# LETTERS

# Total Synthesis of the Potent HIF-1 Inhibitory Antitumor Natural Product, (8*R*)-Mycothiazole, via Baldwin–Lee CsF/Cul sp<sup>3</sup>–sp<sup>2</sup>-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 (+)-(8R)-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  $-13.7^{\circ}$  to  $+42.3^{\circ}$ . The newly developed route to (8R)-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 vinyl-stannanes **8** and **3** to efficiently elaborate the C(1)–C(4) and C(14)–C(18) sectors.

(-)-(8R)-Mycothiazole (1) is a novel naturally occurring thiazolo-polyene first encountered by Crews and co-workers<sup>1</sup> 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<sup>2</sup> being stereoisomer 1 (Figure 1).



Figure 1. Crews' corrected (-)-mycothiazole structure 1 and his originally proposed structure 2 for the (8R)-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 <sup>1</sup>H NMR spectra that were recorded.<sup>1</sup> 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.<sup>3–5</sup>

The first asymmetric total synthesis of (-)-(8R)-2 was accomplished by Shioiri and co-workers in 2003.<sup>3</sup> 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  $[\alpha]_{\rm D}$  that was recorded for (-)-(8R)-2 by Shioiri et al.<sup>3</sup> was  $-26.0^{\circ}$  (c 0.64, CHCl<sub>3</sub>), which differed substantially from the value that had originally been measured for natural (-)-mycothiazole by Crews et al.<sup>1</sup> ( $[\alpha]_D$  -3.8° (c 2.9, CHCl<sub>3</sub>)). Shioiri's <sup>13</sup>C NMR data<sup>3</sup> for (-)-(8R)-2 in CDCl<sub>3</sub> also deviated significantly from the data reported for natural (-)-mycothiazole by Crews.<sup>1</sup> In particular, the <sup>13</sup>C chemical shifts for C(13) and C(16) in 2 were some 5 ppm further downfield<sup>3</sup> than those listed for the same carbons in the natural product (see Figure 1).<sup>1</sup> 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,<sup>3</sup> 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).<sup>4a</sup> As a result, the Cossy group, who also reported a racemic total synthesis of 2 in 2005,<sup>4</sup> again incorrectly concluded<sup>4</sup> that they had achieved a



Received: July 9, 2015

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 <sup>13</sup>C NMR differences that existed between natural mycothiazole and incorrectly formulated synthetic (-)-(8R)-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.<sup>2</sup> The detailed NMR analysis that followed used 600 MHz <sup>1</sup>H NMR NOE difference spectroscopy and spectral simulation to resolve the various spectral inconsistencies, and eventually produced a new structural formulation for (-)-mycothiazole, namely, (-)-(8R)-1, where the C(14)-C(15) alkene was now of (Z)rather than (E)-geometry.<sup>2</sup> 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 (-)-(8R)-2, rather than on a definitive X-ray or Mosher ester NMR determination.

Crews' revision of the (-)-mycothiazole structure<sup>2</sup> and the biological reports that followed<sup>6</sup> have since helped fuel synthetic interest in (-)-(8R)-1 as a target.<sup>7</sup> Yet, despite this, no group has so far achieved a full total synthesis of (-)-(8R)-1, notwithstanding significant progress having been made on the construction of various subregions.<sup>7</sup> Biologically, (-)-(8R)mycothiazole (1) is a molecule of growing pharmacological importance<sup>6</sup> due to the powerful, yet selective, growth inhibitory effects it exerts against human tumor cell lines  $(IC_{50} \text{ values} = 0.36-10 \text{ nM})$ , and for its potent HIF-1 inhibitory effects within hypoxic T47D human breast carcinoma cells (IC<sub>50</sub> = 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 (-)-(8R)-1 must be developed. Herein, we now report success in the latter of these two endeavors.

In our retrosynthetic planning for (-)-(8R)-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) siteselective 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 (-)-(8R)-mycothiazole 1. The known (Z)-vinylstannane  $3^8$  would itself be accessed via Yamamoto's ZrCl<sub>4</sub>-cod alkyne allylstannation procedure.<sup>8</sup>

With this in mind, chiral 2,3-epoxy alcohol 12 (Scheme 2) was prepared following Masamune's published procedure for the opposite enantiomer,<sup>9</sup> and its absolute configuration was unambiguously confirmed by  $[\alpha]_D$  comparison with Masamune's published data.<sup>9</sup> 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<sub>2</sub>

Scheme 1. Our Retrosynthetic Analysis for (-)-(8R)-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 CH2Cl2, 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.<sup>10</sup> 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<sub>3</sub>-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<sub>3</sub>/(CF<sub>3</sub>CO)<sub>2</sub>O,<sup>10</sup> 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





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<sup>11</sup> with known vinylstannane 8 (1.8 equiv), prepared according to Shioiri's method.<sup>3</sup> 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 (-)-(8R)-Mycothiazole (1) and Our Baldwin-Lee Model S<sub>N</sub>2 Study



 $\begin{array}{l} \label{eq:alpha} [\alpha]_D \ -21.3^{\circ} \ (c \ 0.47, \ CHCl_3) & \ Lit. \ [\alpha]_D \ -3.5^{\circ} \ (c \ 0.6, \ CHCl_3) \ (Crews \ et \ al. \ ref \ 2) \\ \ [\alpha]_D \ +42.3^{\circ} \ (c \ 1.13, \ MeOH) & \ Lit. \ [\alpha]_D \ -13.7^{\circ} \ (c \ 0.6, \ MeOH) \ (Crews \ et \ al. \ ref \ 2) \\ \end{array}$ 

Model Study of Whether F<sup>-</sup> S<sub>N</sub>2 Displacement Would Compete in the Baldwin -Lee Stille Cross Coupling Process



bromination of the product alcohol 22 with Ph3P and CBr4 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<sub>4</sub>/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,<sup>11</sup> without F<sup>-</sup> 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  $S_N 2$  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<sup>2</sup>-vinylstannanes with bromides of this sort. However, it is 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<sub>4</sub>Sn under prolonged rt conditions!

The final step in our (8*R*)-mycothiazole synthesis was Odesilylation of **23** with *n*-Bu<sub>4</sub>NF (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,<sup>1</sup> so confirming his 2006 structural revision.<sup>2</sup>

Even so, the  $[\alpha]_{\rm D}$  value that we recorded for synthetic (-)-(8*R*)-mycothiazole (1)  $(-21.4^{\circ}, c \ 0.47 \ \text{CHCl}_3)$  still differed substantially from the value reported by Crews et al. for natural material  $([\alpha]_{\rm D} -3.5^{\circ}, c \ 0.6 \ \text{CHCl}_3)$  in 2006 (see Scheme 3).<sup>2</sup> Even more surprisingly, as well, our synthetic sample of (-)-(8*R*)-mycothiazole (1) also gave rise to a large positive  $[\alpha]_{\rm D}$  of +42.3° in MeOH (*c* 1.13), which contrasted sharply with Crews' 2006  $[\alpha]_{\rm D}$  report of  $-13.7^{\circ}$  in MeOH (*c* 0.6).<sup>2</sup>

Our observations suggest that in a strongly hydrophobic solvent such as CHCl<sub>3</sub> 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.<sup>12</sup> when their  $[\alpha]_D$  was recorded in CHCl<sub>3</sub> in the presence of a small quantity (20%) of the H-bond-disrupter, tetramethylurea.<sup>12</sup>

In conclusion, an unambiguous total synthesis of (-)-(8R)mycothiazole (1) has been accomplished that spectrally confirms Crews' recently revised structure for this natural product. Our  $[\alpha]_D$  measurements for the (8R)-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  $[\alpha]_{\rm D}$  is recorded. Quite clearly, our  $[\alpha]_{\rm D}$  data deviate significantly from the  $[\alpha]_{\rm D}$  values reported by Crews et al. on lower purity natural mycothiazole.<sup>1,2</sup> Given the lack of a good  $[\alpha]_{\rm D}$  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 (8R). 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<sup>11</sup> to the activated bromomethylthiazole 4 constitutes an important new synthetic advance. Our synthesis has also underlined the great synthetic worth of the Yamamoto ZrCl<sub>4</sub>-mediated *cis*-selective alkyne allylstannation reaction<sup>8</sup> and compliments Yamamoto's own elegant efforts at applying this reaction in natural product total synthesis.<sup>8b</sup> 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.or-glett.5b01966.

Full experimental procedures, and copies of the <sup>1</sup>H and <sup>13</sup>C NMR spectra (PDF)

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#### Notes

The authors declare no competing financial interest.

#### ACKNOWLEDGMENTS

We thank QUB for a studentship (to L.W.) and start-up funding.

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