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Total Synthesis of the Potent HIF-1 Inhibitory Antitumor Natural Product, (8*R*)-Mycothiazole, via Baldwin–Lee CsF/CuI sp^3–sp^2-Stille Cross-Coupling. Confirmation of the Crews Reassignment

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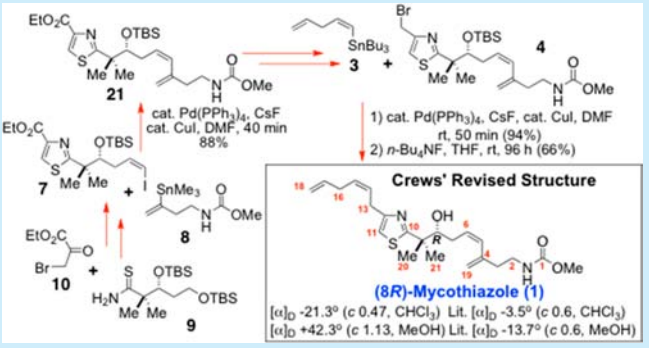
Supporting Information

ABSTRACT: A convenient asymmetric total synthesis of the potent HIF-1 inhibitory antitumor natural product, (−)- or (+)-(8*R*)-mycothiazole (**1**), is described. Not only does our synthesis confirm the 2006 structural reassignment made by Crews (Crews, P., et al. *J. Nat. Prod.* **2006**, *69*, 145), it revises the $[\alpha]_D$ data previously reported for this molecule in MeOH from −13.7° to +42.3°. The newly developed route to (8*R*)-**1** sets the C(8)–OH stereocenter via Sharpless AE/2,3-epoxy alcohol reductive ring opening and utilizes two Baldwin–Lee CsF/cat. CuI Stille cross-coupling reactions with vinylstannanes **8** and **3** to efficiently elaborate the C(1)–C(4) and C(14)–C(18) sectors.



(−)-(8*R*)-Mycothiazole (**1**) is a novel naturally occurring thiazolo-polyene first encountered by Crews and co-workers[1] in anthelmintic extracts of *Spongia mycofijiensis*, a sponge indigenous to coastal waters off Vanuatu island, west of Fiji. The precise chemical structure of (−)-mycothiazole has been the subject of much debate over the years, its most recent structural assignment[2] being stereoisomer **1** (Figure 1).

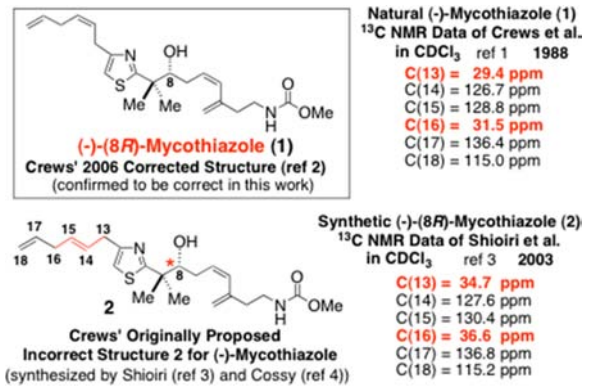


Figure 1. Crews' corrected (−)-mycothiazole structure **1** and his originally proposed structure **2** for the (8*R*)-enantiomer.

However, for more than two decades, (−)-mycothiazole had its structure incorrectly formulated as **2**, due to its complex polyolefinic network giving rise to a series of highly overlapped multiplets in the various ^{1}H NMR spectra that were recorded.[1] This led to an erroneous *J* value of 18 Hz being determined for H14/H15, which inevitably caused an (*E*)-olefin geometry to be incorrectly assigned to the C(14)–C(15)-alkene. Crews also never specified C(8) stereochemistry in his original 1988 structure **2**, which inevitably led to several teams attempting to do this by total synthesis of the individual enantiomers.[3−5]

The first asymmetric total synthesis of (−)-(8*R*)-**2** was accomplished by Shioiri and co-workers in 2003.[3] It stood out for the excellent stereocontrol it imparted to the setting of the target's stereodefined olefins and for its use of a Nagao asymmetric aldol reaction to efficiently control C(8)-hydroxy stereochemistry. Somewhat disconcertingly, however, the final $[\alpha]_D$ that was recorded for (−)-(8*R*)-**2** by Shioiri et al.[3] was −26.0° (*c* 0.64, $CHCl_3$), which differed substantially from the value that had originally been measured for natural (−)-mycothiazole by Crews et al.[1] ($[\alpha]_D$ −3.8° (*c* 2.9, $CHCl_3$)). Shioiri's ^{13}C NMR data[3] for (−)-(8*R*)-**2** in $CDCl_3$ also deviated significantly from the data reported for natural (−)-mycothiazole by Crews.[1] In particular, the ^{13}C chemical shifts for C(13) and C(16) in **2** were some 5 ppm further downfield[3] than those listed for the same carbons in the natural product (see Figure 1).[1] Despite the significant chemical differences that were observed, Shioiri and co-workers still continued to maintain that a confirmatory total synthesis of the natural product had been achieved,[3] attributing these discrepancies to impurities in Crews' original (−)-mycothiazole sample and to several resonance misassignments by Crews (see p S-9 of the Supporting Information of ref 4a).[4a] As a result, the Cossy group, who also reported a racemic total synthesis of **2** in 2005,[4] again incorrectly concluded[4] that they had achieved a

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total synthesis of natural mycothiazole, only to later find that this collective claim would be challenged by Crews[2] in 2006.

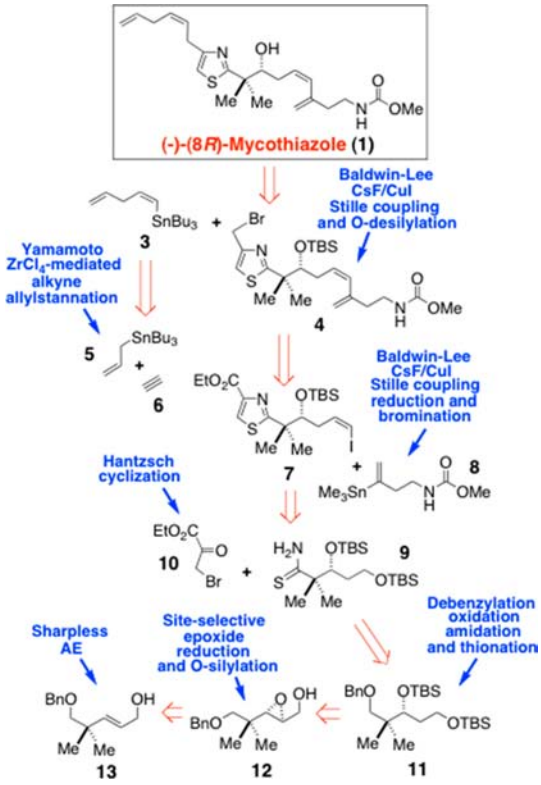
In this connection, and having noted the significant ^{13}C NMR differences that existed between natural mycothiazole and incorrectly formulated synthetic (−)-(8*R*)-**2**, Crews and his team eventually decided to revisit their original 1988 structural assignment, while attempting a second large-scale isolation of the natural product from *Dactylospongia* sp.[2] The detailed NMR analysis that followed used 600 MHz ^1H NMR NOE difference spectroscopy and spectral simulation to resolve the various spectral inconsistencies, and eventually produced a new structural formulation for (−)-mycothiazole, namely, (−)-(8*R*)-**1**, where the C(14)−C(15) alkene was now of (*Z*)- rather than (*E*)-geometry.[2] Despite this important advance, still, the issue of C(8) absolute stereochemistry remained unresolved, with Crews' new assignment resting solely on a tentative $[\alpha]_D$ comparison with Shioiri's (−)-(8*R*)-**2**, rather than on a definitive X-ray or Mosher ester NMR determination.

Crews' revision of the (−)-mycothiazole structure[2] and the biological reports that followed[6] have since helped fuel synthetic interest in (−)-(8*R*)-**1** as a target.[7] Yet, despite this, no group has so far achieved a full total synthesis of (−)-(8*R*)-**1**, notwithstanding significant progress having been made on the construction of various subregions.[7] Biologically, (−)-(8*R*)-mycothiazole (**1**) is a molecule of growing pharmacological importance[6] due to the powerful, yet selective, growth inhibitory effects it exerts against human tumor cell lines (IC_{50} values = 0.36−10 nM), and for its potent HIF-1 inhibitory effects within hypoxic T47D human breast carcinoma cells (IC_{50} = 1 nM). The discovery of these properties for **1** could be of considerable pharmaceutical worth, since **1** does appear to be a potentially tractable new drug lead from where to commence much future discovery effort. However, before drug development can begin in earnest, the issue of (−)-mycothiazole structure and stereochemistry has to be resolved beyond all doubt, and a reliable asymmetric total synthesis of (−)-(8*R*)-**1** must be developed. Herein, we now report success in the latter of these two endeavors.

In our retrosynthetic planning for (−)-(8*R*)-**1** (Scheme 1), the C(8)-hydroxy stereocenter of **1** would be set through a Sharpless asymmetric epoxidation on **13** allied with a C(2) site-selective reductive ring opening of the 2,3-epoxy alcohol **12** and protection. Compound **11** would thereafter be advanced toward thionoamide **9** and thiazole ring construction completed by a Hantzsch cyclization with the bromopyruvate **10**. Subsequent elaboration of the vinyl iodide **7** and Stille cross-coupling with Shioiri's vinylstannane **8**[3] would then provide a product whose ester could be reduced and brominated to yield bromide **4**. A second Stille coupling with the skipped dienylstannane **3** and an O-desilylation would then finalize the synthesis of (−)-(8*R*)-mycothiazole **1**. The known (*Z*)-vinylstannane **3**[8] would itself be accessed via Yamamoto's $ZrCl_4$-cod alkyne allylstannation procedure.[8]

With this in mind, chiral 2,3-epoxy alcohol **12** (Scheme 2) was prepared following Masamune's published procedure for the opposite enantiomer,[9] and its absolute configuration was unambiguously confirmed by $[\alpha]_D$ comparison with Masamune's published data.[9] Although the literature records a 92% ee for formation of the opposite enantiomer, our Mosher ester analysis of **12** revealed that it was virtually optically pure. Epoxy-alcohol **12** was then taken forward and reduced with REDAL in THF/PhMe at −40 °C. Diol **14** was formed almost exclusively and was isolated in 93% yield as an oil after SiO_2

Scheme 1. Our Retrosynthetic Analysis for (−)-(8*R*)-Mycothiazole 1



flash chromatography. It was then doubly O-silylated with TBSOTf (2.2 equiv) and 2,6-lutidine (4.4 equiv) in dry CH_2Cl_2. Although the TBSOTf addition was done at −78 °C, a prolonged reaction period at rt (2 h) was subsequently required to drive the process to completion, whereafter the di-O-silyl ether **11** was obtained in 93% yield. The benzyl ether was then cleaved from **11** by Pd/C catalyzed hydrogenolysis in EtOAc over 30 min. The product alcohol **15**, isolated in 92% yield, was thereafter submitted to a two-stage oxidation under cat. TPAP/NMO and Pinnick conditions. The resulting carboxylic acid **17** was then converted to the pentafluorophenyl ester with DCC/pentafluorophenol in CH_2Cl_2, and ammonolysis was performed with ammonia in THF to give the amide **18**. Amide **18** was subsequently converted into the thionoamide **9** by treatment with Lawesson's reagent in THF at 60 °C, and although this reaction did typically proceed in fair yield (58%), still, this did not markedly hamper overall material throughput.

Thiazole formation was accomplished by implementing either one of the following two Hantzsch protocols.[10] In our slightly lower yielding procedure (56%, 2 steps, one pot), **9** was treated with ethyl bromopyruvate **10** in THF for 2 h. This process also caused a partial O-desilylation of the primary OTBS group, a reaction that could be driven all the way through to completion by exposing the crude product to PPTS in MeOH at rt for 4 h. The great advantage of this protocol lay in its ability to be executed rapidly (6 h overall). However, the alternative, slower, $NaHCO_3$-mediated procedure also procured **19** in a slightly higher yield (61%, 3 steps), but it completed the key thiazole ring-forming step by treatment of the intermediary hydroxy-thiazoline with $NaHCO_3/(CF_3CO)_2O$,[10] prior to performing the PPTS/MeOH induced selective O-desilylation. With alcohol **19** in hand, the synthesis advanced with a Swern oxidation to **20** and a (*Z*)-selective Stork−Zhao Wittig

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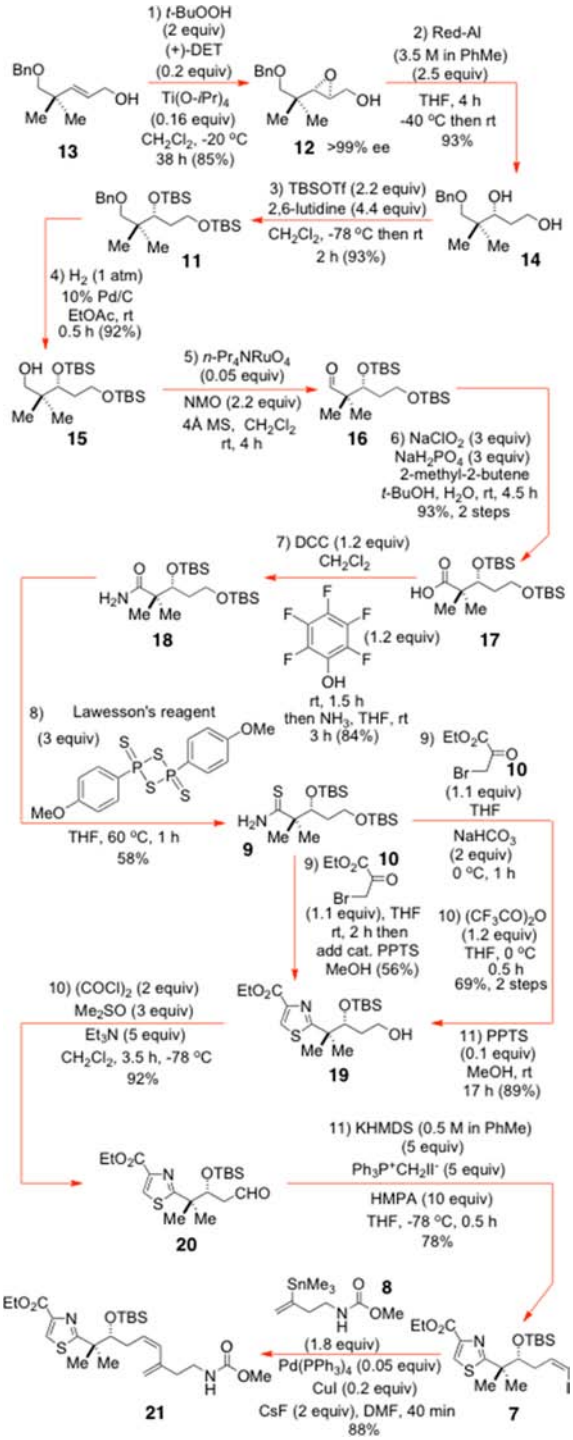
Scheme 2. Our Asymmetric Pathway to Thiazolo-Ester 21

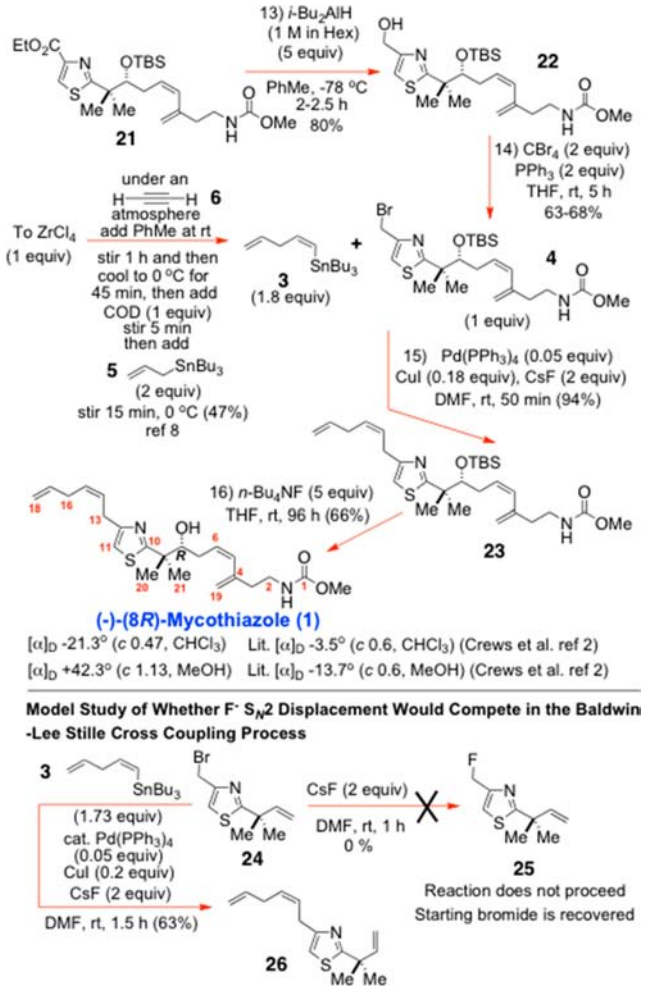


iodoolefination; the latter occurred with high stereocontrol, furnishing **7** as a single stereoisomer without causing damage to the ester in either **20** or **7**.

With the requisite iodoolefin **7** in hand, the C(1)−C(13) sector of (8*R*)-mycothiazole was elaborated by performing a Baldwin−Lee variant of the Stille cross-coupling[11] with known vinylstannane **8** (1.8 equiv), prepared according to Shioiri's method.[3] Importantly, this union worked very well indeed on a quite decent scale (0.65 g with respect to **7**), delivering the desired diene **21** in 88% yield after 40 min of stirring at rt. Significantly, as well, the aforementioned Baldwin−Lee cross-coupling proved to be fully compatible with the carboxyethyl and TBS substituents that were present in **7** and **21**.

Our synthesis progressed further (Scheme 3) with a DIBAL-H reduction of the ester in **21** (73−80% yield) and

Scheme 3. Completion of Our Total Synthesis (−)-(8*R*)-Mycothiazole (1) and Our Baldwin−Lee Model S_N2 Study



bromination of the product alcohol **22** with Ph_3P and CBr_4 in THF, which yielded the bromomethyl thiazole **4** in 63−68% yield. The requisite Stille cross-coupling partner, dienylstannane **3**, was itself stereoselectively prepared by application of Yamamoto's $ZrCl_4$/1,5-cyclooctadiene-promoted alkyne allylstannation addition reaction to acetylene **6**,[8] which afforded the desired *cis*-addition product **3** in 47−57% yield. Vinylstannane **3** efficiently coupled to **4** in 94% yield within 50 min at rt under Baldwin−Lee conditions,[11] without F^- displacement of the bromide compromising the overall success of the process. In the latter regard, prior model studies with the closely related bromomethylthiazole **24** had already shown that CsF (2 equiv) in DMF at rt for 1 h did not S_N2 displace the primary bromide to give **25** (Scheme 3). A subsequent Baldwin−Lee coupling between **24** and **3** was also shown to be viable, a 63% yield of **26** being furnished after 1.5 h at rt. In our view, this novel extension of the CsF/cat. CuI-accelerated Stille protocol to bromomethylthiazoles represents a most useful, and potentially general, advance for coupling activated sp^2-vinylstannanes with bromides of this sort. However, it is

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equally important for us to state that we were unsuccessful when we attempted to perform a similar sp^3–sp^3 cross-coupling between **24** and Me_4Sn under prolonged rt conditions!

The final step in our (8*R*)-mycothiazole synthesis was O-desilylation of **23** with *n*-Bu_4NF (5 equiv) in THF, which required 96 h to reach completion; it afforded **1** in 66% yield. Importantly, the spectral data for synthetic **1** now closely matched those originally reported by Crews' and co-workers in 1988,[1] so confirming his 2006 structural revision.[2]

Even so, the $[\alpha]_D$ value that we recorded for synthetic (−)-(8*R*)-mycothiazole (**1**) (−21.4°, *c* 0.47 $CHCl_3$) still differed substantially from the value reported by Crews et al. for natural material ($[\alpha]_D$ −3.5°, *c* 0.6 $CHCl_3$) in 2006 (see Scheme 3).[2] Even more surprisingly, as well, our synthetic sample of (−)-(8*R*)-mycothiazole (**1**) also gave rise to a large positive $[\alpha]_D$ of +42.3° in MeOH (*c* 1.13), which contrasted sharply with Crews' 2006 $[\alpha]_D$ report of −13.7° in MeOH (*c* 0.6).[2]

Our observations suggest that in a strongly hydrophobic solvent such as $CHCl_3$ strong internal H-bonding exists between the C(8)–OH and the thiazole ring N atom, which helps restrict conformational freedom within the molecule, leading to a substantial negative $[\alpha]_D$. In MeOH, however, this strong internal H-bonding is almost certainly disrupted. Such a significant conformational perturbation could account for the dramatic large positive $[\alpha]_D$ that is now observed for (8*R*)-mycothiazole in this solvent. Similar arguments probably apply to the seemingly anomalous positive $[\alpha]_D$ value that was observed for (−)-(3*R*)-inthomycin C by Taylor et al.[12] when their $[\alpha]_D$ was recorded in $CHCl_3$ in the presence of a small quantity (20%) of the H-bond-disrupter, tetramethylurea.[12]

In conclusion, an unambiguous total synthesis of (−)-(8*R*)-mycothiazole (**1**) has been accomplished that spectrally confirms Crews' recently revised structure for this natural product. Our $[\alpha]_D$ measurements for the (8*R*)-enantiomer of mycothiazole (**1**) have also revealed that it can exhibit both a large negative and a large positive $[\alpha]_D$, depending upon for which solvent the $[\alpha]_D$ is recorded. Quite clearly, our $[\alpha]_D$ data deviate significantly from the $[\alpha]_D$ values reported by Crews et al. on lower purity natural mycothiazole.[1,2] Given the lack of a good $[\alpha]_D$ correlation, our latest findings still do not allow a confident assignment of absolute stereochemistry to be made for natural mycothiazole which must, we believe, remain only tentatively assigned as (8*R*). While future biological testing of synthetic **1**, and its enantiomer, may allow a definitive assignment of absolute stereochemistry for the natural product, a substantial difference in potency will have to be observed for such a protocol to be credible.

As regards chemical highlights of the present synthesis, our seminal application of the Baldwin–Lee cat. CuI/CsF Stille process[11] to the activated bromomethylthiazole **4** constitutes an important new synthetic advance. Our synthesis has also underlined the great synthetic worth of the Yamamoto $ZrCl_4$-mediated *cis*-selective alkyne allylstannation reaction[8] and compliments Yamamoto's own elegant efforts at applying this reaction in natural product total synthesis.[8b] Finally, with the new synthetic route we have developed to (8*R*)-mycothiazole, the gross structure of **1** has been confirmed. Efficient medicinal chemistry exploitation of **1** should now prove possible.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01966.

Full experimental procedures, and copies of the ^1H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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