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Efficient Synthesis of 2′,3′-Dideoxy-2′-amino-3′-thiouridine

Qing Dai[†] and Joseph A. Piccirilli*

Howard Hughes Medical Institute, Department of Biochemistry & Molecular Biology, and Department of Chemistry, The University of Chicago, 5841 South Maryland Avenue, MC 1028, Chicago, Illinois 60637

jpicciri@midway.uchicago.edu

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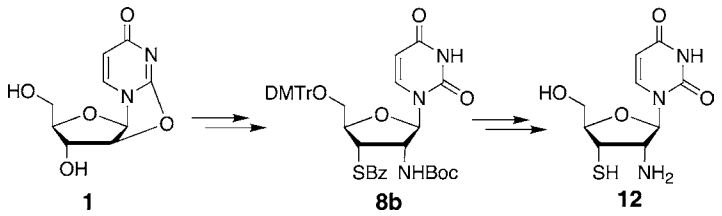
ABSTRACT



Metal ion rescue experiments provide a powerful approach to establish the presence and role of divalent metal ions in the biological function of RNA. The utility of this approach depends on the availability of suitable nucleoside analogues. To expand the range of this experimental strategy, we describe the first synthesis of 2′,3′-dideoxy-2′-amino-3′-thiouridine (12) in 19.5% overall yield starting from 2,2′-anhydrouridine (1).

RNA molecules adopt complex tertiary architectures that perform biological functions.[1] The negatively charged phosphodiester backbone of RNA endows this biopolymer with unique structural and functional properties and may impose a strict requirement for divalent metal ions.[2] To understand how RNA molecules perform their biological functions, the locations and roles of metal ions must be defined. Metal ion rescue experiments provide a powerful means to identify and distinguish specific metal ions, assign their ligands, and test hypotheses regarding the structural and functional roles the metal ions play.[3] Metal ions have varying abilities to coordinate oxygen, nitrogen, and sulfur, and these coordination preferences reflect a metal ion's "hardness" or "softness".[4] Consequently, atomically perturbed RNAs containing

[†] Present address: The Ben May Institute for Cancer Research, The University of Chicago, 5841 S. Maryland Ave. MC 6027, Chicago, IL, 60637.

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sulfur or nitrogen atom substitutions of single oxygen atoms often exhibit rescue behavior by "soft" metal ions,[3] thereby implicating the substituted oxygen atom as a metal ligand. However, the small number of nucleoside analogues currently available[5] limits the scope and applicability of these metal ion rescue experiments.

We previously conducted metal ion rescue experiments together with quantitative analysis to study the exon-ligation step of group II intron self-splicing.[3g,h,o] Using 2′-amino-uridine and 3′-thiouridine, we obtained evidence that both the 2′- and 3′-oxygen atoms at the cleavage site (U_{877} at the 3′-terminus of the ai5γ group II intron) coordinate divalent metal ions in the transition state[3g] (Figure 1). However,

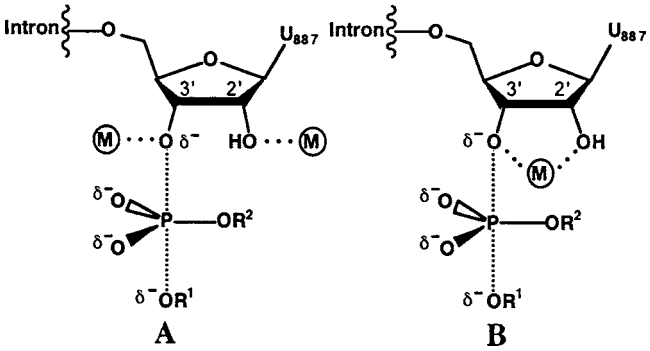

Figure 1. Metal ion ligands in the transition state for the exon ligation step of group II intron self-splicing. R^1 and R^2 are exon 1 and exon 2, respectively. The 3′-hydroxyl group of exon 1 (OR^1) attacks the 5′-splice site, giving rise to spliced exons and releasing the excised intron. Both the 2′- and 3′-oxygen atoms of the cleavage site uridine interact with metal ions,[3g,h,o] but it remains unknown whether two metal ions (as in **A**) or a single metal ion (as in **B**) mediate(s) these interactions. Dots symbolize metal ion coordination.

whether two distinct metal ions mediate these interactions (Figure 1A) or a single metal ion mediates both interactions (Figure 1B) remains unknown. Metal ion rescue experiments using an oligonucleotide substrate containing doubly modified 2′,3′-dideoxy-2′-amino-3′-thiouridine residue at the cleavage site could help to answer this fundamental mechanistic question.[6] Preparation of such a substrate requires synthesis of the parent nucleoside **12** and its incorporation into an oligonucleotide. Although numerous syntheses of 2′ (or 3′)-amino[7] (or thio[8]) single-modified uridines and 2′,3′-doubly modified nucleosides such as 2′,3′-dideoxy-2′,3′-diamino-,[9] 2′,3′-dideoxy-2′,3′-dithio-,[10] and 2′-deoxy-2′-thio-3′-deoxy-3′-aminouridines[11] have been reported, the 2′,3′-dideoxy-2′-amino-3′-thio nucleosides remain unknown. Here, we describe a convenient synthesis of the uridine analogue.

Our synthesis of 2′,3′-dideoxy-2′-amino-3′-thiouridine **12** begins with the commercially available 2,2′-anhydrouridine **1** (Scheme 1). We used the method of McGee et al. to prepare 2′-deoxy-2′-amino-5′-*O*-(4,4′-dimethoxy-trityl)uridine (**2**).[12] Successive mesylation/displacement reactions have enabled installation of a 3′-α-sulfur atom into uridine previously.[13] To employ this strategy in our synthesis, the amino group of **2** must bear a protecting group that fulfills several criteria: (1) it must weaken the nucleophilicity of the 2′ amine so as to allow selective mesylation of the 3′-α hydroxyl group, (2) it must remain unaffected under strongly basic conditions (1−6 N NaOH in ethanol) used for displacement of the 3′-α mesylate, and (3) it must undergo facile removal under appropriate conditions. Based on these considerations, we chose *tert*-butoxycarbonyl (Boc), well-known for its use in peptide chemistry but used infrequently in nucleic acid chemistry. To obtain the Boc-protected nucleoside, 2′-deoxy-2′-amino-5′-*O*-(4,4′-dimethoxytrityl)-uridine (**2**) was treated at 40 °C overnight with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON)[14] in dioxane to afford **3** selectively in 94% yield. Other reagents, such as di-*tert*-butyl dicarbonate,[15] or other solvents, such as methanol (MeOH) or acetonitrile (CH_3CN), gave **3** in significantly lower yields.

To test whether the Boc group meets the aforementioned requirements, we conducted the following control experiments: (i) **3** was treated with methanesulfonyl chloride in pyridine to generate compound **4a** selectively in 90% yield.[16] We could detect no mesylation of the 2′-NHBoc group under these conditions. (ii) Overnight treatment of compound **3** with 6 N NaOH in ethanol (1:2 v/v) at room temperature gave no reaction, suggesting that the Boc group would remain

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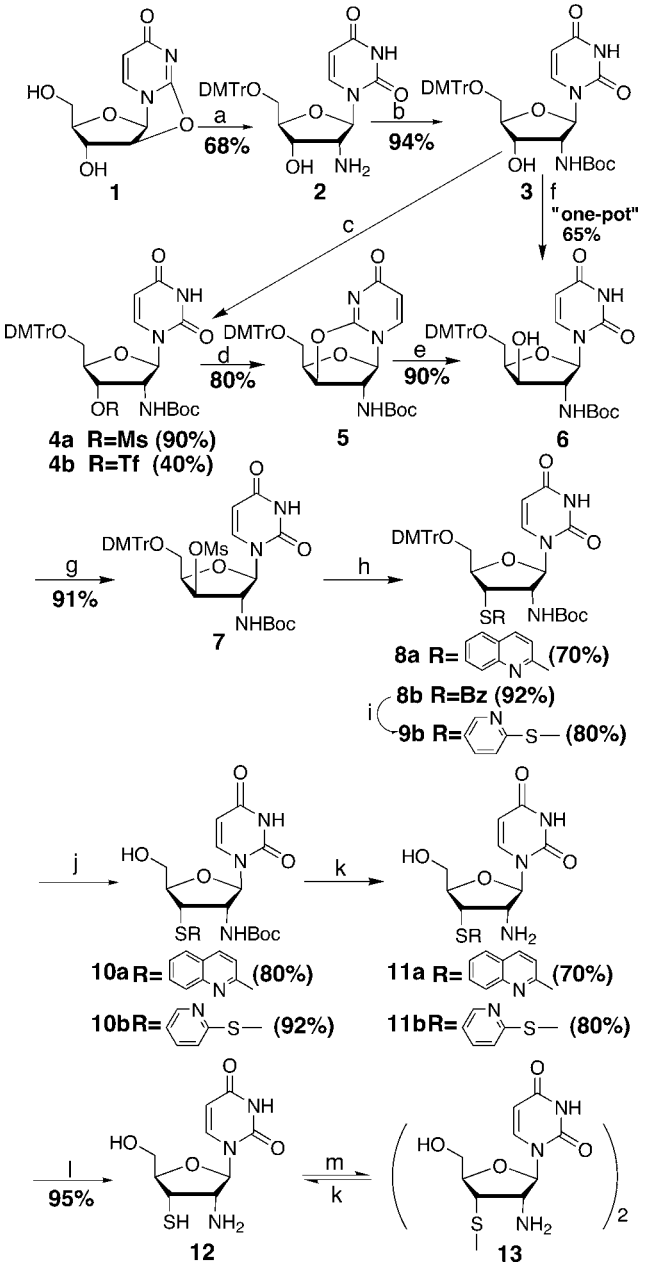
Scheme 1 [a]



[a] Reaction conditions: (a) (1) DMTr-Cl, DMAP, pyridine, 16 h, (2) Cl_3CCN, Et_3N, reflux, 24 h, (3) 6 N NaOH, EtOH, reflux, 16 h; (b) Boc-ON, Et_3N, dioxane, 40 °C, 16 h; (c) for **4a**, $MeSO_2Cl$, pyridine; for **4b**, $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −78 °C to rt; (d) 6 N NaOH (1.1 equiv), EtOH, rt, 16 h; (e) 6 N NaOH (2.0 equiv), EtOH, rt, 16 h; (f) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −78 °C to rt; (d) 6 N NaOH (1.1 equiv), EtOH, rt, 16 h; 6 N NaOH (1.4 equiv), EtOH, rt, 16 h; (g) $MeSO_2Cl$, pyridine, rt, 16 h; (h) for **8a**, 2-quinolinethiol and DBU, DMF, 80 °C, 16 h; for **8b**, PhC(O)SNa, DMF, 100 °C, 8 h; (i) (1) 40% aqueous $MeNH_2$, rt, 16 h, (2) aldrithiol, DMF, 60 °C, 16 h; (j) 3% trichloroacetic acid in acetonitrile, rt, 0.5 h; (k) 30% trifluoroacetic acid in CH_2Cl_2, rt, 1 h; (l) dithiothreitol, THF, rt, 1 h; (m) atmospheric O_2, rt, 3 days.

intact under the strongly basic conditions required in the subsequent steps. (iii) Treatment of **3** with weak acid (3% trichloroacetic acid in dichloromethane, TCA) removed the 5′-dimethoxytrityl (DMTr) group instantly, but the Boc group survived overnight exposure. In contrast, 30% trifluoroacetic acid (TFA) in dichloromethane (CH_2Cl_2) removed both the DMTr and Boc groups within 1 h to give 2′-deoxy-2′-aminouridine[12] quantitatively. These control experiments supported our reasoning that Boc protection of the amino group would enable us to access our target.

We attempted to generate the 3′-β-OH analogue from methanesulfonyl derivative **4a** by treatment with 1 or 6 N ethanolic NaOH (1:1 v/v) at room temperature.[17] No reaction occurred after overnight treatment; overnight reflux with 6 N ethanolic NaOH (1:1 v/v) removed both the Ms and Boc groups, regenerating **2**. As an alternative, we activated **3** as the more reactive trifluoromethanesulfonylate ester[5f,18] by overnight exposure to triflic anhydride in pyridine and CH_2Cl_2 (1:1), initially at −78 °C followed by gradual warming to room temperature. Thin-layer chromatography (TLC) showed that **3** was completely consumed, apparently giving rise to one major product. However, standard workup and column chromatography resulted in a low yield (40%) of **4b**, suggesting that decomposition may have occurred during the purification process. Incubation with ethanolic NaOH (1.0 N, 1.1 equiv) converted **4b** to the 3′-anhydrouridine derivative **5** in 80% yield. Further exposure to ethanolic NaOH (1.0 N, 2.0 equiv) converted **5** to the xylose derivative **6** in 90% yield.[19]

To minimize the possible decomposition of **4b** during isolation, we explored the transformation of **3** to **6** in a "one-pot" reaction. After **3** was completely consumed during trifluoromethanesulfonylation (as monitored by TLC), we quenched the reaction by adding MeOH and removed solvent at low temperature (<30 °C). Without further purification, we treated the residue with ethanolic NaOH, monitoring by TLC the gradual formation of **5** and then **6**. The yields of **6** varied with the number of NaOH equivalents added, presumably due to the formation of a byproduct by E2 elimination.[5f,20] When 2.5 equiv of NaOH was used, the "one-pot" reaction gave **6** in 65% overall yield.[21]

To install the 3′ sulfur on the α face of the nucleoside, we converted **6** to the corresponding 3′-β-mesylate ester **7** in 91% yield. To avoid spectral overlap, NOE experiments were conducted on compound **7** instead of **6**. Irradiation of H-3′ enhanced H-4′ strongly (8.6%) and H-2′ weakly (1.8%), confirming that the 3′-OMs resides on the β-face of the ribose ring. Mesylate **7** reacted with 2-quinolinethiol in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 80 °C to afford **8a** in 70% yield[22] or with sodium thiobenzoate in *N*,*N*-

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dimethylformamide (DMF) at 100 °C to afford **8b** in 92% yield.[23] Attempts to generate the 3′-thionucleoside directly from 2′-amino-3′-anhydrouridine **5** by treatment with *tert*-butyl thiol[24] or sodium thiobenzoate gave no reaction.

We attempted to acquire the target nucleoside **12** by removing the protecting groups from **8a**. TCA in acetonitrile (3%) selectively removed the 5′-DMTr to afford **10a**, which upon exposure to TFA in CH_2Cl_2 (30%) removed the Boc group and generated **11a**. However, attempts at various temperatures to remove the quinoline moiety by reduction with $NaBH_3CN$ in acetic acid and subsequent hydrolysis[22] failed to give 2′,3′-dideoxy-2′-amino-3′-thiouridine **12**.

The 3′-thiobenzoyl derivative **8b** allowed access to the target nucleoside. We converted **8b** to the 3′-pyridyl disulfide **9b** before removing the Boc group, thereby circumventing benzoyl migration to the amino group.[25] Compound **8b** was treated with 40% aqueous methylamine to generate the 3′-mercaptan analogue.[26] Solvent was removed, and the residue was treated with aldrithiol at 60 °C for 16 h in DMF[27] to give the disulfide **9b** in 80% yield. Incubation with 30% TFA in CH_2Cl_2 removed DMTr immediately followed by Boc within 1 h to give the corresponding trifluoroacetate salt. Addition of triethylamine (Et_3N) to neutralize the nucleoside caused partial regeneration of the 5′-DMTr ether. To eliminate this problem, we removed DMTr selectively by treatment with 3% TCA in CH_3CN for 0.5 h and isolated **10b** in 92% yield. Subsequent incubation of **10b** with 30% TFA in CH_2Cl_2 for 1 h removed the Boc group.[28] Following evaporation of the volatile components, we treated the residue with Et_3N in CH_2Cl_2 to afford the neutral nucleoside **11b** in 80% yield. Subsequent reduction of the disulfide bond with dithiothreitol (DTT) gave the target nucleoside, 2′,3′-dideoxy-2′-amino-3′-thiouridine **12** in 95% yield.[29] In dimethyl sulfoxide (DMSO), the nucleoside **12** undergoes gradual aerial oxidation to its dimeric disulfide **13**.[30] In the presence of DTT, **13** undergoes quantitative reduction back to **12**.

In conclusion, we have developed an efficient method for the first synthesis of 2′,3′-dideoxy-2′-amino-3′-thiouridine **12** in 19.5% overall yield starting from 2,2′-anhydrouridine. Our synthetic strategy included four stages: (i) conversion of the starting material to 5′-DMTr-2′-deoxy-2′-aminouridine using the method of McGee et al.;[12] (ii) protection of the 2′ amine as the *tert*-butoxycarbamate; (iii) installation of the 3′ sulfur by stereochemical inversion of the 3′-hydroxyl group, mesylation and displacement with thiobenzoate and (iv) deprotection by consecutive removal of the protecting groups to generate the target nucleoside. This work expands the arsenal of nucleoside analogues with which to investigate the role of metal ions in RNA catalysis. Current efforts are directed toward incorporating **12** into the 3′-splice site of the group II intron.

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Supporting Information Available: Full experimental and analytical data and the ^1H and ^{13}C NMR spectra for compounds **2**, **3**, **4b**, **5**−**7**, **8b**−**11b**, **12,** and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(30) Immediately after column purification, compound **12** was dissolved in DMSO-d_6. ^1H NMR indicated that the sample already contained detectable amounts of the disulfide **13**. After the solution was exposed to the air for 48 h, only 10% of **12** remained. After 72 h, **12** had oxidized quantitatively to **13**.