Nucleosides

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Synthesis of the Transfer-RNA Nucleoside Queuosine by Using a Chiral Allyl Azide Intermediate**

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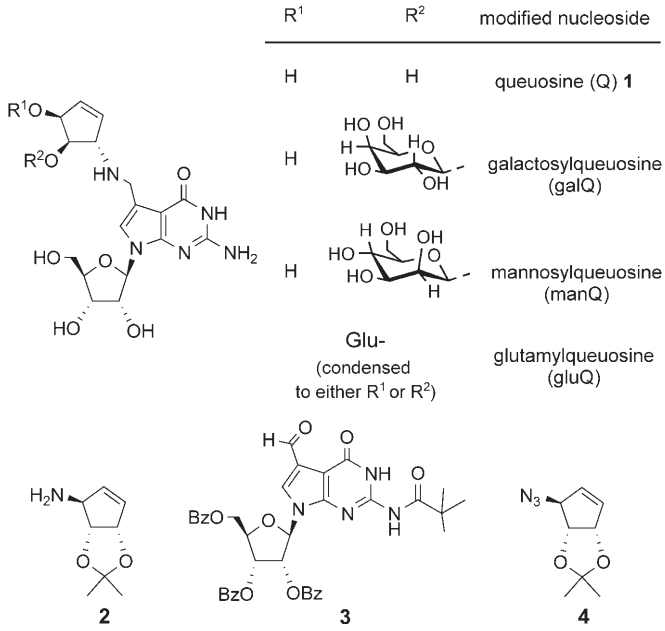
Transfer RNAs (tRNAs) are key molecules needed for the decoding of the genetic information at the ribosome.[1] The tRNAs contain the four canonical RNA bases plus a large set of modified nucleotides whose function in the tRNA charging and decoding process is largely unknown.[2] Queuosine (Q; **1**)[3] and its galactosylated, mannosylated, or amino acid modified derivatives[4] (Scheme 1) are important hypermodified RNA bases present in the anticodon stem loop of various tRNAs. It is currently hypothesized that these base derivatives are needed to fine-regulate the translational process.[5] To enable investigation of the role that these hypermodified bases play during the translational process and to study their largely unknown biosynthesis,[6] efficient synthetic procedures for the modified bases and ultimately of tRNA containing such modified bases are a prerequisite.

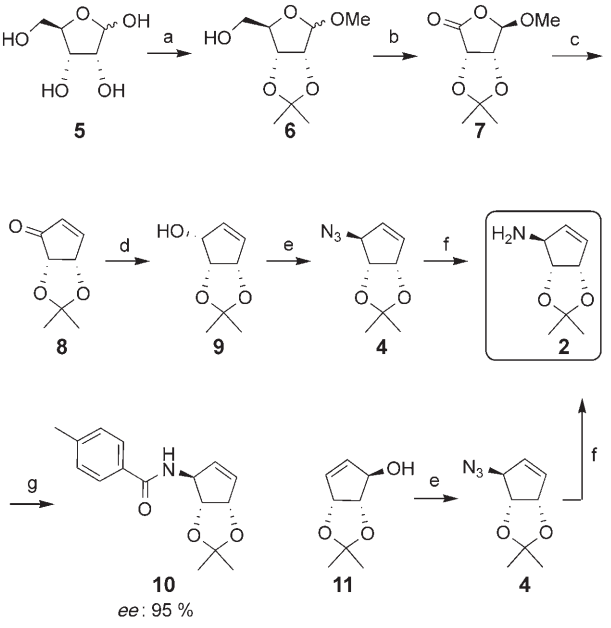


Scheme 1. Chemical structures of queuosine (**1**), its derivatives, and key synthetic intermediates. Bz = benzoyl.

Herein we describe an efficient stereoselective synthesis of the hypermodified tRNA nucleoside queuosine[7] using a reductive amination of 7-deazaguanosine-aldehyde **3** with the cyclopentenylamine **2**,[8] which is prepared from an allyl azide precursor (Scheme 1).

The short synthesis of the allyl amine **2** from the allyl azide precursor **4**, however, requires that the Mitsunobu reaction (Scheme 2, step e)[9] proceeds with a defined regio- (S_N2 vs. S_N2') and stereochemistry (inversion of configuration). In addition, the [3.3] sigmatropic rearrangement, which allyl azides such as **4** are prone to undergo, has to be controlled.[10] Indeed, owing to the efficient [3.3] sigmatropic rearrangement of allylic azides and the reported lack of regiocontrol of Mitsunobu aminations of allylic alcohols,[19] such a synthetic approach looks, at first, rather unattractive.

The cyclopentenyl building block **2** was synthesized starting from D-(−)-ribose **5**. Protection of the 2′ and 3′ hydroxy groups as an acetonide, conversion into the mono-



Scheme 2. Synthesis of the cyclopentenylamine **2**: a) 2,2-dimethoxypropane, MeOH, $HClO_4$, acetone, 94%; b) PCC (4.0 equiv), benzene, reflux, 46%; c) $(MeO)_2P(O)CH_3$, *n*BuLi, THF, 0°C, 56%; d) $NaBH_4$, $CeCl_3$, MeOH, 0°C, 95%; e) DIAD, PPh_3, HN_3 (1.3 M in toluene), THF, 0°C; f) PPh_3, THF, 0°C, 72% (65% from **11**) in two steps; g) toluoyl chloride, NEt_3, CH_2Cl_2, 60%. PCC = pyridinium chlorochromate, DIAD = diisopropylazodicarboxylate.

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methylacetal **6**, and subsequent oxidation with excess pyridinium chlorochromate gave, under C–C bond cleavage lactone **7**. An intramolecular Wittig reaction with dimethylmethylphosphonate and *n*BuLi furnished pentenone **8**.[11] Stereospecific reduction of **8** with sodium borohydride allowed the preparation of the key allyl alcohol intermediate **9** in just four steps with an overall yield of 23%. Subsequent Mitsunobu reaction and then a Staudinger reduction at room temperature gave exclusively **2**. To analyze the optical purity of the allyl amine **2**, we converted it into the toluoyl protected derivative **10**. Analysis of **10** by chiral HPLC, showed to our disappointment that compound **2** was obtained with an unsatisfactory *ee* value of $<80\%$. The reason could be either a partial *anti*-S_N2'-type reaction, as a result of the lack of regiocontrol, or racemization that occurs because of the allyl azide rearrangement.[12] To analyze the allyl azide rearrangement pathway in more detail, we heated the allyl azide **4** to about 60 °C. Indeed, within a few hours the optical rotation dropped to zero, showing that the allyl azide [3.3] sigmatropic rearrangement is indeed a problem at room temperature.

To discover how to control the unwanted allyl azide rearrangement we measured the disappearing optical rotation of a solution of **4** in pure ethanol at various temperatures (Figure 1). From this plot it is evident that the allyl azide equilibration, which is indeed quite fast at room temperature, can be efficiently suppressed at just 0 °C. After checking that the racemization rate in THF and EtOH was identical, we carried out the Mitsunobu reaction **9** to **4** at 0 °C. A Staudinger reduction, also performed at 0 °C, provided compound **2**, which was again converted into the derivative **10** for analysis by chiral HPLC. This time, the key allyl amine intermediate **2** was indeed obtained with an excellent *ee* value of 95% in 72% yield over both steps.

In contrast to recently published procedures, in which the fast allyl azide equilibrium was exploited for synthesis,[10a,13] we show herein that the rearrangement can also be efficiently suppressed, which allows chiral allyl azides to be utilized as convenient synthetic intermediates. In addition, the ability to suppress the racemization demonstrates that the Mitsunobu reaction proceeds, in our case, unexpectedly under strict control of the regiochemistry:[9] we observe exclusively an S_N2 and not *anti*-S_N2'-attack of the azide on the allylic alcohol.

The unusual selectivity of the Mitsunobu amination is further demonstrated by the observation that the use of the diastereomeric allylic alcohol **11**[14] provides the same allyl azide **4** and, after Staudinger reduction, the allylic amine **2**. This result is only explainable if, in this case, we assume an interesting, and for the Mitsonobu amination untypical, *syn*-S_N2' reactivity.[15,16,19]

For the preparation of 7-formyl-7-deazaguanosine as the second key building block needed for the synthesis of queuosine (**1**), a second short synthesis was developed (Scheme 3). Condensation of chloroacetaldehyde (**12**) and 2,4-diamino-6-hydroxypyrimidine furnished 7-deaza-guanine (**13**). Chlorination of the 5-position with phosphoryl chloride, protection of the exocyclic amino function with pivaloyl chloride, and subsequent iodination with NIS gave the deazapurine derivative **14**. This compound was coupled to protected D-(−)-ribose under Vorbrüggen conditions to give the iodonucleoside **15** in good yields.[17]

After dechlorination of **15** with DABCO and triethylamine, the aldehyde group was introduced by a palladium-

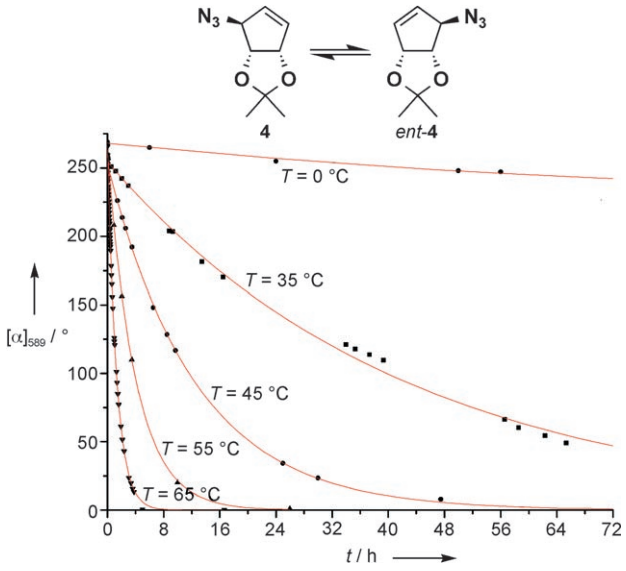
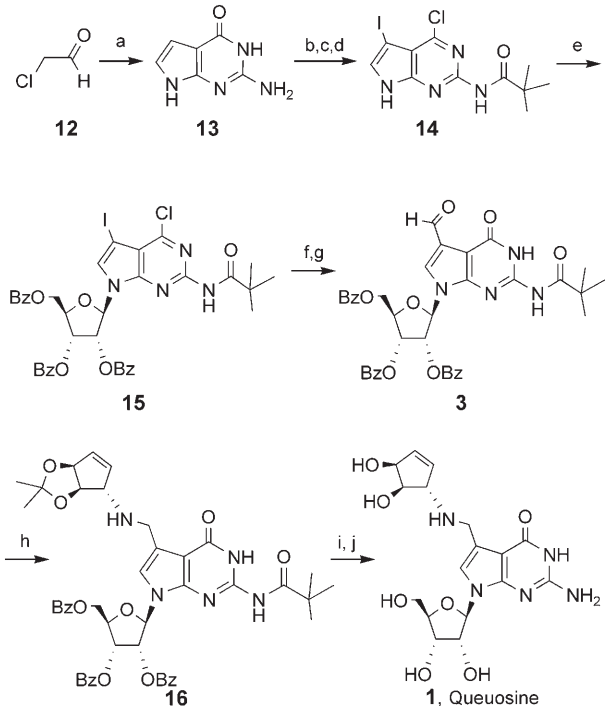


Figure 1. [3.3] sigmatropic rearrangement of **4** at $T = 0$, 35, 45, 55, and 65 °C in EtOH.



Scheme 3. Synthesis of queuosine (**1**): a) 1) NaOMe, H_2O, DMF; 2) 2,4-diamino-6-hydroxypyrimidine, NaOAc, 86%; b) $POCl_3$, 120 °C, 78%; c) PivCl, pyridine, 64%; d) NIS, THF, 81%; e) BSA, TMSTf, 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose, MeCN, 60%; f) DABCO, NEt_3, CsOAc, DMF, 63%; g) $[Pd_2(dba)_3]$ PPh_3, CO, Bu_3SnH, 73%; h) **2**, benzene, then $NaBH_4$, EtOH, 85%; i) NaOH, MeOH; j) HCl (2 N), 45%. Piv = pivaloyl, NIS = *N*-iodosuccinimide, BSA = bis(trimethylsilyl)-acetamide, TMSTf = trimethylsilyltriflate, DABCO = 1,4-diazabicyclo-[2.2.2]octane.

catalyzed CO insertion and subsequent quenching of the organometallic intermediate with tributyltin hydride. The final reductive amination of the product **3** with cyclopentenylamine **2** proceeded in excellent yields with $NaBH_4$ as the hydride source. Final deprotection using NaOH to cleave the pivaloyl and benzoyl protecting groups and subsequent treatment with acid to cleave the acetonide protecting group furnished the hypermodified nucleoside queuosine (**1**) in only 15 synthetic steps in an overall yield of 6.4% (the analytical data are identical to the data published for the natural product[18]).

In summary, we report a short convergent synthesis of the hypermodified tRNA nucleoside queuosine (**1**), which is found in the anticodon stem loop of tRNAs. We show that the synthesis is efficiently performed using the allyl azide intermediate **4**. The [3.3] sigmatropic rearrangement of the allyl azide intermediate, which causes racemization of this key intermediate, could be efficiently suppressed at just 0°C. This result shows that chiral allyl azides can be used as highly valuable intermediates in natural-product synthesis. The ability to prepare queuosine now paves the way for a detailed analysis of its function during the decoding process.

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[1] a) R. Lührmann, H. Eckhardt, G. Stoeffler, *Nature* **1979**, *280*, 423–425; b) J. W. Lee, K. Beebe, L. A. Nangle, J. Jang, C. M. Longo-Guess, S. A. Cook, M. T. Davisson, J. P. Sundberg, P. Schimmel, S. L. Ackerman, *Nature* **2006**, *443*, 50–55.

[2] a) P. F. Agris, *Nucleic Acids Res.* **2004**, *32*, 223–238; b) T. Daviter, F. V. Murphy IV, V. Ramakrishnan, *Science* **2005**, *308*, 1123–1124.

[3] a) S. Yokoyama, T. Miyazawa, Y. Iitaka, Z. Yamaizumi, H. Kasai, S. Nishimura, *Nature* **1979**, *282*, 107–109; b) H. Kasai, H. Nakanishi, R. D. Macfarlane, D. F. Torgerson, Z. Ohashi, J. A. McCloskey, H. J. Gross, S. Nishimura, *J. Am. Chem. Soc.* **1976**, *98*, 5044–5046; c) M. Okada, S. Nishimura, *Nucleic Acids Res.* **1977**, *4*, 2931–2937.

[4] a) M. Blaise, H. D. Becker, G. Keith, C. Cambillau, J. Lapointe, R. Giege, D. Kern, *Nucleic Acids Res.* **2004**, *32*, 2768–2775; b) J. Salazar, A. Ambrogelly, P. F. Crain, J. A. McCloskey, D. Söll, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 7536–7541; c) M. Blaise, H. D. Becker, J. Lapointe, C. Cambillau, R. Giege, D. Kern, *Biochimie* **2005**, *87*, 847–861.

[5] a) F. V. Murphy IV, V. Ramakrishnan, A. Malkiewicz, P. F. Agris, *Nat. Struct. Biol.* **2004**, *11*, 1186–1191; b) C. Höbartner, M. Ebert, B. Jaun, R. Micura, *Angew. Chem.* **2002**, *114*, 619–623; *Angew. Chem. Int. Ed.* **2002**, *41*, 605–609.

[6] a) E. A. Meyer, R. Brenk, R. K. Castellano, M. Furler, G. Klebe, F. Diederich, *ChemBioChem* **2002**, *3*, 250–253; b) E. A. Meyer, M. Furler, F. Diederich, R. Brenk, G. Klebe, *Helv. Chim. Acta* **2004**, *87*, 1333–1356.

[7] T. Ohgi, T. Kondo, T. Goto, *J. Am. Chem. Soc.* **1979**, *101*, 3629–3633.

[8] a) K. H. Kim, M. J. Miller, *Tetrahedron Lett.* **2003**, *44*, 4571–4573; b) B. M. Trost, M. T. Sorum, *Org. Process Res. Dev.* **2003**, *7*, 432–435.

[9] D. Gagnon, S. Lauzon, C. Godbout, C. Spino, *Org. Lett.* **2005**, *7*, 4769–4771.

[10] a) A. K. Feldman, B. Colasson, K. B. Sharpless, V. V. Fokin, *J. Am. Chem. Soc.* **2005**, *127*, 13444–13445; b) A. Gagneux, S. Winstein, W. G. Young, *J. Am. Chem. Soc.* **1960**, *82*, 5956–5957.

[11] M. S. Ali, K. Ramesh, R. T. Borchardt, *Tetrahedron Lett.* **1990**, *31*, 1509–1512.

[12] G. Cardillo, S. Fabbroni, L. Gentilucci, R. Perciaccante, F. Piccinelli, A. Tolomelli, *Org. Lett.* **2005**, *7*, 533–536.

[13] H. Takasu, Y. Tsuji, H. Sajiki, K. Hirota, *Tetrahedron* **2005**, *61*, 11027–11031.

[14] H. Ovaa, J. D. C. Codee, B. Lastdrager, H. S. Overkleft, A. Gijsvert, J. H. van Boom, *Tetrahedron Lett.* **1998**, *39*, 7987–7990.

[15] B. K. Shull, T. Sakai, J. B. Nichols, M. Koreeda, *J. Org. Chem.* **1997**, *62*, 8294–8303.

[16] R. M. Magid, *Tetrahedron* **1980**, *36*, 1901–1930.

[17] F. Seela, X. Peng, *J. Org. Chem.* **2006**, *71*, 81–90.

[18] T. Ohgi, T. Kondo, T. Goto, *J. Am. Chem. Soc.* **1979**, *101*, 3629–3633.

[19] J. Mulzer, G. Funk, *Synthesis* 1995, 101–112.

