A Chemical Method for Site-Specific Modification of RNA: The Convertible Nucleoside Approach

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Abstract: Knowledge of RNA structure can greatly facilitate the understanding of its biological function. However, the physical properties of RNA, especially its conformational heterogeneity, present an impediment to high-resolution structural analysis. Thus, lower resolution methods such as biochemical probing, phylogenetic analysis, and molecular modeling have come to serve an important role in RNA science. This situation has created the need for a means by which to constrain RNA structure, either to reduce its conformational flexibility or to help distinguish between alternative structural models. To address this need, we have developed chemistry that permits the site-specific introduction of functionalizable tethers into RNA. Here we report the design and synthesis of reagents for use in solid-phase RNA synthesis that allow the functionalization of the base moiety of G, C, and A residues. Upon incorporation into oligonucleotides and subsequent treatment with alkylamines, the convertible nucleoside derivatives reported here give rise to functionally tethered N^4-alkyl-C, N^6-alkyl-A, and N^2-alkyl-G residues in RNA. The derivatized RNAs can then be used to target the attachment of chemical probes or the placement of disulfide cross-links as structural constraints. The attachment of nonnatural functional groups to RNA in this fashion provides a powerful means of both probing its structural environment and constraining its conformation. The size and functionality of the *N*-alkyl modification is determined solely by the choice of alkylamine, thereby permitting the preparation of a wide range of functionally tethered RNAs.

Introduction

A necessary step toward understanding the function of RNA involves obtaining a detailed knowledge of its structure. However, determining the structure of RNA by X-ray or NMR has proven difficult, especially for polyribonucleotides that fold into complex three-dimensional arrangements. Thus, only quite recently have structures become available of conformationally complex RNA species, both naked[1−4] and bound to organic ligands.[5−11] Much of the difficulty inherent in structural studies of RNA arises from the same property that makes it such a versatile and interesting biomolecule, namely, the propensity to self-assemble through base-pairing interactions. In the best of cases, RNA folding spontaneously produces a single, well-defined, homogenous structure; however, more commonly the folding process furnishes a mixture of conformationally isomeric products that are poorly suited to structure determination.[12] *In vivo*, RNA chaperones may facilitate the attainment of a properly folded structure.[13]

In the absence of a detailed molecular structure, lower resolution methods such as phylogenetic comparisons, biochemical probing, and RNA folding algorithms are often used to construct a secondary structure map,[14,15] and in rare cases can even permit the construction of a three-dimensional structural model.[16] Site-specific chemical modification also represents a potentially powerful approach for studying the structure, folding, and function of RNA.[17,18] In particular, disulfide cross-linking[19] holds great promise not only for locking the RNA into a predetermined conformer, but also for discriminating among various folding models.[20−23] While it is possible to incorporate modified nucleosides in a site-specific fashion using RNA polymerases,[24] the most general way to address the issue of site selectivity is through the use of the solid-phase synthesis methods. Although the chemical synthesis of RNA is currently limited to oligonucleotides of about 70 nucleotides or less, the combination of chemical synthesis,[25] enzymatic synthesis,[26] and splinted ligation[27] together make it

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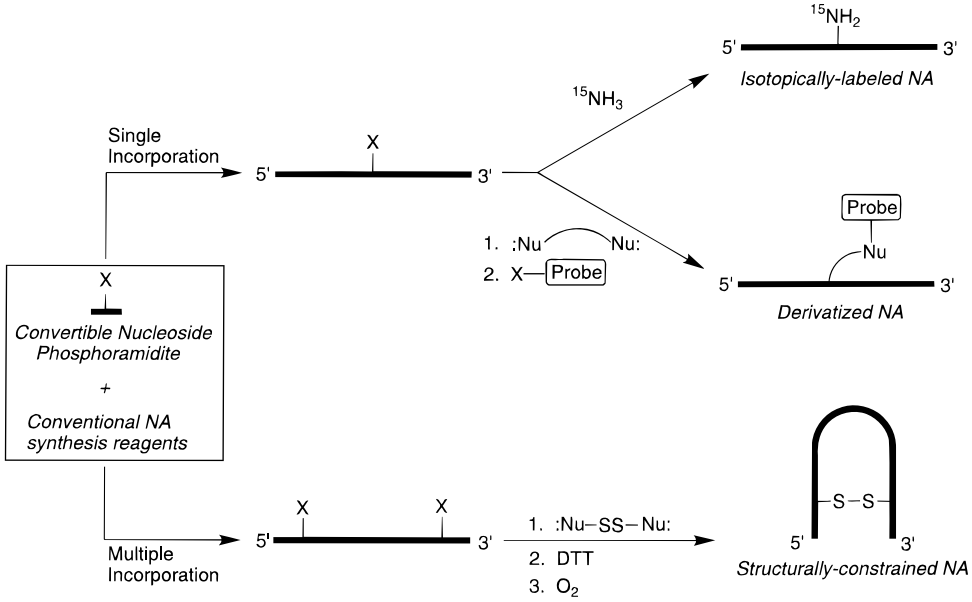

Figure 1. Convertible nucleoside approach. Convertible nucleosides can be site-specifically incorporated into oligonucleotides (either DNA or RNA) during solid-phase synthesis. Postsynthetic treatment with a nucleophile results in substitution at the leaving group (X), generating a functionally tethered oligonucleotide. These tethers can be further elaborated with chemical reagents to make derivatized or cross-linked nucleic acids. NA = nucleic acid.

possible to produce site-specifically-modified RNAs at least several hundred nucleotides in length.

Our objective in the present study was to develop a general method by which to attach functionalized tethers to RNA. Ideally, we envisioned a method that would not only allow the attachment of chemical probes to RNA, but also permit the placement of chemical devices, such as disulfide cross-links, that can be used to constrain RNA folding in an effort to facilitate structural analysis by NMR or crystallography. We felt it important that the modification not disrupt Watson−Crick base-pairing, because any such disruption would limit its placement to positions at the ends of duplex regions. Finally, a truly general method should be fully compatible with standard solid-phase RNA synthesis technology and should permit ligation into larger RNA assemblies using splinted-ligation methodologies.[27,28]

We[19,29−34] and others[35−39] have developed a general method for the post-synthetic modification of DNA known as the convertible nucleoside approach (Figure 1). In this method, a nucleoside derivative containing a leaving group on its nucleobase (a convertible nucleoside) is incorporated into DNA site-specifically through solid-phase synthesis. Following elongation of the nucleic acid polymer, the full-length chain is treated with a nucleophile, which displaces the leaving group and thereby becomes attached to the nucleobase. When the displacing nucleophile is a primary alkylamine, the product is an A, G, or C residue bearing a tether attached to its exocyclic amine (Figure 2); such modified nucleosides are fully capable of Watson−

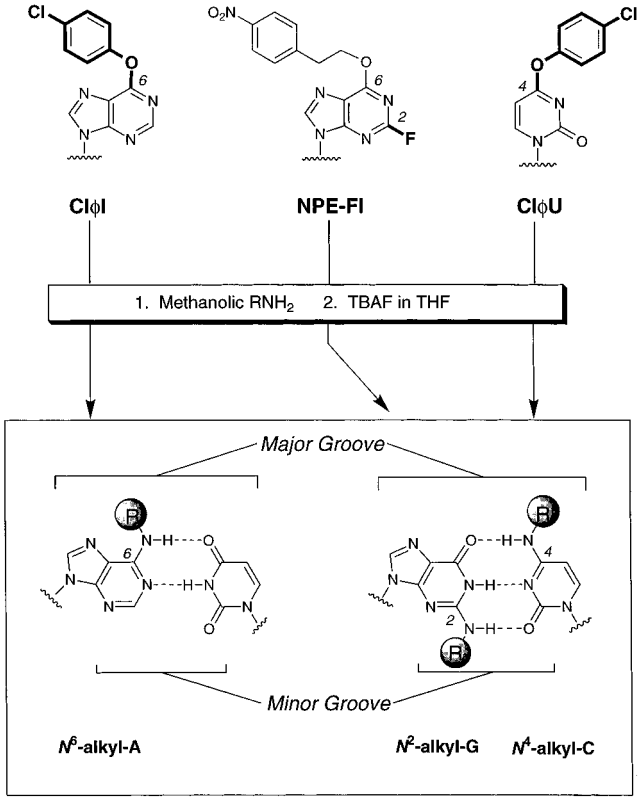

Figure 2. Convertible nucleoside bases for RNA. The base moiety is equipped with a leaving group (bold) that can be substituted by amines. This process results in the generation of *N*-alkyl tethers attached to the exocyclic amines of A, G, or C, allowing both the major and minor grooves to be targeted without interfering with Watson−Crick base-pairing.

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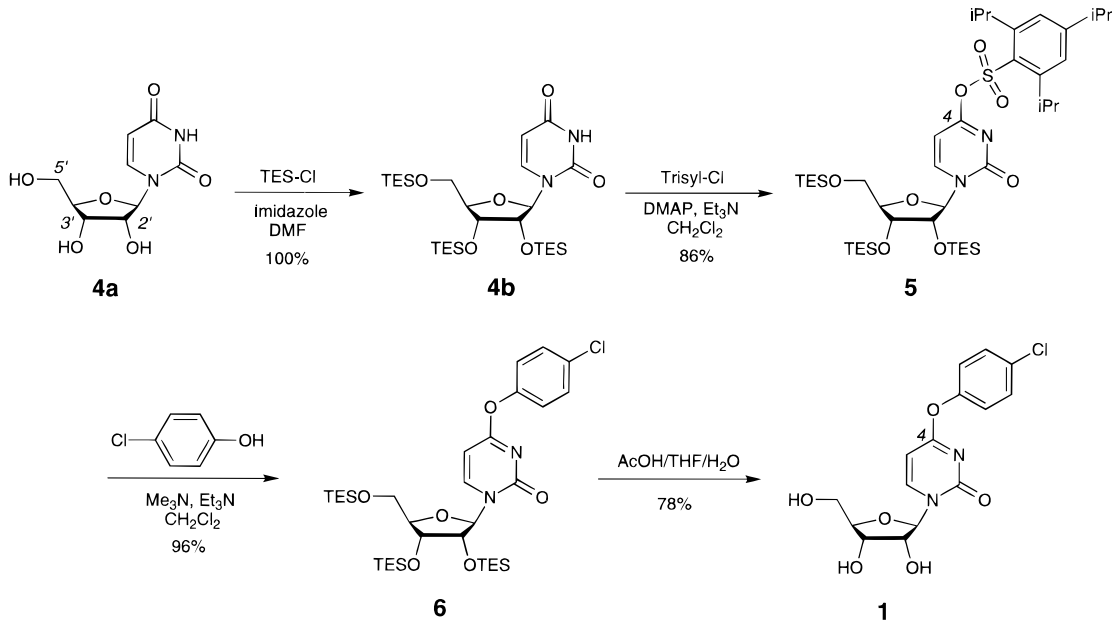
Scheme 1



Crick base-pairing. Depending on the base chosen, one can tether novel functionality to the floor of either the major (A and C) or minor (G) groove. The convertible nucleoside method has been used *inter alia* for the synthesis of thermostabilized DNA and destabilized (bent) DNA helices,[19,40,41] mechanistic investigations of enzymatic DNA methylation,[31,33,42] the preparation of DNA-affinity purification columns,[43−45] and the synthesis of isotopically-labeled DNA for studies of molecular recognition in protein−DNA interfaces.[32] In a preliminary paper, we reported the development of a convertible nucleoside suitable for the functionalization of C residues in RNA, and its use in studying the recognition mode of the RNA glycosylase ricin.[22] Here we provide additional details on the convertible-C reagent and also extend the method to include functionalization of A and G residues in RNA.

Results

Synthesis and Screening of Convertible Nucleosides. The synthesis and methodology described here are an extension of earlier reports from this laboratory on the convertible nucleoside approach as applied to DNA.[19,29−34] Though we hoped to transfer the methodology as seamlessly as possible from DNA to RNA, differences in the chemistry of the two polymers suggested some changes might be necessary. For instance, treatment of chemically synthesized RNA with concentrated aqueous solutions of amines, the preferred conditions for DNA convertible nucleoside conversion, is known to result in reduced yields, presumably owing to base-promoted hydrolysis of the 2′-*O*-silyl ether bonds and concomitant 2′-hydroxyl attack on the neighboring phosphodiester,[46] resulting in oligonucleotide scission. It has been reported that deprotection of synthetic RNA with alcoholic solutions of ammonia, particularly methanol or ethanol, can reduce base-promoted RNA degradation,[46,47] which led us to the use of methanolic solutions of amines, instead of aqueous solutions, for our convertible nucleoside displacement reactions.

Preliminary experiments with 4-*O*-(2,4,6-trimethylphenyl)-uridine (TMPU), the ribose analog of the convertible nucleoside used for the preparation of functionally tethered deoxycytidines (TMPdU),[30,48] showed that it was converted only very slowly by methanolic solutions of amines.[49] To overcome this problem, we screened a number of 4-*O*-aryluridine derivatives in an attempt to identify one that would react with amines efficiently in methanol, preferably at temperatures less than 45 °C, in under 24 h.[49] This led to the selection of 4-*O*-(4-chlorophenyl)uridine (**1**) as a suitable convertible nucleoside in the preparation of functionally modified cytidines.

The synthesis of **1** proceeds in four steps starting with uridine (Scheme 1) and is analogous to the reported synthesis of TMPdU,[30,48] differing only in the use of 2′-OH protecting groups. Use of the acid-labile triethylsilyl ethers in place of base-labile acetate esters was mandated by the increased reactivity of the aryl ether to amine substitution. Specifically, reaction of the ribose hydroxyls of uridine (**4a**) with chlorotriethylsilane gave the tris(triethylsilyl) ether-protected uridine **4b**. Activation of the O^4 position was achieved by formation of the O^4-[(2,4,6-triisopropylphenyl)sulfonyl] ester **5**, which underwent trimethylamine-mediated nucleophilic substitution with 4-chlorophenol to generate the triethylsilyl (TES)-protected 4-*O*-(4-chlorophenyl)uridine **6**. Removal of the TES ethers with AcOH in THF/water proceeded cleanly to afford the convertible nucleoside ClϕU (**1**).

In the preparation of the convertible nucleoside used to generate functionalized adenosines, we were again uncertain as to which aryl ether of inosine would be optimal for our system. Earlier work had demonstrated that 6-*O*-phenyldeoxyinosine (6ϕI) is less reactive with amines than TMPdU, even in aqueous solution.[34] With the additional knowledge that the reactivity of TMPU with amines was itself diminished in going from

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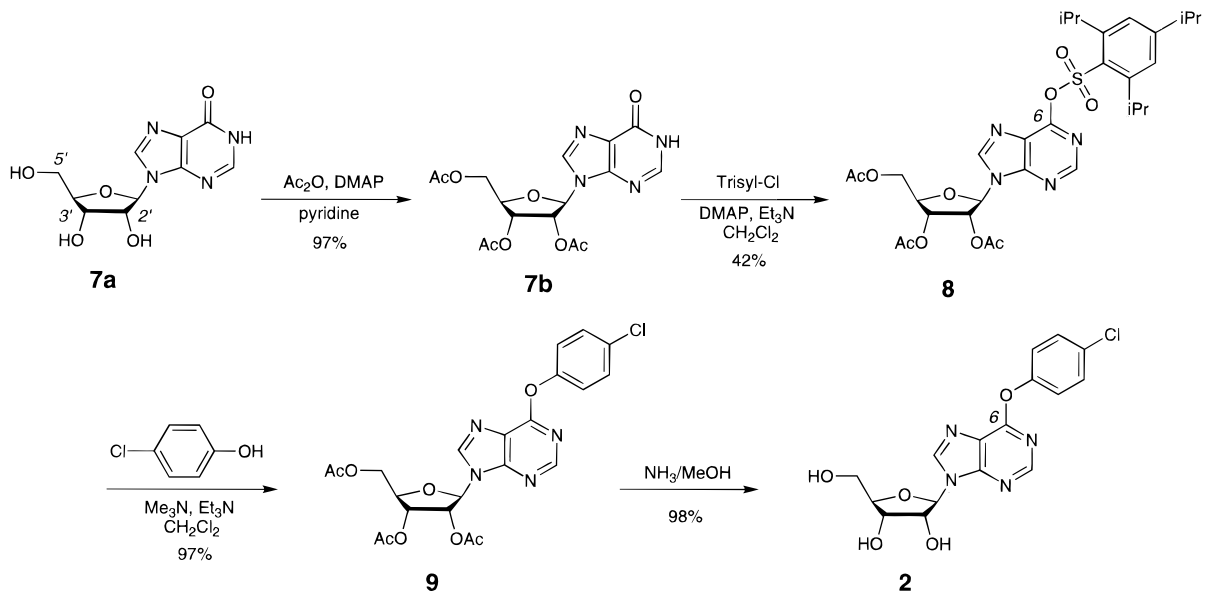
Scheme 2



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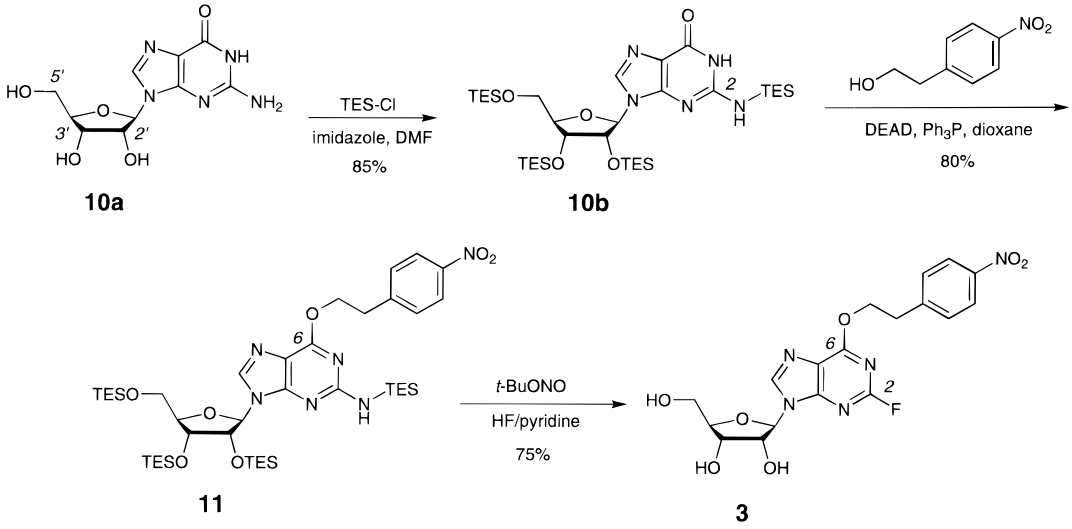

aqueous to methanolic solutions, we expected that a more reactive leaving group on our inosine derivative would be required as well. After screening several O^6-arylinosine derivatives,[49] we settled again on the use of the 4-chlorophenyl derivative 6-*O*-(4-chlorophenyl)inosine (**2**). The synthesis of **2** proceeds in four steps from inosine (**7a**; Scheme 2), closely following the reported synthesis of 6ϕI,[32] starting with tri-*O*-acetylation of the ribose hydroxyl groups (**7b**). Activation of the O^6 position for nucleophilic substitution was achieved through formation of the O^6-[(2,4,6-triisopropylphenyl)sulfonyl] ester **8**. A major side product of this reaction, the N-sulfonated inosine, was easily separated from the desired product by flash chromatography. The sulfonate ester was again displaced in a trimethylamine-mediated reaction with 4-chlorophenol to produce **9**. Aminolysis of the acetate esters in methanol yielded the free nucleoside ClϕI (**2**).

The conversion of 2-fluoro-substituted inosine nucleosides to N^2-substituted guanine derivatives through reactions with amines is known to occur rapidly in aqueous solution[33] when the O^6 position is protected as the 4-nitrophenethyl (NPE) ether.[50] Therefore, we prepared 2-fluoro-6-*O*-(4-nitrophenethyl)inosine (NPE-FI, **3**) without further investigation of alternative leaving groups at the 2-position. We improved on the reported synthesis of NPE-Fdl[51] by reducing it to an efficient three-step process (Scheme 3). Starting with guanosine (**10a**), the tetrakis(triethylsilyl)-protected guanosine **10b** was generated by treatment with chorotriethylsilane/imidazole in *N*,*N*-dimethylformamide (DMF). The O^6 position was protected with the 4-nitrophenethyl moiety through a Mitsunobu process[50,51] to yield **11**. Subsequent diazotization with *tert*-butyl nitrite in the presence of 60% HF/pyridine resulted in fluorination at the 2-position and concomitant removal of the triethylsilyl ethers, thus affording the nucleoside NPE-FI (**3**).

Model Studies of Free Nucleosides. Before incorporating the convertible nucleosides into an oligoribonucleotide, each of the convertible nucleosides was reacted with a number of amines, namely, ammonia, cystamine, 1,4-diaminobutane, ethylenediamine, and ethanolamine, and the progress of conversion was followed by HPLC. From these data, we determined pseudo-first-order rate constants (k_{obs}) and calculated the corresponding reaction half-lives ($t_{1/2}$) for the reaction of each convertible nucleoside with each amine (Table 1). We observed

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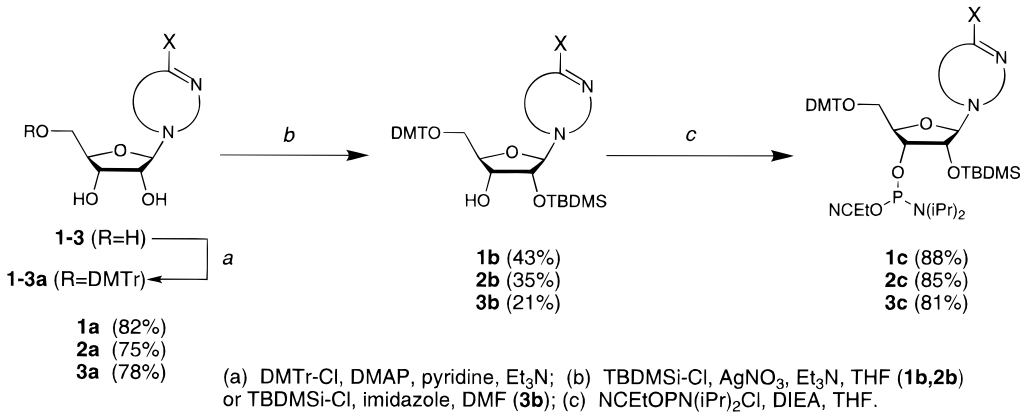
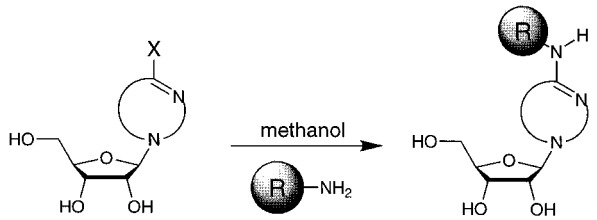
Scheme 4



(a) DMTr-Cl, DMAP, pyridine, Et_3N; (b) TBDMSi-Cl, $AgNO_3$, Et_3N, THF (**1b**,**2b**) or TBDMSi-Cl, imidazole, DMF (**3b**); (c) $NCEtOPN(iPr)_2Cl$, DIEA, THF.

Table 1. Model Studies: Amine Substitution Characteristics of Free Nucleosides[a]



R	concn (M)	$t_{1/2}$ (h) 1	2	3
H	7.0	0.55	31.0	2.00
$H_2NCH_2CH_2SSCH_2CH_2$–	2.0	0.70	1.8	0.35
$H_2NCH_2CH_2CH_2CH_2$–	4.0	0.30	n/d	n/d
$H_2NCH_2CH_2CH_2CH_2$–	2.0	0.56	0.65	0.12
$H_2NCH_2CH_2$–	2.0	0.70	0.70	0.25
$HOCH_2CH_2$–	2.0	0.66	1.57	0.55

[a] n/d = not determined.

that, in each case, with the one exception of the reaction of **2** with ammonia, that an amine concentration of 2 M (or 7 M for ammonia) led to efficient conversion at 42 °C. These conditions are milder than those used for amine displacement on the corresponding DNA reagents.

Preparation of the Nucleoside Phosphoramidites. Having demonstrated that the convertible nucleosides themselves posses sufficient reactivity, we prepared the corresponding phosphoramidites (Scheme 4) for use in RNA synthesis by solid-phase methods. The standard protecting groups were used: 4,4′-dimethoxytrityl (DMT) ethers to block the 5′-OH and *tert*-butyldimethylsilyl (TBDMS) ethers to protect the 2′-hydroxyls. Preparation of the 5′-*O*-DMT nucleosides **1a**, **2a**, and **3a** proceeded cleanly and in high yield using standard chemistry.[52] Formation of the 2′-*O*-TBDMS-protected compounds proved to be somewhat more difficult. In addition to the lack of discrimination between the 2′- and 3′-hydroxyls during silylation, the DMT-protected convertible nucleosides **1a** and **2a** appeared to undergo some degradation under standard TBDMS-Cl/imidazole/DMF conditions. We were able to improve significantly upon these low-yielding reactions by using alternative reaction conditions in the preparation of **1b** and **2b**, which utilized $AgNO_3$/triethylamine (TEA)/TBDMS-Cl in methylene chloride.[53] Under these conditions, little or no degradation of the convertible nucleoside appeared to occur. Phosphitylation of the 3′-position using standard conditions (2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite and *N*,*N*-diisopropylethylamine in THF) resulted in clean, high-yield formation of the corresponding nucleoside phosphoramidites **1**−**3c**.

Phosphoramidites in Solid-Phase Synthesis. For use in automated RNA synthesis, the convertible nucleoside phosphoramidites were prepared in the usual fashion as 0.1 M solutions in dry acetonitrile. A slightly modified synthesis cycle was used in which the coupling time was extended to 12 min. The convertible nucleoside phosphoramidites were used in the synthesis of three 11-mer oligonucleotides, each containing one of the modified nucleosides. The sequences synthesized, 5′-GAC UU**1** GUA CC-3′ (**I**), 5′-AGU CC**2** GCU AG-3′ (**II**), and 5′-GCU AA**3** CCU AU-3′ (**III**), were selected simply to be non-self-complementary. The resin-bound oligonucleotides **I**−**III** were treated in parallel with each of eight amines. As described above, the displacement reactions were carried out using 2 M solutions of amine in methanol at 42 °C with the exception of ammonia, which employed a concentration of 7 M, and methylamine, which was used as an 8 M solution in ethanol. For the reactions carried out on the oligonucleotides containing ClϕU and ClϕI, the reactions were maintained at 42 °C for 18 h. In the case of the oligonucleotide containing NPE-FI, the reactions were run at 42 °C for 12 h. Without neutralizing the amine, the displacement reaction solutions were separated from the resin and immediately run through a cation-exchange column (NH_4^+ form) to exchange the alkylamine with ammonia. Upon elution from the column, collected fractions that contained RNA (as determined by UV absorbance) were combined and dried down to a white residue, which was then treated with 1 M tetrabutylammonium fluoride (TBAF) in THF for 20 h to effect removal of the 2′-*O*-silyl ethers. Alternatively, in the case of oligonucleotides containing ClϕU and ClϕI, now converted to their respective *N*-alkyl forms, desilylation could be carried out using $Et_3N{\cdot}3HF$,[54] which had the advantage of decreasing the reaction time required for deprotection (1 h). In the case of NPE-protected N^2-alkylguanosines, treatment with TBAF was used exclusively, because it removes both the NPE protecting group from the O^6 position and the TBDMS from the 2′-position. Regardless of the method of desilylation, the fully deprotected crude RNA is then desalted on a C18 SepPak cartridge, purified by denaturing PAGE, and quantified by UV absorbance. The yields for the conversions of **I** and **III** were calculated relative to the ammonia reaction. On the basis of subsequent nucleotide composition analysis, however, the conversion yields of **II** were

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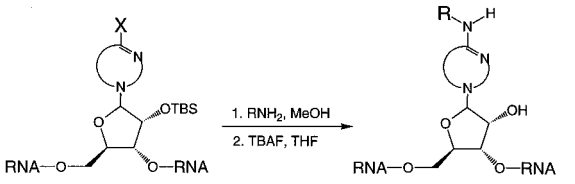
(a)



(b)

Reaction	Amine	Relative Yield I	II	III
1	NH_3	1.0	0.24	1.0
2	CH_3NH_2	1.0	1.0	0.85
3	$H_2NCH_2CH_2NH_2$	0.96	1.0	0.90
4	$H_2NCH_2CH_2CH_2CH_2NH_2$	1.0	1.04	0.88
5	$H_2NCH_2CH_2SSCH_2CH_2NH_2$	0.96	0.96	0.65
6	$HOCH_2CH_2NH_2$	1.04	1.10	1.0
7	$C_6H_5CH_2NH_2$	0.79	0.67	0.65
8	$H_3CSCH_2CH_2NH_2$	0.67	0.38	0.35

(c)

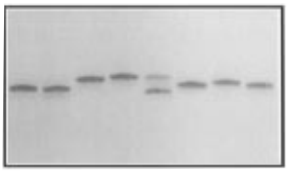


Figure 3. RNA studies of amine substitution reactions. (a) A generalized depiction of the RNA displacement studies. Oligonucleotides **I**, **II**, and **III**, which each contain one of the three convertible nucleosides with a leaving group X (corresponds to bold group in Figure 2), were treated with a methanol solution of amine (RNH_2). This resulted in displacement of the leaving group and substitution with the amine, with concomitant removal of amine and phosphate protecting groups. Subsequent treatment with tetrabutylammonium fluoride (TBAF) results in complete deprotection of the tethered RNA. (b) The results of the amine displacement reactions are summarized for each oligonucleotide. Note that, in the cases of **I** and **III**, the yield is calculated relative to the reaction with ammonia (reaction 1), for which the yield was assigned as 1.0. In the case of **II**, the yield is relative to the methylamine reaction (reaction 2). (c) A representative denaturing PAGE of the unpurified functionally tethered RNAs resulting from the treatment of **III** with the amines listed above. The lane numbers correspond to the reaction numbers in (b), and the products in the column labeled **III**. (RNA was visualized by staining with methylene blue.)

calculated relative to the methylamine reaction. The purity and completeness of conversion was confirmed by complete enzymatic digestion followed by HPLC nucleoside composition analysis. The reactions performed, relative yields, and a representative denaturing PAGE of unpurified tethered RNAs are shown in Figure 3. Most of the conversion reactions proceeded to completion and yielded a single major product (Figure 3c). The only exceptions were the displacement/deprotection reactions employing cystamine (see Figure 3c, lane 5), and the reaction of oligonucleotide **II** with ammonia. The impure products of cystamine displacement appear to result from disulfide disproportionation during workup, since treatment with tris(2-carboxyethyl)phosphine reduced the mixture to a single band on denaturing PAGE (data not shown). Nucleotide composition analysis confirmed the presence of a single nonnatural nucleoside in each RNA; in several cases the identity of the nonnatural nucleoside was directly confirmed by comparison with authentic standards of tethered nucleosides.[49] Nucleotide composition analysis of the reaction product of **II** with ammonia, however, showed the presence of ~75% unreacted ClϕI (data not shown).

Duplex Stability of Tethered Oligonucleotides. To investigate the effect of these tethers on duplex RNA, we synthesized the complementary RNA strands of each modified oligonucleotide. These oligonucleotides, 5′-GGU ACG AAG UC-3′, 5′-CUA GCU GGA CU-3′, and 5′-AUA GGC UUA GC-3′, were deprotected and synthesized using standard procedures. The duplexes were formed by combining equimolar amounts (typically 5 nmol) of each strand and annealing them together by heating to 90 °C followed by slow cooling to room temperature. Melting temperatures were observed by following the change in UV absorbance as the temperature was increased and determination of the first derivative of the UV versus temperature plot. The results are listed in Table 2. In the case of both N^4-alkylcytidines and N^6-alkyladenosines, oligonucleotide tethers that bear a positive charge under the conditions of these studies did not destabilize the duplex, and in some cases even seemed to confer some additional stability (Table 2, entries c and d). The tethers on A and C destabilized the duplex by a modest amount, although the relatively nonpolar tethers of methylamine, benzylamine, and 2-(methylthio)ethylamine perturbed stability more than the polar tether of ethanolamine. In the case of N^2-guanosine tethers, none of the tethers exerted a strong effect on duplex stability.

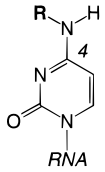
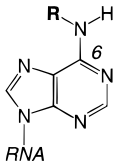
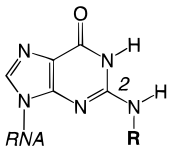
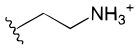
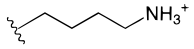
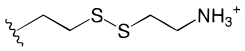
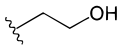
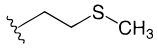
Discussion

Here we have reported the synthesis of convertible nucleosides suitable for functionalizing the exocyclic amines of A, C, and G residues in RNA. We have also demonstrated that these convertible nucleosides can be readily incorporated into RNA and are amenable to amine substitution that results in functionally tethered oligoribonucleotides in an operationally simple process. Futhermore, we have observed that, in most cases, the attachment of the functional tether at the exocyclic amine of the nucleobase only minimally affects duplex stability, and in several cases appears to confer extra stability to the duplex. With the set of convertible nucleosides that we have designed, it is now possible to target modifications to both the major and minor grooves of A-form duplex RNA, as well as single-stranded regions. In cases where both hydrogens on the exocyclic amine participate in hydrogen-bonding, the functional tether can be targeted to the opposite groove. Thus, the present method complements alternative approaches that utilize the 2′-position of the sugar moiety as a site for attachment of functional tethers.[21,23]

We anticipate that the convertible nucleoside methodology demonstrated here should be of utility in the study of RNA structure and function. We have already reported the use of ClϕU (**1**) in the preparation of a conformationally constrained RNA.[22] Though the tethers reported in this work are functionally simple, more complex amines, such as amino acids, may also prove to be useful as conversion reagents.[34,55] Alternatively, simple functionalized tethers can be further modified by group-specific reagents following RNA synthesis. For instance, we have reported the use of thiol-specific reagents to further modify thiol-tethered adenosines in RNA.[28] Although any modification strategy utilizing solid-phase RNA synthesis is limited to the preparation of short oligonucleotides, splinted-

(55) Ferentz, A. E.; Verdine, G. L. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: New York, 1994; pp 14−40.

Table 2. Stabilities of Tethered Duplex RNA Oligomers[a]

Entry	R	T_m (°C)		
				
a		50.5	56.5	50.4
b		48.0	53.2	49.6
c		50.5	57.9	48.2
d		54.3	57.6	46.6
e		n/d	n/d	n/d
f		48.7	52.6	47.5
g		42.3	51.4	46.5
h		41.3	45.6	49.2

[a] The sequences of oligonucleotides are as follows: 5′-GAC UU-N[4]-substituted-C-GUA CC-3′•5′-GGU ACG AAG UC-3′ (derivatives of oligonucleotide **I**, left-hand row); 5′-AGU CC-N[6]-substituted-A-GCU AG-3′•5′-CUA GCU GGA CU-3′ (derivatives of oligonucleotide **II**, middle row); and 5′-GCU AA-N[2]-substituted-G-CCU AU-3′•5′-AUA GGC UUA GC-3′ (derivatives of oligonucleotide **III**, right-hand row). T_m refers to the temperature (°C) at which 50% of the duplex has been denatured.

ligation methods[56] can be used in combination with the convertible nucleoside approach to circumvent these limitations, permitting the preparation of tethered RNA molecules of great size and complexity.

Experimental Section

General Methods. All chemical reagents were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI), with the exception of 2-cyanoethyl(*N*,*N*-diisopropylamino)chlorophosphite, which was purchased from Peninsula Laboratories (Belmont, CA), and 2-(methylthio)-ethylamine, which was purchased from Fluka. Solvents were freshly dried as follows: CH_2Cl_2, pyridine, triethylamine, and *N*,*N*-diisopropylethylamine were distilled from CaH_2; tetrahydrofuran was distilled from Na/benzophenone; methanol was distilled over $Mg/Mg(OCH_3)_2$; and *N*,*N*-dimethylformamide (DMF) was dried over 3 Å activated molecular sieves. All other reagents were used without further purification. Unless otherwise noted, all reactions were run under an inert atmosphere of nitrogen or argon. TLC analyses were carried out using Merck silica gel 60 F_{254} TLC plates, 0.25 mm thickness. Flash column chromatography was performed using Merck silica gel 60 (230−40 mesh). ^{1}H NMR spectra were obtained on one of three Bruker instruments: AM300, AM400, or AM500. ^{13}C NMR spectra were obtained on either a Bruker AM400 or a Bruker AM500. ^{1}H and ^{13}C chemical shifts are reported as δ values (ppm) relative to TMS (δ 0.0) as an external standard. ^{31}P NMR spectra were collected on a Bruker AM500, with 85% H_3PO_4 (δ 0.0) as an external reference. UV spectra and absorbances were measured with an HP 8452A UV−vis spectrophotometer equipped with a photodiode array detector. All HPLC analyses were performed using an HP1090 liquid chromatograph equipped with a photodiode array detector and a C18 reversed-phase column. All FAB-HRMS analyses were performed by the Harvard University Mass Spectrometry Facility on either a JEOL AX-505 or a JEOL SX-102.

2′, 3′, 5′-Tris(*O*-triethylsilyl)uridine (4b). Uridine (1.0 g, 4.095 mmol) and imidazole (1.39 g, 20.48 mmol, 5 equiv) were dissolved in 10 mL of dry DMF. The resulting solution was cooled to 0 °C, and chlorotriethylsilane (2.75 mL, 16.38 mmol, 4.0 equiv) was added dropwise over 10 min. The solution was then allowed to warm to room temperature for 6 h. The reaction solution was poured into 200 mL of Et_2O and was washed with 10% (w/v) aqueous LiBr (4 × 75 mL) to remove DMF. The ethereal solution was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to a clear, colorless oil. Silica gel flash chromatography in 8:2 hexanes/EtOAc (R_f(**4b**) = 0.30, 75:25 hexanes/EtOAc) yielded 2.39 g (4.08 mmol, 99.6%) of a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.8 (s, 1H, H3-imino), 8.09 (d, 1H, H6), 5.85 (d, 1H, H1′), 5.67 (dd, 1H, H5), 4.10 (m, 2H, H2′/H3′), 4.05 (m, 1H, H4′), 3.95 (m, 1H, H5′/H5″), 3.74 (m, 1H, H5′/H5″), 0.93−0.99 (m, 27H, TES methyls), 0.58−0.68 (m, 18H, TES methylene). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 163.3 (C4), 150.2 (C2), 140.4 (C6), 101.5 (C5), 89.0 (C1′), 84.2 (C4′), 76.3 (C2′), 70.7 (C3′), 61.1 (C5′), 6.78, 6.66, 6.55, 4.84, 4.75, 4.12. FAB-HRMS: calcd for $C_{27}H_{54}N_2O_6Si_3$ (M + Na^+) 609.3184, obsd 609.3187.

2′, 3′, 5′-Tris(*O*-triethylsilyl)-4-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]uridine (5). 2′, 3′, 5′-Tris(*O*-triethylsilyl)uridine (**4b**) (2.35 g, 4.01 mmol) and 4-(dimethylamino)pyridine (50 mg, 0.4 mmol, 0.10 equiv) were dissolved in 20 mL of dry, distilled CH_2Cl_2. The solution was chilled to 0 °C, followed by the addition of triethylamine (3.4 mL, 24.1 mmol, 6 equiv). After 30 min, 2,4,6-triisopropylbenzenesulfonyl chloride (2.07 g, 6.82 mmol, 1.7 equiv) was added. The reaction was stirred at 0 °C for 1 h and then at room temperature for an additional 8 h. Over this time the reaction turned a deep crimson color, and a slushy precipitate formed. The crude mixture was poured into 1:1 hexanes/EtOAc and filtered through a glass frit. The filtrate was concentrated in vacuo to yield a brown-yellow oil. Silica gel chromatography in 95:5 hexanes/Et_2O (R_f(**5**) = 0.45, 9:1 hexanes/Et_2O) yielded 2.942 g (3.453 mmol, 86.3%) of a white flaky solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.67 (d, 1H, H6), 7.20 (s, 2H, (2,4,6-triisopropylphenyl)sulfonyl trisyl) aromatic), 6.00 (d, 1H, H5), 5.67 (s, 1H, H1′), 4.22 (septet, 2H, trisyl 2,6-methines), 4.12−4.03 (m, 3H, H2′/H3′/H4′), 4.05 (m, 1H, H5′/H5″), 3.77 (m, 1H, H5′/H5″), 2.90 (heptet, 1H, trisyl 4-methine), 1.30 (d, 6H, trisyl 4-methyls), 1.26 (d, 12H, trisyl 2,6-methyls), 0.99−0.91 (m, 27H, TES methyls), 0.70−0.58 (m, 18H, TES methylenes). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 166.9 (C4), 154.4 (C2), 153.8 (trisyl C2), 151.2 (trisyl C4), 146.4 (C6), 130.8 (trisyl C1), 124.0 (trisyl C3), 94.3 (C5), 91.4 (C1′), 82.9 (C4′), 76.5 (C2′), 68.6 (C3′), 59.9 (C5′), 34.3, 29.7, 24.7, 24.3, 23.5,

(56) Moore, M. J.; Sharp, P. A. *Science* **1992**, *256*, 992−997.

23.4, 6.78, 6.73, 6.70 (TES methyls), 4.83, 4.79, 4.17 (TES methylenes). FAB-HRMS: calcd for $C_{42}H_{76}N_2O_8SSi_3$ (M + Na^+) 875.4524, obsd 875.4528.

2′, 3′, 5′-Tris(*O*-triethylsilyl)-4-*O*-(4-chlorophenyl)uridine (6). 2′, 3′, 5′-Tris-*(O*-triethylsilyl)-4-*O*-[(2,4,6-triisopropylphenylsulfonyl]-uridine (**5**) (2.916 g, 3.423 mmol) and 4-chlorophenol (2.20 g, 17.1 mmol, 5.0 equiv) were dissolved in 20 mL of dry, distilled CH_2Cl_2 at 0 °C. Anhydrous trimethylamine gas was bubbled through the solution at 0 °C for 15 min. While the solution was still cold, freshly distilled triethylamine (1.9 mL, 13.69 mmol, 4 equiv) was added. After 1.5 h the solution was allowed to warm to room temperature. The flask was kept sealed to ensure retention of the trimethylamine. Within 3 h a white slushy precipitate had formed. After a total of 6 h, the excess trimethylamine was allowed to boil off and the remaining solvent was removed in vacuo. Silica gel chromatography in 9:1 hexanes/Et_2O (R_f(**6**) = 0.15, 9:1 hexanes/Et_2O) yielded 2.277 g (3.270 mmol, 95.5%) as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.66 (d, 1H, H6), 7.29 (d, 2H, phenyl H3), 7.11 (d, 2H, phenyl H2), 6.05 (d, 1H, H5), 5.72 (s, 1H, H1′), 4.11 (m, 4H, H2′/H3′/H4′/H5′), 3.82 (m, 1H, H5″), 1.04−0.92 (m, 27H, TES methyls), 0.75−0.59 (m, 18H, TES methylenes). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 171.1 (C4), 155.4 (phenyl C1), 150.1 (C2), 144.9 (C6), 131.1 (phenyl C4), 129.3 (phenyl C3), 123.1 (phenyl C2), 94.2 (C5), 91.5 (C1′), 82.6 (C4′), 76.6 (C2′), 68.4 (C3′), 59.8 (C5′), 6.77, 6.73, 6.40 (TES methyls), 4.81, 4.76, 4.43 (TES methylenes). FAB-HRMS: calcd for $C_{33}H_{57}ClN_2O_6Si_3$ (M + Na^+) 719.3107, obsd 719.3111.

4-*O*-(4-Chlorophenyl)uridine (1). 2′, 3′, 5′-Tris-*(O*-triethylsilyl)-4-*O*-(4-chlorophenyl)uridine (**6**) (8.35 g, 12.0 mmol) was dissolved in 90 mL of THF. Both distilled−deionized water (30 mL) and glacial acetic acid (30 mL) were added. This solution was allowed to stir at room temperature for 13 h until reaction appeared complete by TLC. The crude reaction solution was concentrated in vacuo to yield a clear, colorless oil. Silica gel chromatography with 11:1 CH_2Cl_2/methanol (R_f(**1**) = 0.19, 9:1 CH_2Cl_2/methanol) yielded 3.311 g (9.335 mmol, 77.8%) of **1** as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.51 (d, 1H, H6), 7.49 (d, 2H, phenyl H3), 7.22 (d, 2H, phenyl H2), 6.32 (d, 1H, H5), 5.72 (d, 1H, H1′), 3.98 (m, 2H, H2′/H3′), 3.91 (m, 1H, H4′), 3.75 (m, 1H, H5′/H5″), 3.61 (m, 1H, H5′/H5″). ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 173.2 (C4), 157.8 (phenyl C1), 151.9 (C2), 147.1 (C6), 132.4 (phenyl C4), 130.7 (phenyl C3), 124.6 (phenyl C2), 96.4 (C5), 93.1 (C1′), 85.9 (C4′), 76.5 (C2′), 70.1 (C3′), 61.3 (C5′). FAB-HRMS: calcd for $C_{15}H_{15}ClN_2O_6$ (M + H^+) 355.0696, obsd 355.0697.

5′-*O*-(4,4′-Dimethoxytrityl)-4-*O*-(4-chlorophenyl)uridine (1a). 4-*O*-(4-Chlorophenyl)uridine (**1**) (1.04 g, 2.93 mmol) and 4-(dimethylamino)pyridine (40 mg, 0.29 mmol, 0.10 equiv) were dissolved in 18 mL of dry pyridine. The solution was cooled to 0 °C, followed by the addition of 4,4′-dimethoxytrityl chloride (1.193 g, 3.52 mmol, 1.2 equiv) and triethylamine (1.63 mL, 11.72 mmol, 4 equiv). The solution was stirred at room temperature for 8 h, after which time it was cooled back to 0 °C and quenched with the addition of 10 mL of methanol. The solution was concentrated in vacuo and coevaporated with toluene (2 × 150 mL) to a dark yellow oil. The oil was redissolved in 250 mL of CH_2Cl_2 and was washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 150 mL). The organic portion was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to yield a golden oil. Silica gel chromatography (silica was pretreated with 3% triethylamine in CH_2Cl_2) first with neat CH_2Cl_2 to remove bright yellow impurities and then with 98:2 CH_2Cl_2/methanol (R_f(**1a**) = 0.49, 9:1 CH_2Cl_2/methanol) yielded 1.420 g (2.163 mmol, 73.8%) as a pale foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.34 (d, 1H, H6), 7.33 (d, 2H, phenyl H3), 7.38−7.23 (m, 5H, DMT H2″/H3″/H4″), 7.26 (d, 4H, DMT H2/H2′), 7.07 (d, 2H, phenyl H2), 6.83 (d, 4H, DMT H3/H3′), 5.82 (d, 1H, H1′), 5.76 (d, 1H, H5), 4.38 (m, 1H, H2′), 4.28−4.25 (m, 2H, H3′/H4′), 3.79 (s, 6H, DMT methoxy), 3.52−3.43 (m, 2H, H5′/H5″). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 171.3 (C4), 158.6, 156.1 (phenyl C1), 150.0 (C2), 144.7 (C6), 144.3, 135.3, 135.1, 131.2 (phenyl C4), 130.0 (phenyl C3), 129.6, 128.1, 127.9, 127.0, 123.1 (phenyl C2), 113.2, 94.6 (C5), 92.7 (C1′), 86.9, 84.6 (C4′), 76.0 (C2′), 70.2 (C3′), 62.1 (C5′), 55.2 (DMT methoxy). FAB-HRMS: calcd for $C_{36}H_{33}ClN_2O_8$ (M + Na^+) 679.1821, obsd 679.1823.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-4-*O*-(4-chlorophenyl)uridine (1b). 5′-*O*-(4,4′-Dimethoxytrityl)-4-*O*-(4-chlorophenyl)uridine (**1a**) (3.433 g, 5.23 mmol) was dissolved in 20 mL of dry THF, to which had been added triethylamine (1.0 mL, 7.17 mmol, 2.0 equiv). After 20 min of stirring, *tert*-butyldimethylsilyl chloride (946 mg, 6.28 mmol, 1.2 equiv) and $AgNO_3$ (978 mg, 5.75 mmol, 1.1 equiv) were added to the solution. After stirring for 4 h, the reaction mixture was filtered through a glass frit to remove Ag salts. Then, the filtrate was diluted to 250 mL with ethyl acetate and was washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to yield a pale foam. Silica gel chromatography (silica was pretreated with 3% triethylamine in hexanes) with 75:25 hexanes/EtOAc (R_f(**1b**) = 0.28, 75:25 hexanes/EtOAc) yielded 1.0591 g (1.375 mmol, 26.3%) of 1b as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.51 (d, 1H, H6), 7.33 (d, 2H, phenyl H3), 7.31 (d, 4H, DMT H2/H2′), 7.43−7.26 (m, 5H, DMT H2″/H3″/H4″), 7.07 (d, 2H, phenyl H2), 6.85 (d, 4H, DMT H3/H3″), 5.81 (s, 1H, H1′), 5.64 (d, 1H, H5), 4.39 (m, 1H, H2′), 4.26 (m, 1H, H3′), 4.07 (m, 1H, H4′), 3.80 (s, 6H, DMT methoxy), 3.62−3.53 (m, 2H, H5′/H5″), 0.90 (TBDMS *t*-Bu methyls), 0.30 (s, 3H, TBDMS methyl), 0.18 (s, 3H, TBDMS methyl). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 171.1 (C4), 158.7, 158.6, 155.1 (phenyl C1), 150.1 (C2), 144.7 (C6), 144.4, 135.4, 135.2, 131.2 (phenyl C4), 130.2 (phenyl C3), 129.5, 128.2, 128.0, 127.1, 123.1 (phenyl C2), 113.3, 94.7 (C5), 91.0 (C1′), 87.0, 83.0 (C4′), 76.5 (C2′), 68.8 (C3′), 61.1 (C5′), 55.2, 25.8 (TBDMS *t*-Bu methyls), −4.4, −5.5 (TBDMS methyls). FAB-HRMS: calcd for $C_{42}H_{47}ClN_2O_8Si$ (M + Na^+) 793.2685, obsd 793.2688.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(2-cyanoethoxy)(*N*,*N*-diisopropylamino) phosphino]-2′-*O*-(*tert*-butyldimethylsilyl)-4-*O*-(4-chlorophenyl)uridine (1c). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-4-*O*-(4-chlorophenyl)uridine (**1b**) (1.0748 g, 1.394 mmol) was dissolved in 10 mL of dry THF. Freshly distilled *N*,*N*-diisopropylethylamine (1.46 mL, 8.364 mmol, 6 equiv) was added to the solution at room temperature. 2-Cyanoethyl (*N*,*N*-diisopropylamino)chlorophosphite (0.62 mL, 2.79 mmol, 2 equiv) was added to the solution dropwise over 10 min. The solution was allowed to stir at room temperature. Within 1 h, a white precipitate had formed. After 6 h, the reaction mixture was poured into 200 mL of EtOAc and subsequently washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to a pale oil. Silica gel chromatography (silica was pretreated with 3% triethylamine in hexanes) in 6:4 hexanes/EtOAc (R_f(diastereomers of **1c**) = 0.70, 0.68, 1:1 hexanes/EtOAc). Concentration in vacuo yielded 1.1063 g (1.14 mmol, 81.8%) of **1c** as an off-white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.57 (d, H6, diastereomer 1), 8.49 (d, H6, diastereomer 2), 7.33 (d, 2H, phenyl H3), 7.29 (d, 4H, DMT H2/H2′), 7.46−7.24 (m, 5H, DMT H2″/H3″/H4″), 7.07 (d, 2H, phenyl H2), 6.84 (d, 4H, DMT H3/H3′), 5.82 (s, H1′, diastereomer 2), 5.73 (s, H1′, diastereomer 1), 5.61 (d, H5, diastereomer 1), 5.50 (d, H5, diastereomer 2), 4.36−4.31 (m, 3H, H2′/H3′/H4′), 3.80 (s, 6H, DMT methoxy), 3.72 (m, 2H, cyanoethyl H1/H1′), 3.53 (m, 2H, H5′/H5″), 3.72−3.50 (m, 2H, diisopropylamino methines), 2.40 (t, 2H, cyanoethyl H2/H2′), 1.24−1.00 (m, 12H, diisopropylamino methyls), 0.89, 0.84 (s, 9H, TBDMS *t*-Bu methyl), 0.25, 0.24, 0.13, 0.12 (TBDMS methyls). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 171.1, 171.0 (C4), 158.7, 158.6, 155.2, 155.1 (phenyl C1), 150.2, 150.1 (C2), 144.7, 144.6 (C6), 144.4, 144.2, 135.3, 135.0, 131.0, 130.9 (phenyl C4), 130.4, 130.3, 130.2, 130.1 (phenyl C3), 129.5, 129.4, 128.4, 127.9, 123.1, 123.2, 123.1 (phenyl C2), 117.3 (CN), 113.3, 113.2, 113.1, 94.7, 94.6 (C5), 91.9, 91.5 (C1′), 87.1, 87.0, 81.1 (C4′), 75.6, 75.0 (C2′), 71.3, 71.1, 69.5, 69.4 (C3′), 61.2, 60.8 (C5′), 58.1, 57.9, 55.2, 43.2, 43.1, 43.0 (diisopropylamino methines), 25.9, 25.8 (TBDMS *t*-Bu methyls), 24.8, 24.7, 24.5 (diisopropylamino methyls), 20.4, 20.1, 18.0, −4.0, −4.4, −5.2, −5.3. FAB-HRMS: calcd for $C_{51}H_{64}ClN_4O_9PSi$ (M + Na^+) 993.3763, obsd 993.3766.

2′, 3′, 5′-Tris-*O*-acetylinosine (7b). Inosine (7a) (3.0 g, 11.2 mmol) and 4-(dimethylamino)pyridine (135 mg, 1.12 mmol, 0.10 equiv) were suspended in 20 mL of dry pyridine. The suspension was cooled to 0 °C, followed by the addition of acetic anhydride (10.6 mL, 112 mmol, 10 equiv). The solution was allowed to stir at 0 °C for 1 h and then at room temperature for 6 h, during which time all solids dissolved.

The solution was quenched with 5 mL of cold methanol and then concentrated in vacuo to a pale yellow oil. Silica gel chromatography in 95:5 CH_2Cl_2/methanol (R_f(**7b**) = 0.42, 9:1 CH_2Cl_2/methanol) yielded 4.2976 g (10.9 mmol, 97.0%) of a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.30 (s, 1H, H8), 8.08 (s, 1H, H2), 6.17 (d, 1H, H1′), 5.86 (t, 1H, H2′), 5.59 (m, 1H, H3′), 4.37−4.45 (m, 3H, H4′/H5′/H5″), 2.14 (s, 3H, acetate), 2.13 (s, 3H, acetate), 2.08 (s, 3H, acetate). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 169.5, 169.3 (acetate carbonyls), 158.7 (C6), 148.7 (C4), 145.8 (C2), 138.6 (C8), 125.1 (C5), 86.6 (C1′), 80.4 (C4′), 73.3 (C2′), 70.5 (C3′), 63.0 (C5′), 20.7, 20.5, 20.3 (acetate methyls). FAB-HRMS: calcd for $C_{16}H_{18}N_4O_8$ (M + Na^+) 417.1021, obsd 417.1022.

2′, 3′, 5′-Tri-*O*-acetyl-6-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-inosine (8). 2′, 3′, 5′-Tri-*O*-acetylinosine (**7b**) (5.40g, 13.6 mmol) and 4-(dimethylamino)pyridine (170 mg, 1.36 mmol, 0.10 equiv) were dissolved in 150 mL of dry, distilled CH_2Cl_2. The resulting solution was cooled to 0 °C. Freshly distilled triethylamine (11.4 mL, 81.6 mmol, 6 equiv) was added to the solution, followed by 2,4,6-triisopropylbenzenesulfonyl chloride (7.41 g, 24.48 mmol, 1.8 equiv). The solution was stirred at 0 °C for 90 min and then at room temperature for 5 h. The reaction mixture was poured into 200 mL of 1:1 hexanes/EtOAc. This mixture was filtered and then concentrated in vacuo to a crimson oil. Silica gel chromatography in 6:4 hexanes/EtOAc (R_f(**8**) = 0.33, 1:1 hexanes/EtOAc), followed by concentration in vacuo, yielded 3.70 g (5.59 mmol, 41.1%) of **8** as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.56 (s, 1H, H2), 8.20 (s, 1H, H8), 7.21 (s, 2H, trisyl aromatic), 6.22 (d, 1H, H1′), 5.94 (t, 1H, H2′), 5.65 (t, 1H, H3′), 4.47−4.31 (m, 5H, H4′/H5′/H5″/trisyl 2,6-methines), 2.92 (hep, 1H, trisyl 4-methine), 2.15 (s, 3H, acetate), 2.12 (s, 3H, acetate), 2.09 (s, 3H, acetate), 1.27 (t, 18H, trisyl methyls). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 170.2, 169.5, 169.3 (acetate carbonyls), 155.1 (C6), 154.5 (C2), 153.6, 151.8 (C4), 151.1, 143.0 (C8), 131.1, 124.0, 123.0 (C5), 86.7 (C1′), 80.5 (C4′), 73.1 (C2′), 70.5 (C3′), 62.9 (C5′), 34.3, 29.8, 24.5, 24.3 (trisyl 2,6-methyls), 23.5 (trisyl 4-methyl), 20.7, 20.5, 20.3 (acetate methyls). FAB-HRMS: calcd for $C_{31}H_{40}N_4O_{10}S$ (M + Na^+) 683.2360, obsd 683.2363.

2′, 3′, 5′-Tri-*O*-acetyl-6-*O*-(4-chlorophenyl)inosine (9). 2′, 3′, 5′-Tri-*O*-acetyl-6-*O*-[(2,4,6-triisopropylphenylsulfonyl]inosine (**8**) (3.50 g, 5.30 mmol) was dissolved in 80 mL of dry, distilled CH_2Cl_2. To this stirring solution was added 4-chlorophenol (3.40 g, 26.5 mmol, 5.0 equiv). Upon cooling to 0 °C, anhydrous trimethylamine gas was bubbled through the solution for 15 min. Following the addition of trimethylamine, triethylamine (4.43 mL, 31.8 mmol, 6.0 equiv) was added. The solution was allowed to stir at 0 °C for 1 h and then at room temperature for 6 h, during which time a white precipitate formed. Excess trimethylamine was allowed to boil off, followed by filtration. The remaining solution was concentrated in vacuo to a pale oil. Silica gel chromatography in 1:1 hexanes/EtOAc (R_f(**9**) = 0.23, 1:1 hexanes/EtOAc), followed by concentration in vacuo, yielded 2.60 g (5.16 mmol, 97.4%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.52 (s, 1H, H2), 8.18 (s, 1H, H8), 7.41 (d, 2H, phenyl H3), 7.20 (d, 2H, phenyl H2), 6.23 (d, 1H, H1′), 5.97 (t, 1H, H2′), 5.67 (t, 1H, H3′), 4.48 (m, 1H, H4′), 4.47−4.37 (m, 2H, H5′/H5″), 2.15 (s, 3H, acetate), 2.13 (s, 3H, acetate), 2.10 (s, 3H, acetate). ^{13}C ($CDCl_3$, 100 MHz): δ (ppm) 170.3, 169.5, 169.3 (acetate carbonyls), 160.0 (phenyl C1), 152.5 (C2), 152.4 (C4), 150.7 (C6), 141.6 (C8), 131.3 (phenyl C4), 129.7 (phenyl C3), 123.2 (phenyl C2), 121.6 (C5), 86.8 (C1′), 80.4 (C4′), 73.2 (C2′), 70.5 (C3′), 62.9 (C5′), 20.7, 20.5, 20.4 (acetate methyls). FAB-HRMS: calcd for $C_{22}H_{21}ClN_4O_8$ (M + Na^+) 505.1124, obsd 505.1126.

6-*O*-(4-Chlorophenyl)inosine (2). 2′, 3′, 5′-Tri-*O*-acetyl-6-*O*-(4-chlorophenyl)inosine (**9**) (2.07 g, 4.10 mmol) was dissolved in 20 mL of anhydrous methanol. The solution was cooled to 0 °C, followed by the addition of 20 mL of a methanol solution of ammonia, saturated at 0 °C (∼7 M). The solution was allowed to stir at 0 °C for 3 h, at which time only one major band appeared by TLC (R_f(**2**) = 0.22, 9:1 CH_2Cl_2/methanol). The ammonia and methanol were removed in vacuo to yield a pale yellow foam. Silica gel chromatography in 92:8 CH_2Cl_2/methanol yielded 1.518 g (4.01 mmol, 97.8%) of a white foam. ^{1}H NMR (CD_3OD, 300 MHz): δ (ppm) 8.67 (s, 1H, H2), 8.42 (s, 1H, H8), 7.47 (d, 2H, phenyl H3), 7.28 (d, 2H, phenyl H2), 6.11 (d, 1H, H1′), 4.74 (t, 1H, H2′), 4.36 (m, 1H, H3′), 4.18 (m, 1H, H4′), 3.92−3.73 (2dd, 2H, H5′/H5″). ^{13}C NMR(DMSO-d_6, 100 MHz): δ (ppm) 161.1 (phenyl C1), 153.9 (C6), 152.9 (C2), 152.4 (C4), 145.0 (C8), 132.2 (phenyl C4), 130.7 (phenyl C3), 124.5 (phenyl C2), 123.0 (C5), 91.0 (C1′), 87.7 (C4′), 75.9 (C2′), 72.2 (C3′), 63.0 (C5′). FAB-HRMS: calcd for $C_{16}H_{15}ClN_4O_5$ (M + H^+) 379.0808, obsd 379.0809.

5′-*O*-(4,4′-Dimethoxytrityl)-6-*O*-(4-chlorophenyl)inosine (2a). 6-*O*-(4-Chlorophenyl)inosine (**1**) (2.837 g, 7.29 mmol) and 4-(dimethylamino)pyridine (90 mg, 0.73 mmol, 0.10 equiv) were dissolved in 40 mL of dry pyridine. To the solution was added freshly distilled triethylamine (4.1 mL, 29.2 mmol, 1.1 equiv) and 4,4′-dimethoxytrityl chloride (3.68 g, 8.75 mmol, 1.2 equiv). The reaction appeared complete by TLC after 6 h. Following the addition of 10 mL of cold methanol, the solution was concentrated in vacuo and coevaporated with toluene (2 × 100 mL) to yield an amber oil. This oil was redissolved in 300 mL of CH_2Cl_2 and was washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 200 mL). The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to a yellow oil. Silica gel chromatography (silica was pretreated with 3% triethylamine in CH_2Cl_2) first with neat CH_2Cl_2 to remove bright yellow impurities and then with 98:2 CH_2Cl_2/methanol (R_f(**2a**) = 0.14, 9:1 CH_2Cl_2/methanol) yielded 2.9 g (4.26 mmol, 58.5%) as a light-brown foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.48 (s, 1H, H2), 8.34 (s, 1H, H8), 7.46−7.22 (m, 5H, DMT H2″/H3″/H4″), 7.41 (d, 2H, phenyl H3), 7.31 (d, 4H, DMT H2/H2′), 7.23 (d, 2H, phenyl H2), 6.81 (d, 4H, DMT H3/H3′), 6.17 (d, 1H, H1′), 4.89 (t, 1H, H2′), 4.55 (t, 1H, H3′), 4.40 (m, 1H, H4′), 3.81 (s, 6H, DMT methoxy), 3.51 (dd, 1H, H5′/H5″), 3.42 (dd, 1H, H5′/H5″). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 159.8 (phenyl C1), 158.4, 152.5 (C6), 151.7 (C2), 150.7 (C4), 144.4, 142.0 (C8), 135.5, 131.1 (phenyl C4), 130.1, 130.0, 129.6 (phenyl C3), 128.0, 127.9, 126.8, 123.1 (phenyl C2), 122.0 (C5), 113.3, 113.1, 90.0 (C1′), 86.5, 85.2 (C4′), 75.0 (C2′), 71.4 (C3′), 63.4 (C5′), 55.1 (DMT methoxy). FAB-HRMS: calcd for $C_{37}H_{33}ClN_4O_7$ (M + Na^+) 703.1934, obsd 703.1935.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-6-*O*-(4-chlorophenyl)inosine (2b). 5′-*O*-(4,4′-Dimethoxytrityl)-6-*O*-(chlorophenyl)inosine (**2a**) (2.6 g, 3.82 mmol) was dissolved in 18 mL of dry, distilled THF. Freshly distilled triethylamine (1.0 mL, 7.17 mmol, 1.9 equiv) was added to the solution, followed by the addition of *tert*-butyldimethylsilyl chloride (772 mg, 5.12 mmol, 1.2 equiv). Within 5 min, $AgNO_3$ (716 mg, 4.21 mmol, 1.1 equiv) was added to the solution. After 4 h of stirring at room temperature, the reaction mixture was filtered through a glass frit to remove Ag solids. The filtrate was then diluted to 250 mL with ethyl acetate and washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 150 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and then concentrated in vacuo to a light yellow oil. Silica gel chromatography (silica was pretreated with 90:8:2 hexanes/EtOAc/Et_3N) with 75:25 hexanes/EtOAc (R_f(**2b**) = 0.26, 7:3 hexanes/EtOAc) yielded 919 mg (1.16 mmol, 30.3%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.44 (s, 1H, H2), 8.27 (s, 1H, H8), 7.48−7.22 (m, 5H, DMT H2″/H3″/H4″), 7.45 (d, 2H, phenyl H3), 7.36 (d, 4H, DMT H2/H2″), 7.24 (d, 2H, phenyl H2), 6.83 (d, 4H, DMT H3/H3′), 6.14 (d, 1H, H1′), 5.04 (t, 1H, H2′), 4.41 (m, 1H, H3′), 4.31 (m, 1H, H4′), 3.81 (s, 6H, DMT methoxy), 3.55 (dd, 1H, H5′/H5″), 3.45 (dd, 1H, H5′/H5″), 2.76 (br, 1H, 3′-OH), 0.88 (s, 9H, TBDMS *tert*-Bu methyls), 0.04 (s, 3H, TBDMS methyl), −0.11 (s, 3H, TBDMS methyl). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 159.9 (phenyl C1), 158.6, 153.0 (C6), 152.1 (C2), 150.9 (C4), 144.5, 142.1 (C8), 135.6, 131.2 (phenyl C4), 130.1, 130.1, 129.7 (phenyl C3), 128.2, 127.9, 127.0, 123.2 (phenyl C2), 122.1 (C5), 113.2, 88.6 (C1′), 86.8, 84.3 (C4′), 75.9 (C2′), 71.6 (C3′), 63.4 (C5′), 55.2 (DMT methoxy), 25.7 (TBDMS *tert*-Bu methyls), 17.9, −5.0, −5.1 (TBDMS methyls). FAB-HRMS: calcd for $C_{43}H_{47}ClN_4O_7Si$ (M + Na^+) 817.2798, obsd 817.2800.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-[(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphino] 2′-*O*-(*tert*-butyldimethylsilyl)-6-*O*-(4-chlorophenyl)inosine (2c). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-6-*O*-(4-chlorophenyl)inosine (**2b**) (857.7 mg, 1.080 mmol) was dissolved in 8 mL of dry, distilled THF. Freshly distilled *N*,*N*-diisopropylethylamine (1.2 mL, 6.48 mmol, 6 equiv) was added to the stirring solution at room temperature. 2-Cyanoethyl (*N*,*N*-diisopropylamino)chlorophosphite (0.48 mL, 2.16 mmol, 2 equiv) was carefully added to the solution dropwise over 10 min. After approximately 1 h at room temperature, a white precipitate had formed. The reaction was

allowed to stir for an additional 3 h, and was then poured into 250 mL of EtOAc. This solution was washed with 5% (w/v) aqueous $NaHCO_3$ (2 × 150 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to yield a pale yellow oil. Silica gel chromatography (silica was pretreated with 90:8:2 hexanes/EtOAc/Et_3N) with 7:3 hexanes/EtOAc (R_f(**2c**) = 0.15, 0.12 (two diastereomers) in 8:2 hexanes/EtOAc) yielded 894 mg (0.899 mmol, 83.3%) as a white foam. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.40 (s, H2, diastereomer 1), 8.38 (s, H2, diastereomer 2), 8.27 (s, H8, diastereomer 1), 8.24 (s, H8, diastereomer 2), 7.47−7.32 (m, 5H, DMT H2″/H3″/H4″), 7.40 (m, 2H, phenyl H3), 7.33 (m, 4H, DMT H2/H2′), 7.21 (m, 2H, phenyl H2), 6.80 (m, 4H, DMT H3/H3′), 6.11 (d, H1′, diastereomer 2), 6.05 (d, H1′, diastereomer 1), 5.07 (m, 1H, H2′), 4.44−4.36 (m, 2H, H3′/H4′), 3.92−3.58 (m, 2H, diisopropyl methines), 3.77, 3.76 (s, 6H, DMT methoxy), 3.61 (t, 2H, cyanoethyl H1), 3.52, 3.35 (m, 2H, H5′/H5″), 2.30 (t, 2H, cyanoethyl H2), 1.25−1.05 (m, 12H, diisopropyl methyls), 0.76, 0.73 (2s, 9H, TBDMS *t*-Bu methyls), −0.02, −0.03, −0.19, −0.21 (4s, 6H, TBDMS methyls). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 159.8 (phenyl C1), 158.6, 153.1 (C6), 152.0 (C2), 150.8 (C4), 144.5, 144.3, 142.4, 142.3 (C8), 135.7, 135.6, 135.5, 135.4, 131.1 (phenyl C4), 130.1, 130.0, 129.7 (phenyl C3), 128.2, 127.9, 127.0, 123.2 (phenyl C2), 122.1 (C5), 117.5, 117.2 (CN), 113.2, 113.1, 88.5, 88.3 (C1′), 86.9, 86.6, 84.3, 83.9 (C4′),75.8, 74.7 (C2′), 73.5, 73.4, 72.8, 72.7 (C3′), 63.3, 63.2 (C5′), 58.9, 58.7, 57.7, 57.5 (cyanoethyl C1), 55.2 (DMT methoxy), 43.5, 43.4, 43.0, 42.9, 25.6, 25.5 (TBDMS *t*-Bu methyls), 24.7, 24.6, 24.5 (diisopropyl methyls), 20.4, 20.1 (cyanoethyl C2), 17.9, −4.67, −5.16 (TBDMS methyls). ^{31}P NMR ($CDCl_3$, 202 MHz): δ (ppm) 151.9, 149.8. FAB-HRMS: calcd for $C_{52}H_{64}ClN_6O_8PSi$ (M + H^+) 995.4055, obsd 995.4059.

2′,3′,5′-Tris*(O*-triethylsilyl)-*N*^{2}-(triethylsilyl)guanosine (10b). To a suspension of guanosine (**10a**) (2.86g, 10 mmol) and imidazole (8.5 g, 120 mmol, 12 equiv) in 12 mL of dry DMF was added chlorotriethylsilane (10.1 mL, 60 mmol, 6 equiv) in three portions over 2 h at 0 °C. The reaction mixture was allowed to warm to room temperature over 8 h and then was poured into 75 mL of Et_2O and washed with 10% (w/v) aqueous LiBr (3 × 100 mL). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated to a pale oil. Silica gel chromatography of this residue (14:5:1 CH_2Cl_2/EtOAc/MeOH) (R_f(**10b**) = 0.45, 9:1 CH_2Cl_2/MeOH) afforded 4.7 g of **10b** (64%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 7.89 (s, 1H, H8), 5.97 (d, 1H, H1′), 4.78 (brs, 1H, N2-H), 4.43 (m, 1H, H2′), 4.24 (m, 1H, H3′), 4.06 (m, 1H, H4′), 3.82−3.72 (m, 2H, H5′ and H5″), 1.02−0.96 (m, 27H), 0.89−0.86 (m, 6H), 0.81−0.78 (m, 9H), 0.71−0.63 (m, 12H), 0.43−0.35 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 160.0, 154.4, 152.8, 135.4, 117.1, 87.1, 85.4, 77.1, 63.0, 7.0, 6.8, 6.7, 6.5, 5.0, 4.6, 4.2, 4.0. FAB-HRMS: calcd for $C_{34}H_{69}N_5O_5Si_4$ (M + Na^+) 762.4269, obsd 762.4274.

2′,3′,5′-Tris*(O*-triethylsilyl)-*N*^{2}-(triethylsilyl)-6-*O*-(4-nitrophenethyl)guanosine (11). A mixture of 2′,3′,5′-Tris*(O*-triethylsilyl)-*N*^{2}-(triethylsilyl)guanosine (**10b**) (102 mg, 0.16 mmol), 4-nitrophenethyl alcohol (44.7 mg, 0.24 mmol, 1.5 equiv), and triphenylphosphine (88.5 mg, 0.33 mmol, 2 equiv) was suspended in 1.6 mL of dry dioxane in the dark. To this suspension was added diethyl azodicarboxylate (52 mL, 0.32 mmol, 2 equiv). After stirring at room temperature overnight, the mixture was concentrated to a white solid suspended in a pink-orange oil. The residue was dissolved in 20 mL of $CHCl_3$, washed with saturated NaCl (2 × 20 mL) and 5% (w/v) aqueous $NaHCO_3$ (2 × 20 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to a pink-orange oil. Silica gel flash chromatography with 3:2 hexanes/EtOAc (R_f(**11**) = 0.89, 1:1 hexanes/EtOAc) afforded 108.2 mg (87%) of 11 as a yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 8.16 (d, 2H), 7.94 (s, 1H, H8), 7.47 (d, 2H), 5.94 (d, 1H, H1′), 4.72 (t, 2H), 4.60 (t, 1H, H2′), 4.47 (s, 1H, N2-H), 4.29 (m, 1H, H3′), 4.07 (m, 1H, H4′), 3.90−3.73 (m, 2H, H5′ and H5″), 3.28 (t, 2H), 1.00−0.94 (m, 27H), 0.82−0.77 (m, 15H), 0.69−0.62 (m, 12H), 0.48−0.36 (m, 6H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 160.5, 160.3, 154.3, 146.1, 137.9, 129.8, 123.7, 87.1, 85.4, 75.9, 72.6, 65.7, 62.3, 35.2, 7.0, 6.8, 6.7, 6.6, 4.9, 4.6, 4.5, 4.4, 4.3, 4.2. FAB-HRMS: calcd for $C_{42}H_{76}N_6O_7Si_4$ (M + H^+) 889.4927, obsd 889.4931.

2-Fluoro-6-*O*-(4-nitrophenethyl)inosine (3). A solution of 60% HF in pyridine was generated by diluting 19 mL of 70% HF/pyridine with 3.3 mL of distilled pyridine in a 50 mL polypropylene tube. In a separate tube, 2′,3′,5′-tris*(O*-triethylsilyl)-*N*^{2}-(triethylsilyl)-6-*O*-(4-nitrophenethyl)guanosine (**11**) (1.13 g, 1.3 mmol) was dissolved in 5.5 mL of the 60% HF/pyridine solution at −42 °C. To this solution was added *t*-butyl nitrite (0.215 mL, 1.8 mmol, 1.4 equiv) in a dropwise fashion. After stirring for 3 h, the reaction mixture was diluted with 20 mL of $CHCl_3$, poured slowly onto 20 g of K_2CO_3, and diluted with water. The aqueous layer was separated and extracted with $CHCl_3$ (2 × 20 mL). The combined organic layers were washed with $NaHCO_3$ (40 mL) and water (40 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo to an orange oil. Silica gel flash chromatography of this residue with 12:1 CH_2Cl_2/MeOH (R_f(**3**) = 0.45, 9:1 CH_2Cl_2/MeOH) afforded 424 mg (0.97 mmol, 75%) of **3** as a yellow solid. ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 8.53 (s, 1H, H8), 8.15 (d, 2H), 7.59 (d, 2H), 5.98 (d, 1H, H1′), 4.85 (t, 2H), 4.62 (t, 1H, H2′), 4.32 (m, 1H, H3′), 4.12 (m, 1H, H4′), 3.89−3.73 (m, 2H, H5′ and H5″), 3.32 (t, 2H). ^{13}C NMR (CD_3OD, 100 MHz): δ (ppm) 163.5, 163.4, 160.3, 158.2, 154.4, 154.2, 148.3, 147.3, 144.1, 131.3, 124.5, 90.1, 87.4, 75.8, 71.9, 62.8, 35.7. FAB-HRMS: calcd for $C_{18}H_{18}N_5O_7F$ (M + H^+) 436.1268, obsd 436.1269.

5′-*O*-(4,4′-Dimethoxytrityl)-2-fluoro-6-*O*-(4-nitrophenethyl)-inosine (3a). 2-fluoro-6-*O*-(4-nitrophenethyl)inosine (**3**) (1.5 g, 3.4 mmol) and 4-(dimethylamino)pyridine (43.3 mg, 0.34 mmol, 0.10 equiv) were dissolved in 30 mL of dry pyridine. To this solution was added 4,4′-dimethoxytrityl chloride (1.5 g, 4.4 mmol, 1.3 equiv) and triethylamine (2.8 mL, 20.4 mmol, 6 equiv). After stirring at room temperature for 16 h, the solution was quenched with 15 mL of methanol and concentrated in vacuo to an orange oil. This residue was dissolved in 50 mL of CH_2Cl_2 and washed with 5% (w/v) aqueous $NaHCO_3$ (3 × 50 mL). The combined aqueous layers were back-extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated in vacuo to an orange oil. Silica gel flash chromatography (silica was pretreated with 3% triethylamine in CH_2Cl_2) with 19:1 CH_2Cl_2/MeOH (R_f(**3a**) = 0.75, 9:1 CH_2Cl_2/MeOH) afforded 1.88 g (2.55 mmol, 74%) of **3a** as an off-white foam. ^{1}H NMR ($CDCl_3$ and TEA, 400 MHz): δ (ppm) 8.16 (d, 2H), 8.11 (s, 1H, H8), 7.47 (d, 2H), 7.36 (d, 2H), 7.28−7.14 (m, 7H), 6.75 (m, 4H), 5.97 (d, 1H, H1′), 4.82 (t, 2H), 4.70 (t, 1H, H2′), 4.44 (m, 1H, H3′), 4.24 (m, 1H, H4′), 3.75 (s, 6H), 3.45−3.28 (m, 4H, H5′ and H5″ and *p*-NPE CH_2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (ppm) 162.1, 162.0, 158.8, 158.6, 156.7, 153.2, 153.0, 147.0, 145.3, 144.6, 141.7141.7, 135.7, 130.1, 130.0, 128.1, 127.9, 126.9, 123.8, 120.1, 120.0, 113.2, 89.6, 86.5, 84.8, 77.3, 74.6, 70.8, 67.6, 63.4, 55.2, 35.0. FAB-HRMS: calcd for $C_{39}H_{36}N_5O_9F$ (M + Na^+) 760.2392, obsd 760.2395.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-6-*O*-(4-nitrophenethyl)inosine (3b). A mixture of 5′-*O*-(4,4′-dimethoxytrityl)-2-fluoro-6-*O*-(4-nitrophenethyl)inosine (**3a**) (1.58 g, 2.14 mmol) and imidazole (1.02 g, 15 mmol, 7 equiv) was dissolved in 3 mL of dry DMF at 0 °C. To this solution was added *tert*-butyldimethylsilyl chloride (386 mg, 2.57 mmol, 1.2 equiv). After stirring for 9 h at room temperature, the reaction mixture was diluted with 30 mL of ether and washed with 10% (w/v) aqueous LiBr (3 × 30 mL). The combined aqueous layers were back-extracted with ether (2 × 40 mL). The combined organic layers were then dried over Na_2SO_4, filtered, and concentrated in vacuo to a yellow oil. Silica gel flash chromatography of this residue (silica gel was pretreated with 3% triethylamine in hexanes) with a gradient of 2:1 to 1:1 hexanes/EtOAc (R_f(**3b**) = 0.85, 19:1 CH_2Cl_2/MeOH) afforded 346 mg (0.41 mmol, 19%) of **3b** as a white foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.17 (d, 2H), 8.13 (s, 1H), 7.49 (d, 2H), 7.42 (d, 2H), 7.33−7.18 (m, 7H), 6.81 (d, 4H), 6.00 (d, 1H, H1′), 4.83 (m, 3H), 4.32 (m, 1H), 4.25 (m, 1H), 3.77 (s, 6H), 3.46 (dd, 2H, H5′/H5″), 3.32 (t, 2H), 0.84 (s, 9H), 0.01 (s, 3H), −0.13 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 162.2, 162.0, 159.0, 158.6, 156.9, 153.4, 153.3, 147.0, 145.3, 144.5, 141.3, 135.5, 130.1, 129.9, 128.1, 128.0, 127.0, 123.8, 120.0, 119.9, 113.3, 88.3, 86.8, 84.4, 76.0, 71.6, 67.6, 63.4, 55.2, 35.0, 25.5, 17.9, −5.0, −5.1. FAB-HRMS: calcd for $C_{45}H_{50}N_5O_9FSi$ (M + Na^+) 874.3256, obsd 874.3260.

5′-*O*-(4,4′-Dimethoxytrityl)-3′-*O*-(2-cyanoethoxy)-*N*,*N*-diisopropylamino)phosphino] 2′-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-6-*O*-(4-nitrophenethyl)inosine (3c). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-2-fluoro-6-*O*-(4-nitrophenethyl)inosine (**3b**) (360

mg, 0.42 mmol) was dissolved in 3 mL of dry THF. To this solution was added *N*,*N*-diisopropylethylamine (0.45 mL, 2.54 mmol, 6 equiv) and 2-cyanoethyl (*N*,*N*-diisopropylamino)chlorophosphite (0.189 mL, 0.85 mmol, 2 equiv). Within 10 min a white precipitate had formed. The solution was allowed to stir for 12 h at room temperature, and was subsequently diluted with 20 mL of EtOAc. The resulting solution was washed with 5% (w/v) aqueous $NaHCO_3$ (3 × 15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to a yellow oil. Silica gel flash chromatography (silica gel was pretreated with 3% triethylamine in hexanes) with 2:1 hexanes/EtOAc (R_f(**3c**) = 0.16, 3:1 hexanes/EtOAc) afforded 360 mg (0.34 mmol, 81%) of **3c** as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.16 (d, 2H), 8.12 (s, 1H), 7.50−7.41 (m, 4H), 7.34−7.19 (m, 8H), 6.80 (m, 4H), 5.98 (d, 1H, H1′ diastereomer 1), 5.97 (d, 1H, H1′ diastereomer 2), 4.90−4.81 (m, 3H), 4.42−4.33 (m, 2H), 3.77 (s, 6H), 3.62−3.54 (m, 4H), 3.40−3.30 (m, 4H), 2.58−2.53 (m, 4H), 2.31 (t, 1H), 1.17 (d, 6H), 1.05 (d, 6H), 0.77 (s, 9H), −0.01 (s, 3H), −0.18 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 162.2, 161.9, 158.9, 158.6, 156.8, 153.5, 153.3, 147.0, 145.3, 145.2, 144.5, 144.4, 141.7, 135.6, 135.5, 135.4,130.2, 130.1, 130.0, 128.2, 128.1, 127.9,127.0, 123.8, 120.1, 120.0, 117.6, 117.3, 113.2, 88.47, 88.28, 86.80, 86.68, 84.35, 84.18, 83.83, 83.80, 73.35, 73.25, 72.78, 72.63, 71.56, 67.58, 67.50, 63.3, 63.2, 58.7, 57.6, 55.2, 43.4, 43.0, 35.0, 25.6, 24.6, 22.5, 21.3, 20.5, 20.1, 17.9, −4.66, −4.98, −5.14. FAB HRMS: calcd for $C_{54}H_{67}N_7O_{10}SiPF$ (M + Na^+) 1074, obsd 1074.

Conversion Reactions of Free Convertible Nucleosides. In a typical reaction, 0.6 mmol of convertible nucleoside was treated with either 7 M methanolic NH_3 (methanol saturated with NH_3 at 0 °C) or a 2 M methanolic solution of amine at 42 °C for various lengths of time ranging from 2 to 18 h. The reactions were neutralized with acetic acid (or, in the case of the NH_3 reactions, evaporated on a Speed-vac concentrator) and then analyzed by reversed-phase HPLC with a gradient of 0−50% CH_3CN in 0.1 M triethylammonium acetate over 20 min. A minimum of four data points were collected for each reaction, plus an assumed 0 min time point. The relative amounts of product and starting material were determined by the integration of A_{254} peak areas. Graphical plots of $\ln(1 + A_{prod}/A_{sm})$ versus time, where A_{prod} is the area under the product peak and A_{sm} is the area under the starting material peak, yielded values of the pseudo-first-order observed rate constant, k_{obs}, from which $t_{1/2}$ was calculated.

Oligonucleotide Synthesis. All RNA syntheses were carried out using an ABI 392 DNA/RNA synthesizer with a conductance trityl monitor. Synthesizer reagents were supplied by Perkin-Elmer-ABI (Foster City, CA), with the exception of the N-PAC ribonucleoside phosphoramidites, which were obtained from Biogenex (San Ramon, CA). The standard 1 μmol RNA synthesis cycle was modified to have an extended coupling time of 12 min. All phosphoramidites, including the convertible nucleoside phosphoramidites ClϕU, ClϕI, and NPE-FI, were dissolved to a concentration of 0.1 M in anhydrous CH_3CN. Syntheses of oligonucleotides containing convertible nucleosides proceeded with average stepwise yields of greater than 97%.

Oligonucleotide Displacements and Deprotection. For displacement reactions involving ammonia, the resin-bound oligonucleotides were treated with 1.5 mL of methanolic ammonia solution (7 M, saturated at 0 °C), at 42 °C for 18 h. In displacement reactions involving methylamine, the resin-bound oligonucleotides were treated with 1.5 mL of ethanolic methylamine solution (8 M) at 42 °C for 18 h. The resulting solutions for both ammonia and methylamine were concentrated under vacuum using a Speed-vac concentrator (Savant). For oligonucleotide displacement reactions involving all other amines, the resin-bound oligonucleotides were treated with 0.2−0.4 mL of a 2 M solution of amine in methanol at 42 °C for 18 h. The resulting solutions were then separated from the resin and immediately eluted through a 20 mL Dowex 50 × 8−100 cation exchange column (NH_4^+ form) with 9:1 methanol/water. The RNA-containing fractions were pooled and concentrated on a Speed-vac concentrator to a dry residue.

Removal of the 2′-*O*-silyl ethers (and the NPE from NPE-FI) was afforded by treating each of the oligonucleotides with 0.6 mL of 1 M tetrabutylammonium fluoride (TBAF) in THF for 20 h at room temperature. These reactions were quenched by the addition of 0.8 mL of 1 M TEAA, pH 7.5. The oligonucleotides were then desalted by loading onto a C18 SepPak cartridge (Waters/Millipore), followed by elution with 30% CH_3CN/0.1 M triethylammonium bicarbonate and lyophilization to a dry residue.

Oligonucleotide Purification. The crude deprotected oligonucleotides were purified using denaturing polyacrylamide gel electrophoresis. All gels were 20% polyacrylamide (19:1 acrylamide/bisacrylamide) using a Tris-borate/EDTA running buffer (90 mM Tris free base, 90 mM boric acid, and 2 mM Na_2EDTA, pH 8.3). The product oligonucleotides were visualized on the gel by UV-shadowing over a fluorescent-active TLC plate, excised from the gel, crushed, and then soaked overnight at 37 °C with 10 mL of 1 M ammonium acetate. The resulting solution was desalted on a C18 SepPak cartridge to afford purified RNA oligomer as a dry, off-white solid. Oligonucleotides were quantified by UV absorbance at 260 nm, assuming an extinction coefficient of 1.0×10^4 M^{-1} cm^{-1} per nucleotide.

Nucleoside Composition Analysis. In a typical digestion analysis, 8 nmol of oligonucleotide was dissolved in 60 mL of a solution containing 0.2 mM $ZnCl_2$, 16 mM $MgCl_2$, 250 mM Tris−HCl (pH 6.0), 0.2 unit of snake venom phosphodiesterase (Pharmacia), and 4 units of calf-intestinal alkaline phosphatase (Boehringer Mannheim), and was heated at 37 °C for 8 h. Samples were then injected onto a reversed-phase C18 HPLC column (Waters/Millipore) with a gradient elution from 100% 0.1 M TEAA to 50% acetonitrile/50% 0.1 M TEAA over 15 min (flow rate 1.5 mL/min). The peaks corresponding to the four natural nucleosides C, U, G, and A were identified by coinjection with nucleoside standards. The modified nucleosides were identified as peaks not corresponding to known nucleosides, which in all samples except for oligonucleotides treated with 2-(methylthio)ethylamine was a single peak.

Determination of Thermal Denaturation Temperatures (T_m). The RNA heterodimers were prepared by mixing 5 nmol of each strand (as determined by UV quantitation) in a solution that was 10 mM Tris−HCl (pH 7.5) and 50 mM NaCl. Following brief heat denaturation at 85 °C for 2 min, the oligonucleotides were allowed to anneal by slowly cooling to room temperature over a span of 1 h and subsequent refrigeration at 4 °C. Melting curves were obtained with an HP8452 UV−vis spectrophotometer equipped with a photodiode array detector and a Peltier temperature controller by measuring A_{260} at 2 °C increments. The values for T_m were determined by calculating the first derivative of the melting curve.

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