Synthetic Studies on Mycolactones: Synthesis of the Mycolactone Core Structure through Ring-Closing Olefin Metathesis

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Abstract: The mycolactone core structure **2a** has been prepared through ring-closing olefin metathesis from diene **3** with exquisite *E* selectivity. The preparation of diene **3** included a highly efficient stereoselective synthesis of carboxylic acid **5**. The mycolactone core structure **2a** may serve as a versatile intermediate for the synthesis of mycolactone and analogues thereof.

Key words: Buruli, mycolactone, ring-closing olefin metathesis, natural products, stereoselectivity, total synthesis

Mycolactones A and B [**1** (Figure 1); 'mycolactone'[2]] are secondary metabolites of polyketide origin, and are believed to be the toxic agents responsible for the pathogenic effects of *Mycobacterium ulcerans*.[3] Infection with *M. ulcerans* leads to Buruli ulcer, a disfiguring disease associated with tissue destruction and suppression of the immune system that affects large parts of tropical and subtropical regions of Africa, Asia, Latin America and the Western Pacific. Mycolactone has been shown to possess cytotoxic, analgesic and immunosuppressive activity[4–6] and intradermal inoculation of the purified toxin in guinea pigs leads to lesions similar to those of Buruli ulcer in humans. Treatment of Buruli ulcer with antibiotics has been unsuccessful so far and the only treatment option currently available consists in surgery (to remove the lesion) followed by a skin graft if necessary.

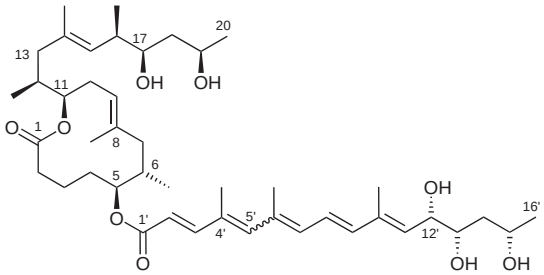

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Figure 1

The total synthesis of mycolactones A, B, and C has been reported by Kishi and co-workers in a series of three publications[7–9] and is based on the construction of the macrocyclic core structure through macrolactonization of an appropriately protected seco acid comprising the C1−C20 fragment. No other syntheses of mycolactone have yet been described in the literature, although several reports have addressed the synthesis of the unsaturated fatty acid portion attached to the macrolactone ring via an ester bond (C1′−C16′).[10–12]

In spite of the biological importance of mycolactones, no systematic studies on structure–activity relationships have been reported so far for this class of natural products.[9,13] Apart from elucidating the importance of individual structural elements for the pathogenic potential of the naturally produced toxins, such studies could conceivably lead to analogues with antibiotic activity against Buruli, e.g. by blocking specific steps in the biosynthesis of mycolactones. Based on these considerations, we have embarked on the development of a new, flexible route to mycolactones that would enable the incorporation of various structural modifications, in particular as part of the macrolactone core or the C12−C20 side chain. In this paper we would like to report on the successful construction of the C13-functionalized macrolactone core structure of mycolactones (C1−C13) based on ring-closing olefin metathesis (RCM) and some preliminary work on the elaboration of the entire C12−C20 side chain on this intermediate. The disclosure of these results at an intermediate stage of our work towards mycolactones and their analogues is prompted by a recent report by Alexander et al. on the synthesis of the mycolactone core with a pendant 4-ketopent-2-yl moiety on C11.[14] As in our case, ring closure was achieved through RCM, but a distinctly different route was followed for the construction of the requisite RCM precursor (including a completely different synthesis of carboxylic acid **5**).

At the most advanced level, our approach to mycolactones (as in Kishi's synthesis) foresees ester formation between an appropriately protected version of the C1−C20 core alcohol and the C1′−C16′ fatty acid side chain (or between independently modified versions of these building blocks) followed by protecting-group removal. The C1−C20 fragment should be accessible from macrocyclic precursors **2b** or **2a** (Scheme 1) through cross-metathesis with olefin **6** or transition-metal-catalyzed cross-coupling with vinyl iodide **7**,[7–9] respectively (or any other appropriate olefin or vinyl iodide, in the case of analogue synthesis). While **2b** could be directly derived from **2a**, a suitable precursor for the latter appeared to be the diene **3**, provided that macrocyclization of **3** through RCM would proceed with

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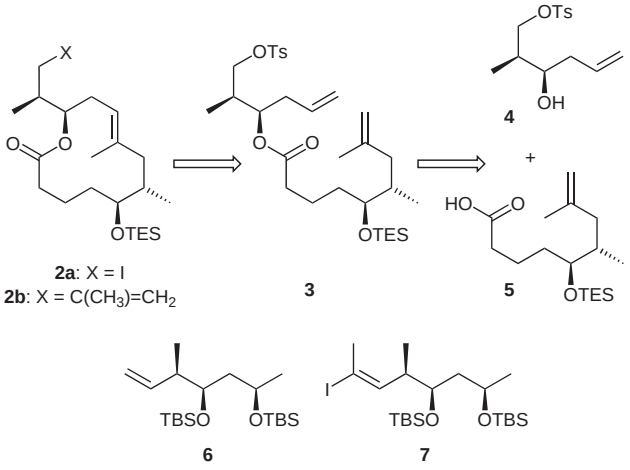
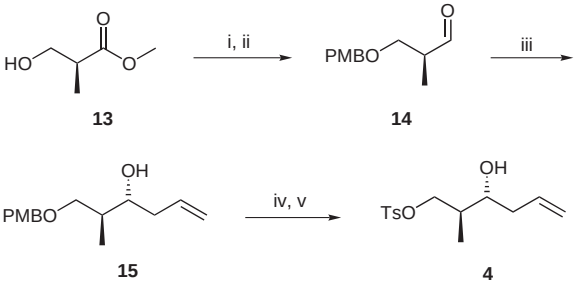


Scheme 1

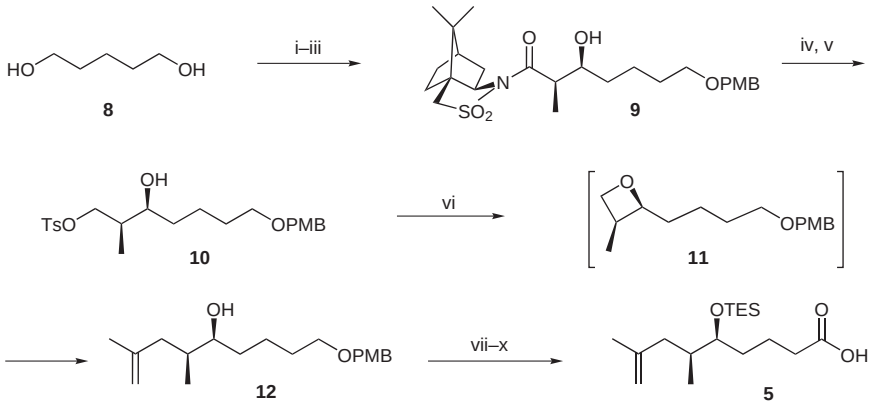
acceptable selectivity.[15] Although this was not clear at the outset of our work, we felt strongly attracted to this strategy, as it would provide very efficient access to the desired core structures **2a** or **2b**. Diene **3** would in turn be obtained through esterification of carboxylic acid **5** with alcohol **4**.

Scheme 2 summarizes the synthesis of carboxylic acid **5**, which starts from cheap 1,5-pentanediol **8**. Monoprotection of **8** as a *p*-methoxybenzyl (PMB) ether followed by Swern oxidation and subsequent aldol reaction of the resulting aldehyde with *N*-propionyl-(2*R*)-bornane-(10,2)-sultam[16] provided the aldol product **9** in excellent yield and with >95% diastereomeric excess. Reductive removal of the auxiliary and subsequent selective tosylation of the primary hydroxyl group gave tosylate **10**, which could be directly converted into olefin **12**, employing methodology that had been previously developed by Carreira and co-workers for phenyllithium reagents.[17] Thus, treatment of **10** with an excess of isopropenyllithium gave **12** in 61% yield, presumably through the intermediacy of oxetane **11**.[17] In contrast, attempts to prepare **12** through Cu-catalyzed coupling of the primary alkyl iodide derived from TES-protected **10** via Finkelstein exchange and isopropenylmagnesium bromide[18] proved to be unsuccessful. Olefin **12** was then elaborated into the desired building block **5** via TES protection of the secondary hydroxyl group, oxidative cleavage of the PMB ether with DDQ and finally conversion of the resulting primary alcohol into the carboxylic acid. The latter transformation was based on a two-step protocol involving (i) oxidation to the aldehyde with Dess–Martin periodinane (DMP)[19] followed by (ii) aldehyde oxidation with buffered $NaClO_2$ in the presence of 2-methyl-2-butene as chlorine scavenger.[20] Carboxylic acid **5** could thus be obtained from **8** in ten steps and 20% overall yield.



Scheme 3 *Reagents and conditions*: (i) PMB-trichloroacetimidate, TfOH, Et_2O, 0 °C, 2 h, 58%; (ii) DIBAL-H, toluene, −90 °C, 1.5 h, quant. (crude); (iii) $Bu_3SnCH_2CH=CH_2$, $SnCl_4$, CH_2Cl_2, −90 °C, 1 h, 82%, >95% de; (iv) DDQ, CH_2Cl_2, buffer pH 7.2, r.t., 1.75 h; (b) $LiAlH_4$, Et_2O, 0 °C → r.t., 1 h, 76%; (v) TsCl, Et_3N, DMAP, CH_2Cl_2, 35 °C, 5 h, 67%.

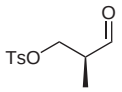
The synthesis of alcohol **4** proceeded via PMB-protected aldehyde **14,** which was readily obtained in two steps from (*S*)-Roche ester **13** (Scheme 3). Allylstannylation of **14** with allyltributyltin gave the desired *anti* product **15** in 82% yield and with excellent diastereoselectivity (>20:1).



Scheme 2 *Reagents and conditions*: (i) PMBCl, NaH, benzene, reflux, 6 h, 75%; (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, −78 °C → r.t., 1 h, 94%; (iii) (a) *N*-propionyl-(2*R*)-bornane-(10,2)-sultam, Et_2BOTf, CH_2Cl_2, −5 °C, 20 min; (b) addition of aldehyde, −78 °C, 1.25 h, 83%, >95% de; (iv) $LiAlH_4$, THF, 0 °C → r.t., 2.5 h, 77%; (v) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C → r.t., 5 h, 91%; (vi) (a) $LiC(CH_3)=CH_2$ (1.5 equiv), Et_2O, −20 °C → r.t., 45 min, r.t., 30 min; (b) $LiC(CH_3)=CH_2$ (3 equiv), $BF_3{\cdot}Et_2O$ (3 equiv), −78 °C, 25 min, 61%; (vii) TESOTf, 2,6-lutidine, CH_2Cl_2, −78 °C → r.t., 1 h, 98%; (viii) DDQ, CH_2Cl_2, buffer pH 7.2, r.t., 45 min, 92%; (ix) DMP, CH_2Cl_2, 0 °C → r.t., 2 h; (x) $NaClO_2$, 2-methyl-2-butene, *t*-BuOH, NaH_2PO_4, H_2O, r.t., 30 min, 85% (two steps).

Subsequent treatment of **15** with DDQ produced a mixture of the free diol and the corresponding *p*-methoxybenzoate ester as the major product. This mixture was not separated, but directly treated with $LiAlH_4$ to provide the desired free alcohol in 76% yield. Selective tosylation of the primary hydroxyl group then gave **4**. The tosyl group simultaneously serves as a protecting group (during ester formation) and as an activating group for nucleophilic substitution reactions at C13 subsequent to macrocycle formation. The incorporation of this group prior to RCM obviates the need for a deprotection/activation sequence at a more advanced stage of the synthesis, which we felt could be more problematic.

The stereochemical outcome of the allylstannylation reaction of **14** is in line with previous results reported by White and co-workers for the allylstannylation of the benzyl-protected variant of **14**.[21] Unfortunately, the direct allylstannylation of tosylated aldehyde **14a** (Figure 2; prepared from **13** by tosylation and DIBAL-H reduction in 74% yield[22]) gave only a 4:6 mixture of *anti* and *syn* isomers (79% chemical yield, including traces of residual Bu_3Sn-derivatives) and thus did not represent a viable alternative to the route depicted in Scheme 3.

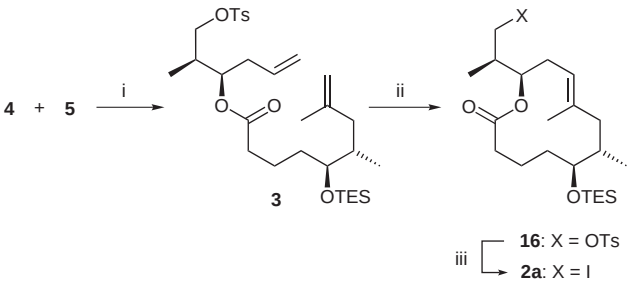


14a

Figure 2

A ca. 10:1 mixture of **4** and its (2*S*,3*S*)-*syn* isomer could be obtained after allylboration of **14a** with (+)-$Ipc_2B(CH_2CH{=}CH_2)$. However, this material was only obtained in 19% yield and after extensive chromatographic purification, thus significantly limiting the practicability of the allylboration approach.[23]

As depicted in Scheme 4, esterification of **4** with **5** under Höfle–Steglich conditions[24] proceeded smoothly to provide the RCM substrate diene **3** in 82% yield.



Scheme 4 *Reagents and conditions*: (i) DCC, DMAP, CH_2Cl_2, 0 °C → r.t., 15 h, 82%; (ii) Grubbs' II catalyst (8 mol%), CH_2Cl_2, reflux, 4 h, 72% (only *E*-isomer); (iii) NaI, acetone, r.t. → 65 °C, 6.5 h, 73%.

Gratifyingly, treatment of this diene with second-generation Grubbs catalyst[15] under reflux conditions in CH_2Cl_2 for 4 hours resulted in the efficient formation of the 12-membered ring, with *E*-isomer **16** being the only isolable product.[25] As indicated above, similar findings have recently been reported by Alexander et al. for a somewhat different substrate,[14] thus indicating that the *trans* preference of the RCM is not unique for a specific (exocyclic) C11-substituent. Conversion of tosylate **16** into iodide **2a** could be achieved in good yield, but reaction time proved to be a highly critical variable for the successful execution of this transformation. Thus, treatment of **16** with NaI in acetone at reflux for 24 hours (rather than ca. 6 hours) produced none of the desired **2a**, but only other unidentified reaction products. Preliminary attempts at the conversion of **2a** into **2b** {treatment of **2a** with $LiCu[C(CH_3){=}CH_2]_3$; Pd(0)-catalyzed Negishi coupling with $ClZnC(CH_3){=}CH_2$[26]} have been unsuccessful, with most of the starting **2a** being recovered unchanged.[27] However, other options for this transformation will be explored as will be the Negishi coupling of **2a** with vinyl iodide **7**. The results of these experiments will be reported in due course.

In summary, we have achieved a highly efficient stereoselective synthesis of functionalized macrolactones **16** and **2a**, which should be versatile precursors for the synthesis of natural mycolactones and structurally modified analogues thereof.

Acknowledgment

We are indebted to Dr. Bernhard Pfeiffer for help with the NMR experiments to determine the geometry of the C8–C9 double bond.

References and Notes

(1) Current Address: CARBOGEN AMCIS AG, 5502 Hunzenschwil, Switzerland.
(2) The term 'mycolactone' usually refers to the naturally occurring mixture of mycolactones A and B, which are geometric isomers at the C4′=C5′ double bond. Although both isomers can be isolated separately, each of them rapidly isomerizes to the mixture of mycolactones A and B.
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(23) The reaction of **14a** with (+)-$Ipc_2B(CH_2CH{=}CH_2)$ represents the mismatched reactant combination. The matched case [reaction of *ent*-**14a** with (+)-$Ipc_2B(CH_2CH{=}CH_2)$] has been reported by Fürstner and co-workers to produce the *syn* product in good yield and with excellent selectivity (see ref. 22).
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(25) **Preparation of 16**: To a solution of diene **3** (565 mg, 0.97 mmol) in CH_2Cl_2 (320 mL, 0.003 M) was added [1,3-bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene] dichloro(phenylmethylene)(tricyclohexylphosphine)Ru (Grubbs' II catalyst; 42 mg, 0.075 mmol) and the mixture was heated to reflux for 4 h (with additional 21 mg of catalyst being added after 2 h). After cooling to r.t., H_2O (50 mL) was added to the reaction mixture and a part of the solvent was removed under reduced pressure. The layers were separated and the aqueous solution was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic extracts were dried over $MgSO_4$ and the solvent was evaporated in vacuo. Purification of the residue by flash chromatography in EtOAc−hexane (1:10) gave **16** (391 mg, 72%) as a faintly yellow, viscous oil; $[\alpha]^{20}_D$ −44.0° (*c* = 0.61, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.79 (d, *J* = 8.3 Hz, 2 H), 7.35 (d, *J* = 8.0 Hz, 2 H), 4.85−4.97 (m, 2 H), 4.02−4.08 (m, 1 H), 3.80−3.86 (m, 1 H), 3.37−3.42 (m, 1 H), 2.46 (s, 3 H), 2.35−2.43 (m, 2 H), 2.00−2.11 (m, 2 H), 1.58−1.94 (m, 6 H), 1.65 (s, 3 H), 1.30−1.45 (m, 2 H), 0.90−0.99 (m, 15 H), 0.60 (q, *J* = 8.0 Hz, 6 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.2, 144.8, 138.1, 132.9, 129.8, 128.0, 120.4, 77.6, 72.5, 71.6, 45.3, 37.6, 35.6, 33.5, 33.3, 31.4, 21.7, 21.6, 18.7, 15.7, 13.4, 7.0, 5.1. IR (film): 2954, 2912, 2876, 1731, 1366, 1244, 1176, 1022, 969, 815, 672 cm^{-1}. HRMS (ESI, +ve): *m*/*z* $[M + Na]^+$ calcd for $C_{29}H_{48}O_6SSi$: 575.2833; found: 575.2827.
The assignment of the newly formed double bond as *E* was based on the absence of cross peaks between the C8-methyl group and C9-H in both NOESY and ROSY experiments. As expected for an *E* configured double bond between C8 and C9 a strong NOE cross peak was observed between C9-H and the 7-CH_2 moiety.
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(27) Encouraged by the efficiency and selectivity of ring closure observed with RCM substrate **3**, we have also investigated triene **17** as a possible substrate for RCM-mediated formation of the 12-membered ring (Figure 3). However, treatment of this compound with Grubbs' second-generation catalyst did not produce any of the desired 12-membered macrolactone. Instead, and perhaps not too surprisingly, the major product formed in the reaction appears to be cyclohexene **18** (based on MS analysis of the reaction mixture).

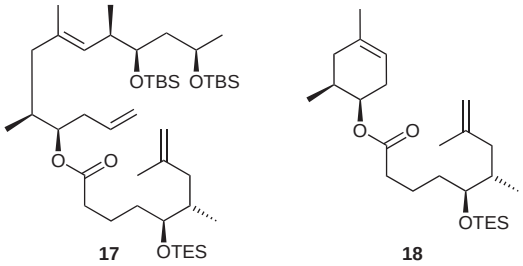

Figure 3