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Total Synthesis of the Mycolactones

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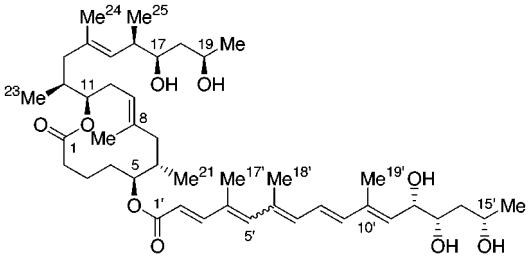
ABSTRACT



The first total synthesis of the mycolactones is reported. This work unambiguously confirms our earlier relative and absolute stereochemical assignment of the mycolactones.

The mycolactones were isolated in 1999 by Small and co-workers from *Mycobacterium ulcerans*, the causative pathogen of Buruli ulcer. This disease is characterized by the formation of large, painless, necrotic ulcers and the lack of an acute inflammatory response. Evidence from animal studies suggests that the mycolactones are directly responsible for the observed pathology, and they have attracted considerable attention for their highly potent apoptotic activity as well as for being the first examples of polyketide macrolides to be isolated from a human pathogen.[1,2] The gross structure of these natural products was elucidated through 2D NMR experiments.[3] Via an NMR database approach, we recently established the complete structure of the mycolactones (Figure 1).[4,5] In this letter, we report a total synthesis of the mycolactones, thereby confirming unambiguously our earlier relative and absolute stereochemical assignment.

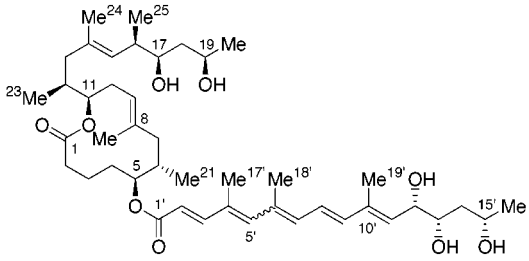


Figure 1. **1a** (4′*Z*): Mycolactone A; **1b** (4′*E*): Mycolactone B.

We envisioned that an obvious synthetic route to the mycolactones would proceed through esterification of the C5 hydroxyl group in the mycolactone core moiety with the unsaturated fatty acid (Scheme 1).[6] To achieve the proposed

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(1) (a) George, K. M.; Chatterjee, D.; Gunawardana, G.; Welty, D.; Hayman, J.; Lee, R.; Small, P. L. C. *Science* **1999**, *283*, 854. (b) George, K. M.; Pascopella, L.; Welty, D. M.; Small, P. L. C. *Infect. Immun.* **2000**, *68*, 877. (c) Rohr, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2847.

(2) For biological activities of the mycolactones, see also: Dobos, K. M.; Small, P. L.; Deslauriers, M.; Quinn, F. D.; King, C. H. *Infect. Immun.* **2001**, *69*, 7182 and references therein.

(3) Gunawardana, G.; Chatterjee, D.; George, K. M.; Brennan, P.; Whittern, D.; Small, P. L. C. *J. Am. Chem. Soc.* **1999**, *121*, 6092.

(4) (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) We recently noticed that the chemical shift assignment for the C19 carbon of **1c** and **1d** in ref 4a should be revised. Because of this, the graphs shown in Figure 2 in ref 4a should be corrected as included in the Supporting Information of this paper.

(6) For the numbering adopted in this paper, see the structures in Figure 1.

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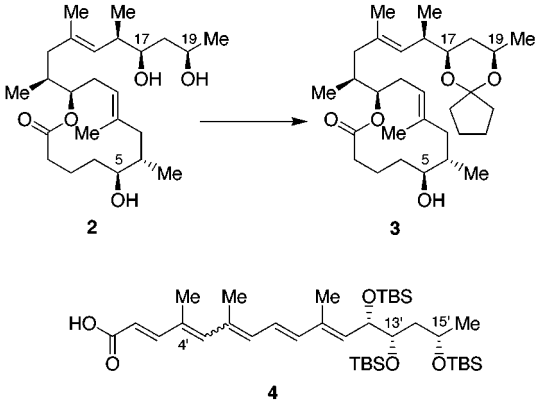
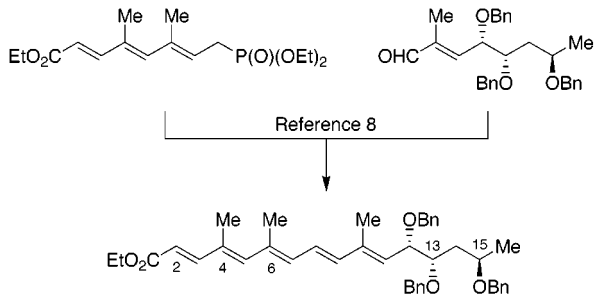
Scheme 1



transformation selectively, we opted to block the C17 and C19 as well as the C12′, C13′, and C15′ hydroxyls with suitable protecting groups. Considering the anticipated chemical instability associated with the unsaturated fatty acid moiety (vide infra), we chose TBS ethers to protect the C12′, C13′, and C15′ hydroxyl groups. With respect to the core moiety, we previously reported an effective synthesis of the triol **2**[4a] and anticipated a selective protection of the C17 and C19 hydroxyls via a cyclic ketal. Among the cyclic ketals commonly used in synthesis, we selected a cyclopentylidene primarily because of its easier hydrolysis compared to that of acetonides and cyclohexylidenes.[7] In practice, **2** was effectively converted to **3** by treatment with 1,1-dimethoxycyclopentane in the presence of *p*-TsOH.

Gurjar and Cherian recently reported a synthesis of ethyl (2*E*,4*Z*/*E*,6*E*,8*E*,10*E*)-12,13,15-tribenzyloxy-4,6,10-trimethyl-2,4,6,8,10-hexadecapentaenoate (Scheme 2).[8] This synthetic

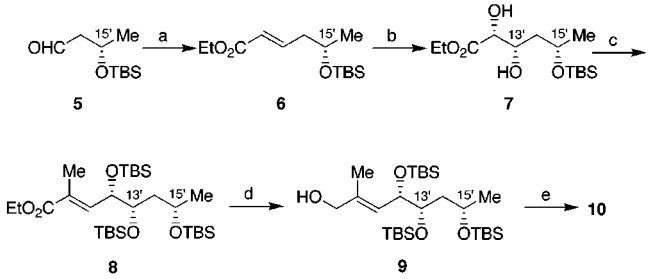
Scheme 2



route appeared to meet well with our needs with suitably protected C12′, C13′, and C15′ hydroxyl groups possessing the correct configuration.

Schemes 3 and 4 outline a synthesis of the requisite unsaturated fatty acid **4**. The known aldehyde **5**,[9] readily

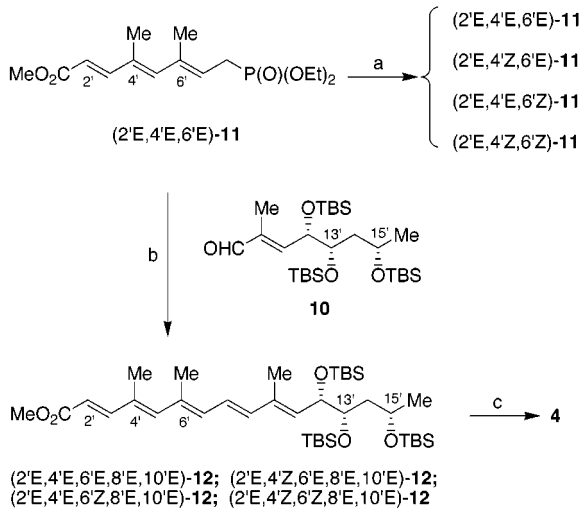
Scheme 3[a]



[a] Reagents and Conditions: (a) NaH, $(EtO)_2P(O)CH_2CO_2Et$, THF, rt, 1 h 64%. (b) AD-mix-α, $MeSO_2NH_2$, 1:1 *t*-BuOH/H_2O, 0 °C, 40 h, 70%, α:β = 3.8:1. (c) (1) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C, 1 h, 99%. (2) DiBAl-H, CH_2Cl_2, −78 °C, 1.5 h, 89%. (3) SO_3·Py, *i*-Pr_2NEt, 3:2 CH_2Cl_2/DMSO, rt, 1 h. (4) $Ph_3P{=}C(Me)CO_2Et$, PhMe, 110 °C, 12 h, 83% (2 steps). (d) (1) DiBAl-H, CH_2Cl_2, −78 °C, 1.5 h. (2) Separation of diastereomers by flash chromatography; major isomer, 57%, minor isomer, 15%. (e) SO_3·Py, *i*-Pr_2NEt, 3:2 CH_2Cl_2/DMSO, rt, 1 h, quant.

available from ethyl (*S*)-3-hydroxy-*n*-butyrate in two steps, was converted to **6**. Catalytic asymmetric dihydroxylation

Scheme 4[a]



[a] Reagents and Conditions: (a) LDA, THF, −78 °C, then 2,4,6-trimethylphenol. (b) LDA, THF, −78 °C → rt, 1 h, 94%. (c) LiOH, 4:1:1 THF/MeOH/H_2O, rt, 18 h, quant.

of **6** with AD-mix-α gave a 3.8:1 mixture of the expected diol **7** and its diastereomer.[10,11] This mixture was then

(7) For an example, see: van Heeswijk, W. A. R.; Goedhart, J. B.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1977**, *58*, 337.
(8) Gurjar, M. K.; Cherian, J. *Heterocycles* **2001**, *55*, 1095.
(9) Paterson, I.; Craw, P. A. *Tetrahedron Lett.* **1989**, *30*, 5799.

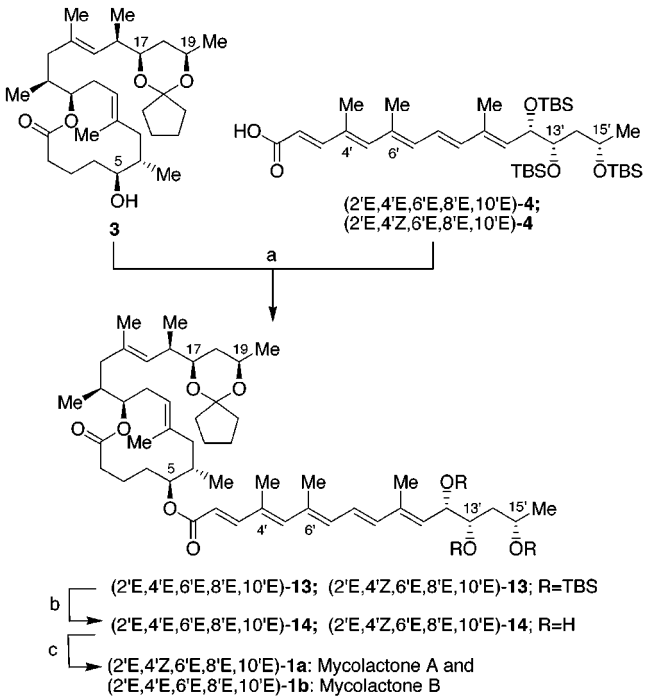
(10) (a) Jacobsen, E. N.; Markó, I.; Mungall, W. S.; Schröder, G.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 1968. (b) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768.
(11) In the catalytic dihydroxylation series, the following stereoselectivities were observed: 3.8:1 with AD-mix-α, 3.8:1 with $(DHQ)_2PYR$, 3.5:1 with DHQ-PHN, 2.5:1 with DHQ-MEQ, and 1.5:1 with DHQ-IND. In the stoichiometric dihydroxylation series, oxidation in the presence of the Corey ligand (Corey, E. J.; Jardine, P. D.; Virgil, S.; Yuen, P.-W.; Connell, R. D. *J. Am. Chem. Soc.* **1989**, *111*, 9243) yielded the desired product exclusively (^1H NMR analysis).

transformed to the corresponding mixture of α,β-unsaturated esters **8** in four steps. Despite extensive efforts, this diastereomeric mixture was found to be chromatographically inseparable at any step in the sequence. Fortunately, the diastereomeric mixture of the primary alcohols derived from **8** was found to be readily separable with column chromatography to give the stereochemically pure **9** and its diastereomer in approximately 30% and 8% overall yield from **6**. Via correlation with the sample previously prepared,[12] the relative and absolute configuration of the major alcohol **9** was established as indicated, which matched with the major diastereomer predicted for AD-mix-α dihydroxylation.[10]

Employing a minor modification of the procedure reported by Gurjar and Cherian,[8] the phosphonate (2′*E*,4′*E*,6′*E*)-**11** with a methyl ester at C1′ was prepared.[13] The anion generated from **11** was expected to exist as a mixture of geometric isomers. Indeed, deprotonation of **11** with LDA in THF at −78 °C, followed by quenching with 2,4,6-trimethylphenol, gave a mixture composed of 55% [(2′*E*,4′*E*,6′*E*)-**11**], 25% [(2′*E*,4′*E*,6′*Z*)-**11**], 14% [(2′*E*,4′*Z*,6′*E*)-**11**], and 6% [(2′*E*,4′*Z*,6′*Z*)-**11**].[14] Upon treatment (−78 °C → rt) with aldehyde **10**, the anion gave a mixture of (2′*E*,4′*E*,6′*E*,8′*E*,10′*E*)-**12** (73%), (2′*E*,4′*Z*,6′*E*,8′*E*,10′*E*)-**12** (17%), (2′*E*,4′*E*,6′*Z*,8′*E*,10′*E*)-**12** (7%), and (2′*E*,4′*Z*,6′*Z*,8′*E*,-10′*E*)-**12** (3%). The structure of these geometric isomers was deduced from analysis of ^{1}H NMR spectra.[14,15] The composition of this mixture varied from experiment to experiment, but the ratio indicated was representative. By photolysis (acetone-d_6, tungsten lamp) or during isolation under common laboratory conditions, this composition changed to a mixture of (2′*E*,4′*E*,6′*E*,8′*E*,10′*E*)-**12** (36%), (2′*E*,4′*Z*,6′*E*,8′*E*,10′*E*)-**12** (52%), (2′*E*,4′*E*,6′*Z*,8′*E*,10′*E*)-**12** (4%), (2′*E*,4′*Z*,6′*Z*,8′*E*,10′*E*)-**12** (5%), and a fifth isomer (3%). Close examination of the ^{1}H NMR spectrum revealed that the fifth isomer was a mixture of two 10′*Z* geometric isomers corresponding to the two major isomers.[15] As noted by others,[3,8] 4′*Z* and 4′*E* geometric isomers were interconvertible, and the 3:2 ratio appeared to represent the ratio at the steady state under photochemical conditions and common laboratory conditions. Although these geometric isomers were chromatographically inseparable at the ester stage, the corresponding acids (2′*E*,4′*E*,6′*E*,8′*E*,10′*E*)-**4** and (2′*E*,4′*Z*,6′*E*,8′*E*,10′*E*)-**4** could be separated from the other geometric isomers by silica gel chromatography [hexanes−ethyl acetate (6:1)].

Esterification of the mycolactone core alcohol **3** with the unsaturated fatty acids **4** was accomplished via the Yamaguchi protocol to furnish the protected mycolactones **13** as an approximately 3:2 mixture of 4′*Z* and 4′*E* isomers in 90% yield (Scheme 5).[16,17] It was hoped that all of the protecting

Scheme 5[a]



[a] Reagents and Conditions: (a) $Cl_3C_6H_2COCl$, *i*-Pr_2NEt, DMAP, PhH, rt, 20 h, 90%. (b) TBAF, THF, rt, 1 h, 81%. (c) 2:2:1 THF/HOAc/H_2O, rt, 10 h, and the recovered starting material was recycled (once), 67%.

groups in **13** could be removed in a single step. Indeed, treatment of **13** with HF·Py in MeCN gave the synthetic mycolactones, but only in 5−10% yield. The low yield was attributed largely to the instability of the products under the deprotection conditions employed.[18] To eliminate or suppress the undesired side reactions, deprotection was carried out in two separate steps. The three TBS groups were removed under standard conditions (TBAF/THF/rt), to yield the triol **14** as an approximately 3:2 mixture of 4′*Z* and 4′*E* isomers in 81% yield. The cyclopentylidene ketal of **14** was then hydrolyzed by treatment with aqueous acetic acid [AcOH/H_2O/THF (2:1:2)] at room temperature. It should be noted that, even under these conditions, the side reactions on the

(12) The details for structural correlation of **9** with the authentic sample reported in ref 4b is included in Supporting Information.

(13) ^{1}H NMR ($CDCl_3$, 500 MHz) of (2′*E*,4′*E*,6′*E*)-**11**: δ 7.35 ppm (1H, d, *J* = 16.0 Hz), 6.28 (1H, s), 5.89 (1H, d, *J* = 15.5), 5.52 (1H, m), 4.12 (4H, m), 3.77 (3H, s), 2.72 (2H, dd, *J* = 22.5, 7.5), 1.95 (3H, s), 1.86 (3H, d, *J* = 4.0), 1.33 (6H, t, *J* = 7.0).

(14) The stereochemistry of geometric isomers was assigned via NOE experiments on the olefinic protons.

(15) The H3′ signal in the ^{1}H NMR spectrum (acetone-d_6) is diagnostic to differentiate these geometric isomers: δ 7.35 ppm (d, *J* = 15.5 Hz) for (2′*E*,4′*E*,6′*E*,8′*E*,10′*E*)-**12**; 7.92 (d, *J* = 15.5) for (2′*E*,4′*Z*,6′*E*,8′*E*,10′*E*)-**12**; 7.36 (d, *J* = 15.5) for (2′*E*,4′*E*,6′*Z*,8′*E*,10′*E*)-**12**; 7.43 (d, *J* = 15.5) for (2′*E*,4′*Z*,6′*Z*,8′*E*,10′*E*)-**12**. The fifth isomer observed after photolysis seemed to be a mixture of two geometric isomers, corresponding to (2′*E*,4′*E*,6′*E*,8′*E*,10′*Z*)-**12** and (2′*E*,4′*Z*,6′*E*,8′*E*,10′*Z*)-**12**. In addition to the H3′ proton, the H11′ resonance is diagnostic to assign the structure for these isomers: δ 5.58 ppm (d, *J* = 8.5 Hz) for (2′*E*,4′*E*,6′*E*,10′*Z*)-**12** and 5.62 (d, *J* = 8.5) for (2′*E*,4′*Z*,6′*E*,10′*Z*)-**12**; 5.67 (d, *J* = 8.5) for (2′*E*,4′*E*,6′*E*,10′*E*)-**12** and 5.66 (d, *J* = 8.5) for (2′*E*,4′*Z*,6′*E*,10′*E*)-**12**.

(16) (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Sakurai, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1990**, *31*, 6367.

(17) No esterification was observed with EDCI/DMAP or BOP/DMAP.

(18) Byproduct formation appeared to be initiated by a Michael addition of a hydroxyl group, followed by a secondary Michael addition(s), and resulted in a complex mixture of the side products. Attempted base- and acid-treatments yielded only a trace amount of the desired products.

unsaturated fatty acid moiety were not completely eliminated, but the formation of side products was significantly slower than hydrolysis of the cyclopentylidene group. Thus, the deprotection was quenched at approximately 60% completion, and the recovered **14** was resubjected to the aqueous acetic acid treatment. After one recycle, the synthetic mycolactones (**1a,b**) were isolated in 67% yield as an approximately 3:2 mixture of 4′*Z* and 4′*E* isomers.

The synthetic mycolactones were fully characterized [^{1}H and ^{13}C NMR (acetone-d_6), HR-MS, UV, α_D]. Rigorous comparison of ^{1}H NMR (acetone-d_6), ^{13}C NMR (acetone-d_6), TLC [silica gel, $CHCl_3$/MeOH/H_2O (90:10:1)], bioassay, and $\Delta\delta$ of ^{1}H NMR in DMBA[4b] demonstrated that the synthetic mycolactones were identical to the natural mycolactones, thereby confirming unambiguously our earlier relative and absolute stereochemical assignment of the mycolactones.

Finally, we should comment on the purity of the synthetic and natural mycolactones. Having observed the chemical behavior of **12**, it was naturally assumed that the synthetic and natural mycolactones might be contaminated with other geometric isomers corresponding to those found in **12**.[15] Indeed, close examination of the ^{1}H NMR spectrum[3] revealed that the natural mycolactones contained a small amount (<5%) of apparently (2′*E*,4′*Z*,6′*Z*,8′*E*,10′*E*)-geometric isomer.[19] It was anticipated that contamination with this minor geometric isomer could be eliminated by performing synthetic operations in the dark. Coupling of **3** with an 11:1 mixture of chromatographically purified (2′*E*,4′*Z*,6′*E*,8′*E*,10′*E*)-**4** and (2′*E*,4′*E*,6′*E*,8′*E*,10′*E*)-**4**, followed by deprotection, furnished an approximately 1.8:1 mixture of the mycolactones A and B that was indeed found to be free from minor geometric isomers (^{1}H NMR analysis).[20]

Acknowledgment. We thank Professor Pam Small at University of Tennessee for a generous gift of natural mycolactones and also for performing biological tests. We are grateful to the National Institutes of Health for generous financial support (CA 22215). A.B.B. gratefully acknowledges support in the form of an American Cancer Society postdoctoral fellowship (PF-99-117-01-CDD, partially funded by the National Fisheries Institute).

Supporting Information Available: Complete experimental details for the syntheses outlined in Schemes 1 and 3−5 and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) The H3′ resonance in the ^{1}H NMR spectrum (acetone-d_6) was found at 7.92 ppm (d, J = 15.6 Hz) for mycolactone A, 7.37 (d, J = 15.6) for mycolactone B, and 7.43 (d, J = 15.6) for the (2′*E*,4′*Z*,6′*Z*,8′*E*,10′*E*)-isomer.[15]

(20) The ^{1}H NMR spectrum of this product is included in Supporting Information.