ORGANIC LETTERS

2003
Vol. 5, No. 18
3233−3236

Synthesis of Proposed Oxidation–Cyclization–Methylation Intermediates of the Coumarin Antibiotic Biosynthetic Pathway

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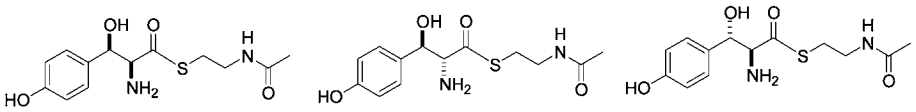
Received June 16, 2003

ABSTRACT



A chemoenzymatic synthesis was described to prepare proposed oxidation–cyclization–methylation intermediates of the coumarin antibiotic biosynthetic pathway. The successful synthesis of these fragile molecules relies heavily on mild enzymatic deprotection and efficient enzymatic kinetic resolution to minimize epimerization, decomposition, multiple orthogonal protections, and retro aldol reactions often encountered in their chemical synthesis.

Coumarin-containing antibiotics such as novobiocin (**1**, Figure 1, aminocoumarin core highlighted), clorobiocin (**2**), coumermycin A_1 (**3**), and simocyclinone (**4**), produced by *Streptomyces* species, have gained renewed interest since the discovery that they are potent inhibitors of bacterial DNA gyrase, which is essential for cell viability.[1a,b] In addition, these coumarin antibiotics are potent against methicilin-resistant strains of S*taphylococci* species, currently one of the major concerns in the treatment of bacterial infections.[2]

Biosynthetically, it is proposed that the conserved aminocoumarin core is produced via an oxidation−cyclization−methylation sequence from thioester **5** (Scheme 1),[3] a substrate (L-β-OH-Tyr)−carrier protein (T) conjugate via a phosphopantetheine linker reminiscent of classic nonribosomal peptide synthetase (NRPS) systems (squiggly line, see box in Scheme 1).[4] The first oxidation is predicted to be catalyzed by enzymes NovJ/K to yield keto derivative **6**,

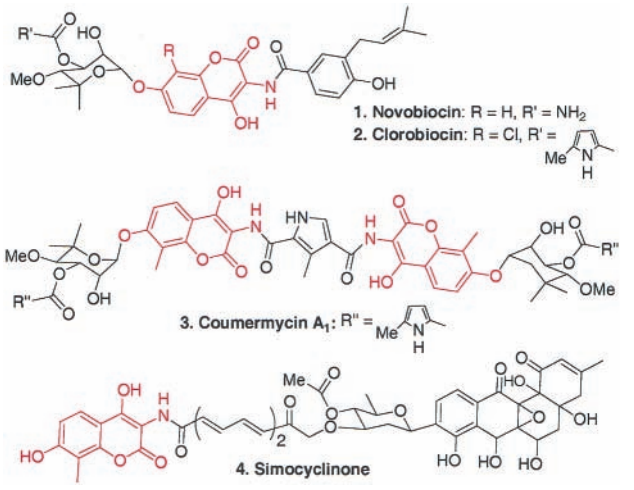


Figure 1. Coumarin-containing antibiotics.

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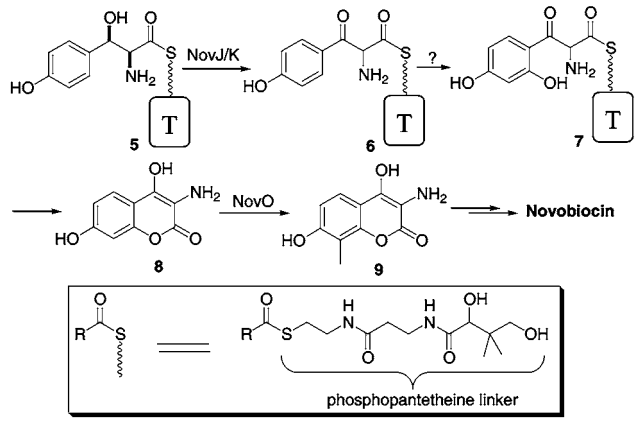
(1) (a) Maxwell, A. *Trends Microbiol.* **1997**, *5*, 102. (b) Calia, H.; Hoerman, L.; Schultz, P.; Lebeau, L.; Mallouh, V.; Wigley, D. B.; Wang, J. C.; Mioskowski, C.; Oudet, P. *J. Mol. Biol.* **1994**, *236*, 618.
(2) Boehm, H. J.; Boehringer, M.; Bur, D.; Gmeunder, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. *J. Med. Chem.* **2000**, *43*, 2664.
(3) Chen, H.; Walsh, C. T. *Chem. Biol.* **2001**, *8*, 301.

10.1021/ol035101r CCC: $25.00
Published on Web 08/02/2003

Scheme 1



followed by another oxidation by a yet unidentified protein to produce thioester **7**.[3] At this stage, aminocoumarin **8** could be released from the carrier protein via intramolecular cyclization and converted to methyl aminocoumarin **9** by the methyltransferase NovO in novobiocin or to chloro aminocoumarin by a halogenase in clorobiocin (not shown, see **2** in Figure 1).

Currently, however, the evaluation of the proposed aminocoumarin biosynthesis is hampered by the lack of availability of *N*-acetyl cysteamine (NAC) thioester (2*S*,3*R*)-*β*-OH-Tyr−SNAC (**10**) and its diastereomers (2*S*,3*S*)-**11** and (2*R*,3*R*)-**12** (Figure 2), which are small-molecule surrogates

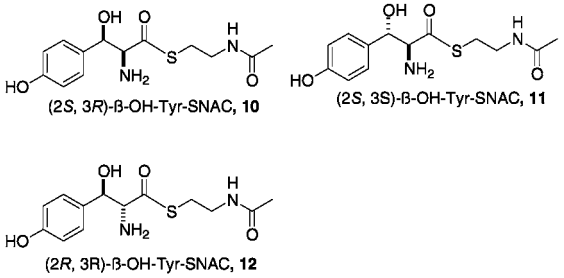
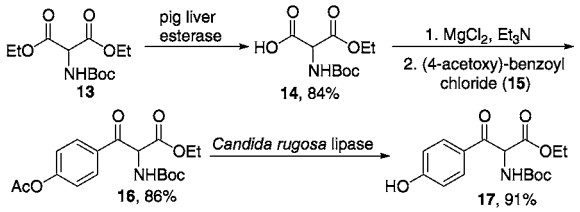


Figure 2. Stereoisomeric thioester precursors for NovJ/K.

of the substrate−protein conjugate **5** and needed to characterize the function of NovJ/K. Despite the apparent structural simplicity, the range of densely functional groups together with multiple chiral centers in these inherently unstable intermediates requires mild conditions for efficient synthesis.[5a,b] For example, in general, thioesters are labile toward even weak nucleophiles such as MeOH, and yet α-amino-*β*-hydroxy thioester **10** contains one primary amine and two hydroxyl groups. We disclose here a chemoenzymatic approach to the preparation of **10**−**12**. This method can also be adapted to the synthesis of aminocoumarin **8**, the proposed substrate of NovO (Scheme 1).

The synthesis started with commercially available N-Boc diethyl malonate **13** (Scheme 2). Upon selective enzymatic

Scheme 2



hydrolysis by pig liver esterase (PLE), monoacid **14** was obtained in good yields (84%) at gram scale. In the presence of Et_3N and $MgCl_2$, the monoacid was then condensed with (4-acetoxy)-benzoyl chloride (**15**) to produce the desired *β*-keto ester **16** (86%), which was carried over to the next step without further purification.[6] It should be noted that vigorous agitation is essential for this reaction to avoid low yields (20−30%). The acetyl group in **16** was then removed using *Candida rugosa* lipase (CRL) to provide the key intermediate **17** in excellent yields (91%) without hydrolyzing the ethyl ester. In this scheme, both PLE and CRL were identified by a high-throughput screening protocol reported recently.[7] Nonselective chemical hydrolysis causes extensive decomposition of the starting material due to the instability of the resulting *β*-keto acid (not shown).

To create the desired (2*S*,3*R*) stereochemistry in **10**, initial efforts were directed toward the microbial reduction of **17** using yeast libraries.[8a,b] Preliminary screening showed promising results with 10∼30% conversions for some strains. To further optimize the reaction conditions, however, a reference standard of syn amino alcohol **21** was needed, and an independent synthesis of **21** was initiated, beginning with cinnamic ester **19** prepared from **18** by O-benzylation (>98%) (Scheme 3). Compound **20**, whose stereochemistry is known, was produced as the major regioisomer in 40−45% yields by Sharpless aminohydroxylation[9] and subsequently converted to **21** by switching protecting groups. By this method, the desired product was isolated in high yields (98%) and dr (syn/anti > 100:1) and good enantiomeric excess (89% ee by chiral HPLC). At this stage, it appears that the current route could be used to replace enzymatic

(4) Marahiel, M.; Stachelhaus, T.; Mootz, H. D. *Chem. Rev.* **1997**, *97*, 2651.

(5) For representative syntheses of thioesters with no amino groups: (a) Cheung, K.-M.; Coles, S. J.; Hursthouse, M. B.; Johnson, N. J.; Shoolingin-Jordan, P. M. *Angew. Chem., Int. Ed.* **2001**, *41*, 1199. (b) Hunziker, D.; Wu, N.; Kenoshita, K.; Cane, D. E.; Khosla, C. *Tetrahedron Lett.* **1999**, *40*, 635.

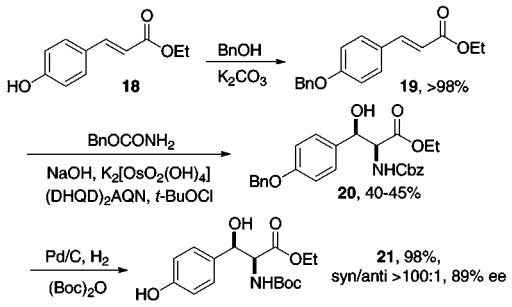
(6) Krysan, D. J. *Tetrahedron Lett.* **1996**, *37*, 3303.

(7) Yazbeck, D. R.; Tao, J.; Martinez, C. A.; Kline, B. J.; Hu, S. *Adv. Synth. Catal.* **2003**, *345*, 524−532.

(8) For enzymatic reduction on similar substrates, see: (a) Fadnavis, N. W.; Vadivel, S. K.; Sharfuddin, M.; Bhalerao, U. T. *Tetrahedron: Asymmetry* **1997**, *8*, 4003. (b) Dillon, M. P.; Hayes, M. A.; Simpson, T. J.; Sweeney, J. B. *Biorg. Med. Chem. Lett.* **1991**, *1*, 223.

(9) (a) Dong, L.; Miller, M. J. *J. Org. Chem.* **2002**, *67*, 4759. (b) Tao, B.; Schlingloff, G.; Sharpless, K. B. *Tetrahedron Lett.* **1998**, *39*, 2507.

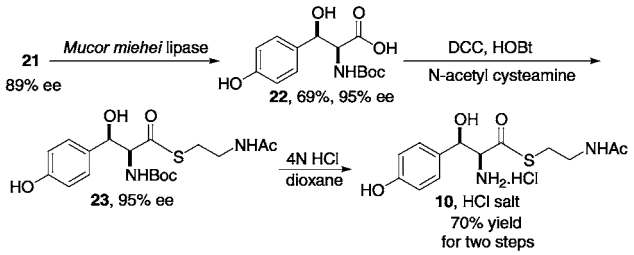
Scheme 3



reduction if the enantiomeric purity could be further improved in subsequent synthetic steps toward **10**.

Since both the retro aldol reaction and epimerization at C-2 chiral center were observed when **21** underwent chemical hydrolysis (syn/anti = 10:1 vs >100:1), we decided to remove the ester group enzymatically (Scheme 4). Plate

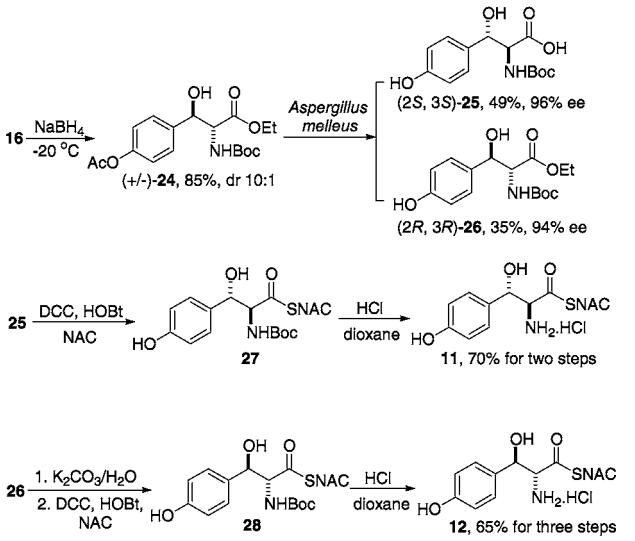
Scheme 4



screening showed that *Mucor miehei* lipase (MML) was able to produce acid **22** (69%) at pH 6−7. Interestingly, the desired (2*S*,3*R*)-enantiomer was enriched by chiral HPLC analysis (95% ee for **22** vs 89% for **21**) as a result of kinetic resolution during hydrolysis. To complete the synthesis of **10**, the key step is thioester formation between *N*-acetyl cysteamine and **22**. Remarkably, in the presence of DCC/HOBt, both free hydroxyl groups in **22** did not interfere with the desired coupling to give thioester **23** and the reaction was finished within 2 h as shown by HPLC. However, the product is not stable under the reaction conditions and should be isolated within this time frame before decomposition into an unknown byproduct. In this fashion, **23** was generated with excellent ee (95% by chiral HPLC), indicating that no epimerization occurred during thioester formation. Other coupling conditions tested were not as successful.[5a,b] Without further purification, **23** was deprotected using dry HCl and converted to **10** as an HCl salt in high yields (70% over two steps from **22**) and good UV purity (90−95%). It should be noted that this compound has limited stability at −20 °C (2−3 weeks) and lower temperatures are preferred for prolonged storage. In addition, the thioester is prone to alcoholysis in MeOH, and enzyme assays should be performed in weakly nucleophilic buffers, e.g., phosphate over Tris.

Encouraged by this success, we decided to prepare the other two enantiomeric stereoisomers **11** and **12** through enzymatic kinetic resolution.[10a−d] First, a racemic amino alcohol **24** was prepared from *β*-keto ester **16** (85%) in high diastereoselectivity (dr > 10:1) using $NaBH_4$ at −20 °C (Scheme 5). The key step is an enzymatic kinetic resolution

Scheme 5

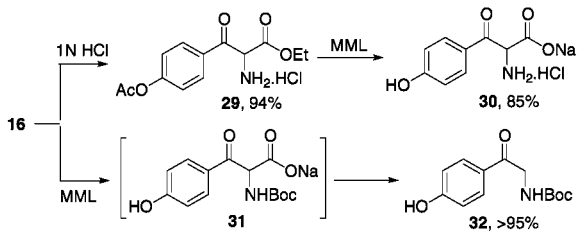


where *Aspergillus melleus* protease (AMP) was identified from library screening to be able to hydrolytically resolve the ester precursor **24** to give both the (2*S*,3*S*)-acid **25** (49% yield, 96% ee) and (2*R*,3*R*)-ester **26** (35% yield, 94% ee) in good yields and high optical purity. The anti 2-amino to 3-hydroxy relationship in **26** was unambiguously determined by comparing it to the syn amino alcohol **21**. The acid **25** was subsequently converted to **11** as an HCl salt via the intermediate **27** with 70% yield over two steps upon coupling and deprotection. As for the ester **26**, it was hydrolyzed and then turned into **12** via the intermediate **28** (65% over three steps) by the same coupling and deprotection procedure. The ees for both **27** (96% ee) and **28** (94% ee) were measured by chiral HPLC, indicating that no loss of enantiomeric purity took place during coupling.

With targets **10**−**12** in hand, we decided to turn our attention to the preparation of other intermediates in the biosynthesis. First, *β*-keto ester **16** could be readily converted to α-amino *β*-keto acid salt **30** (Scheme 6), the proposed product of NovJ/K upon thioester cleavage (Scheme 1),[3] by a two step sequence: removal of the Boc group to give α-amino *β*-keto ester **29** (94%) followed by enzymatic hydrolysis catalyzed by MML (85%). The Boc group in **16** has to be removed before enzymatic hydrolysis since N-Boc *β*-keto acid **31** from enzymatic hydrolysis decarboxylates

(10) For some recent applications of enzymatic resolution, see: (a) Demir, A. S.; Sesenoglu, O. *Org. Lett.* **2002**, *4*, 2021. (b) Taber, D. F.; Xu, M.; Hartnett, J. C. *J. Am. Chem. Soc.* **2002**, *124*, 13121. (c) Delhaes, L.; Biot, C.; Berry, L.; Delcourt, P.; Maciejewski, L. A.; Camus, D.; Brocard, J. S.; Dive, D. *ChemBiochem* **2002**, *3*, 418. (d) Luna, A.; Gotor, V. *Org. Lett.* **2002**, *4*, 3627.

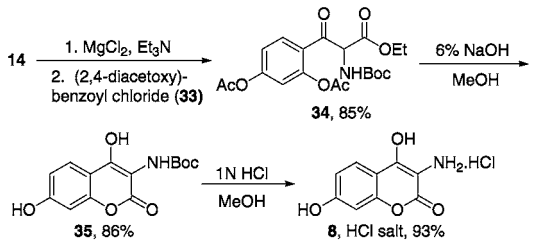
Scheme 6



spontaneously to amino ketone **32**. It should be noted that **30** should be kept at −80 °C for prolonged storage to minimize decomposition.

In addition, the aminocoumarin core **8** could be prepared from β-keto ester precursor **34**, a condensed product (85%) from monoacid **14** and acid chloride **33** (Scheme 7). Upon

Scheme 7



treatment of substrate **34** with 6% NaOH in MeOH, N-Boc aminocoumarin **35** was isolated (86%) in one pot through selective hydrolysis and intramolecular cyclization. Without further purification, the crude product was deprotected to give the target **8** as an HCl salt, which was collected as a precipitate from the reaction in 93% yield and high purity (>95% by HPLC and ^{13}C NMR).

In conclusion, the intrinsically unstable stereoisomers **10**−**12** were synthesized as the proposed substrates for NovJ/K in high optical purity (94−96% ees) over six steps. The method can be readily adapted to the preparation of the keto acid salt **30**, a product of NovJ/K, and aminocoumarin **8**, a proposed substrate for NovO. While the successful synthesis of these intermediates may expedite the elucidation of the coumarin antibiotic biosynthetic pathway, the chemoenzymatic approach described here could also be modified to synthesize other structurally similar thioester intermediates in many NRPS systems, e.g., chloramphenicol and vancomycin.[4] Not only can enzymes facilitate deprotection by minimizing epimerization, decomposition, and multiple orthogonal protection−deprotection and retro aldol reactions, which are often associated with the chemical synthesis of these fragile molecules, they can also be applied to prepare two enantiomers convergently with high individual chirality from one racemate through kinetic resolution. We are currently using these intermediates to evaluate the function of heterologously expressed proteins NovJ/K and NovO.

Acknowledgment. Research at HMD was supported by NIH grant GM20011 (to C.T.W) and a National Defense Science & Engineering Graduate (NDSEG) fellowship (to M.P.). J.T. and S.H. thank Kim Albizati and Van Martin for helpful discussions and Shirley Wang for HRMS support.

Supporting Information Available: Experimental procedures, ESI-MS, HRMS, IR, ^{1}H and ^{13}C NMR for compounds **8**, **10**, **16**, **21**, **22**, **24**, and **32**, and related chiral HPLC methods. This material is available free of charge via the Internet at http://pubs.acs.org.

OL035101R