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A Diverted Total Synthesis of Mycolactone Analogues: An Insight into Buruli Ulcer Toxins

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Abstract: Mycolactones are complex macrolides responsible for a severe necrotizing skin disease called Buruli ulcer. Deciphering their functional interactions is of fundamental importance for the understanding, and ultimately, the control of this devastating mycobacterial infection. We report herein a diverted total synthesis approach of mycolactones analogues and provide the first insights into their structure–activity relationship based on cytopathic assays on L929 fibroblasts. The lowest concentration inducing a cytopathic effect was determined for selected analogues, allowing a clear picture to emerge by comparison with the natural toxins.

Keywords: Buruli ulcer · metathesis · mycolactones · structure–activity relationships · total synthesis

Introduction

Isolation of mycolactones A and B from Mycobacterium ulcerans MU1615 by Small and co-workers in 1999[1] led to a new paradigm in polyketide natural products. These enigmatic macrolides constitute the first and only examples of polyketides that are a virulence determinant of a human pathogen.[2] Indeed, mycolactones are responsible for Buruli ulcer, a devastating necrotizing skin disease present in more than thirty countries in the world, located mainly in West and Central Africa but also in Australia and now in Japan.[3] In addition, it has been recently proposed that other genetically related mycolactone-producing mycobacteria such as *Mycobacterium shinshuense*, "*liflandii*" and *pseudoshottsii* or *marinum* (except *M. marinum* M) that are pathogenic agents for human, frogs, and fish, respectively, should be now reclassified as M. ulcerans strains, thus highlighting the worldwide distribution and broad host range of these mycobacteria.[4] To date, progress has been made in the antibiotherapy of Buruli ulcer; the use of a combination of streptomycin and rifampicin recommended by the World Health Organization is effective in early and limited infections[5] but still needs to be combined with wide surgical excision in severe cases.[6]

The compelling structures of mycolactones have immediately triggered considerable interest from the synthetic community. Seminal studies by Kishi and co-workers have established the relative and absolute stereochemistry of mycolactone in 2001.[7]

In the following years, elegant syntheses of almost all the members of these seducing macrolides have then been disclosed by Kishi's group.[8] Besides unveiling highly unusual features such as stereochemical heterogeneity in mycolactones F,[8g] these studies have recently been extended to the efficient detection of traces amounts of mycolactones by a fluorogenic chemosensor.[9] In 2011, the groups of Negishi[10] and Altmann[11] reported on the second and third stereoselective total synthesis of mycolactone A/B. Partial syntheses have also been disclosed since 2001.[12–14]

However, the understanding of mycolactones functional interactions at a molecular level is still missing and would

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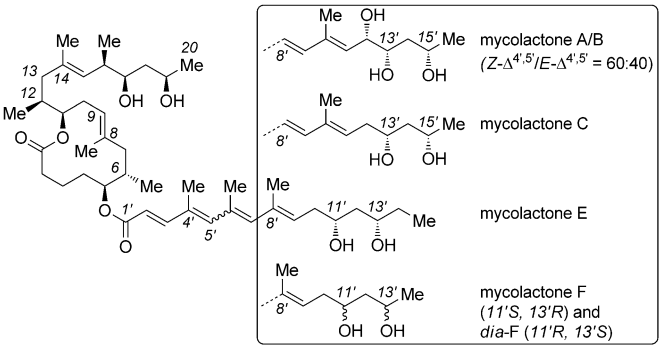


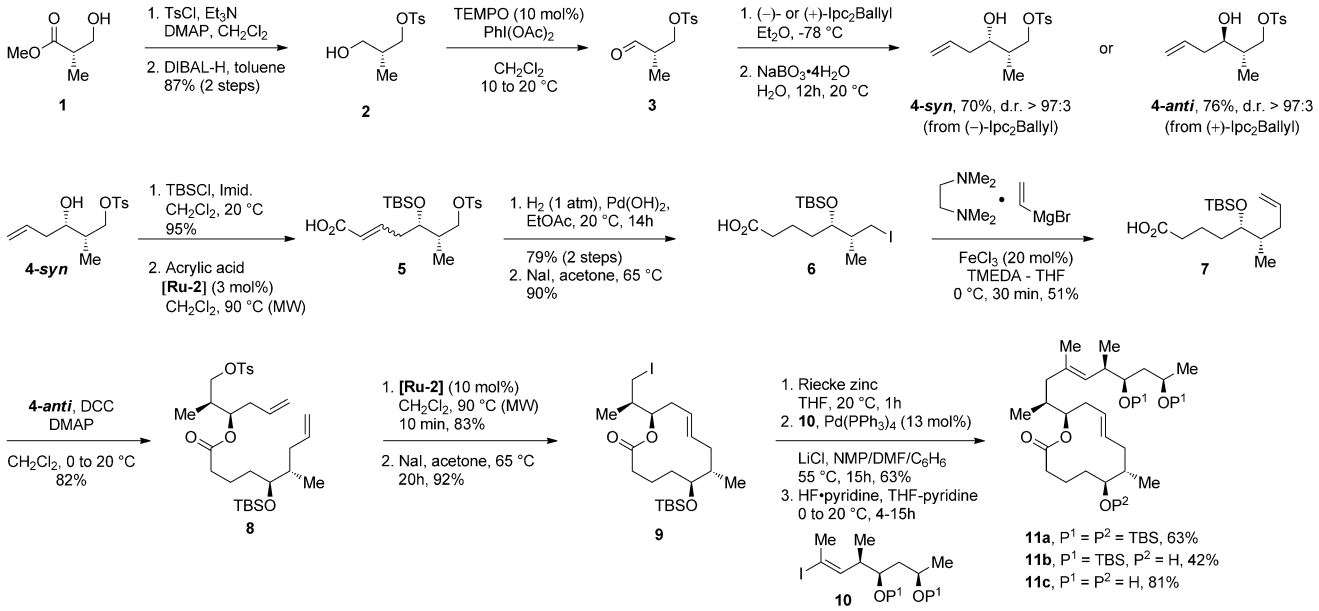
certainly pave the way for the control of this dramatic mycobacterial infection. For the past years, we have been engaged in a research program at the frontiers of immunology and cell biology trying to decipher the functional interplay of mycolactones by use of diverted total synthesis.[15] Herein, we wish to report a flexible synthetic Scheme that allows for rapid elaboration of a panel of simplified C8-desmethyl mycolactones analogues. The cytopathic activity of the various mycolactones analogues on L929 fibroblasts was determined and led to the first structure–activity relationship of these potent toxins. The synthesis of a fluorescent mycolactone variant is also reported based on this key structural information.

Results and Discussion

The efficiency and logic of a retrosynthetic analysis of mycolactones into three fragments, C1–C13, C14–C20, and C1′–C16′, originally proposed by Kishi, has been widely accepted by the community.[11,13] We adopted these key disconnections for the elaboration of mycolactone analogues and developed distinct synthetic approaches to the C1–C13 (C8-desmethyl) undecenolide and C1′–C16′ fragments. The synthesis of these fragments has to be flexible thereby allowing a rapid elaboration of simplified toxin analogues. Three elements of structural diversity were more specifically targeted: the northern fragment C14–C20, the trisubstituted alkene C8–C9, and the C12′,C13′,C15′-stereotriad. Based on investigations by the groups of Small[16] and Leadlay,[17] the latter appears to be a key structural element for both cytopathic and immunosuppressive activity. A synthetic access to natural mycolactones A/B was not the highest priority in this program because these macrolides were obtained by purification of extracts of M. ulcerans 1615 (ATCC35840) cultures at the Institut Pasteur.

The synthesis of the C1–C13 core structure of mycolactone could be efficiently traced back to a single chiral retron, (*S*)-Roche ester **1**, displaying the required absolute stereochemistry for the C6- and C12-stereocenters (Scheme 1). Asymmetric allylboration of aldehyde **3** obtained in three steps from **1** with (−)- or (+)-Ipc$_2$Ballyl[18] afforded the corresponding homoallylic alcohols **4-*syn***[19] and **4-*anti*** as single diastereomers. This diastereoselective allylboration reaction has to be performed at a strictly controlled temperature (−78 °C) to ensure a high diastereomeric ratio.[11,19] The nature of the oxidative work-up was also found to be crucial: the use of diethanolamine as recommended[19] required extensive chromatographic separation and led to low yields,[11] whereas sodium perborate,[20] an inexpensive oxidant, afforded **4** reproducibly and in high yields (70–76 % over two steps). Starting from **4-*syn***, four trivial steps, including an efficient cross-metathesis with acrylic acid, gave the desired ω-iodocarboxylic acid **6**. Displacement of the iodo leaving group by a variety of alkenyl nucleophiles proved to be very difficult. After an extensive screening, only the iron-catalyzed alkenylation reaction developed by Cossy[21] led to the desired ω-alkenylcarboxylic acid **7** (Scheme 1). It is worthwhile noticing that this is the first example of an iron-catalyzed alkenylation reaction of a free carboxylic acid. Compound **7** was then submitted to an esterification reaction with alcohol **4-*anti*** using Steglich conditions.

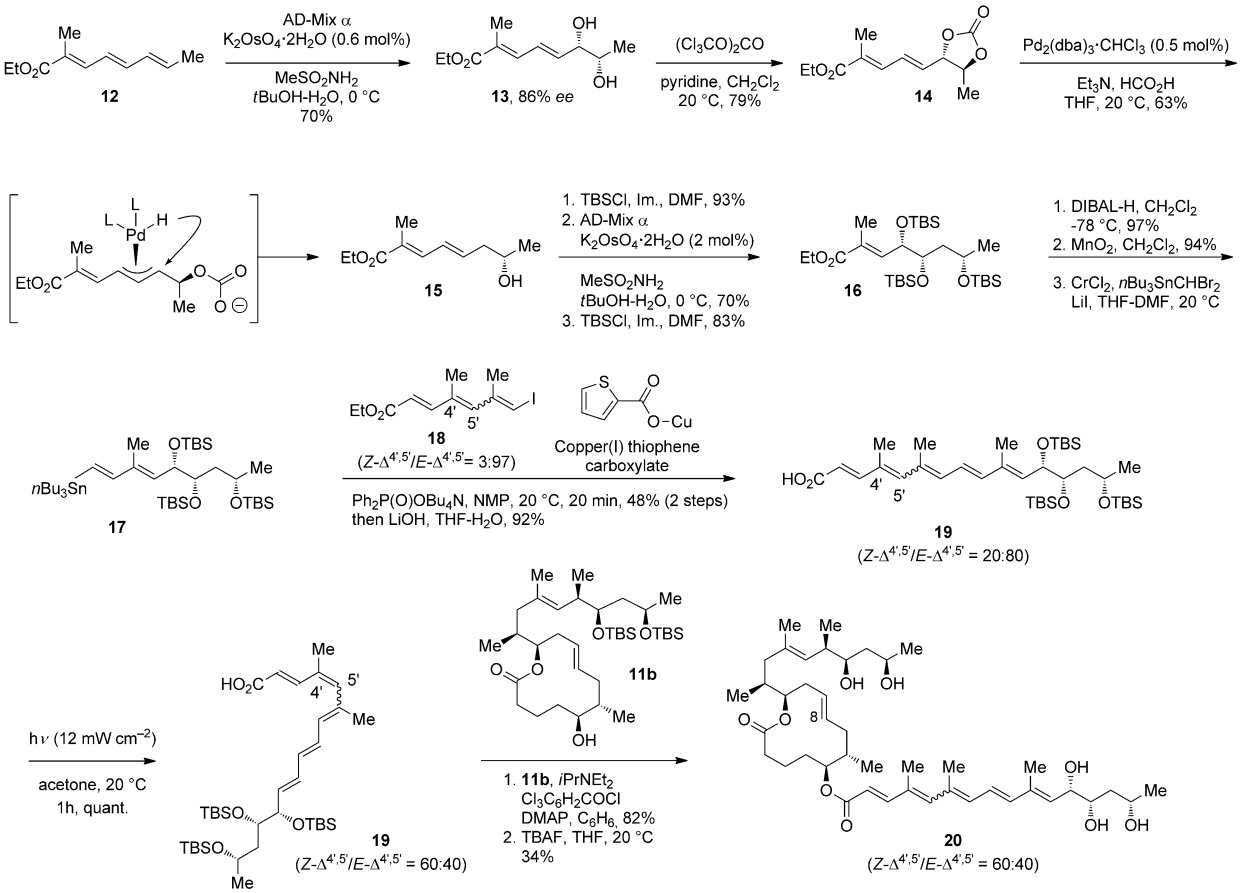
The resulting ester **8** underwent an *E*-selective ring-closing metathesis (RCM) using 10 mol % of second-generation Grubbs catalyst.[11,13] Iodide **9** could then be obtained by using a Finkelstein reaction. Treatment of iodomacrolactone **9** with Riecke zinc allowed a smooth conversion to the cor-



Scheme 1. Synthesis of the C1–C20 fragment of C8-desmethyl mycolactones.

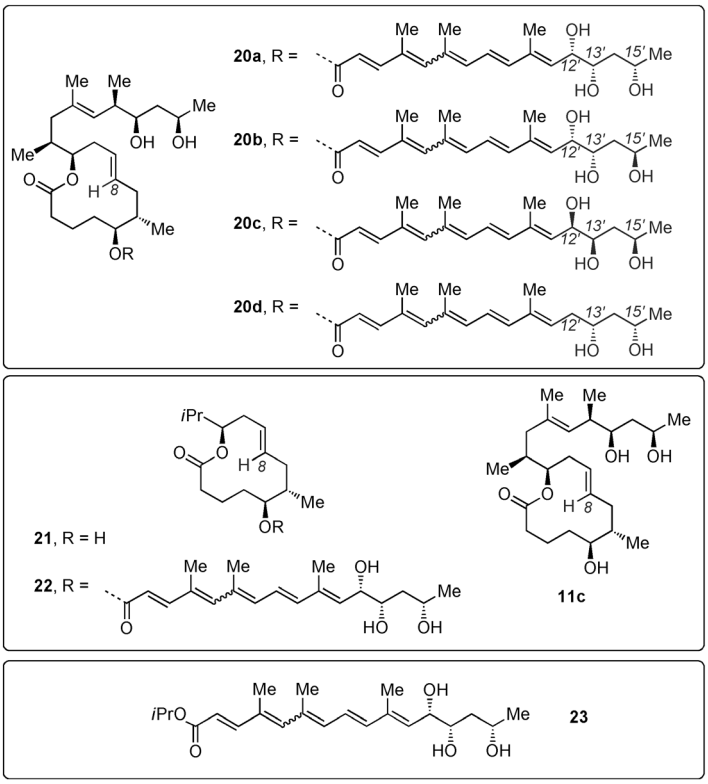
responding organozinc derivative, which could be coupled with vinyliodide **10** (prepared according to Kishi's route[8]) by a Negishi coupling. The C1–C20 fragment of C8-desmethyl mycolactones A/B was thus obtained in 63% yield. This very efficient coupling strategy was previously developed by the group of Kishi[8] and also used by the groups of Altmann[11] and Burkart[13] during their synthetic studies on the mycolactones C1–C20 fragment. Previous disconnections for the synthesis of the C1–C20 fragment investigated earlier in our laboratories involved the formation of the C15–C16 carbon–carbon bond through organometallic couplings. However, all these efforts proved unsuccessful.[22] Finally, we were able to selectively access the C5-hydroxy macrolactone **11b** or the fully deprotected C1–C20 fragment **11c** with HF·pyridine.

A flexible approach to the C1′–C16′ fragment was then devised based on the highly efficient catalytic asymmetric oxidation/reduction sequence developed by O'Doherty[23] (Scheme 2). Regio- and enantioselective asymmetric dihydroxylation of trienoate **12** (prepared in one step from *trans*,*trans*-2,4-hexadienal) delivered dihydroxyester **13** (70% yield, 86% enantiomeric excess (*ee*)). Carbonate formation followed by allylic reduction led cleanly to the δ-hydroxyester **15** with the C15′ absolute configuration matching that of natural mycolactones A/B. Asymmetric dihydroxylation then proceeded with complete regio- and diastereoselectivity affording ester **16** in good yield after protection of the diol moiety as a silyl ether. A three-step sequence including a chromium-mediated one-carbon homologation of the α,β-unsaturated aldehyde led to dienylstannane **17**.[24] The latter could be engaged in a highly practical copper thiophene carboxylate-promoted cross-coupling reaction with iodotrienoate **18** (prepared in ten steps from diethyl methylmalonate), at room temperature in only 45 min. The corresponding sensitive ester was obtained in 48% yield over two steps thus highlighting the reactivity of Liebeskind and Srogl's mediator in challenging transformations.[25] Saponification and photoisomerisation of the pentaenoic acid **19** (green house bulb, 12 mW cm^{-2}) afforded a Z-$\Delta^{4',5'}$/E-$\Delta^{4',5'}$ = 60:40 mixture as in natural mycolactones A/B. Macrolactone **11b** and pentaenoic acid **19** were then coupled in 82% yield. As previously noticed by Kishi,[8h] global deprotection of the resulting pentakis-silylether by tetra-*n*-butylammonium fluoride (TBAF) is a very challenging transformation with dramatic scale-up issues. The C12′,C13′,C15′-silylethers are readily deprotected with TBAF at room temperature, contrary to the C17 and C19 silylethers. An anhydrous work-up procedure[26] and the recycling of the partially deprotected material were keys to the isolation of the C8-desmethyl mycolactones A/B **20a**.



Scheme 2. Synthesis of the southern fragment of mycolactones A/B and its assembly with the C8-desmethyl core **11b**.

The synthetic strategy presented in Scheme 1 and Scheme 2 is flexible and allowed the rapid elaboration of a panel of C8-desmethyl mycolactone variants, eight of which are represented in Scheme 3.[27] The activity of these simplified mycolactones analogues was then studied in a cytopathic assay on L929 mouse fibroblasts. These cells are considered as a standard for the evaluation of the cytopathic activity (CPA) of lipidic extracts of M. ulcerans strains since 1974.[1]



Scheme 3. A panel of C8-desmethyl mycolactones analogues obtained through DTS.

The cytopathic activity (determined as the number of rounded cells compared to the total number of cells) of these eight variants was first measured at 10 and 50 μM in serum-free media[28] on L929 mouse fibroblasts. Then, the lowest concentration inducing a cytopathic effect was determined for selected analogues, unraveling clear structure–activity relationships by comparison with the natural toxins. The results obtained are summarized in Figure 1. The simplified fragments **21** and **23** induced less than 20% of cell rounding in 48 h, both at 10 and 50 μM. The C1–C20 fragment **11c** and the analogue **22** lacking the C14–C20 northern fragment were also moderately cytopathic at 10 μM, with less than 30 and 60%, respectively, of cell rounding but demonstrated 100% of cell rounding at 50 μM. The deoxy-C12′ derivative **20d** induced a cell rounding in the 50% range at 10 μM, whereas analogues **20a–b** and natural mycolactones A/B induced 100% of cell rounding within 48 h.

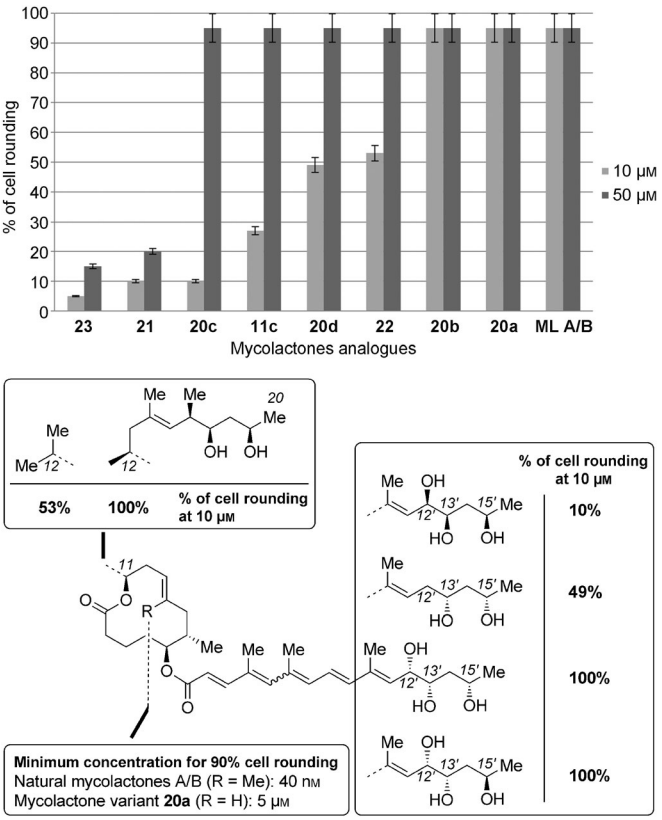


Figure 1. Cytophatic activity of C8-desmethyl mycolactone analogues on L929 mouse fibroblasts at 10 and 50 μM.

Overall, these cellular assays provided significant information on the structural requirements for cytotoxicity. Neither the undecenolide core **21** alone nor the unsaturated fatty acid side chain of mycolactones **23** are sufficient for cytopathicity. Compounds **11c** and **22** possessing, respectively, the northern fragment (C14–C20) or the southern fragment (C1′–C16′) of the toxin combined with the undecenolide core showed limited cytopathicity at 10 μM, suggesting that the combination of the northern, central, and southern fragment of the toxins is required for potency. Particularly noteworthy is the role of the C12′-hydroxyl: when this functional group is deleted as in compound **20d**, only 49% of cell rounding is observed, whereas 100% cell rounding is obtained under the same conditions for compound **20a**, the C8-desmethyl mycolactones A/B.[29] Interestingly, the same phenomenon has been reported for natural mycolactone C: this macrolide induced 90% of cell rounding at 1.1 μM, a 50 fold decrease compared to natural mycolactones A/B.[30] In line with these results, the fully *epi*-stereotriad in **20c** led to a dramatic decrease in cytopathicity (10% at 10 μM). The stereochemistry at C15′ appeared less important since 100% of CPA was obtained for **20b** at 10 μM.

The lowest concentration inducing a cytopathic effect was then determined for natural mycolactones A/B and for the structurally closest analogue **20a**, lacking the C8-methyl substituent of the natural toxins. At 40 nM, mycolactones A/B induce 90% of cell rounding of L929 fibroblasts within 24 h,

which is in agreement with studies by Small and co-workers.[30] By comparison the minimal concentration for such an effect for compound **20a** is 5 μM. The lack of the C8-methyl substituent thus reduces the CPA by a factor 125, stressing the importance of seemingly simple structural simplifications on structure–activity relationships.

Deciphering the functional interactions of mycolactones is of fundamental importance for the understanding and ultimately the control of Buruli ulcer. The design of a fluorescent mycolactone analogue would also constitute an additional tool that could allow for the identification of the yet unknown cellular target of Buruli ulcer toxins. Based on the results of the cytopathic assays presented above, we chose to substitute the northern fragment of mycolactone with a bodipy fluorophore, thus avoiding the chemical suppression of the crucial C12′,C13′,C15′-triol motif, which was reported by Small and Snyder in the design of a related probe.[16] Compound **28** was synthesized in five steps from macrolactone **24** through a copper-catalyzed azide–alkyne cycloaddition between azide **26** and the ω-alkynyl-bodipy **27** (Figure 2). To our delight, probe **28** presented a cytopathic activity close to **20a** with a minimum concentration inducing 90% of cell rounding of 10 μM. As desired, no cell rounding was measurable at 0.5 μM, the concentration used in the uptake experiments illustrated in Figure 2. Bodipy mycolactone **28** was detected and localized in less than two minutes in the cytoplasm, without any visible binding with the nucleus, therefore pointing towards a cytoplasmic target (Figure 2, e and f). Thus, derivative **28** is structurally related

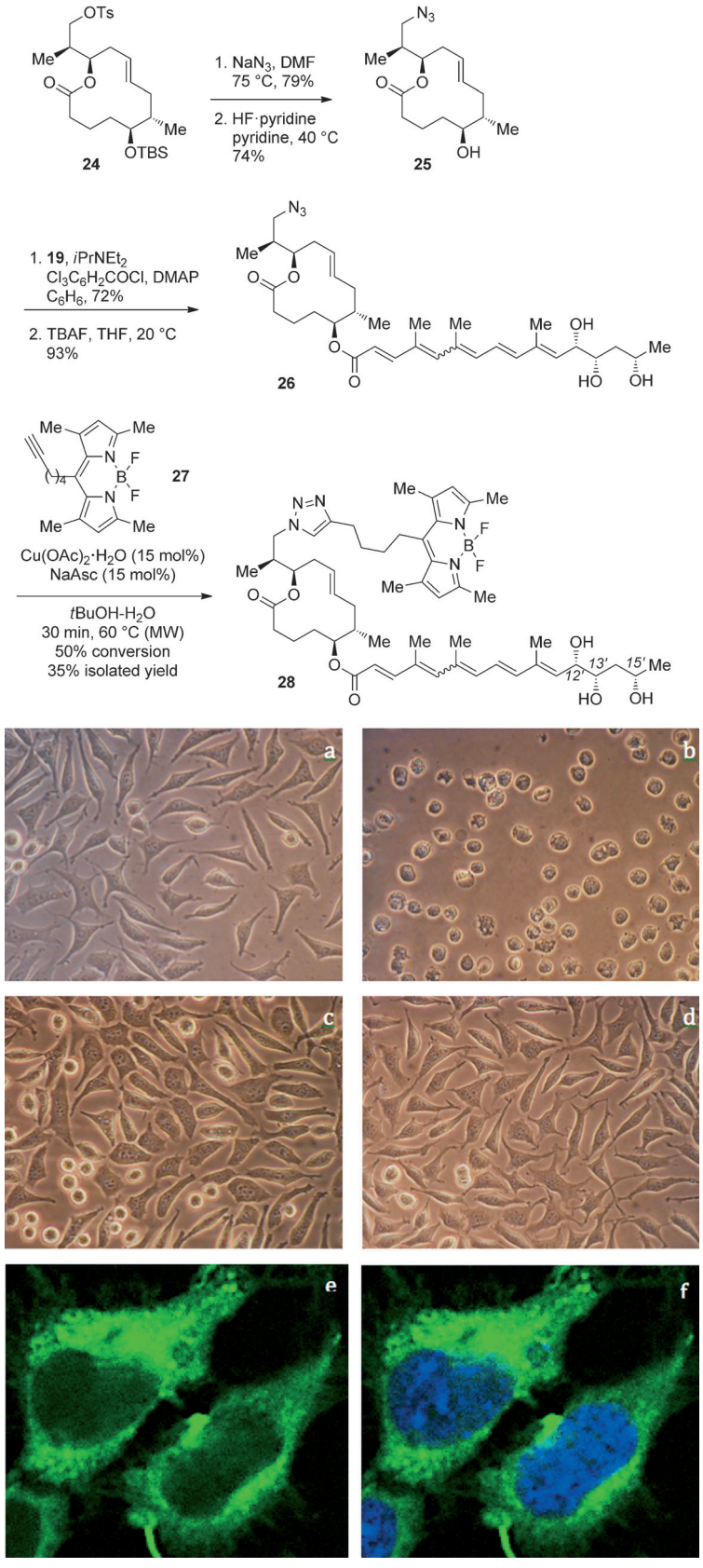


Figure 2. Top: Synthesis of bodipy-mycolactone **28**. Bottom: L929 fibroblasts incubated for 48 h in serum-free medium with DTS analogues (10 μM, 0.1% DMSO); a) **21**; b) **20a**; c) **20c**; d) serum-free medium/DMSO (0.1%); e) uptake of bodipymycolactone variant **28** (0.5 μM) after 2 h of incubation and f) after staining of the nuclei with 4′,6-diamidino-2-phenylindole (DAPI, magnification ×40).

to mycolactones, non-toxic at submicromolar concentrations and presents an interesting cellular penetration. This molecular probe will prove useful for further mechanistic studies.

Conclusion

In conclusion, a diverted total synthesis approach of Buruli ulcer toxins analogues has been designed, featuring a diversity of metal-mediated transformations including Cossy's iron-catalyzed alkenylation reaction,[21a] a cross-metathesis, and a ring-closing metathesis for the construction of the C8–C9 unsaturation. In addition, a modular approach of the C12′,C13′,C15′ stereotriad has been devised based on the highly stereoselective catalytic asymmetric oxidation/reduction sequence developed by O'Doherty.[23] A copper-mediated Liebeskind–Srogl coupling allowed the elaboration of the sensitive pentaenic moiety, thus completing the synthesis of the challenging southern fragment of mycolactones. The implementation of this modular strategy allowed a rapid synthesis of a panel of analogues including a fluorescent variant. Their cytopathicities have been measured on L929 mouse fibroblasts, thus providing the first structure–activity relationship of these potent toxins. In addition, the lowest concentration inducing a cytopathic effect was determined for natural mycolactones A/B and for the structurally closest analogue **20a**.

The structural information gathered during these studies, such as the central role of the C8-methyl substituent and of the C12′,C13′,C15′ stereotriad, will be central to the understanding of the functional interactions of mycolactones and ultimately to the deciphering of their mode of action.

Acknowledgements

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[27] See the Supporting Information for the detailed synthesis of mycolactones variants **20b–d**, **21**, **22**, and **23**.
[28] To evaluate the relevance of plasma protein binding on in vitro potency, cytopathic assays were run in complete medium (Eagle's MEM supplemented with 10% horse serum). A negligible shift in potency was observed (data not shown). For a review, see: D. A. Smith, L. Di, E. H. Kerns, *Nat. Rev.* **2010**, *9*, 929.
[29] The presence of the C12′-hydroxy motif is also critical for the immunosuppressive effect of mycolactones, see ref. [17].
[30] A. Mve-Obiang, R. E. Lee, F. Portaels, P. L. C. Small, *Infect. Immun.* **2003**, *71*, 774.

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