Tetrahedron Letters 51 (2010) 6463–6465

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

An efficient synthesis of futalosine

Xu Li, Martin E. Tanner *

University of British Columbia, Department of Chemistry, Vancouver, British Columbia, Canada V6T 1Z1

ARTICLE INFO

Article history:
Received 24 July 2010
Accepted 3 October 2010

ABSTRACT

Futalosine is a naturally occurring nucleoside comprised of an inosine core with a 3-carboxyphenyl methylene ketone functionality replacing the C-5′ hydroxyl. Recent studies have shown that it is a key intermediate in an alternative biosynthetic pathway that generates menaquinone in a variety of bacterial species. Here we report the first synthesis of futalosine in seven steps from inosine in an overall 17% yield. This work will enable further studies on menaquinone biosynthesis in pathogenic bacteria.

© 2010 Elsevier Ltd. All rights reserved.

Futalosine is a nucleoside that was first isolated in 1999 from the fermentation broth of *Streptomyces* sp. MK359-NF1 (Fig. 1).[1] It is comprised of an inosine core with a 3-carboxyphenyl methylene ketone functionality replacing the C-5′ hydroxyl. More recently, futalosine has been identified as a key intermediate in an alternative biosynthetic pathway for menaquinone (vitamin K_2).[2,3] Studies on *Streptomyces coelicolor* showed that the *men* genes normally responsible for menaquinone biosynthesis in species such as *Escherichia coli* were lacking, and instead this organism produces the vitamin using a completely novel pathway. Using gene knockouts it was possible to isolate and identify several of the intermediates in the pathway, including futalosine. It was demonstrated that futalosine serves as a substrate for futalosine nucleosidase (futalosine hydrolase) in both *S. coelicolor* and *Thermus thermophilus* and is converted into dehypoxanthine futalosine (Fig. 1).[4,3] This compound is ultimately converted into menaquinone. Bioinformatic analyses indicated that the pathogenic organisms *Helicobacter pylori* and *Campylobacter jejuni* also lacked the *men* genes, but possessed homologs to the genes of the futalosine pathway. Since these organisms are the causative agents of duodenal ulcers and food poisoning, and since menaquinone biosynthesis is required for their survival, the futalosine pathway enzymes represent attractive targets for antibiotic design.[5–7] Furthermore, the absence of the pathway in both humans and beneficial intestinal bacteria, promises drug treatments with a lack of undesirable side effects. Further efforts to identify the enzymes of the futalosine pathway for menaquinone biosynthesis and to develop inhibitors against them are hampered by the low availability of the substrates. In past studies, modest amounts of futalosine were isolated from fermentations of mutant strains of *S. coelicolor* supplemented with menaquinone.[4,3] In order to produce larger amounts of fualosine and to open the doors for analog generation, an efficient synthesis of futalosine was desired. Here we report the first synthesis of futalosine in seven steps from inosine in an overall 17% yield. This work will enable further studies on menaquinone biosynthesis in pathogenic bacteria.

Our first attempt at the synthesis of futalosine involved protection of the hypoxanthine N1 with a benzyl group and the ribose 2° hydroxyls as a dimethylacetonide (Scheme 1).

Accordingly, treatment of inosine with benzyl bromide and $NaHCO_3$ selectively benzylated the N1 position as described previously.[8] Treatment with 2,2-dimethoxypropane and *p*-toluenesulfonic acid then gave compound **1**. In order to introduce the aromatic side chain, a one-pot oxidation/Wittig procedure previously described by Ikejiri et al. was employed.[9] This procedure avoids the isolation of the C5′ aldehyde that can lead to decomposition or epimerization. Phosphorane **2** was first prepared by

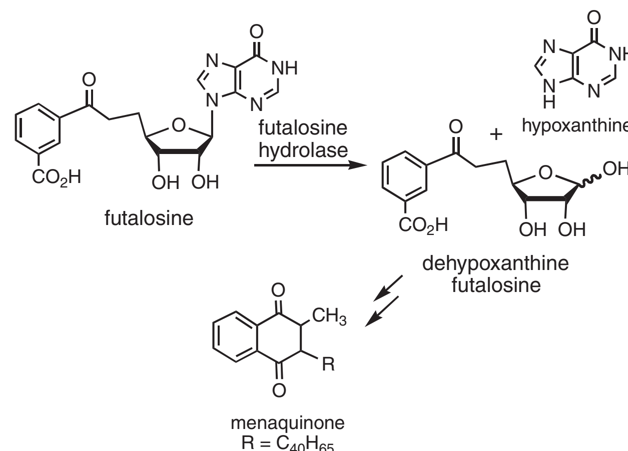
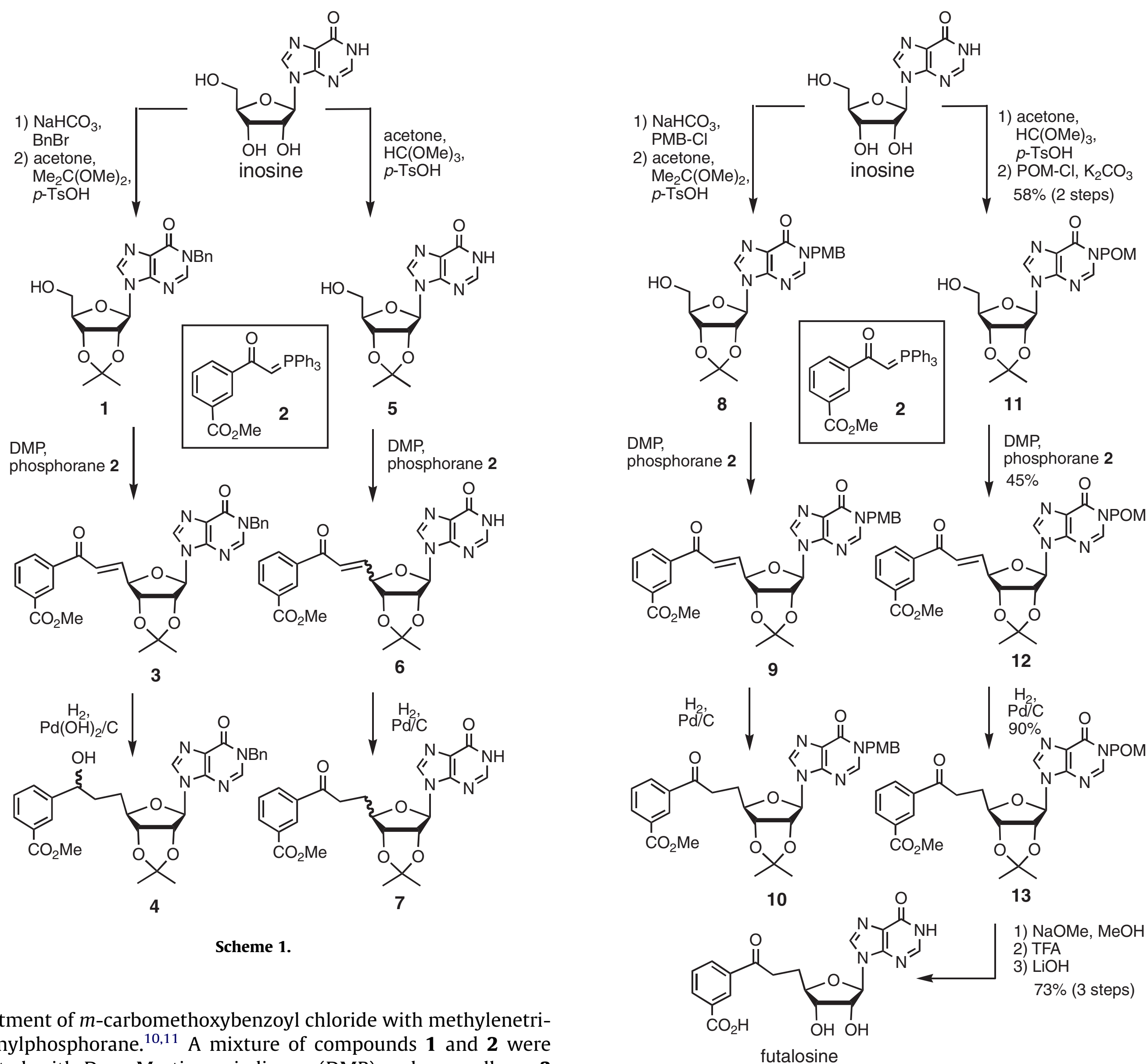


Figure 1. The reaction catalyzed by futalosine hydrolase.

* Corresponding author. Tel.: +1 604 822 9453; fax: +1 604 822 2847.
E-mail address: mtanner@chem.ubc.ca (M.E. Tanner).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.10.010



Scheme 1.

Scheme 2.

treatment of *m*-carbomethoxybenzoyl chloride with methylenetriphenylphosphorane.[10,11] A mixture of compounds **1** and **2** were treated with Dess–Martin periodinane (DMP) and gave alkene **3** predominantly as the trans isomer. We then envisioned that reduction of the alkene and the removal of the benzyl protecting group could be accomplished in a single step. Treatment of compound **3** with hydrogen and Pd/C cleanly reduced the alkene to give a single isomer, but the benzyl group remained intact. When the reduction was carried out with Pearlman's catalyst, compound **4** was obtained in which both the alkene and the ketone were reduced, but again the benzyl group remained intact. The difficulty in removing the N1 benzyl group led us to pursue a synthesis in which the N1 position of hypoxanthine was left unprotected. Acetonide **5** was prepared by treatment of inosine with acetone, triethylorthoformate, and *p*-toluenesulfonic acid as described previously.[12] When acetonide **5** and phosphorane **2** were treated with DMP, however, a mixture of two isomers of compound **6** was obtained. Hydrogenation of compound **6** also gave an approximately equimolar mixture of isomers of compound **7** that were inseparable by conventional silica gel chromatography. It would, therefore, appear that in the absence of a protecting group on N1, epimerization at C4′ occurs during the oxidative coupling procedure.

We, therefore, sought to utilize an N1 protecting group that would be easier to remove at the end of the synthesis. A literature report describing the advantages of using a *p*-methoxybenzyl (PMB) group instead of a benzyl group in protecting inosine,[13] prompted us to prepare compound **8** (Scheme 2). The oxidative coupling of compound **8** with phosphorane **2** produced the trans isomer of alkene **9**. Hydrogenation of the alkene proceeded smoothly to give a single isomer of compound **10**. Unfortunately, our attempts to remove the *N*-*p*-methoxybenzyl group using either cerric ammonium nitrate or aluminum trichloride/anisole gave low product yields and/or decomposition. In our final successful synthesis of futalosine, a pivaloyloxymethyl (POM) group was used to protect the inosine N1.[14] The protection of inosine as an acetonide followed by treatment with chloromethyl pivalate and potassium carbonate gave compound **11** in a 58% yield. Oxidative coupling with phosphorane **2** gave trans alkene **12** in a 45% yield. Hydrogenation produced compound **13** in a 90% yield. Deprotection of the POM group proceeded smoothly in sodium methoxide/methanol and removal of the acetonide and methyl ester was achieved using trifluoroacetic acid and lithium hydroxide, respectively. The acidic form of futalosine was generated by treatment

with Amberlite 120H resin (hydrogen form) to give a 73% yield of futalosine after the three deprotection steps (17% overall yield from inosine). The spectral properties of synthetic futalosine were identical to those reported for the natural product (see supplementary data).[3,1]

In summary, a convenient synthesis of futalosine is now available and can be used to prepare useful amounts of this biosynthetic intermediate. This work will be useful in enabling studies on menaquinone biosynthesis in pathogenic organisms and in the development of inhibition assays for the enzyme futalosine hydrolase.

Acknowledgments

This research was supported by the Canadian Institutes of Health Research (CIHR).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.10.010.

References and notes

1. Hosokawa, N.; Naganawa, H.; Kasahara, T.; Hattori, S.; Hamada, M.; Takeuchi, T.; Yamamoto, S.; Tsuchiya, K. S.; Hori, M. *Chem. Pharm. Bull.* **1999**, *47*, 1032–1034.
2. Dairi, T. *J. Antibiot.* **2009**, *62*, 347–352.
3. Hiratsuka, T.; Furihata, K.; Ishikawa, J.; Yamashita, H.; Itoh, N.; Seto, H.; Dairi, T. *Science* **2008**, *321*, 1670–1673.
4. Hiratsuka, T.; Itoh, N.; Seto, H.; Dairi, T. *Biosci. Biotech. Biochem.* **2009**, *73*, 1137–1141.
5. Marcelli, S. W.; Chang, H.-T.; Chapman, T.; Chalk, P. A.; Miles, R. J.; Poole, R. K. *FEMS Microbiol. Lett.* **1996**, *138*, 59–64.
6. Moss, C. W.; Lambert-Fair, M. A.; Nicholson, M. A.; Guerrant, G. O. *J. Clin. Microbiol.* **1990**, *28*, 395–397.
7. Collins, M. D.; Costas, M.; Owen, R. J. *Arch. Microbiol.* **1984**, *137*, 168–170.
8. Szarek, W. A.; Pinto, B. M.; Iwakawa, M. *Can. J. Chem.* **1985**, *63*, 2149–2161.
9. Ikejiri, M.; Ohshima, T.; Fukushima, A.; Shimotohno, K.; Maruyama, T. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4638–4641.
10. Shah, B. K.; Neckers, D. C. *J. Am. Chem. Soc.* **2004**, *126*, 1830–1835.
11. Gompper, R.; Polborn, K.; Seifert, C.; Wagner, H.-U. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 332–334.
12. Davidson, J. G.; Fiore, P. J. *Nucleosides Nucleotides* **1991**, *10*, 1477–1483.
13. Torii, T.; Izawa, K.; Cho, D. H.; Jang, D. O. *Nucleosides Nucleotides* **2007**, *26*, 985–988.
14. Brown, J. M.; Christodoulou, C.; Jones, S. S.; Modak, A. S.; Reese, C. B.; Sibanda, S.; Ubasawa, A. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1735–1750.