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Monomers for Preparation of Amide-Linked RNA: Asymmetric Synthesis of All Four Nucleoside 5′-Azido 3′-Carboxylic Acids

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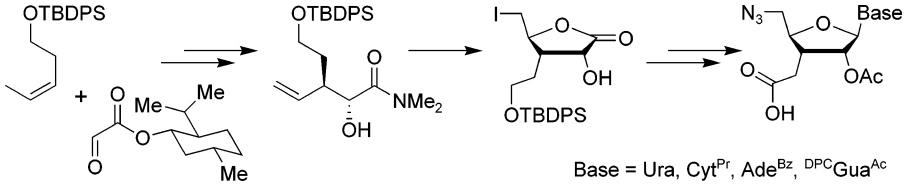

Recent discovery of RNA interference as an efficient and naturally occurring mechanism of gene regulation has reinvigorated the interest in chemically modified RNA. For potential in-vivo applications small interfering RNAs require chemical modifications to fine-tune the thermal stability and increase the cellular delivery and potency and in vivo half-life of the RNA duplexes. From this perspective, amides as neutral and hydrophobic internucleoside linkages in RNA are highly interesting modifications for RNA interference. Amides are remarkably good mimics of the phosphodiester backbone of RNA and can be prepared using a relatively straightforward peptide coupling chemistry. However, the progress in the field has been hampered by the shortage of efficient methods to synthesize the monomeric building blocks for such couplings, the nucleoside amino acid equivalents. Herein, we report enantioselective synthesis of 5′-azido 3′-carboxylic acid derivatives of all four natural ribonucleosides. The key transformations in our synthesis are a double asymmetric ene reaction and a stereoselective iodolactonization that form the basic carbon skeleton of the modified ribose. Standard nucleoside synthesis is followed by a short and highly efficient protecting group manipulation to give the enantiomerically pure (>98%) title compounds in 9−10 steps and 15−19% overall yields starting from small achiral molecules. The present results are a significant improvement over our first-generation racemic synthesis and compare favorably with the previously reported synthesis from nucleoside and carbohydrate precursors.

Introduction

Nucleic acid analogues where the phosphodiesters are replaced with nonionic non-phosphorus linkages began to attract wide interest about a decade ago as potential nuclease-resistant antisense compounds.[1−3] Short hybrid DNA−RNA A-form duplexes, where the DNA strands have a few selected phosphodiesters replaced by neutral synthetic linkages, have been the most extensively studied model systems for testing the properties of such linkages.[2,3] Of many nonionic DNA analogues tested, amide[3−5] (Figure 1, 3′-CH_2−CO−NH-5′ (**1a**) and 3′-CH_2−NH−CO-5′ (**2a**)), methylene(methylimino)[6] (3′-CH_2−N(CH_3)−O-5′), formacetal[7,8] (3′-O−CH_2−O-5′), and thio-

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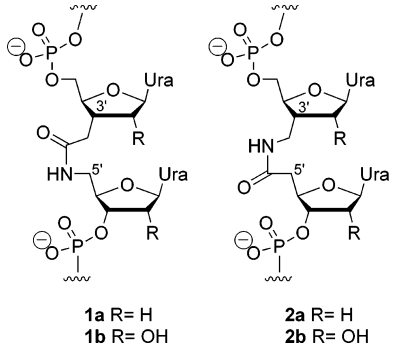

FIGURE 1. Amides as internucleoside linkages.

formacetal[8] (3′-S−CH_2−O-5′) analogues form thermally stable double helices.

Emerging RNA-based gene control technologies,[9] especially, the discovery of RNA interference,[10] have invigorated the interest in chemically modified RNA.[11,12] Modifications of the sugar−phosphate backbone such as phosphorothioates,[12a] 2′-*O*-alkyl,[12a−c] 2′-F,[12a−c] and 4′-thio[12d] RNA, and even locked nucleic acid (LNA)[12a,e] are generally well-tolerated in RNA interference. Moreover, small interfering RNAs (siRNAs) having boranophosphate-modified internucleoside linkages are at least as active as the native siRNAs.[12f] Despite the potentially interesting applications in RNA interference, there are relatively few reports on synthesis and properties of RNA analogues having dephospho linkages. Ribonucleoside dimers having thioformacetal[13] and sulfide[14] (3′-CH_2−CH_2−S-5′) linkages have been prepared, incorporated in short DNA fragments, and shown to destabilize short DNA−RNA duplexes. The analysis of these data, however, is complicated because the ribonucleoside dimers were incorporated in DNA, which may cause complex interplay of alternating (DNA and RNA) sugar compositions.[15] Benner and co-workers[16] studied dimethylene sulfone (3′-CH_2−SO_2−CH_2-5′) linked DNA and RNA. Substitution of a single phosphodiester with the sulfone linkage strongly destabilized DNA−DNA and DNA−RNA duplexes.[16a] Although the crystal structure of dinucleoside analogue r(G_{SO2}C) revealed a slightly distorted Watson−Crick miniduplex,[16b] all sulfone-linked octamers did not base pair with complementary oligonucleotides but self-associated and formed a very stable self-folded structure instead.[16c,d] Recently, we showed that formacetal[17] and both isomeric amide[18] (Figure 1, **1b** and **2b**) linkages were well-accommodated in all RNA−RNA duplexes. Whereas the **1b**-modified duplexes have thermal stability similar to the nonmodified controls, the **2b** modification remarkably stabilizes the RNA−RNA duplexes (Δt_m up to over 2 °C per modification).[18] This is in contrast to the results by De Mesmaeker and co-workers in the DNA series where both **1a**- and **2a**-modified duplexes show thermal stability similar to the nonmodified DNA.[4,5]

Except for the extensively studied peptide nucleic acids, which form stable Watson−Crick base paired helices with complementary DNA, RNA, and itself,[19,20] only a few studies report on nucleic acid analogues having uniformly modified amide backbones. All amide-linked DNA (Figure 1, analogue of **1a**) forms stable duplexes with the complementary unmodified RNA and DNA.[21] Robins and co-workers[22] prepared all amide-linked uridine pentamer (Figure 1, analogue of **1b**) but did not disclose the biochemical properties of this analogue.

A practical advantage of oligoamide analogues of nucleic acids is that they could be prepared using a relatively straightforward peptide coupling chemistry. The synthetic challenge, that so far has hindered the progress in the field, is the synthesis of the highly modified monomers, C-branched nucleoside amino acids (such as **3**, Figure 2) required for the peptide type couplings.

Traditional syntheses of modified nucleosides analogous to **3** start from readily available chiral pool compounds: nucleosides or carbohydrates. Several research groups have synthesized compounds related to **3**. Von Matt and co-workers synthesized 2′-deoxy[21] and 2′-OMe[23] derivatives of **3** starting from nucleosides. Robins and co-

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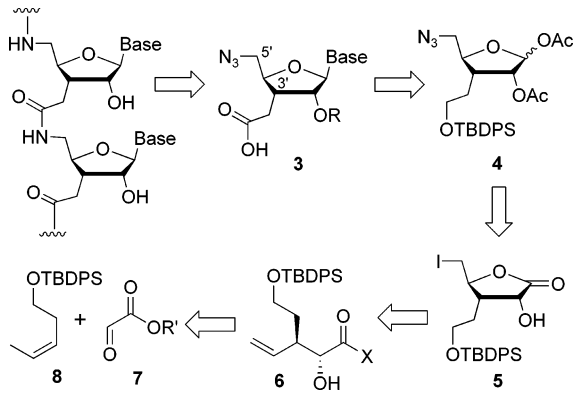


FIGURE 2. Retrosynthetic analysis of amide-linked RNA.

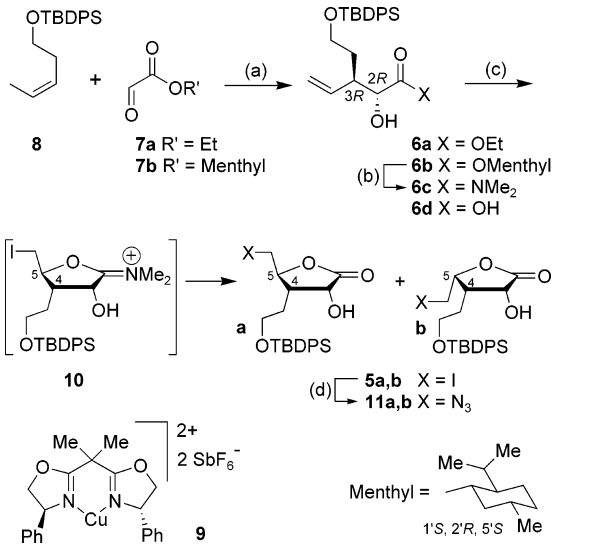
workers[24] prepared a series of ribonucleosides **3** (base = adenine and thymine) starting from nucleosides[24a,b] or protected xylose.[24c] Both groups used the Wittig reaction followed by a stereoselective hydrogenation to form the new carbon−carbon bond at C3′. The advantage of traditional carbohydrate-based routes is that the stereochemical relationships are already set or can be easily adjusted in the chiral pool starting materials. However, such routes are frequently lengthy and laborious because the multifunctional and sensitive starting materials limit the synthetic methodology that can be used and require extensive protecting group manipulations.

In a preliminary paper, we recently reported de novo synthesis of the racemic uridine derivative of **3** (Figure 2, base = Ura, R = *tert*-butyldimethylsilyl (TBS)).[25] Although conceptually similar approaches have been frequently used to prepare carbocyclic nucleosides,[26] applications of total synthesis principles to modify natural nucleosides are relatively rare.[27,28] The most relevant precedent is of Lavallee and Just[27] who synthesized 3′-carbomethoxymethyl thymidine using a radical cyclization as the key step. In the present paper we report a full account on further developments of our total synthesis route that culminated in asymmetric synthesis of all four protected nucleoside azido acids (Figure 2, **3**, base = Ura, Cyt, Ade, Gua, R = Ac). The synthesis was accomplished in 9−10 steps and 15−19% overall yield, which constitutes a significant improvement over the previously reported syntheses.[24,25]

Results and Discussion

Retrosynthetic Analysis. Our retrosynthetic analysis of **3** (Figure 2) disconnects the heterocyclic base first.

SCHEME 1[a]



[a] Conditions: (a) for **7b**:**9**, CH_2Cl_2, rt, 3 days, 89% (dr 98:2); (b) Me_2NH−HCl, $AlMe_3$, toluene, 70−90 °C, 2 days, 79%; (c) I_2, $NaHCO_3$, THF/H_2O (2:1), 0 °C, 26 h, 65%; (d) NaN_3, DMF, 50 °C, 23 h, 78%.

Such an approach allows all four nucleoside derivatives to be made from the same modified glycosyl donor **4**. Adjustment of functional groups in **4** reveals the iodolactone **5** as the key intermediate. Opening of the five-membered tetrahydofuran ring (iodolactonization) identifies the unsaturated carboxylic acid **6** (or a derivative thereof) as the next intermediate. These disconnections reduce the complexity from two heterocycles and four stereogenic centers in **3** to only two stereogenic centers in the acyclic **6**, which can be further disconnected to simple organic compounds **7** and **8** to be joined in a stereoselective ene reaction.

Double Asymmetric Ene Reaction. In our preliminary paper,[25] reaction of ethyl glyoxylate **7a** with alkene **8** in the presence of the bis(oxazolinyl)copper(II) catalyst **9**, developed by Evans and co-workers,[29] gave the chiral hydroxy ester **6a** in 70% yield after 4 days (Scheme 1). The use of anhydrous **9** was essential; the reaction catalyzed by the aqua complex of **9** was unacceptably slow (30% after 7 days). Enantioselective HPLC (Chiralcel OD-H) established that **6a** was formed along with its enantiomer in a ratio of 95:5.

In the present study, this result was substantially improved by performing the reaction in a double asymmetric mode. Whitesell and co-workers[30] have described a series of menthol-derived auxiliaries for carbonyl ene reactions. We chose the modestly selective (1′*S*,2′*R*,5′*S*)-menthyl glyoxalate **7b** because the low cost and easy large-scale preparation of **7b** were considered more important than a higher asymmetric induction achievable by more expensive alternatives (such as 8-phenylmenthyl

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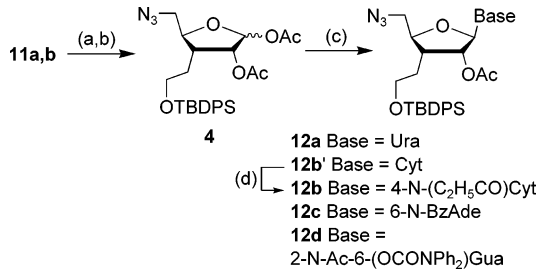
glyoxylate). Interestingly, the reaction of **7b** with alkene **8** in the presence of catalyst **9** gave **6b** in a higher yield (89%) over a shorter period of time (3 days) than in the case of ethyl glyoxylate (Scheme 1). In the ^{1}H NMR spectrum, the peaks due to the (2*S*,3*S*) diastereomer of **6b** were barely visible and could not be reliably integrated (see Supporting Information). Assuming a 2% cutoff for the sensitivity of NMR integration, the NMR suggested that the diastereomer ratio (dr) was better than 98:2. This result was later confirmed by enantioselective HPLC. The work of Mikami et al. has established good precedent for the regioselectivity and the anti diastereoselectivity in the carbonyl ene reactions of this type.[31] The ^{1}H NMR spectrum of **6b** also indicated the presence of <10% of a minor compounds, possibly the syn diastereomers, which were separated by flash chromatography at later stages.

Stereoselective Iodolactonization and Synthesis of Modified Ribose. Next we planned to construct the tetrahydofuran ring by stereoselective iodolactonization. From the literature precedents[32] we anticipated that under thermodynamic control the C4−C5 trans isomer would be the major product. Iodolactonization of ethyl (**6a**), menthyl (**6b**), or trimethylsilyl esters gave complex mixtures that did not contain the desired lactone. Iodolactonization (under conditions given in Scheme 1) of carboxylic acid **6d** gave a mixture of C4−C5 trans and cis lactones **5a** and **5b** in a ratio of 1.5:1 (56% yield). Eventually, the best results were achieved with amides as the cyclization precursors.[33] Thus, treatment of ester **6b** with the aluminum amide reagent prepared from $AlMe_3$ and dimethylamine hydrochloride gave the dimethylamide **6c** in 79% yield. Iodolactonization of **6c** gave the desired iodolactone as a nonseparable mixture of C4−C5 trans (**5a**) and cis (**5b**) diastereomers in a ratio of 4:1. Providing that the hydrolysis of the intermediate iminium ion **10** is slow enough to allow for thermodynamic control in the iodolactonization of amide **6c**, it is conceivable that the observed 4:1 stereoselectivity reflects the sterically most favorable trans arrangement of the two alkyl substituents on the relatively flat five-membered ring in **10**.[32,33] A brief study of reaction conditions revealed that a tetrahydrofuran (THF)/water mixture was the best solvent; MeCN, CH_2Cl_2, MeOH, dimethylformamide (DMF), dimethoxyethane, and diethyl either alone or with water present reduced the yield. Sodium bicarbonate was the best base; the use of $NHEt_3^+HCO_3^-$, NEt_3, K_2CO_3, and $NBu_4^+OH^-$ reduced both yield and stereoselectivity. Whereas dimethyl and pyrrolidinyl amides performed equally well, other amide derivatives gave inferior results: EtNH−, low yield and selectivity; PhNH−, no product; MeO(Me)N−, yield 50%. The best results were achieved at 0 to −10 °C; lower or higher temperatures reduced the yield and selectivity. Finally, the use of *N*-iodosuccinimide instead of iodine gave the mixture of **5a** and **5b** in 58% yield and 2:1 ratio. Because we could not efficiently separate the trans and cis isomers of **5** (or later intermediates) by flash column chromatography, the following synthetic steps were carried out on mixtures (for clarity only the major diastereomer is shown in Scheme 2). Separation of isomers was achieved at later steps after the synthesis of nucleosides. Treatment of iodolactones **5** with sodium azide gave the mixture of trans and cis azidolactones **11a,b**. Selective reduction of the lactone group[34] was immediately followed by acetylation of the hydroxyl groups to give the glycosyl acetate **4** as a mixture of four diastereomers (Scheme 2).

SCHEME 2[a]



[a] Conditions: (a) DIBAL-H, toluene, −78 °C, 110 min; (b) Ac_2O/pyridine (1:1), rt, 23 h, 86% (two steps); (c) for **12a**, *O*,*O*′-bis(trimethylsilyl)uracil, TMSOTf, CH_2Cl_2, rt, 3 h, > 99%; for **12b**′, *O*,*N*-bis(trimethylsilyl)cytosine, TMSOTf, $ClCH_2CH_2Cl$, reflux, 50 min, >99%; for **12c**, 6-*N*-benzoyladenine, $SnCl_4$, CH_3CN, rt, 30 min, 60%; for **12d**, BSA, 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine, $ClCH_2CH_2Cl$, reflux, 10 min, add to **4** in $ClCH_2CH_2Cl$, add TMSOTf, reflux 1 h, 56%; (d) TMSCl, 1 h, propionic anhydride, 16 h, H_2O, 20 min, pyridine, rt, 61%.

Synthesis of Modified Nucleosides. Following the standard Vorbrüggen methodology,[35] coupling of bis(trimethylsilyl) heterocycles with **4** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave the modified pyrimidine nucleosides **12a** and **12b**′. For the synthesis of adenosine derivative **12c**, we used the tin tetrachloride mediated reaction of **4** with 6-*N*-benzoyladenine.[36] To achieve a regioselective synthesis of guanosine derivative **12d**, we used the methodology developed by Zou and Robins.[37] Thus, the coupling of **4** with persilylated 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine gave **12d** as a single 9-*N*-isomer. At this stage the 3′,4′-cis and -trans isomers of purine nucleosides were separated by silica gel chromatography, giving **12c** and **12d** as single diastereomers. The somewhat lower yield of the purine nucleosides reflected the removal of the undesired 3′,4′-cis diastereomers. The pyrimidine nucleosides **12a** and **12b**′ were still inseparable mixtures of isomers.

Protecting Group Manipulation and Final Steps. The most important issue after the synthesis of nucleosides was the design of a protecting group scheme for the final compounds, azido acids **3a**−**d**. The need for extensive protection−deprotection of hydroxyl and amino

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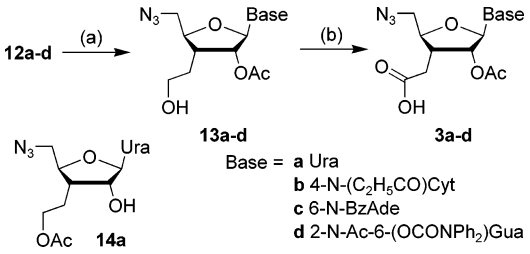
SCHEME 3[a]

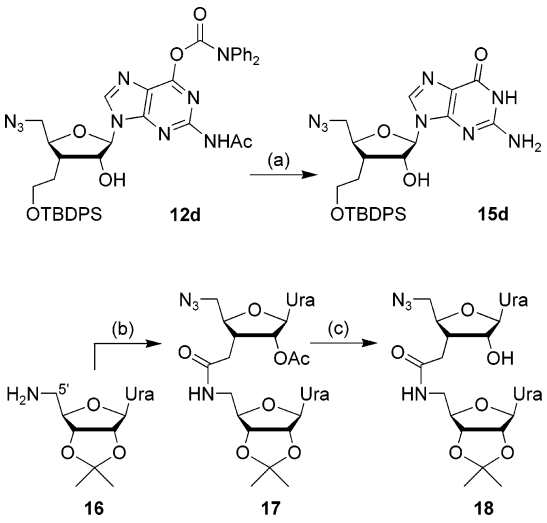


[a] Conditions: (a) TBAF/AcOH (1:1), THF, rt, (**13a**) 2.75 h, 79%, (**13b**) 5 h, 99%, (**13c**) 4 h, 93%, (**13d**) 5 h, 98%; (b) TEMPO (cat.), (diacetoxyiodo)benzene, CH_3CN/H_2O (1:1), (**3a**) rt, 41 h, 80%, (**3d**) 0 °C, 4 days, 87% or (i) Dess−Martin periodinane, CH_2Cl_2, 1.5 h; (ii) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, *tert*-butyl alcohol/H_2O (1:1), rt, 25 min, (**3b**) 86%, (**3c**) 86%.

functions is one of the most severe problems in carbohydrate chemistry. In our initial design, published in the preliminary paper,[25] we removed both the TBDPS ether and the 2′-*O*-Ac groups from **12a** and used a two-step silylation-selective deprotection sequence to install the 2′-*O*-TBS protection. The overall yield of this sequence was 37%, reflecting the difficulty of protecting group manipulations on advanced carbohydrate intermediates. Herein we describe a second-generation design that retains the base labile 2′-*O*- and *N*-acyl protections and minimizes the protection−deprotection steps. Because the 2′-*O*-Ac group was required for the stereoselective synthesis of nucleosides and the 2-*N*-acetyl and 6-*O*-diphenylcarbamoyl (DPC) groups were required for the regioselective synthesis of the guanosine derivative **12d**, we envisioned that the most efficient design would keep these base labile protections in the final products. We were also encouraged by our previous successful experience with 2′-*O*- and *N*-acyl groups in synthesis of native[38] and modified[17,18] RNA. Thus, the cytidine **12b′** was further converted into 4-*N*-propionyl derivative **12b**, which was isolated as a single diastereomer after silica gel chromatography.

Next we investigated selective removal of the TBDPS ether in the presence of the 2′-*O*-Ac group in **12a**, which was a problem during our preliminary work.[25] Treatment of **12a** with tetrabutylammonium fluoride (TBAF) in THF resulted in a mixture of compounds. Along with the expected **13a** and its 3′,4′-cis isomer, the mixture contained the fully deprotected diol (ca 5%) and another byproduct (25%). The NMR spectrum of the latter was consistent with structure **14a**, where the acetyl group had migrated to the primary alcohol (Scheme 3). It is conceivable that the spatial 1,7 relationship of the acetyl and the TBDPS ether cause this unexpected side reaction. Similar results were obtained using the less basic NH_4F in methanol[39] and the weakly acidic HF in pyridine and $HSiF_6$ in acetonitrile.[40] The strongly acidic 1% HCl in methanol caused complete deprotection. After considerable experimentation we found that a mixture of TBAF and acetic acid (1:1) in THF cleanly removed the TBDPS group in **12a** without any side reactions at the 2′-*O*-Ac.[41] At this stage the 3′,4′-isomers of **13a** were separated by silica gel chromatography. The buffered TBAF reagent was also successful with the base-protected nucleosides **12b**−**d**, yielding all four primary alcohols **13a**−**d** as pure diastereomers.

SCHEME 4[a]



[a] Conditions: (a) NH_3/MeOH, rt, 24 h, >90%; (b) **3a**, HCTU, DIEA, DMF, rt, 50 min, 83%; (c) NH_3/MeOH, rt, 20 min, >99%

The last step, oxidation of alcohol to carboxylic acid, was achieved for **13b** and **13c** in a two-step process using Dess−Martin periodinane followed by $NaClO_2$. For **13a** and **13d**, a combination of TEMPO (catalytic) and (diacetoxyiodo)benzene gave somewhat better results.

Stability of Protecting Groups and Internucleoside Amide Linkage. The conditions for removal of the base labile groups were established using the guanosine derivative **12d** (Scheme 4). Thus treatment of **12d** with an anhydrous solution of NH_3 in methanol (saturated at 0 °C) resulted in a clean and complete removal of all three protecting groups (2′-*O*-Ac, 2-*N*-Ac, and 6-*O*-DPC) in less than 24 h at room temperature. The estimated half-life (from thin-layer chromatography (TLC)) of formation of the fully deprotected guanosine derivative **15d** was less than 5 h. Under the same conditions cytidine **12b** and adenosine **12c** were rapidly and fully deprotected within 3 and 6 h, respectively.

To check the stability of the 2′-*O*-Ac group and the internucleoside amide linkage under the deprotection conditions, we prepared dimer **17** (Scheme 4). 2-(6-Chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) mediated coupling of uridine azido acid **3a** with amine **16** gave the dimer **17** in good yield. Exposure of **17** to a saturated solution of NH_3 in methanol at room temperature resulted in a rapid (<20 min) removal of the 2′-*O*-Ac group to form **18**. Further exposure of **18** to the same deprotection solution

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(41) (a) Hayward, C. M.; Yohannes, D.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1993**, *115*, 9345−9346. (b) Smith, A. B.; Chen, S. S.-Y.; Nelson, F. C.; Reichert, J. M.; Salvatore, B. A. *J. Am. Chem. Soc.* **1995**, *117*, 12013−12014. (c) Panek, J. S.; Liu, P. *J. Am. Chem. Soc.* **2000**, *122*, 11090−11097.

resulted in no observable cleavage (TLC and NMR) of the internucleoside amide even after 6 days at room temperature. However, treatment of **18** with a mixture of aqueous NH_3 and methanol (3:1) at 65 °C resulted in about 80% degradation of the amide after 16 h. Thus, the internucleoside amide linkages **1b** (as in dimer **18**) are notably more reactive than regular alkyl amides. This must be a result of neighboring group assistance due to a spatial 1,5 relationship of amide and 2′-OH. Nevertheless, anhydrous NH_3 in methanol at room temperature provides a safe reactivity window for removal of all protecting groups without detectable cleavage of the internucleoside amides.

Conclusions

We have developed the asymmetric synthesis of all four enantiomerically pure (>98%) nucleoside 5′-azido 3′-carboxylic acids **3a**−**d** in nine steps (10 for cytidine) starting from **7b** and **8**. The number of steps and the overall yields (**3a**, 19%; **3b**, 16%; **3c**, 15%; **3d**, 15%) compare favorably with the previously reported synthesis from D-xylose[24] and our first-generation racemic synthesis.[25] It is conceivable that our synthesis may be further improved by employing an alternative and more efficient electrophilic cyclization. The asymmetric synthesis has several important advantages over the traditional routes that start from sugars or nucleosides. Carbohydrate routes are constrained by the choice of the chiral pool starting material and, once developed, are difficult to change and improve. In contrast, the asymmetric synthesis is extremely flexible for optimization. Because the upstream starting materials are usually small simple molecules, it is much easier to find a new reaction that provides a better synthesis of a key intermediate. We have recently demonstrated such an improvement in the asymmetric synthesis of modified nucleosides related to **3a**−**d**, the isomeric 3′-azido 5′-carboxylic acids.[42] Because the key 3′-C alkyl substituent does not directly participate in the chemical transformations, it is conceivable that the synthesis of **3a**−**d** reported herein could be modified to provide other nucleoside analogues with different 3′-C alkyl substitution patterns. Finally, the asymmetric synthesis provides access to both enantiomers of the modified nucleosides by choosing the appropriate chiral catalyst and auxiliary, which is not possible starting from sugars or nucleosides. Such an option may be highly useful for potential applications of amide-modified RNA in biotechnology, biomedicine, and nanotechnology. Current work in our laboratory is focused on solid-phase synthesis, properties, and applications of oligoamides derived from the nucleoside 5′-azido 3′-carboxylic acids **3a**−**d** reported herein.

Experimental Section

(2*R*,3*R*)-3-[2-(*tert*-Butyldiphenylsilanoxy)ethyl]-2-hydroxy-4-pentenoic Acid (1′*S*, 2′*R*, 5′*S*)-Menthyl Ester (6b). The reaction was done in a glovebox under an atmosphere of dry nitrogen. 2,2′-Isopropylidenebis[(*R*)-4-phenyl-2-oxazoline] (**9**; 0.45 g, 1.35 mmol) and $CuCl_2$ (0.18 g, 1.35 mmol) in dry CH_2Cl_2 (10 mL) were stirred for 4 h. The green solution was filtered through a 25 mm hydrophobic poly(tetrafluoroethylene) (PTFE) membrane filter (0.45 μm, Waters). $AgSbF_6$ (0.93 g, 2.7 mmol) in CH_2Cl_2 (40 mL) was added to the clear filtrate, the mixture was stirred for 3 h, carefully decanted from the majority of precipitate, and filtered through a 25 mm hydrophobic PTFE membrane filter (0.45 μm, Waters). To the clear green solution were added 1-(*tert*-butyldiphenylsilanoxy)-3-pentene[25] (**8**; 4.32 g, 13.32 mmol) and freshly distilled (1′*S*,2′*R*,5′*S*)-menthyl glyoxalate[30b] (**7b**; 28 g, 132 mmol), and the mixture was stirred for 3 days at room temperature. The solvent was evaporated, and the residue was purified by silica gel column chromatography (5−20% of ethyl ether in hexanes, stepwise gradient by 5%) to afford **6b** as oil. Yield: 6.36 g, 89%. TLC R_f = 0.33 hexanes/ethyl ether (7:3). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.69−7.66 (m, 4H), 7.45−7.34 (m, 6H), 5.72−5.60 (m, 1H), 5.18−5.00 (m, 2H), 4.83−4.74 (m, 1H), 4.24−4.21 (m, 1H), 3.80−3.65 (m, 2H), 2.89−2.81 (m, 2H), 1.98−1.66 (m, 6H), 1.54−1.35 (m, 2H), 1.06 (s, 9H),1.10−0.75 (overlapping m, 2H), 0.90 (t, J = 6.7 Hz, 6H), 0.76 (d, J = 7.2 Hz, 3H). TLC R_f = 0.33 hexanes/ether (7:3). HRMS (ESI). Calcd for $C_{33}H_{48}O_4Si$ $[M + H]^+$: 537.3400. Found: 537.3348. The ^{1}H NMR spectrum contained minor impurities: <2% of the (2*S*,3*S*) diastereomer (well-separated doublet at 0.72 ppm) and <10% total of not identified compounds, presumably the syn diastereomers (see Supporting Information). The following two experiments (for NMR spectra, see Supporting Information) were used to clarify the sense of asymmetric induction by menthyl auxiliaries and catalyst **9** (matched vs mismatched combinations) and to assign the ^{1}H NMR spectra of the diastereomers. Reaction of (1′*R*,2′*S*,5′*R*)-menthyl glyoxylate (the enantiomer of **7b**) catalyzed by $SnCl_4$ gave the products with dr of 3:1 (the major product was the enantiomer of **6b**). The mismatched double asymmetric reaction of (1′*R*,2′*S*,5′*R*)-menthyl glyoxylate (the enantiomer of **7b**) catalyzed by **9** gave the same products with dr of 1:5. In all cases, the observed stereoselectivity was in good agreement with our previous study[25] and literature precedents.[29,30] ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 174.2, 135.7, 133.9, 129.7, 127.8, 118.2, 75.9, 72.7, 61.4, 47.1, 43.9, 41.1, 34.2, 33.9, 31.5, 27.0, 26.3, 23.4, 22.1, 20.9, 19.4, 16.4.

For experimental procedure for synthesis of **6c**, see Supporting Information.

(3*R*,4*S*,5*S*)-4-[2-(*tert*-Butyldiphenylsilanoxy)ethyl]-3-hydroxy-5-iodomethyl-2-dihydrofuranone (Mixture of 5a and 5b). Iodine (0.47 g, 1.85 mmol) was added to the solution of (2*R*,3*R*)-3-[2-(*tert*-butyldiphenylsilanoxy)ethyl]-2-hydroxy-4-pentenoic acid dimethylamide (**6c**; 0.20 g, 0.47 mmol) in THF (2 mL) and saturated aqueous $NaHCO_3$ (1 mL) at 0 °C. The mixture was stirred for 26 h at 0 °C. Saturated aqueous $Na_2S_2O_3$ (3 mL) and ether (3 mL) were added to quench the reaction. The aqueous layer was extracted with ether (5 × 3 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography (5−20% of ethyl acetate in hexanes, stepwise gradient by 5%) to afford a nonseparable mixture of C4−C5 trans (**5a**) and cis (**5b**) iodolactones in a ratio of 4:1. Yield: 0.16 g, 65%. TLC R_f = 0.27 CH_2Cl_2/2-propanol (100:1). IR: 3430, 1778 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz; major diastereomer (C3−C4 trans)): δ 7.68−7.65 (m, 4H), 7.48−7.38 (m, 6H), 4.54−4.48 (m, 1H), 4.32−4.26 (m, 1H), 3.78−3.65 (m, 3H), 3.44−3.28 (m, 2H), 2.63−2.54 (m, 1H), 2.07−1.96 (m, 1H), 1.72−1.61 (m, 1H), 1.07 (s, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 175.6, 135.7, 132.8, 132.8, 130.1, 128.0, 81.7, 69.1, 62.1, 43.7, 28.6, 27.0, 19.2, 6.2.

For experimental procedures for synthesis of **4**, **11a**, and **11b**, see Supporting Information.

2′-*O*-Acetyl-5′-azido-3′-[2-(*tert*-butyldiphenylsilanoxy)-ethyl]-3′,5′-dideoxyuridine (12a). A solution of 1,2-di-*O*-acetyl-5-azido-3-[2-(*tert*-butyldiphenylsilanoxy)ethyl]-3,5-dideoxyribofuranose (**4**; 0.31 g, 0.58 mmol), *O*,*O*′-bis(trimethylsilyl)uracil[35a] (0.30 g, 1.17 mmol), and trimethylsilyl trifluoromethanesulfonate (0.26 g, 1.17 mmol) in CH_2Cl_2 (20 mL) was

(42) Compare the routes in: (a) Rozners, E.; Xu, Q. *Org. Lett.* **2003**, *5*, 3999−4001. (b) Xu, Q.; Rozners, E. *Org. Lett.* **2005**, *7*, 2821−2824.

stirred for 3 h. CH_2Cl_2 (80 mL) and saturated aqueous $NaHCO_3$ (80 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography (1−3% of 2-propanol in CH_2Cl_2 stepwise gradient by 1%) to afford **12a**. Yield: 0.34 g, 99%. TLC R_f = 0.37 CH_2Cl_2/2-propanol (94:6). IR: 2104, 1745 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz; major diastereomer): δ 10.02 (s, H), 7.65−7.61 (m, 4H), 7.50−7.35 (m, 7H), 5.82−5.72 (m, 2H), 5.26 (d, J = 6.3 Hz, 1H), 4.05−4.00 (m, 1H), 3.80−3.62 (m, 3H), 3.47 (dd, J = 4.2 Hz, 13.5 Hz, H), 2.68−2.58 (m, 1H), 2.02 (s, 3H), 1.72−1.48 (m, 2H), 1.06 (s, 9H). MS (ESI). Calcd for $C_{29}H_{35}N_5O_6Si$: 577.2. Found $[M + H]^+$: 578.3.

2′-*O*-Acetyl-5′-azido-3′-[2-(*tert*-butyldiphenylsilanoxy)-ethyl]-3′,5′-dideoxycytidine (12b′). Trimethylsilyl trifluoromethanesulfonate (0.34 g, 1.52 mmol) was added to a solution of **4** (0.40 g, 0.76 mmol) and *O*,*N*-bis(trimethylsilyl)-cytosine[43] (0.39 g, 1.52 mmol) in 1,2-dichloroethane (10 mL) at 0 °C. The solution was refluxed for 50 min, cooled to room temperature, and diluted with cold CH_2Cl_2 (100 mL). Saturated aqueous $NaHCO_3$ (100 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (4 × 100 mL). The combined organic layers were dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (4−8% of methanol in CH_2Cl_2 stepwise gradient by 4%) to afford **12b′**. Yield: 0.44 g, 99%. TLC R_f = 0.14 CH_2Cl_2/2-propanol (94:6). IR: 2102, 1643 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz; major diastereomer): δ 7.66−7.57 (m, 5H), 7.43−7.32 (m, 6H), 5.87 (d, J = 7.5 Hz, H), 5.80 (s, H), 5.34 (d, J = 5.7 Hz, H), 4.05−4.00 (m, 1H), 3.78−3.59 (m, 3H), 3.49 (dd, J = 4.7 Hz, 13.7 Hz, H), 2.50−2.40 (m, 1H), 2.02 (s, 3H), 1.61−1.45 (m, 2H), 1.03 (s, 9H). MS (ESI). Calcd for $C_{29}H_{36}N_6O_5Si$: 576.3. Found $[M + H]^+$: 577.4.

2′-*O*-Acetyl-5′-azido-3′-[2-(*tert*-butyldiphenylsilanoxy)-ethyl]-3′,5′-dideoxy-6 -*N*-benzoyladenosine (12c). $SnCl_4$ (86.3 mg, 0.33 mmol) was added to a solution of **4** (87 mg, 0.17 mmol) and 6-*N*-benzoyladenine (39.6 mg, 0.17 mmol) in CH_3CN (5 mL). After stirring for 30 min, CH_2Cl_2 (25 mL) and saturated aqueous $NaHCO_3$ (25 mL) were added. The aqueous layer was extracted with CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried (Na_2SO_4), concentrated and purified by silica gel column chromatography (2−10% of 2-propanol in 2:1 hexanes/ethyl acetate, stepwise gradient by 2%) to afford **12c** as a single 3′,4′-trans diastereomer. Yield: 70 mg, 60%. TLC R_f = 0.39 CH_2Cl_2/2-propanol (94:6). IR: 2102, 1736 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.79 (s, H), 8.26 (s, H), 8.06−8.03 (m, 2H), 7.65−7.20 (m, 13H), 6.09 (s, 1H), 5.59 (d, J = 5.7 Hz, 1H), 4.23−4.17 (m, 1H), 3.79−3.55 (m, 4H), 3.28−3.18 (m, 1H), 2.10 (s, 1H), 1.85−1.57 (m, 2H), 1.04 (s, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 170.0, 164.7, 152.7, 151.3, 149.7, 141.9, 135.6, 135.6, 133.8, 133.5, 133.3, 132.9, 130.0, 129.0, 128.0, 127. 9, 123.4, 89.9, 83.9, 78.4, 61.5, 52.3, 39.1, 27.7, 26.9, 20.8, 19.2. MS (ESI). Calcd for $C_{37}H_{40}N_8O_5Si$ + Na: 727.3. Found $[M + Na]^+$: 727.2.

2′-*O*-Acetyl-5′-azido-3′-[2-(*tert*-butyldiphenylsilanoxy)-ethyl]-3′,5′-dideoxy-2-*N*-acetyl-6-*O*-diphenylcarbamoylguanosine (12d). Bis(trimethylsilyl)acetamide (BSA; 0.62 g, 3.06 mmol) was added to a solution of 2-*N*-acetyl-6-*O*-diphenylcarbamoylguanine[37] (0.59 g, 1.53 mmol) in 1,2-dichloroethane (22 mL). The mixture was refluxed for 10 min, cooled to room temperature, and added to a solution of **4** (0.40 g, 0.76 mmol) in 1,2-dichloroethane (8 mL). Trimethylsilyl trifluoromethanesulfonate (0.34 g, 1.53 mmol) was added dropwise; the brown solution was refluxed for 1 h, cooled to room temperature, and diluted with CH_2Cl_2 (150 mL). Saturated aqueous $NaHCO_3$ (150 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The white residue was dissolved in CH_2Cl_2 (100 mL) and filtered. The filtrate was concentrated and purified by silica gel column chromatography (7−21% of 2-propanol in hexanes, stepwise gradient by 7%) to afford **12d** as a single 3′,4′-trans diastereomer. Yield: 362 mg, 56%. TLC R_f = 0.42 CH_2Cl_2/2-propanol (94:6). IR: 2102, 1745 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 8.18 (s, H), 8.01(s, H), 7.62−7.57 (m, 4H), 7.45−7.22 (m, 16H), 5.95 (s, H), 5.43 (d, J = 5.7 Hz, H), 4.19−4.13 (m, 1H), 3.79−3.56 (m, 4H), 3.08 (s, H), 2.42(s, 3H), 2.06 (s, 3H), 1.84−1.57 (m, 2H), 1.01 (s, 9H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 169.8, 156.4, 154.2, 152.2, 150.4, 142.4, 141.9, 135.6, 135.6, 133.5, 133.4, 129.9, 129.3, 127.9, 127.9, 127.1, 121.4, 89.7, 83.8, 78.4, 61.8, 55.9, 52.3, 39.1, 27.7, 26.9, 25.2, 20.7, 19.2. MS (ESI). Calcd for $C_{45}H_{47}N_9O_7Si$: 853.3. Found $[M + H]^+$: 854.3.

2′-*O*-Acetyl-5′-azido-3′-(2-hydroxyethyl)-3′,5′-dideoxyuridine (13a). Acetic acid (33 μL, 0.58 mmol) was added to 1 M TBAF solution in THF (0.58 mL). To this solution of 1 M TBAF/HOAc (1:1 mol/mol) was added **12a** (67 mg, 0.12 mmol) dissolved in THF (34 mL). The reaction mixture was stirred for 2.75 h, diluted with ethyl acetate (100 mL), and applied directly to silica gel chromatography (1−3% of methanol in ethyl acetate, stepwise gradient by 2%) to give 41 mg of crude product, which was purified by silica gel column (6−15% of 2-propanol in CH_2Cl_2 stepwise gradient by 3%) to give the desired *trans*-alcohol product. This product was further purified by preparative HPLC (SUPELCOSIL PLC-SI, silica gel, 12 μm, 25 cm × 21.1 cm, elution with 4% methanol in CH_2Cl_2) to afford **13a** as a single 3′,4′-trans diastereomer**.** Yield: 29 mg, 75%. TLC R_f = 0.37 CH_2Cl_2/2-propanol (90:10). IR: 2108, 1680 cm^{-1}, ^1H NMR ($CDCl_3$, 300 MHz): δ 9.23 (s, 1H), 7.54 (d, J = 8.1 Hz, 1H), 5.75 (d, J = 8.1 Hz, 1H), 5.69 (s, 1H), 5.53 (d, J = 5.7 Hz, 1H), 4.10−4.06 (m, 1H), 3.86−3.55 (m, 4H), 2.68−2.58 (m, 1H), 2.15 (s, 3H), 1.76−1.52 (m, 2H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 170.2, 163.6, 150.2, 140.7, 102.6, 91.9, 83.0, 77.9, 60.3, 51.7, 39.0, 27.2, 20.9. MS (ESI). Calcd for $C_{13}H_{17}N_5O_6$ + Na: 362.1. Found $[M + Na]^+$: 362.1.

For experimental procedures for synthesis of **13b**−**d**, see Supporting Information.

2′-*O*-Acetyl-5′-azido-3′-carboxymethyl-3′,5′-dideoxyuridine (3a). (Diacetoxyiodo)benzene (96.7 mg, 0.3 mmol) and TEMPO (6.2 mg, 0.04 mmol) were added to the solution of **13a** (34 mg, 0.1 mmol) in CH_3CN/H_2O (1.2 mL/1.2 mL). After stirring for 41 h, the mixture was freeze-dried and purified by silica gel column chromatography using a gradient (3−9%, stepwise by 2%) of methanol (containing 8% H_2O) in CH_2Cl_2 to afford **3a.** Yield: 28 mg, 80%; ee was not determined because no baseline separation was achieved (see Supporting Information). HPLC: R_t= 76.7 min (major enantiomer) and 81.1 min (minor enantiomer) on Chiralcel OD-H 4.6 × 150 mm column equipped with Chiralcel OD 4.6 × 50 mm precolumn; eluent, 0.5% of acetic acid and 10% of ethanol in hexanes; flow rate, 0.75 mL/min. TLC R_f = 0.31 MeOH/CH_2Cl_2 (10:90). IR: 2108, 1711 cm^{-1}. Anal. Calcd for ($C_{13}H_{15}N_5O_7 + H_2O$): C, 42.05; H, 4.61; N, 18.86. Found: C, 42.05; H, 4.28; N, 18.46. ^1H NMR ($CDCl_3/CD_3OD$, 5:1, 300 MHz): δ 7.56 (d, J = 8.1 Hz, 1H), 5.78 (d, J = 8.1 Hz, 1H), 5.78 (d, J = 2.4 Hz, 1H), 5.48 (dd, J = 1.8 Hz, J = 6.9 Hz, 1H), 4.11−4.06 (m, 1H), 3.79 (dd, J = 2.7 Hz, J = 13.2 Hz, 1H), 3.56 (dd, J = 4.2 Hz, J = 13.5 Hz, 1H), 2.96−2.86 (m, 1H), 2.59−2.35 (m, 2H), 2.13 (s, 3H). ^{13}C NMR ($CDCl_3/CD_3OD$, 75.4 MHz): δ 173.1, 170.0, 164.0, 150.6, 140.4, 102.8, 90.5, 82.0, 77.6, 51.8, 38.4, 30.2, 20.3. HRMS (ESI). Calcd for $C_{13}H_{15}N_5O_7$ $[M + H]^+$: 354.1049. Found: 354.1028.

2′-*O*-Acetyl-5′-azido-3′-carboxymethyl-3′,5′-dideoxy-4-*N*-propionylcytidine (3b). Dess−Martin periodinane (15 wt % solution in CH_2Cl_2, 0.38 mL, 0.18 mmol) was added to the solution of 2′-*O*-acetyl-5′-azido-3′-(2-hydroxyethyl)-3′,5′-dideoxy-4-*N*-propionylcytidine (**13b**; 36 mg, 0.091 mmol) in CH_2Cl_2 (1.9 mL). The solution was stirred for 1.5 h. Saturated aqueous $Na_2S_2O_3$ (0.6 mL) was added to quench the reaction. The aqueous layer was extracted with CH_2Cl_2 (4 × 2 mL). Combined organic layers were washed by aqueous phosphate buffer (pH = 9.2; 4 × 1 mL). The organic layer was concentrated to give the intermediate aldehyde as a white residue that was used in the next step without further purification.

(43) Nishimura, T.; Iwai, I. *Chem. Pharm. Bull.* **1964**, *12*, 352−356.

To the solution of aldehyde in *tert*-butyl alcohol/H_2O (1:1, 5.5 mL) were added a mixture of 2-methyl-2-butene (2 M in THF, 0.23 mL, 0.46 mmol), NaH_2PO_4 (27.4 mg, 0.23 mmol), and $NaClO_2$ (technical 80%, 36.1 mg, 0.32 mmol). After stirring for 25 min, saturated aqueous $Na_2S_2O_3$ (1 mL) was added. The solution was freeze-dried and purified by silica gel column chromatography starting with 3% of methanol in CH_2Cl_2 followed by a gradient (3−9%, stepwise by 2%) of methanol (containing 8% H_2O) in CH_2Cl_2 to afford **3b.** Yield: 32 mg, 86%, ee 97.8% (see Supporting Information). HPLC: $R_t = 45.2$ min (major enantiomer) and 64.2 min (minor enantiomer) on Chiralcel OD-H 4.6 × 150 mm column equipped with Chiralcel OD 4.6 × 50 mm precolumn; eluent, 0.5% of acetic acid and 10% of ethanol in hexanes; flow rate, 0.75 mL/min. TLC $R_f =$ 0.38 MeOH/CH_2Cl_2 (10:90). IR: 2106, 1728 cm^{-1}. Anal. Calcd for ($C_{16}H_{20}N_6O_7$ + 0.5H_2O): C, 46.04; H, 5.07; N, 20.14. Found: C, 45.83; H, 4.88; N, 19.93. ^1H NMR ($CDCl_3$/CD_3OD, 5:1, 300 MHz): δ 8.08 (d, $J = 7.5$ Hz, H), 7.55 (b, 1H), 5.84 (s, 1H), 5.56(d, $J = 6.0$ Hz, H), 4.19−4.13 (m, 1H), 3.90−3.84 (m, 1H), 3.67−3.62 (m, 1H), 2.89−2.79 (m, 1H), 2.57−2.32 (m, 1H), 2.15 (s, 3H), 1.21 (t, $J = 7.5$ Hz, 3H). ^{13}C NMR ($CDCl_3$/CD_3-OD, 75.4 MHz): δ 174.9, 169.8, 163.0, 155.8, 150.6, 144.4, 97.3, 91.6, 82.7, 77.8, 51.7, 38.3, 30.6, 20.4, 8.7. HRMS (ESI). Calcd for $C_{16}H_{20}N_6O_7$ [M + H]$^+$: 409.1471. Found: 409.1442.

2′-*O*-Acetyl-5′-azido-3′-carboxymethyl-3′,5′-dideoxy-6-*N*-benzoyladenosine (3c). Dess−Martin periodinane (15 wt % solution in CH_2Cl_2, 0.25 mL, 0.12 mmol) was added to the solution of 2′-*O*-acetyl-5′-azido-3′-(2-hydroxyethyl)-3′,5′-dideoxy-6-*N*-benzoyladenosine (**13c**; 28 mg, 0.06 mmol) in CH_2Cl_2 (1.3 mL). The reaction mixture was stirred for 1 h and diluted by CH_2Cl_2 (5 mL). Saturated aqueous $NaHCO_3$ containing an excess of $Na_2S_2O_3$ (4 mL) was added to quench the reaction. This mixture was stirred for 20 min. The organic layer was washed by saturated aqueous $NaHCO_3$ containing an excess of $Na_2S_2O_3$ (4 mL) and aqueous phosphate buffer (pH = 9.2; 4 mL), dried (Na_2SO_4), concentrated to give the intermediate aldehyde as a white residue that was used in the next step without further purification.

To the solution of aldehyde in *tert*-butyl alcohol/H_2O (1:1, 3.8 mL) were added a mixture of 2-methyl-2-butene (2 M in THF, 0.15 mL, 0.3 mmol), NaH_2PO_4 (18 mg, 0.15 mmol), and $NaClO_2$ (technical 80%, 23.7 mg, 0.21 mmol). After stirring for 1 h, saturated aqueous $Na_2S_2O_3$ (2 mL) was added. The solution was freeze-dried and purified by silica gel column chromatography starting with 3% of methanol in CH_2Cl_2 followed by a gradient (3−9%, stepwise by 2%) of methanol (containing 8% H_2O) in CH_2Cl_2 to afford **3c.** Yield: 25 mg, 86%; ee was not determined because no separation was achieved (see Supporting Information). HPLC: $R_t = 83.2$ min (single peak) on Chiralcel OD-H 4.6 × 150 mm column equipped with Chiralcel OD 4.6 × 50 mm precolumn; eluent, 0.5% of acetic acid and 10% of ethanol in hexanes; flow rate, 0.75 mL/min. TLC $R_f = 0.43$ MeOH/CH_2Cl_2 (10:90). IR: 2106, 1705 cm^{-1}. Anal. Calcd for ($C_{21}H_{22}N_8O_7$ + H_2O): C, 50.60; H, 4.45; N, 22.48. Found: C, 50.97; H, 4.14; N, 22.14. ^1H NMR ($CDCl_3$/CD_3OD, 5:1, 300 MHz): δ 8.04 (s, 1H), 8.38 (s, 1H), 8.10−8.07 (m, 2H), 7.65−7.51(m, 3H), 6.17 (s, 1H), 5.82 (d, t, $J = 6$ Hz, 1H), 4.29−4.23 (m, 1H), 3.82−3.60 (m, 2H), 3.43−3.33 (m, 1H), 2.69−2.46 (m, 2H), 2.18 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.4 MHz): δ 174.8, 170.0, 165.5, 152.3, 151.8, 149.9, 142.3, 133.4, 133.0, 128.8, 128.4, 123.6, 89.3, 83.2, 78.4, 52.1, 38.9, 30.2, 20.7. HRMS (ESI). Calcd for $C_{21}H_{20}N_8O_6$ [M + H]$^+$: 481.1584. Found: 481.1573

2′-*O*-Acetyl-5′-azido-3′-carboxymethyl-3′,5′-dideoxy-2-*N*-acetyl-6-*O*-diphenylcarbamoylguanosine (3d). (Diacetoxyiodo)benzene (73.8 mg, 0.23 mmol), TEMPO (4.76 mg, 0.03 mmol), and NaOAc (12.5 mg, 0.15 mmol) were added to the solution of 2′-*O*-acetyl-5′-azido-3′-(2-hydroxyethyl)-3′,5′-dideoxy-2-*N*-acetyl-6-*O*-diphenylca rbamoylguanosine (**13d**; 47 mg, 0.076 mmol) in CH_3CN/H_2O (1.2 mL/1.2 mL). After stirring for 4 days at 0 °C, the mixture was freeze-dried and purified by silica gel column chromatography starting with 3% methanol in CH_2Cl_2 followed by a gradient (3−9%, stepwise by 2%) of methanol (containing 8% H_2O) in CH_2Cl_2 to afford **3d.** Yield: 42 mg, 87%; ee 95% (see Supporting Information). HPLC: $R_t = 51.0$ min (minor enantiomer) and 66.8 min (major enantiomer) on Chiralcel OD-H 4.6 × 150 mm column equipped with Chiralcel OD 4.6 × 50 mm precolumn; eluent, 0.5% of acetic acid and 10% of ethanol in hexanes; flow rate, 0.75 mL/min. TLC $R_f = 0.5$ MeOH/CH_2Cl_2 (10:90). IR: 2104, 1741 cm^{-1}. Anal. Calcd for ($C_{29}H_{27}N_9O_8$ + H_2O): C, 53.79; H, 4.51; N, 19.47. Found: C, 53.95; H, 4.32; N, 19.36. ^1H NMR ($CDCl_3$/CD_3OD, 5:1, 300 MHz): δ 8.12 (s, H), 7.35−7.14 (m, 10H), 5.23 (s, H), 5.70 (d, $J = 5.7$ Hz, 1H), 4.14−4.08 (m, 1H), 3.66−3.50 (m, 2H), 3.41(b, 1H), 2.57−2.36 (m, 2H), 2.31(s, 3H), 2.05(s, 3H). ^{13}C NMR ($CDCl_3$/CD_3OD, 75.4 MHz): δ 174.4, 170.3, 156.2, 154.1, 151.8, 150.3, 143.8, 141.8, 129.4, 127.3, 121.5, 90.3, 83.6, 78.7, 52.6, 39.2, 30.4, 25.2, 20.7. HRMS (ESI). Calcd for $C_{29}H_{27}N_9O_8$ [M + H]$^+$: 630.2061. Found: 630.2003.

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

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