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Design, synthesis, and cytotoxicity of stabilized mycolactone analogs

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ABSTRACT

On exposure to visible light, mycolactone A/B, the causative toxin of Buruli ulcer, rearranges to a mixture of four photo-mycolactones apparently via a rare photochemically-induced [$_4\pi_s + {}_2\pi_a$] cycloaddition. In order to prevent the rearrangement, two C6′-C7′ dihydromycolactone analogs 6′α-**15** and 6′β-**15** were designed and synthesized. 6′α-**15** and 6′β-**15** were shown to be stable under not only photochemical, but also acidic and basic conditions. Cytotoxicity was tested against arbitrarily chosen four cell lines (human Hek-293, human lung carcinoma A-549, human melanoma LOX-IMVI, and mouse L-929), thereby revealing that: (1) both analogs maintain potent cytotoxicity; (2) 6′β-**15** exhibits significantly higher potency against human cell lines than 6′α-**15**; (3) in comparison with parent mycolactone A/B, 6′β-**15** exhibits equal potency against human Hek-293, whereas significantly lower potency against human lung carcinoma A-549 and human melanoma LOX-IMVI.

Mycolactone A/B, the causative toxin of Buruli ulcer, was isolated from *Mycobacterium ulcerans* by Small and co-workers in 1999.[1] This devastating disease results in progressive necrotic lesions that, if untreated, can extend up to 15% of a patient's skin surface. Surgical intervention was the only practical curative therapy for Buruli ulcer. Encouragingly, combination treatments with rifampicin and either streptomycin or amikacin have recently been reported to prevent the growth of the bacteria, especially in early lesions.[2] Evidence from animal studies suggests that mycolactone A/B is directly responsible for the observed pathology, and recent studies have shed light on the mode of action of mycolactone A/B.[3–5] The gross structure of mycolactones A and B was elucidated with spectroscopic methods, whereas the stereochemistry was predicted via the universal NMR database approach and confirmed by total synthesis.[6–9] Under standard laboratory conditions, mycolactones A and B exist as a rapidly equilibrating 3:2 mixture of $\Delta^{4',5'}$-*Z* (major) and $\Delta^{4',5'}$-*E* (minor) isomers, and are referred to as mycolactone A/B in this paper (Scheme 1).

We have been interested in the chemical and biological properties of mycolactones and recently reported the photochemically-induced rearrangement of mycolactone A/B into four photo-mycolactones A1, A2, B1, and B2 (Scheme 1).[10] Interestingly, all four photo-mycolactones were found to exhibit significantly reduced cytotoxicity, compared with parent mycolactone A/B.

On exposure to light through a 365 nm filter at 30 °C in acetone, mycolactone A/B (**1**) rapidly yields an approximately 2:7:1:1 mixture of 4′*E*, 6′*E*, 8′*E*, 10′*E*, 4′*Z*, 6′*E*, 8′*E*, 10′*E*, 4′*E*, 6′*Z*, 8′*E*, 10′*E*, and 4′*Z*, 6′*Z*, 8′*E*, 10′*E* geometrical isomers. The facile *E*⇆*Z* isomerization is then followed by a slower photochemically induced [$_4\pi_s + {}_2\pi_a$] cyclization, to furnish the four photo-mycolactones (see: the structures depicted in the bracket in Scheme 1). According to the proposed mechanism, the C6′ double bond is required for the photochemical cyclization to proceed, thereby suggesting a possibility of synthesizing a photochemically-stabilized mycolactone analog with a replacement of the C6′-C7′ double bond for a C—C single bond. This operation of structure-modification results in two dihydromycolactones 6′α-**15** and 6′β-**15** (Scheme 2). In this letter, we report a synthesis of these mycolactone-A/B analogs and their photochemical and chemical stability, as well as cytotoxicity, in comparison with parent mycolactone A/B.

Scheme 2 outlines a retrosynthetic analysis of 6′α-**15** and 6′β-**15**. This analysis largely relies on our previous work, including: (1) final esterification step[11]; (2) choice of protecting groups for five hydroxyl groups[8]; (3) use of the two previous synthetic intermediates, i.e., the protected form of mycolactone core **14** and aldehyde **12**.[8] Thus, the major remaining question is concerned with the coupling of the C1′-C8′ building block with aldehyde **12**, to form the *E*-olefin **13**. For this case, Julia-Kocienski olefination[12] appears to be an obvious choice, i.e., 6′α-**10**/6′β-**10** + **12** → 6′α-**13**/6′β-**13**, respectively.

Scheme 3 summarizes the synthesis of sulfones 6′α-**10** and 6′β-**10** from commercially available (*S*)- and (*R*)-glycidols, respectively. For this synthesis, we planned to incorporate a chiral methyl group at C6′ into the fatty-acid backbone. Among several options, we chose to adopt the method developed for the synthesis of the

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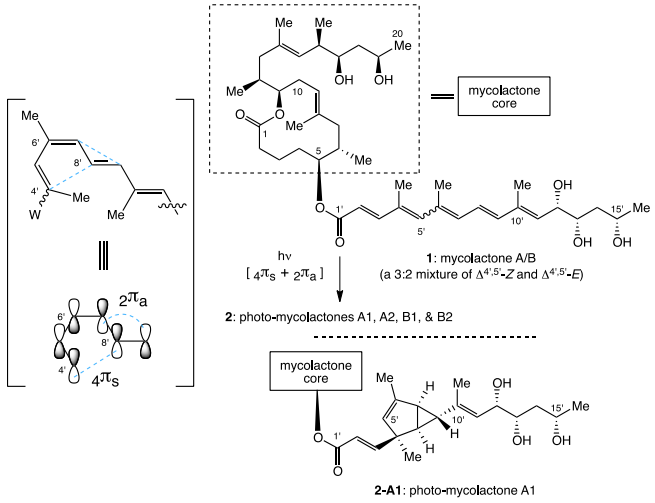
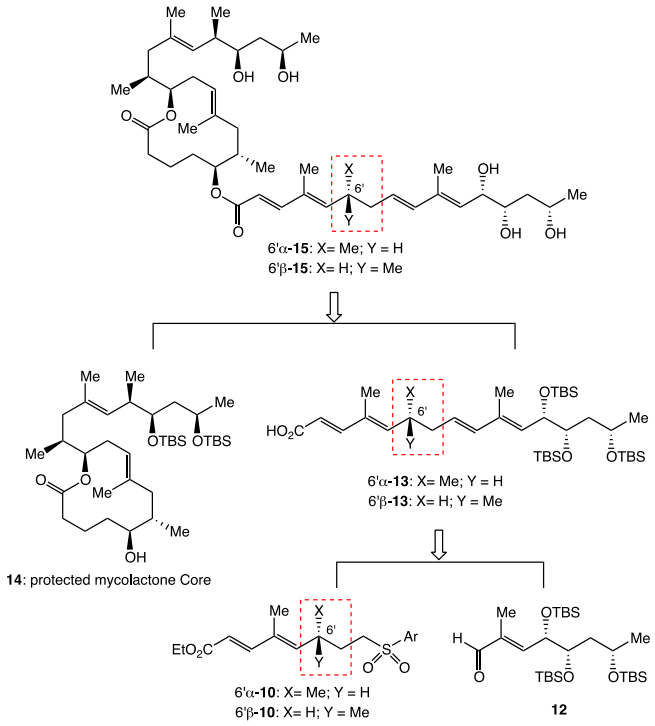


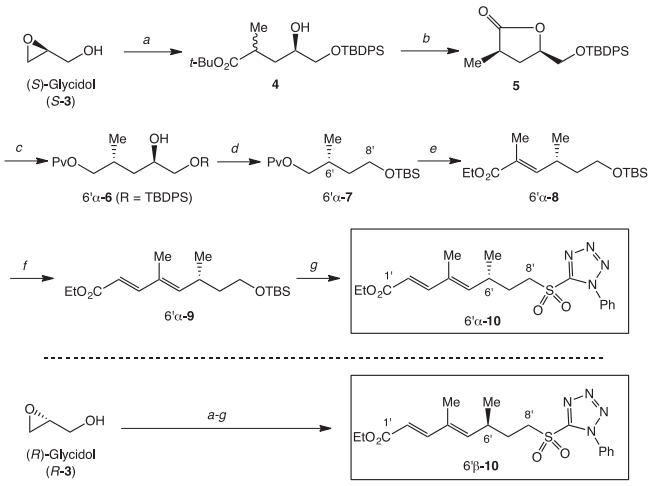
Scheme 1. Photochemical rearrangement of mycolactone A/B (**1**) into photo-mycolactones A1, A2, B1, & B2 (only one structure of the photo-mycolactones shown).



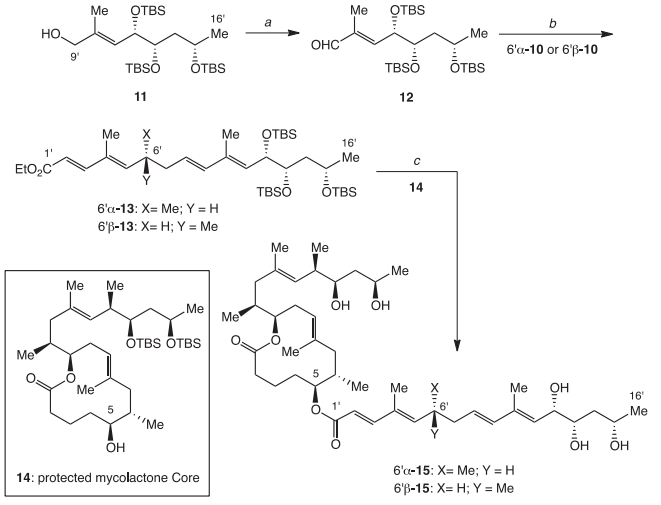
Scheme 2. Retrosynthetic analysis of C6′-C7′ dihydromycolactones 6′β-**15** and 6′α-**15**.

C23-C26 building block of halichondrins.[13] Thus, after protection of the primary alcohol with TBDPS ether, (*S*)-glycidol was treated with *tert*-butyl propionate under the condition reported by Taylor, to give *tert*-butyl ester **4** which, upon treatment with PTSA, resulted in the γ-lactone as a diastereomeric mixture.[14] Treatment with LDA, then with 2,6-di-*tert*-butylphenol, gave an 8–10:1 diastereomeric mixture of γ-lactones as a white solid. On single recrystallization from hexanes, the diastereomeric ratio was improved up to 50–100:1, to give γ-lactone **5**. Based on the consideration that the protonation took place preferentially from the direction opposite to the $CH_2OTBDPS$ group, we assigned, and proved, the stereochemistry of the major diastereomer as indicated.[15] $LiBH_4$-reduction of γ-lactone **5**, selective primary alcohol protection, TBDPS-deprotection, $NaIO_4$-oxidation, $NaBH_4$-reduction, and then TBS-protection treatment straightforwardly gave C5′-C8′ building block 6′α-**7**. We then followed the step-wise chain elongation route used in the previous work[16] to transform 6′α-**7** to 6′α-**9** via 6′α-**8**. TBS-deprotection of 6′α-**9**, followed by treatment with 1-phenyl-1H-tetrazole-5-thiol under the Mitsunobu condition,[17] gave the sulfide, which was oxidized with $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/30% H_2O_2,[17] to furnish sulfone ester 6′α-**10**, required for the proposed Julia-Kocienski olefination.

Using the same sequence of reactions, 6′β-**10** was also prepared from (*R*)-glycidol.



Scheme 3. Stereoselective synthesis of sulfones 6′β-**10** and 6′α-**10**. Reagents and conditions: (a) 1. TBDPS-Cl, imidazole, CH_2Cl_2 (96%); 2. $MeCH_2CO_2Bu$-*t*, LiHMDS, $AlEt_3$, THF (95%). (*b*) 1. PTSA, $CHCl_3$, reflux (96%); 2. LDA, 2,6-di-*tert*-butylphenol, THF (*dr* = 8–10:1), then recrystallization (*dr* = 50–100:1; 65%). (*c*) 1. $LiBH_4$, THF, MeOH (97%); 2. Pv-Cl, Py, CH_2Cl_2 (90%). (*d*) 1. TBAF, THF (96%); 2. $NaIO_4$, aq·THF (93%); 3. $NaBH_4$, MeOH (96%); 4. TBS-Cl, imidazole, CH_2Cl_2. (94%) (e) 1. DIBAL, CH_2Cl_2 (92%); 2. SO_3·Py, $(i\text{-}Pr)_2(Et)N$, DMSO, CH_2Cl_2 (90%); 3. $Ph_3P=C(Me)CO_2Et$, CH_2Cl_2 (90%). (*f*) 1. DIBAL, CH_2Cl_2 (94%); 2. MnO_2, CH_2Cl_2 (92%); 3. $(EtO)_2P(O)CH_2CO_2Et$, *n*-BuLi, THF (93%); (*g*) 1. PPTS, EtOH (90%); 5. 1-phenyl-1H-tetrazole-5-thiol, DIAD, TPP, THF (94%); 2. H_2O_2, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, EtOH (90%). With use of the same sequence of reactions, sulfone ester 6′β-**10** was prepared from (*R*)-glycidol.



Scheme 4. Completion of the synthesis. Reagents and conditions: (a) MnO_2, CH_2Cl_2 (96%); (b) KHMDS, THF, −78 °C (90%); (c) 1. LiOH, THF, MeOH, Water (92%), 2. **14**, 2,4,6-Trichlorobenzoyl chloride, DMAP, DIPEA, Toluene (86%), 3. TBAF, THF (80%); Prepared 6′β-**15** using the same series of reaction sequence starting from 6′β-**10**.

Synthesis of the unsaturated fatty acid ester is summarized in the upper half of Scheme 4. MnO_2-oxidation of allylic alcohol **11** gave aldehyde **12**, which was then subjected to the one-pot version of Julia-Kocienski olefination with 6′α-**10**, to furnish *E*-olefin ester 6′α-**13**. The product was chromatographically isolated in 90% yield and fully characterized (HR-MS, ^{1}H NMR, ^{13}C NMR, UV, and IR). The stereochemistry of newly introduced olefin was established as *E* from $J_{8',9'}$ = 15.7 Hz.

With use of the protocol developed for the synthesis of mycolactone A/B,[8] *E*-olefin ester 6′α-**13** was uneventfully converted to dihydromycolactone 6′α-**15** in 3 steps in 63% overall yield. The final product was isolated with preparative TLC (500 μM silica gel; 5% MeOH/EtOAc) and fully characterized (HR-MS, ^{1}H NMR, ^{13}C NMR, UV, and IR).

Similarly, dihydromycolactone 6′β-**15** was synthesized from 6′β-**10** and was fully characterized. As expected, 6′α-**15** and 6′β-**15** exhibited very similar, but distinctly different ^{1}H NMR properties.

With dihydromycolatones 6′α-**15** and 6′β-**15** in hand, we studied their stability, relative to parent mycolactone A/B, under the photochemical, as well as acidic, basic, and thermal conditions (Table 1). It should be noted that mycolactone A/B is stable to isolate and fully characterize. However, it gradually decomposes, particularly in neat. For this reason, we store mycolactone A/B as an EtOAc solution in a sealed brown ampoule at −20 °C; under this condition, mycolactone A/B has been shown to be stable for at least five years.[19]

Mycolactone A/B shows UV absorption at 362 nm (log ε: 4.35 in MeOH) and, on exposure to light through a 365 nm filter, it is cleanly transformed into a mixture of 4 photo-mycolactones (*vide ante*). Dihydromycolactones 6′α-**15** and 6′β-**15** exhibit an expected blue shift in UV absorption (UV (MeOH) λ_{max} 268 nm (log ε 4.53), λ_{max} 238 nm (log ε 4.58), λ_{max} 230 nm (log ε 4.59)). Importantly, they show virtually no UV absorption at the region uncovered by a 365 nm filter. As expected, on exposure to light through a 365 nm filter, dihydromycolactones 6′α-**15** and 6′β-**15** were found to be stable. Sunlight is also known to induce the transformation of mycolactone A/B to photomycolactones.[10a] Therefore, the photochemical stability of dihydromycolactones 6′α-**15** and 6′β-**15** was also tested under sunlight; on exposure to sunlight in acetone at rt, for 2 days, dihydromycolactones 6′α-**15** and 6′β-**15** exhibited only geometrical isomerization of double bonds, but no skeletal rearrangement.[18,20]

We then studied the acidic, basic, and thermal stability of dihydromycolactones 6′α-**15** and 6′β-**15**, relative to parent mycolactone A/B. For the acid-stability test, we chose the condition of methanol containing 0.1 M aq. HCl (15 eq) at rt, and the stability was monitored by ^{1}H NMR spectroscopy. For 2 h, no significant decomposition was detected for dihydro-mycolactones 6′α-**15** and 6′β-**15**, as well as mycolactone A/B. However, for 8 h, parent mycolactone A/B extensively decomposed (∼80%), whereas dihydromycolactones 6′α-**15** and 6′β-**15** were stable. Similarly, the base-stability under the condition of methanol containing 0.1 M aq. NaOH (15 eq) at rt was tested. Once again, for 2 h, no significant decomposition was detected for dihydromycolactones 6′α-**15** and 6′β-**15**, as well as mycolactone A/B. However, for 8 h, parent mycolactone A/B extensively decomposed (∼90%), whereas dihydromycolactones 6′α-**15** and 6′β-**15** were stable. Lastly, thermal stability was studied in acetone at 60 °C; mycolactone A/B existed as a rapidly equilibrating 3:2 mixture of $\Delta^{4',5'}$-*Z* and $\Delta^{4',5'}$-*E* isomers, whereas 6′α-**15** and 6′β-**15**, were stable even after 10 days.

With photochemically and chemically stabilized mycolactone analogs in hand, we studied their biological activity. For this purpose, we chose to measure their cytotoxicity against arbitrarily chosen Hek-293, A-549, LOX-IMVI, and L-929 cell lines as the first step (Table 2). It is worth noting that: (1) both 6′α-**15** and 6′β-**15** preserve potent cytotoxicity; (2) 6′β-**15** exhibits a practically same potency against human Hek-293 as parent mycolactone A/B, but significantly less potency against human cancer cell lines; (3) 6′α-**15** and 6′β-**15** exhibit a significant difference in cytotoxicity against human-cell lines, but not mouse-cell lines.

We are interested in the observation that an inversion in the stereochemistry of the C6′ methyl group caused a significant difference in cytotoxicity against human cell lines, thereby hinting an importance of a specific stereostructure presented by the unsaturated fatty acid moiety. In this connection, it is worthwhile mentioning recent reports on the cytotoxicity of mycolactone analogs by Altmann and by Blanchard[21]; changes in the unsaturated fatty acid moiety affected the cytotoxic activity against mammalian cells more profoundly than changes in the mycolactone core moiety.

In summary, we have synthesized dihydromycolactones 6′α-**15** and 6′β-**15**, and shown their stability under not only the photochemical, but also acidic, basic, and thermal conditions. Using arbitrarily chosen 4 cell lines, we have shown that both 6′α-**15** and 6′β-**15** exhibit potent cytotoxicity. In addition, we have

Table 1
Stability comparison of mycolactone A/B vs. dihydro-mycolactones.

Conditions	Time	Mycolactone A/B	Dihydro- mycolactones
Photolysis at 365 nm in acetone	2 h ∼ 3 days	Unstable (initial *E*/*Z* double- bond isomerization, followed by photocyclization to [3.1.0]-products)	Stable
Acid (0.1 M HCl (15 eq) in MeOH)	2 h	Stable	Stable
	8 h	∼80% decomposition	Stable
Base (0.1 M NaOH (15 eq) in MeOH)	2 h	Stable	Stable
	14 h	∼90% decomposition	Stable
Thermal (60 °C in acetone)	1 day	Stable (*E*/*Z* double-bond Isomerisation)	Stable
	10 days	Stable (*E*/*Z* double-bond Isomerisation)	Stable

Table 2
Cytotoxicity of mycolactone A/B and dihydro-mycolactones 6′α-**15** and 6′β-**15** (quadruple experiments for each case).

Compound	Cell line			
	IC_{50} (nM)			
	Human Hek-293	Human lung carcinoma A-549	Human mela-noma LOX-IMVI	Mouse L-929
Mycolactone A/B (**1**)	3.2 ± 0.9	0.77 ± 0.15	6.9 ± 0.7	13 ± 4
C6′α-Me dihydro-mycolactone 6′α-**15**	83 ± 31	400 ± 229	120 ± 14	63 ± 4
C6′β-Me dihydro-mycolactone 6′β-**15**	3.0 ± 0.6	77 ± 29	29 ± 4	53 ± 4

made an interesting observation that an inversion in the stereochemistry of the C6′ methyl group caused a significant difference in cytotoxicity against human cell lines, hinting an importance of a specific stereostructure presented by the unsaturated fatty acid moiety. Overall, the chemically stabilized mycolactone analogs might serve us as a valuable tool for study on the biology of mycolactone A/B.

Acknowledgements

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.01.036.

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18. On exposure to sunlight in acetone at rt for 2 days yields an approximately 2:1:1 mixture of 2′*E*, 4′*E*, 8′*E*, 10′*E*, 2′*E*, 4′*E*, 8′*E*, 10′*Z* and 2′*E*, 4′*Z*, 8′*E*, 10′*Z* geometrical isomers.
19. Approximately 20–30% mycolactone A/B decomposed in neat at rt for 10 days. Mycolactone A/B was also stored as a DMSO or EtOH solution in sealed brown ampoules at −20 °C. Under this condition, mycolactone A/B was stable at least for 1 month. However, it was found that ~20% mycolactone A/B in DMSO and EtOH decomposed over three years.
20. Mycolactone A/B is insoluble in water. However, it can be solubilized by addition of 0.1% Triton X-100 or NP-40. The stability of mycolactone A/B, dihydromycolactones 6′α-**15** and 6′β-**15** in water containing 0.1% Triton X-100 and 1 N HCl (15 eq) or IN NaOH (15 eq) was found to be very similar to that in methanol, respectively.
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