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An efficient synthetic approach to 6,5′-(*S*)- and 6,5′-(*R*)-cyclouridine†

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Here we present new routes for the efficient syntheses of 6,5′-(*S*)- and 6,5′-(*R*)-cyclouridine. The syntheses utilize readily accessible uridine as a starting material. This route to the *R* diastereomer is significantly more efficient than previous synthetic efforts, allowing us to obtain large amounts of pure material for future biological testing.

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Cyclonucleosides have been interesting targets of the nucleoside community due to their rigid geometry. A second linkage between the 5′-carbon of the sugar and the nucleobase fixes the base in the *anti* conformation. Such a linkage results in two unique diastereomers at the 5′-position in regards to the 5′-OH (Fig. 1).

6,5′-cyclouridine (**1*S*** and **1*R***) and similar compounds are unable to bind uridine phosphorylase (UrdPase), an enzyme involved in the anabolism and catabolism of pyrimidines, that has been shown to be upregulated in multiple human cancer cell lines.[1–3] Nucleosides locked in a *syn* conformation are known to be potent inhibitors of UrdPase. Cyclouridine has been shown to reduce the rate of hydrolysis in Ribonuclease A.[5] Ribonucleases degrade unprotected single stranded RNA and some homologs, most notably Ranpirnase, have been shown to have cytotoxic effects on cancer cells.[6] To date, information relating to the specific binding events of cyclouridine is sparse, due to low amounts of pure, stereometrically defined, samples. An efficient synthesis of the *S* and *R* diastereomers of 6,5′-cyclouridine will allow for further investigation of the interactions that these enzymes have with cyclouridine, and allow for stability studies to be performed with oligonucleotide strands. Cyclonucleosides also provide unique opportunities for template studies, where the cyclonucleoside could be used in the template strand or as a substrate to test the promiscuity of polymerases.

Several syntheses of 6,5′-cyclo-5′-deoxyuridine have been undertaken, but access to the 5′-OH derivative has been problematic.[7–11] The *R* 5′-OH derivative has been particularly difficult to make, with only one previously reported synthesis, which relied on the epimerization of the *S* diastereomer and resulted in an unfavorable 2 : 1 ratio of *S* to *R* after a 24 h reaction.[7]

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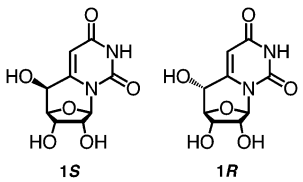

Fig. 1 6,5′-(*S*)- and 6,5′-(*R*)-cyclouridine.

Our synthesis is a modification of the path established by Ueda *et al.* to obtain the protected 6,5′-cyclo-5′-deoxyuridine compound, **7**. We have been able to achieve higher yields with fewer steps while synthesizing compound **7**. Our synthesis begins by protecting the 2′, 3′, and 5′ OH positions on uridine with acetate groups (Scheme 1). The 5 position on the uridine base is then chlorinated with CAN and LiCl using Asakura's method.[12] Deprotection of the acetates and subsequent acetonide protection of the 2′ and 3′ hydroxyls yields **5**. At this point we perform a one-step iodination of the 5′ position using Moffatt's chemistry, instead of the two-step procedure previously utilized.[10,13] Radical cyclization with AIBN and Bu_3SnH and dehydrohalogenation with NaOMe produces **7** in very good yield.

Compound **7** can be directly oxidized to ketone, **8**, *via* an allylic oxidation with SeO_2 (Scheme 2). Reduction with $NaBH_4$ yields exclusively the *S* diastereomer at the 5′ position. Acidic removal of the acetonide gives 6,5′-(*S*)-cyclouridine, **1*S***. This represents the most efficient pathway that has been reported to obtain the *S* diastereomer.

Meanwhile the acetonide of **7** can be replaced with acetates to give **10**. Oxidation with SeO_2 and *t*-BuOOH, using methods established by Sharpless, affords a mixture of the *R* and *S* products, with the *R* diastereomer being the dominant product in a better than 3/1 ratio.[14] The two diastereomers can be separated with purification by column chromatography. A justification for this reactivity is that when *t*-BuOOH is added suring SeO_2 oxidation of **7**, it results in a mixture of the *S* diastereomer and starting material. Likely, the rigid nature of the acetonide protected sugar prevents the *R* diastereomer from forming, while the acetates allow the sugar to be more flexible during the oxidation and the *R* diastereomer can be obtained. The acetates on **11*S*** and **11*R*** were removed with 7N NH_3 in MeOH to yield the cyclonucleosides.

Crystal structures of compounds **9** and **11*R*** were obtained to confirm the stereochemistry at the 5′ position (Fig. 2 and ESI†). Attempts to crystallize the fully deprotected products, **1*R*** and **1*S***, yielded poor quality crystals, however, the crystal

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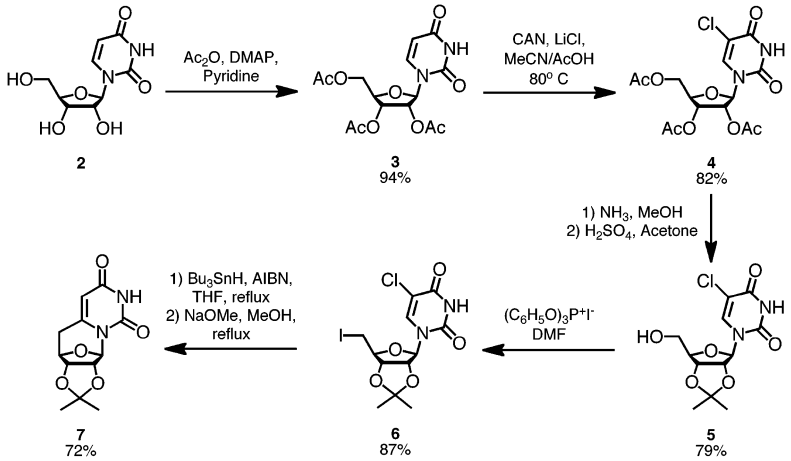
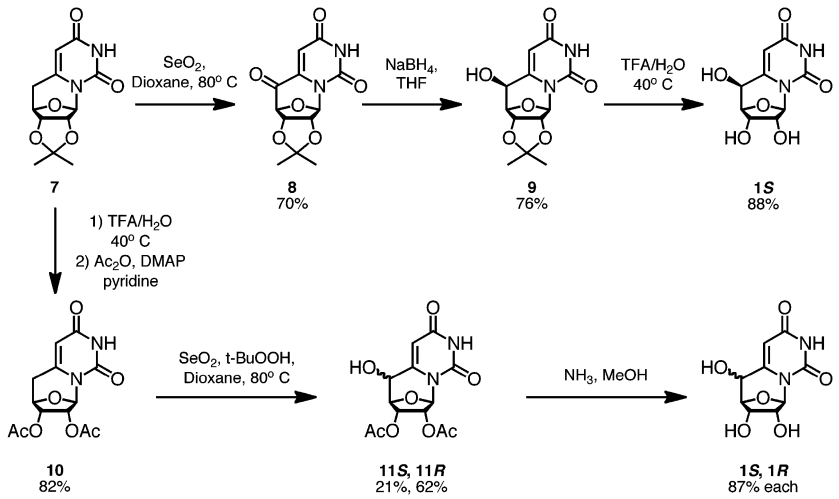


Scheme 1 The synthesis of 2′, 3′-isopropylidine 6-5′-cyclo-5′-deoxyuridine.



Scheme 2 Synthesis of *R* and *S* diastereomers of 6,5′-cyclouridine.

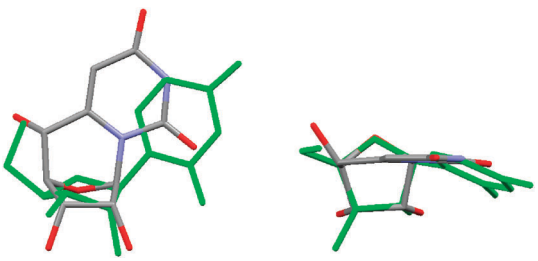


Fig. 2 A front and overhead view of the crystal structure of **11*R*** with the acetates removed overlaid with a crystal of uridine, excised from an RNA strand.[4]

of **11*R*** is a good approximation of **1*R*** when the acetate groups are hidden. When overlaying the crystal of **11*R*** and a uridine nucleotide whose structural parameter were obtained from a crystal of an RNA dodecamer, several differences are observed between the cyclo and native nucleosides.[4] The additional bond between the 5′ carbon and the 6 carbon causes the U base to be "pulled" back quite dramatically. This may explain why the rate of hydroysis in RNase A is slowed, since the shape and conformation of the nucleoside is distorted. Pulling the base away from the Watson–Crick interactions could be detrimental to interstrand base pairing. Studies relating to the strength of base pairing interactions are ongoing in the lab.

One beneficial characteristic of the compounds was seen with regards to the glycosidic torsion angle, χ, of **11*R***. The angle is -148.80°, very close to ribonucleosides, which have a χ value near -160°. Cyclouridine provides an effective substrate for studies requiring a nucleoside in the *anti* conformation.

The sugar pucker of the cyclouridine resides in the envelope configuration rather than the C_3'-*endo* half chair adopted by the native nucleoside. This change in the sugar conformation has implications for the structure of an oligomer of cyclonucleotides, as the 3′ OH is shifted further down from the *endo* position. Further investigation is needed to understand if this is the preferred sugar pucker that is adopted when cyclouridine is inserted into an oligonucleotide strand.

The 5′ OH in **11*R*** is in a *gauche*, *trans* position in relation to the O_4' and the C_3', while the *S* diastereomer is locked in a *trans*, *gauche* position. Native nucleosides usually adopt the *gauche*, *gauche* position, where the 5′ OH points back towards the furanose ring, as seen in the crystal structure overlay. The implication of the sugar pucker will be taken into consideration in our continued investigations.

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In summary a new and improved route to the *S* and *R* diastereomers of 6,5′-cyclouridine is presented. A key oxidation step with SeO_2 and *t*-BuOOH affords the *R* diastereomer in a 3 : 1 ratio compared with the *S* diastereomer, greatly increasing the yield and lowering the reaction time previously reported. These syntheses will allow for the production of large quantities of each diastereomer of cyclouridine to be obtained for further biological studies. Additionally, the crystal structures of cyclouridine have helped to elucidate the unique structural features caused by the introduction of the C6–C5′ bond.

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