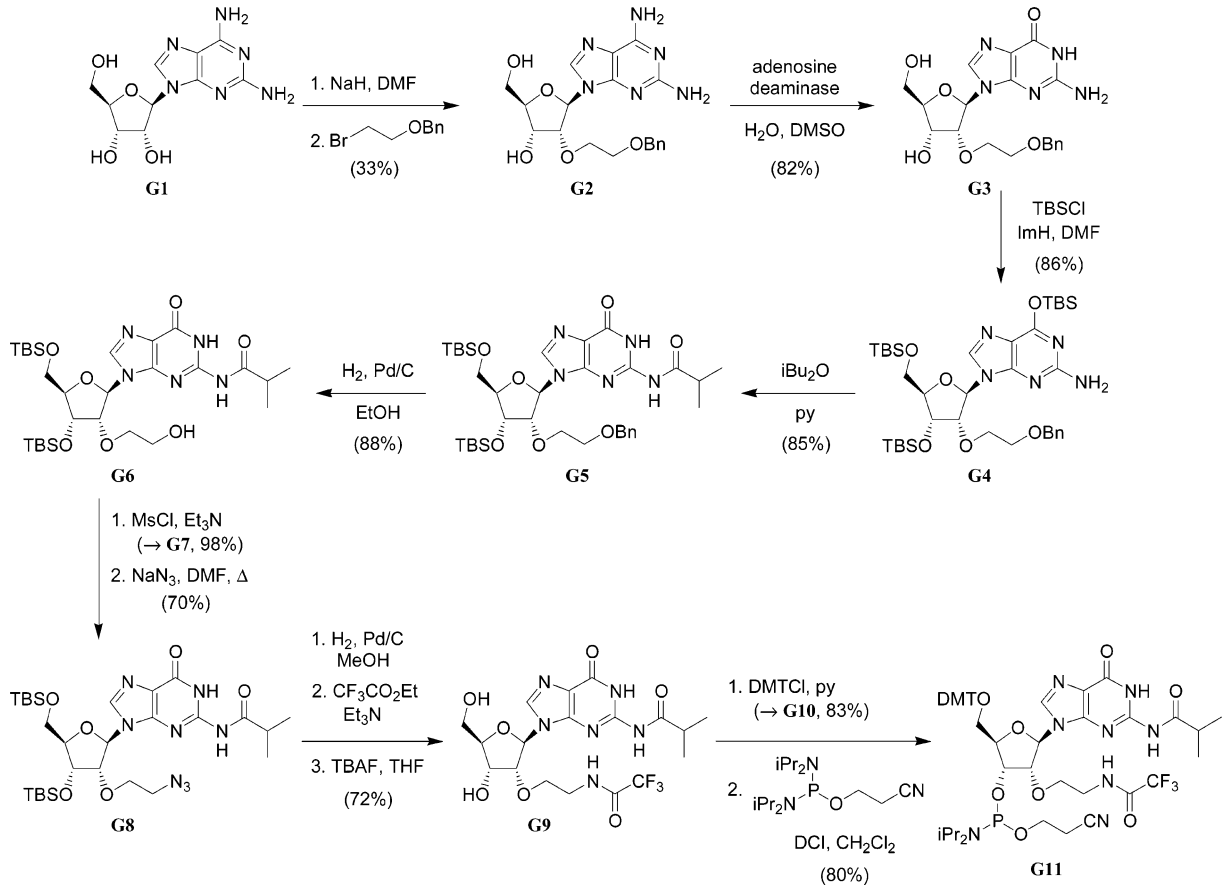
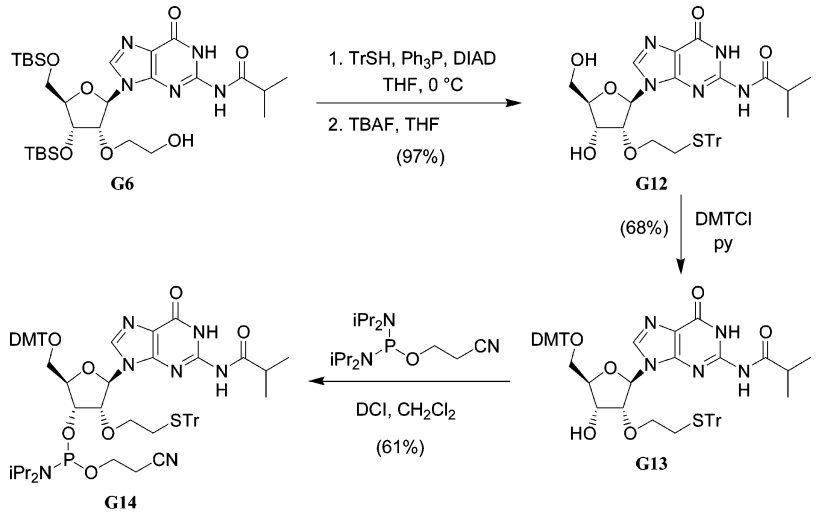
SCHEME 5



SCHEME 6



strates that the free thiol moiety can be readily unmasked after solid-phase synthesis.

For practical purposes, we generally store large amounts of all nucleosides as the immediate precursors to the phosphoramidites (i.e., as **U8**, **U13**, **A10**, **A14**, **G10**, and **G13**), which obviates the issue of long-term phosphoramidite stability. Nevertheless, we have not noticed substantial decomposition of any of the phosphoramidites upon storage for months at −20 °C or below when the compounds are suitably protected from moisture.

Conclusions

We have described a comprehensive and streamlined set of experimental procedures for synthesizing all four standard RNA nucleoside phosphoramidites, each with either a 2′-tethered amine or a 2′-tethered thiol functional group. The synthetic routes are maximally convergent and provide sufficient amounts of phosphoramidite for

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several solid-phase synthesis coupling reactions. The resulting oligonucleotides are immediately useful in ongoing studies of RNA structure, folding, and catalysis.

Experimental Section

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-*N*^{3}-(methoxycarbonylvinyl)uridine (U2). $TIPDSCl_2$ (2.88 mL, 9.01 mmol) was added dropwise over 5 min to a solution of uridine **U1** (2.00 g, 8.20 mmol) in pyridine (50 mL) at 0 °C. (Alternatively, the $TIPDSCl_2$ can be generated in situ as described in the text; we have successfully used the procedure of ref 18 on the 10−25 mmol scale.) The colorless solution was warmed to room temperature, stirred for 10 h, and then quenched with water (5 mL). Pyridine was removed under vacuum, and the residual white solid was partitioned between EtOAc (100 mL) and water (100 mL). The organic layer was washed with cold 1 N HCl (2 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl (50 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum to produce 4.20 g of a crude white solid that was used without further purification. This solid was dissolved in 42 mL of 1:5 CH_2Cl_2/CH_3CN and cooled to ∼13−15 °C (chilled water bath; at higher temperature, methyl propiolate reacts considerably with the 2′-hydroxyl group, whereas at lower temperature, the overall reaction rate is very low). DMAP (0.30 g, 2.40 mmol) was added to the solution; this was followed by the addition of methyl propiolate (0.25 mL, 2.40 mmol) dropwise over a period of 2 min. An additional aliquot of methyl propiolate (2.40 mmol) was added every 30 min until TLC indicated complete consumption of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine; three additional aliquots were required. The solution was quenched with MeOH (8 mL) and concentrated under vacuum. The residual red viscous oil was purified via chromatography using 0−30% EtOAc in hexanes as the eluant to yield 3.20 g (68%) of **U2** as a white foam: R_f 0.28 (3:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, J = 15.0 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 15.0 Hz, 1H), 5.76 (d, J = 8.0 Hz, 1H), 5.74 (br s, 1H), 4.37 (dd, J = 9.0, 4.7 Hz, 1H), 4.22 (ABqd, J = 13.3, 1.5 Hz, 1H), 4.17 (d, J = 5.0 Hz, 1H), 4.14−4.09 (m, 1H), 4.00 (ABqd, J = 13.5, 3.0 Hz, 1H), 3.78 (s, 3H), 2.84 (s, 1H), 1.10−1.00 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.6, 161.1, 149.2, 138.2, 134.0, 113.6, 101.0, 91.4, 82.0, 75.3, 68.8, 60.0, 51.8, 17.4, 17.3, 17.21, 17.20, 16.98, 16.91, 16.8, 16.7, 13.3, 12.8, 12.7, 12.4. FAB-HRMS M^+ calcd for $C_{25}H_{42}N_2O_9Si_2$ 570.7801, found 570.7804.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-allyl-*N*^{3}-(methoxycarbonylvinyl)uridine (U3). A solution of **U2** (3.20 g, 5.60 mmol) and allyl methyl carbonate (1.30 g, 11.20 mmol) in THF (20 mL) was added dropwise via cannula over 15 min, rinsing the flask containing **U2** with THF (3 × 2 mL), to a suspension of 1,4-bisphosphinobutane (92 mg, 0.20 mmol) and $Pd_2(dba)_3$ (52 mg, 0.06 mmol) in THF (6 mL). After the mixture was stirred at room temperature for 12 h, the solution was quenched with MeOH (4 mL) and concentrated under vacuum. The residual green viscous oil was purified via chromatography with 20% EtOAc in hexanes as the eluant to produce 2.90 g (85%) of **U3** as a white foam: R_f 0.50 (4:1 hexanes/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 15 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.08 (d, J = 15 Hz, 1H), 5.92 (ddt, J = 17.3, 10.5, 5.2 Hz, 1H), 5.74 (s, 1H), 5.73 (d, J = 8.3 Hz, 1H), 5.39 (dd, J = 17.3, 1.7 Hz, 1H), 5.21 (dd, J = 10.5, 1.2 Hz, 1H), 4.39 (m, 2H), 4.25 (ABqd, J = 14 Hz, 1H), 4.19 (ABqd, J = 9.6, 1.6 Hz, 1H), 4.14 (ABqd, J = 9.6, 4.0 Hz, 1H), 3.96 (ABqd, J = 14.0, 2.0 Hz, 1H), 3.86 (d, J = 4 Hz, 1H), 3.77 (s, 3H), 0.99−1.10 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.5, 161.1, 149.1, 137.8, 134.13, 134.10, 113.5, 117.6, 100.7, 89.6, 81.9, 80.9, 71.2, 67.9, 59.2, 51.7, 17.44, 17.40, 17.25, 17.20, 17.0, 16.9, 16.7, 13.4, 13.0, 12.8, 12.3. FAB-HRMS M^+ calcd for $C_{28}H_{47}N_2O_9Si_2$ 611.2821, found 611.2820.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-hydroxyethyl)uridine (U4). A solution of **U3** (2.80 g, 4.58 mmol) in CH_2Cl_2 (50 mL) was cooled to −78 °C. Ozone was bubbled through the solution for 20 min until a faint blue color persisted. The ozonide was reduced by the addition of $NaBH_4$ (0.976 g, 25.8 mmol) dissolved in EtOH (40 mL). After the mixture was stirred for 3.5 h, the solution was quenched with saturated aqueous NH_4Cl (100 mL), and the cloudy white mixture was diluted with EtOAc (200 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl (75 mL), dried over Na_2SO_4, and concentrated under vacuum. The residual colorless viscous oil was purified via chromatography with 0−7% MeOH in CH_2Cl_2 as the eluant to yield 1.60 g (62%) of **U4** as a white foam: R_f 0.42 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.08 (br s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 5.73 (br s, 1H), 5.69 (dd, J = 8.0, 1.6 Hz, 1H), 4.26 (ABq, J = 13.6 Hz, 1H), 4.19 (ABqd, J = 10.0, 4.0 Hz, 1H), 4.15 (ABqd, J = 10.0, 2.0 Hz, 1H), 4.03−3.88 (m, 4H), 3.74−3.70 (m, 2H), 2.92 (br s, 1H), 1.10−1.02 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.2, 150.1, 139.2, 101.7, 89.4, 82.9, 81.8, 73.0, 68.1, 61.6, 59.2, 17.4, 17.3, 17.2, 17.1, 17.0, 16.92, 16.90, 16.7, 13.4, 13.0, 12.8, 12.5. FAB-HRMS $[M + H]^+$ calcd for $C_{23}H_{43}N_2O_8Si_2$ 531.2558, found 531.2557.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-methanesulfonylethyl)uridine (U5). A portion of **U4** (2.20 g, 4.14 mmol, combined from two preparations of **U4** as described above) was coevaporated with pyridine (10 mL) and dissolved in CH_2Cl_2 (35 mL). The solution was cooled to 0 °C, and Et_3N (0.69 mL, 4.96 mmol) and methanesulfonyl chloride (0.38 mL, 4.96 mmol) were added. After 45 min, the solution was quenched with MeOH (5 mL) and concentrated under vacuum. The residual colorless viscous oil was purified via chromatography with 0−50% EtOAc in CH_2Cl_2 as the eluant to produce 2.10 g (82%) of **U5** as a white foam: R_f 0.36 (1:4 EtOAc/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.60 (br s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 5.72 (s, 1H), 5.68 (d, J = 8.0 Hz, 1H), 4.45−4.25 (dt, J = 4.2, 2.0 Hz, 2H), 4.24 (ABq, J = 13.6 Hz, 1H), 4.18 (ABqd, J = 9.6, 4.5 Hz, 1H), 4.13−4.09 (m, 3H), 3.95 (ABqd, J = 13.6, 2.0 Hz, 1H), 3.87 (d, J = 4.0 Hz, 1H), 3.09 (br s, 3H), 1.01 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.8, 150.2, 139.2, 101.7, 88.5, 82.9, 81.6, 69.4, 68.9, 68.1, 59.2, 37.6, 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8, 16.7, 13.3, 13.0, 12.8, 12.3. EI-HRMS $[M + H]^+$ calcd for $C_{24}H_{45}N_2O_{10}Si_2S$ 609.2336, found 609.2333.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-azidoethyl)uridine (U6). NaN_3 (0.88 g, 13.5 mmol) and 18-crown-6 (5 mg, 0.019 mmol) were added to a solution of **U5** (1.65 g, 2.71 mmol) in DMF (25 mL). The solution was stirred at 60 °C for 6 h. The solvents were removed under vacuum, and the residual colorless viscous oil was partitioned between EtOAc (75 mL) and water (75 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic extracts were washed with saturated aqueous NaCl (75 mL), dried over Na_2SO_4, and concentrated under vacuum to yield 1.57 g (104% of the theoretical amount) of **U6** as a pale yellow foam. Most of this crude sample was used in the next reaction step without further purification: R_f 0.50 (3:1 CH_2Cl_2/EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.84 (br s, 1H), 7.91 (d, J = 8.0 Hz, 1H), 5.74 (s, 1H), 5.68 (d, J = 8.0 Hz, 1H), 4.25 (ABq, J = 14.0 Hz, 1H), 4.193 (d, J = 4.0 Hz, 1H), 4.190 (m, 1H), 4.03 (ddd, J = 6.4, 4.0, 2.8 Hz, 2H), 3.97 (ABqd, J = 13.6, 1.6 Hz, 1H), 3.86 (d, J = 3.2 Hz, 1H), 3.41 (ABqdd, J = 13.2, 6.0, 4.0 Hz, 2H), 1.10−0.93 (m, 28H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.3, 149.9, 139.5, 101.6, 88.9, 82.8, 81.6, 70.5, 68.1, 59.3, 50.8, 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 16.8, 16.7, 13.4, 13.0, 12.8, 12.4. EI-HRMS $[M + H]^+$ calcd for $C_{23}H_{42}N_5O_7Si_2$ 556.2623, found 556.2622.

2′-*O*-(2-(Trifluoroacetamido)ethyl)uridine (U7). Triphenylphosphine (1.38 g, 5.26 mmol) and water (0.24 mL, 13.2

mmol) were added to a solution of crude **U6** (1.46 g, assumed 2.63 mmol) dissolved in THF (25 mL). The solution was stirred at 45 °C for 4.5 h. After the solution was cooled to room temperature, ethyl trifluoroacetate (1.56 mL, 13.2 mmol) and Et_3N (1.83 mL, 13.2 mmol) were added, and the mixture continued to be stirred at room temperature for 10 h. The solution was cooled to 0 °C, and 1 M TBAF in THF (5.25 mL, 5.25 mmol) was added. The solution was warmed to room temperature over 5 h, and the solvents were removed under vacuum. The residual colorless viscous oil was purified via chromatography with 5% MeOH in EtOAc as the eluant to produce 0.95 g (94%) of **U7** as a white foam: R_f 0.35 (1:19 MeOH/EtOAc); ^{1}H NMR (400 MHz, acetone-d_6) δ 10.06 (br s, 1H), 8.59 (br s, 1H), 8.07 (d, J = 8.0 Hz, 1H), 5.92 (d, J = 4.0 Hz, 1H), 5.57 (d, J = 8.0 Hz, 1H), 4.39 (br s, 1H), 4.34 (br t, J = 5.2 Hz, 1H), 4.23 (br s, 1H), 4.13 (dd, J = 4.8, 3.2 Hz, 1H), 3.99−3.93 (m, 2H), 3.89 (br s, 1H), 3.84−3.78 (m, 2H), 3.65−3.49 (m, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 163.8, 157.9, 151.4, 141.1, 117.2, 102.4, 88.5, 85.7, 83.3, 69.5, 69.3, 61.2, 40.5. ^{19}F NMR (470 MHz, acetone-d_6) δ −76.9. EI-HRMS $[M + H]^+$ calcd for $C_{13}H_{17}N_3O_7F_3$ 384.1022, found 384.1018.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)-ethyl)uridine (U8). 4,4′-Dimethoxytrityl chloride (0.53 g, 1.56 mmol) and DMAP (2 mg, 0.016 mmol) were added to a solution of **U7** (0.50 g, 1.3 mmol) in pyridine (10 mL). The solution was stirred at room temperature for 20 h, and the solvents were removed under vacuum. The orange viscous oil was purified by column chromatography. The silica gel was swirled with 10% Et_3N in hexanes (∼1 mL of solvent per 1 g of silica gel) for several minutes, and the solvents were removed under vacuum until a freely flowing powder was obtained. The column was packed with this prewashed silica gel using CH_2Cl_2. After the sample had been loaded, the column was eluted with a gradient of 0−5% MeOH in CH_2Cl_2 to produce 0.67 g (75%) of **U8** as a white foam: R_f 0.32 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, J = 8.0 Hz, 1H), 7.63 (br s, 1H), 7.39−7.37 (m, 2H), 7.32−7.27 (m, 7H), 6.86−6.82 (m, 4H), 5.85 (d, J = 1.2 Hz, 1H), 5.31 (d, J = 8.5 Hz, 1H), 4.42 (dd, J = 8.4, 5.2 Hz, 1H), 4.05−4.01 (m, 2H), 3.96 (dd, J = 6.4, 4.0 Hz, 1H), 3.93 (d, J = 6.4 Hz, 1H), 3.79 (s, 6H), 3.64 (br s, 1H), 3.62−3.59 (m, 2H), 3.53 (ABqd, J = 11.2, 2.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.1, 158.73, 158.69, 150.8, 144.2, 139.8, 135.2, 135.0, 130.1, 130.0, 128.1, 128.0, 127.2, 113.3, 102.4, 88.2, 87.1, 83.3, 83.1, 69.2, 68.4, 60.8, 55.2, 39.6; ^{19}F NMR (470 MHz, $CDCl_3$) δ −76.1. FAB-HRMS $[M + H]^+$ calcd for $C_{34}H_{34}N_3O_9F_3$ 685.2248, found 685.2247.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)-ethyl)uridine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (U9). A portion of **U8** (0.42 g, 0.60 mmol) was coevaporated with pyridine (2 × 10 mL) and dissolved in CH_2Cl_2 (10 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.27 mL, 0.82 mmol) and 4,5-dicyanoimidazole[27] (DCI, 90 mg, 0.76 mmol) were added. The cloudy mixture was warmed to room temperature, stirred for 10 h, and diluted with CH_2Cl_2 (75 mL) and saturated aqueous $NaHCO_3$ (100 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl (100 mL), dried over Na_2SO_4, and concentrated under vacuum. The residual colorless viscous oil was purified via chromatography; the column was packed with silica gel using 5% Et_3N in hexanes. After the sample had been loaded in CH_2Cl_2, the column was eluted with 0−3% Et_3N in CH_2Cl_2 to produce 0.44 g (81%) of **U9** as a colorless oil: R_f 0.80 (1:1 EtOAc/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 8.67 and 8.64 (each br s, total 1H), 7.98 and 7.94 (each d, J = 8.0 Hz, total 1H), 7.52−7.47 (m, 2H), 7.41−7.31 (m, 7H), 7.28−7.24 (m, 1H), 6.93−6.89 (m, 4H), 5.94 and 5.93 (each d, J = 3.0 Hz, total 1H), 5.22 and 5.21 (each d, J = 8.0 Hz, total 1H), 4.67 and 4.59 (each ddd, J = 9.5, 7.0, 5.0 Hz, total 1H), 4.31 and 4.27 (each dd, J = 5.0, 2.5 Hz, total 1H), 4.26 and 4.21 (each td, J = 7.5, 3.0 Hz, total 1H), 3.98−3.80 (m, 3H), 3.79 and 3.78 (each s, total 6H), 3.75−3.45 (m, 7H), 2.80 and 2.63 (t with J = 6.0 Hz and m, total 2H), 1.20, 1.19, 1.18, 1.17, and 1.08 (each d, J = 7.0 Hz, total 12H); ^{13}C NMR (100 MHz, acetone-d_6) δ 163.5, 159.8, 159.7, 151.3, 145.7, 140.5, 136.3, 136.1, 131.13, 131.10. 129.10, 129.07, 128.7, 127.8, 114.0, 102.4, 89.1, 87.5, 83.07, 83.00, 81.95, 81.90, 70.8, 69.0, 62.2, 59.3, 59.1, 55.5, 43.9, 43.8, 40.3, 25.00, 24.96, 24.90, 24.80, 20.72, 20.70; ^{19}F NMR (376 MHz, acetone-d_6) δ −76.71 and −76.68; ^{31}P NMR (202 MHz, acetone-d_6) δ 151.2 and 150.1. FAB-HRMS $[M + Na]^+$ calcd for $C_{43}H_{51}N_5O_{10}F_3NaP$ 908.3224, found 908.3223.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)-ethyl)-4-(1,2,4-triazolyl)uridine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (U10). A portion of 1,2,4-triazole (0.80 g, 11.6 mmol) was dissolved in acetonitrile (15 mL) and cooled to 0 °C. $POCl_3$ (200 μL, 2.0 mmol) was added dropwise over 10 min to the cloudy white mixture, and the mixture was stirred at 0 °C for 20 min. A portion of Et_3N (8.0 mL, 57.4 mmol) was added, and the mixture was stirred at 0 °C for 3 h. A solution of **U9** (0.200 g, 0.22 mmol) in CH_3CN (0.5 mL) was transferred into the reaction flask via cannula, rinsing the flask containing **U9** with CH_3CN (2 × 0.3 mL). The mixture was stirred at 0 °C for 1.5 h and diluted with 10% Et_3N in EtOAc (25 mL). Saturated aqueous $NaHCO_3$ (25 mL) was added to the mixture resulting in two clear layers. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (25 mL), dried over Na_2SO_4, and concentrated under vacuum. The residual pale yellow foam was purified via chromatography with the column packed as described for **U9** (the sample was loaded in 1:4 hexanes/CH_2Cl_2). The column was eluted with 1:4 hexanes/CH_2Cl_2 containing 3% Et_3N to yield 0.100 g (45%) of **U10** as a white foam: R_f 0.42 (1:5:4 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 9.241 and 9.240 (each s, total 1H), 8.90 and 8.86 (each d, J = 7.0 Hz, total 1H), 8.23 and 8.22 (each s, total 1H), 7.56 and 7.52 (each d, J = 7.5 Hz, total 2H), 7.45−7.31 (m, 9H), 6.94 (m, 4H), 6.56 and 6.50 (each d, J = 7.5 Hz, total 1H), 5.92 (s, 1H), 4.81 and 4.72 (td, J = 9.5, 4.5 Hz, total 1H), 4.38−4.28 (m, 2H), 4.15 (m, 1H), 4.00 (m, 1H), 3.81 and 3.78 (each s, total 6H), 3.75−3.57 (m, 8H), 2.65 (m, 1H), 1.22 (d, J = 6.5 Hz, 6H), 1.17 (d, J = 6.5 Hz, 6H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.5, 159.2, 154.2, 154.0, 147.6, 144.8, 143.3, 135.9, 135.5, 130.56, 130.46, 128.6, 128.2, 127.3, 118.5, 113.4, 106.8, 94.2, 91.4, 90.9, 87.1, 87.2, 82.2, 81.9, 81.1, 69.2, 69.1, 68.7, 60.5, 60.3, 58.6, 58.4, 54.9, 46.3, 43.3, 43.2, 43.1, 39.7, 39.6, 39.5, 26.1, 24.3, 20.2; ^{19}F NMR (470 MHz, acetone-d_6) δ −76.75 and −76.72; ^{31}P NMR (202 MHz, acetone-d_6) δ 151.7 and 149.5. ESI-HRMS $[M + H]^+$ calcd for $C_{45}H_{53}N_8O_9F_3P$ 937.3625, found 937.3594.

3′,5′-*O*-(Tetraisopropyldisiloxane-1,3-diyl)-2′-*O*-(2-(triphenylmethylthio)ethyl)uridine (U11). Diisopropyl azodicarboxylate (1.1 mL, 5.6 mmol) was added to a solution of triphenylphosphine (1.48 g, 5.6 mmol) in THF (30 mL) cooled to 0 °C. After 0.5 h, a solution of **U4** (1.50 g, 2.82 mmol) and trityl mercaptan (1.55 g, 5.6 mmol) in THF (20 mL) was added via cannula, and the flask containing the latter reagents was rinsed with THF (2 × 5 mL). After 40 min, the solution was quenched with MeOH (2 mL) and concentrated under vacuum. The resulting colorless viscous oil was purified via chromatography with 10−30% EtOAc in hexanes as the eluant to produce 1.90 g (85%) of **U11** as a white foam: R_f 0.35 (3:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.42−7.18 (m, 15H), 5.67 (dd, J = 6.0, 2.0 Hz, 1H), 5.66 (s, 1H), 4.22 (ABq, J = 13.5 Hz, 1H), 4.12 (s, 1H), 3.95 (dd, J = 13.5, 2.0 Hz, 1H), 3.76−3.68 (m, 4H), 2.47−2.44 (m, 2H), 1.10−0.97 (m, 28H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.1, 150.0, 145.1, 139.9, 129.9, 128.1, 126.8, 101.6, 89.2, 82.6, 81.8, 70.0, 68.4, 66.7, 59.6, 32.2, 17.7, 17.6,

17.5, 17.4, 17.3, 17.2. 17.1. 17.0, 13.6, 13.3, 13.1, 12.7. ESI-HRMS $[M + Na]^+$ calcd for $C_{42}H_{56}N_2O_7SSi_2Na$ 811.3245, found 811.3296.

2′-*O*-(2-(Triphenylmethylthio)ethyl)uridine (U12). TBAF (1 M) in THF (5.6 mL, 5.6 mmol) was added dropwise over 5 min to a solution of **U11** (1.90 g, 2.41 mmol) in THF (30 mL) at 0 °C. The solution was stirred at room temperature for 6 h, diluted with EtOAc (20 mL), washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), dried over Na_2SO_4, and concentrated under vacuum. The resulting colorless viscous oil was purified via chromatography with 0−5% MeOH in CH_2Cl_2 as the eluant to produce 0.96 g (72%) of **U12** as a white foam: R_f 0.33 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.81 (br s, 1 H), 7.75 (d, J = 8.0 Hz, 1H), 7.41−7.19 (m, 15H), 5.69 (d, J = 8.0 Hz, 1H), 5.65 (d, J = 4.0 Hz, 1H), 4.20 (t, J = 5.2 Hz, 1H), 4.01 (br dt, J = 5.6, 2.4 Hz, 1H), 3.94 (dd, J = 5.2, 3.5 Hz, 1H), 3.93 (ABqd, J = 12.2, 1.5 Hz, 1H), 3.77 (ABqd, J = 11.2, 2.0 Hz, 1H), 3.60 (dt, J = 10.0 Hz, 5.6 Hz, 1H), 3.38 (br s, 1H), 3.23 (ddd, J = 10.5, 8.0, 5.0 Hz, 1H), 3.12 (br s, 1H), 2.54 (ddd, J = 13.0, 7.4, 5.4 Hz, 1H), 2.40 (dt, J = 13.0, 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.6, 150.4, 144.7, 142.2, 129.8, 128.3, 127.1, 102.6, 90.7, 85.3, 81.2, 69.6, 68.9, 61.6, 32.3. ESI-HRMS $[M + Na]^+$ calcd for $C_{30}H_{30}N_2O_6SNa$ 569.1722, found 569.1714.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)uridine (U13). A portion of **U12** (0.90 g, 1.65 mmol) was coevaporated from pyridine (3 mL) and dissolved in pyridine (3 mL). The solution was cooled to 0 °C, and 4,4′-dimethoxytrityl chloride (1.00 g, 2.95 mmol) was added. After the mixture was stirred at room temperature for 12 h, the solvents were removed under vacuum. The residual orange foam was purified via chromatography with the column packed as described for **U9**. The column was eluted with a gradient of 0−5% MeOH in CH_2Cl_2 to produce 0.95 g (68%) of **U13** as a pale yellow foam: R_f 0.45 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, acetone-d_6) δ 10.07 (br s, 1H), 7.733 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.48−7.21 (m, 24H), 6.92−6.90 (m, 4H), 5.89 (d, J = 3.0 Hz, 1H), 5.26 (d, J = 8.0 Hz, 1H), 4.41 (q, J = 5.5 Hz, 1H), 4.06 (dt, J = 6.5, 3.0 Hz, 1H), 3.95 (dd, J = 4.5, 3.0 Hz, 1H), 3.71 (br q, J = 7.5 Hz, 1H), 3.65 (ABqd, J = 10.5, 7.0 Hz, 1H), 3.54 (ABqt, J = 10.5, 6.5 Hz, 1H), 3.79 (s, 6H), 3.47 (ABqd, J = 11.0, 3.0 Hz, 1H), 3.34 (ABqd, J = 11.0, 3.0 Hz, 1H), 2.86 (br s, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 163.0, 159.1, 150.6, 145.2, 135.9, 135.7, 130.4, 129.8, 128.4, 128.2, 128.1, 127.1, 126.9, 113.4, 101.8, 87.8, 86.8, 83.3, 82.4, 69.1, 67.1, 62.6, 54.9, 32.0, 18.5. ESI-HRMS $[M + Na]^+$ calcd for $C_{51}H_{48}N_2O_8SNa$ 871.3029, found 871.3060.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)uridine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)-phosphoramidite (U14). A portion of **U13** (0.30 g, 0.35 mmol) was coevaporated with pyridine (2 × 5 mL) and dissolved in CH_2Cl_2 (4 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (0.15 mL, 0.45 mmol) and 4,5-dicyanoimidazole (50 mg, 0.42 mmol) were added. The cloudy mixture was warmed to room temperature and stirred for 6 h. The mixture was diluted with 5% Et_3N in EtOAc (25 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residual pale yellow foam was purified via chromatography with the column packed as described for **U9** (the sample was loaded in 1:15:4 Et_3N/hexanes/CH_2Cl_2). The column was eluted with a gradient from 1:15:4 to 1:7:12 Et_3N/hexanes/CH_2Cl_2 to produce 0.31 g (84%) of **U14** as a white foam: R_f 0.51 (1:7:12 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 10.10 (br s, 1H), 7.93 and 7.86 (each d, J = 8.0 Hz, total 1H), 7.53−7.22 (m, 24H), 6.87 (m, 4H), 5.93 and 5.91 (each d, J = 3.0 Hz, total 1H), 5.23 and 5.21 (each d, J = 2.5 Hz, total 1H), 4.57 and 4.49 (each m, total 1H), 4.21 and 4.15 (each m, total 1H), 4.08 (m, 1H), 3.88 (m, 1H), 3.79 and 3.78 (each s, total 6H), 3.68 (m, 1H), 3.63 (m, 4H), 3.55−3.43 (m, 2H), 2.71 (m, 1H), 2.62 (m, 1H), 2.45 (m, 2H), 1.18 (m, 12H); ^{13}C NMR (125 MHz, acetone-d_6) δ 162.5, 159.0, 150.5, 145.2, 140.2, 130.5, 129.8, 128.4, 128.3, 127.1, 126.9, 113.3, 101.7, 88.0, 87.0, 82.2, 82.3, 82.1, 70.3, 69.3, 69.1, 66.2, 62.2, 43.2, 32.1, 24.2, 20.1; ^{31}P NMR (202 MHz, acetone-d_6) δ 150.8 and 150.4. ESI-HRMS $[M + H]^+$ calcd for $C_{60}H_{66}N_4O_9SP$ 1049.4288, found 1049.4281.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-4-(1,2,4-triazolyl)uridine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (U15). A portion of 1,2,4-triazole (0.65 g, 9.4 mmol) was dissolved in acetonitrile (13 mL) and cooled to 0 °C. $POCl_3$ (165 μL, 1.8 mmol) was added dropwise over 10 min to the cloudy white mixture, and the mixture was stirred at 0 °C for 20 min. A portion of Et_3N (5.1 mL, 36.6 mmol) was added, and the mixture was stirred at 0 °C for 3 h. A solution of **U14** (0.197 g, 0.188 mmol) in CH_3CN (1.5 mL) was transferred into the reaction flask via cannula; the flask containing **U14** was rinsed with CH_3CN (2 × 0.2 mL). The mixture was stirred at 0 °C for 1.5 h and diluted with 10% Et_3N in EtOAc (25 mL). Saturated aqueous $NaHCO_3$ (25 mL) was added to the mixture resulting in two clear layers. The organic layer was separated, washed with saturated aqueous $NaHCO_3$ (25 mL), dried over Na_2SO_4, and concentrated under vacuum. The residual pale yellow foam was purified via chromatography with the column packed as described for **U9** (the sample was loaded in 3:2 hexanes/CH_2Cl_2). The column was eluted with 1:11:8 to 1:8:11 Et_3N/hexanes/CH_2Cl_2 to yield 0.112 g (54%) of **U15** as a white foam: R_f 0.55 (1:11:8 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 9.24 and 9.23 (each s, total 1H), 8.89 and 8.83 (each d, J = 7.0 Hz, total 1H), 8.22 and 8.21 (each s, total 1H), 7.57−7.23 (m, 24H), 6.93 (m, 4H), 6.53 and 6.46 (each d, J = 7.0 Hz, total 1H), 5.91 and 5.90 (each s, total 1H), 4.77 and 4.64 (each td, J = 9.0, 5.0 Hz, total 1H), 4.30 (m, 1H), 4.15 (ABqd, J = 10.5, 4.5 Hz, 1H), 3.96−3.82 (m, 2H), 3.81 and 3.80 (each s, total 6H), 3.78−3.56 (m, 6H), 2.85 and 2.82 (each s, total 1H), 2.68 (m, 1H), 2.62 (m, 1H), 2.53 (m, 1H), 1.19 (d, J = 7.5 Hz, 3H), 1.14 (d, J = 7.0 Hz, 3H), 1.10 (d, J = 7.0 Hz, 3H). 1.05 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 159.4, 159.1, 154.2, 147.7, 145.3, 144.8, 143.2, 135.5, 135.4, 130.6, 130.5, 130.4, 130.3, 129.8, 128.6, 128.2, 127.3, 126.9, 113.3, 94.0, 90.7, 87.1, 82.2, 69.1, 68.9, 60.5, 60.3, 58.6, 58.4, 54.9, 43.3, 43.2, 32.1, 24.5, 20.0, 18.6; ^{31}P NMR (202 MHz, acetone-d_6) δ 151.2 and 149.6. ESI-HRMS $[M + Na]^+$ calcd for $C_{62}H_{66}N_7O_8PSNa$ 1122.4329, found 1122.4363.

2′-*O*-(Methoxycarbonylmethyl)adenosine (A2). A 60% dispersion of sodium hydride in mineral oil (1.26 g, 32 mmol) was added to a suspension of adenosine (6.15 g, 23.0 mmol) in DMF (150 mL) at 0 °C. The suspension was stirred at 0 °C for 1 h, and methyl bromoacetate (3.3 mL, 34.5 mmol) was added. The mixture was warmed to room temperature, stirred for 8 h, and quenched with acetic acid (3 mL) and methanol (10 mL). The solvents were removed under vacuum, and the resulting paste was purified via chromatography (dry-load) with 1−10% MeOH in CH_2Cl_2 as the eluant to produce 3.65 g (47%) of **A2** as a white foam: R_f 0.35 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.87 (s, 1H), 6.58 (br s, 1H), 5.96 (br s, 2H), 5.94 (d, J = 7.5 Hz, 1H), 4.73 (dd, J = 7.6, 4.3 Hz, 1H), 4.43 (d, J = 5.5 Hz, 1H), 4.40−4.31 (m, 4H), 3.97−3.91 (m, 2H), 3.74 (s, 3H), 3.72 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.2, 156.1, 152.3, 148.3, 141.0, 121.1, 89.0, 87.4, 84.6, 70.8, 69.1, 63.2, 52.5. ESI-HRMS $[M + H]^+$ calcd for $C_{13}H_{18}N_5O_6$ 340.1257, found 340.1266. The HMBC spectrum was also recorded (see text).

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(methoxycarbonylmethyl)adenosine (A3). *tert*-Butyldimethylsilyl chloride (5.08 g, 33.7 mmol) and imidazole (2.27 g, 16.2 mmol) were added to a stirred solution of **A2** (3.65 g, 10.8 mmol) in DMF (50 mL). After 16 h, the solvents were removed under vacuum, and the resulting paste was partitioned between water (100 mL) and CH_2Cl_2 (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 70 mL). The combined organic extracts were

washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$, and concentrated under vacuum. The resulting oil was purified via chromatography with 0−3% MeOH in CH_2Cl_2 as the eluant to produce 5.67 g (93%) of **A3** as a white solid: R_f 0.54 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.20 (s, 1H), 6.22 (d, 1H, J = 4.1 Hz), 5.78 (br s, 2H), 4.58 (dd, J = 4.6, 3.5 Hz, 1H), 4.57 (dd, J = 4.7, 3.3 Hz, 1H), 4.28 (ABq, J = 16.7 Hz, 1H), 4.20 (ABq, J = 16.8 Hz, 1H), 4.14 (q, J = 3.4 Hz, 1H), 3.96 (ABqd, J = 11.4, 3.6 Hz, 1H), 3.77 (ABqd, J = 11.5, 3.1 Hz, 1H), 3.64 (s, 3H), 0.93 (s, 9H), 0.91 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H), 0.09 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.1, 155.3, 152.9, 149.7, 139.7, 120.1, 86.7, 85.1, 82.1, 70.2, 67.6, 61.9, 51.2, 25.9, 25.7, 18.4, 18.1, −4.7, −4.9, −5.4, −5.5. FAB-HRMS $[M + H]^+$ calcd for $C_{25}H_{46}N_5O_6Si_2$ 568.2984, found 568.2986.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-hydroxyethyl)adenosine (A4). Lithium aluminum hydride (0.99 g, 25 mmol) was added to a solution of **A3** (5.67 g, 9.98 mmol) in ether (45 mL) cooled to 0 °C. After 2 h, the solution was quenched at 0 °C by the slow addition of saturated aqueous NH_4Cl (100 mL). After the solution was warmed to room temperature, it was diluted with ether (100 mL). The aqueous layer was extracted with ether (6 × 100 mL). The combined organic extracts were washed with saturated aqueous NaCl (200 mL), dried over $MgSO_4$, and concentrated under vacuum to produce 5.07 g (94%) of **A4** as a colorless oil: R_f 0.61 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 8.19 (s, 1H), 6.18 (d, J = 3.4 Hz, 1H), 6.04 (br s, 2H), 4.49 (dd, J = 5.4, 5.0 Hz, 1H), 4.32 (dd, J = 4.6, 3.6 Hz, 1H), 4.12 (dd, J = 5.80, 2.8 Hz, 1H), 3.99 (ABqd, J = 11.6, 3.2 Hz, 1H), 3.80−3.70 (m, 5H), 3.65 (br s, 1H), 0.92 (s, 9H), 0.91 (s, 9H), 0.10 (s, 9H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 152.9, 149.1, 138.9, 119.9, 87.5, 84.6, 82.9, 72.6, 69.6, 61.7, 61.5, 26.0, 25.7, 18.4, 18.0, −4.6, −4.8, −5.4, −5.5. EI-HRMS M^+ calcd for $C_{24}H_{45}N_5O_5Si_2$ 539.2959, found 539.2953.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-hydroxyethyl)-*N*^{6}-isobutyryladenosine (A5). Trimethylsilyl chloride (3.0 mL, 23.6 mmol) was added to a solution of **A4** (2.41 g, 4.46 mmol) in pyridine (20 mL) resulting in a cloudy mixture. After 1 h, isobutyric anhydride (3.0 mL, 18.1 mmol) was added. After an additional 5 h, the mixture was cooled to 0 °C, and water (4 mL) was added. After an additional 30 min, concentrated NH_4OH (4 mL) was added, and the sample was concentrated under vacuum. The resulting paste was partitioned between CH_2Cl_2 (100 mL) and water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and saturated aqueous NaCl (50 mL), dried over $MgSO_4$, and concentrated under vacuum. The residual colorless viscous oil was purified via chromatography with 40−80% EtOAc in CH_2Cl_2 as the eluant to yield 2.44 g (90%) of **A5** as a white foam: R_f 0.39 (4:1 EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.70 (s, 1H), 8.43 (br s, 1H), 8.37 (s, 1H), 6.22 (d, J = 3.5 Hz, 1H), 4.51 (dd, J = 5.6, 5 Hz, 1H), 4.34 (dd, J = 4.3, 3.3 Hz, 1H), 4.15 (dt, J = 4.5, 2.5 Hz, 1H), 4.02 (ABqd, J = 11.6, 2.8 Hz, 1H), 3.81−3.74 (m, 5H), 3.24 (septet, J = 6.7 Hz, 1H), 2.55 (br s, 1H), 1.32 (d, J = 6.7 Hz, 3H), 1.30 (d, J = 6.7 Hz, 3H), 0.93 (s, 9H), 0.92 (s, 9H), 0.12 (s, 6H), 0.11 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 152.7, 150.4, 149.7, 141.0, 121.8, 87.6, 84.7, 83.0, 72.4, 69.3, 61.7, 61.3, 36.0, 26.0, 25.6, 19.2, 18.8, 18.4, 18.0, −4.7, −4.9, −5.4, −5.6. EI-HRMS M^+ calcd for $C_{28}H_{51}N_5O_6Si_2$ 609.3377, found. 609.3376.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-methanesulfonylethyl)-*N*^{6}-isobutyryladenosine (A6). Et_3N (0.7 mL, 5 mmol) was added to a solution of **A5** (0.61 g, 1.0 mmol) in CH_2Cl_2 (10 mL) cooled to 0 °C and followed by the addition of methanesulfonyl chloride (0.23 mL, 3.0 mmol). After 1 h, the solution was diluted with CH_2Cl_2 (50 mL), and washed with saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL), and the combined organic extracts were washed with saturated aqueous NaCl (50 mL), dried over $MgSO_4$, and concentrated under vacuum. The residual yellow viscous oil was purified via chromatography with 1:1 EtOAc/CH_2Cl_2 as the eluant to produce 0.57 g (82%) of **A6** as a colorless oil: R_f 0.52 (1:1 EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.84 (br s, 1H), 8.67 (s, 1H), 8.36 (s, 1H), 6.16 (d, J = 3.5 Hz, 1H), 4.52 (dd, J = 5.3, 4.8 Hz, 1H), 4.34 (dd, J = 4.5, 3.8 Hz, 1H), 4.33 (t, J = 4.0 Hz, 2H), 4.09 (ddd, J = 6.2, 3.0, 2.4 Hz, 1H), 3.98 (ABqd, J = 11.6, 3.3 Hz, 1H), 3.89 (m, 2H), 3.76 (ABqd, J = 11.6, 2.6 Hz, 1H), 3.16 (m, 1H), 2.98 (s, 3H), 1.27 (d, J = 6.8 Hz, 3H), 1.27 (d, J = 6.8 Hz, 3H), 0.90 (s, 9H), 0.89 (s, 9H), 0.09 (s, 3H), 0.08 (s, 3H), 0.07 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 152.5, 150.8, 149.3, 141.4, 122.5, 86.7, 84.8, 82.7, 69.6, 68.5, 68.5, 61.5, 37.5, 35.9, 25.9, 25.6, 19.1, 18.3, 17.95, −4.7, −5.0, −5.5. EI-HRMS M^+ calcd for $C_{29}H_{53}N_5O_8SSi_2$ 687.3153, found. 687.3143.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-azidoethyl)-*N*^{6}-isobutyryladenosine (A7). NaN_3 (0.26 g, 4.0 mmol) was added to a solution of **A6** (0.55 g, 0.80 mmol) in DMF (8 mL), and the mixture was heated at 50 °C for 3 h. The solvents were removed under vacuum, and the resulting pale orange residue was partitioned between CH_2Cl_2 (100 mL) and water (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were washed with saturated aqueous NaCl (50 mL), dried over $MgSO_4$, and concentrated under vacuum to produce 0.49 g (97%) of **A7** as a colorless oil: R_f 0.78 (1:1 EtOAc/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 8.45 (br s, 1H) 8.38 (s, 1H), 6.20 (d, J = 4.0 Hz, 1H), 4.49 (dd, J = 5.3, 4.9 Hz, 1H), 4.37 (dd, J = 4.4, 3.8 Hz, 1H), 4.16, (ddd, J = 5.9, 3.3, 2.9 Hz, 1H), 4.03 (ABqd, J = 11.5, 3.5 Hz, 1H), 3.84−3.78 (m, 3H), 3.45 (ABqt, J = 13.6, 5.1 Hz, 1H), 3.37 (ABQt, J = 13.4, 4.8 Hz, 1H), 3.21 (br septet, J = 6.7 Hz, 1H), 1.31 (d, J = 6.8 Hz, 6H), 0.94 (s, 9H), 0.93 (s, 9H), 0.11 (s, 6H,), 0.12 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.2, 152.4, 150.7, 149.3, 141.6, 122.6, 87.1, 84.6, 82.2, 69.6, 69.6, 61.4, 50.6, 35.8, 25.8, 25.5, 19.0, 18.2, 17.9, −4.8, −5.1, −5.6. FAB-HRMS $[M + H]^+$ calcd $C_{28}H_{51}N_8O_5Si_2$ 635.3520, found 635.3523.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-(trifluoroacetamido)ethyl)-*N*^{6}-isobutyryladenosine (A8). Pd/C (10%, 0.20 g, 0.19 mmol) was added to a solution of **A7** (0.48 g, 0.76 mmol) in MeOH (9 mL). The flask was evacuated and placed under an atmosphere of hydrogen. The consumption of **A7** was monitored by TLC. After 1.5 h, ethyl trifluoroacetate (1.0 mL, 8.4 mmol) and Et_3N (0.7 mL, 5.0 mmol) were added. After 4 h, silica gel (3 g) was added for dry loading, and the solvents were removed under vacuum. The sample was purified via chromatography with 1:4 EtOAc/CH_2Cl_2 as the eluant to produce 0.44 g (81%) of **A8** as a pale yellow oily solid: R_f 0.75 (1:1 EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.17 (br s, 1H), 8.62 (s, 1H), 8.35 (s, 1H), 7.38 (br t, J = 4.8 Hz, 1H), 6.12 (d, J = 3.6 Hz, 1H), 4.48 (dd, J = 5.3, 4.9 Hz, 1H), 4.28 (dd, J = 4.4, 3.9 Hz, 1H), 4.08 (ddd, J = 5.3, 2.7, 2.6 Hz, 1H), 3.94 (ABqd, J = 11.6, 3.1 Hz, 1H), 3.80−3.75 (m, 1H), 3.73 (ABqd, J = 11.7, 2.5 Hz, 1H), 3.72−3.68 (m, 1H), 3.60−3.50 (m, 1H), 3.50−3.40 (m, 1H), 3.20 (m, 1H), 1.23 (d, J = 6.9 Hz, 3H), 1.22 (d, J = 6.8 Hz, 3H), 0.86 (s, 9H), 0.84 (s, 9H), 0.06 (s, 3H), 0.04 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.4, 157.2, 152.3, 150.6, 149.4, 141.3, 122.5, 115.6, 87.3, 84.9, 82.4, 69.9, 68.1, 61.4, 39.6, 35.8, 25.8, 25.4, 19.0, 18.2, 17.8, −4.9, −5.1, −5.6, −5.7; ^{19}F NMR (470 MHz, $CDCl_3$) δ −76.2. FAB-HRMS $[M + H]^+$ calcd for $C_{30}H_{52}N_6O_6F_3Si_2$ 705.3439, found 704.3437.

2′-*O*-(2-(Trifluoroacetamido)ethyl)-*N*^{6}-isobutyryladenosine (A9). TBAF (1 M) in THF (1.4 mL, 1.36 mmol) was added dropwise over 5 min to a solution of **A8** (0.440 g, 0.62 mmol) in THF (7 mL) cooled to 0 °C. The solution was warmed to room temperature. After 3 h, silica gel (2 g) was added for dry loading, and the solvents were removed under vacuum. The sample was purified via chromatography with 0−6% MeOH in CH_2Cl_2 as the eluant to produce 0.253 g (86%) of **A9** as a clear colorless oil: R_f 0.17 (1:1 EtOAc/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.65 (br s, 1H), 8.12 (s, 1H),

6.00 (d, J = 6.9 Hz, 1H), 5.87 (br s, 1H), 4.80 (dd, J = 6.9, 4.7 Hz, 1H), 4.64 (br d, J = 3.8 Hz, 1H), 4.36 (d, J = 1.6 Hz, 1H), 3.99 (d, J = 12.9 Hz, 1H), 3.80 (d, J = 12.5 Hz, 1H), 3.70−3.63 (m, 2H), 3.60−3.54 (m, 1H), 3.47−3.40 (m, 1H), 3.26 (septet, J = 6.1 Hz, 1H), 1.32 (d, J = 6.8 Hz, 3H), 1.31 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 177.3, 157.7, 151.9, 150.3, 149.8, 143.1, 123.3, 115.8, 89.1, 87.7, 81.7, 70.1, 68.5, 62.4, 39.9, 35.9, 19.0; ^{19}F NMR (470 MHz, $CDCl_3$) δ −76.1. FAB-HRMS $[M + H]^+$ calcd for $C_{18}H_{24}N_6O_6F_3$ 477.1709, found 477.1707.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)-ethyl)-*N*^{6}-isobutyryladenosine (A10). 4,4′-Dimethoxytrityl chloride (0.19 g, 0.57 mmol) was added to a solution of **A9** (0.249 g, 0.52 mmol) in pyridine (5 mL) cooled to 0 °C. The resulting solution was warmed to room temperature and stirred for 24 h, and the solvents were removed under vacuum. The residual yellow gum was purified via chromatography with the column packed as described for **U8**. The column was eluted with a gradient of 0−6% MeOH in CH_2Cl_2 to yield 0.292 g (71%) of **A10** as a yellow foam: R_f 0.35 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.88 (br s, 1H), 8.60 (s, 1H), 8.22 (s, 1H), 7.37−7.15 (m, 9H), 6.75 (d, J = 8.9 Hz, 4H), 6.13 (d, J = 2.6 Hz, 1H), 4.58−4.55 (m, 2H), 4.25 (br q, J = 4.0 Hz, 1H), 3.92 (ABqdd, J = 10.0, 5.0, 3.5 Hz, 1H), 3.81 (ABqdd, J = 11.0, 7.5, 3.5 Hz, 1H), 3.72 (s, 6H), 3.54 (m, 1H), 3.51 (dd, J = 11.0, 3.5 Hz, 1H), 3.48 (m, 1H), 3.37 (dd, J = 10.5, 4.0 Hz, 1H), 3.18 (septet, J = 7.5 Hz, 1H), 1.24 (d, J = 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.7, 158.8, 157.8, 152.6, 151.1, 149.6, 144.7, 141.9, 135.9, 135.8, 130.3, 130.2, 128.4, 128.1, 127.2, 123.0, 116.2, 113.4, 88.2, 86.7, 84.0, 82.2, 69.9, 69.0, 63.1, 55.4, 40.2, 36.3, 19.4; ^{19}F NMR (470 MHz, $CDCl_3$) δ −76.1. ESI-HRMS $[M + H]^+$ calcd for $C_{39}H_{42}N_6O_8F_3$ 779.3016, found 779.3013.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)-ethyl)-*N*^{6}-isobutyryladenosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (A11). A portion of **A10** (0.214 g, 0.30 mmol) was coevaporated with pyridine (5 mL) and dissolved in CH_2Cl_2 (6 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (220 μL, 0.69 mmol) and 4,5-dicyanoimidazole (40 mg, 0.34 mmol) were added. The cloudy mixture was warmed to room temperature, stirred for 4 h, and diluted with CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL), dried over Na_2SO_4, and concentrated under vacuum. The residual colorless viscous oil was purified via chromatography on Et_3N-washed silica gel with 1:6:3 to 1:3:6 Et_3N/hexanes/CH_2Cl_2 as the eluant to yield 0.198 g (73%) of **A11** as a yellow gum. TLC revealed the presence of both diastereomers: R_f 0.48 and 0.38 (1:3:6 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy also revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 9.31 (br s, 1H), 8.54 and 8.53 (each s, total 1H), 8.46 and 8.45 (each s, total 1H), 7.47−7.21 (m, 9H), 6.87−6.81 (m, 4H), 6.25 and 6.24 (each d, J = 4.5 Hz, total 1H), 5.08−5.05 (m, total 1H), 4.94 and 4.88 (each dt, J = 10.5, 5.0 Hz, total 1H), 4.41 and 4.36 (each td, J = 4.5, 3.5 Hz, total 1H), 3.93 (m, total 1H), 3.84 (m, total 1H), 3.78 and 3.77 (each s, total 3H), 3.75−3.73 (m, total 1H), 3.72−3.67 (m, total 2H), 3.55−3.53 (m, total 3H), 3.44−3.40 (m, total 2H), 2.89 and 2.86 (br s, total 1H), 2.82−2.79 (m, total 1H), 2.62 (t, J = 6.0 Hz, total 2H), 1.24−1.12 (m, 18H); ^{13}C NMR (125 MHz, acetone-d_6) δ 175.8, 158.97, 158.95, 152.1, 151.6, 150.1, 145.3, 143.1, 142.9, 136.08, 136.02, 135.98, 130.39, 130.32, 130.29, 128.40, 128.33, 127.9, 126.9, 123.5, 118.2, 113.2, 88.1, 87.8, 86.4, 83.5, 80.47, 80.44, 71.63, 71.53, 68.63, 68.49, 63.3, 63.1, 58.82, 58.66, 54.8, 43.33, 43.24, 39.73, 39.61, 35.2, 24.41, 24.35, 24.22, 24.16, 20.04, 19.98, 18.9; ^{19}F NMR (470 MHz, acetone-d_6) δ −76.77 and −76.80; ^{31}P NMR (202 MHz, acetone-d_6) δ 150.7 (just one peak observed). FAB-HRMS $[M + Na]^+$ calcd for $C_{48}H_{58}N_8O_9PNaF_3$ 1001.3914, found 1001.3914.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-*N*^{6}-isobutyryladenosine (A12). Diethyl azodicarboxylate (DEAD, 1.13 mL, 7.1 mmol) was added to a solution of triphenylphosphine (1.86 g, 7.1 mmol) in THF (30 mL) cooled to 0 °C. The DEAD was prepared as described;[41] the use of commercially available diisopropyl azodicarboxylate (see procedures for **U11** and **G12**) was not tested. After 1 h, a solution of **A5** (2.88 g, 4.72 mmol) and trityl mercaptan (1.95 g, 7.1 mmol) in THF (20 mL) was added through a cannula; the flask containing the latter reagents was rinsed with THF (6 × 5 mL). After 1 h, the solution was quenched with MeOH (10 mL) and concentrated under vacuum. The resulting oil was purified via chromatography with 10−40% EtOAc in hexanes as the eluant to produce 3.29 g (80%) of **A12** as an off-white foam: R_f 0.38 (1:2 EtOAc/hexanes); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.63 (br s, 1H), 8.31 (s, 1H), 7.37−7.19 (m, 15H), 6.07 (d, J = 3.8 Hz, 1H), 4.44 (dd, J = 5.1, 4.9 Hz, 1H), 4.18 (dd, J = 4.4, 4.1 Hz, 1H), 4.10 (ddd, J = 5.1, 3.4, 2.7 Hz, 1H), 3.99 (ABqd, J = 11.5, 3.9 Hz, 1H), 3.77 (ABqd, J = 11.5, 2.7 Hz, 1H), 3.47 (dt, J = 9.8, 6.8 Hz, 1H), 3.34 (dt, J = 9.9, 6.8 Hz, 1H), 3.23 (m, 1H), 2.42 (t, J = 6.9 Hz, 2H), 1.33 (d, J = 6.8 Hz, 3H), 1.32 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 0.91 (s, 9H), 0.11 (s, 6H), 0.10 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 152.5, 150.8, 149.2, 144.6, 141.6, 129.5, 127.8, 126.6, 122.4, 87.0, 85.0, 82.0, 69.6, 69.4, 66.6, 61.7, 36.1, 31.6, 26.0, 25.7, 19.2, 19.2, 18.4, 18.0, −4.6, −4.9, −5.5. EI-HRMS M^+ calcd for $C_{47}H_{65}N_5O_5SSi_2$ 867.4244, found 867.4242.

2′-*O*-(2-(Triphenylmethylthio)ethyl)-*N*^{6}-isobutyryl adenosine (A13). TBAF (1 M) in THF (8.4 mL, 8.4 mmol) dropwise over 10 min was added to a solution of **A12** (3.29 g, 3.8 mmol) in THF (40 mL) cooled to 0 °C. After 2 h, the solvents were removed under vacuum. The resulting oil was purified via chromatography with 0−5% MeOH in CH_2Cl_2 as the eluant to yield 2.08 g (85%) of **A13** as a white foam: R_f 0.23 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.45 (br s, 1H), 7.99 (s, 1H), 7.40−7.21 (m, 15H), 6.11 (dd, J = 12, 2 Hz, 1H), 5.84 (d, J = 7.5 Hz, 1H), 4.59 (dd, J = 7.5, 4.4 Hz, 1H), 4.35 (d, J = 3.5 Hz, 1H), 4.34 (br s, 1H), 3.96 (td, J = 12.5, 2.5 Hz, 1H), 3.76 (dt, J = 12.5, 1.5 Hz, 1H), 3.26 (septet, J = 6.5 Hz, 1H), 3.19 (dt, J = 10.0, 5.0 Hz, 1H), 3.10 (br s, 1H), 2.86 (ddd, J = 9.7, 8.6, 4.6 Hz, 1H), 2.54 (ddd, J = 13.6, 8.4, 4.9 Hz, 1H), 2.32 (dt, J = 13.8, 4.4 Hz, 1H), 1.33 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.3, 151.9, 150.2, 149.9, 144.2, 143.0, 129.3, 128.0, 126.8, 123.4, 89.4, 88.0, 81.0, 70.4, 69.2, 67.0, 63.1, 36.0, 31.8, 19.2, 19.1. FAB-HRMS M^+ calcd for $C_{35}H_{38}N_5O_5S$ 640.2593, found 640.2596.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-*N*^{6}-isobutyryladenosine (A14). Et_3N (1 mL) and 4,4′-dimethoxytrityl chloride (0.58 g, 1.68 mmol) were added to a solution of **A13** (0.98 g, 1.53 mmol) in CH_2Cl_2 (15 mL) cooled to 0 °C. The solution was warmed to room temperature, stirred for 24 h, and quenched with MeOH (2 mL). The sample was purified via chromatography using the dry loading technique (4 g of silica gel) with the column packed as described for **U8** (the sample was loaded in 10:9 hexanes/CH_2Cl_2). The column was eluted with a gradient of Et_3N/hexanes/CH_2Cl_2 from 1:10:9 to 1:1:18 to produce 0.905 g (63%) of **A14** as a yellow foam: R_f 0.17 (1:10:9 Et_3N/hexanes/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H), 8.35 (br s, 1H), 8.10 (s, 1H), 7.42−7.16 (m, 24H), 6.81−6.80 (m, 4H), 6.04 (d, J = 4.3 Hz, 1H), 4.43 (t, J = 4.5 Hz, 1H), 4.36 (q, J = 5.0 Hz, 1H), 4.20 (q, J = 4.2 Hz, 1H), 3.78 (s, 6H), 3.55 (ABqt, J = 10.5, 5.0 Hz, 1H), 3.49 (ABqd, J = 10.6, 3.5 Hz, 1H), 3.38 (ABqd, J = 10.7, 4.4 Hz, 1H), 3.18 (br septet, J = 6.0 Hz, 1H), 3.12 (ABqdd, J = 10.1, 7.9, 5.3 Hz, 1H), 2.87 (br d, J = 5.5 Hz, 1H), 2.57 (m, 1H), 2.42 (ABqt, J = 13.5, 5.5 Hz, 1H), 1.31 (d, J = 6.9 Hz, 3H), 1.30 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 158.6, 152.5, 151.0, 149.2, 144.5, 144.4, 141.5, 135.6, 135.5, 130.0, 129.5, 128.1, 128.0, 127.9, 127.0, 126.8, 122.6,

(41) Simionescu, C. I.; Comanita, E.; Pastravanu, M.; Comanita, B. *Acta Chim. Hung.* **1990**, *127*, 41−44.

113.2, 86.8, 86.6, 84.0, 81.4, 69.6, 69.5, 67.0, 62.9, 55.2, 36.1, 31.9, 19.1. FAB-HRMS [M + Na]$^+$ calcd for $C_{56}H_{55}N_5O_7NaS$ 964.3720, found 964.3718.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-*N*^{6}-isobutyryladenosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (A15). A portion of **A14** (0.116 g, 0.12 mmol) was coevaporated with pyridine (3 mL) and dissolved in CH_2Cl_2 (3.4 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (50 μL, 0.16 mmol) and 4,5-dicyanoimidazole (19 mg, 0.16 mmol) were added. The cloudy mixture was warmed to room temperature, stirred for 4 h, and diluted with CH_2Cl_2 (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic extracts were washed with saturated aqueous NaCl (25 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified via chromatography with the column packed as described for **U8** (the sample was loaded in 8:1 hexanes/CH_2Cl_2). The column was eluted with 1:8:1 to 1:5:4 Et_3N/hexanes/CH_2Cl_2 to produce 0.102 g (73%) of **A15** as a white foam: R_f 0.46 (1:5:4 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 and 8.56 (each s, total 1H), 8.29 and 8.28 (each br s, total 1H), 8.14 and 8.09 (each s, total 1H), 7.42−7.15 (m, 24H), 6.81−6.77 (m, 4H), 6.04 and 6.01 (each d, J = 5.6 and 4.8 Hz, total 1H), 4.67 and 4.56 (each t, J = 4.2 and 4.8 Hz, total 1H), 4.53−4.43 (m, 1H), 4.36 and 4.30 (each q, J = 4.0 Hz, total 1H), 3.77 and 3.76 (each s, total 6H), 3.70−3.48 (m, 4H), 3.34−3.21 (m, 3H), 2.57−2.33 (m, 6H), 1.31 (d, J = 6.8 Hz, 6H), 1.15 (d, J = 6.8 Hz, 3H), 1.16 (d, J = 6.8 Hz, 3H), 1.11 (d, J = 6.8 Hz, 3H), 1.02 (d, J = 6.8 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.9, 158.5, 158.5, 152.4, 151.1, 151.0, 149.2, 149.2, 144.6, 144.5, 144.5, 144.4, 144.4, 142.0, 141.8, 135.6, 135.5, 130.1, 130.0, 129.5, 129.5, 128.3, 128.1, 127.9, 127.8, 126.7, 126.6, 122.6, 117.7, 117.3, 113.1, 87.2, 87.0, 86.6, 86.5, 83.6, 80.7, 80.3, 71.1, 71.0, 70.6, 69.6, 69.3, 66.6, 62.9, 62.4, 58.8, 58.7, 57.9, 57.8, 55.2, 55.1, 52.6, 46.8, 43.3, 43.2, 43.1, 43.0, 36.0, 31.7, 31.6, 24.7, 24.6, 24.5, 24.5, 20.8, 20.3, 20.2, 20.1, 20.0, 19.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ 151.5 and 150.9. FAB-HRMS [M + H]$^+$ calcd for $C_{65}H_{73}N_7O_8PS$ 1142.4979, found 1142.4982.

2′-*O*-(2-Benzyloxyethyl)-2-aminoadenosine (G2). A 60% dispersion of sodium hydride in mineral oil (0.27 g, 6.6 mmol) was added to a suspension of 2,6-diaminopurine riboside (1.56 g, 6.0 mmol) in DMF (20 mL). After the mixture was stirred for 1 h, benzyl 2-bromoethyl ether (0.94 mL, 6.0 mmol) was added dropwise over 5 min, and the pale yellow mixture was stirred for 18 h. The solvents were removed under vacuum, and the residual yellow solid was purified via chromatography with 5−30% MeOH in CH_2Cl_2 as the eluant to produce 0.81 g (33%) of **G2** as a white solid: R_f 0.65 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.94 (s, 1H), 7.29−7.17 (m, 5H), 6.79 (br s, 2H), 5.84 (d, J = 6.5 Hz, 1H), 5.74 (br s, 2H), 5.45 (dd, J = 6.0, 5.0 Hz, 1H), 5.07 (dd, J = 4.5, 1.5 Hz, 1H), 4.47 (dd, J = 6.5, 5.5 Hz, 1H), 4.36 (s, 2H), 4.26 (m, 1H), 3.92 (m, 1H), 3.72 (ABqd, J = 11.1, 14.1 Hz, 1H), 3.60−3.52 (m, 3H), 3.49 (t, 4.5 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.9, 156.9, 152.2, 138.9, 136.7, 128.9, 128.2, 128.0, 114.1, 86.8, 85.7, 81.6, 72.0, 69.8, 69.7, 69.5, 62.3. FAB-HRMS [M + H]$^+$ calcd for $C_{19}H_{25}N_6O_5$ 417.1886, found 417.1888.

2′-*O*-(2-Benzyloxyethyl)guanosine (G3). A solution of adenosine deaminase type II (20 mg, 30 units) in 0.1 M Tris, pH 7.5 (4 mL), 0.1 M sodium phosphate, pH 7.4 (0.2 mL), and DMSO (1.0 mL) was added to a solution of **G2** (0.65 g, 1.6 mmol) in 0.1 M Tris, pH 7.5 (6 mL), 0.1 M sodium phosphate, pH 7.4 (0.4 mL), and DMSO (1.5 mL). The cloudy white mixture was stirred at room temperature for 36 h, and the solvents were removed under vacuum. The pale yellow viscous oil was purified via chromatography with 5−15% MeOH in CH_2Cl_2 as the eluant to yield 0.53 g (82%) of **G3** as white powder: R_f 0.35 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.62 (br s, 1H), 7.95 (s, 1H), 7.27−7.17 (m, 5 H), 6.45 (br s, 2H), 5.80 (d, J = 6.4 Hz, 1H), 5.08−5.04 (m, 2H), 4.37 (s, 1H), 4.35 (m, 1H), 4.23 (dd, J = 4.5, 3.0 Hz, 1H), 3.88 (q, J = 3.2 Hz, 1H), 3.72 (m, 1H), 3.62−3.55 (m, 2H), 3.55−3.44 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.9, 154.2, 151.5, 138.2, 135.6, 128.3, 127.6, 127.5, 116.7, 86.0, 84.6, 81.5, 72.1, 69.2, 69.1, 69.0, 61.5. ESI-HRMS [M + H]$^+$ calcd for $C_{19}H_{24}N_5O_6$ 418.1727, found 418.1735.

3′,5′,*O*^{6}-Tris(*tert*-butyldimethylsilyl)-2′-*O*-(2-benzyloxyethyl)guanosine (G4). *tert*-Butyldimethylsilyl chloride (0.72 g, 4.8 mmol) and imidazole (0.55 g, 7.9 mmol) were added to a solution of **G3** (0.53 g, 1.3 mmol) in DMF (15 mL). The solution was stirred at room temperature for 12 h, diluted with CH_2Cl_2 (15 mL), and quenched with MeOH (1 mL) and water (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with saturated aqueous NH_4Cl (20 mL) and saturated aqueous NaCl (20 mL) and dried over $MgSO_4$. The solvents were removed under vacuum to produce 0.78 g (73%) of **G4** as a white powder: R_f 0.55 (1:9 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.30−7.24 (m, 5H), 6.36 (br s, 2H), 5.97 (d, J = 4.0 Hz, 1H), 4.52 (s, 2H), 4.48 (t, J = 4.8 Hz, 1H), 4.24 (t, J = 4.0 Hz, 1H), 4.08 (dt, J = 5.6, 2.9 Hz, 1H), 3.93 (ABqd, J = 11.4, 3.3 Hz, 1H), 3.81−3.74 (m, 3H), 3.61 (t, J = 4.0 Hz, 2H), 0.92 (s, 18H), 0.91 (s, 9H), 0.11 (s, 3H), 0.10 (s, 6H), 0.09 (s, 3H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 153.7, 151.5, 138.3, 136.2, 128.6, 127.9, 127.8, 117.7, 86.7, 84.8, 82.8, 73.4, 70.4, 70.2, 69.6, 62.1, 26.3, 26.0, 25.9, 18.7, 18.4, −4.3, −4.6, −5.1, −5.2. ESI-HRMS [M + H]$^+$ calcd for $C_{37}H_{66}N_5O_6Si_3$ 760.4321, found 760.4298.

3′,5′-Bis(*tert*-butyldimethylsilyl)-2′-*O*-(2-benzyloxyethyl)-*N*^{2}-isobutyrylguanosine (G5). Isobutyryl chloride (0.50 mL, 5.0 mmol) was added to a solution of **G4** (0.78 g, 1.03 mmol) in pyridine (5 mL) cooled to 0 °C. After the mixture was stirred for 45 min, the colorless solution was quenched with saturated aqueous $NaHCO_3$ (25 mL) and extracted with EtOAc (2 × 25 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under vacuum. The residual yellow foam was purified via chromatography with 1−5% MeOH in CH_2Cl_2 as the eluant to yield 0.73 g (98%) of **G5** as a white foam: R_f 0.75 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (br s, 1H), 8.05 (s, 1H), 7.29 (s, 1H), 7.28−7.24 (m, 5H), 5.98 (d, J = 4.0 Hz, 1H), 4.52 (ABq, J = 12.4 Hz, 1H), 4.43 (ABq, J = 12.0 Hz, 1H), 4.42 (d, J = 3.0 Hz, 1H), 4.10 (t, J = 4.0 Hz, 1H), 4.07 (dt, J = 5.5, 3.0 Hz, 1H), 3.91 (ABqd, J = 11.6, 2.8 Hz, 1H), 3.76 (ABqd, J = 11.6, 3.0 Hz, 1H), 3.74 (br q, J = 3.0 Hz, 2H), 3.55 (t, J = 5.0 Hz, 2H), 2.52 (septet, J = 7.0 Hz, 1H), 1.16 (d, J = 7.0 Hz, 3H), 1.14 (d, J = 6.5 Hz, 3H), 0.91 (s, 9H), 0.90 (s, 9H), 0.10−0.09 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.9, 155.9, 148.2, 147.8, 138.2, 137.2, 128.6, 127.9, 127.8, 121.6, 86.5, 85.3, 83.6, 73.5, 70.1, 69.7, 62.1, 36.5, 25.9, 25.6, 18.8, 18.8, 18.3, 18.0, −4.7, −4.9, −5.4, −5.5. ESI-HRMS [M + H]$^+$ calcd for $C_{35}H_{58}N_5O_7Si_2$ 716.3875, found 716.3860.

3′,5′-Bis(*tert*-butyldimethylsilyl)-2′-*O*-(2-hydroxyethyl)-*N*^{2}-isobutyrylguanosine (G6). Pd/C (10%, 1.0 g, 0.94 mmol) was added to a solution of **G5** (0.73 g, 1.02 mmol) in EtOH (10 mL). The flask was evacuated and placed under an atmosphere of hydrogen. The consumption of **G5** was monitored by TLC. After 12 h, 10 g of Celite was added, and the mixture was filtered and concentrated under vacuum. The residue was purified via chromatography with 0−5% MeOH in CH_2Cl_2 as the eluant to produce 0.56 g (88%) of **G6** as white foam: R_f 0.45 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 8.04 (s, 1H), 6.01 (s, 1H), 4.38 (t, J = 5.5 Hz, 1H), 4.07 (d, J = 7.0 Hz, 1H), 4.05 (d, J = 5.0 Hz, 1H), 3.91 (q, J = 5.07 Hz, 1H), 3.75 (ABq, J = 5.0 Hz, 1H), 3.79−3.71 (br m, 6H), 2.75 (septet, J = 6.5 Hz, 1H), 1.18 (d, J = 7.0 Hz, 6H), 0.87 (s, 9H), 0.86 (s, 9H), 0.06 (s, 6H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.1, 156.3, 148.4, 147.9, 137.1, 121.3, 88.2, 84.4, 84.2, 72.7, 69.3, 61.7, 61.2, 36.3, 26.3, 25.6, 19.3, 19.2, 19.1, 19.0, 18.6, 18.2, −4.4, −4.8, −5.2, −5.3. ESI-HRMS [M + H]$^+$ calcd for $C_{28}H_{52}N_5O_7Si_2$ 626.3405, found 626.3394.

3′,5′-Bis(*tert*-butyldimethylsilyl)-2′-*O*-(2-methanesulfonylethyl)-*N*²-isobutyrylguanosine (G7). A solution of **G6** (0.50 g, 0.80 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C. Portions of Et_3N (0.15 mL, 1.07 mmol) and methanesulfonyl chloride (84 μL, 1.09 mmol) were added. After 1 h, the colorless solution was diluted with CH_2Cl_2 (25 mL), washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), dried over Na_2SO_4, and concentrated under vacuum to produce 0.55 g (98%) of **G7** as a pale yellow foam: R_f 0.45 (1:24 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.18 (br s, 1H), 9.90 (br s, 1H), 8.06 (s, 1H), 5.90 (s, 1H), 4.38−4.31 (m, 4H), 3.99 (t, J = 8.8 Hz, 1H), 3.94 (m, 1H), 3.90 (d, J = 4.8 Hz, 1H), 3.73 (d, J = 11.2 Hz, 1H), 3.07 (s, 3H), 2.67 (septet, J = 6.8 Hz, 1H), 1.14 (d, J = 6.8 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 0.87 (s, 9H), 0.82 (s, 9H), 0.08 (s, 3H), 0.06 (s, 3H), 0.02 (s, 3H), 0.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 156.0, 148.4, 147.8, 136.8, 121.6, 88.1, 84.4, 83.7, 69.7, 69.5, 68.8, 60.9, 51.0, 37.7, 36.3, 26.3, 25.8, 19.2, 19.1, 18.7, 18.2, −4.4, −4.7−5.1, −5.2. ESI-HRMS $[M + H]^+$ calcd for $C_{29}H_{54}N_5O_9Si_2S$ 704.3181, found 704.3171.

3′,5′-Bis(*tert*-butyldimethylsilyl)-2′-*O*-(2-azidoethyl)-*N*²-isobutyrylguanosine (G8). NaN_3 (0.25 g, 4.0 mmol) and 18-crown-6 (50 mg, 0.19 mmol) were added to a solution of **G7** (0.55 g, 0.78 mmol) in DMF (6 mL). The cloudy pale yellow mixture was stirred at 60 °C for 5 h, diluted with EtOAc (25 mL), washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), dried over Na_2SO_4, and concentrated under vacuum. The pale yellow foam was purified via chromatography using a gradient of 20−40% EtOAc in CH_2Cl_2 as the eluant to produce 0.35 g (70%) of **G8** as a white foam: R_f 0.75 (1:2 EtOAc/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.37 (s, 1H), 10.35 (s, 1H), 8.14 (s, 1H), 5.98 (d, J = 2.4 Hz, 1H), 4.39 (dd, J = 7.4, 5.2 Hz, 1H), 4.05 (td, J = 9.2, 2.4 Hz, 1H), 3.98 (dd, J = 12.0, 2.4 Hz, 1H), 3.89 (dt, J = 9.2, 2.4 Hz, 1H), 3.99 (ABqd, J = 11.6, 2.4 Hz, 1H), 3.85 (dd, J = 4.4, 2.0 Hz, 1H), 3.85 (m, 1H), 3.75 (ABqd, J = 11.2, 2.4 Hz, 1H), 3.71 (m, 1H), 2.76 (septet, J = 6.8 Hz, 1H), 1.15 (d, J = 6.8 Hz, 3H), 1.12 (d, J = 6.8 Hz, 3H), 0.87 (s, 9H), 0.85 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H), 0.031 (s, 3H), 0.028 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.2, 156.2, 148.4, 148.3, 137.3, 121.4, 87.0, 84.1, 83.7, 69.7, 69.0, 68.8, 61.3, 51.0, 36.2, 26.3, 25.8, 19.2, 18.7, 18.2, −4.4, −4.7, −5.2. ESI-HRMS $[M + H]^+$ calcd for $C_{28}H_{51}N_8O_6Si_2$ 651.3470, found 651.3467.

2′-*O*-(2-(Trifluoroacetamido)ethyl)-*N*²-isobutyrylguanosine (G9). Pd/C (10%, 0.37 g, 0.35 mmol) was added to a solution of **G8** (0.350 g, 0.53 mmol) in EtOH (13 mL). The flask was evacuated and placed under an atmosphere of hydrogen. The consumption of **G8** was monitored by TLC. After 12 h, 10 g of Celite was added, and the mixture was filtered and concentrated under vacuum. The pale yellow foam was dissolved in THF (5 mL), and ethyl trifluoroacetate (0.28 mL, 2.4 mmol) and Et_3N (0.34 mL, 2.4 mmol) were added. After 12 h, the solution was cooled to 0 °C, and 1 M TBAF in THF (1.24 mL, 1.24 mmol) was added dropwise over 5 min. The solution was warmed to room temperature and stirred for 6 h. The solvents were removed under vacuum, and the residual yellow foam was purified via chromatography using a gradient of 5−15% MeOH in CH_2Cl_2 as the eluant to yield 0.190 g (73%) of **G9** as a white foam: R_f 0.23 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (br s, 1H), 11.62 (br s, 1H), 9.25 (s, 1H), 5.88 (d, J = 6.0 Hz, 1H), 5.26 (d, J = 4.8 Hz, 1H), 5.07 (t, J = 5.2 Hz, 1H), 4.38 (t, J = 4.8 Hz, 1H), 4.30 (ABq, J = 4.8 Hz, 1H), 3.92 (ABq, J = 4.8 Hz, 1H), 3.67 (m, 1H), 3.55 (m, 4H), 2.74 (septet, J = 6.8 Hz, 1H), 1.11 (s, 3H), 1.09 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.8, 155.5, 149.5, 148.9, 138.1, 120.8, 86.7, 85.2, 82.2, 69.5, 67.9, 61.8, 35.4, 19.49, 19.48l; ^{19}F NMR (376 MHz, DMSO-d_6) δ −74.9. ESI-HRMS $[M + H]^+$ calcd for $C_{18}H_{24}N_6O_7F_3$ 493.1659, found 493.1664.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)ethyl)-*N*²-isobutyrylguanosine (G10). A portion of **G9** (0.180 g, 0.37 mmol) was coevaporated from pyridine (2 × 5 mL) and dissolved in pyridine (3 mL). The solution was cooled to 0 °C, and 4,4′-dimethoxytrityl chloride (0.15 g, 0.44 mmol) was added. After the solution was stirred at room temperature for 18 h, the solvents were removed under vacuum. The residual yellow foam was purified via chromatography with the column packed as described for **U9**. The column was eluted with a gradient of 0−5% MeOH in CH_2Cl_2 to produce 0.240 g (83%) of **G10** as a white foam: R_f 0.45 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, acetone-d_6) δ 10.68 (br s, 1H), 8.91 (br s, 1H), 8.01 (s, 1H), 7.45−7.17 (m, 9H), 6.81 (m, 4H), 6.03 (d, J = 3.0 Hz, 1H), 4.70 (t, J = 11.0 Hz, 2H), 4.61 (t, J = 7.5 Hz, 1H), 4.21 (m, 1H), 3.98 (m, 2H), 3.74(s, 6H), 3.60 (m, 2H), 3.46−3.37 (m, 2H), 3.20 (q, J = 7.0 Hz, 1H), 2.88 (septet, J = 7.0 Hz, 1H), 1.22 (d, J = 4.0 Hz, 3H), 1.20 (d, J = 3.5 Hz, 3H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.3, 159.0, 158.9, 155.4, 148.7, 148.5, 145.4, 137.7, 136.1, 136.0, 130.3, 128.4, 128.0, 127.0, 121.3, 113.2, 87.3, 86.3, 83.8, 82.3, 70.1, 68.8, 63.9, 54.8, 46.4, 40.0, 35.9, 29.7, 18.7, 18.6; ^{19}F NMR (470 MHz, acetone-d_6) δ −76.7. ESI-HRMS $[M + H]^+$ calcd for $C_{39}H_{42}N_6O_9F_3$ 795.2965, found 795.2978.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(trifluoroacetamido)ethyl)-*N*²-isobutyrylguanosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (G11). A portion of **G10** (0.160 g, 0.20 mmol) was coevaporated with pyridine (2 × 5 mL) and dissolved in CH_2Cl_2 (2 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (87 μL, 0.27 mmol) and 4,5-dicyanoimidazole (27 mg, 0.24 mmol) were added. The colorless solution was warmed to room temperature, stirred for 6 h, diluted with 5% Et_3N in EtOAc (25 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residual pale yellow solid was purified by chromatography with the column packed as described for **U9** (the sample was loaded in 4:15 hexanes/CH_2Cl_2). The column was eluted with a gradient from 1:4:15 Et_3N/hexanes/CH_2Cl_2 to 1:19 Et_3N/CH_2Cl_2 to produce 0.160 g (80%) of **G11** as a white foam: R_f 0.46 (1:19 Et_3N/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 8.87 (br s, 1H), 7.99 and 7.94 (each s, total 1H), 7.50−7.23 (m, 9H), 6.87 (m, 4H), 6.01 and 6.00 (each d, J = 4.5 Hz, total 1H), 4.73 (dt, J = 8.5 Hz, 5.0 Hz, 1H), 4.66 and 4.59 (each dt, J = 9.5 Hz, 5.5 Hz, total 1H), 4.38 and 4.37 (each m, total 1H), 4.04 (m, 1H), 3.98 (m, 2H), 3.90 (m, 1H), 3.78 and 3.77 (each s, total 6H), 3.74−3.40 (m, 8H), 2.86 (septet, J = 7.0 Hz, 1H), 2.79 (t, J = 10.5 Hz, 1H), 2.59 (m, 1H), 1.18 (m, 16H), 1.08 (s, 1H), 1.07 (s, 1H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.2, 159.0, 155.2, 148.7, 148.5, 145.3, 145.2, 136.9, 136.8, 136.0, 135.9, 130.3, 128.4, 128.3, 127.0, 121.6, 118.6, 118.3, 113.3, 87.5, 86.5, 83.5, 83.2, 81.5, 81.2, 71.4, 71.3, 68.6, 68.4, 63.5, 63.2, 59.1, 59.0, 58.5, 58.4, 54.9, 52.8, 43.3, 43.2, 39.8, 35.8, 24.5, 20.0, 18.6; ^{19}F NMR (470 MHz, acetone-d_6) δ −76.7 (just one peak observed); ^{31}P NMR (202 MHz, acetone-d_6) δ 151.2 and 150.6. ESI-HRMS $[M + H]^+$ calcd for $C_{48}H_{59}N_8O_{10}F_3P$ 995.4044, found 995.4040.

2′-*O*-(2-(Triphenylmethylthio)ethyl)-*N*²-isobutyrylguanosine (G12). Diisopropyl azodicarboxylate (123 μL, 0.64 mmol) was added to a solution of triphenylphosphine (0.124 g, 0.50 mmol) in THF (4 mL) cooled to 0 °C. After 30 min, a solution of **G6** (0.153 g, 0.24 mmol) and trityl mercaptan (0.178 g, 0.62 mmol) in THF (2 mL) was added via cannula; the flask containing the latter reagents was rinsed with THF (2 × 2 mL). After 30 min, 1 M TBAF in THF (0.48 mL, 0.48 mmol) was added to the solution. After 3 h, the solvents were removed under vacuum, and the residue was purified via chromatography with 0−2% MeOH in CH_2Cl_2 as the eluant to yield 0.156 g (97%) of **G12** as a white foam: R_f 0.33 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.69 (br s, 1H), 8.12 (s, 1H), 7.31−7.13 (m, 15H), 5.86 (d, J = 6.0 Hz, 1H), 5.23 (br s, 1H), 4.43 (br s, 1H), 4.39 (q, J = 5.0 Hz, 1H), 4.17 (br s, 1H), 3.95 (br s, 1H), 3.88 (ABq, J = 12.5 Hz, 1H), 3.74 (br ABqd, J = 12.0, 1.5 Hz, 1H), 3.19 (m, 3H), 2.77 (septet, 1H, J = 7.0 Hz), 2.41 (ABqt, J = 13.5, 6.0 Hz, 1H), 2.32 (ABqt, J = 13.5, 5.5 Hz, 1H), 1.21 (d, J = 6.5 Hz, 3H), 1.20 (d, J = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 178.9, 155.1, 147.6, 147.2, 144.2, 139.1, 129.3,

127.9, 126.8, 122.0, 88.1, 86.8, 81.7, 70.2, 69.2, 66.5, 62.5, 36.2, 31.9, 18.9. ESI-HRMS $[M + H]^+$ calcd for $C_{35}H_{38}N_5O_6S$ 656.2543, found 656.2543.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-*N*²-isobutyrylguanosine (G13). A portion of **G12** (0.150 g, 0.23 mmol) was coevaporated from pyridine (3 mL) and dissolved in pyridine (3 mL). The solution was cooled to 0 °C, and 4,4′-dimethoxytrityl chloride (0.089 g, 0.26 mmol) was added. After the mixture was stirred at room temperature for 12 h, the solvents were removed under vacuum. The residual pale yellow foam was purified via chromatography with the column packed as described for **U9**. The column was eluted with a gradient of 0−5% MeOH in CH_2Cl_2 to yield 0.150 g (68%) of **G13** as a white foam: R_f 0.55 (1:19 MeOH/CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.46 (dd, J = 7.0, 1.5 Hz, 2H), 7.36−7.11 (m, 24H), 6.82−6.75 (m, 4H), 5.83 (d, J = 6.5 Hz, 1H), 4.54 (m, 1H), 4.34 (m, 1H), 4.15 (d, J = 2.0 Hz, 1H), 3.75 (s, 3H), 3.74 (s, 3H), 3.40 (ABqd, J = 10.5, 2.0 Hz, 1H), 3.39 (ABqt, J = 10.5, 5.0 Hz, 1H), 3.15 (ABqd, J = 10.5, 3.5 Hz, 1H), 3.07−3.02 (m, 1H), 2.61 (m, 2H), 2.37 (ABqt, J = 13.0, 5.5 Hz, 1H), 1.91 (septet, J = 7.0 Hz, 1H), 0.91 (d, J = 7.0 Hz, 3H), 0.81 (d, J = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.1, 158.9, 156.7, 148.5, 147.5, 145.1, 144.6, 138.7, 136.1, 135.8, 130.3, 130.2, 129.7, 128.2, 127.3, 127.1, 122.2, 113.5, 86.5, 84.5, 69.7, 67.5, 55.5, 36.4, 32.1, 18.9. ESI-HRMS $[M + H]^+$ calcd for $C_{56}H_{56}N_5O_8S$ 958.3850, found 958.3817.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(2-(triphenylmethylthio)ethyl)-*N*²-isobutyrylguanosine-3′-*O*-(2-cyanoethyl-*N*,*N*-diisopropyl)phosphoramidite (G14). A portion of **G13** (0.150 g, 0.16 mmol) was coevaporated with pyridine (2 × 5 mL) and dissolved in CH_2Cl_2 (1 mL). The solution was cooled to 0 °C, and 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (65 μL, 0.21 mmol) and 4,5-dicyanoimidazole (24 mg, 0.22 mmol) were added. The colorless solution was warmed to room temperature, stirred for 6 h, diluted with 5% Et_3N in EtOAc (25 mL), and washed with saturated aqueous $NaHCO_3$ (2 × 25 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The residual pale yellow solid was purified via chromatography with the column packed as described for **U9** (the sample was loaded in 14:6 hexanes/CH_2Cl_2). The column was eluted with a gradient from 1:14:6 to 1:3:16 Et_3N/hexanes/CH_2Cl_2 to produce 0.109 g (61%) of **G14** as a white foam: R_f 0.44 (1:3:16 Et_3N/hexanes/CH_2Cl_2). NMR spectroscopy revealed the presence of both diastereomers: ^{1}H NMR (500 MHz, acetone-d_6) δ 7.98 and 7.96 (each s, total 1H), 7.50 and 7.49 (each s, 2H), 7.36−7.23 (m, 24H), 6.87 (m, 4H), 5.96 and 5.92 (each d, J = 6.0 Hz, total 1H), 4.63 and 4.57 (each t, J = 5.5 Hz, total 1H), 4.53 (m, 1H), 4.35 and 4.28 (each ABqd, J = 7.5, 4.0 Hz, total 1H), 3.78 (s, 6H), 3.72−3.59 (m, 5H), 3.45−3.32 (m, 3H), 2.77 (septet, J = 7.0 Hz, 1H), 2.72 (m, 1H), 2.60 (t, J = 6.0 Hz, 1H), 2.36 (m, 2H), 1.16 (m, 16H), 1.07 (d, J = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, acetone-d_6) δ 180.2, 159.0, 155.2, 148.7, 148.5, 145.3, 145.2, 136.9, 136.8, 136.0, 135.9, 130.3, 128.4, 128.3, 127.0, 121.6, 118.6, 118.3, 113.3, 87.5, 86.5, 83.5, 83.2, 81.5, 81.2, 71.4, 71.3, 68.6, 68.4, 63.5, 63.2, 59.1, 59.0, 58.5, 58.4, 54.9, 52.8, 46.1, 43.3, 43.2, 39.8, 35.8, 24.5, 20.0, 18.6; ^{31}P NMR (202 MHz, acetone-d_6) δ 151.13 and 151.06. FAB-HRMS $[M + H]^+$ calcd for $C_{65}H_{73}N_7O_9PS$ 1158.4928, found 1158.4929.

Solid-Phase RNA Oligonucleotide Synthesis. Acetonitrile (synthesis grade), the standard 2′-TOM-phosphoramidetes for A, C, G, and U, 5-ethylthio-1*H*-tetrazole, and other standard solutions were from a commercial supplier. RNA oligonucleotides were synthesized on the 1 μmol scale. The standard coupling time of 6 min was used for the four standard phosphoramidites, and an increased coupling time of 10 min was used for the modified phosphoramidites. After the solid-phase synthesis, the solid support was transferred to a screw-cap glass vial and incubated at room temperature for 16 h with 1.5 mL of methylamine solution, prepared by mixing equal volumes of 40% aqueous methylamine and 33% methylamine in ethanol. After the vial was cooled briefly on ice, the supernatant was transferred by pipet into two 1.5 mL eppendorf tubes; the solid support and vial were rinsed with water (2 × 0.25 mL). The combined solutions were evaporated to dryness using an evaporating centrifuge. The residue was dissolved in a total volume of 1.0 mL of 1 M TBAF in THF and stirred at 37 °C for 12 h. One milliliter of 1 M Tris, pH 7.5, was added to the solution, and the solution was evaporated to half the original volume. Additional 1 M Tris, pH 7.5, was added to a total volume of 2.0 mL, and the oligonucleotide was desalted on a NAP-25 column using water as the eluant. The fractions containing the desired RNA oligonucleotide (as judged by UV absorbance at 260 nm) were combined and evaporated to dryness. The oligonucleotide was then purified by 20% denaturing PAGE using 1× TBE (89 mM each Tris and boric acid, pH 8.3, containing 2 mM EDTA) as the running buffer. The gel electrophoresis and oligonucleotide extraction and precipitation procedures have been described previously.[4e] The solid-phase coupling efficiency for each of the modified phosphoramidites was >90% as judged by the appearance of abort bands on the polyacrylamide gels. The yield of the purified oligoribonucleotide was typically 150−200 nmol, which is similar to the yields for unmodified oligoribonucleotides that are synthesized in a similar fashion.

Oligonucleotide Sequences and Mass Spectrometry Data. Five RNA oligonucleotides were prepared using the above procedure incorporating phosphoramidites **U9**, **U10**, **A11**, **G11**, and **A15**. The first four oligonucleotides have one of the four 2′-tethered amine nucleotides (U, C, A, or G), and the fifth oligonucleotide has the adenosine 2′-tethered thiol nucleotide with the thiol group protected as the *S*-trityl derivative. The oligonucleotide sequences and mass spectrometry data are given below. 5′-^{32}P radiolabeling of each oligonucleotide after PAGE purification revealed that each oligonucleotide is >99% pure (see Supporting Information). The oligonucleotide with the 2′-tethered thiol was ligated to a larger strand of RNA with T4 DNA ligase and a DNA splint,[40] and the *S*-trityl group was subsequently deprotected with $AgNO_3$ essentially as described[31] (data not shown).

Oligonucleotide with 2′-Tethered Amine U. The sequence is 5′-UUCUGUUGAXAUGGAUGCAGUUCA-3′, where X denotes the modified nucleotide from incorporation of phosphoramidite **U9**. MALDI-MS calcd 7675.6, found 7677.2.

Oligonucleotide with 2′-Tethered Amine C. The sequence is 5′-UUCUGUUGAUAUGGAUGXAGUUCA-3′, where X denotes the modified nucleotide from incorporation of phosphoramidite **U10**. MALDI-MS calcd 7675.6, found 7672.9.

Oligonucleotide with 2′-Tethered Amine A. The sequence is 5′-GGAAUUGCGGGAXAG-3′, where X denotes the modified nucleotide from incorporation of phosphoramidite **A11**. MALDI-MS calcd 4961.1, found 4962.1.

Oligonucleotide with 2′-Tethered Amine G. The sequence is 5′-GGAAUUXCGGGAAAG-3′, where X denotes the modified nucleotide from incorporation of phosphoramidite **G11**. MALDI-MS calcd 4961.1, found 4961.9.

Oligonucleotide with 2′-Tethered Thiol A. The sequence is 5′-GGAAUZGCGGGAXAG-3′, where X denotes the modified nucleotide (*S*-trityl protecting group remaining) from incorporation of phosphoramidite **A15** and Z denotes a 2′-amino-2′-deoxyuridine nucleotide (C. V. Miduturu and S. K. Silverman, manuscript in preparation). MALDI-MS calcd 5219.3, found 5218.7.

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Supporting Information Available: General experimental procedures, ^{1}H and ^{31}P NMR spectra, and 5′-^{32}P radiolabeling to demonstrate oligoribonucleotide purity. This material is available free of charge via the Internet at http://pubs.acs.org.

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