ORGANIC LETTERS

2002
Vol. 4, No. 22
3859−3862

A Flexible Route to (5*R*)-Thiolactomycin, a Naturally Occurring Inhibitor of Fatty Acid Synthesis

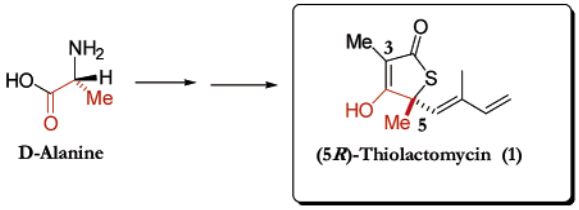
Jill M. McFadden, Gojeb L. Frehywot, and Craig A. Townsend*

Department of Chemistry, The Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218

ctownsend@jhu.edu

Received August 6, 2002

ABSTRACT



A new and efficient asymmetric synthesis of naturally occurring (5*R*)-thiolactomycin (1) using D-alanine as the source of chirality is described.

Naturally occurring thiolactomycin is a unique molecule that exerts selective activity against only the dissociable type II fatty acid synthase (FAS) enzymes.[1] Isolated from *Nocardia* sp., thiolactomycin is the most well studied of the reported naturally occurring thiotetronic acids.[2] (Figure 1) It is active in vitro against Gram-positive, Gram-negative, and anaerobic bacteria. Furthermore, thiolactomycin is nontoxic in mice and can be used to treat urinary tract and intraperitoneal bacterial infections.[2]

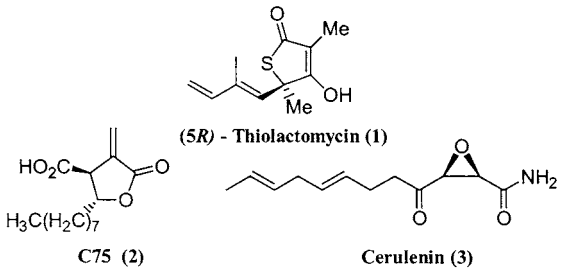


Figure 1.

Thiolactomycin (**1**, TLM) is a reversible inhibitor[1a] of the β-ketoacyl synthase (KAS) of bacterial FAS systems including KAS I−III in *E. coli*. Recently, a crystal structure of KAS I (FabB from *E. coli*) with thiolactomycin bound reveals the essential enzyme−ligand binding interactions and establishes the existence of hydrophobic and pantetheine binding pockets that are both unoptimally filled.[3] Indeed, structure−activity studies demonstrate that TLM analogues with an extended C5 hydrocarbon chain exhibit enhanced antimycobacterial activity[4a] and effective inhibition of pea (*Pisum sativum*) FAS.[4b] Also, C3-acetyl analogues of thiolactomycin with C5 aryl or alkyl functionality display

(1) (a) Nishida, I.; Kawaguchi, A.; Yamada, M.; *J. Biochem.* **1986**, *99*, 1447−1454. See reviews: (b) Heath, R. J.; White, S. W.; Rock, C. O. *Prog. Lipid Res.* **2001**, *40*, 467−497. (c) Campbell, J. W.; Cronan, J. E. *Annu. Rev. Microbiol.* **2001**, *55*, 305−332.

(2) (a) Sasaki, H.; Oishi, H.; Hayashi, T.; Matsuura, I.; Ando, K. Sawada, M. *J. Antiobiotics* **1982**, *35*, 396−400. (b) Noto, T.; Miyakawa, S.; Oishi, H.; Endo, H.; Okazaki, H. *J. Antibiotics* **1982**, *35*, 401−410. (c) Miyakawa, S.; Suzuki, K.; Noto, T.; Harada, Y.; Okazaki H. *J. Antibiotics* **1982**, *35*, 411−419. (d) Oishi, H.; Noto, T.; Sasaki, H.; Suzuki, K. *J. Antibiotics* **1982**, *35*, 391−395. (e) Sato, T.; Suzuki, K.; Kadota, S.; Abe, K.; Takamura, S.; Iwanami, M. *J. Anibiotics* **1989**, 890−896. (f) Omura, S.; Nakagawa, A.; Iwata, R.; Hatano, A. *J. Antibiotics* **1983**, *36*, 1781−1782. (g) Sato, T.; Suzuki, K.; Kadota, S.; Abe, K.; Takamura, S.; Iwanami, M. *J. Antibiotics* **1989**, *42*, 890−896. (h) Omura, S.; Iwai, Y.; Nakagawa, A.; Iwata, R.; Takahashi, Y.; Shimizu, H.; Tanaka, H. *J. Antibiotics* **1983**, *36*, 109−114. (i) Nishida, I.; Kawaguchi, A.; Yamada, M. *J. Biochem.* **1986**, *99*, 1447−1454.

(3) Price, A. C.; Choi, K. H.; Heath, R. J.; Li, Z.; White, S.; Rock, C. O. *J. Biol. Chem.* **2001**, 276, *9*, 6551−6559.

10.1021/ol026685k CCC: $22.00
Published on Web 10/08/2002

effective activity against *Staphylococcus aureus* and *Pasteurella multocida*.[4c]

Importantly, inhibition of mammalian type I FAS by C75 (**2**) and cerulenin (**3**) leads to selective cytotoxicity against various cancer cells in vitro.[5] Xenografts of MCF7 breast cancer cells in nude mice treated with C75 show FAS inhibition, followed by apoptosis and reduction in tumor size. Reports also demonstrate that C75 causes significant weight loss by reducing hypothalamic NPY expression and stimulating CPT-1 activity and fatty acid (FA) oxidation.[6]

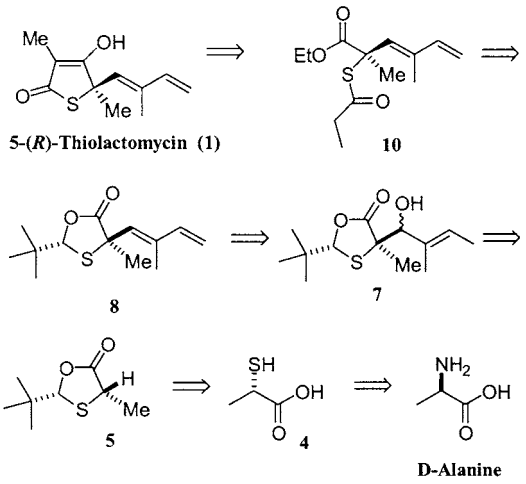
Indeed these studies suggest that inhibition of FAS can be used not only as a strategy to develop new antibacterial agents (e.g., thiolactomycin) but also as a selective route to treat cancer and obesity (e.g., C75 and cerulenin). Perhaps, even more striking and not understood is the high specificity of TLM for only the β-ketoacyl synthases of type II FAS, while C75 and cerulenin are inactivators of both type I and II FAS systems. Intrigued by TLM's unique selectivity and the inherent potential for analogues of TLM to be potent therapeutic agents, we have developed an efficient asymmetric synthesis of naturally occurring (5*R*)-thiolactomycin.

There have been only a few reports on the synthesis of thiolactomycin. Salvino et al. described the first racemic total synthesis of thiolactomycin, which involved the alkylation of a thiotetronic acid dianion with an isoprene cation equivalent (3-ethoxy-2-methyl-2-propenal).[7a] Treatment of the resulting aldehyde with methylene triphenyl-phosphorane afforded thiolactomycin. Thomas and co-workers developed an asymmetric synthesis of (5*S*)-thiolactomycin and other thiotetronic acids. The key step in this route used a stereoselective [3,3]-rearrangement of an allyl xanthate to the corresponding dithiocarbonate.[7b−d] To our knowledge, these are the only reported syntheses of thiolactomycin, with only the latter addressing enantiomeric purity.

Our synthesis employs Seebach's self-regeneration of chirality method, which utilizes amino acids as the chiral building blocks.[8] This approach is potentially quite versatile, enabling selective functionalization of both the C3 and C5 positions of the thiolactone ring.

Retrosynthetic analysis envisioned thiolactomycin (**1**) to be derived through a thio-Dieckman condensation of **10** (Scheme 1). Carefully controlled conditions would enable

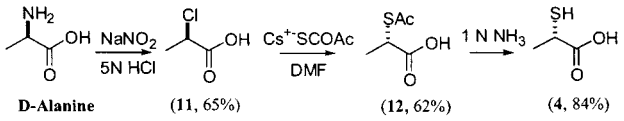
Scheme 1. Retrosynthesis of (5*R*)-Thiolactomycin



10 to be synthesized from the corresponding oxathiolanone **8**. Conversion of allylic alcohol **7** through a sulfenate-sulfoxide [2,3]-sigmatropic rearrangement accompanied by a thermal syn-elimination was anticipated to provide diene **8**. The allylic alcohol **7** can be obtained directly and likely with complete 1,3-diastereoinduction from addition of optically pure oxathiolanone **5**. Oxathiolanone **5** of high enantiomeric purity can be prepared from (2*S*)-thiolactic acid **4.**[8,9b]

Optically pure (2*S*)-thiolactic acid was obtained by the method of Kellogg as previously described (Scheme 2).[9]

Scheme 2. Synthesis of (2*S*)-Thiolactic Acid[9]



Briefly, (2*R*)-alanine was converted to (2*R*)-chloropropionic acid (**11**, 65%) with retention of configuration using the classical diazotization−chlorination protocol.[9c] Chloride displacement with clean inversion of stereochemistry was achieved with cesium thioacetate in DMF **12** (62%, Scheme 2). Deacylation was carried out without loss of optical purity in 1 N NH_3, providing (2*S*)-thiolactic acid (**4**, 84%).

A mixture of cis and trans (2.5:1) (*S*)-oxathiolanones **5** and **6** (99%) was prepared from the acid-catalyzed acetalization of (2*S*)-thiolactic acid (**4**) with pivalaldehyde as previously reported.[8,9] Recrystallization (8:1 pentane/ether)

(4) (a) Kremer, L.; Douglas, J. D.; Baulard, A. R.; Morehouse, C.; Guy, M. R.; Alland, D.; Dover: L. G.; Lakey, J. H.; Jacobs, W. R.; Brennan, P. J.; Minnikin, D. E.; Besra, G. S. *J. Biol. Chem.* **2000**, *275*, 22, 16857−16864. (b) Jones, A. L.; Herbert, D.; Rutter, A. J.; Dancer, J. E.; Harwood: J. L. *Biochem. J.* **2000**, *347*, 205−209. (c) Sakya, S. M.; Suarez-Contreras, M.; Dirlam, J. P.; O'Connell, T. N.; Hayashi, S. F.; Santoro, S. L.; Kamicker, B. J.; George, D. M.; Ziegler, C. B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2751−2754.

(5) (a) Pizer, E. S.; Thupari, J.; Han, W. F.; Pinn, M. L.; Chrest, F. J.; Frehywot, G. L.; Townsend, C. A.; Kuhajda, F. P. *Cancer Res.* **2000**, *60*, 213−218. (b) Kuhajda, F. P.; Pizer, E.; Li, J. N.; Mani, N. S.; Frehywot, G. L.; Townsend, C. A. *PNAS* **2000**, *97*, 3450−3454. (c) Pizer, E. S.; Lax, S. F.; Kuhajda, D. P.; Pasternack, G. R.; Kurman, R. J. *Cancer* **1998**, 528−537.

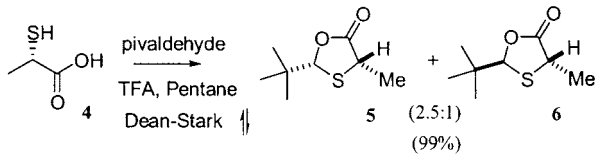
(6) (a) Thupari, J. N.; Landree, L. E.; Ronnett, G. V.; Kuhajda, F. P.; *PNAS* **2002**, *99*, 14, 9498−9502. (b) Loftus, T. M.; Jaworsky, D. E.; Frehywot, Gojeb L.; Townsend, Craig, A.; Ronnett, Gabriele, V.; Lane, Daniel M.; Kuhajda, Francis P. *Science* **2000**, *288*, 2379−2381.

(7) (a) Wang, C. J.; Salvino, J. M. *Tetrahedron Lett.* **1984**, *25*, 46, 5243−5246. (b) Chambers, M. S.; Thomas, E. J.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1987**, 1228−1230. (c) Chambers, M. S.; Thomas, E. J. *J. Chem. Soc. Chem. Commun.* **1989**, 23−24. (d) Chambers, M. S.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 417−431.

(8) (a) Seebach, D.; Naef, R.; Calderari, G. *Tetrahedron* **1984**, *40*, 8, 1313−1324. (b) Seebach, D.; Sting, A. R.; Hoffman, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2708−2748.

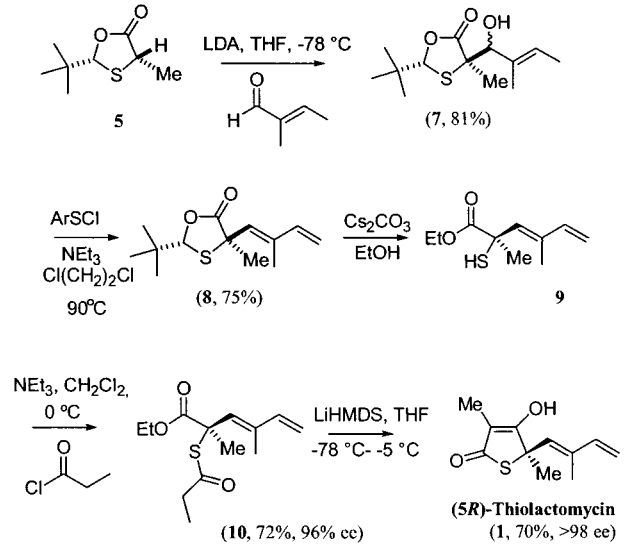
(9) (a) Strijtveen, B.; Kellogg, R. M. *J. Org. Chem.* **1986**, *51*, 3664−3671. (b) Strijtveen, B.; Kellogg, R. M. *Tetrahedron* **1987**, *43*, 21, 5039−5054. (c) Fu, S. C. J.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1954**, 6054−6058.

Scheme 3. Synthesis of 2(*R*),5(*S*)-Oxathiolanone



at −78 °C afforded optically pure **5** (54%, Scheme 3). Formation of the lithium-enolate of **5** was achieved with LDA at −78 °C, and addition of tiglic aldehyde (*trans*-2-methyl-2-butenal) was specific to the *re*-face, yielding **7** (81%) as a 2:1 mixture of diastereomeric alcohols (Scheme 4).

Scheme 4. Asymmetric Synthesis of 5(*R*)-Thiolactomycin

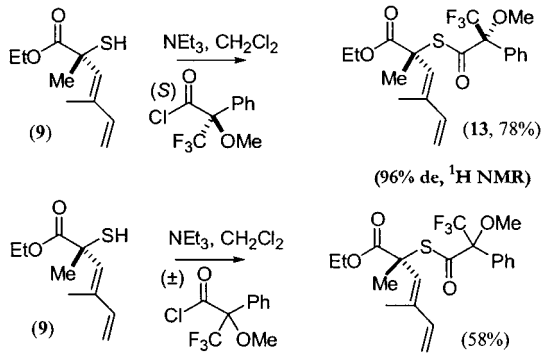


Reich and co-workers reported a method for the 1,4 dehydration of allylic alcohols to provide 1,3-dienes.[10] This sequence consists of treating the allylic alcohol with 2,4-dinitrobenzenesulfenyl chloride to provide the sulfenate ester, which undergoes a [2,3]-sigmatropic rearrangement to the allylic sulfoxide. Thermal syn-elimination of the sulfoxide gives the diene.[10] Therefore, treatment of **7** with 2,4-dinitrobenzenesulfenyl chloride and NEt_3 in refluxing dichloroethane provided **8** (75%) in good yield (Scheme 4).

This method is particularly advantageous in our synthesis since the [2,3]-sigmatropic rearrangement provides almost exclusively the trans stereochemistry at the C1′ alkene (trans:cis = 14:1). Dissolved in ethanol, the oxathiolanone ring of **8** was opened upon the addition of Cs_2CO_3. The released thiol **9** is particularly sensitive to both acidic and basic conditions because decomposition was commonly observed. Cesium carbonate proved to be the most effective base able to both catalyze ethanolysis and allow labile **9** to be isolated in good yield. Immediately, **9** was acylated with propionyl chloride to give **10** (72%, two-step yield). Enolate formation was accomplished at −78 °C with LiHMDS, and Dieckman condensation provided optically pure (5*R*)-thiolactomycin **1** (70%, = 96% ee, Scheme 4). Deacylation of the thioester competes with the Dieckman condensation even at low concentration. The use of LiHMDS as the base minimized this side reaction. Other bases, including KOtBu, NaH, LDA, and LiTMP, all gave a significant amount of deacylation.

The optical purity was assessed by formation of Mosher esters **13** from thiol **9** and found to be 96% ee [The vinylic C1′ hydrogens are resolved (^{1}H NMR) and could be readily integrated; ^{19}F NMR displayed two resolved ^{19}F peaks, Scheme 5]. Recrystallization of **1** (96% ee) from 3:1 hexanes/

Scheme 5. Enantiomeric Purity by Mosher's Method



acetone provided optically pure (5*R*)-thiolacomycin $[\alpha]_D^{24}$ +174 (*c* 0.6, MeOH); mp 119.5−121 °C (lit.[2a] $[\alpha]_D^{20}$ +176 (*c* 1.0, MeOH), mp 120 °C).

Herein we have described an efficient and versatile asymmetric synthesis of (5*R*)-thiolactomycin. This route offers several important advantages over other existing syntheses. First, optically enriched thiolactomycin was synthesized in nine steps from D-alanine. Second, **5** and **9** are modular intermediates capable of selective functionalization at both the C3 and C5 positions of the thiolactone ring. In comparison, Thomas et al. also used benzyl-protected **9** in their asymmetric synthesis.[7c,d] They assembled the C2, C3, and C3′−CH_3 carbons of the thiolactomycin skeleton by preparation of the α-methyl-*β*-ketoester of benzyl-protected **9**. Unfortunately, from this intermediate it was not possible to directly obtain TLM due to the instability of the 1,3-diene. Additional steps were required to protect the terminal alkene as an aryl-selenide. The route described herein enables the 1,3-diene to be obtained directly. A [2,3]-sigmatropic rearrangement of the performed aryl sulfenate (from **7**) followed by a thermal syn elimination provided **8** with almost exclusively the trans stereochemistry at the C1′−C2′ alkene (trans:cis = 14:1). We installed the C2, C3, and C3′−CH_3 by preparing thiopropionate **10** from **9**. Subsequent thio-Dieckman condensation provided (5*R*)-thiolacomycin.

(10) (a) Reich, H. J.; Wollowitz, S. *J. Am. Chem. Soc.* **1982**, *104*, 7051−7059. (b) Tang, R.; Mislow, K. *J. Am. Chem. Soc.* **1970**, *92*, 2100. (c) Bickart, P.; Carson, F. W.; Jacobus, J.; Miller, E. G.; Mislow, K. *J. Am. Chem. Soc.* **1968**, *90*, 4869.

The flexibility of this route opens the thiolactomycin class to investigation and optimization as inhibitors of fatty acid synthesis. The γ-thiolactone can be varied in a combinatorial sense at C3, C5, and, in principle, at the C4-enol to develop more potent antibacterial agents. Manipulation of functionality around the thiolactone ring can be used to probe the interaction of thiolactomycin with β-ketoacyl synthases to gain an understanding of its unique specificity for only type II FAS systems. These insights could reveal structural alterations that engender inhibitory activity against type I human FAS and enable the preparation of agents useful in the treatment of cancers and obesity.

Acknowledgment. We are grateful to the National Institutes of Health (CA 91634 to J.M.M. and in part AI 43846 to C.A.T.) and FASgen, Inc., for financial support.

Supporting Information Available: Spectral and analytical data for compounds for **1**, **5**, **7**, **8**, **10**, and **13** and copies of ^{1}H NMR spectra for compounds **1**, **5**, **7**, **8**, **10**, and **13**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL026685K