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A short, concise synthesis of queuine

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ABSTRACT

A short, concise synthesis of queuine was accomplished in a 36% overall yield through a convergent scheme utilizing a reductive amination as the penultimate step. The synthesis demonstrates the utility of silylation to facilitate reactions of various pyrrolo[2,3-*d*]pyrimidine intermediates, and offers the possibility of easily accessing related pyrrolo[2,3-*d*]pyrimidines as well as making additional analogues of queuine.

Queuine (**1**; Fig. 1) is one of the approximately one hundred modified nucleotides found in RNA.[1] It was first discovered in bovine amniotic fluid and later determined to be conserved throughout the eukaryl and eubacterial kingdoms with very few exceptions.[2,3] Queuine is unusual in that, unlike the majority of modified nucleotides that result from modifications of the genetically encoded nucleotides, it is incorporated into RNA by transglycosylation. Queuine modification differs depending on the type of organism under consideration.[2] Eukaryae incorporate queuine into RNA, whereas eubacteria incorporate preQ$_1$ (**2**; Fig. 1), which then undergoes modification to yield queuine. Queuine has long been known to occur in the wobble position of four tRNAs: aspartic acid, asparagine, histidine, and tyrosine.[4,5] However, recent in vitro studies of the *Escherichia coli* transglycosylase have demonstrated the potential for queuine to exist in other RNA species.[6–8] To more fully probe the occurrence of queuine and to utilize it in mechanistic studies of eukaryl transglycosylases requires an efficient, straightforward synthesis.

There are three previous reports on the synthesis of queuine.[9] That of Kondo et al.[9a] proceeds via a Schiff base between a protected pyrrolopyrimidine aldehyde and protected queuine side chain amine in a total of 19 steps. The synthesis of Akimoto et al.[9b] is much shorter and proceeds via a Mannich reaction to incorporate the protected side chain of queuine regioselectively to the C-5 position of a pyrrolopyrimidine precursor. Its main drawback is the requirement of several equivalents of the side chain in a key exchange reaction. The more recent synthesis of Barnett and Grubb[9c] employs a different disconnection of the molecule, proceeding via a key Mitsunobu reaction to introduce the cyclopentenylamine side chain and a subsequent cyclocondensation reaction to build up the core heterocyclic moiety. While relatively straightforward and efficient, it requires 11 linear steps. In this Letter, we report on an exceptionally short and high yielding synthesis of queuine from readily available precursors.

Our synthesis is shown in Scheme 1 and utilizes a strategy wherein easily accessed side chain amine and core heterocycle moieties are condensed via a reductive amination step followed by a global deprotection step. The most direct route to the desired heterocyclic moiety of queuine was determined to be through preQ$_0$ (**3**),[10] which was difficult to enter into further reaction due to its poor organic solubility. A solution to this was to draw from the procedure of Barnett and Kobierski[11] to first silylate **3** with excess *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and after removing volatiles in vacuo, treat the residual oil with trityl chloride in pyridine to provide tritylated preQ$_0$ (**4**). The site of tritylation, while not important for subsequent reactions, was determined by ^{1}H NMR to be on the N-2 position.

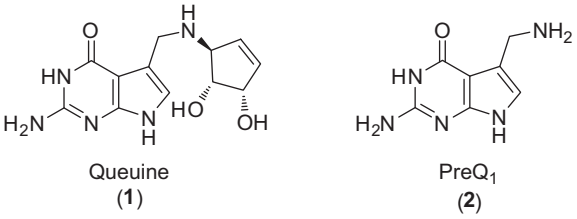

Figure 1. Structures of queuine and preQ$_1$.

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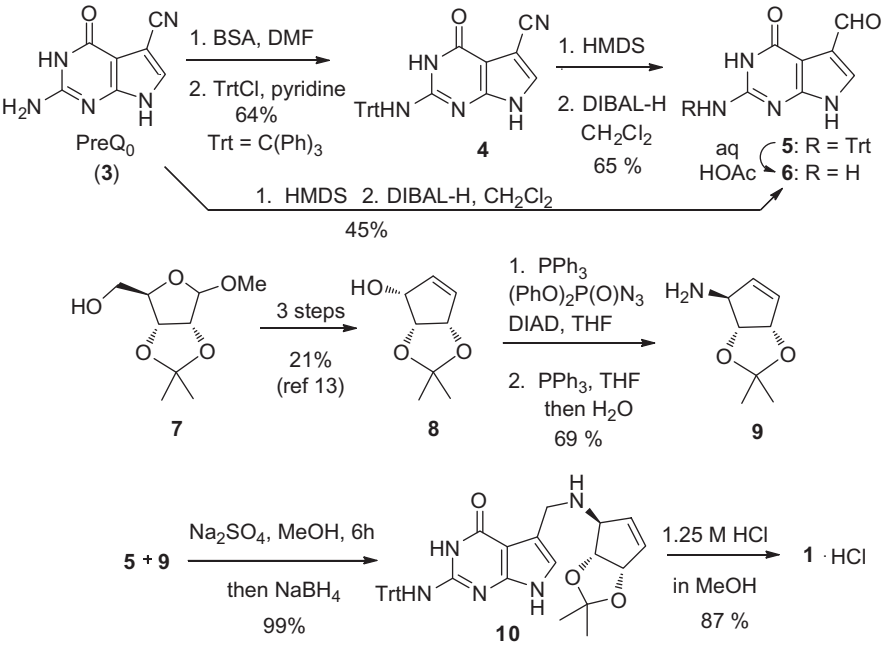

Scheme 1. Convergent synthesis of queuine (**1**) via reductive amination of pyrrolo[2,3-*d*]pyrimidine (**5**) and amino alcohol (**9**).

Several trials were attempted to transform nitrile **4** to desired aldehyde **5** with DIBAL-H before a reproducible method with an acceptable yield was found. A key breakthrough was to utilize silylation of the heterocycle to impart sufficient solubility such that reduction could be conducted at a lower temperature. Hence, protected nitrile **4** was silylated with HMDS in refluxing toluene, and upon removal of volatiles the remaining glass-like solid was dissolved in dichloromethane and cooled to −78 °C. DIBAL-H was then added to complete the transformation to **5** after mild acidic workup. Acid hydrolysis of **5** then provided highly insoluble aldehyde **6**.[12] We found that this could also be obtained directly from unprotected nitrile **3** under similar DIBAL-H reduction conditions described above, albeit in modest yield. The chemistry shown in Scheme 1 completed a two-pot sequence in an overall 42% yield to key heterocyclic aldehyde **5** for subsequent entry into a reductive amination reaction.

With the aldehyde in hand, our attention focused on synthesizing the amine side chain of queuine. A recent synthesis by Klepper et al.[13] was deemed to be the best available approach and was utilized in our synthesis with some modifications. Our decision to follow this route was based on its length, overall yield, and the fact that it addresses a [3.3] sigmatropic rearrangement of an allylic azide intermediate that leads to racemization, which likely occurs in earlier routes employing similar intermediates. Klepper et al. were able to suppress this by conducting the Mitsunobu reaction and subsequent Staudinger reduction in the same pot at a reduced temperature.

As shown in Scheme 1, the synthesis of the cyclopentenylamine side chain begins with commercially available **7**. This contains the requisite chirality for the two alcohol functions in queuine. The reactions from **7** to alcohol **8** were conducted similarly as previously described,[13] although minor adjustments were made in several steps (see Supplementary data). For the key final step (**8**–**9**), we modified the procedure of Klepper et al. to avoid the use of hazardous hydrazoic acid in the Mitsunobu reaction. Utilizing diphenylphosphoryl azide (DPPA) instead, **9** was procured in good yield with careful control of temperature followed by in situ Staudinger reduction. The four-pot route from **7** to **9** proceeded in 14.5% overall yield and was highly reproducible.

Before completing the synthesis, the chiral purity of amine **9** needed to be verified due to possible racemization of the azide intermediate derived from **8**. This was accomplished by generating the Mosher amide[14] under standard amidation conditions. Subsequent ^{19}F NMR demonstrated that only one enantiomer was present. This was confirmed by comparing the spectrum of the Mosher amide of **9** to that of racemic **9**, synthesized via an alternate route that failed to suppress the [3.3] sigmatropic rearrangement of the allylic azide (see Supplementary data).

With key reaction partners aldehyde **5** and amine **9** in hand, the synthesis of queuine was completed. As shown in Scheme 1, this was done under standard reductive amination conditions to provide penultimate adduct **10** in near quantitative yield. This was entered directly into a global deprotection with methanolic HCl to provide queuine (**1**) which precipitated out of solution as the monohydrochloride salt in 87% yield.

In conclusion, we have developed a concise synthesis of queuine, which is considerably shorter than previously reported syntheses. Our synthesis proceeds in an overall 36% yield in a four-pot linear sequence from starting pyrimidine **3**. Our route demonstrates the utility of silylation to facilitate reactions of various pyrrolo[2,3-*d*]pyrimidine intermediates, which otherwise would be difficult to conduct reliably. Furthermore, our synthesis, especially in the facile generation of aldehydes **5** and **6**, offers the possibility of easily accessing related pyrrolo[2,3-*d*]pyrimidines as well as making further analogues of queuine. Future publications will report on the synthesis of radiolabeled queuine, wherein $NaBT_4$ is utilized in the reductive amination step, and the application of this compound to study the prevalence of queuine as well as to conduct kinetic studies of the eukaryl transglycosylase.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.06.008.

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