Communication

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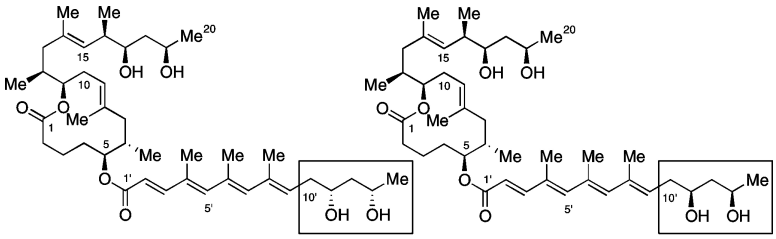

9: Remote Diastereomer of Mycolactone F

10: Mycolactone F

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Total Synthesis and Stereochemistry of Mycolactone F

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Buruli ulcer is a severe necrotizing skin disease caused by *Mycobacterium ulcerans*, but it is one of the most neglected diseases.[1] Infection with *M. ulcerans*, probably carried by aquatic insects,[2] results in progressive necrotic lesions that, if untreated, can extend to 15% of a patient's skin surface. Currently, surgical intervention is the only realistic therapy. In 1999, Small and co-workers isolated the toxic metabolites, named mycolactones A and B, from *M. ulcerans*. Evidence from animal studies suggested that mycolactones A and B are directly responsible for the observed pathology.[3]

The gross structure of mycolactones A and B was elucidated primarily through 2-D NMR experiments.[4] Their stereochemistry was predicted via the NMR database approach and then confirmed by total synthesis.[5,6] Through these studies, mycolactones A and B are now described as a mixture of *Z*-$\Delta^{4',5'}$- and *E*-$\Delta^{4',5'}$-geometric isomers (Scheme 1). Mycolactones A and B constitute the major metabolites produced by West African strains of *M. ulcerans*. However, several mycolactone congeners, including mycolactone C,[7] mycolactone D,[7b] and C2′-methyl mycolactones A and B,[8] have recently been isolated from clinical isolates of *M. ulcerans* from Africa, Malaysia, Asia, Australia, and Mexico. With use of organic synthesis as the main tool, the structure of mycolactone C was also elucidated (Scheme 1).[9]

Recently, a mycolactone congener, called mycolactone F, was isolated from the fish pathogen *M. marinum*,[10] and its gross structure was proposed as *Z*-$\Delta^{4',5'}$-geometric isomer of the structure shown in Scheme 1. Intriguingly, mycolactone F was reported to exhibit a significantly lower toxicity.

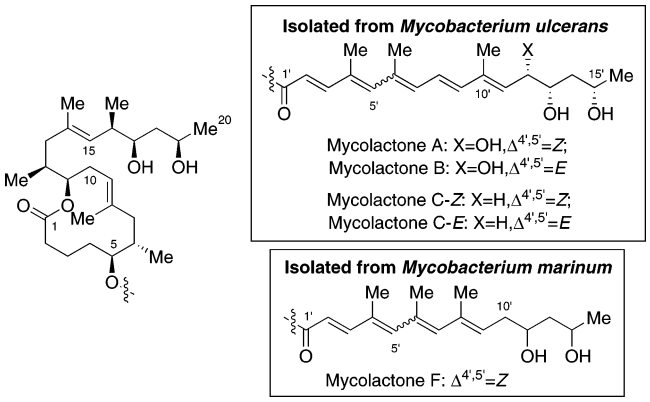
The mycolactones have attracted considerable attention from the synthetic community not only for their highly potent biological activity but also for being the first examples of polyketide macrolides to be isolated from a human pathogen.[11−16] In this communication, we report the total synthesis and the stereochemistry assignment of mycolactone F.

Because of its structure similarity with mycolactones A/B and C, we recognized that a small modification of the previous synthetic route should allow us to obtain mycolactone F; in particular, we planned to assemble mycolactone F via esterification of the known core alcohol **7** (Scheme 4) with unsaturated acid **6** (Scheme 2).[6] The reported fragmentation pattern of MS spectrum supports the proposed gross structure for the unsaturated fatty acid portion, but there are no data available to suggest its stereochemistry. However, considering its structure similarity with mycolactones A/B and C, we speculated **6** to be the likely candidate and carried out its synthesis (Scheme 2). In this synthesis, we purposely adopted a stepwise chain elongation approach because we desired to establish the stereochemistry of each olefinic bond in a stepwise manner.

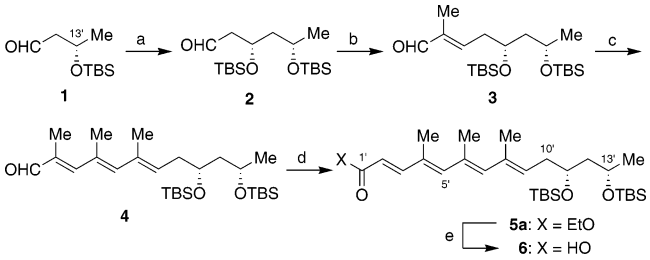
Tetraenoate **5a** exhibited the UV absorption maximum at 323 nm (log $\epsilon = 4.29$, MeOH) but virtually no absorption in the visible region. Thus, unlike the corresponding unsaturated fatty acid ester in the mycolactone A/B series,[4−6] **5a** was found to be stable under the standard laboratory conditions. On irradiation at 300 nm in acetone, **5a** smoothly isomerized to furnish a 4:3:3 mixture[17] of the geometric isomers (Scheme 3). A NOESY experiment was conducted on this mixture, thereby suggesting that they are (2′*E*,4′*E*,6′*E*)-**5a** (4 parts), (2′*E*,4′*E*,6′*Z*)-**5b** (3 parts), and (2′*E*,4′*Z*,6′*E*)-**5c** (3 parts). This assignment was further confirmed through an independent synthesis of **5b** and **5c**.[18] Through this study, the diagnostic chemical shifts (^{1}H NMR in acetone) were identified to assign the geometric stereochemistry of the tetraenoate, namely, **5a**: 6.47 ppm (H-5′) and 7.38 (H-3′); **5b**: 6.66 (H-5′) and 7.39 (H-3′); **5c**: 6.35 (H-5′) and 7.93 (H-3′).

On aqueous base treatment, **5a** gave the corresponding acid **6** without noticeable geometric isomerism. Following the procedure reported for mycolactones A/B and C, **6** was coupled with the core alcohol **7** under the Yamaguchi condition[19] (**6** + **7** → **8**), followed by global deprotection (**8** → **9**), to furnish the synthetic mycolactone F (Scheme 4). The ^{1}H NMR spectrum (Figure 1) revealed that the product thus obtained was predominantly (2′*E*,4′*E*,6′*E*)-**9** but was contaminated with approximately 4% each of two geometric

Scheme 1. Structures of Mycolactones A/B and C and Proposed Gross Structure of Mycolactone F



Scheme 2. Synthesis of Side Chain[a]



[a] Reagents and conditions: (a) (1) Cr-mediate, catalytic asymmetric allylation, 83% (de 14:1); (2) TBAF, 84% (single stereoisomer); (3) TBSCl, im., DMF, rt, 2.5 h, quant.; (4) O_3, CH_2Cl_2, −78 °C, PPh_3, 83%; (b) (1) $(EtO)_2P(O)CH(Me)CO_2Et$, *n*-BuLi, THF, 0 °C; (2) DIBAL, CH_2Cl_2, −78 °C, 1.5 h; (3) MnO_2, CH_2Cl_2, 72% (3 steps); (c) (1) $(EtO)_2P(O)CH(Me)CO_2Et$, *n*-BuLi, THF, 0 °C; (2) DIBAL, CH_2Cl_2, −78 °C, 1.5 h; (3) MnO_2, CH_2Cl_2, 62% (3 steps); (4−6) repeat steps 1−3, 36% (3 steps); (d) $(EtO)_2P(O)CHCO_2Et$, *n*-BuLi, THF, 0 °C, 90%; (e) LiOH, 4:1:1 THF/MeOH/H_2O, rt, 16 h, 89%.

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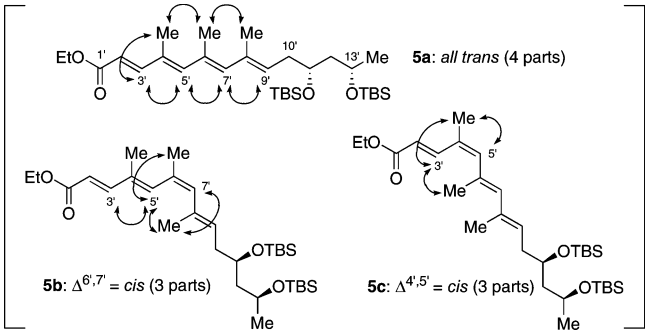
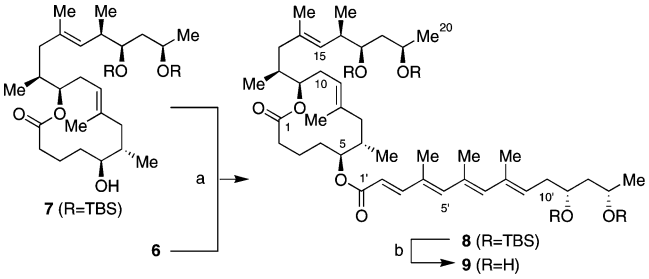
Scheme 3. Photochemical Isomerism of **5a**



[a] Photochemical isomerization was done in acetone with a Reonet photoreactor at 300 nm to furnish a 4:3:3 mixture of **5a**, **5b**, and **5c** as the major products. The arrows indicate the detected NOEs. For the synthesis of **5b** and **5c**, see Supporting Information.

Scheme 4. Completion of Total Synthesis[a]



[a] Reagents and conditions: Yamaguchi esterification: (a) $Cl_3C_6H_2COCl$, *i*-Pr_2NEt, DMAP, PhH, rt, 24 h, 75%; (b) TBAF, THF, rt, 18 h, 83%.

isomers. Once again, under the standard laboratory conditions, the degree of geometric isomerism was negligibly slow. However, under the photochemical condition (300 nm, acetone, 8 min), a facile geometric isomerism was observed to furnish a 5:2:2 mixture of three predominant isomers. Interestingly, the product ratio in the photochemical isomerism of **9** appeared to be different from that of **5a**. With use of the diagnostic chemical shifts identified in the **5a** series, the three dominant products were assigned as 2′*E*,4′*E*,6′*E* (5 parts), 2′*E*,4′*E*,6′*Z* (2 parts), and 2′*E*,4′*Z*,6′*E* (2 parts) isomers.[20]

The ^{1}H NMR spectrum of synthetic mycolactone F (**9**) was found to match the reported ^{1}H NMR spectrum of natural mycolactone F,[10a] except that the content of geometric isomers in natural mycolactone F is higher than that in synthetic material; namely, natural mycolactone F is a mixture of 2′*E*,4′*E*,6′*E* (ca. 8 parts), 2′*E*,4′*E*,6′*Z* (ca. 1 part), 2′*E*,4′*Z*,6′*E* (ca. 1 part), and an additional geometric isomer (trace).[20] This conclusion was further supported by comparison of the ^{1}H NMR spectrum of the photochemically isomerized synthetic mycolactone F. The fact that the ^{1}H NMR spectrum of synthetic material matched well that of natural mycolactone F did suggest the structure of mycolactone F to be **9**. At the same time, we recognized that this comparison alone could not exclude the possibility of **10** as mycolactone F (Scheme 5).

In our terminology, **10** is a remote diastereomer of **9**.[21] As demonstrated in the universal NMR database work,[22] remote diastereomers exhibit virtually identical or very similar NMR spectra in an achiral NMR solvent. However, physicochemical properties in a chiral environment are useful to distinguish remote diastereomers. Indeed, the chiral NMR solvent method was applied to establish the absolute configuration of the unsaturated fatty acid portion of mycolactones A and B.[5b]

Back to the case of mycolactone F, we recognized the need for an analytical method that can distinguish the remote diastereomers **9** and **10**. In order to address this specific issue, we synthesized

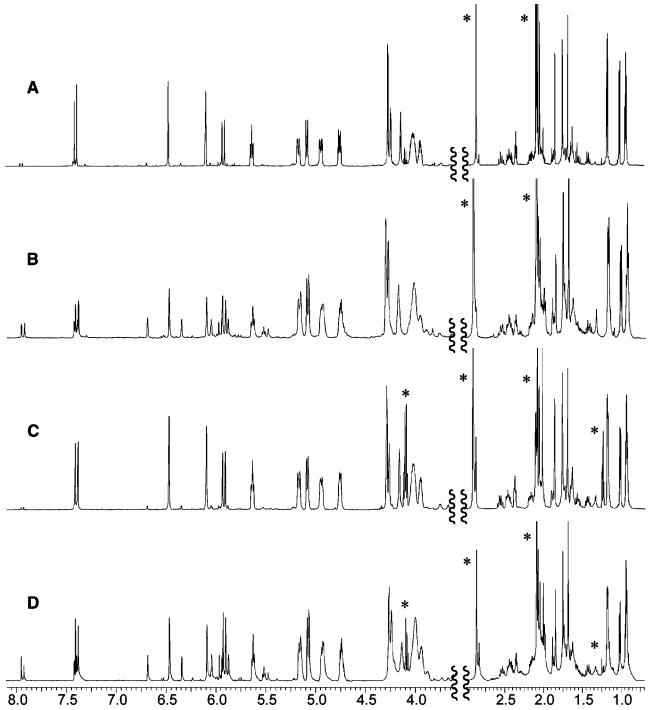
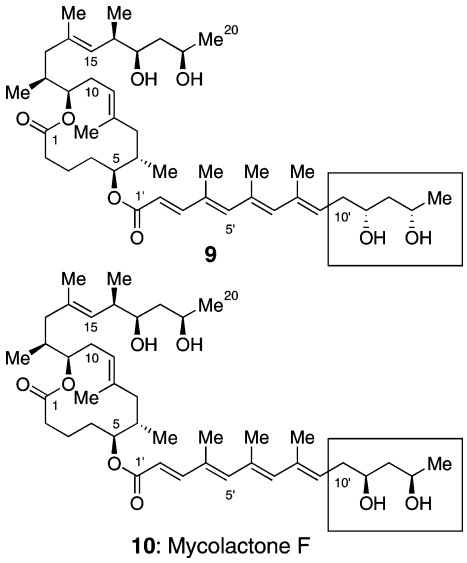


Figure 1. ^{1}H NMR spectrum (600 MHz, acetone-d_6) of synthetic mycolactone F (**10**) and its remote diastereomer (**9**). Panels A and B: before and after photochemical isomerization of **9**, respectively. Panels C and D: before and after photochemical isomerization of **10**, respectively. *Signals from contaminated acetone (2.09 ppm), EtOAc (4.09, 2.00, 1.24), and H_2O (2.88).

Scheme 5. Remote Diastereomers



the remote diastereomer **10** from the enantiomer of **1**, following the synthetic route outlined in Schemes 2 and 4.[18] As anticipated, the ^{1}H NMR spectrum of **10** (Figure 1) was found to be indistinguishable from that of **9**.

With remote diastereomers **9** and **10** in hand, we began to search for an analytical method to distinguish them. As the case of mycolactones A and B, we initially intended to adopt the chiral NMR solvent method[23] but did not pursue this approach for two reasons. First, as only a very minute amount of natural mycolactone F was available for our study,[24] we were concerned with its applicability to the present case. Second, in a preliminary study on **9**, we noticed the resonance of 11′-H is overlapped with other

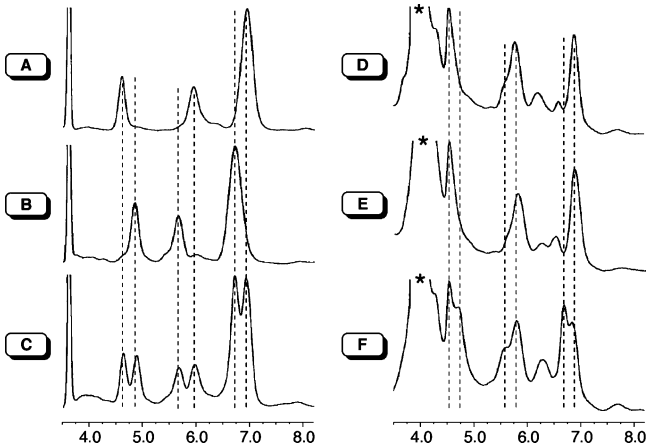


Figure 2. HPLC analysis of synthetic, photochemically isomerized mycolactone F (**10**), and its remote diastereomer (**9**). Column: Chiral Tech, Chiralpak IA (5 μm), 250 × 4.6 mm; solvent (isocratic): *i*-PrOH/PhMe = 6/94; flow rate 1 mL/min; detection: absorption at 323 nm. Panel A: **10**. Panel B: **9**. Panel C: a ca. 1:1 mixture of **9** and **10**. Panel D: the lipid extract containing natural mycolactone F. *Less polar materials contained in the lipid extract. Panel E: a ca. 1:1 mixture of mycolactone F and **10**. Panel F: a ca. 1:1 mixture of natural mycolactone F and **9**.

resonances in DMBA.[23] With these considerations, we focused on the HPLC method with chiral columns. For this search, we purposely used the photochemically isomerized **9** and **10**, with the hope that each of their geometric isomers may give a distinct retention time. Thus, comparison could be performed on the basis of six, instead of two, distinct retention times. After numerous attempts, we found that a Chiralpak IA chiral column in a mixture of toluene and isopropanol can distinguish all of the six remote diastereomers, that is, (2′*E*,4′*E*,6′*E*)-, (2′*E*,4′*Z*,6′*E*)-, and (2′*E*,4′*E*,6′*Z*)-isomers of both **9** and **10** (Figure 2). Finally, the natural product[24] was subjected to this analytical method, thereby demonstrating that the complete structure of natural mycolactone F is **10**, instead of **9**. We should note that there is the possibility that the antipode of **9** might accidentally elute from the same Chiralpak IA column at the same retention time as that observed for **10**, and this question should experimentally be addressed. However, considering the fact that the HPLC comparison is conducted on **9** and its two geometric isomers, we anticipate that it is a very remote possibility.

The bioactivity of synthetic mycolactone F and its remote diastereomer was studied by Professor P. L. C. Small at University of Tennessee, thereby revealing that (1) both synthetic mycolactone F and its remote diastereomer exhibit an overall biological profile similar to the one found on natural mycolactone F, but (2) synthetic mycolactone F is significantly more potent (ca. 1000-fold) than its remote diastereomer. The details of bioactivities will be published in a separate account.

In conclusion, we have synthesized mycolactone F and its remote diastereomer. As anticipated, they gave virtually identical ^{1}H and ^{13}C NMR spectra. With use of the synthetic materials, we have developed an HPLC method with a high sensitivity to distinguish mycolactone F from its remote diastereomer. This analytical method has allowed us to conclude that natural mycolactone F is represented by the structure **10**. Surprisingly, the absolute configuration of its unsaturated fatty acid corresponds to the antipode of that of natural mycolactones A/B and C. Related to this, we should point out that the HPLC chart (panel D, Figure 2) indicates that natural mycolactone F might contain a trace amount of the remote diastereomer **9**. In our view, the result reported here raises several intriguing questions. For instance, clinical isolates of *M. ulcerans* from Africa were reported to be more virulent than clinical isolates of *M. ulcerans* from Asia, Mexico, and Australia. The noted difference in severity might be related to the heterogeneity of the absolute configuration discussed. With the synthesis as well as the analytical method well-developed, we should be able to address this and related questions on the mycolactone class of natural products.

Acknowledgment. With great respect, we dedicate this work to Professor Dieter Seebach on the occasion of his 70th birthday. We thank Professor P. L. C. Small at the University of Tennessee for a generous gift of the lipid extract containing natural mycolactone F and also for performing biological tests. We are grateful to the National Institutes of Health (CA 22215) and to the Eisai Research Institute for generous financial support.

Supporting Information Available: Experimental details and ^{1}H and ^{13}C NMR spectra of key compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) For the HPLC work, the crude lipid extract was directly used because only a very limited amount of natural mycolactone F was available. The total amount of material used for this study was approximately five times of one injection shown in Figure 2.

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