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Toward Amide-Modified RNA: Synthesis of 3′-Aminomethyl-5′-carboxy-3′,5′-dideoxy Nucleosides

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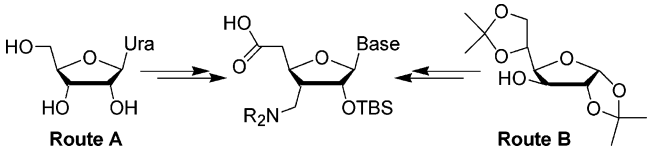

Recent discovery of RNA interference has reinvigorated the interest in chemically modified RNA. Chemical approaches may be used to optimize properties of small interfering RNAs, such as thermal stability, cellular delivery, in vivo half-life, and pharmacokinetics. From this perspective, amides as neutral and hydrophobic internucleoside linkages in RNA are highly interesting modifications that so far have not been tested in RNA interference. Amides are remarkably good mimics of the phosphodiester backbone of RNA and can be prepared using a relatively straightforward peptide coupling chemistry. The synthetic challenge that has hampered the progress in this field has been preparation of monomeric building blocks for such couplings, the nucleoside amino acid equivalents. Herein, we report two synthetic routes to enantiomerically pure 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy nucleosides, monomers for preparation of amide-modified RNA. Modification of uridine, a representative of natural nucleosides, using nitroaldol chemistry gives the target amino acid in 16 steps and 9% overall yield. The alternative synthesis starting from glucose is somewhat less efficient (17 steps and 6% yield of 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy uridine), but provides easier access to modified nucleosides having other heterocyclic bases. The syntheses developed herein will allow preparation of amide-modified RNA analogues and exploration of their potential as tools and probes for RNA interference, fundamental biochemistry, and bio- and nanotechnology.

Introduction

RNA interference (RNAi) is an evolutionarily conserved gene regulation mechanism in response to double-stranded RNA (dsRNA).[1,2] RNAi has already become a major tool in bioinformatics and biochemistry for studying biological mechanisms and protein function through the loss-of-function analysis. Moreover, the discovery that the dsRNA step of RNAi may be bypassed by directly introducing synthetic short interfering RNAs (siRNAs) into human cells[3] has revitalized the idea of gene therapy using short synthetic oligonucleotides.[4−6] However, for siRNAs to be useful in vivo they need to be chemically modified to optimize their enzymatic stability, cellular uptake, biodistribution, and pharmacokinetics while minimizing toxicity and undesired off-target effects of siRNAs.

Several RNA analogues initially designed to increase the nuclease resistance of antisense oligonucleotides have already shown promising results in RNAi.[7,8] In general, modifications that maintain and reinforce the A-type geometry of RNA are relatively well tolerated in RNAi, the most prominent examples being 2′-*O*-Me,[9−14] 2′-F,[11−16] locked nucleic acids (LNA),[14,17]

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and 4′-thio RNA.[18] However, extensive modification that changes the overall shape of RNA results in a sharp decrease in RNAi activity.[11,14] For example, increasing the size of the alkyl groups at the 2′-position to allyl or methoxyethyl strongly inhibits RNAi.[10,13] Most remarkably, modifications of the phosphate backbone are also well tolerated. Phosphorothioates, the most popular modifications in antisense research, have shown promising results,[10,11,16,19] and even a fully modified siRNA duplex retains some RNAi activity.[14] SiRNAs with boranophosphate modifications have demonstrated similar potential in RNAi.[20] Several studies have demonstrated in vivo silencing of therapeutically relevant genes using different combinations of carbohydrate and backbone modifications.[19,21]

However, none of the currently used modifications offers ideal properties for in vivo RNAi applications. The 2′-modifications give only limited improvements in enzymatic stability and may inhibit RNAi activity if used extensively. Phosphorothioates and boranophosphates are typically heterogeneous mixtures of diastereomers with lower thermal stability than the native RNA. Phosphorothioates also exhibit nonspecific binding to proteins and inhibit a variety of enzymes, which is a likely cause of cytotoxicity and side effects in clinical trials.[10,16] On the other hand, it is conceivable that RNAi may tolerate even more radical backbone modifications, provided that the modified linkages closely mimic the A-type geometry of the RNA duplex. In particular, hydrophobic non-phosphorus backbones will ensure high (if not complete) enzymatic resistance and may offer other important advantages for in vivo applications, such as favorable cellular uptake, biodistribution, and pharmacokinetics.

However, most of the non-phosphorus backbones are poor mimics of nucleic acid structure as can be judged from the dramatically reduced thermal stability of the modified double helices.[22,23] Among the few exceptions are amides (3′-CH_2-CONH-5′ and 3′-CH_2NHCO-5′),[24−26] methylene(methylimino) (3′-$CH_2N(CH_3)O$-5′),[27,28] formacetal (3′-OCH_2O-5′),[29−32] and thioformacetal (3′-SCH_2O-5′)[31,32] backbones. Recently, we showed that the two isomeric amide linkages (Figure 1, **1** and **2**) were excellent mimics of the phosphodiester backbone in RNA duplexes.[26] Whereas the duplexes having amide **1** modification have thermal stability similar to the nonmodified controls, the amide **2** remarkably stabilizes the RNA duplexes (Δt_m up to over 2 °C per modification).[26] This is in contrast to the results by De Mesmaeker and co-workers in the DNA series where both amides **1** and **2** have thermal stability similar to the nonmodified DNA.[24] A practical advantage of amides is that they can be prepared using a relatively straightforward peptide coupling chemistry. The synthetic challenge that so far has hindered the progress in this field has been the synthesis of the highly modified C-branched nucleoside amino acids (such as **3**, Scheme 1), monomers for the peptide type couplings.

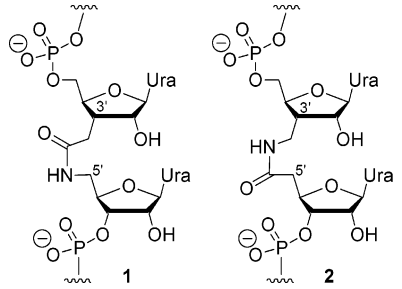

FIGURE 1. Amides as internucleoside linkages.

Whereas several research groups have developed synthetic routes for introduction of amides **1** in RNA,[26,33−35] preparation of amide **2** modified nucleic acid has been a challenging and little attempted goal. Before our first report[26] on synthesis of 3′-azidomethyl-3′-deoxyuridine and 5′-carboxy-5′-deoxyuridine using the nitroaldol−reduction sequences, there had been only one precedent of similar separate one carbon homologation in the DNA series using a variant of radical alkylation at the C3′ and Wittig reaction at the C5′.[24] In a series of two preliminary papers,[36,37] we recently reported asymmetric synthesis of 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy uridine **4a** (Scheme 1,

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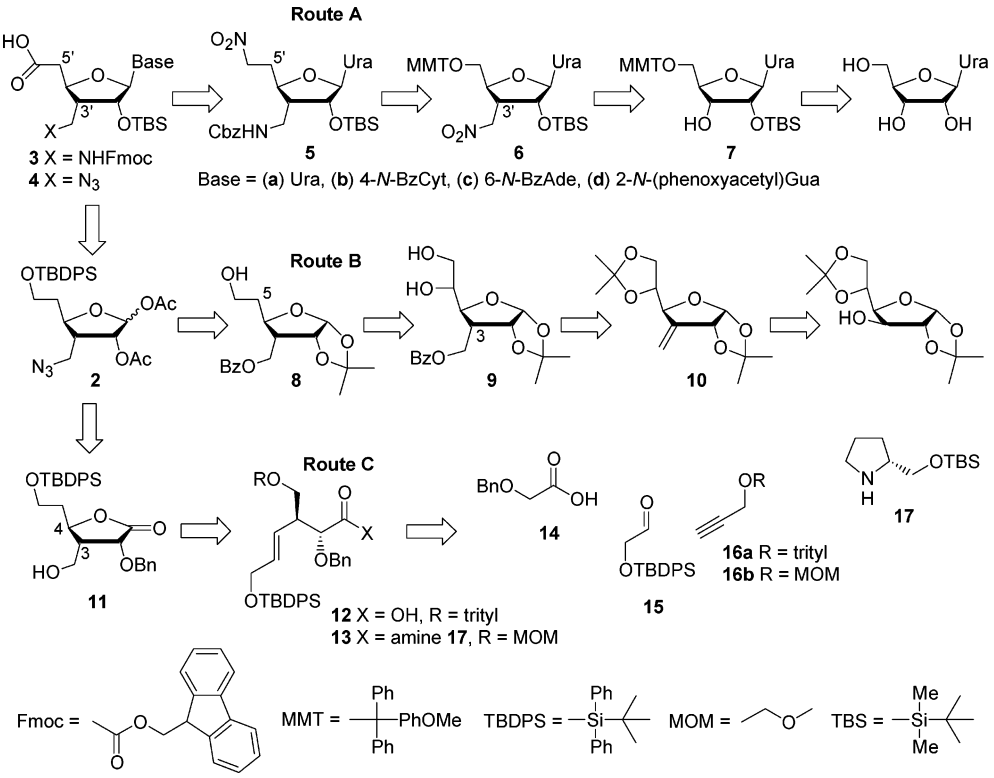
SCHEME 1. Retrosynthetic Analysis of Amide-Linked RNA



Base = Ura) starting both from a common carbohydrate precursor and from simple achiral organic compounds. In the present paper we report extension of these preliminary studies to preparation of all four protected nucleoside azido acids **4a**−**d** (Scheme 1, Base = Ura, Cyt, Ade, Gua). We have also developed synthesis of Fmoc protected uridine amino acid **3a** starting from protected uridine and using a modification of our nitroaldol−reduction chemistry.[26] Each of the two routes, starting either from carbohydrates or from nucleosides, has unique advantages and shortcomings.

Results and Discussion

1. Retrosynthetic Analysis. There are three general strategies to synthesize nucleosides that have modifications in their sugar moiety. The traditional routes take the advantage of chiral pool starting materials, nucleosides and carbohydrates, which have most of the carbon skeleton and stereochemical relationships already set or easily adjustable. The most straightforward route starts with the corresponding natural nucleoside. Such an approach works best if the modification is a relatively minor one and can be installed using mild chemistry tolerated by the multifunctional nucleosides. The main drawback is that four separate syntheses have to be done if the modification of all four natural nucleosides is required. Moreover, due to distinct chemical properties of the heterocycles, reaction conditions developed for a specific nucleoside may not be transferable to other nucleosides. Our retrosynthetic analysis of 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy uridine **3a** following the nucleoside route (Scheme 1, Route A) involves sequential use of nitroaldol reaction for one carbon homologation at C3′ (**6**) and C5′ (**5**) followed by conversion of the nitroalkyl groups into amine and carboxylic acid, respectively.[26] The starting material for this sequence is the selectively protected uridine **7**.

Another popular approach to synthesis of modified nucleosides is to use a common carbohydrate as a glycosyl donor (such as **2**, Scheme 1), which is coupled with the desired heterocycle to give the modified nucleoside. The advantages are that a variety of heterocyclic bases can be installed late in the synthesis and that the carbohydrate intermediates are generally less sensitive than nucleosides. However, such routes are frequently lengthy and laborious because the multifunctional carbohydrates require extensive protecting group manipulations. We recognized that a common glycosyl donor **2** can be derived from the 3,5-C-homologated ribose **8**, an advanced intermediate in Benner's synthesis of sulfone-modified RNA (Scheme 1, Route B).[38] In Benner's synthesis,[38a] an elimination−hydroboration sequence on glucose-derived **9** established the C5 homologation in **8**, and a Wittig reaction to give **10** followed by stereoselective hydroboration established the C3 homologation.

The third approach is de novo asymmetric construction of the modified nucleosides using the principles of total synthesis. Although such an approach traditionally has been more popular in preparation of carbocyclic nucleosides,[39] few examples of de novo synthesis of natural and carbohydrate-modified nucleosides have been reported.[36,40−42] We recently developed a very

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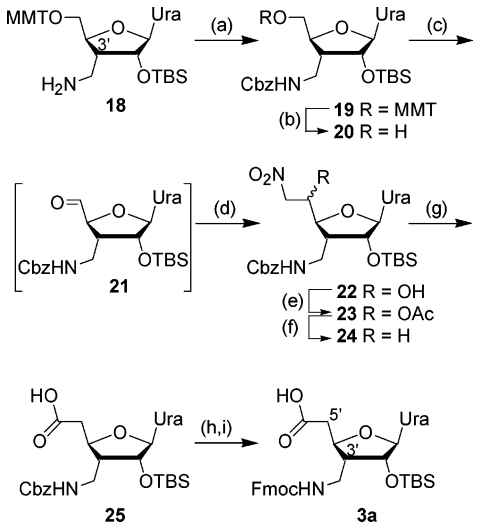
SCHEME 2[a]



[a] Conditions: (a) CbzCl, 2 N NaOH, dioxane/H_2O (4:1), pH 9, rt, 20 min, 93%; (b) 10% $CHCl_2COOH$ in CH_2Cl_2, Et_3SiH, rt, 15 min, 77%; (c) DMSO, EDC, pyridine, TFA, toluene, rt, 1 h; (d) CH_3NO_2, $NaOCH_3$, MeOH, rt, 1.5 h; (e) Ac_2O, cat. p-TsOH, rt, 1.5 h; (f) $NaBH_4$, abs. EtOH, rt, 40 min, 65% (4 steps); (g) $NaNO_2$, AcOH, DMSO, 40 °C, 28 h, 67%; (h) H_2, Pd/C, MeOH, rt, 3 h; (i) Fmoc-succinimide, $NaHCO_3$, dioxane/acetone/H_2O (6:1:1), rt, 3 h, 90% (2 steps).

efficient asymmetric synthesis of all four 5′-azido-3′-carboxymethyl-3′,5′-dideoxy nucleosides[41,42] (constitutional isomers of **4a**−**d**) and reported preliminary results toward the more challenging 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d** (Scheme 1).[36,37] In our retrosynthetic analysis (Scheme 1, Route C) the common glycosyl donor **2** is derived from lactone **11**. In our first design,[36] lactone **11** was obtained by iodolactonization of unsaturated carboxylic acid **12**, which in turn was made in several steps from the small organic compounds **14**, **15**, and **16a**. Recently, we reported preliminary results toward an improved de novo route, which proceeds through unsaturated amide **13**, available from **14**, **15**, **16b**, and chiral amine **17**.[37] The main advantage of de novo synthesis is greater flexibility in choosing chemical reactions and starting materials, which should allow easier optimization and future development of more efficient synthesis of **4a**−**d**.

2. Modification of Natural Nucleosides (Route A): The Nitroaldol Approach. The synthesis of 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy uridine derivative **3a** (Scheme 2) starts with the protected 3′-aminomethyl-3′-deoxy uridine **18**, which was prepared following our previously developed nitroaldol−reduction sequence.[26] Protection of the amino group as benzyloxycarbamate (Cbz) was followed by cleavage of the 5′-(4-methoxytrityl) group (MMT) using dichloroacetic acid in the presence of triethylsilane to give **20**, the key starting material for the following 5′-homologation steps. Oxidation of the primary alcohol and addition of the nitromethane was done in a one-pot mode without isolation of the intermediate aldehyde **21**. Oxidation conditions were optimized to simplify work up and isolation of the products. In particular, the use of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (EDC) allowed for removal of the urea byproducts via simple aqueous extraction.[43]

The resulting mixture of diastereomeric aldol aducts (**22**) was used directly (without characterization) in the next acetylation and reductive elimination steps to give nitroalkane **24** in good yield. For acetylation, the use of *para*-toluenesulfonic acid (p-TsOH) as the catalyst (previously we used $HClO_4$ in a similar procedure[26]) was important to retain the 2′-*O*-TBS protection. Nitroalkane **24** was converted into carboxylic acid **25** using the procedure developed by Mioskowski and co-workers[44] and also used in our earlier studies to prepare the 5′-carboxy-5′-deoxy uridine derivative.[26] Finally, the temporary Cbz protection, which served very well during the 5′-homologation, was replaced with the Fmoc protection, which we expected to be better suited for the solid phase oligoamide synthesis. The protecting group replacement maneuver was done in a one-pot two-step sequence of hydrogenation and carbamate formation under standard conditions, thus completing the synthesis of the protected 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy nucleoside **3a** in 16 steps and 9% overall yield starting from uridine.

3. Synthesis from D-Glucose (Route B). In our preliminary paper,[36] we reported the synthesis of glycosyl donor **2** starting from the known C-homologated carbohydrate derivative **8** (Scheme 3).[38] In summary, protection of the primary alcohol, installation of the azido group, and replacement of the 1,2-isopropylidene group with acetates gave the glycosyl donor **2** in six steps and 36% yield starting from **8** (overall 14 steps and ∼9% yield from the commercially available 1,2:5,6-di-*O*-isopropylidene-D-glucose). Intermediate **8** features the complete carbon skeleton and correct stereochemical relationships required for the sugar moiety of our target 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d**. Herein we report extension of these preliminary studies to prepare 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d** bearing all four natural heterocyclic bases.

Synthesis of the modified pyrimidine nucleosides was done following the standard Vorbrüggen methodology.[45] Thus, coupling of bis(trimethylsilyl) heterocycles with **2** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave **26a** and **26b** in good yields. The adenosine derivative **26c** was made using the tin tetrachloride mediated reaction of **2** with unprotected adenine as described by Saneyoshi and Satoh.[46] The guanosine derivative **26d** was prepared as single N9-isomer using the TMSOTf mediated coupling of **2** with persilylated 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine as described by Zou and Robins.[47]

Our initial design of the final steps was to selectively cleave the 6′-*O*-TBDPS group, oxidize the primary alcohol, and, finally, replace the 2′-*O*-Ac with the 2′-*O*-TBS group. In contrast to our previous synthesis of the isomeric 5′-azido-3′-carboxymethyl-3′,5′-dideoxy nucleosides,[42] the 2′-*O*-Ac cannot be used as a protecting group in the final azido acids because reduction of the adjacent azide to amine would trigger 1,5-migration of the acetyl to form acetamide. We decided to use the 2′-*O*-TBS protection because the 2′-*O*-triethylsilyl (TES) group used in our preliminary study[36] was found to be too labile. Whereas the first two steps (selective desilylation and oxidation of the 6′-OH) on the uridine derivative **26a** did not cause any problems,

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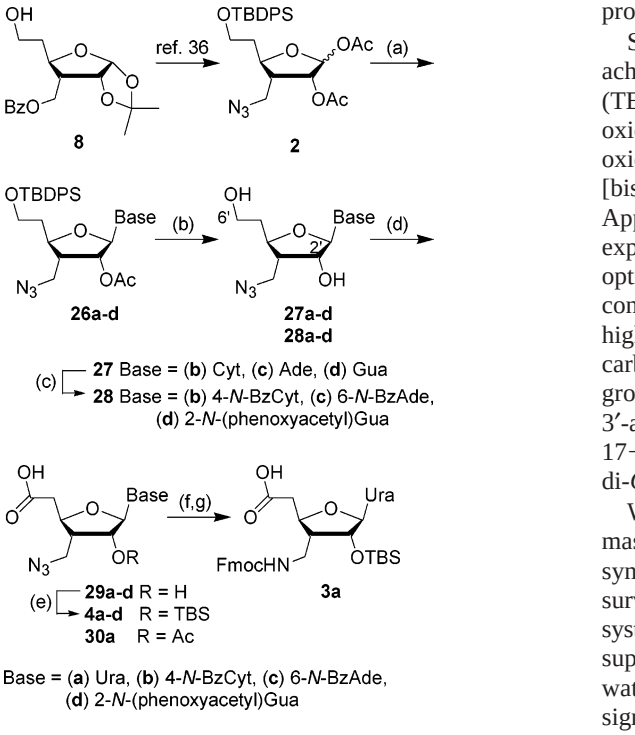
SCHEME 3[a]



[a] Conditions: (a) for **26a**: 2,4-*O*,*O*′-bis(trimethylsilyl)uracil, TMSOTf, CH_2Cl_2, rt, 1.5 h, 86%; for **26b**: 2,4-*O*,*N*-bis(trimethylsilyl)cytosine, TMSOTf, $ClCH_2CH_2Cl$, reflux, 1 h, 85%; for **26c**: adenine, $SnCl_4$, CH_3CN, rt, 3 min, 79%; for **26d**: bis(trimethylsilyl)acetamide, 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine, $ClCH_2CH_2Cl$, reflux, 10 min, add to **2** in $ClCH_2CH_2Cl$, add TMSOTf, reflux 1 h, 83%; (b) for **27a** and **27b**: HCl, methanol, rt, 5 days, 91 and 97%; for **27c** and **27d**: NaOH, H_2O/methanol, rt, 3−5 days, 91%; (c) for **28b**: benzoic anhydride, DMF, rt, 24 h, 94%; for **28c**: TMSCl, pyridine/CH_2Cl_2, rt, 1 h, add benzoyl chloride, 0 °C, 4 h, add methanol, rt, overnight, 89%; for **28d**: (i) TMSCl, pyridine/CH_2Cl_2, rt, 1 h, add phenoxyacetyl chloride, 0 °C, overnight, (ii) H_2O, 0 °C, 1 h, 93%; (d) TEMPO, $NaClO_2$, NaClO, Bu_4NHCl, MeCN, rt, 5 h; (e) for **4a**: TBSCl, DMF/pyridine (1:2), rt, 5 days, 85% (2 steps); for **4b**−**d**: TBSOTf, 2,6-lutidine, CH_2Cl_2, rt, 2−3 days, **4b** 85%, **4c** 67%, **4d** 47% (2 steps); (f) Bu_3SnH, AIBN, toluene, 90 °C, 25 min; (g) Fmoc-succinimide, $NaHCO_3$, acetone/H_2O (1:1), 0 °C, 2 h, rt, overnight, 83% (two steps).

cleavage of the 2′-*O*-Ac group (in resulting **30a**) with NaOH was surprisingly slow and gave byproducts after prolonged reaction times. We speculate that the increased steric hindrance around the C2′ (especially the branching at the C3′) and the deprotonation of the 5′-carboxylate, which makes **30a** negatively charged, had a mutually negative effect on the rate of the hydrolysis. Therefore, we chose an alternative design based on selective oxidation to distinguish the primary and secondary alcohols after complete deprotection of **26a**−**d** (Scheme 3).

After some experimentation, we found that treatment of the pyrimidine nucleosides **26a** and **26b** with methanolic HCl for 5 days resulted in a clean removal of both 6′-*O*-TBDPS and 2′-*O*-Ac groups. At this point, the heterocyclic amino group of cytidine was selectively protected using benzoic anhydride in DMF.[48] For purine nucleosides **26c** and **26d**, NaOH in aqueous methanol was the preferred reagent because the acidic conditions led to some depurination. The heterocyclic amino groups of adenosine and guanosine were protected as benzoyl and phenoxyacetyl amides, respectively, using the temporary TMS protection method.[49]

Selective oxidation of the primary 6′-hydroxyl group was achieved using the 2,2,6,6-tetramethylpiperidilyloxy radical (TEMPO), which is a versatile reagent often used for selective oxidation of carbohydrates.[50] Widlanski et al.[51] have reported oxidation of nucleoside 5′-hydroxyl groups using TEMPO and [bis(acetoxy)iodo]benzene (BAIB) as a stoichiometric oxidant. Application of the TEMPO/BAIB system to **28a** gave the expected carboxylic acid **29a** in 80% yield.[36] After careful optimization of reaction conditions, we found that the system consisting of TEMPO, $NaClO_2$ and bleach[52] gave cleaner and higher yielding oxidation of **28a**−**d** to the corresponding carboxylic acids **29a**−**d**. Protection of the remaining 2′-OH group as TBS ether completed the syntheses of all four 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d** in 17−18 steps and 2−6% overall yields starting from 1,2:5,6-di-*O*-isopropylidene-D-glucose.

We envisioned that the azide in **4a**−**d** might serve as a masked amino function in our planned solid phase oligoamide synthesis. Using uridine **4a** as a model compound we briefly surveyed different methods for azide reduction. Two reagent systems that have been previously used to reduce azides on solid support—$SnCl_2$, PhSH, triethylamine[53,54] and $P(Me)_3$, toluene, water[55]—gave low yields of the target amine accompanied by a significant amount of byproducts. Catalytic hydrogenation over 10% Pd on carbon was relatively fast and good yielding (∼80%). The best result was achieved with Bu_3SnH and AIBN in toluene at 90 °C (conditions previously used by us[26] for reduction of 5′-azido-5′-deoxyuridine) which gave clean and high yielding (>90%) reduction of **4a** to the corresponding amine (Scheme 3). Protection of the newly formed amine as Fmoc carbamate gave **3a**, which was identical to the material previously prepared form uridine using Route A (Scheme 2). The use of Fmoc protected amines is an alternative strategy for synthesis of amide-modified RNAs, especially useful if the solid phase reduction of azide encounters problems. Synthesis starting from 1,2:5,6-di-*O*-isopropylidene-D-glucose (Scheme 1, Route B) required 19 steps and gave the Fmoc protected amino acid **3a** in 5% overall yield. Although currently inferior to Route A that starts from uridine, the synthetic strategy that uses the modified furanoside **2** as an intermediate may be more practical if synthesis of all four modified natural nucleosides is required.

4. Future Outlook: Toward de Novo Asymmetric Synthesis of 3,5-C-Branched Furanoside 2 (Route C). Applying the principles of total synthesis to develop shorter and higher yielding routes to the key intermediate **2** (see Route C) may further enhance the synthetic efficiency of Route B. To this end, we have reported de novo synthesis of **2** using addition of alkynylzinc derivative of **16a** to aldehyde **15**, Lindlar reduction, esterification with **14** followed by an Ireland−Claisen rearrangement (to give **12**), and iodolactonization as the key steps (Scheme 4).[36] Although the synthesis could be rendered asym-

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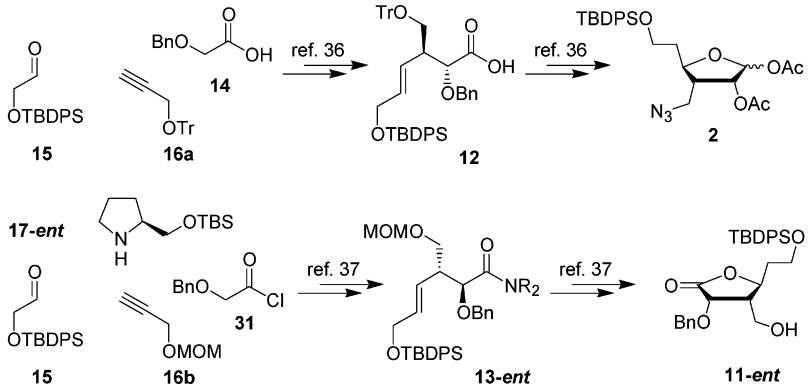
SCHEME 4



metric by employing a chiral ligand in the alkynylzinc addition, the ee achieved (92%) was modest for the planned multiple couplings of the **4a**−**d** to form amide-linked RNA. Furthermore, the iodolactonization of the carboxylic acid **12** was not stereoselective and required laborious recycling of the undesired stereoisomer. Overall, the glycosyl donor **2** was prepared in 12 steps and 11.5% yield starting from **15** and **16a**. Thus, our first generation de novo synthesis was already slightly more efficient than Route B, which produces **2** in 14 steps and 9% overall yield.

Literature precedents[56] and recent studies in our laboratory[37,41,42] suggested that iodolactonization of amides provided a more stereoselective route to the required *trans*-3,4-dialkyl-γ-butyrolactones (e.g., **11**) and, eventually, to the desired furanoside **2**. After some experimentation, we were able to improve our first generation de novo synthesis by using acyl−Claisen rearrangement[58,59] to access the required unsaturated amide **13-*ent*** (Scheme 4).[37] The allylic amine required for the acyl−Claisen rearrangement was prepared in an enantiomerically pure form using a three-component coupling of **15**, **16b**, and **17**.[57] Because of the initially arbitrary choice of the chiral amine **17**, we actually obtained amide **13-*ent*** that had the absolute stereochemistry opposite to the one required for synthesis of modified RNA with the natural chirality. This is not a problem as both enantiomers of proline are commercially available and can be used to synthesize **17** and its enantiomer **17-*ent***.[60] The ability to produce both enantiomers of amide-linked RNA is a unique advantage of the de novo synthesis (Route C) that is not available if starting either from nucleoside (Route A) or carbohydrate (Route B) precursors.

Iodolactonization of **13-*ent*** proceeded with good trans stereoselectivity (3.5:1) and after reductive removal of iodine and cleavage of the MOM protection gave lactone **11-*ent***. At this stage, the acyl−Claisen[37] and the previously designed Ireland−Claisen[36] routes converge upon lactone **11** (or its enantiomers **11-*ent***), which can be advanced to furanoside **2** (or **2-*ent***) in five steps and 49% yield as previously described by us.[36] Applying the acyl−Claisen rearrangement route and starting with the correct enantiomers of chiral amine auxiliary should give the common intermediate **2** in overall 11 steps and 13% yield. The work is in progress in our lab to further improve and optimize the de novo synthesis of **2** which, combined with the final nucleoside synthesis steps (as described in Route B), may provide a truly superior route to the highly modified 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d**.

Conclusions

Two synthetic routes for preparation of protected 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy nucleosides have been developed (Scheme 1, Routes A and B). Each route has its unique advantages and shortcomings. Synthesis starting from nucleosides (Route A) is the most direct and currently the most efficient in terms of synthetic steps and overall yield−16 steps, 9% of Fmoc protected 3′-aminomethyluridine derivative **3a**. However, four parallel syntheses would be required for preparation of **3a**−**d** with all four natural heterocyclic bases. Moreover, reaction conditions developed for uridine may need extensive optimization to be applicable for other nucleosides. The advantage of the synthesis starting from glucose (Route B) is that a common glycosyl donor, furanoside **2**, allows introduction of a variety of natural and synthetic nucleobases. However, carbohydrates are highly functionalized and sensitive intermediates that require extensive protecting group manipulation. As a result, Route B requires 17−18 steps for preparation of 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d** in 2−6% overall yields (19 steps, 5% to make **3a**).

It is conceivable that the shortcomings of Route B can be alleviated by future development of de novo synthesis of the key common intermediate **2** (Route C). In its current status, de novo synthesis has the potential to increase the efficiency of **4a**−**d** preparation to 15−16 steps and 5−9% overall yields. An important advantage of de novo synthesis is that it may be further improved by optimization of the key reactions, e.g., Claisen rearrangement and iodolactonization, or by introduction of entirely new syntheses of any of the intermediates. Finally, the access to both enantiomers of **4a**−**d**, and ultimately the stereoisomers of amide-linked RNA, may be highly useful for potential applications in biotechnology, biomedicine, and nanotechnology. Current work in our laboratory is focused on optimization of de novo synthesis of **2** and on application of protected 3′-aminomethyl-5′-carboxy-3′,5′-dideoxy nucleosides

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3a−**d** and 3′-azidomethyl-5′-carboxy-3′,5′-dideoxy nucleosides **4a**−**d** in the solid phase synthesis of amide-linked RNA.

Experimental Section

3′-[*N*-(Benzyloxycarbonyl)aminomethyl]-2′-*O*-*tert*-butyldimethylsilyl-5′,3′-dideoxy-5′-nitromethyluridine (24). 3′-[*N*-(Benzyloxycarbonyl)aminomethyl]-2′-*O*-*tert*-butyldimethylsilyl-3′-deoxy uridine **20** (1.03 g, 2.04 mmol) was dried by evaporation of added dry toluene (2 × 50 mL) and then dissolved in dry toluene (10 mL). DMSO (5 mL), 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide (EDC) (1.19 g, 6.2 mmol), pyridine (3.06 mmol, 247 μL), and trifluoroacetic acid (2.6 mmol, 196 μL) were added, and the mixture was stirred at room temperature for 1 h. Sodium (328 mg, 14.3 mmol) was dissolved in methanol (6 mL), mixed with nitromethane (10 mL), and added to the reaction mixture. After being stirred at room temperature for 1.5 h the mixture was diluted with ethyl acetate (100 mL) and extracted with aqueous 10% citric acid solution (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue was purified by silica gel column chromatography using a stepwise gradient of ethyl acetate in hexane (50 to 70%, 5% steps). The resultant diastereomeric mixture of **22** (0.97 g, 1.72 mmol) was dissolved in acetic anhydride (5 mL), *p*-toluenesulfonic acid monohydrate (82 mg, 0.43 mmol) was added, and the mixture was stirred at room temperature for 1.5 h. The mixture was diluted with ethyl acetate (75 mL) and extracted with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4), evaporated, and coevaporated with dry toluene (2 × 30 mL). The residue (crude **23**) was dissolved in absolute ethanol (5 mL). $NaBH_4$ (260 mg, 6.9 mmol) was added, and the mixture was stirred at room temperature for 40 min, diluted with ethyl acetate (75 mL), and extracted with aqueous 10% citric acid solution (30 mL), saturated aqueous $NaHCO_3$ (30 mL), and brine (30 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue was purified by silica gel column chromatography using a stepwise gradient of ethyl acetate in hexane (40 to 75%, 5% steps) to afford **24** as a white foam. Yield 725 mg, 65%. TLC R_f = 0.57 (CH_2Cl_2/ethanol, 19:1). Elemental analysis calculated for $C_{25}H_{36}N_4O_8Si$: C, 54.73; H, 6.61; N, 10.21. Found: C, 54.54; H, 6.63; N, 10.05. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (major rotamer) 11.39 (s, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.39−7.28 (m, 5H), 7.23 (t, J = 4.9 Hz, 1H), 5.63 (dd, J = 8.1 Hz, J = 1.9 Hz, 1H), 5.53 (s, 1H), 5.06−4.98 (m, 2H), 4.72−4.63 (m, 2H), 4.46 (d, J = 4.8 Hz, 1H), 3.99 (td, J = 9.8 Hz, J = 2.2 Hz, 1H), 3.28−3.21 (m, 1H), 3.08−3.01 (m, 1H), 2.40−2.32 (m, 1H), 2.31−2.23 (m, 1H), 2.09−2.01 (m, 1H), 0.85 (s, 9H), 0.09, 0.04 (2s, 6H). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 163.9, 156.6, 151.0, 140.8, 137.8, 129.0, 128.5, 128.4, 102.1, 91.9, 80.4, 76.7, 73.5, 66.0, 46.2, 37.7, 31.6, 26.3, 18.4, −4.1, −4.9.

3′-[*N*-(Benzyloxycarbonyl)aminomethyl]-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-5′,3′-dideoxyuridine (25). 3′-[*N*-(Benzyloxycarbonyl)aminomethyl]-2′-*O*-*tert*-butyldimethylsilyl-5′,3′-dide oxy-5′-nitromethyluridine **24** (725 mg, 1.32 mmol) was dried by evaporation with added dry toluene (30 mL) and acetonitrile (30 mL) and then dissolved in dry DMSO (4 mL). $NaNO_2$ (475 mg, 6.88 mmol) and acetic acid (1.25 mL, 21.65 mmol) were added, and the mixture was stirred at 40 °C for 28 h. The pH was adjusted to ca. 4.5 with 0.1 M HCl, and water (50 mL) was added. The precipitate was filtered off, washed with water (100 mL), dissolved in ethyl acetate (100 mL), and extracted with aqueous 10% citric acid solution (50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4), evaporated, and purified by silica gel column chromatography using a stepwise gradient of ethanol in CH_2Cl_2 (0−7%, 1% steps containing 1% of acetic acid) to afford **25** as a white foam. Yield 470 mg, 67%. TLC R_f = 0.33 (CH_2Cl_2/ethanol, 4:1). Elemental analysis calculated for $C_{25}H_{35}N_3O_8Si$: C, 56.27; H, 6.61; N, 7.87. Found: C, 55.97; H, 6.60; N, 7.77. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (major rotamer) 9.67−9.59 (broad, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.37−7.28 (m, 5H), 5.78 (d, J = 8.1 Hz, 1H), 5.59 (s, 1H), 5.16−5.21 (m, 1H), 5.15−5.08 (m, 2H), 4.49−4.38 (m, 2H), 3.50−3.38 (m, 1H), 3.37−3.29 (m, 1H), 2.93−2.84 (m, 1H), 2.67 (dd, J = 16.0 Hz, J = 7.1 Hz, 1H), 2.40−2.31 (m, 1H), 0.90 (s, 9H), 0.16, 0.10 (2s, 6H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 174.2, 164.1, 156.6, 150.1, 140.6, 136.3, 128.5, 128.2, 128.1, 102.1, 93.0, 78.7, 77.3, 67.0, 45.6, 37.9, 37.6, 25.8, 18.0, −4.4, −5.4.

2′-*O*-*tert*-Butyldimethylsilyl-5′-carboxy-5′,3′-dideoxy-3′-[*N*-(fluorenylmethoxycarbonyl)aminomethyl]uridine (3a). 3′-[*N*-(Benzyloxycarbonyl)aminomethyl]-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-5′,3′-dideoxyuridine **25** (440 mg, 0.82 mmol) was dissolved in methanol (30 mL), and 10% Pd/C (140 mg) was added. The mixture was stirred at room temperature for 3 h under an atmosphere of H_2. Dioxane (20 mL) was added, and the catalyst was filtered off and washed with dioxane (10 mL) and acetonitrile (10 mL). The filtrate was concentrated under reduced pressure, and the residue was dissolved in acetone/H_2O (1:1, 15 mL). Dioxane (10 mL) and $NaHCO_3$ (172 mg, 2.05 mmol) were added. A solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (329 mg, 1.02 mmol) in dioxane (30 mL) was added dropwise over 30 min. The mixture was stirred at room temperature for 3 h and concentrated to almost dry. The residue was dissolved in ethyl acetate (75 mL) and extracted with 10% citric acid solution (30 mL) and brine (30 mL). The organic layer was dried (Na_2SO_4), evaporated, and purified by silica gel column chromatography using stepwise gradient of ethanol in CH_2Cl_2 (0−5.5%, 0.5% steps containing 1% of acetic acid) to afford **3a** as a white foam. Yield 460 mg, 90%. TLC R_f = 0.39 (CH_2Cl_2/ethanol, 9:1). Elemental analysis calculated for $C_{32}H_{39}N_3O_8Si$: C, 61.82; H, 6.32; N, 6.76. Found: C, 61.44; H, 6.30; N, 6.54. ^{1}H NMR ($CDCl_3$, 600 MHz): δ (major rotamer) 9.84−9.75 (broad, 1H), 7.73 (d, J = 7.7 Hz, 2H), 7.59−7.52 (m, 2H), 7.50 (d, J = 8.1 Hz, 1H), 7.45−7.33 (m, 2H), 7.32−7.22 (m, 2H), 5.79 (d, J = 8.1 Hz, 1H), 5.58 (s, 1H), 5.24−5.15 (m, 1H), 4.49−4.33 (m, 4H), 4.22−4.15 (m, 1H), 3.47−3.39 (m, 1H), 3.36−3.24 (m, 1H), 2.86 (dd, J = 16.1 Hz, J = 3.3 Hz, 1H), 2.70−2.52 (m, 1H), 2.43−2.27 (m, 1H), 0.90 (s, 9H), 0.14, 0.09 (2s, 6H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ 174.3, 164.3, 156.6, 150.1, 143.8, 143.7, 141.3, 140.75, 127.7, 127.0, 125.0, 120.0, 102.1, 93.0, 78.6, 77.4, 66.8, 47.1, 45.5, 37.9, 37.6, 31.6, 25.7, 18.0, −4.5, −5.4. MS (ESI) calculated for $C_{32}H_{39}N_3O_8Si$ 621.3, found [M + 1] 621.8.

2′-*O*-Acetyl-3′-azidomethyl-5′-[(*tert*-butyldiphenylsilyloxy)-methyl]-3′,5′-dideoxyuridine (26a) was prepared as previously reported.[36]

2′-*O*-Acetyl-3′-azidomethyl-5′-[(*tert*-butyldiphenylsilyloxy)-methyl]-3′,5′-dideoxycytidine (26b). Trimethylsilyl trifluoromethanesulfonate (1.08 g, 0.88 mL, 4.86 mmol) was added to a solution of **2** (1.27 g, 2.42 mmol) and 2,4-*O*,*N*-bis(trimethylsilyl)cytosine (1.24 g, 4.86 mmol) in 1,2-dichloroethane (35 mL) at 0 °C. The solution was refluxed at 80 °C for 1 h, cooled to room temperature, and diluted with cold CH_2Cl_2 (150 mL). Saturated aqueous $NaHCO_3$ (100 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (CH_2Cl_2/MeOH, 10:1) to afford **26b**. Yield 1.18 g, 85%. TLC R_f = 0.30 (CH_2Cl_2/MeOH, 10:1). IR: 2102, 1731 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.68−7.65 (4H, m), 7.43−7.36 (6H, m), 7.23 (1H, s), 5.82 (1H, d), 5.65 (1H, d), 5.49 (1H, dd), 4.14 (1H, m), 3.87 (2H, m), 3.46 (1H, dd), 3.28 (1H, dd), 2.32 (1H, m), 2.11 (3H, s), 2.03 (1H, m), 1.83 (1H, m), 1.07 (9H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.9, 166.4, 155.8, 150.7, 140.8, 135.8, 133.9, 133.9, 130.0, 127.9, 95.6, 92.0, 79.4, 77.8, 60.8, 48.0, 45.5, 37.2, 27.1, 21.1, 19.4. HRMS (ESI) calculated for $C_{29}H_{36}N_6O_5Si$ 576.2516, found 577.2594 [M + 1].

2′-*O*-Acetyl-3′-azidomethyl-5′-[(*tert*-butyldiphenylsilyloxy)-methyl]-3′,5′-dideoxyadenosine (26c). $SnCl_4$ (0.24 g, 0.11 mL, 0.91 mmol) was added to a mixture of **2** (0.24 g, 0.46 mmol) and adenine (0.068 g, 0.50 mmol) in CH_3CN (5 mL). After being stirred for 3 min, the cloudy solution turned clear. CH_2Cl_2 (20 mL) and saturated aqueous $NaHCO_3$ (10 mL) were added. The aqueous layer

was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (CH_2Cl_2/MeOH, 25:1) to afford **26c**. Yield 0.22 g, 79%. TLC R_f = 0.33 (CH_2Cl_2/MeOH, 25:1). IR: 2104, 1743 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.21 (1H, s), 7.79 (1H, s), 7.66−7.62 (4H, m), 7.41−7.33 (6H, m), 5.85 (1H, d), 5.89 (3H, m), 4.25 (1H, m), 3.81 (2H, m), 3.58 (1H, dd), 3.43 (1H, dd), 3.15 (1H, m), 2.17 (3H, s), 2.01−1.90 (3H, m), 1.06 (9H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.2, 155.8, 153.4, 149.6, 139.8, 135.8, 133.9, 129.9, 129.9, 127.9, 120.6, 89.6, 79.9, 78.1, 60.6, 55.9, 48.0, 45.4, 37.2, 27.1, 21.0, 19.4. HRMS (ESI) calculated for $C_{30}H_{36}N_8O_4Si$ 600.2629, found 601.2707 [M + 1].

2-*N*,2′-*O*-Diacetyl-3′-azidomethyl-5′-[(*tert*-butyldiphenylsilyloxy)methyl]-3′,5′-dideoxy-6-*N*-diphenylcarbamoylguanosine (26d). Bis(trimethylsilyl)acetamide (BSA) (1.46 g, 1.77 mL, 7.19 mmol) was added to a solution of 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine (1.40 g, 3.61 mmol) in 1,2-dichloroethane (20 mL). The mixture was refluxed for 10 min, cooled to room temperature, and added to a solution of **2** (0.95 g, 1.81 mmol) in 1,2-dichloroethane (6 mL). Trimethylsilyl trifluoromethanesulfonate (0.80 g, 0.72 mL, 3.60 mmol) was added dropwise. The brown solution was refluxed for 1 h, cooled to room temperature, and diluted with CH_2Cl_2 (100 mL). Saturated aqueous $NaHCO_3$ (100 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (hexane/ethyl acetate, 1:1) to afford **26d**. Yield 1.28 g, 83%. TLC R_f = 0.62 (hexane/ethyl acetate, 1:2). IR: 2101, 1710 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.04 (1H, s, br), 7.91 (1H, s, br), 7.64 (4H, m), 7.44−7.33 (14H, m), 7.26 (2H, m), 5.74 (2H, m), 4.25 (1H, m), 3.80 (2H, m), 3.59−3.43 (2H, m), 3.35 (1H, s, br), 2.39 (3H, s), 2.17 (3H, s), 2.02 (1H, m), 1.87 (1H, m), 1.05 (9H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.4, 156.5, 154.3, 152.2, 150.6, 143.5, 142.0, 135.8, 133.9, 133.9, 130.0, 129.9, 129.5, 127.9, 127.9. 127.3, 121.8, 90.1, 80.1, 78.4, 60.7, 47.9, 45.1, 37.1, 27.2, 25.2, 21.0, 19.5. HRMS (ESI) calculated for $C_{45}H_{47}N_9O_7Si$ 853.3368, found 854.3446 [M + 1].

3′-Azidomethyl-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-3′,5′-dideoxyuridine (4a). A phosphate buffer (pH 7, 0.2 mL) was added to a mixture of **28a** (300 mg, 1.01 mmol), 2,2,6,6-tetramethylpiperidilyloxy radical (TEMPO, 79 mg, 0.51 mmol), sodium chlorite (454 mg, 5.04 mmol), and tetrabutylammonium chloride (10 mg, 0.034 mmol) in CH_3CN (0.2 mL). The mixture was stirred at 40 °C for 2 min. One drop of sodium hypochlorite solution (0.05 mL, available chlorine 10−13%) was added. The solution turned deep brown immediately. After being stirred for 5 h, the reaction solution turned light yellow. The solvent was evaporated, and the residue was loaded onto a C-18 reverse phase column and eluted with water (50 mL) to remove inorganic residue and then with methanol to recover the product (**29a**). Methanol was evaporated, and the residue was dissolved in DMF (2 mL) and pyridine (4 mL). *tert*-Butyldimethylsilyl chloride (831 mg, 5.50 mmol) was added. After the mixture was stirred for 5 days, the reaction was quenched by adding saturated $NaHCO_3$ solution (2 mL). The organic layer was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (CH_2Cl_2/MeOH, 12:1) to afford **4a**. Yield 365 mg, 85% for two steps. TLC R_f = 0.45 (CH_2Cl_2/MeOH, 12:1). IR: 2101, 1725 cm^{-1}. Elemental analysis calculated for $C_{17}H_{27}N_5O_6Si$ + 0.5 H_2O: C, 47.03; H, 6.27; N, 16.13. Found: C, 47.04; H, 6.30; N, 15.83. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.99 (1H, s), 7.58 (1H, d), 5.83 (1H, d), 5.59 (1H, S), 4.56 (2H, m), 3.62 (1H, dd), 3.41 (1H, dd), 2.93 (1H, dd), 2.34 (1H, m), 0.91 (9H, s), 0.15 (6H, d). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.7, 164.7, 150.4, 140.8, 102.2, 93.6, 79.3, 48.0, 45.9, 38.2, 26.0, 18.3, −4.21, −5.25. HRMS (ESI) calculated for $C_{17}H_{27}N_5O_6Si$ 425.1731, found [M + 1] 426.1809.

3′-Azidomethyl-4-*N*-benzoyl-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-3′,5′-dideoxycytidine (4b). A phosphate buffer (pH 7, 0.1 mL) was added to a mixture of **28b** (145 mg, 0.36 mmol), 2,2,6,6-tetramethylpiperidilyloxy radical (TEMPO, 28 mg, 0.18 mmol), sodium chlorite (162 mg, 1.8 mmol), and tetrabutylammonium chloride (5 mg, 0.018 mmol) in CH_3CN (0.1 mL). The mixture was stirred at 40 °C for 2 min. One drop of sodium hypochlorite solution (0.05 mL, available chlorine 10−13%) was added. The solution turned deep brown immediately. After being stirred for 5 h, the reaction solution turned light yellow. The solvent was evaporated, and the residue was loaded onto a C-18 reverse phase column and eluted with water (50 mL) to remove inorganic salts and then with methanol to recover the product (**29b**). Methanol was evaporated, and the residue was dissolved in CH_2Cl_2 (5 mL). Dry 2,6-lutidine (270 mg, 2.52 mmol) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (475 mg, 1.8 mmol) were added. After the mixture was stirred for 2 days, the reaction was quenched by adding phosphate buffer (pH 6.7, 2 mL) and extracted with CH_2Cl_2 (5 × 10 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel chromatography (CH_2Cl_2/MeOH, 12:1) to afford **4b**. Yield 162 mg, 85% for two steps. TLC R_f = 0.46 (CH_2Cl_2/MeOH, 12:1). IR: 2101, 1757 cm^{-1}. Elemental analysis calculated for $C_{24}H_{34}N_6O_6Si$: C, 54.32; H, 6.46; N, 15.84. Found: C, 54.32; H, 6.01; N, 15.76. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.01 (2H, m), 7.64−7.51 (3H, m), 5.68 (1H, m), 3.64 (1H, dd), 3.43 (1H, dd), 2.91 (1H, dd), 2.71 (1H, dd), 1.99 (1H, m), 0.95 (9H, s), 0.32 (3H, s), 0.20 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 172.8, 167.6, 163.5, 156.0, 144.0, 133.3, 133.2, 129.0, 128.2, 97.2, 94.1, 80.0, 47.5, 46.2, 38.8, 26.1, 18.3, −4.1, −5.6. HRMS (ESI) calculated for $C_{24}H_{34}N_6O_6Si$ 528.2153, found [M + 1] 529.2231.

3′-Azidomethyl-4-*N*-benzoyl-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-3′,5′-dideoxyadenosine (4c). Compound **28c** (22 mg, 0.07 mmol) was converted into **4c** using the same procedure as described above for **4b**. Yield 21 mg, 67% for two steps. TLC R_f = 0.41 (CH_2Cl_2/MeOH, 12:1). IR: 2102, 1731 cm^{-1}. Elemental analysis calculated for $C_{25}H_{32}N_8O_5Si$ + 0.5 H_2O: C, 53.46; H, 5.93; N, 19.95. Found: C, 53.24; H, 5.67; N, 19.01. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.68 (1H, s), 8.36 (1H, s), 8.00 (1H, m), 7.60−7.45 (3H, m), 5.95 (1H, s), 4.78 (1H, s), 4.50 (1H, s), 3.59 (1H, m), 3.45 (1H, m), 2.83−2.47 (3H,m), 0.91 (9H,s), 0.17 (3H, s), 0.13 (3H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 165.9, 152.4, 151.5, 149.4, 142.1, 133.5, 133.0, 132.9, 128.9, 128.3, 123.3, 91.9, 47.7, 45.9, 25.9, 18.2, −4.26, −5.24.

3′-Azidomethyl-2′-*O*-*tert*-butyldimethylsilyl-5′-carboxy-3′,5′-dideoxy-2-*N*-phenoxyacetylguanosine (4d). Compound **28d** (150 mg, 0.32 mmol) was converted into **4d** using the same procedure as described above for **4b**. Yield 90 mg, 47% for two steps. TLC R_f = 0.43 (CH_2Cl_2/MeOH, 12:1). IR: 2102, 1731 cm^{-1}. Elemental analysis calculated for $C_{26}H_{34}N_8O_7Si$ + 0.5 H_2O: C, 51.39; H, 5.81; N, 18.44. Found: C, 51.20; H, 5.60; N, 17.99. ^1H NMR ($CDCl_3$, 300 MHz): δ 9.11, (1H, s), 8.47 (1H, s), 7.37 (2H, m), 7.10 (1H, m), 6.98 (2H, m), 5.84 (1H, s), 4.72 (2H, s), 4.65 (1H, m), 4.40 (1H, m), 3.62−3.47 (2H, m), 3.05−2.79 (3H, m), 0.91 (9H, s). 0.08 (6H, s). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 173.6, 170.0, 156.7, 155.2, 150.7, 147.2, 146.5, 138.9, 130.2, 123.1, 121.1, 114.9, 91.4, 79.3, 67.0, 47.9, 44.7, 37.5, 25.9, 18.3, −4.4, −5.0. HRMS (ESI) calculated for $C_{26}H_{34}N_8O_7Si$ 598.2320, found [M + 1] 599.2398.

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Supporting Information Available: Experimental procedures, spectral data, and copies of ^1H and ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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