# LETTERS

# Total Synthesis and Structural Revision of Sekothrixide

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# **Supporting Information**

**ABSTRACT:** The first total synthesis of 14-membered macrolide sekothrixide and the originally proposed structure are reported. Seven contiguous asymmetric centers in the side chain were constructed using ring-openings of several kinds of epoxide. Assembly of the left segment and right segment was performed on the basis of the RCM reaction to generate 14-membered lactones having an *E*-trisubstituted olefin. These synthetic results led to a revision of C4, C6, and C8 stereochemistry in the structure of natural sekothrixide.

M ultidrug resistance (MDR) is a serious problem in cancer chemotherapy. Cellular desensitization to a broad range of chemotherapeutic agents is often associated with overexpression in tumor-cell membranes of P-glycoprotein, which excretes drugs from the cell interior through a molecular pump.<sup>1</sup> Seto et al. reported that sekothrixide, isolated from *Saccharothrixide* sp. CF24, exhibited cytocidal activity against colchicine-resistant KB cells (KB-C2) with an IC<sub>50</sub> of 6.5  $\mu$ g/ mL in EAGLE's minimum essential medium.<sup>2</sup> Furthermore, it showed synergistic activity against KB-C2 with an IC<sub>50</sub> of 1.0  $\mu$ g/mL in the presence of colchicines (1.5  $\mu$ g/mL). Their first report showed a planar structure, composed of a 14-membered  $\beta$ -ketolactone possessing an *E*-10,11-trisubstituted olefin and a highly oxygenated long side chain (Figure 1). Five years later,



Figure 1. Revised and proposed structures of sekothrixide.

the relative configuration of the seven continuous stereocenters in the side chain was determined by chemical degradation and careful analyses of <sup>1</sup>H and <sup>13</sup>C NMR spectra of the compound obtained.<sup>3</sup> In contrast, stereocenters at the C4, C6, and C8 positions were assigned on the basis of results of computational chemistry.<sup>3</sup> Although the absolute configuration of sekothrixide was not verified by any other experiment, Seto et al. proposed the three-dimensional structure of **2** by also considering that four stereocenters in the 14-membered lactone ring were in accordance with Celmer's rule.<sup>4</sup> Interesting biological properties as well as the unique structure of sekothrixide stimulated us to set about its total synthesis. As described below, the stereoisomer **2** obtained by our synthetic effort was not in



agreement with natural sekothrixide, and eventually, the absolute configuration of sekothrixide was revised as structure 1. We report herein the first total synthesis of sekothrixide (1) by means of acyclic stereocontrol based on several types of epoxy ring opening and revision of the proposed structure of sekothrixide.

We designed a convergent synthetic route consisting of the left segment A and right segment B (Scheme 1). These





segments were expected to be joined by esterification and ringclosing metathesis (RCM) reaction.<sup>5,6</sup> Segment **B** could be obtained using stereoselective alkylation<sup>7</sup> and enzymatic desymmetrization of *meso*-2,4-dimethyl-1,5-pentadiol.<sup>8</sup> Segment **A** could be synthesized from **C** through selective cleavage of silylene<sup>9</sup> and subsequent homologation. Construction of

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### **Organic Letters**

seven contiguous asymmetric centers in C could be synthesized from G (via D, E, and F) based on ring-openings of several kinds of epoxide. This plan requires that the ring-opening of D occurs adjacent to the secondary alcohol.

Initially, we aimed at the total synthesis of the originally proposed sekothrixide **2**. Reduction of epoxy alcohol  $3^{10}$  with Red-Al<sup>11</sup> resulted in epoxide opening to generate a mixture of 1,3-diol and 1,2-diol with a ratio of ca. 2:1. After oxidative degradation of the undesired 1,2-diol with NaIO<sub>4</sub>, purified 1,3-diol was converted to alcohol **4** by protection with a di-*tert*-butylsilylene group<sup>12</sup> and removal of a *p*-methoxybenzyl group (Scheme 2). Oxidation of **4** with IBX followed by Wittig

#### Scheme 2. Synthesis of Enone 9



reaction afforded the conjugated ester **5**, which was transformed to *trans*-epoxy sulfide **6** by sequential DIBAH reduction, Katsuki–Sharpless (KS) epoxidation,<sup>13</sup> and sulfide formation upon treatment with PhSSPh/<sup>n</sup>Bu<sub>3</sub>P/pyridine.<sup>14</sup> The key alkylation of epoxy sulfide **6** with Me<sub>3</sub>Al occurred successfully with double inversion via episulfonium ion to afford sulfide **7** as a single stereoisomer in 80% yield.<sup>15</sup> Protection of **7** with a MOM group followed by m-CPBA oxidation produced sulfone **8** in excellent yield, followed by conversion to **9** in three steps: (1) coupling with 1,2-epoxybutane under basic conditions, (2) IBX oxidation, and (3) elimination upon treatment with DBU.

Three asymmetric centers at C17-C19 have been introduced by a sequential method consisting of Corey-Bakshi-Shibata (CBS) reduction,16 KS epoxidation of the secondary allyl alcohol,<sup>17</sup> and ring-opening of anti-epoxy alcohol with an organocopper reagent (Scheme 3). When a stoichiometric amount of chiral oxazaborolidine was used, CBS reduction of 9 furnished the desired alcohol 10 as a major component with an epimeric ratio of 15:1.18 The absolute configuration at the C19 position was confirmed using a modified Mosher's method.<sup>19</sup> Secondary allylic alcohol **10** was subjected to KS epoxidation with L-DIPT at -30 °C, which was nicely matched with the S configuration at the C19 position, generating  $\beta$ -epoxide 11 as a single isomer in 83% yield. Next, our attention was focused on regiochemical control in the ringopening of epoxide with a Gilman reagent. At first, substitution of epoxy alcohol 11 with Me<sub>2</sub>CuLi proceeded nonselectively to give a mixture of 1,3-diol 13 and 1,2-diol 14 with a ratio of ca. 1:1, which was treated with NaIO<sub>4</sub> and subsequently purified by column chromatography on silica gel leading to pure 13 in 39%





overall yield. To our delight, when the secondary alcohol was protected with a trimethylsilyl group, nucleophilic attack occurred preferentially adjacent to the TMS silyl ether. Thus, treatment of 12 with Me<sub>2</sub>CuLi resulted in a highly regioselective opening to generate 13 in high yield after treatment with NaIO<sub>4</sub>. Diol 13 was converted to 15 via removal of the MOM group upon treatment with anisole and  $\mathrm{AlCl}_{2},^{20}$ regioselective acetalization, and protection with ethyl vinyl ether. The next task was regioselective cleavage of silvlene. Treatment of 15 with MeLi in Et<sub>2</sub>O did not result in any reaction. Interestingly, addition of TMEDA promoted preferential cleavage of the Si-O bond at the terminal side, leading to primary alcohol 16 in 85% yield. After oxidation of 16 with TPAP, the aldehyde obtained was converted to terminal alkene 17 through Wittig reaction. Removal of the di-tert-butylmethylsilyl group, which was highly stable, was accomplished by CsF in DMSO at 120 °C to afford the left segment 18.

Scheme 4 shows the synthesis of right segment 24. Optically active alcohol 19,<sup>21</sup> which was prepared by a chemoenzymatic method,<sup>8</sup> initially was converted to 21 through oxidation with IBX, Horner–Emmons elongation with phosphonate 20,<sup>22</sup> and catalytic hydrogenation. Stereoselective methylation<sup>7</sup> of 21

Scheme 4. Synthesis of Right Segment 24



### **Organic Letters**

produced trimethyl compound 22 in 83% yield, which was transformed into  $23^{23}$  in six steps: (1) reduction with LiBH<sub>4</sub>,<sup>24</sup> (2) silylation, (3) removal of the MPM group, (4) iodination, (5) coupling with a propenyl Grignard reagent in the presence of CuI, and (6) desilylation. Furthermore, 23 was converted to 24 by (1) IBX oxidation, (2) a Mukaiyama aldol reaction with ketene silyl acetal,<sup>25</sup> and (3) hydrolysis of the resulting ester. Also *ent*-24, the right segment of 1, was synthesized from *ent*-23<sup>23</sup> in a similar way.

With the left segment 18 and right segment 24 prepared, assembly of the 14-membered lactone was performed on the basis of macrocyclic RCM. Treatment of both segments with 2methyl-6-nitrobenzoic anhydride, Et<sub>3</sub>N, and DMAP provided 25 in 72% yield.<sup>26</sup> Upon treatment with Grubbs' second catalyst in refluxing CH<sub>2</sub>Cl<sub>2</sub>, RCM of 25 occurred E-selectively to afford 14-membered lactone, which was subjected to removal of the silyl group using TBAF. The alcohol 26 obtained was finally converted to the proposed sekothrixide structure 2 by the sequence of oxidation with TPAP and removal of the protective group. The <sup>13</sup>C NMR spectrum showed a characteristic *E*-allyl methyl carbon peak at approximately  $\delta$  18.0.<sup>27</sup> However, the <sup>1</sup>H and <sup>13</sup>C NMR spectra of 2 did not match that of natural sekothrixide, with the greatest differences involving peaks from the ketolide portion. The methylene protons for C2 of natural sekothrixide gave signals at  $\delta$  3.50 and 3.29, while peaks from the corresponding protons in 2 were observed at  $\delta$ 3.49 and 3.40. The C4 proton of **2** gave a peak  $\delta$  0.19 upfield of that for the corresponding proton ( $\delta$  2.91) in the natural product. A characteristic peak for C7-H in the natural product appeared at  $\delta$  0.51 but was not observed in 2. In contrast, peaks assigned to the side chain were very similar between 2 and natural sekothrixide, suggesting that errors existed in the 14membered lactone. These findings led to an attempt at convergent assembly of 18 and ent-24 in a manner similar to that used to produce 2. As a result, we succeeded in the synthesis of 1, for which the <sup>1</sup>H and <sup>13</sup>C NMR spectra and the high-resolution mass spectrum were identical to those for natural sekothrixide. The optical rotation of synthetic sekothrixide was  $[\alpha]_D = -46.4$  (c = 0.18, MeOH), which had the same sign as that for natural sekothrixide  $[\alpha]_{\rm D} = -45.1$  (c = 1.00, MeOH)]. Consequently, the relative and absolute configurations of sekothrixide were unequivocally determined to be that shown as structure 1.

In conclusion, we have accomplished the first total synthesis of sekothrixide (1) (Scheme 5). The left segment 18, which possesses seven contiguous asymmetric centers, was constructed stereoselectively using stereospecific alkylation of epoxysulfide 6 with Me<sub>3</sub>Al, regioselective substitution of an epoxide linked to silyloxy group 12 with Me<sub>2</sub>CuLi and regioselective cleavage of silylene 15. Notably, the key RCM reaction proceeded stereoselectively to generate 14-membered lactones having an *E*-trisubstituted olefin. Total synthesis of 1 revised the C4, C6, and C8 stereochemistry of the originally proposed structure 2, which was against Celmer's rule. The established route would be applicable to the syntheses of other stereoisomers of the lactone portion. These findings will contribute to the development of effective chemotherapy agents against MDR.





# ASSOCIATED CONTENT

# **Supporting Information**

Experimental procedures along with experimental and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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# Notes

The authors declare no competing financial interest.

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