Stereochemistry of the Core Structure of the Mycolactones

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The mycolactones (Figure 1) were isolated in 1999 by Small and co-workers[1] 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,[1a] and they have attracted considerable attention for their highly potent apoptotic activity[2] as well as for being the first examples of polyketide macrolides to be isolated from a human pathogen.[3] The gross structure of these compounds was elucidated through 2-D NMR experiments; however, the stereochemistry remained undetermined.

We recognized that the concepts recently advanced in our laboratory related to the structural elucidation of natural products[4,5] were uniquely suited to address the stereochemical assignment of the mycolactones. To this end, the use of a combined approach employing both an NMR database and the preparation of model compounds to determine the relative and absolute configuration of the mycolactone core structure is herein reported.

It was envisioned that the stereochemical elucidation of the mycolactones would begin by preparing an NMR database of model compounds corresponding to C.13−C.20. Comparison of this database with the reported NMR data for the mycolactones was expected to allow us to predict the relative stereochemistry at C.16, C.17, and C.19. This database was prepared beginning with methyl (*R*)-3-hydroxybutyrate to set the C.19 stereocenter. The C.16 and C.17 stereocenters were subsequently installed via the Brown crotylboration protocol[6] employing separately each enantiomer of Ipc_2BOMe with each of *Z*- and *E*-2-butene. Subtraction of the chemical shift values of this database (i.e., **1a**−**d**) from the reported NMR data for the mycolactones (Figure 2) clearly demonstrates the relative configuration of C.16, C.17, and C.19 to be all-syn (i.e., **1a**).

Having predicted the relative stereochemistry at C.16, C.17, and C.19, we next sought to determine the relative stereochemistry at C.5, C.6, and C.11, C.12. However, we speculated that, in contrast to the desertomycins/oasomycins,[5] a database approach that treated these two stereochemical clusters independently was not likely to be successful as three of these four stereocenters were likely to interact with each other within the 12-membered lactone. We therefore explored the possibility of logically preparing a series of C.1−C.25 synthetic diastereomers for comparison with the mycolactones.

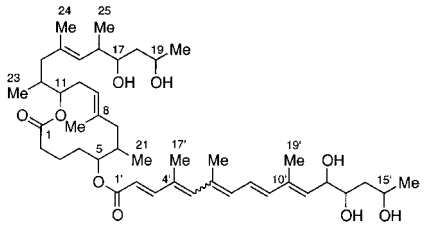


Figure 1. Mycolactone A, *Z*-$\Delta^{4',5'}$; mycolactone B, *E*-$\Delta^{4',5'}$.

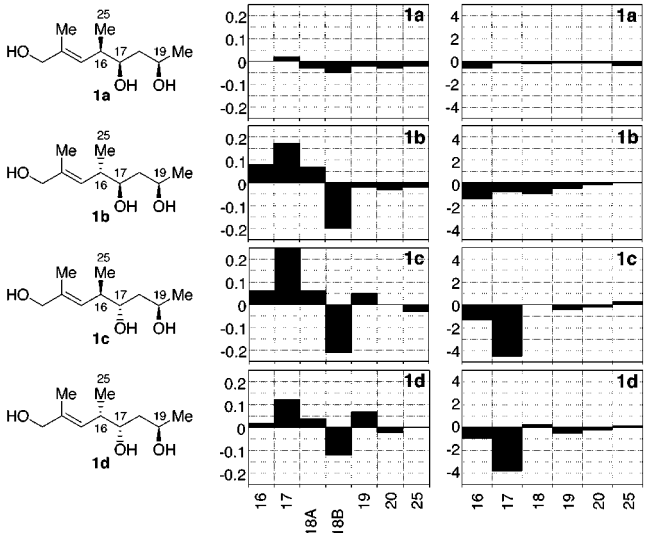
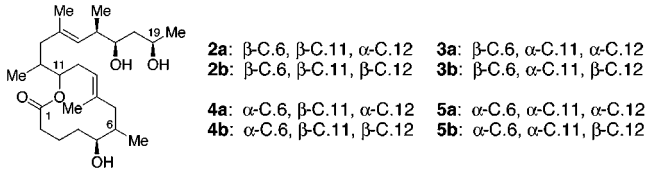


Figure 2. Graphs representing chemical shift difference (*y*-axis, ppm) of ^1H (500 MHz, left column) and ^{13}C (125 MHz, right column) spectra between each corresponding database compound **1a**−**d** atom (*x*-axis) and the reported values for the mycolactones (CD_3COCD_3).



2a: β-C.6, β-C.11, α-C.12 **3a**: β-C.6, α-C.11, α-C.12
2b: β-C.6, β-C.11, β-C.12 **3b**: β-C.6, α-C.11, β-C.12

4a: α-C.6, β-C.11, α-C.12 **5a**: α-C.6, α-C.11, α-C.12
4b: α-C.6, β-C.11, β-C.12 **5b**: α-C.6, α-C.11, β-C.12

Figure 3. Four subgroups of C.1 to C.25 diastereomers.

Several comments should be made regarding this strategy. First, in principle, a series of C.1−C.14 model compounds should be sufficient to determine the relative stereochemistry at the C.5−C.12 cluster. However, we opted to employ diastereomers of the entire core structure both to correlate the relative stereochemistry of the C.5−C.12 cluster to the C.16−C.19 cluster and also to determine the absolute configuration of these stereocenters through correlation with the natural core structure. Second, eight C.1−C.25 diastereomers should be required to determine the relative stereochemistry at the C.5−C.12 cluster, plus one additional diastereomer to correlate this cluster to the C.16−C.19 cluster. However, the amount of required synthetic work can be reduced by assuming that the interactions within the ring will be manifest primarily at C.5, C.6, and C.11. Therefore, the eight possible diastereomers can be logically divided into four subgroups consisting of the four possible C.5, C.6, and C.11 diastereomers (holding C.5 constant); the two diastereomers contained in each subgroup will differ only in the configuration at C.12 (Figure 3). Holding the C.16, C.17, and C.19 stereocenters constant, we expect that the preparation of one member of each subgroup would yield a compound with an NMR spectrum that should correlate

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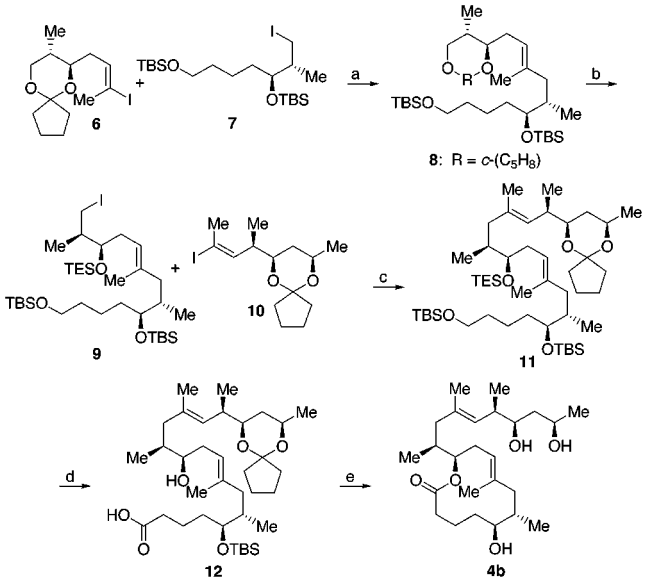
Scheme 1[a]



[a] Conditions: (a) *t*-BuLi (3 equiv), $ZnCl_2$, $Pd(Ph_3P)_4$, THF, 60%; (b) (1) $CH_2Cl_2-H_2O-TFA$ (8:2:0.5), 77%; (2) PivCl, pyr., 99%; (3) TESCl, imid., CH_2Cl_2, 91%; (4) DiBAl-H, CH_2Cl_2, −78 °C, 98%; (5) I_2, Ph_3P, imid., $Et_2O-MeCN$ (3:1), 91%; (c) *t*-BuLi (3 equiv), $ZnCl_2$, $Pd(Ph_3P)_4$, THF, 50%; (d) (1) HF·pyr.−pyr.−THF (1:1:4), THF, 72%; (2) TEMPO, NCS, Bu_4NCl, CH_2Cl_2-pH 8.6 buffer (1:1), 95%; (3) $NaClO_2$, NaH_2PO_4, *m*-$(MeO)_2$-C_6H_4, DMSO−*t*-BuOH (1:1), 94%; (e) (1) $Cl_3C_6H_2COCl$, *i*-Pr_2NEt, PhH; DMAP, PhH, 70%; (2) $CH_2Cl_2-H_2O-TFA$ (8:2:0.5), 62%; (3) HF·pyr., MeCN, 77%.

closely to that of the core structure of the mycolactones. Finally, the C.16,C.17,C.19 epimer of the closest matching diastereomer would be prepared to determine the relative stereochemistry of the mycolactone core structure.

We envisioned preparing each core structure diastereomer via a convergent strategy employing fragments corresponding to C.1−C.7, C.8−C.13, and C.14−C.20 (i.e., **7**, **6**, and **10**, Scheme 1). This approach affords an extremely flexible method for the preparation of each diastereomer through the appropriate choice of stereochemistry in each fragment.

In the event, preparation of diastereomer **4b** was accomplished (Scheme 1) by coupling the C.8−C.13 vinyl iodide **6** with the C.1−C.7 iodide **7** employing a modification[7] of Negishi's conditions[8] to give **8**. This compound was next subjected to a five-step sequence to generate iodide **9**,[9] which was then coupled with the C.14−C.20 vinyl iodide fragment **10** via a second modified Negishi coupling to give **11**.[7, 8]

Both the TES ether and the primary TBS ether were then selectively removed with buffered HF·pyr., and the primary alcohol was selectively oxidized first to the aldehyde with TEMPO,[10] and then to the carboxylic acid with buffered $NaClO_2$ to give **12**. Yamaguchi macrolactonization[11] proceeded smoothly to give the lactone, and a two-step deprotection protocol afforded the C.1−C.25 diastereomer **4b**.

Preparation of one member of each additional subgroup (i.e., **2b**, **3a**, and **5a**) proceeded in an analogous manner as the synthesis of **4b**, employing the appropriate asymmetric starting materials and reagents for each diastereomer. ^{1}H NMR comparison (500 MHz, CD_3OD) of each core structure diastereomer with an authentic sample prepared via hydrolysis of the natural mycolactones (K_2CO_3, MeOH) revealed that only **4b** corresponded exactly to the natural material; the other diastereomers were significantly different. As expected, preparation and NMR comparison of the other member of this subgroup (i.e., **4a**) showed that this diastereomer corresponded very closely, but not exactly, to the natural core structure.

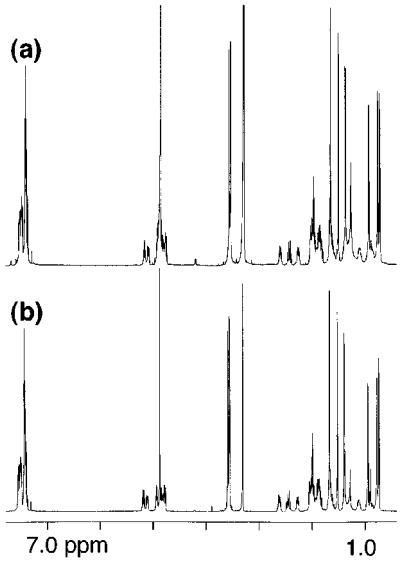


Figure 4. (a) ^{1}H NMR (500 MHz, CD_3OD) of the tri-(*S*)-Mosher ester of the core structure of the natural mycolactones. (b) ^{1}H NMR (500 MHz, CD_3OD) of the tri-(*S*)-Mosher ester of **4b**.

To determine the relative stereochemical relationship between the C.5−C.12 cluster and the C.16−C.19 cluster, the (C.16,C.17, C.19)-*epi*-**4b** diastereomer was prepared; as expected, the ^{1}H NMR spectrum (500 MHz, CD_3OD) of this compound was almost identical to that of the natural core structure. Therefore, the tri-(*S*)- and tri-(*R*)-Mosher esters of both **4b** and (C.16,C.17,C.19)-*epi*-**4b** were prepared and compared to the tri-(*S*)-Mosher ester prepared from the natural material. ^{1}H NMR comparison showed that only the tri-(*S*)-Mosher ester prepared from **4b** was identical to the tri-(*S*)-Mosher ester prepared from the natural mycolactone core structure (Figure 4), thereby proving that **4b** represents both the relative and absolute configuration of the core structure of the natural mycolactones.

In summary, we have employed a combined NMR database and model compound synthesis approach to determine that **4b** represents the relative and absolute stereochemistry of the core structure of the mycolactones. Additionally, the NMR database approach has been employed to determine the relative stereochemistry at C.12′, C.13′, C.15′ as β, α, α,[12] and full details of these results will be reported in due course.

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Supporting Information Available: Complete experimental details for the synthesis of **4b** as well as ^{1}H NMR comparisons (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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