Efficient Synthesis of 5-(Thioalkyl)uridines *via* Ring Opening of α-Ureidomethylene Thiolactones

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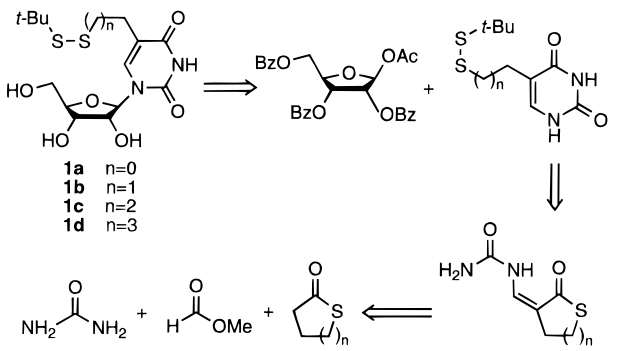
Disulfide cross-linking of thiols is a potentially valuable tool for probing RNA structure and function. The approach has proven useful in the study of DNA[1] and DNA−protein interactions,[2] and chemical strategies for its application to RNA are beginning to emerge.[3] Disulfide bonds have been site-specifically engineered into RNA molecules to constrain a stem-loop conformation,[3a] to determine spatial proximity of hammerhead ribozyme−substrate complexes,[3b] and to engineer a minimal substrate for an RNA glycosylase.[3c] While these examples use the disulfide crosslink to impose conformational constraints, it may also be possible to obtain information about folding pathways and dynamics of RNA molecules, analogous to studies with proteins.[4] Extension of these approaches to RNA requires the incorporation of thiol groups at specific regions in an RNA tertiary structure with minimal perturbation of native conformation. This requirement necessitates the synthesis of appropriate nucleoside monomers.

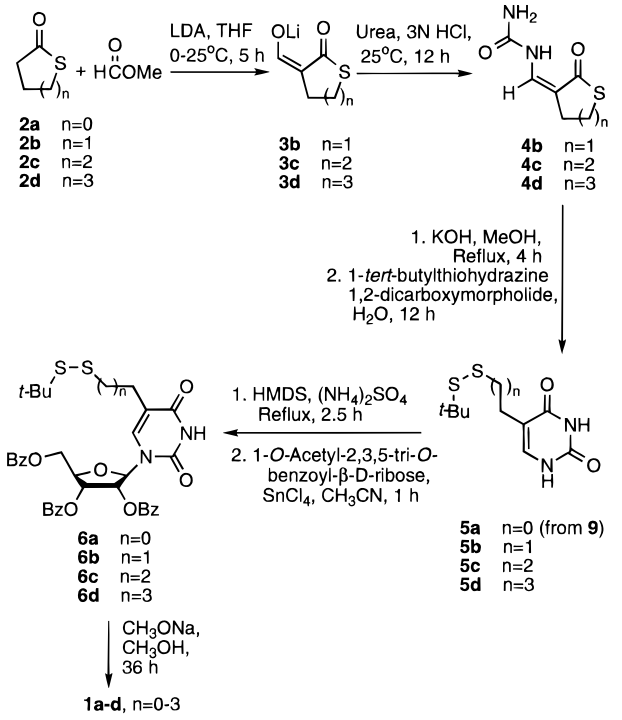
5-(Thioalkyl)uridines may be particularly useful for this purpose. Substituents at the C-5 position of pyrimidine nucleosides are likely to have little effect on glycosidic torsion angle and overall nucleoside conformation[5] and do not interfere directly with the hydrogen-bonding functional groups, thereby allowing Watson-Crick base pairing and other tertiary contacts. Synthesis of 5-(thioalkyl)-2′-deoxyuridines from 5-(hydroxyalkyl)-2′-deoxyuridines has recently been reported by Goodwin and Glick.[6] They introduced hydroxyalkyl substituents at C-5 of 2′-deoxyuridines using palladium-catalyzed addition of alkenes or alkynes to either 5-halogenated or 5-mercurio-2′-deoxyuridines, followed by appropriate redox chemistry. The hydroxyalkyl groups were then transformed to thioalkyl groups in several steps. Synthesis of the corresponding ribonucleosides has not yet been described.

We report here an efficient preparation of 5-(thioalkyl)-uridines from simple, readily available starting materials. Our approach (Scheme 1) is based on two observations: (1) the Hilbert−Johnson glycosylation reaction of peracylated ribose with pyrimidines is highly efficient and proceeds with high regio- and stereoselectivity in the preparation of pyrimidine nucleosides,[7] and (2) α-ureidomethylene lactones are readily isomerized to 5-(hydroxyalkyl)uracils.[8] The possibility that the corresponding thiolactones might analogously rearrange to 5-(thioalkyl)uracils suggests a convenient route to (thioalkyl)uridines that does not require multistep thiol-incorporation reaction sequences.[6,9]

Scheme 1



Scheme 2



Ureidomethylene thiolactones were prepared from thiolactones and subsequently isomerized to uracil derivatives according to Scheme 2. Butyrothiolactone (**2b**) is commercially available, and valeryl lactone (**2c**)[10] and thiepanone (**2d**)[11] can be easily prepared using literature methods. While the Claisen condensations of the corresponding normal lactones with methyl formate worked adequately with sodium methoxide as a base to give the

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sodium salt of α-hydroxymethylene lactone,[8] no reaction of **2b** with methyl formate was observed under identical conditions. A stronger base, magnesium diisopropylamide, was reported to afford low yields for the condensation reactions of **2b** and **2c**.[12] We observed that lithium diisopropylamide (LDA) improved the condensations considerably. Thus, addition of LDA to a mixture of **2b** and methyl formate in dry THF produced the lithium salt **3b**, which precipitated from the reaction mixture in 90% yield (crude). Without further purification, compound **3b** was added to a stirred solution of urea in 3 N HCl at 0 °C. After the solution was stirred for 1 h at room temperature and allowed to stand overnight, a solid formed that was collected by filtration to give **4b** in 67% yield.

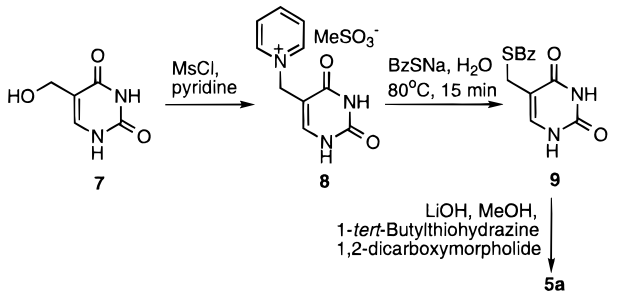
Treatment of **4b** with potassium hydroxide in methanol at reflux for 4 h under nitrogen yielded a solid precipitate. To this suspension were added water (to dissolve the solid) and 1-(*tert*-butylthio)hydrazine 1,2-dicarboxymorpholide.[13] The mixture was stirred for 1 h at room temperature and allowed to stand overnight to give again a solid precipitate, which was collected by filtration to yield **5b** in 79% yield.

The condensation of **2c** with methyl formate under the same conditions as for **2b** failed to give **3c** as a precipitate, possibly because of increased solubility of the product in THF due to the extra methylene group. Thus, this condensation reaction mixture was concentrated and directly treated with urea in 3 N HCl. The product **4c** precipitated from the mixture, was collected by filtration, and was found to be analytically pure by elemental analysis and ^{1}H NMR. The overall yield for the two-step conversion of **2c** to **4c** was 48%. Compound **4c** was converted to **5c** in 75% yield using the same procedure as that for **4b** to **5b**.

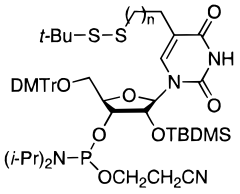
Analogous transformations of thiepanone **2d** using procedures similar to those described for conversion of **2b** and **2c** to **5b** and **5c**, respectively, generated product **5d** in very low overall yields. This is not surprising as base-induced polymerization of **2d** has been well documented[11] and can compete with the desired condensation between **2d** and methyl formate. We tested a different base, lithium bis(trimethylsilyl)amide at −78 °C, which proved to be very effective for the desired condensation, presumably by minimizing polymerization and other side reactions. The condensation reaction mixture was concentrated to a solid and treated with a solution of urea in absolute ethanol. After the mixture was refluxed overnight, water and 1-(*tert*-butylthio)hydrazine 1,2-dicarboxymorpholide were sequentially added at room temperature. Two hours of stirring gave the product **5d** as a solid. It is likely that condensation intermediate **3d** reacts with urea, leading directly to formation of the pyrimidine ring. While possible intermediates **3d** and **4d** were not isolated, the overall procedure was simplified, and the overall yield from **2d** to **5d** was 56%, higher than those from **2b** and **2c** to **5b** and **5c**, respectively. We are currently investigating whether it is possible to further improve the conversions of **5b** and **5c** according to this modified procedure and whether it can be applied to higher homologs of the thiolactones.

Attempts to convert the 4-membered thiolactone **2a** to **5a** using these procedures proved unsuccessful. However, **5a** was easily prepared from commercially available 5-(hydroxymethyl)uracil according to Scheme 3. Thus, treatment of 5-(hydroxymethyl)uracil with methanesulfonyl chloride in pyridine gave the pyridinium salt **8** in 69% yield after recrystallization from methanol. Heating an aqueous mixture of **8** and sodium thiobenzoate at 80 °C for 15 min resulted in precipitation of the benzoylthio derivative **9** in greater than 90% yield. Debenzoylation of **9** with lithium hydroxide in methanol in the presence of 1-(*tert*-butylthio)hydrazine 1,2-dicarboxymorpholide resulted in efficient conversion to the *tert*-butyl disulfide **5a** (93%).

Scheme 3



The modified bases **5a**−**d** were converted to the corresponding nucleosides using the Hilbert-Johnson reaction (Scheme 2).[7] These bases **5a**−**d** were heated in hexamethyldisilazane at reflux, subsequently treated *in situ* with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-*β*-D-ribofuranose and tin chloride in acetonitrile. Although these reactions were extremely air- and moisture-sensitive, products **6a**−**d** were obtained in yields ranging from 75−93%. Debenzoylation by treatment with 25% sodium methoxide in methanol at room temperature, followed by acidification with Dowex-resin (50WX8−200), afforded the unprotected nucleosides **1a**−**d** in yields exceeding 90%.



10a-d, n=0-3

In summary, we have described a short, practical route to 5-(thioalkyl)uridines from readily available starting materials. These modified uridines **1a**−**d** have been converted to the phosphoramidite products **10a**−**d** using standard procedures[14,15] and have been incorporated site-specifically into RNA molecules with coupling yields identical to those of normal nucleoside phosphoramidites. Furthermore, it has been possible to convert nucleosides **6a**−**d** directly to the corresponding cytidine derivative *via* the 4-triazole intermediate.[16] These results will be reported in due course.

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Supporting Information Available: Experimental details and characterizations of all new compounds (9 pages).

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