Tetrahedron Letters 51 (2010) 1782–1785

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis and structure assignment of the minor metabolite arising from the frog pathogen *Mycobacterium liflandii*

Thomas Spangenberg, Sylvain Aubry, Yoshito Kishi *

Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 8 January 2010
Revised 25 January 2010
Accepted 28 January 2010
Available online 2 February 2010

ABSTRACT

Total synthesis and structure assignment of the minor metabolite present in lipid extracts of the frog pathogen *Mycobacterium liflandii* are reported.

Buruli ulcer is a devastating but neglected necrotizing skin disease caused by *Mycobacterium ulcerans* infection, the third most common mycobacteriosis in humans after tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*). It is noted that the occurrence of Buruli ulcer is increasing and spreading in tropical countries, and that the incidence of the disease may exceed that of leprosy and tuberculosis in highly affected areas. Infection with *M. ulcerans*, probably carried by aquatic insects, results in progressive necrotic lesions that can extend to 15% of a patient's skin surface if left untreated. At present, surgical intervention is the only realistic curative therapy for Buruli ulcer.[1]

Unlike the two most recognized pathogenic mycobacteria, *M. turberculosis* and *M. leprae*, *M. ulcerans* was recognized to produce a small molecule toxin. In 1999, Small and co-workers isolated two toxins, designated as mycolactones A and B, from *M. ulcerans*.[2] Mycolactones A and B are now known to exist as an equilibrating mixture of geometric isomers (Fig. 1), and are referred to as mycolactone A/B in this Letter.[3–5] Intradermal inoculation of mycolactone A/B into guinea pigs produces a lesion similar to that of Buruli ulcer in humans, demonstrating their direct correlation with Buruli ulcer.[6]

Mycolactone A/B constitutes the major metabolite produced by West African strains of *M. ulcerans*. Several mycolactone congeners, including mycolactones C and D, were isolated from clinical isolates of *M. ulcerans* from Africa, Malaysia, Asia, Australia, and Mexico.[7a,8] Interestingly, a mycolactone-like metabolite was isolated from the fish pathogen *Mycobacterium marinum* as well as *Mycobacterium pseudoshottsii*, and displays stereochemical heterogeneity depending upon its living environment.[9a]

Figure 1 lists all the mycolactones whose complete structure has been established.[3–5,7b,9b,c,11b] Interestingly, all the mycolactones known to date are composed of a conserved 12-membered macrolactone core and a highly unsaturated fatty acid side chain that differs among the members of this class of natural products.

In 2004, a Buruli ulcer-like disease was reported in laboratory colonies of the West African clawed frog *Xenopus tropicalis* that had been imported to the United States.[10] This disease, lethal to the frogs, is caused by infection with the pathogenic *Mycobacterium liflandii*. In 2005, Small and co-workers succeeded in the isolation of a new mycolactone, called mycolactone E, and proposed its

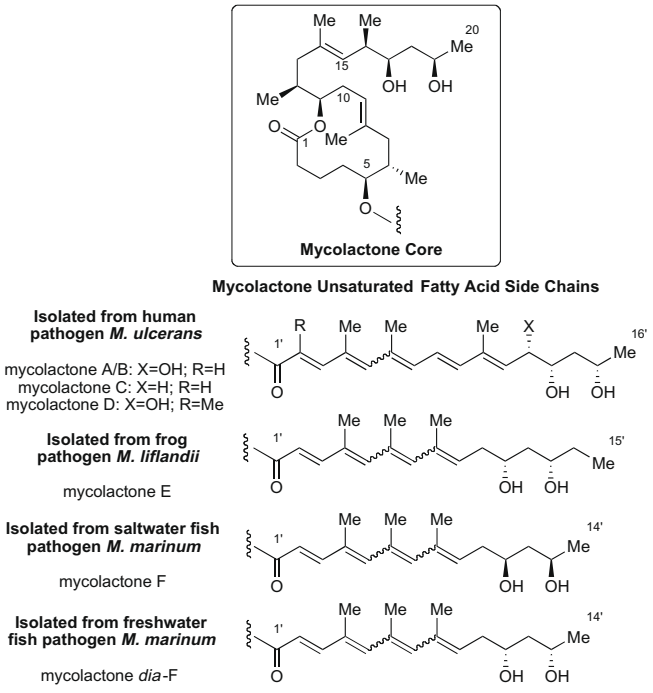


Figure 1. Structures of the mycolactones.

* Corresponding author.
E-mail address: kishi@chemistry.harvard.edu (Y. Kishi).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.01.105

gross-structure. Based on MS/MS analysis in combination with deuterium exchange and chemical transformation, Leadlay and co-workers suggested an alternative gross-structure.[11a] Using organic synthesis as the major tool, we established the complete structure of mycolactone E as shown in Figure 1.[11b]

Interestingly, both Leadlay and Small noticed the presence of a minor metabolite present in lipid extracts of mycolactone E.[11a] Mass spectroscopy analysis suggested that this minor metabolite shares the core structure with mycolactone E, but contains the polyunsaturated fatty acid in which one of the two hydroxyl groups found in mycolactone E is replaced by a ketone. Based on the MS/MS fragmentation pattern, in particular the fragment resulting from a characteristic McLafferty rearrangement, Leadlay and co-workers proposed the C13′-ketone structure for the minor metabolite. Coupled with the stereochemistry of mycolactone E recently established,[11b] the proposed C13′-ketone is now translated to **1** (Fig. 2). We are interested in confirming the proposed structure. In connection with this, we should mention a significant challenge encountered in the structure determination of mycolactone E; only a very minute amount of mycolactone E was available for that study.[12] Obviously, we have to face the same obstacle in the structure confirmation reported in this Letter.

To establish the proposed structure, we decided to adopt the approach previously used for mycolactones.[5,7b,9b,11b] This approach consisted of two steps, that is, (1) to synthesize the proposed structure and (2) to compare the synthetic substance with the natural product. For a synthesis of **1**, we opted to follow the previous synthetic route; specifically we planned: (1) to couple the protected mycolactone core **2** with the suitably functionalized fatty acid **3** via Yamaguchi esterification, (2) to use a 1,3-dithiane as a synthetic equivalent with the C13′-ketone, and (3) to adopt an iterative Horner–Emmons olefination approach for introduction of the tetraenoic acid moiety (Scheme 1). We then recognized that the multicomponent anion-relay-chemistry is ideally suited for a synthesis of the key fragment **4** from commercially available (−)-epichlorohydrin.[13]

The synthesis began with the reaction of (−)-epichlorohydrin (AK Scientific; optical purity: 99%) with vinylmagnesium bromide in the presence of a catalytic amount of copper iodide to yield the homoallylic alcohol which was converted to epoxide **5** by a KOH-treatment (83%, two steps). TBS-1,3-dithiane[14] was deprotonated with *t*-BuLi and then reacted with epoxide **5** to initiate the anion-relay-chemistry. In the same pot, a [1,4]-Brook rearrangement was triggered upon addition of HMPA, and the newly generated dithiane anion was trapped with ethyl iodide to furnish the key fragment **4** in 64% yield. Dihydroxylation with OsO_4, followed by oxidative cleavage of the 1,2-diol, gave aldehyde **6** without significantly disturbing the dithiane group.[15] Next, a chain-elongation protocol consisting of (1) Horner–Emmons olefination, (2) DIBAl-H reduction, and (3) MnO_2 oxidation was repeatedly applied to obtain the protected unsaturated fatty acid ester **16** in excellent overall yield. Although cumbersome, we purposely adopted the iterative chain-elongation approach to isolate, and characterize, a stereochemically homogeneous *E*-unsaturated ester in each cycle.[9b,11b] In this synthesis, Horner–Emmons olefination gave the product with *E*/*Z*: 94/6 (first cycle, **7**), 98/2 (second cycle, **10**), 98/2 (third cycle, **13**), and 95/5 (fourth cycle, **16**). Finally, saponification of **16** with LiOH furnished the stereochemically homogeneous **3** in quantitative yield (Scheme 2).

Acid **3** was coupled with the protected core **2**[16] under Yamaguchi esterification conditions, to furnish the fully protected mycolactone **17** in 91% yield (Scheme 3).

To complete the synthesis, we needed to deprotect the 1,3-dithiane and three TBS-groups. Through the first- and second-generation syntheses of mycolactone A/B,[5] we learned that this natural product is relatively labile under acidic conditions, but perfectly stable under the conditions of TBAF-promoted TBS-deprotection. Based on this knowledge, we planned to cleave first the 1,3-dithiane and then the TBS-groups. Experimentally, we found that, upon treatment with silver nitrate and *N*-chlorosuccinimide,[17] the 1,3-dithiane was cleanly removed to give the desired ketone **18** in 87% yield. However, deprotection of the three TBS-groups was

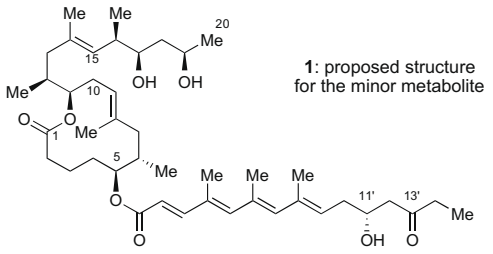
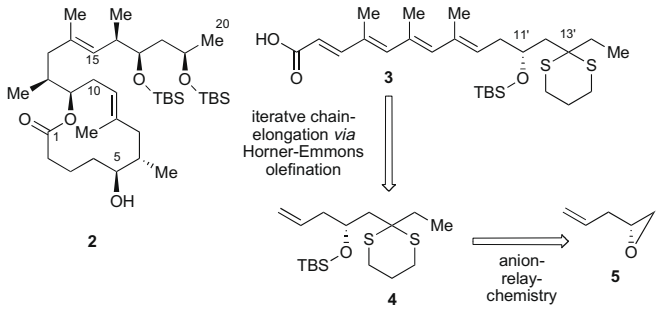
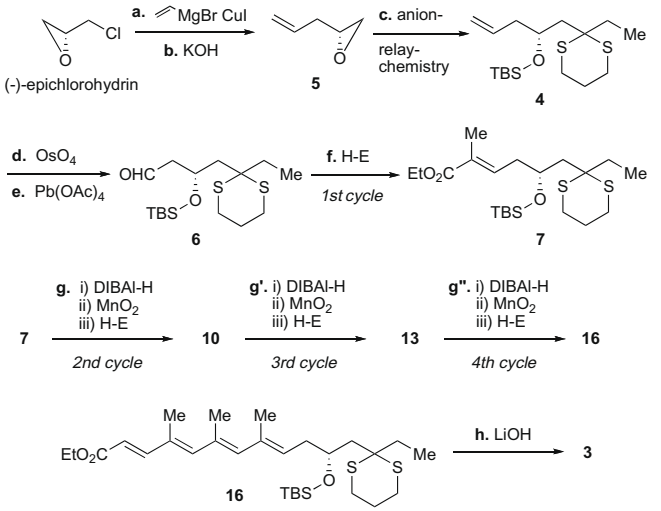


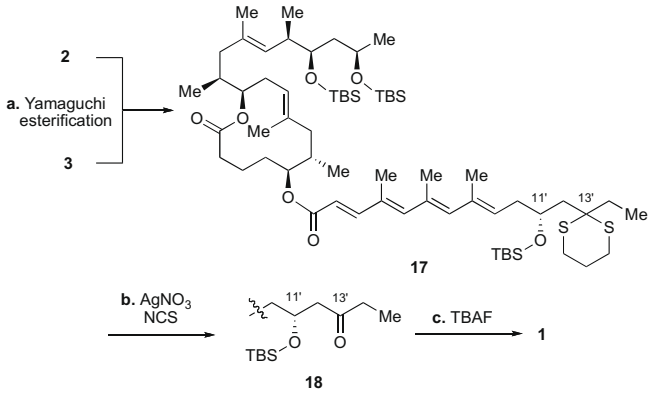
Figure 2. Proposed complete structure of the minor metabolite present in a crude lipid extract of the frog pathogen *M. liflandii*.



Scheme 1. Overall plan for a total synthesis of the minor metabolite **1**. *Abbreviation*: TBS = *t*-Bu(Me)$_2$Si-.



Scheme 2. Synthesis of the protected unsaturated fatty acid. Reagents and conditions: (a) VinylMgBr, CuI cat. Et_2O, −78 °C to −40 °C, 92%, ee: >95% estimated from ^{1}H NMR of its Mosher ester; (b) KOH, distillation (90 °C, atm pressure), 91%; (c) *t*-BuLi, TBS-1,3-dithiane, HMPA, EtI, −78 °C to −25 °C, 64%; (d) OsO_4, $K_3Fe(CN)_6$, DABCO, $MeSO_2NH_2$, *t*-BuOH/H_2O, 0 °C; (e) $Pb(OAc)_4$, PhH, 0 °C, 57% (two steps); (f) $EtO_2CCH(Me)P(O)(OEt)_2$, *n*-BuLi, LiBr, MeCN, 0 °C to rt, 94% (*E*/*Z*: 94/6); (g) and (g′) DIBAl-H, DCM, −78 °C; MnO_2, DCM, rt; $EtO_2CCH(Me)P(O)(OEt)_2$, *n*-BuLi, THF, 0 °C to rt, 72–92% over three steps (*E*/*Z*: 98/2); (g″) DIBAl-H, DCM, −78 °C; MnO_2, DCM, rt; $EtO_2CCH_2P(O)(OEt)_2$, *n*-BuLi, THF, 0 °C to rt, 95% over three steps (*E*/*Z*: 95/5); (h) LiOH, THF/MeOH/H_2O, rt, quant. *Abbreviations*: TBS = *t*-Bu(Me)$_2$Si-; H–E = Horner–Emmons olefination.



Scheme 3. Completion of the synthesis. Reagents and conditions: (a) 1,3,5-trichlorobenzoylchloride, DMAP, *i*-Pr_2NEt, PhH, rt, 91%; (b) NCS, $AgNO_3$, 2,6-lutidine, $MeCN/H_2O$, 0 °C, 10–15 s, 87%; (c) TBAF, THF, rt, 44%. *Abbreviation*: TBS = *t*-$Bu(Me)_2Si$-.

problematic; under the standard TBAF conditions, deprotection stopped mainly at the mono-TBS stage or resulted in complete decomposition.[18] After numerous attempts, we finally found that the desired deprotection was realized by treatment with excess TBAF (30 equiv) in THF (2 mM), followed by a $CaCO_3$/Dowex resin workup, to furnish completely deprotected **1** in 44% yield.[19] Unfortunately, this procedure was found problematic in a slightly larger scale (4 mg). Therefore, we conducted several small-scale experiments to obtain a sufficient amount of the synthetic **1** for full characterization and comparison with the natural product. Notably, the synthetic **1** was found to give a MS/MS spectrum, with the predominant peak being a loss of 2-butanone from the molecular ion via a McLafferty rearrangement, as reported for the natural product.

Our next task was to prove, or disprove, that the synthetic **1** is identical with the natural minor metabolite. Given only a very minute amount of the authentic sample dispensable for our study,[12] we needed a highly sensitive and reliable method. For the stereochemistry assignment of mycolactone F, we have developed such a method that relies on the chiral HPLC profile. In short, this method consisted of two steps, that is, (1) to prepare a photochemically-equilibrated mixture of geometric isomers for a given mycolactone and (2) to perform structure analysis based on the distinct *HPLC profile* comprised of a set of peaks with different retention times, each peak corresponding to one geometric isomer present in the mixture.[9b,9c]

In this work, we subjected the synthetic **1** to photochemically-induced equilibration in acetone (Reonet, 300 nm for 2 min), to yield a ca. 1:1:1 mixture of three major geometric isomers (all-*trans*-, *Z*-$\Delta^{4',5'}$-, and *Z*-$\Delta^{6',7'}$-isomers, thick red dashed lines),[9b,11b] along with three minor isomers (thin red dashed lines). We found that Chiralpak IA chiral column gave an excellent separation of all the geometric isomers, yielding its HPLC profile (Fig. 3, panel A). Under the same conditions, synthetic mycolactone E exhibited a different HPLC profile (Fig. 3, panel B, blue dashed lines). In addition, a co-injection of synthetic **1** and mycolactone E demonstrated that their HPLC profiles are clearly distinguishable from each other (Fig. 3, panel C).

With this foundation, we compared the synthetic **1** with the minor metabolite of the frog pathogen *M. liflandii*. For this comparison, we used the same batch of *M. liflandii* lipid extract as the one used for the mycolactone E study.[9b,12] By MS analysis, this lipid extract was shown to contain both mycolactone E and the minor metabolite. In addition, mycolactone E contained in this extract was demonstrated to be a mixture of geometric isomers with the ratio virtually identical to that found in the photochemically-equilibrated synthetic sample. Thus, we assumed that the minor metabolite in this mixture is also a mixture of geometric isomers. Panel D in Figure 3 shows the HPLC behavior of this sample, demonstrating that both HPLC profiles, one characteristic to mycolactone E and the other characteristic to the minor metabolite, are clearly detectable. This conclusion is further supported by co-injection of the synthetic **1** and the crude lipid extract (Fig. 3, panel E). Overall, these experiments establish that the minor metabolite present in the crude lipid extract of the frog pathogen *M. liflandii* is represented by the structure **1**.

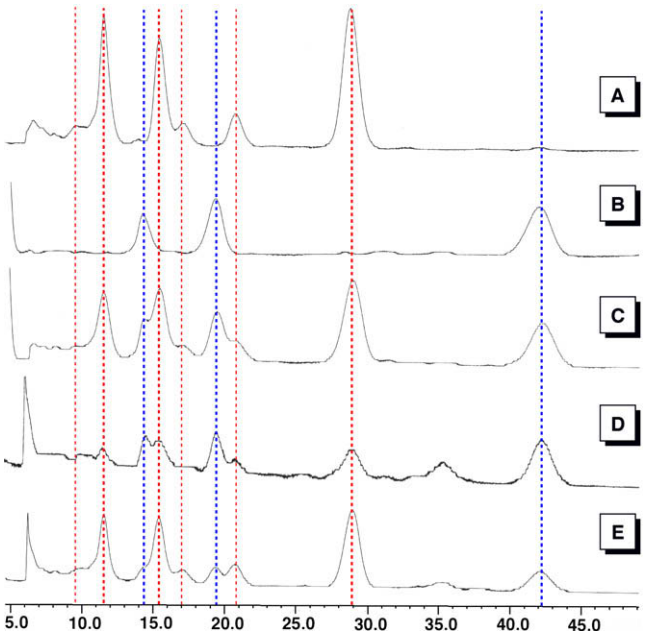


Figure 3. Chiral HPLC analysis. Analytical conditions: column: Chiral Tech, Chiralpak IA (5 μm), 250 × 4.6 mm; solvent (isocratic): toluene/ethanol 98.5/1.5;[21] flow rate: 1 mL/min; detection: UV at 323 nm. Panel A: synthetic **1**; Panel B: synthetic mycolactone E; Panel C: ca. 1:1 mixture of synthetic **1** and mycolactone E; Panel D: lipid extract of *M. liflandii*; Panel E: ca. 1:1 mixture of synthetic **1** and lipid extract of *M. liflandii*.

The mycolactone class of natural products is known to exhibit a variety of biological activities.[1f] Synthetic **1** exhibited cytotoxicity (GI_{50}: 15 nM) against L929 murine fibroblast cells that is practically identical to that of synthetic mycolactone E.[20]

In conclusion, we have established the complete structure of the minor metabolite present in lipid extracts of the frog pathogen *M. liflandii*. In this work, we have used synthetic **1** to characterize the HPLC profile, which is then used for structure analysis of the natural product.

Acknowledgments

We thank Professor Pam Small at University of Tennessee for the generous gift of lipid extracts of the frog pathogen *M. liflandii*. We are grateful to the National Institutes of Health (CA 22215) and Eisai USA Foundation for generous financial support.

Supplementary data

Supplementary data (experimental details and ^1H and ^{13}C NMR spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.01.105.

References and notes

1. For general reviews on Buruli ulcer for example see: (a) Asiedu, K.; Scherpbier, R. In *Buruli ulcer: Mycobacterium ulcerans infection*; Ravinglione, M., Ed.; World

Health Organization: Geneva, Switzerland, 2000; (b) van der Werf, T. S.; Stinear, T.; Stienstra, Y.; van der Graaf, W. T. A.; Small, P. L. *Lancet* **2003**, *362*, 1062; (c) Johnson, P. D. R.; Stinear, T.; Small, P. L. C.; Pluschke, G.; Merritt, R. W.; Portaels, F.; Huygen, K.; Hayman, J. A.; Asiedu, K. *PLoS Med.* **2005**, *2*, e108; (d) van der Werf, T. S.; Stienstra, Y.; Johnson, R. C.; Phillips, R.; Adjei, O.; Fleischer, B.; Wansbrough-Jones, M. H.; Johnson, P. D. R.; Portaels, F.; van der Graaf, W. T. A.; Asiedu, K. *Bull. World Health Org.* **2005**, *83*, 785; (e) Hong, H.; Demangel, C.; Pidot, S. J.; Leadlay, P. F.; Stinear, T. *Nat. Prod. Rep.* **2008**, 447; (f) Demangel, C.; Stinear, T. P.; Cole, S. T. *Nat. Rev. Microbiol.* **2009**, *7*, 50.

2. George, K. M.; Chatterjee, D.; Gunawardana, G.; Welty, D.; Hayman, J.; Lee, R.; Small, P. L. C. *Science* **1999**, *283*, 854.
3. For the gross-structure of mycolactone A/B, see: Gunawardana, G.; Chatterjee, D.; George, K. M.; Brennan, P.; Whittern, D.; Small, P. L. C. *J. Am. Chem. Soc.* **1999**, *121*, 6092.
4. For the stereochemistry of mycolactone A/B, see: (a) Benowitz, A. B.; Fidanze, S.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 5128; (b) Fidanze, S.; Song, F.; Szlosek-Pinaud, M.; Small, P. L. C.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 10117.
5. For the total synthesis of mycolactone A/B. See: Song, F.; Fidanze, S.; Benowitz, A. B.; Kishi, Y. *Org. Lett.* **2002**, *4*, 647. and *Tetrahedron*, **2007**, *63*, 5739.
6. George, K. M.; Pascopella, L.; Welty, D. M.; Small, P. L. C. *Infect. Immun.* **2000**, *68*, 877.
7. For mycolactone C, see: (a) the isolation and gross-structure: (a) Mve-Obiang, A.; Lee, R. E.; Portaels, F.; Small, P. L. C. *Infect. Immun.* **2003**, *71*, 774; the stereochemistry and total synthesis: (b) Judd, T. C.; Bischoff, A.; Kishi, Y.; Adusumilli, S.; Small, P. L. C. *Org. Lett.* **2004**, *6*, 4901.
8. Hong, H.; Spencer, J. B.; Porter, J. L.; Leadlay, P. F.; Stinear, T. *ChemBioChem* **2005**, *6*, 643.
9. For mycolactone F, see: (a) isolation and gross-structure: (a) Ranger, B. S.; Mahrous, E. A.; Mosi, L.; Adusumilli, S.; Lee, R. E.; Colorni, A.; Rhodes, M.; Small, P. L. C. *Infect. Immun.* **2006**, *74*, 6037; Hong, H.; Stinear, T.; Porter, J.; Demangel, C.; Leadlay, P. F. *ChemBioChem* **2007**, *8*, 2043; stereochemistry and total synthesis of mycolactone F isolated from the saltwater fish pathogen *M. marinum*: (b) Kim, H.-J.; Kishi, Y. *J. Am. Chem. Soc.* **2008**, *130*, 1842; stereochemistry and total synthesis of mycolactone F isolated from the freshwater fish pathogen *M. marinum*: (c) Kim, H.-J.; Jackson, K. L.; Kishi, Y.; Williamson, H. R.; Mosi, L.; Small, P. L. C. *Chem. Commun.* **2009**, 7402.
10. Trott, K. A.; Stacy, B. A.; Lifland, B. D.; Diggs, H. E.; Harland, R. M.; Khokha, M. K.; Grammer, T. A.; Parker, J. M. *Comp. Med.* **2004**, *54*, 309.
11. For mycolactone E, see: (a) isolation and gross-structure: (a) Hong, H.; Stinear, T.; Skelton, P.; Spencer, J. B.; Leadlay, P. F. *Chem. Commun.* **2005**, 4306; and Mve-Obiang, A.; Lee, R. E.; Umstot, E. S.; Trott, K. A.; Grammer, T. C.; Parker, J. M.; Ranger, B. S.; Grainger, R.; Mahrous, E. A.; Small, P. L. C. *Infect. Immun.* **2005**, *73*, 3307; stereochemistry and total synthesis: (b) Aubry, S.; Lee, R. E.; Mahrous, E. A.; Small, P. L. C.; Beachboard, D.; Kishi, Y. *Org. Lett.* **2008**, *10*, 5385.
12. We used the lipid extract from fermentation broth of *M. liflandii* donated by Professor Pam Small.[11a] The total amount of mycolactones present in this extract was estimated to be equivalent with ~10 injections for HPLC analysis.
13. For a recent review on anion-relay-chemistry see: Smith, A. B., III; Wuest, W. M. *Chem. Commun.* **2008**, 5883.
14. For the preparation of TBS-1,3-dithiane see: Silverman, R. B.; Lu, X.; Banik, G. M. *J. Org. Chem.* **1992**, *57*, 6617.
15. For dihydroxylation of sulfur containing substrates, see for example: (a) Walsh, P. J.; Ho, P. T.; King, S. B.; Sharpless, K. B. *Tetrahedron Lett.* **1994**, *35*, 5129; (b) Sammakia, T.; Hurley, B.; Sammond, D. M.; Smith, R. S.; Sobolov, S. B.; Oeschger, T. R. *Tetrahedron Lett.* **1996**, *37*, 4427.
16. The core alcohol **2** was synthesized by Dr. Han-Je Kim in this laboratory.
17. Corey, E. J.; Erickson, B. W. *J. Org. Chem.* **1971**, *36*, 3553.
18. TBAF tested included: TBAF (Aldrich) as supplied and TBAF buffered with imidazole hydrochloride and AcOH.
19. Kaburagi, Y.; Kishi, Y. *Org. Lett.* **2007**, *9*, 723.
20. This test was performed by Dr. Katrina L. Jackson in this laboratory. The complete data will be reported elsewhere.
21. Using the initial solvent conditions described for mycolactone E (benzene/EtOH 98.5:1.5) resulted in the overlap of several peaks, see Supplementary data.