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Efficient Synthesis of Deazaguanosine-Derived tRNA Nucleosides $PreQ_0$, $PreQ_1$, and Archaeosine Using the Turbo-Grignard Method

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The natural products $PreQ_1$, $PreQ_0$, and archaeosine are deazaguanosine-derived natural products of immense biological importance. All three are critical components of tRNAs and, as such, are involved in decoding genetic information. We were able to consolidate and shorten the syntheses of all three compounds by using a novel Turbo-Grignard-based approach. The reported iodine/magnesium exchange followed by a Grignard reaction with the deazaguanosine skeleton showed a high tolerance towards important functional groups such as esters and amides.

Introduction

Naturally occurring RNAs like messenger RNA (mRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA) contain modified nucleosides, which structurally deviate from the canonical ones.[1] The found changes range from simple methylations of the base or of the C2′–OH of the sugar to hypermodifications featuring large side chains and additional rings. The most complex hypermodifications are probably those derived from deazaguanosines. These bases not only contain an additional side chain but also have an altered ring system, in which the N7 of guanosine is replaced by a carbon atom.

In archaea, the deazaguanosine nucleoside archaeosine (G^+) occurs in the 15-position of nearly all tRNAs,[2] where it is suspected to stabilize their structural integrity (Figure 1).[3] Under physiological pH it is positively charged and placed in a cleft with high negative electrostatic potential.[2] Queuosine (Q) occurs in the wobble position of the anticodon in $tRNA^{His}$, $tRNA^{Asn}$, $tRNA^{Asp}$, and $tRNA^{Tyr}$ of both eukaryotes and prokaryotes (Figure 1).[4] It is involved in numerous key processes such as translational fidelity,[5] development,[6] proliferation,[7] and bacterial virulence.[8] There is a multitude of excellent reviews discussing the individual aspects of queuosine biosynthesis[2,9,10,11] and functions.[9,12] Furthermore, the influence of the Q base on the activity of proto-oncogenes[13] and correlations between a decreased Q-content and malignancy as well as tumor differentiation implies a participation of Q-deficiency in the neoplastic processes.[14] $PreQ_1$ and $PreQ_0$ (Figure 1) are central biosynthetic precursors of queuosine and archaeosine, respectively.[2,15] They are incorporated into tRNA by tRNA guanine transglycosylases (TGTs),[11] one of which has been identified as a target for the treatment of shigellosis.[16] In addition, deazaguanosine-derived nucleosides are of significant interest from a medicinal chemistry point of view, as they have antibacterial, antifungal, antiviral, and anticancer activity.[17]

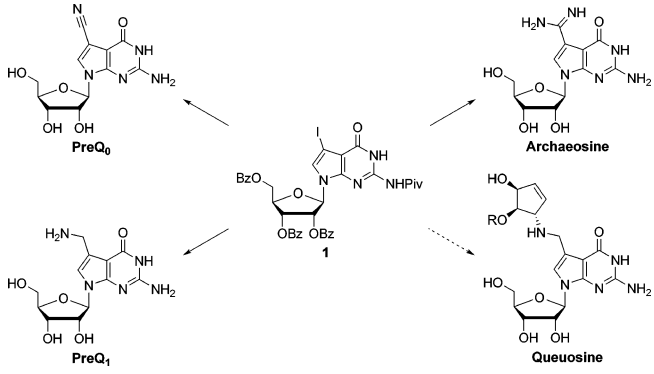

Figure 1. The hypermodified deazanucleosides $PreQ_0$, archaeosine, $PreQ_1$, and queuosine are all available from iodonucleoside **1**. The synthesis of queuosine has been presented before.[18]

Results and Discussion

The high biological importance of deazaguanosines prompted us to establish a chemo- and regioselective approach for the functionalization of deazaguanosines in the critical 7-position starting from the common key intermediate **1** (Figure 1). The method was intended to tolerate a broad range of functionalities such as protecting groups,

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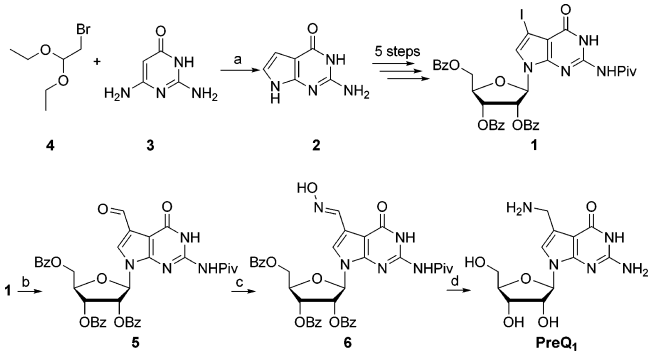
both at the sugar moiety and the heterocycle. For this study, we chose the naturally occurring nucleosides $PreQ_0$, $PreQ_1$, and archaeosine.

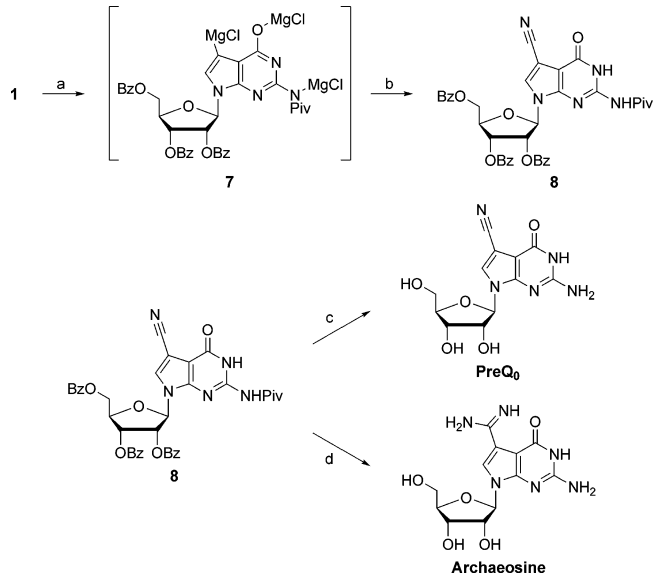
The synthesis of nucleoside **1** was reported in preceding publications.[18,19] For the initial step of the synthesis, which requires preparation of nucleobase **2**,[20] we found that condensation of pyrimidinone **3** with bromoacetaldehyde, in situ generated from bromoacetaldehyde diethyl acetal **4** is superior to the procedure published before (Scheme 1). Subsequent steps towards nucleoside **1** were performed as published[18] yielding critical intermediate **1** in six steps and 10% overall yield.



Scheme 1. Synthesis of the natural deazaguanosine derivative $PreQ_1$. Reagents and conditions: (a) HCl, NaOAc, water, 90 °C and 80 °C, 4 h, 74%; (b) $Pd_2(dba)_3$, triphenylphosphane, CO, tributyltin hydride, toluene, 50 °C, 2 h, 73%; (c) hydroxylamine hydrochloride, $NaHCO_3$, H_2O, EtOH, –20 °C, 12 h, 65%; (d) $NiCl_2{\cdot}H_2O$, $NaBH_4$, MeOH, 0 °C, 1 h, then NaOH in MeOH (0.5 M), room temp., 12 h, 42%.

Starting from nucleoside **1** we recently reported a formylation strategy that led to the hypermodified nucleoside queuosine in nine steps.[18] This approach was also used for the preparation of $PreQ_1$ (Scheme 1). After palladium-catalyzed formylation of nucleoside **1** with CO and Bu_3SnH, the resulting aldehyde **5** was converted into the corresponding oxime **6**. Reduction of the oxime moiety was initially attempted with $NaBH_4$, which however proved to be too unreactive. Functional groups that are inert to treatment with $NaBH_4$ have been shown to gain susceptibility to reduction after addition of $NiCl_2{\cdot}H_2O$.[21] Application of these conditions indeed allowed clean reduction of the oxime to furnish the benzoylated and pivaloylated $PreQ_1$. The crude product was deprotected with 0.5 M NaOH in MeOH to yield $PreQ_1$ in excellent purity after HPLC. In summary, formylation and subsequent reduction of nucleoside **1** allows preparation of $PreQ_1$ in only nine straightforward steps and in 1.9% overall yield. This is significantly shorter and higher yielding than previous routes.[22,23]

For the synthesis of C7-substituted deazaguanosine derivatives such as $PreQ_0$ and archaeosine, we expanded the scope of the novel and versatile reagent *i*PrMgCl·LiCl (Turbo-Grignard)[24] from uracil[25,26] and adenosines[26,27] to a further class of nucleosides: the deazaguanosines (Scheme 2). Using the Turbo-Grignard enables mild conditions at low temperature compared to a reaction using CuCN.[28] To this end, iodo compound **1** in toluene at –78 °C was first deprotonated with MeMgCl (2 equiv.) and then converted into Grignard compound **7** by addition of *i*PrMgCl·LiCl. This procedure leads to almost quantitative formation of the corresponding metallated species **7**. This was shown by treatment of intermediate **7** with water, which caused complete deiodination of the starting material. The most likely structure of compound **7** is shown in Scheme 2. Interestingly, the iodine/magnesium exchange reaction takes place despite the presence of two negatively charged positions in the starting material after deprotonation with MeMgCl. Grignard reagent **7** was subsequently trapped by addition of TosCN. The corresponding nitrile nucleoside **8** was isolated by using a standard extraction procedure followed by column chromatography.



Scheme 2. Synthesis of the deazaguanosine-containing tRNA bases $PreQ_0$ and archaeosine. Reagents and conditions: (a) MeMgCl, *i*PrMgCl·LiCl, toluene, –65 °C, 6 h; (b) TosCN, 65 °C to room temp., 18 h; (c) NH_3 in H_2O (28%), 60 °C, 16 h, 78%; (d) HCl (g), MeOH, then NH_3 in MeOH (7 M), room temp., 19 h, 40%. Complexed LiCl in intermediate **7** has been omitted for the sake of clarity.

The established iodine/magnesium exchange reaction has the great advantage of tolerating various functional groups including ester and amide moieties present during the conversion. Furthermore, the reactive C8-H position, which has been used for C–H activation before,[29] is not attacked under the conditions presented here. This high tolerance towards functional groups is strictly limited to the Turbo-Grignard reagent. Experiments with *i*PrMgCl alone resulted in decomposition of nucleoside **1**, which showed that the presence of LiCl is essential for enabling the transformation.[30]

Starting from nitrile nucleoside **8** we prepared $PreQ_0$, the biosynthetic precursor of archaeosine, by simple deprotection with NH_3 in water. $PreQ_0$ is available by this route in only eight steps and 4.7% overall yield.[22,31]

Finally, nitrile nucleoside **8** was converted into archaeosine by using Pinner conditions. This approach not only converted the nitrile efficiently into the amidinium moiety

but simultaneously removed all protecting groups. For this reaction, we dissolved nucleoside **8** in MeOH and treated the solution with gaseous HCl. After 3 h the solvent was removed, and the resulting oil was stirred in 7 N ammonia in MeOH. The crude product was purified by HPLC as described before.[31] The described approach furnished archaeosine in only eight steps with an overall yield of 2.4%.

Conclusions

To the best of our knowledge this report describes the first Grignard and iodine/magnesium exchange reaction performed with deazapurines. Application of the Turbo-Grignard reagent allowed us to perform functionalization of the C7 position in the presence of benzoyl and pivaloyl protecting groups. This facilitated the synthesis of the deazaguanosine-derived tRNA nucleosides archaeosine, $PreQ_0$, and $PreQ_1$, which should now enable detailed biochemical investigation of their functions in vivo.[32] In the course of this study, introduction of the versatile nitrile moiety through the presented Turbo-Grignard reaction has been established. Expansion of the scope of this reaction to other electrophiles is currently under investigation and might turn the here-established reaction into a general tool for the manipulation of deazaguanosines.

Supporting Information (see footnote on the first page of this article): Experimental procedures and spectroscopic data for the compounds prepared.

Acknowledgments

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