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Toward Amide-Linked RNA Mimics: Total Synthesis of 3′-C Branched Uridine Azido Acid via an Ene–Iodolactonization Approach

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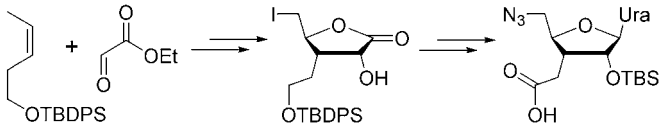
ABSTRACT



A novel synthesis of 3′-C branched uridine azido acid has been accomplished by a sequence of stereoselective ene and iodolactonization reactions. Compared to traditional routes that start from carbohydrates, the present methodology is more flexible and can be further optimized by incorporation of novel future developments of synthetic chemistry. Because the key chemistry does not involve the 3′-C substituent, our route is a general approach to 3′-C alkyl nucleoside analogues.

Gene therapy with ribozymes, small nucleic acids that can catalytically cleave disease related messenger RNA molecules, is a promising alternative to conventional chemotherapy.[1] However, the relatively low catalytic efficiency of ribozymes currently prohibits clinical realization of this potential. Compared to proteins, RNA is inherently less suited for catalysis because of its limited folding, backbone flexibility, and low diversity of functional groups.[2] RNA has negatively charged phosphates that are located on the surface of the molecule in double helical form, and electrostatic repulsion prevents helixes from forming the closely packed tertiary structures needed for RNA to function as a catalyst.

We propose that replacement of the negatively charged phosphates by a neutral amide backbone will eliminate the unfavorable electrostatic repulsion, and will considerably improve the folding of RNA. Because of the improved folding, we envision that the ribozymes built of amide-linked RNA will be more efficient catalysts than their natural counterparts.

The amide backbone has been used successfully in synthetic biopolymer mimics. Peptide nucleic acids (PNA) formed stable sequence-specific helixes with complementary DNA and PNA.[3] Short hybrid DNA−RNA duplexes, where the DNA strand has a few selected phosphodiesters replaced by neutral amide linkages, had thermal stability similar to the nonmodified controls.[4,5] One of us previously found that amide linkages are equally well tolerated at some selected positions in analogous RNA−RNA duplexes.[6] Short uniformly modified amide-linked DNA also formed stable duplexes with the complementary unmodified RNA and DNA.[7] These results suggest that the overall conformation

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(3) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497. Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norden, B.; Nielsen, P. E. *Nature* **1993**, *365*, 566. Wittung, P.; Nielsen, P. E.; Buchardt, O.; Egholm, M.; Norden, B. *Nature* **1994**, *368*, 561.
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of the amide linkage fits well in a nucleic acid double helix causing no major disturbance.

Amide-linked RNA can be prepared adopting the well-developed peptide synthesis protocols (Figure 1). The

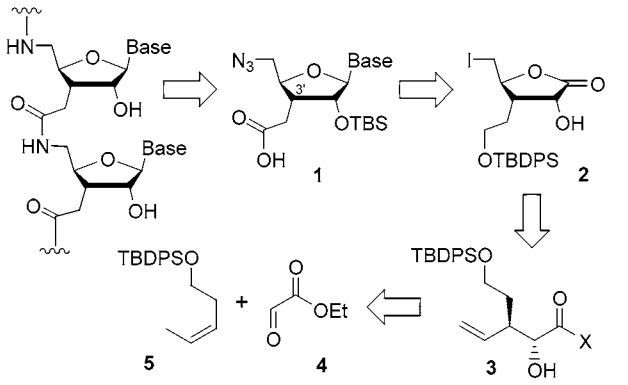


Figure 1. Retrosynthetic analysis of amide-linked RNA.

synthetic challenge is the preparation of modified nucleosides **1**, the amino acid equivalents required for the peptide type couplings. Traditional syntheses of modified nucleosides analogous to **1** focus on readily available chiral pool starting materials: nucleosides and carbohydrates.[4−8] The advantage is that the stereochemical relationships are already set or can be easily adjusted. However, such routes are frequently lengthy and inefficient because the multifunctional and sensitive starting materials limit the synthetic methodology that can be used and require extensive protecting group manipulations.

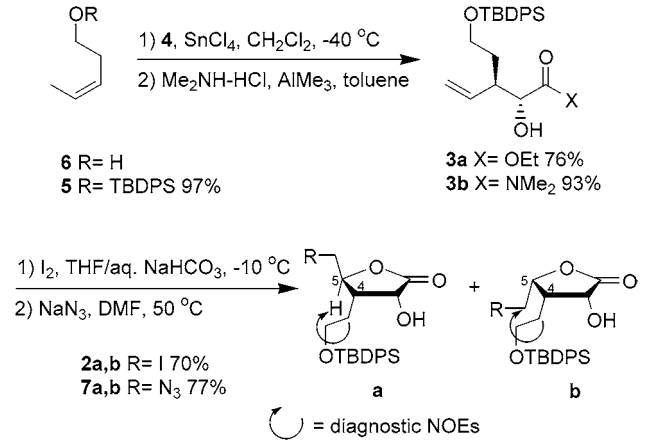
Several groups have reported syntheses of modified nucleosides analogous to **1**. Von Matt and co-workers synthesized 2′-deoxy[7] and 2′-OMe[8b] analogues of **1** starting from nucleosides. Robins, Peterson, and co-workers[8a] prepared a series of ribonucleosides **1** (base = adenine, thymine) starting from protected xylose. Both groups used the Wittig reaction followed by stereoselective hydrogenation to form the new carbon−carbon bond at C3′. Robins et al.[9] also reported a synthesis of an amide-linked uridine pentamer.

In this letter we report a novel total synthesis approach to 3′-C branched ribonucleosides **1**. Our approach focuses on reliable and stereoselective reactions that can be used to *de novo* synthesize the modified nucleosides **1** from simple starting materials. Although conceptually similar approaches have been used to prepare carbocyclic nucleosides,[10] applications of total synthesis principles to modify natural nucleosides are relatively rare.[11,12] Lavallee et al.[11] reported a total synthesis of 3′-carbomethoxymethyl thymidine using radical cyclization as the key step.

Our retrosynthetic analysis of **1** (Figure 1) first disconnects the heterocyclic base and after adjustment of functional groups reveals the iodolactone **2** as the first key intermediate. Opening of the five-membered tetrahydofuran ring (iodolactonization) identifies the unsaturated carboxylic acid **3** (or derivative thereof) as the next key intermediate. These disconnections reduce the complexity from two heterocycles and four stereogenic centers in **1** to only two stereogenic centers in the acyclic **3**. The key intermediate **3** can be further disconnected to simple organic compounds **4** and **5** to be joined in a stereoselective ene reaction.

The synthetic realization of this plan started with protection of the commercially available (*Z*)-3-penten-1-ol **6** as the *tert*-butyldiphenylsilyl (TBDPS) ether **5** (Scheme 1). The tin

Scheme 1



tetrachloride promoted ene reaction of **5** with ethyl glyoxalate **4** gave the racemic ester **3a**. The work of Mikami et al.[13] has established good precedent for the regioselectivity and the *anti* diastereoselectivity in the carbonyl ene reactions of this type. The ^{1}H NMR spectrum of **3a** indicated the presence of 5−10% of a minor compound, possibly the *syn* diastereomer (not separable by flash chromatography at this stage).

Because the iodolactonization[14] of **3a** was impracticably slow we transformed the ester into dimethyl amide **3b**. Iodolactonization of **3b** in aqueous tetrahydrofuran in the presence of sodium bicarbonate gave a mixture of *trans* and *cis* iodolactones **2** in a ratio of 4:1. Attempts to optimize temperature, solvent, base, and amide substituents did not improve the *trans*/*cis* ratio. From literature precedents, it is conceivable that the 4:1 preference for the *trans* isomer

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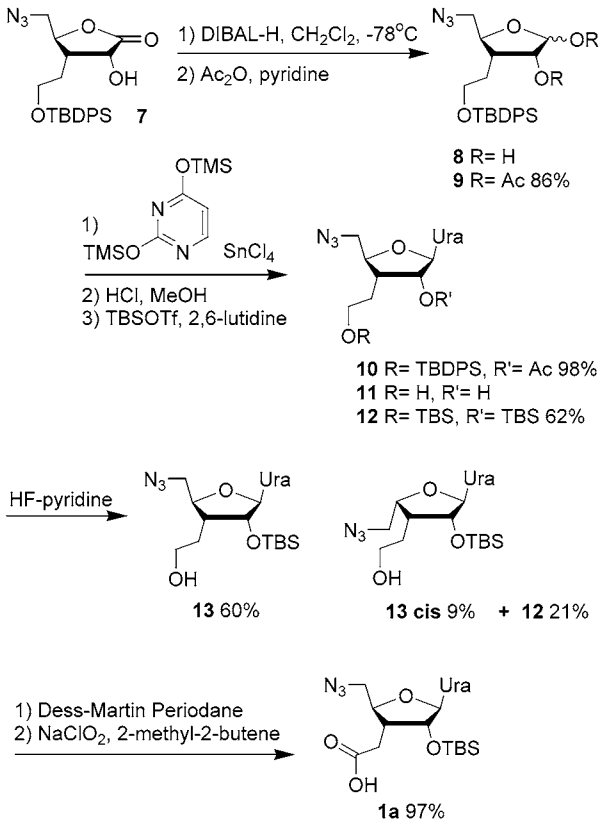
reflects the thermodynamic control[14] in iodolactonization of amide **3b**.[15] Similar cyclization of the carboxylic acid (X = OH), expected to be under kinetic control, gave **2a** and **2b** in a ratio close to 1:1. Because we could not efficiently separate the *trans* and *cis* isomers of **2** (or later intermediates) by flash column chromatography the following steps were carried out on mixtures (for clarity only the major diastereomer is shown in Scheme 2). Separation of isomers was

Scheme 2



achieved after synthesis of nucleoside and removal of the TBDPS protection.

Treatment of iodolactones **2** with sodium azide led to azidolactones **7**. Although inseparable by flash column chromatography, **7** could be separated by HPLC on silica gel column. The configuration of the diastereomeric products *trans* **7a** and *cis* **7b**, initially assigned based on literature precedents,[15] was confirmed by NOESY experiments on the separated diastereomers. We observed diagnostic NOEs between the C4-methylene group and either the H5 of **7a** or the C5-azidomethylene protons of **7b** (Scheme 1).

Using modifications of the published procedures,[16] we selectively reduced the lactone group of **7** (2.5 equiv of DIBAL-H) and immediately acetylated the hydroxyl groups to give the glycosyl acetates **9** in 86% yield for two steps without purification of the intermediate lactols **8** (Scheme 2). Treatment of **9** with trimethylsilyl uracil in the presence of tin tetrachloride gave the modified uridine nucleoside **10**.

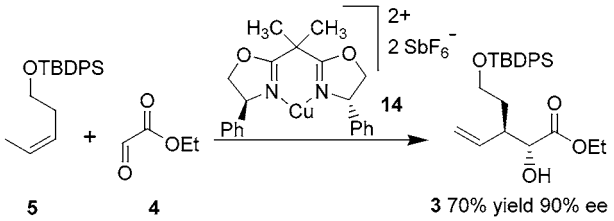
Although the 2′-O-acetyl group in principle would be compatible with our planned synthesis of amide-linked RNA, our initial experiments showed that it was too labile for the final steps toward **1**. Migration and cleavage of the acetyl group complicated our attempts of selective removal of TBDPS. Therefore, a protecting group change was necessary to reveal the primary alcohol for the final oxidation and to install the 2′-OTBS. Acid-catalyzed cleavage of both the TBDPS and the acetyl group followed by disilylation with *tert*-butyldimethylsilyl triflate (TBSOTf) gave **12** in 62% yield for two steps without purification of the intermediate diol **11**. Treatment with HF-pyridine selectively removed the primary TBS group and revealed the alcohol **13**, setting the stage for the final oxidation. At this point, flash silica gel chromatography efficiently separated the products of the deprotection reaction giving **13** (60%), **13**-*cis* (9%), and unreacted starting material **12** (21%). The isomeric purity of **13** was confirmed by ^{1}H NMR.

A two-step oxidation of alcohol **13** gave the target carboxylic acid **1a** in a 97% yield after column chromatography. An analytical sample of **1a** was obtained by repeated crystallization from hexanes or toluene containing small amounts of methylene chloride.[17]

The identity of our final product was confirmed by comparison of **1a** and **1a**-*cis*[18] with a sample of chiral carboxylic acid **1a** that we prepared following the procedures published by Robins and Peterson.[8a] The ^{1}H NMR spectrum of **1a** prepared by our route matched exactly the spectrum of independently prepared **1a**, whereas the spectrum of **1a**-*cis* was distinctly different (see Supporting Information).

Our racemic synthesis of **1** (Schemes 1 and 2) can be rendered asymmetric by using either chiral Lewis acid catalyst[19,20] or chiral auxiliary[21] or a combination of both in a double asymmetric mode. In preliminary experiments we tested the chiral copper(II) bis(oxazolinyl) catalyst **14** developed by Evans and co-workers (Scheme 3).[19] In contrast

Scheme 3



to original reports, the use of anhydrous **14** was essential to obtain chiral nonracemic **3** in acceptable yields (70% after 4 days) and good enantiomeric purity (90% ee). The reaction

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(17) The synthetic intermediates in Schemes 1 and 2 were characterized by ^{1}H and ^{13}C NMR and FT-IR spectroscopy. Elemental analysis of **1a** was in excellent agreement with theoretical calculation. See Supporting Information for more detail.

catalyzed by the aqua complex of **14** was unacceptably slow (30% after 7 days). This result demonstrates the feasibility of asymmetric synthesis of **1** using our route. The enantiomeric purity can be further improved either by crystallization of the final product **1** or by double asymmetric induction using auxiliaries developed by Whitesell and co-workers.[21]

The total synthesis approach has the advantage that both enantiomers of **1** can be prepared by choosing the appropriate chiral catalyst or auxiliary thereby providing access to both enantiomers of amide-linked RNA. This is important for development of enantioselective ribozyme catalysis. For example, Seelig et al.[22] showed that artificially selected ribozymes built of D- and L-nucleosides catalyzed Diels−Alder reaction yielding enantiomeric products.

In summary, our synthetic route (Schemes 1 and 2) provides racemic 3′-C branched uridine azido acid **1a** in 13 steps and 11% overall yield. Our route has possibilities for further improvement and optimization using future developments of synthetic organic chemistry, e.g., improved variants of iodolactonization and alternative syntheses of **3**. Current efforts in our laboratory are focused on enantioselective synthesis of **1a**.

Another feature of our synthesis is the generality. Because the key substituent in the modified nucleoside, the 3′-C alkyl branch, does not directly participate in the chemical transformations, our route can be used to prepare other analogues having different 3′-C substituents. For example, an ongoing project in our laboratory requires modified nucleosides having the 3′-C-CH_2OH substituent. Currently we are working on adopting the present synthesis (Schemes 1 and 2) to prepare these analogues starting from crotyl alcohol.

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Supporting Information Available: Experimental procedures, spectral data, and copies of ^1H and ^{13}C NMR data for compounds **1**−**3**, **5**, **7**, **10**, **12**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) A sample of **1a**-*cis* was prepared from **13**-*cis* in 76% yield by the same two-step oxidation procedure as **1a** (see Scheme 2).

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