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A highly stereocontrolled and efficient synthesis of α- and β-pseudouridines

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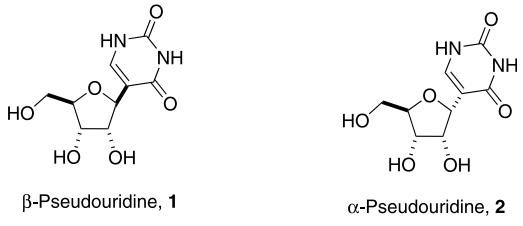
Abstract—A five-step practical and stereocontrolled synthesis of α- and β-pseudouridines from D-ribonolactone is described. The key step involves a highly stereoselective reduction of a hemiketal *C*-nucleoside intermediate in each case. Multi-gram quantities of β-pseudouridine can now be made available.

The ubiquitous presence of β-pseudouridine **1** (Fig. 1), a *C*-5 β-linked uridine in rRNA and tRNA of bacterial and mammalian origins has instigated numerous studies concerned with its functional relevance.[1] The occurrence of pseudouridine in highly conserved regions of the RNA superstructure reflects on its importance in molecular recognition phenomena dealing with polypeptide synthesis for example.[2] This and other observations of biological significance have resulted in studies aimed at the incorporation of synthetic *C*-nucleosides consisting of aromatic and heteroaromatic aglycones in various RNAs and DNAs.[3] The cytotoxic activity of aromatic *C*-nucleosides has been known for some time.[4] β-Pseudouridine has been recently reported as an antimutagenic substance in beer.[5]

As already commented on elsewhere,[6] the exploitation of the potentially important roles that β-pseudouridine can play in the RNA field has been hampered by its limited availability and prohibitive cost. Only recently have methods been developed to access *C*-nucleosides in preparatively significant amounts.[3,4,6] These have been extended to pyrimidine bases resulting in a practical 7-step synthesis of β-pseudouridine from readily available materials such as ribonolactone.[6] In spite of these improvements, a 1:1 mixture of anomeric *C*-nucleosides is obtained as a result of a non-selective reduction of a lactol intermediate with triethylsilane.[7]



β-Pseudouridine, **1** α-Pseudouridine, **2**

Figure 1. Structures of α- and β-pseudouridine.

We report herein, a practical, and most importantly, a highly stereoselective synthesis of β-pseudouridine in 5-steps with an overall yield of 46% starting with D-ribonolactone. The method is also applicable to the synthesis of α-pseudouridine.

Treatment of D-ribonolactone **3** with 2,2-dimethoxypropane in the presence of pyridinium *p*-toluenesulfonate and sodium sulfate gave the protected bis-acetal **4** in 94% isolated yield (Scheme 1).[8] Addition of 5-lithio-2,4-di-*t*-butoxypyrimidine **5**[6b] to **4** in THF at −78°C gave the adduct **6** as a 1:8 α/β-mixture in 89% yield. The latter, in equilibrium with its hydroxyketone isomer (not shown) was reduced with L-Selectride (3.4 equiv.) in the presence of $ZnCl_2$ (1.36 equiv.) to give the acyclic D-*altro*-hexitol **7** as a single isomer in 85% yield. Cycloetherification under Mitsunobu conditions[9] with diisopropyl azodicarboxylate (DIAD) and triphenylphosphine gave **8** in 70% yield. Finally, deprotection with 70% acetic acid gave β-pseudouridine in 93% yield, identical with an authentic sample. This constitutes the shortest synthesis of β-pseudouridine which can now be prepared in multi-gram quantities.[10]

The anomeric α-pseudouridine **2**,[6b] was synthesized essentially using the same protocol, except that the

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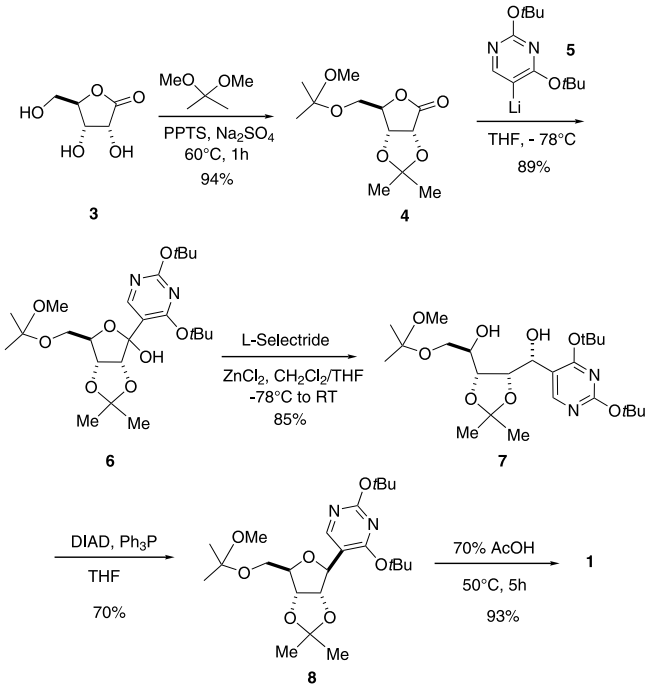
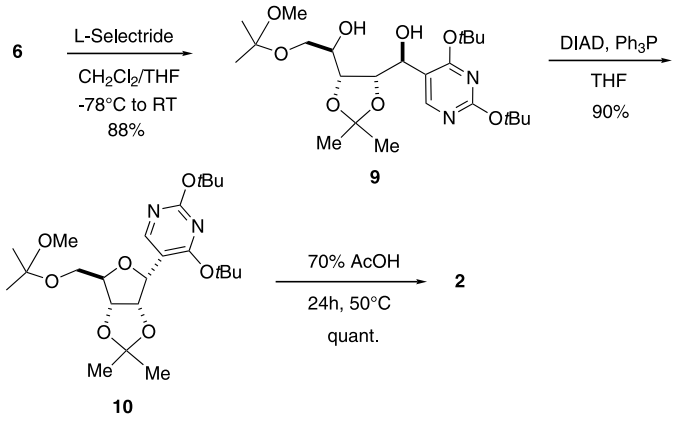


Scheme 1.

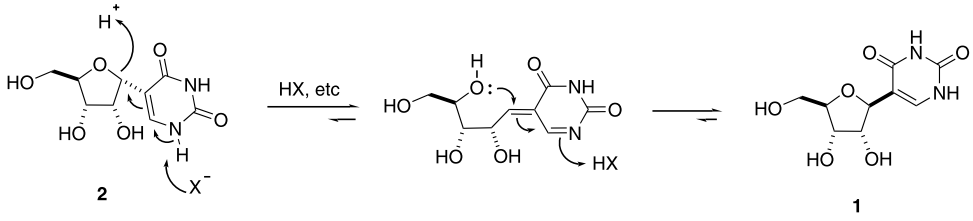
reduction of the lactol intermediate **6** was done with L-Selectride *in the absence of zinc chloride* (Scheme 2). Remarkably, an >20:1 α/β-mixture of the D-*allo*-**9** and D-*altro*-**7** hexitols respectively was obtained in 88% yield. Subsequent cycloetherification and deprotection



Scheme 2.

as described above gave α-pseudouridine **2** with an overall yield of 66%. It is of interest that the Mitsunobu cyclization was faster and more efficient in the case of **9** compared to **7**, probably due to a lower steric barrier.[11]

In an earlier version of the synthesis where 2,4-dimethoxypyrimidine was the aglycone, hydrolysis with NaI/TMSCl (MeCN, 16 h) according to a literature procedure[12] (or with 70% AcOH, 30% HBr in AcOH, reflux 1 h) led to considerable anomerization. Related epimerizations have been recently reported for aromatic *C*-nucleosides under mild acidic conditions.[13] A plausible mechanism is shown in Scheme 3.



Scheme 3.

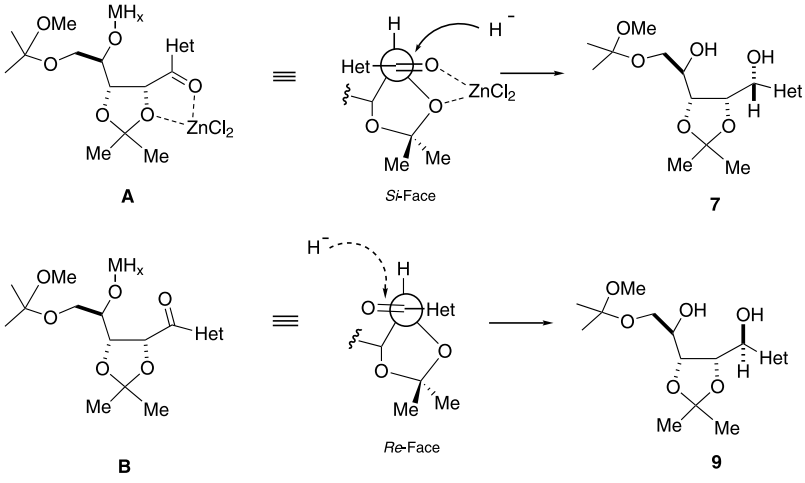


Figure 2. Possible pathways for stereoselective reductions.

The remarkably stereoselective reduction of the lactol **6** with L-Selectride in the presence or absence of zinc chloride can be rationalized by invoking chelated and non-chelated intermediates, respectively as illustrated in Figure 2. Chelation of the divalent zinc with the ketone carbonyl and the acetal oxygen allows hydride delivery from the *Si* (pro-*S*) face to give the observed D-*altro* isomer **7**. In the absence of chelation, the *Re*(pro-*R*)-face of the carbonyl is better exposed to receive the bulky hydride. The influence of the protective groups on the heterocycle (ex. 2-*C*-benzimidazolyl; 2-*C*-imidazolyl, etc.) on the selectivity of reductions of *C*-heterocyclic lactols with $NaBH_4$, has been reported.[9a]

The extension of this new method to the stereoselective synthesis of other *C*-nucleosides is currently in progress.

Acknowledgements

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References

1. For a review, see Maden, B. E. H. *Nature* **1997**, *389*, 129.
2. Lane, B. G.; Ofengand, J.; Gray, M. W. *Biochimie* **1995**, *77*, 7.
3. See, for example: (a) Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, *59*, 7238; (b) Hildbrand, S.; Leumann, C. *Angew. Chem. Int. Ed.* **1996**, *35*, 1968; (c) Matulic-Adamic, J.; Beigelman, L. *Tetrahedron Lett.* **1997**, *38*, 1669; (d) Parsch, J.; Engels, J. W. *Helv. Chim. Acta* **2000**, *83*, 1791.
4. Krohn, K.; Heins, H.; Wielckens, K. *J. Med. Chem.* **1992**, *35*, 511.
5. Yoshikawa, T.; Kimura, S.; Hatano, T.; Okamoto, K.; Hayatsu, H.; Arimoto-Kobayashi, S. *Food Chem. Toxicol.* **2002**, *40*, 1165; see also, Monobe, M.; Arimoto-Kobayashi, S.; Ando, K. *Mutation Res.* **2003**, *538*, 93.
6. (a) Grohen, P. J.; Chow, C. S. *Tetrahedron Lett.* **1999**, *40*, 2049; (b) For earlier syntheses of α- and β-pseudouridines, see Brown, D. M.; Ogden, R. C. *J. Chem. Soc., Perkin Trans. 1* **1981**, 723 and references cited therein.
7. Rolf, D.; Gray, G. R. *J. Am. Chem. Soc.* **1982**, *104*, 3539.
8. The original procedure without the inclusion of sodium sulfate gave **4** in 40% yield, accompanied by the protected δ-lactone and 2,3-*O*-isopropylidene-D-ribonolactone, see Csuk, R.; Kühn, M.; Ströhl, D. *Tetrahedron* **1997**, *53*, 1311. See Scheme 1 for the optimized and modified procedure.
9. (a) Guianvarc'h, D.; Fourrey, J.-L.; Tran Huu Dau, M.-E.; Guérineau, V.; Benhida, R. *J. Org. Chem.* **2002**, *67*, 3724; for reviews, see (b) Mitsunobu, O. *Synthesis* **1981**, 1; (c) Hughes, D. C. *Org. React.* **1992**, *42*, 335.
10. Typical procedure **6**→**8** for β-pseudouridine.
7: To **6** (450 mg, 0.93 mmol) in dry DCM (36 mL) at −78°C, was added $ZnCl_2$ (1.26 mL, 1.26 mmol, 1 M in Et_2O) and the mixture was stirred for 30 min. L-Selectride (3.16 mL, 3.16 mmol, 1 M in THF) was then added dropwise and portionwise for a period of 30 min at −78°. Mixture was then slowly brought to room temperature and stirred overnight. The white heterogeneous solution was quenched with EtOH (0.54 mL), H_2O (0.120 mL), NaOH 6M (0.47 mL) and H_2O_2 30% (0.47 mL). It was then stirred for 30 min and diluted with EtOAc (50 mL) and H_2O (50 mL). Usual workup gave a colorless oil which was purified by flash chromatography (1:1 hexanes/EtOAc) to give **7** as a white foamy solid (385 mg, 85%); R_f=0.35 (1:1 hexanes/EtOAc), m.p. 40–42°C, $[\alpha]_D$ −50.64° (*c* 0.23, MeOH). **8**: To **7** (155 mg, 0.32 mmol) in dry THF (33 mL) was added Ph_3P (168 mg, 0.64 mmol). The colorless mixture was cooled to 0°C and DIAD (0.126 mL, 0.64 mmol) was added, stirred at 4°C for 48 h, then brought to room temperature and concentrated. The yellow oil was purified by flash chromatography (2:1 hexanes/EtOAc) to give **8** as a colorless oil (105 mg, 70%); R_f=0.72 (1:1 hexanes/EtOAc); $[\alpha]_D$ +12.0° (*c* 8.5, MeOH).
11. Typical procedure **6**→**9**→**10** for α-pseudouridine.
9: To **6** (500 mg, 1.03 mmol) in dry THF (40 mL) at −78°C, was added L-Selectride (3.5 mL, 3.5 mmol, 1 M in THF) dropwise and portionwise for a period of 30 min. Mixture was then slowly brought to room temperature and stirred overnight, concentrated and dissolved with DCM (40 mL). EtOH (0.54 mL), H_2O (0.120 mL), NaOH 6M (0.47 mL) and H_2O_2 30% (0.47 mL) were added and stirred for 30 min, then diluted with EtOAc (50 mL) and H_2O (50 mL). Usual workup gave a colorless oil which was purified by flash chromatography (1:1 hexanes/EtOAc) to give **9** as a white foamy solid (430 mg, 88%); R_f=0.32 (1:1 hexanes/EtOAc), m.p. 43–45°C, $[\alpha]_D$ +8.35° (*c* 0.46, MeOH).
10: To **9** (350 mg, 0.72 mmol) in dry THF (75 mL) was added Ph_3P (378 mg, 1.44 mmol). The colorless mixture was cooled to 0°C and DIAD (0.28 mL, 1.44 mmol) was added. Mixture was stirred at 4°C for 24 h then brought to room temperature and concentrated. The yellow oil was purified by flash chromatography (2:1 hexanes/EtOAc) to give **10** as a colorless oil (305 mg, 90%); R_f=0.74 (1:1 hexanes/EtOAc); $[\alpha]_D$ −37.2° (*c* 0.5, MeOH).
12. (a) Kundu, N. G.; Das, B.; Spears, P.; Majundar, A.; Kang, S.-I. *J. Med. Chem.* **1990**, *33*, 1975; (b) Morita, T.; Okamoto, Y.; Sakurai, H. *J. Chem. Soc., Chem. Commun.* **1978**, 874; (c) Silverman, R. B.; Radak, R. E.; Hacker, N. P. *J. Org. Chem.* **1979**, *44*, 4970.
13. Jiang, Y. L.; Stivers, J. T. *Tetrahedron Lett.* **2003**, *44*, 4051.