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Modular total syntheses of mycolactone A/B and its [^{2}H]-isotopologue

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Sarah Saint-Auret,[a] Hajer Abdelkafi,[a] Didier Le Nouen,[b] Laure Guenin-Macé,[c] Caroline Demangel,[c] Philippe Bisseret[a] and Nicolas Blanchard[a*]

A modular total synthesis of mycolactone A/B, the exotoxin produced by *Mycobacterium ulcerans* has been achieved through the orchestration of several Pd-catalyzed key steps. While this route leads to a mixture of the natural product and its C12 epimer (4:1 ratio), this was inconsequential from the biological activity standpoint. Compared to previously reported routes, this synthetic blueprint allows the late-stage modification of the toxin, as exemplified with the preparation of [22,22,22-$^2H_3$]-mycolactone A/B.

Buruli ulcer is a dramatic skin disease in human currently reported in more than 30 countries in Africa, South America and Western Pacific regions, with >2000 new cases in 2015.[1] Since the clinical description of this third most common mycobacterial disease in 1948,[2] intense research efforts have been deployed by the scientific community to understand and control this infection caused by the environmental *Mycobacterium ulcerans*.[1c,3] In 1999, the discovery of mycolactone A/B as the major virulence factor of *M. ulcerans* was a breakthrough as it opened a molecular perspective on this devastating disease.[4] Mycolactone A/B, a complex macrolidic polyketide secreted by *Mycobacterium ulcerans* (Scheme 1), belongs to a larger family comprising nine mycolactones (C, D, E plus its minor metabolite, F, dia-F, S1 and S2) isolated from *M. ulcerans* or *M. marinum* as major or minor species and affecting several animal species.[1c,5] Pioneering studies by Kishi confirmed the gross structure and elucidated the complete stereochemistry of the exotoxin in 2001,[6] and several research groups have since then contributed to elegant total and partial syntheses.[1c,7]

Since 2006, we have been engaged in a Diversity Oriented Synthesis approach to mycolactone A/B analogs that led us to report some structure-activity relationship data and to uncover a fully functional analog of mycolactone A/B mimicking its activity in *in vivo* inflammation disorders models.[8] In addition to its necrotic potential, mycolactone A/B has inhibitory properties on cytokines production by immune cells.[1c,9] Considering the translational potential of mycolactone A/B and its analogues,[8c] a modular synthetic scheme is of prime importance to further advance our understanding of this promising therapeutic agent through *in vivo* studies. A second current challenge is the quantification of mycolactone from biological samples that could be addressed by isotope dilution mass spectrometry (IDMS)[10] using the appropriately stable isotope labeled mycolactone.

We report herein our preliminary results of a flexible synthetic blueprint that culminates in a total synthesis of mycolactone A/B (as a 12*S*/12*R* = 80:20 mixture of diastereomers) that is indistinguishable in its ability to inhibit T-cell interleukin-2 production compared to the toxin isolated from the culture of *M. ulcerans*. In addition, this *de novo* strategy led to the [22,22,22-$^2H_3$]-isotopologue of the natural product, which could be used as an internal standard for IDMS.

The retrosynthetic analysis of mycolactone A/B and its [22,22,22-$^2H_3$]-isotopologue is depicted in Scheme 1 and calls for an esterification reaction between the C1C20 fragments **1a,b** and the fatty carboxylic acid C1'C16' **2**.[1c] The second disconnection relies on a late stage methylation reaction of the C8-Br σ-bond of compound **3** which in turn could be obtained via the formation of the C13C14 σ–bond thanks to a Suzuki cross-coupling of the organoborane **5** with the (*E*)-trisubstituted vinyliodide **4**. Organoborane **5** could be prepared by a chemo- and diastereoselective hydroboration reaction of the isopropenyl-macrolactone **6**, itself obtained from skipped diene **7** by a dihydroxylation reaction followed by elimination. Further disconnection focuses on the (*Z*)-trisubstituted C8C9 vinylbromide motif of **7** that could be prepared in a single step

[a] *Université de Strasbourg, CNRS, Laboratoire de Chimie Moléculaire UMR 7509, 67000 Strasbourg, France. E-mail : n.blanchard@unistra.fr*
[b] *Université de Haute-Alsace, Laboratoire de Chimie Organique et Bioorganique EA 4566, 3bis rue Werner, 68093 Mulhouse Cedex, France.*
[c] *Institut Pasteur, Unité d'Immunobiologie de l'Infection, INSERM U1221, 25 Rue Du Dr Roux, 75724 Paris Cedex 15, France.*
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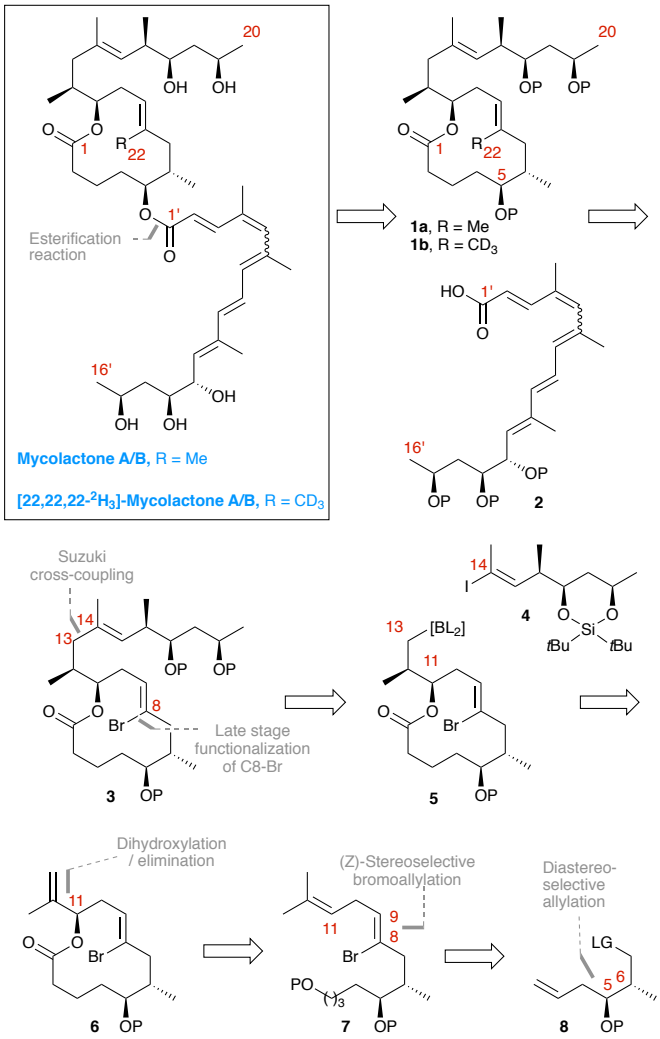
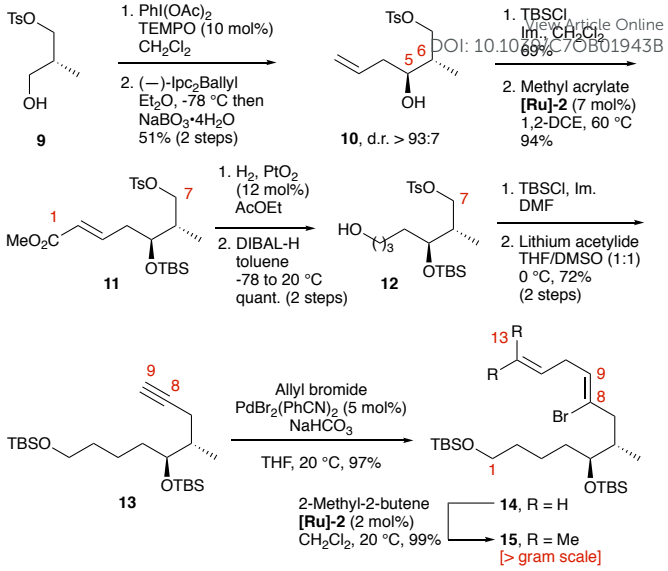


using a Kaneda stereoselective palladium-catalyzed bromoallylation reaction[11] of the corresponding alkyne, itself prepared in a few steps from homoallylic ether **8**.

Scheme 1. Retrosynthetic analysis of mycolactone A/B and its [22,22,22-$^2H_3$]-isotopologue.

In the forward sense, oxidation of primary alcohol **9**[8a,b] to the corresponding aldehyde followed by its reaction with the commercially available (–)-diisopinocampheyl borane proceeded smoothly at -78 °C to deliver homoallylic alcohol **10** in good yield and diastereoselectivity, on a decagram scale (Scheme 2).[8a,b] As noted in our first-generation synthesis of mycolactone analogs, the nature of the oxidative work-up proved crucial, with sodium perborate being the most practical oxidant and leading to the cleanest crude reaction. Protection of the C5-hydroxy group as a silyl ether followed by chain extension towards C1 relied on a cross-metathesis reaction with methyl acrylate using second generation Grubbs precatalyst **[Ru]-2**, leading to the α,β-unsaturated ester **11**.

Reduction of the C2C3-π-deficient system of the latter under PtO_2-catalyzed hydrogenation conditions was then followed by reduction of the ester function to the primary alcohol **12**. Protection as a silyl ether followed by displacement of the tosylate with the commercially available lithium acetylide•1,2-ethylenediamine delivered the desired terminal alkyne **13** in good yield. This seemingly trivial step required a careful optimization as a competitive E2-elimination of the tosylate was a serious concern in a variety of solvents and temperatures.



Scheme 2. Synthesis of the C1C13 Sector of Mycolactone A/B.

Kaneda stereoselective bromoallylation[11] of **13** led exclusively to (*Z*)-**14** (97%) which was then engaged in a cross-metathesis with 2-methyl-2-butene, leading to **15** (99%) on gram scale.

Dihydroxylation of the C11C12-π-system of **15** proceeded in high yield and with an excellent diastereoselectivity (d.r. > 97:3, Scheme 3) as proven by the complementary Mosher esters of the C11-hydroxy function.[12] 1,2-Diol **16** could then be transformed into the desired isopropenyl alcohol **17** in good yield using a straightforward four-step sequence (54%). Oxidation to the carboxylic acid **18** proceeded in good yield, thus setting the stage for the macrolactonization of the seco-acid. Among the different strategies[13] evaluated for the macrolactonization of **18**, Yamaguchi conditions proved superior and led to the desired 12-membered macrolactone **19** in 48% (three steps) reproducibly. We then focused on the forging of the C13C14-σ-bond via a Suzuki cross-coupling between organoborane **5** and vinyliodide **4**.[14] Generation of the intermediate organoborane **5** in such a complex setting should proceed exclusively at the 1,1-disubstitued C12C13-π-system of **19**, although the trisubstituted C8C9-π-system is more electron-deficient. In addition, this hydroboration reaction installs the C12-stereocenter and should thus occur diastereoselectively thanks to the conformational bias imposed by the C11 stereogenic center in the α-position of the C12C13 π-system.[15] The reluctance of the 1,1-disubstituted alkene of **19** to undergo hydroboration with 9-BBN-H in a variety of reaction conditions was quite surprising. After considerable experimentation, it was discovered that the hydroboration proceeded with complete chemoselectivity and correct diastereoselectivity (d.r. = 80:20, unseparable C12 epimers) using 9-BBN-H dimer in THF under ultrasonic irradiation. Although not very common in

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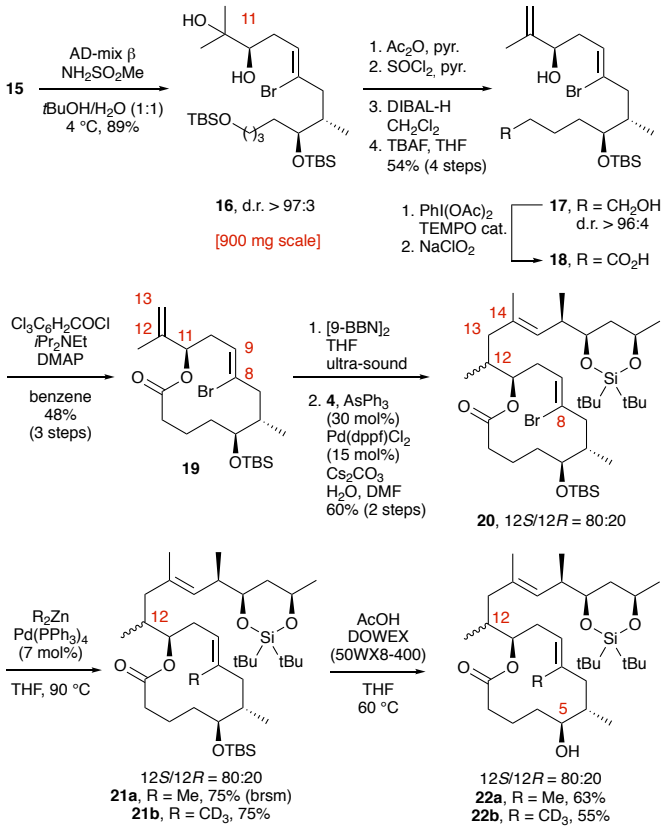
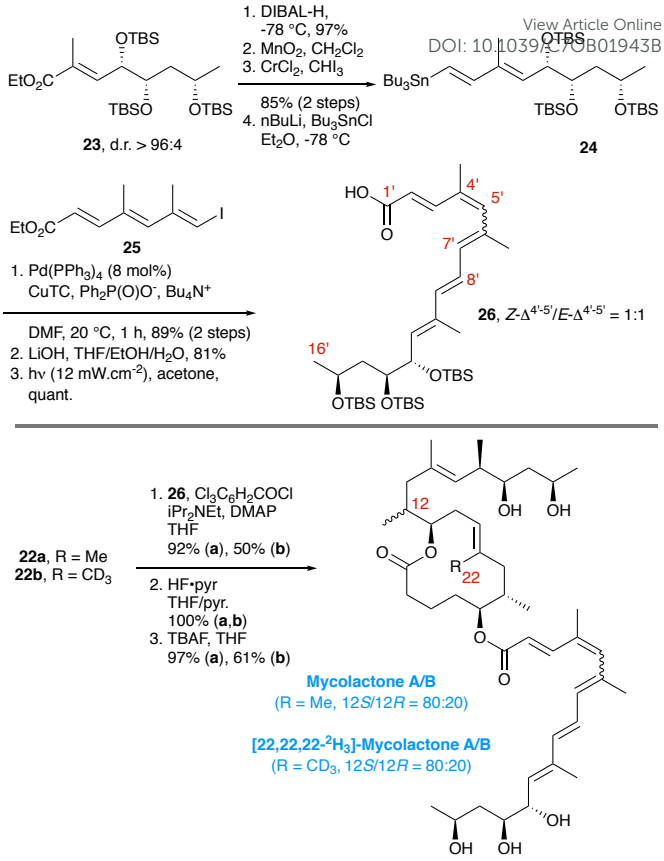


hydroboration of alkenes, these conditions were pioneered by Brown in 1985[16] and have found applications in a few total syntheses.[17]

Scheme 3. C1C20-Fragments syntheses via hydroboration/Suzuki Cross-Coupling.

Under optimized conditions, the hydroboration/Suzuki cascade proceeded smoothly in 60%, delivering the C1C20 fragment **20** (as a 12*S*/12*R* = 80:20 mixture of diastereomers). A late-stage methylation of the C8-position was finally required; this task in a quite complex setting was met by palladium-catalyzed Negishi cross-couplings with dimethylzinc or d_6-dimethylzinc in THF at 90 °C, leading to **21a** and **21b**. Final deprotections of the C5-silyloxy groups under acidic conditions delivered the complete C1C20 fragments **22a** (63%) and **22b** (55%) both as a unseparable 12*S*/12*R* = 80:20 mixture of diastereomers.

Having in hand the complete C1-C20 fragments of the natural mycolactone A/B and of its [22,22,22-$^2H_3$]-isotopologue, we turned our attention to the southern C1'-C16'-fragment **26** with the goal of improving our first-generation synthesis[9a,b] of this crucial sector of the natural product. Indeed, screening of catalytic systems for the key Stille cross-coupling between iodo-trienoate **25** and dienyl stannane **24** pointed to the Fürstner modification[18] ($Pd(PPh_3)_4$, copper(I) thiophene carboxylate (CuTC)[19] and $Bu_4N(Ph_2P(O)O^-$ as a tin salts scavenger[20] in DMF). An excellent 89% yield was obtained for the last two steps, namely formation and cross-coupling of dienylstannane **24**. Hydrolysis of the ester followed by visible-light photoisomerization of the conjugated pentaenyl system led to



the desired C1'-C16' protected fragment **26** as a *Z*-$\Delta^{4',5'}$/*E*-$\Delta^{4',5'}$ = 1:1 mixture. The latter is also found in the natural exotoxin of *M. ulcerans* due to the severe allylic strain[1,21] imposed by the substitution pattern of the C4'C5'-π-system.

Scheme 4. Synthesis of the C1'C16' fragment **26** and completion of the syntheses of mycolactone A/B and of its [22,22,22-$^2H_3$]-isotopologue.

Completion of mycolactone A/B and [22,22,22-$^2H_3$]-mycolactone A/B syntheses was readily accomplished by esterification under Yamaguchi conditions of **22a,b** with **26**, followed by stepwise deprotections of the orthogonal silyl ethers as already reported by Altmann and co-workers.[8i]

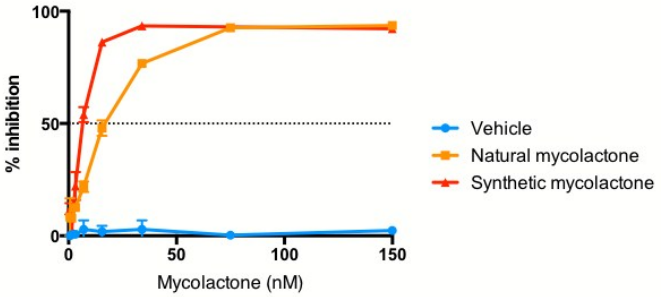


Figure 1. Comparative immunosuppressive activities of synthetic (as a 12*S*/12*R* = 80:20 mixture of diastereomers) and natural mycolactone A/B as evaluated by inhibition of IL-2 production by activated human T cells.

The ability of this synthetic mycolactone (as a 12*S*/12*R* = 80:20 mixture of diastereomers) to inhibit the activation-induced

production of IL-2 by Jurkat cells was measured and compared to results obtained with the toxin isolated from the culture of *M. ulcerans* (Figure 1). In this T cell model, the natural mycolactone dose-dependently suppressed the production of IL-2 upon stimulation using phorbol 12-myristate 13-acetate (PMA) and calcimycin with a half maximal inhibitory concentration (IC_{50}) of 17 nM. The synthetic mycolactone had a comparable dose-dependent suppressive effect with an IC_{50} of 6,7 nM.[12]

In conclusion, we have reported a total synthesis of mycolactone A/B as a 12*S*/12*R* mixture of diastereomers that ultimately proved inconsequential on the biological activity. Indeed, the ability of this synthetic mycolactone A/B to inhibit T-cell interleukin-2 production is indistinguishable from the one observed with the toxin isolated from the culture of *M. ulcerans*. In addition, the flexible synthetic blueprint also allowed the synthesis of [22,22,22-$^2H_3$]-mycolactone A/B as a potential internal standard for isotope dilution mass spectrometry. Considering the translational potential of mycolactone A/B, this work opens new perspectives for the late-stage modification advanced mycolactones intermediates, especially in the macrolactonic core. Advances toward specific probes based on mycolactone A/B chemical structure and able to decipher the biology of this complex disease are underway and will be reported in due course.

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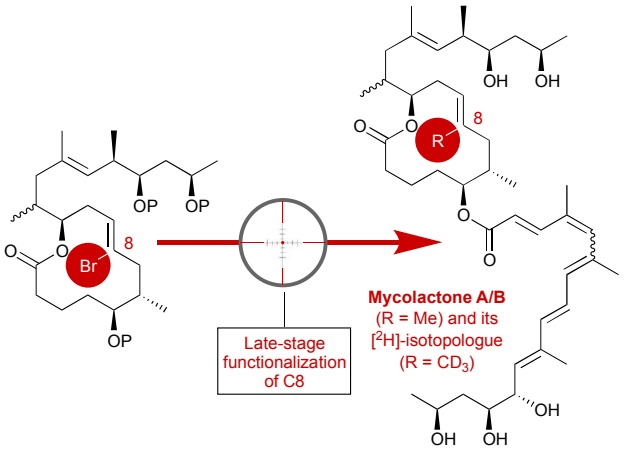

A new synthetic blueprint of mycolactone A/B is reported, granting access to the natural product and its [^{2}H]–isotopologue.

[a] Université de Strasbourg, CNRS, Laboratoire de Chimie Moléculaire UMR 7509, 67000 Strasbourg, France. E-mail : n.blanchard@unistra.fr
[b] Université de Haute-Alsace, Laboratoire de Chimie Organique et Bioorganique EA 4566, 3bis rue Werner, 68093 Mulhouse Cedex, France.
[c] Institut Pasteur, Unité d'Immunobiologie de l'Infection, INSERM U1221, 25 Rue Du Dr Roux, 75724 Paris Cedex 15, France.
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