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Supplementary material to:

History, Biology and Chemistry of *Mycobacterium ulcerans* infection (Buruli ulcer disease)

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1. Total synthesis of Mycolactone C (2004)

Mycolactone C is a minor metabolite of the West African *M. ulcerans* strains and the major component of the Australian strains as discussed in Section 6.4. High resolution mass spectrometry experiments suggested that mycolactone C corresponded to a a deoxy-mycolactone A/B and based on LC-sequential mass spectrometry, Spencer further identified the C12'-position as the only possible deoxygenation site.[1,2] Following these hints, Kishi disclosed in 2004 the total synthesis of the four possible diastereomers of the C12'-deoxy mycolactone A/B and unambiguously demonstrated that mycolactone C corresponded to **SI-1a** (figure SI-1).[3]

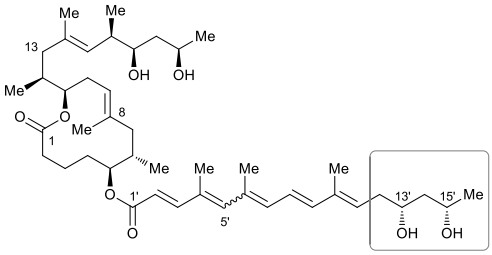
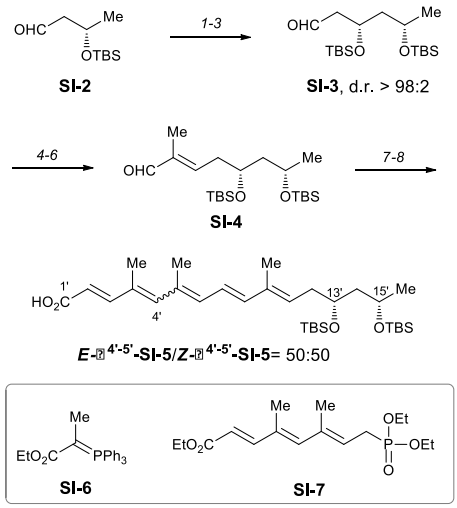


Figure SI-1. Mycolactone C and its three complementary diastereomers.

In a first step, comparison of thin layer chromatography mobilities and of ^{1}H NMR spectrums of the C1-C20 fragment of natural mycolactones A/B and C showed that they were identical. Due to the very limited amount of the natural toxin, it was postulated that the absolute configurations of the two C1-C20 fragments were also identical. Taking into account the similar UV spectrums of mycolactones A/B and C, indicative of a similar pentaneoic ester motif, Spencer's hypothesis was strengthened. Kishi then embarked into the synthesis of the four possible diastereomers of the C1'-C16' fragment, starting from both enantiomers of ethyl 3-hydroxybutyrate (Scheme SI-1). Using both enantiomers of Brown's allylborane, the allylation of aldehyde **SI-2** offered a set of four diastereo- and enantiomerically pure homoallylic alcohols. Further elaboration into the α,β-unsaturated aldehyde **SI-4** set the stage for the olefination reaction with known phosphonate **SI-7**. The resulting ester was saponified with lithine and the carboxylic acid esterified with the C1-C20 fragment, following the strategy adopted for the total synthesis of mycolactone A/B. The four possible diastereomers of mycolactone C were isolated as a *Z*-$\Delta^{4',5'}$:*E*-$\Delta^{4',5'}$ = 50:50 mixture.



Scheme SI-1. Synthesis of the C1'-C16' fragment of mycolactone C. *Reagents and conditions*: (1) (+)-Ipc$_2$BOMe, allylMgBr, Et$_2$O, -78 °C, 67%; (2) TBSCl, DMF, 20 °C, 97%; (3) O_3, CH_2Cl_2, -78 °C, PPh_3, 97%; (4) **SI-6**, toluene, 110 °C, 84%; (5) DIBAL-H, CH_2Cl_2, -78 °C, 89%; (6) Dess-Martin periodinane, CH_2Cl_2, 96%; (7) **SI-7**, LDA, THF, -78 to 20 °C, 94%; (8) LiOH, THF/H_2O/MeOH, 20 °C, 96%.

The crucial comparison with natural mycolactone C was then undertaken by comparison of the thin layer chromatography mobilities and ^{1}H NMR spectrums in an achiral solvent, allowing to easily attribute the C13',C15'-*syn* relationship. Unfortunately, the absolute configurations of the two C13',C15' stereocenters could not be determined using NMR analysis in chiral solvents, as was done with mycolactone A/B, due to the lack of sufficiently pure natural mycolactone C. Eventually, it was found that HPLC

was a reliable method to separate both $\Delta^{4',5'}$ isomers of **SI-1a** and **SI-1b**, with the faster eluting peak corresponding to the *Z*-$\Delta^{4',5'}$ *syn*-C13'*R*/C15'*S*-diol **SI-1a** clearly distinct from the *Z*-$\Delta^{4',5'}$ *syn*-C13'*S*/C15'*R*-diol **SI-1b**. Comparison with the HPLC profile of natural mycolactone C demonstrated that the *Z*-$\Delta^{4',5'}$ *syn*-C13'*R*/C15'*S*-diol **SI-1a** was the correct structure of the toxin, validating the hypothesis of a deoxy-mycolactone A/B formulated by Spencer and further establishing its absolute configuration.

2. Total synthesis of mycolactone E (2008)

Mycolactone E was isolated from a *M. ulcerans*-like mycobacteria originally called *Mycobacterium liflandii* that infected a laboratory colony of the West African clawed frog *Xenopus tropicalis* (see Section 6.4). Based on tandem mass spectrometry measurements, Leadlay proposed the structure **SI-8** that differs from natural mycolactone A/B by the length of the fatty acid side chain and the number of unsaturations and hydroxyl groups (figure SI-2).[4] The complete structure of mycolactone E was reported in 2008 by Kishi through a very elegant combination of total synthesis, NMR analysis and HPLC measurements.[5] From the synthetic perspective, the two postulated structures of mycolactone E were synthesized by esterification of the C1-C20 fragment with the two appropriate fatty acid side chains **SI-9a** and **SI-9b**, that differs only by the absolute configurations of the C11' and C13' stereocenters.

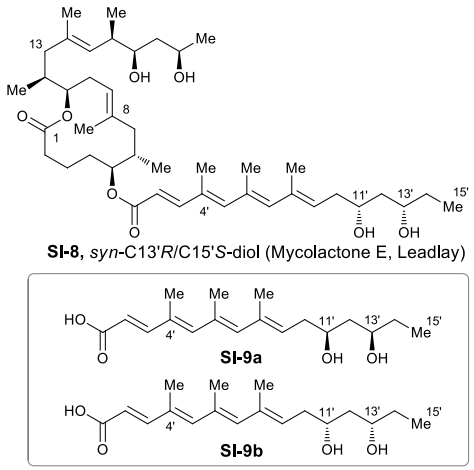
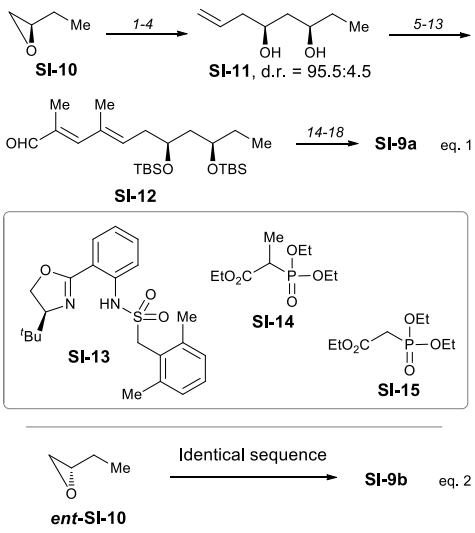


Figure SI-2. Mycolactone E and two diastereomeric C1'-C15' fragments of mycolactone E.

SI-9a,b were prepared from both enantiomers of the chiral epoxide **SI-10** that was first opened with a vinylcopper reagent followed by protection of the alkoxide as a trimethylsilyl ether (Scheme SI-2, eqs. 1 and 2). Oxidative cleavage of the terminal alkene and chromium-mediated asymmetric allylation of the intermediate aldehyde delivered **SI-11** as a single diastereomer in moderate yield. Classical functional group transformations further processed **SI-11** to the complete southern fragment of mycolactone E that was esterified with the C1-C20 fragment. Upon global deprotection, the two possible diastereomers of mycolactone E were obtained in good yields.

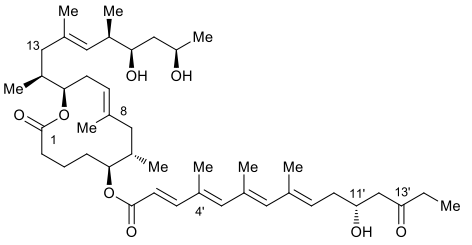


Scheme SI-2. Synthesis of both diastereomers of the mycolactone E C1'-C16' fragment. *Reagents and conditions:* (1) vinylMgBr, CuI, THF, Et_2O, -20 °C, then *i*-Pr_2NEt, TMSCl; (2) OsO_4 (2.5 mol%), NMO, CH_2Cl_2, -20 °C; (3) $Pb(OAc)_4$, benzene, 20 °C, 67% (3 steps); (4) allylbromide, $CrBr_3$ (10 mol%), **SI-13** (11 mol%), Mn, Et_3N, 2,6-lutidine, Cp_2ZrCl_2, THF, 0 °C, then HCl (0.5 N in H_2O), 55%, d.r. = 95.5:4.5; (5) TBSCl, imidazole, DMF, 20 °C, 86%; (6) OsO_4 (2.5 mol%), NMO, CH_2Cl_2, -20 °C; (7) $Pb(OAc)_4$, benzene, 20 °C, 93% (2 steps); (8) *n*-BuLi, **SI-14**, LiBr, MeCN, 0 °C, 95%; (9) DIBAL-H, CH_2Cl_2, -78 °C, 89%; (10) MnO_2, CH_2Cl_2, 20 °C, 94%; (11) *n*-BuLi, **SI-14**, LiBr, MeCN, 0 °C, 96%; (12) DIBAL-H, CH_2Cl_2, -78 °C, 74%; (13) MnO_2, CH_2Cl_2, 20 °C, 96%; (14) *n*-BuLi, **SI-14**, THF, 0 to 20 °C, 95%; (15) DIBAL-H, CH_2Cl_2, -78 °C, 69%; (16) MnO_2, CH_2Cl_2, 20 °C, 96%; (17) *n*-BuLi, **SI-15**, THF, 0 to 20 °C, 87%; (18) LiOH, THF/H_2O/MeOH, 96%.

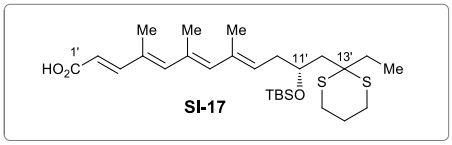
One of the main issues associated with the structural determination of the minor metabolites of *M. ulcerans* is the very limited amount of material extracted from culture broths. In the case of mycolactone E, this amount was estimated to be sufficient for only ten injections in HPLC thus stressing the importance of reliable analytical methods. A first analysis by ^{1}H NMR revealed that natural mycolactone E was composed of three C4',C7'-geometrical isomers, (*E*-$\Delta^{4',5'}$,*E*-$\Delta^{6',7'}$), (*Z*-$\Delta^{4',5'}$,*E*-$\Delta^{6',7'}$) and (*E*-$\Delta^{4',5'}$,*Z*-$\Delta^{6',7'}$) that was established by comparison with the ^{1}H NMR spectrum of photochemically equilibrated **SI-8**. Due to the similar NMR spectrums of **SI-8** and (C11',C13')-*epi*-**SI-8**, an HPLC method was devised to establish the absolute configuration of natural mycolactone E. Using a chiral phase for the HPLC analysis, photochemically equilibrated **SI-8** and (C11',C13')-*epi*-**SI-8** gave three peaks each that could be differentiated. Final comparison with natural mycolactone E followed by co-injections experiments unambiguously established the structure of the natural toxin as **SI-8**.

During isolation studies of mycolactone E, both the laboratories of Small and Leadlay noticed the presence of a minor metabolite whose MS/MS analysis suggested that the southern fragment consisted of a C11',C13'-hydroxy-ketone motif instead of the C11',C13'-diol motif of mycolactone E (figure SI-3).[4,6] To firmly establish the structure of this minor metabolite of *M.*

liflandii, Kishi reported a straightforward total synthesis in 2010 using the synthetic strategy of mycolactone A/B, namely the esterification of the C1-C20 fragment with the appropriate tetraenoic acid **SI-17**.[7]



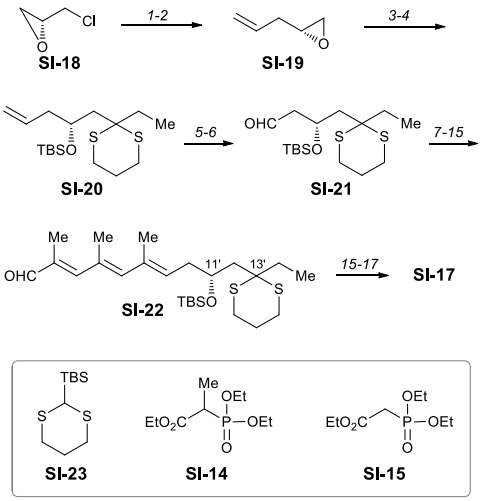
SI-16 (Minor metabolite of mycolactone E)



SI-17

Figure SI-3. Structure of mycolactone E minor metabolite.

Synthesis of the latter started with the copper(I)-catalyzed vinylation reaction of (-)-epichlorhydrin **SI-18**, followed by an intramolecular S_N2 reaction in basic media, delivering epoxide **SI-19** in excellent yield (Scheme SI-3). An anion-relay-chemistry was then used to introduce a masked form of the C13'-ketone via the ring opening of **SI-19** by a 2-lithio-2-silyl-1,3-dithiane.[8] Addition of HMPA triggered a [1,4]-Brook rearrangement and the intermediate 2-lithio-1,3-dithiane was then trapped by ethyl iodide, leading to **SI-20** in good overall yield. An iterative approach was then used to elaborate the targeted tetraenoic acid **SI-17** with a perfectly controlled stereochemistry.

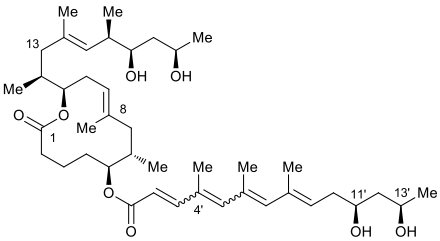


Scheme SI-3. Synthesis of the C1'-C15' fragment of mycolactone E minor metabolite. *Reagents and conditions:* (1) vinylMgBr, CuI (1 mol%), THF, Et_2O, -78 to -40 °C, 92%; (2) KOH, 120 °C, 91%; (3) ^{t}BuLi, **SI-23**, Et_2O, -78 °C; (4) EtI, HMPA, -78 to -20 °C, 64% (2 steps); (5) OsO_4 (5 mol%), $K_3Fe(CN)_6$, K_2CO_3, DABCO, $MeSO_2NH_2$, ^{t}BuOH, H_2O, 0 °C; (6) $Pb(OAc)_4$, benzene, 0 °C, 57% (2 steps); (7) *n*-BuLi, **SI-14**, LiBr, MeCN, 0 to 20 °C, 94%; (8) DIBAL-H, CH_2Cl_2, -78 °C, 85%; (9) MnO_2, CH_2Cl_2, 20 °C, 90%; (10) *n*-BuLi, **SI-14**, THF, 0 to 20 °C, 94%; (11) DIBAL-H, CH_2Cl_2, -78 °C, 99%; (12) MnO_2, CH_2Cl_2, 20 °C, 95%; (13) *n*-BuLi, **SI-14**, THF, 0 to 20 °C, 97%; (14) DIBAL-H, CH_2Cl_2, -78 °C, 99%; (15) MnO_2, CH_2Cl_2, 20 °C, 98%; (16) *n*-BuLi, **SI-15**, THF, 0 to 20 °C, 98%; (17) LiOH, THF/H_2O/MeOH, 99%.

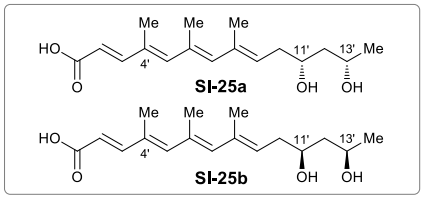
Esterification with the complete C1-C20 fragment and sequential deprotection of the dithiane and of the three silyl ethers led to **SI-16** in a moderate yield, the latter resulting from a difficult deprotection of the silyl ethers. More specifically, it was found that an anhydrous work-up that was developed for the halichondrin total synthesis was critical to the success of the deprotection.[9] In addition, issues with the scale were observed, the deprotection reaction being problematic over four milligrams of fully protected material. Nevertheless, this total synthesis allowed the preparation of a stereochemically homogeneous derivative **SI-16** that was photochemically equilibrated at 300 nm for two minutes, thereby leading to a *E*-$\Delta^{4',5'}$,*E*-$\Delta^{6',7'}$-**SI-16**/*Z*-$\Delta^{4',5'}$,*E*-$\Delta^{6',7'}$-**SI-16**/*E*-$\Delta^{4',5'}$,*Z*-$\Delta^{6',7'}$-**SI-16** = 1:1:1 mixture. Using a chiral HPLC approach similar to the mycolactone E case, Kishi established the structure of the minor metabolite of *M. liflandii* as **SI-16**.

3. Total synthesis of mycolactone F (2008)

Mycolactone F was isolated form the fish pathogen *Mycobacterium marinum* in 2006 (see section 6.4) and its structure was proposed to correspond to **SI-24** (Figure SI-4).[10] To confirm the gross structure proposed by Small and to establish the absolute stereochemistry, Kishi reported the total synthesis of the two possible diastereomers of mycolactone F, using the same synthetic blueprint as for mycolactone A/B.[11]



SI-24, *syn*-C11'*S*/C13'*R*-diol (Mycolactone F)



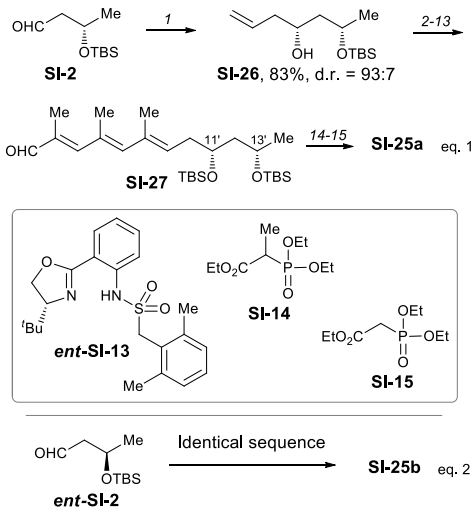
SI-25a

SI-25b

Figure SI-4. Mycolactone F structure and two diastereomeric C1'-C14' fragments of mycolactone F.

The preparation of the two southern fragments **SI-25a** and **SI-25b** relied on an iterative synthesis starting from both enantiomers **SI-2** and ***ent*-SI-2** and featuring a chromium-mediated, catalytic asymmetric allylation reaction to control the absolute stereochemistry of C11' (Scheme SI-4, eqs. 1 and 2). A

final esterification with the complete C1-C20 fragment followed by global deprotection delivered the two mycolactone F candidates.



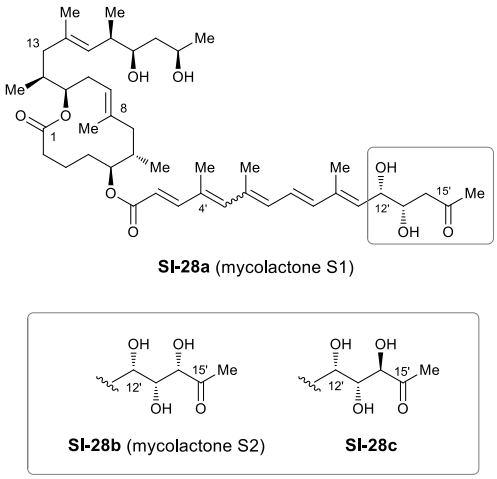
Scheme SI-4. Synthesis of both possible diastereomers of the C1'-C14' fragment of mycolactone F. *Reagents and conditions:* (1) allylbromide, $CrCl_3$•3THF (10 mol%), ***ent*-SI-13** (11 mol%), Mn, Et_3N, 2,6-lutidine, Cp_2ZrCl_2, THF, 0 °C, 83%, d.r. = 93:7; (2) TBAF, THF, 20 °C, 84% (separation of minor diastereomer); (3) TBSCl, imidazole, DMF, 20 °C, 100%; (4) O_3, CH_2Cl_2, -78 °C, then PPh_3, 83%; (5) *n*-BuLi, **SI-14**, LiBr, MeCN, 0 °C, 85%; (6) DIBAL-H, CH_2Cl_2, -78 °C, 92%; (7) MnO_2, CH_2Cl_2, 20 °C, 95%; (8) *n*-BuLi, **SI-14**, THF, 0 to 20 °C, 86%; (9) DIBAL-H, CH_2Cl_2, -78 °C, 78%; (10) MnO_2, CH_2Cl_2, 20 °C, 92%; (11) *n*-BuLi, **SI-14**, THF, 0 to 20 °C, 95%; (12) DIBAL-H, CH_2Cl_2, -78 °C, 40%; (13) MnO_2, CH_2Cl_2, 20 °C, 96%; (14) *n*-BuLi, **SI-15**, THF, 0 to 20 °C, 90%; (15) LiOH, THF/H_2O/MeOH, 89%.

As in the case of mycolactone C, only a tiny amount of the natural toxin was available for comparison with the synthetic derivatives **SI-24** and (C11',C13')-*epi*-**SI-24**, thus excluding a NMR comparison in achiral and chiral solvents. The HPLC strategy that was successfully used in mycolactone C endeavor was therefore exploited. Upon photochemical equilibration of **SI-24** and (C11',C13')-*epi*-**SI-24** and comparison of their HPLC profiles on a chiral phase with mycolactone F, it was established that diastereomer **SI-24** possessed the correct structure for the toxin produced by *M. marinum* (it should be noted here that a minor proportion of (C11',C13')-*epi*-**SI-24** could be detected in the HPLC profile of natural mycolactone F). This result was quite unexpected as the stereochemistry of the southern fragment was opposite to all the other known mycolactones (A/B, C and E), thus pointing out striking stereochemical heterogeneity in the mycolactone family. Even more surprisingly, a detailed study of freshwater fish strain CC240299 (from Koi) and BB170200 (from a silver perch) unambiguously proved that the toxin infecting freshwater fish was mycolactone *dia*-F, the remote diastereomer of mycolactone F **SI-24** infecting saltwater fish.

4. Total synthesis of mycolactones S1 and S2 (2012)

Mycolatones S1 and S2 were isolated from culture agar of *Mycobacterium ulcerans* subsp. *Shinshuense*, a strain that was isolated from cutaneous ulcer lesion in a Japanese patient (see Section 6.4). By using high-resolution mass spectroscopy and MS/MS analysis, Kishi proposed a C15'-keto derivative of mycolactone A/B **SI-28a** as the best candidate for the structure of mycolactone S1, and C14'-hydroxy,C15'-keto derivatives **SI-28b** and **SI-28c** as possible structures for mycolactone S2 (figure SI-5).[12] To confirm these structures, Kishi reported the total syntheses of the three potential mycolactones **SI-28a**, **SI-28b** and **SI-28c** using the same synthetic strategy as for mycolactone A/B.

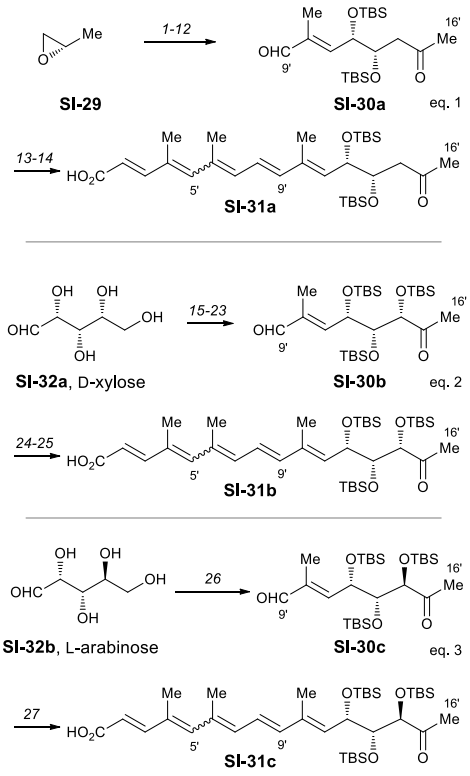
Figure SI-5. Structure of mycolactones S1 and S2.



The synthesis of these three potential new mycolactones was based on the esterification of the corresponding C1'-C16' fatty acid side chains with the C5-hydroxy group of the C1-C20 fragment, as in all the other total syntheses of the mycolactones (Scheme SI-5, eqs. 1 to 3).

The synthesis of mycolactone S1 southern fragment started by the ring-opening reaction of chiral epoxyde **SI-29** using a vinylcopper reagent (eq. 1). A four step sequence then delivered an α,β-unsaturated ester that was submitted to a diastereoselective dihydroxylation reaction using AD mix-α (d.r. = 8:1), that forged the C12' and C13' stereocenters. Further classical transformations led to the key aldehyde intermediate **SI-30a**. The latter was engaged in a Horner-Wadsworth-Emmons olefination reaction using the lithium anion of SI-7 that delivered the C1'-C16' fragment as a C1-methyl ester. A final saponification offered the C14'-dehydroxylated polyunsaturated acid **SI-31a**.

For the two mycolactone S2 candidates (eq. 2 and 3), the strategy relied on the use of the chiral pool to construct the C12'-C14' stereogenic centers. Fragments **SI-30b** and **SI-30c** were respectively synthesized from D-xylose **SI-32a** and L-arabinose **SI-32b** *via* a coupling reaction with ethyl bromopropionate leading to a *E*-$\Delta^{10',11'}$:*Z*-$\Delta^{10',11'}$ = 75:25 mixture of the corresponding α,β-unsaturated esters. Classical functional transformations, selective deprotection, methylation of the C15' terminal ketone and oxidation of the primary alcohol furnished the desired aldehydes **SI-30b** and **SI-30c**.



Scheme SI-5. Synthesis of the three possible diastereomers of the C1'-C16' fragments of mycolactones S1 and S2. *Reagents and conditions*: (1) vinylMgBr, CuI (10 mol%), Et_2O, -20 °C; (2) MPMBr, NaH, THF/DMF, 0 to 20 °C, 87% (2 steps); (3) OsO_4 (1 mol%), $NaIO_4$, 2,6-lutidine, dioxane, H_2O, 20 °C; (4) **SI-36** (methyl ester), toluene, 80 °C, 70% (2 steps); (5) AD mix-α, $MeSO_2NH_2$, dioxane, H_2O, 0 °C, d.r. = 8:1; (6) TBSCl, $AgNO_3$, pyridine, DMF, 20 °C, 87% (2 steps); (7) DIBAL-H, CH_2Cl_2, -78 °C, 80% (separation of minor isomer); (8) SO_3•pyridine, *i*-Pr_2NEt, DMSO, CH_2Cl_2, 20 °C, 95%; (9) **SI-6**, toluene, 90 °C; (10) DIBAL-H, CH_2Cl_2, -78 °C, 78% (2 steps); (11) DDQ, CH_2Cl_2, H_2O, 0 °C, 94%; (12) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 20 °C, 90%; (13) **SI-7** (methyl ester), LDA, THF, -78 to 20 °C; (14) LiOH, THF/H_2O/MeOH; (15) Ethyl 2-bromopropionate, Zn, PBu_3, 100 °C, 56%, *E*/*Z* = 75:25; (16) MMTrCl, pyridine, 0 °C, 70% (separation of isomers); (17) TBSCl, $AgNO_3$, pyridine, DMF, 20 °C, 100%; (18) DIBAL-H, CH_2Cl_2, -78 °C; (19) PivCl, pyridine, DMAP, CH_2Cl_2, 0 °C, 98% (2 steps); (20) HCO_2H, Et_2O, 20 °C, 88%; (21) SO_3•pyridine, *i*-Pr_2NEt, DMSO, CH_2Cl_2, 20 °C, 95%; (22) MeMgCl, Et_2O, 0 to 20 °C, 93%; (23) Dess-Martin periodinane, $NaHCO_3$, CH_2Cl_2, 20 °C, 84%; (24) **SI-7** (methyl ester), LDA, THF, -78 to 20 °C, 72%; (25) LiOH, THF/H_2O/MeOH, 69%; (26) repeat steps 15 to 23 with 31% overall yield; (27) repeat steps 24 and 25.

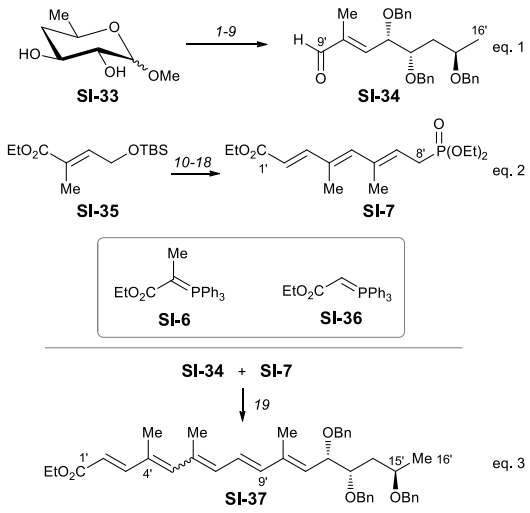
As mentioned above, these three fragments were processed to the complete mycolactone S1 and S2 candidates by esterification with the C5-hydroxy group of the C1-C20 fragment, followed by global deprotection under optimized conditions. Given the very minute amount of natural metabolites available, the synthetic mycolactones **SI-28a-c** were subjected to the HPLC strategy used for mycolactones E and F. Comparison of chiral and achiral HPLC profiles of these synthetic mycolactones with both natural metabolites allowed to clearly establish that mycolactones S1 and S2 corresponded respectively to **SI-28a** and **SI-28b**.

5. Partial syntheses

Several partial syntheses of mycolactone A/B were reported since early 2001. The southern fragment has historically draw the attention first with two synthetic approaches reported by Gurjar in 2001 followed by Feringa and Minnaard in 2005.[13,14] The attention next focused on the undecenolide motif of mycolactone with the RCM approaches, to elaborate the C8-C9 trisubstituted double bond.[15] These different synthetic blueprints are discussed in the following paragraphs.

It should be reminded here that in early 2001, the relative and absolute configuration of mycolactone A/B was not known and in this context, the work of Gurjar has to be considered as the first step towards the elucidation of the stereostructure of these complex toxins.[13] Gurjar's approach relied on the Horner-Wadsworth-Emmons reaction of phosphonate **SI-7** and aldehyde **SI-34** for the creation of the C8'-C9' π bond (Scheme SI-6). Since the relative and absolute configurations of the C12',C13',C15'-stereocluster were not known, Gurjar arbitrarily chose to start from **SI-33**, a readily available derivative from the chiral pool (eq. 1). The latter was converted into the desired aldehyde **SI-34** through successive hydrolysis of methyl glycoside bond and classical functional transformations. On the other hand, phosphonate **SI-7** was prepared thanks to an Arbuzov reaction of the corresponding primary bromide, the latter being synthesized from **SI-35** via an iterative method involving a reduction, oxidation and olefination sequence (eq. 2).

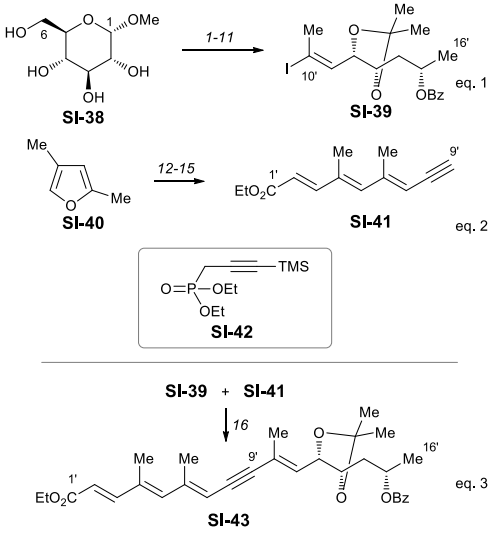
The key olefination reaction relied on phosphonate **SI-7** and delivered **SI-37** as a E-$\Delta^{4',5'}$:Z-$\Delta^{4',5'}$ = 2:3 mixture (eq. 3). No further comparisons with the natural toxins were made and therefore, this report should be considered as the first general synthetic sequence of the southern fragment of mycolactone even if no conclusions could be drawn on mycolactone southern fragment stereostructure. In this regard, Gurjar's work is cited by Kishi as a source of inspiration for the key C8'-C9' π bond disconnection of the fatty acid side chain of mycolactone A/B.



Scheme SI-6. Synthesis of the C1'-C16' fragment of mycolactone A/B by Gurjar. *Reagents and conditions*: (1) H_2SO_4, dioxane-H_2O, 100 °C; (2) $NaBH_4$, MeOH, 0 °C, 54% (2 steps); (3) TBSCl, imidazole, CH_2Cl_2, 20 °C; (4) BnBr, NaH, DMF, 20 °C; (5) TBAF, THF, 20 °C, 75% (3 steps); (6) $(COCl)_2$, DMSO, Et_3N, -78 to 20 °C; (7) **SI-6**, benzene, reflux, 80% (2 steps); (8) DIBAL-H, CH_2Cl_2, -78 °C, 94%; (9) MnO_2, $CHCl_3$, 20 °C; (10) DIBAL-H, CH_2Cl_2, -78 °C; (11) MnO_2, $CHCl_3$, 20 °C; (12) **SI-6**, benzene, reflux, 84% (3 steps); (13) DIBAL-H, CH_2Cl_2, -78 °C, 92%; (14) MnO_2, $CHCl_3$, 20 °C; (15) **SI-36**, benzene, reflux, 83% (2 steps); (16) TBAF, THF, 93%; (17) PBr_3, Et_2O, 0 °C; (18) $P(OEt)_3$, 90 °C, 64% (2 steps); (19) LDA, THF, -78 to 0 °C, 65%.

Another synthetic approach of the C1'-C16' fragment was disclosed by Feringa and Minnaard in 2005 (Scheme SI-7).[14] Several approaches were investigated by these authors with the aim of obtaining the fatty acid side chain but only the more relevant results will be described in this Report. The synthetic strategy relied on the formation of the C9'-C10' σ bond via a sonogashira coupling of the terminal alkyne **SI-41** with the vinyl iodide **SI-39**. The latter was synthesized from methyl-α-D-glucopyranoside **SI-38** by reduction of the 4- and 6-hydroxyl groups in two steps (eq. 1).[16] Protection of the diol as an acetonide, inversion of C15'-stereocenter via a Mitsunobu reaction and dithiane deprotection set the stage for the Corey-Fuchs homologation of the aldehyde into the corresponding terminal alkyne. Methylation of the latter and palladium-catalyzed hydrostannylation followed by iodolysis furnished the vinyl iodide intermediate **SI-39**.

Synthesis of the fragment **SI-41** was achieved in four steps from 2,4-dimethylfuran **SI-40,** using a rhodium-catalyzed reaction leading to a E-$\Delta^{4',5'}$:Z-$\Delta^{4',5'}$ = 72:28 mixture of the corresponding ketoester (eq. 2).[17] The desired (*E*)-isomer was isolated and subjected to a Horner–Wadsworth–Emmons olefination with phosphonate **SI-42** and subsequent TBAF deprotection provided **SI-41** as a E-$\Delta^{6',7'}$:Z-$\Delta^{6',7'}$ = 80:20 mixture. Sonogashira coupling of **SI-39** and **SI-41** led to the internal alkyne **SI-43** (eq. 3).

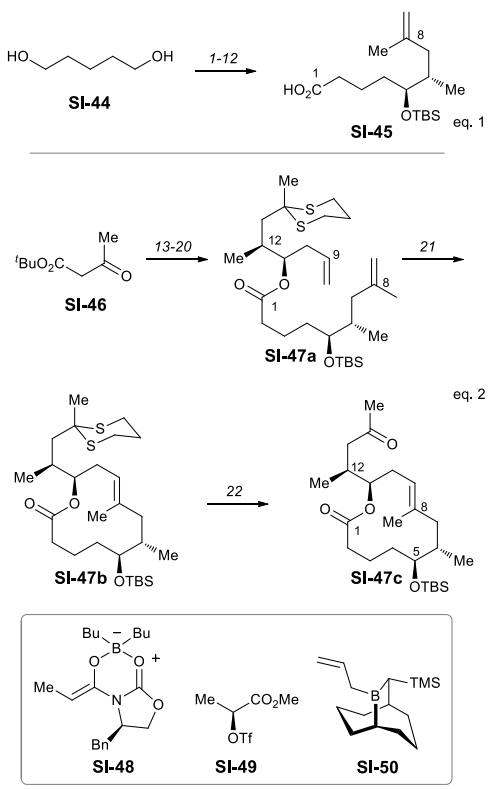


Scheme SI-7. Synthesis of the C1'-C16' fragment of mycolactone A/B by Feringa and Minnaard. *Reagents and conditions*: (1) SO_2Cl_2, pyridine, CH_2Cl_2, -78 to 50 °C, then NaI, MeOH/H_2O, 56%; (2) Bu_3SnH, AIBN, toluene, reflux, 89%; (3) 1,3-propanedithiol, HCl (37% in H_2O), 20 °C, 87%; (4) acetone, H_2SO_4, $CuSO_4$, 20 °C, 95%; (5) PPh_3, $PhCO_2H$, DEAD, THF, 20 °C, 82%; (6) MeI, 2,4,6-collidine, acetone/H_2O, reflux, 97%; (7) PPh_3, CBr_4, CH_2Cl_2, 0 to 20 °C, 72%; (8) LDA, THF, -78 °C, 88%; (9) LDA, HMPA, MeI, THF, -78 to -10 °C, 88%; (10) $PdCl_2(PPh_3)_2$ (5 mol%), Bu_3SnH, pentane, 63%; (11) I_2, CH_2Cl_2, -78 to 20 °C, 99%; (12) ethyl diazoacetate, $Rh_2(OAc)_4$ (0.4 mol%), CH_2Cl_2, 20 °C; (13) I_2 (cat.), CH_2Cl_2, 20 °C, 47% (2 steps); (14) **SI-42**, *n*-BuLi, THF, 0 to 20 °C, 61%; (15) TBAF, THF, EtOAc, 0 °C, 80%; (16) $Pd(PPh_3)_4$ (2 mol%), CuI (2 mol%), *i*-$PrNH_2$, 20 °C, 94%.

Several attempts to partially reduce alkyne **SI-43** into the corresponding (*E*)-alkene were carried out (H_2, Lindlard's catalyst; Zn, $Cu(OAc)_2$•H_2O, $AgNO_3$; H_2, Elsevier's catalyst; $Ni(OAc)_2$•$4H_2O$, hydrazine, $NaBH_4$, H_2) but none has shown sufficient selectivity to be useful on a preparative scale.

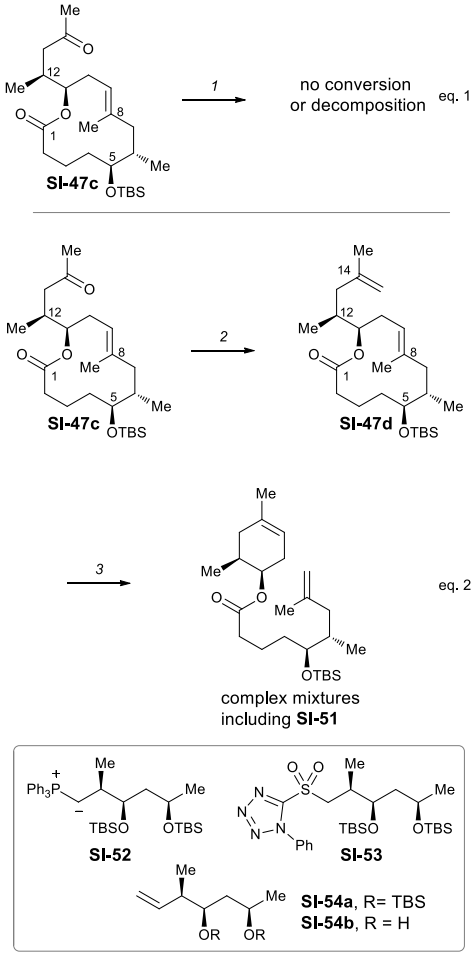
The last partial synthesis of mycolactone that is relevant to this section was reported by Burkart and collaborators who were the first to propose a RCM reaction for the creation of the trisubstituted C8-C9 olefin.[15a] In addition, the formation of the C14-C15 π bond relied on a Wittig, Julia-Kocienski or Julia-Lythgoe olefination reactions, a distinct feature of this strategy.

In a first approach, Burkart elaborated the C8-C9 unsaturation prior to the creation of the C13-C14 σ bond via the RCM of the corresponding dienyl ester **SI-47a** (Scheme SI-8).
The C1-C8 fragment **SI-45** was prepared in 12 steps from 1,5-pentanediol **SI-44** (eq. 1). The C5- and C6-stereogenic centers of this fragment were set up thanks to an Evans *syn*-aldolisation reaction (d.r.>98:2) followed by conversion of the acyloxazolidinone into the corresponding aldehyde. The latter was vinylated with isopropenylmagnesium bromide and the intermediate hydroxy group was acylated. The resulting allylic acetate was then reduced in the presence of palladium(0) and ammonium formate in an efficient sequence on a five millimole scale. However, as noticed later by the authors on a very closely related substrate, scale up of this transformation was not reproducible and required multiple runs on small scale. Final functional group transformations delivered the required C1-C8 portion **SI-45** of mycolactone A/B. Meanwhile, the C9-C13 fragment was prepared from *tert*-butyl acetoacetate **SI-46** in 7 steps (eq. 2), the C12-stereocenter being first controlled via a chiral alkylation by a 2-triflyloxy ester and the C11-stereocenter being set up *via* a totally diastereoselective Soderquist allylation reaction.[18]
Esterification of the C1-C8 and C9-C13 fragments led to the RCM precursor **SI-47a** that could be smoothly converted to the desired undecenolide **SI-47b** using 5 mol% of Grubbs-2 catalyst in refluxing dichloromethane. A single (*E*)-isomer was formed during this key reaction as proved by nOe experiments and X-ray diffraction studies of the corresponding ketone **SI-47c** obtained by deprotection of the dithiane motif.



Scheme SI-8. First approach to the mycolactone core by Burkart. *Reagents and conditions*: (1) TBDPSCl, imidazole, DMF, 20 °C, 86%; (2) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -70 °C, 85%; (3) **SI-48**, Bu_2BOTf, Et_3N, CH_2Cl_2, -78 to 0 °C, 95%; (4) TBSOTf, *i*-Pr_2NEt, CH_2Cl_2, 0 to 20 °C, 98%; (5) $LiBH_4$, MeOH, THF, 0 °C, 74%; (6) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 to 20 °C, 95%; (7) isopropenylmagnesium bromide, THF, 0 °C; (8) Ac_2O, DMAP, pyridine, 20 °C, 89% (2 steps); (9) $Pd(PPh_3)_4$ (5 mol%), PBu_3 (50 mol%), HCO_2NH_4, dioxane, reflux, 88% ; (10) NaOH (5 M in MeOH), MeOH, reflux, 96%; (11) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 to 20 °C, 97%; (12) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, ^{t}BuOH, H_2O, 20 °C, 88%; (13) NaH, THF, 0 °C; (14) **SI-49**, THF, 0 to 20 °C; (15) CF_3CO_2H, CH_2Cl_2, 20 °C, (see reference 18); (16) 1,3-propanedithiol, $BF_3{\cdot}OEt_2$, CH_2Cl_2, -10 °C, 96%; (17) $LiAlH_4$, THF, 0 to 20 °C, 98%; (18) TPAP (5 mol%), NMO, MS 4Å, CH_2Cl_2, 20 °C, 81%; (19) **SI-50**, Et_2O, -78 °C, 88%, d.r. = 98:2; (20) **SI-45**, DCC, DMAP, CSA (90 mol%), CH_2Cl_2, 95%; (21) **[Ru]-2** (5 mol%), CH_2Cl_2, (7 mM), reflux, 60%; (22) NCS, $AgNO_3$, CH_3CN, H_2O, 20 °C, 81%.

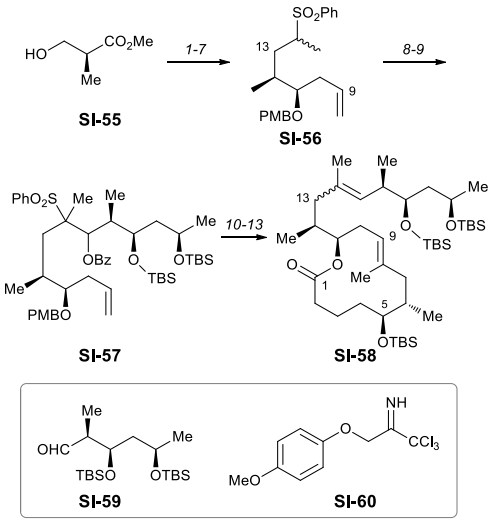
However, olefination reactions of ketone **SI-47c**, in order to elaborate the full C1-C20 portion of mycolactone A/B, proved impossible and required a revision of the synthetic strategy (Scheme SI-9, eq. 1). **SI-47c** was therefore transformed into the corresponding C14-*exo*-methylene derivative **SI-47d** that was submitted to various cross-metathesis reactions (eq. 2). Only cyclohexene **SI-51** was identified from a complex mixture, even in the presence of an excess of the C14-C20 alkenyl derivatives **SI-54a** or **SI-54b**.



Scheme SI-9. Unsuccessful strategies *en route* to the C1-C20 fragment of mycolactone A/B. *Reagents and conditions*: (1) **SI-52** or **SI-53**, base; (2) $Ph_3P{=}CH_2$, THF, 82%; (3) **SI-54a** or **SI-54b**, variations of metathesis catalysts, solvents and temperatures.

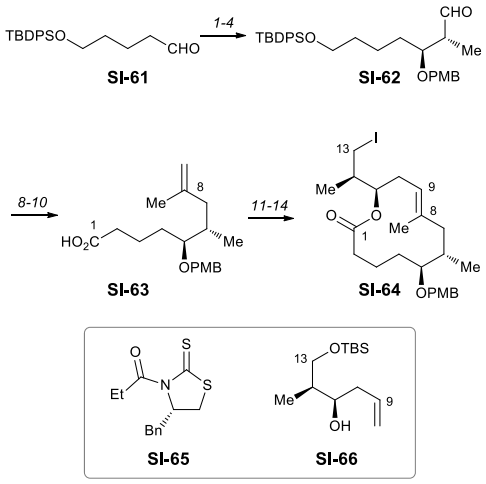
Later on, Burkart investigated the formation of the C8-C9 π bond on a partially elaborated C1-C20 fragment (Scheme SI-10).[15b] To avoid the formation of cyclohexenyl derivatives during the metathetic step, a precursor of the C13-C14 σ bond was introduced thanks to an interrupted Julia-Lythgoe olefination between sulfone **SI-56** and aldehyde **SI-59**. The sulfonyl derivative was prepared in seven steps from methyl (*S*)-(+)-3-hydroxy-2-methylpropionate **SI-55** using a diastereoselective Keck allylation reaction to control the C11-stereocenter. Condensation of the lithiosulfone, obtained by deprotonation of **SI-56**, with aldehyde **SI-59** followed by trapping of the lithium alkoxyde with benzoyl chloride led to a diastereomeric mixture of benzoyloxysulfones **SI-57** in 57% yield. The latter mixture was processed to ester, precursor of the RCM reaction. In the presence of 5 mol% of Grubbs-2 catalyst in refluxing dichloromethane for three days, a 64% yield of the desired undecenolide was obtained as a single (*E*)-isomer. Unfortunately, unravelling the C13-C14 olefinic motif could not performed selectively since a *E*-$\Delta^{13,14}$:*Z*-$\Delta^{13,14}$ = 2:1 mixture was obtained using sodium amalgam in

methanol, regardless of the diastereomeric composition of the precursor. Furthermore, the selective deprotection of the C5-silyloxy motif could not be achieved using a variety of deprotection conditions, thus compromising this synthetic approach.



Scheme SI-10. Second approach to complete C1-C20 fragment of mycolactone A/B. *Reagents and conditions*: (1) TBSCl, imidazole, DMF, 0 to 23 °C, 97%; (2) DIBAL-H-H, CH_2Cl_2, -78 °C, 90%; (3) allyltributyl-stannane, $SnCl_4$, THF, -78 °C, 80%, d.r.> 97.5:2.5; (4) **SI-60**, BF_3·OEt_2, cyclohexane, CH_2Cl_2, 0 °C, 73%; (5) TBAF, THF, 20 °C, 93%; (6) I_2, PPh_3, imidazole, toluene, 0 °C, 98%; (7) $PhSO_2Et$, *n*-BuLi, HMPA, THF, -78 to 20 °C, 95%; (8) *n*-BuLi, THF, -78 to -20 °C then **SI-59**, -78 to -20 °C; (9) BzCl, -78 to 20 °C, 57% (2 steps); (10) DDQ, CH_2Cl_2, H_2O, 20 °C, 95%; (11) **SI-45**, DCC, DMAP, CSA, CH_2Cl_2, 96%; (12) **[Ru]-2** (5 mol%), CH_2Cl_2, reflux, 64%; (13) Na/Hg, MeOH, -20 to 0 °C, 90%.

In a last revision of this RCM synthetic strategy, Burkart proposed to intercept one of Kishi's advanced intermediate, the C1-C20 fragment **SI-64**, therefore accomplishing a formal synthesis of mycolactone A/B (Scheme SI-11).[15b] The synthesis of the RCM precursor closely paralleled the 2006 route, the only modification being the use of a Crimmins aldolization reaction to control the C5,C6-stereocenters. Further functional groups transformation led to the RCM precursor that underwent cyclization to the desired macrolide as a single geometrical isomer using only 1 mol% of Grubbs-2 catalyst in refluxing dichloromethane for a day. A last C13-deprotection and iodation afforded compound **SI-64** whose identity was confirmed by comparison with the spectroscopic data reported by Kishi.



Scheme SI-11.Synthesis of C1-C13 fragment of mycolactone A/B. *Reagents and conditions*: (1) **SI-65**, $TiCl_4$, *i*-Pr_2NEt, CH_2Cl_2, -78 °C, 96%; (2) (MeO)MeNH•HCl, imidazole, CH_2Cl_2, 95%; (3) PMBBr, NaH, DMF, 20 °C, 89%; (4) DIBAL-H, toluene, -78 °C, 99%; (5) isopropenylmagnesium bromide, THF, -78 to 20 °C; (6) Ac_2O, DMAP, pyridine, 87%; (7) $Pd(PPh_3)_4$ (1 mol%), PBu_3 (10 mol%), HCO_2NH_4, dioxane, reflux, 87%; (8) TBAF, THF, 20 °C, 90%; (9) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 to 20 °C; (10) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, ^{t}BuOH, H_2O, 20 °C, 97%; (11) **SI-66**, DCC, DMAP, pyridine, CH_2Cl_2, 87%; (12) **[Ru]-2** (1 mol%), CH_2Cl_2, reflux, 60%; (13) TBAF, THF, 85%; (14) PPh_3, I_2, imidazole, toluene, 0 °C, 98%.

6. Conclusions

The seminal total syntheses of mycolactones C, E, F, *dia*-F, S1 and S2 by Kishi demonstrate once again the power of organic synthesis and the elegance that can be reached in its exercise. These studies have been central in structure determination, structure confirmation or in establishing unusual features such as the stereochemical heterogeneity of mycolactones F and *dia*-F. The partial syntheses of mycolactone A/B also validated synthetic strategies, such as ring-closing metathesis, that were used successfully in structure-activity relationship studies.

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