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## RECENT ADVANCES IN THE SYNTHESSES OF BIOLOGICALLY ACTIVE NATURAL PRODUCTS USING BIOCATALYST

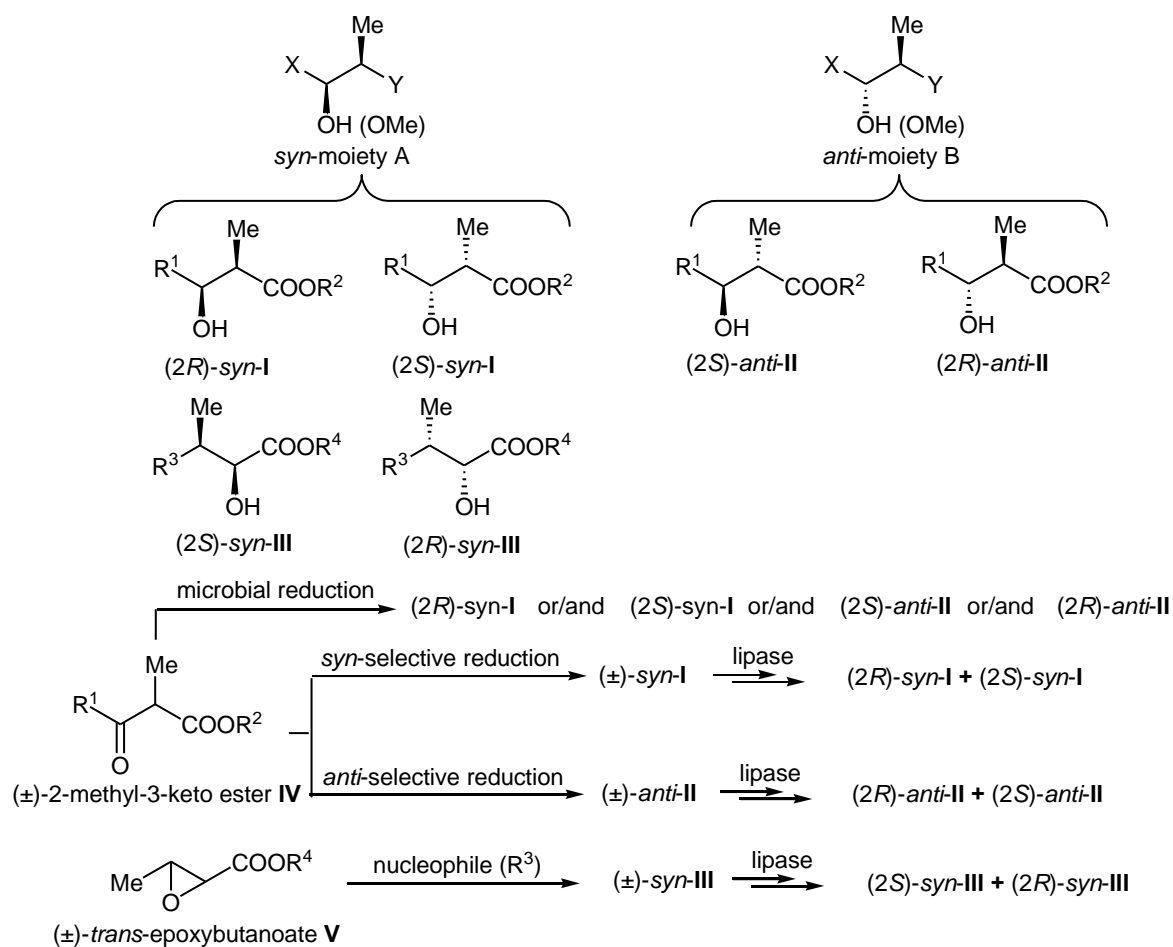
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**Abstract** - This review summarizes the chemoenzymatic synthesis of biologically active natural products based on a combination of chemical diastereoselectivity and enzymatic enantioselectivity using lipase. Diastereoselective synthesis of ( $\pm$ )-(2,3)-*syn*-2-methyl-3-hydroxy ester **I** or ( $\pm$ )-(2,3)-*syn*-3-methyl-2-hydroxy ester **III** was achieved based on diastereoselective reduction of ( $\pm$ )-2-methyl-3-keto ester **IV** or the reaction of ( $\pm$ )-*trans*-epoxybutanoate **V** and carbon-nucleophile, respectively. These racemic alcohols were subjected to enzymatic resolution to afford the corresponding enantiomers. Each enantiomerically pure compound was converted to biologically active natural products such as oudemansins, chuangxinmycin, asperlin, indolmycin, cystothiazoles, melithiazols and myxothiazols possessing antifungal and cytotoxic activities, inhibition of NADH oxidation, etc.

### 1. INTRODUCTION

In connection with synthetic studies of polyketides such as polyoxomacrolide and polyether antibiotics, stereocontrolled reaction in acyclic systems have been extensively investigated. Among them, the stereoselective synthesis of these natural products has attracted the attention of many synthetic organic chemists, since the *syn*-moiety **A** or *anti*-moiety **B** repeatedly appears in the framework of the above antibiotics. Moreover, these moieties are an important building block for the synthesis of a complex array of methyl and oxygen functional groups involved in these natural products. Efforts have been focused mainly on the development of the regio- and stereocontrolled aldol reaction, and excellent results have been documented. Alternatively, we focused on the synthesis of chiral 2-methyl-3-hydroxy esters [(2*R*)-*syn*-**I**, (2*S*)-*syn*-**I**, (2*S*)-*anti*-**II** and (2*R*)-*anti*-**II**] and 3-methyl-2-hydroxy esters [(2*S*)-*syn*-**III**,

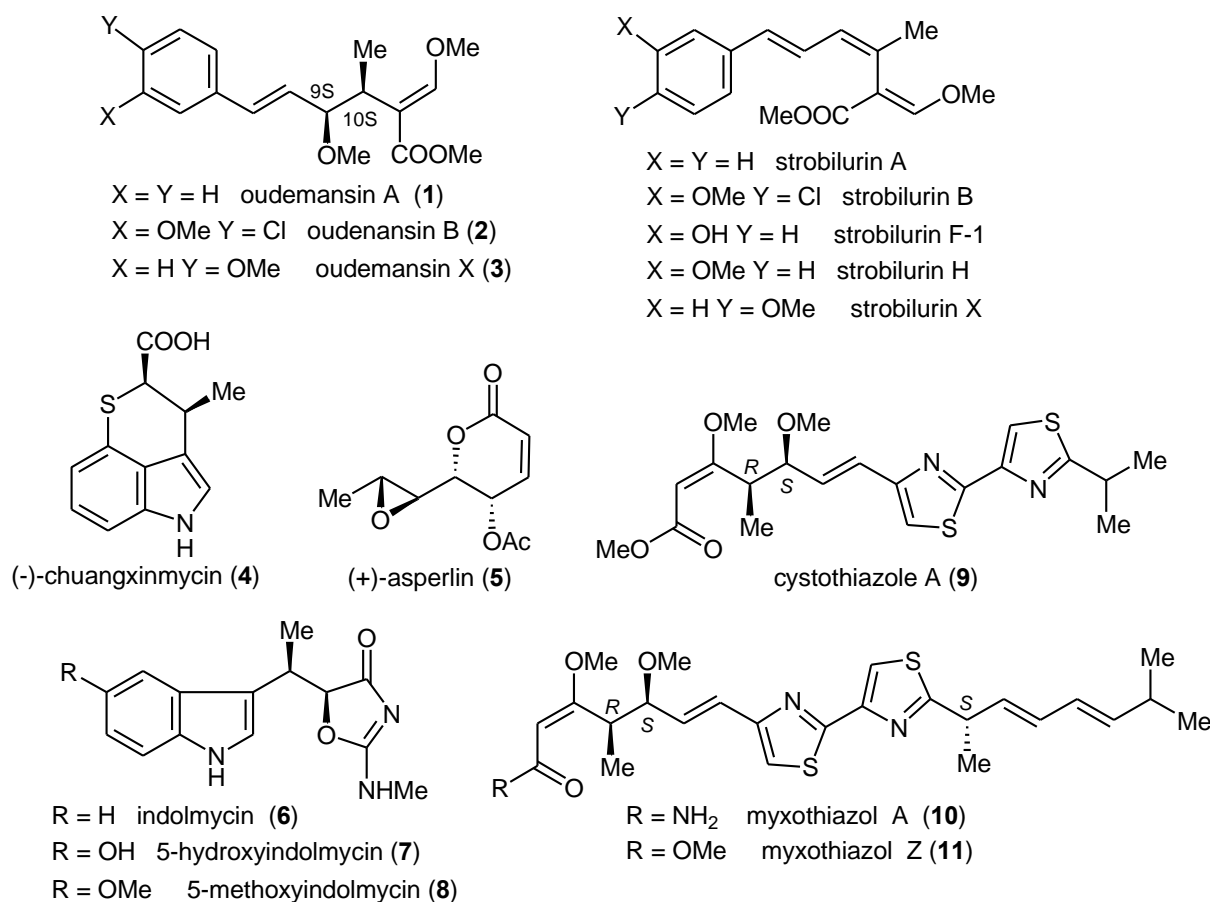


Scheme 1

(2*R*)-*syn*-III] based on the stereoselective reduction of the corresponding (±)-2-methyl-3-keto ester IV and the stereoselective epoxy ring opening cleavage of *trans*-epoxybutanoate V with nucleophile (R<sup>3</sup>), respectively. Optically active 2-methyl-3-hydroxy esters could be obtained by the microbial reduction of the (±)-2-methyl-3-keto ester IV. The above-mentioned enantiomers [(2*R*)-*syn*-I and (2*S*)-*syn*-I] of 2-methyl-3-hydroxy esters could be obtained based on the lipase-catalyzed resolution of the racemic alcohol (±)-*syn*-I. Likewise, two coupled enantiomers [(2*S*)-*anti*-II and (2*R*)-*anti*-II, (2*S*)-*syn*-III and (2*R*)-*syn*-III] could be obtained by the same strategy from the racemic alcohols [(±)-*anti*-II, (±)-*syn*-III] respectively (Scheme 1). In this review, a highly stereoselective syntheses of the natural products such as oudemansins [A (1) and B (2)], (-)-chuangxinmycin (4), (+)-asperlin (5), indolmycins [(6) and (8)], cystothiazole A (9) and myxothiazols [A (10) and Z (11)] were achieved based on a combination of chemical diastereoselectivity and enzymatic enantioselectivity (Scheme 2).

## 2. SYNTHESIS OF OUDEMANSINS<sup>6</sup>

The strobilurins,<sup>1,2</sup> oudemansins [A (1),<sup>3</sup> B (2),<sup>4</sup> C (3)<sup>5</sup>] are naturally occurring fungidal substances possessing a β-methoxyacrylate moiety. These compounds are produced by various fungi and bacteria

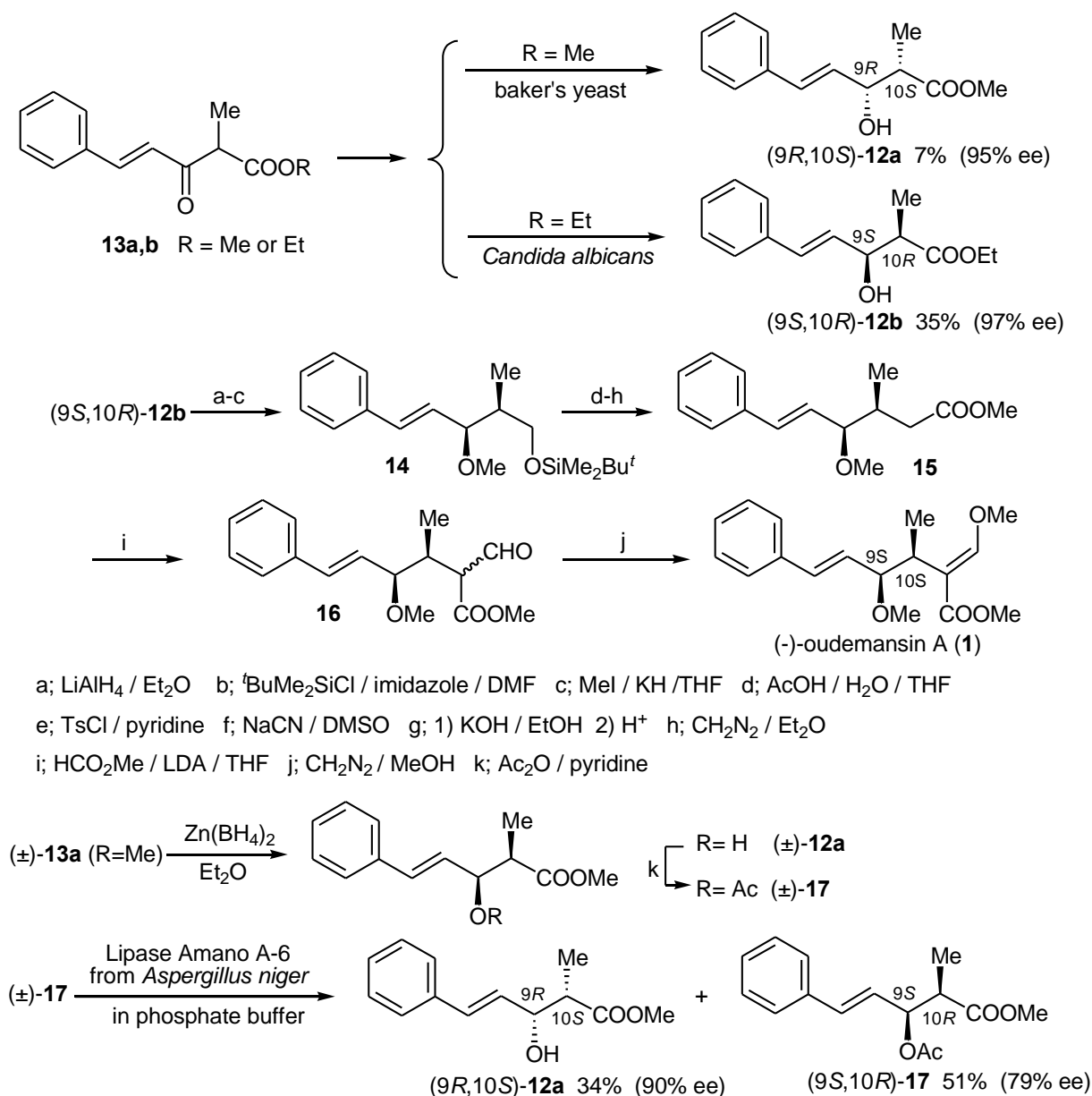


Scheme 2

and have attracted considerable interest from the viewpoint of not only their novel structure but also various kinds of biological activity. For example, they are able to control the growth of fungi, or exhibit insecticidal, antiviral or antitumor activity. Furthermore, they inhibit mitochondrial respiration by binding at a specific site on cytochrome b, and this biological characteristic has made them valuable biochemical tools.

### 2.1. Synthesis of oudemansin A<sup>6</sup>

Oudemansin A (**1**)<sup>3</sup>, an antibiotic isolated from mycelial cultures of *Oudemansiella radicata* exhibits strong antifungal activities. The structure and the relative configuration of **1** have been determined by X-ray analysis, but the absolute configuration of its two chiral centers is not known yet. The first synthetic approaches to racemic oudemansin A (**1**) have been described.<sup>7</sup> The absolute structure of **1** was unequivocally determined as 9S, 10S by the synthesis of the optically active form. The most intriguing point of the present synthesis is the preparation of the optically active *syn*- $\beta$ -hydroxy esters (**12a** or **12b**). This was successfully achieved by the use of microbiological asymmetric reduction of the corresponding  $\alpha$ -methyl- $\beta$ -keto esters (**13a,b**). This type of microbiological reduction has been extensively studied by us in a related system.<sup>8</sup> Reduction of the  $\beta$ -keto methyl ester (**13a**) with baker's



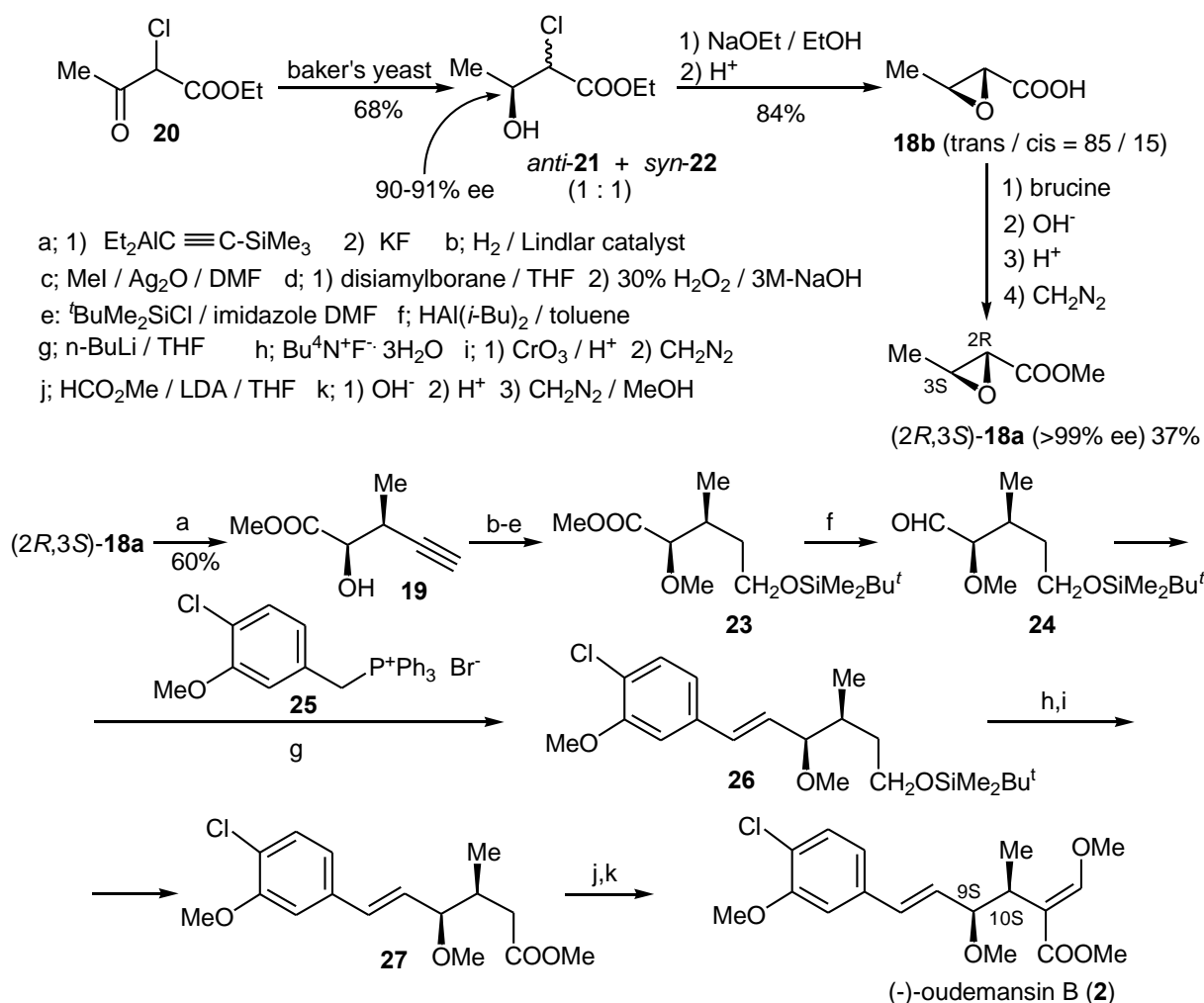
Scheme 3

yeast (*Saccharomyces cerevisiae*) gave the  $\beta$ -hydroxy methyl ester (**12a**), although the yield was poor (7%). The structure of the reduction product was assigned as **12a** ( $9R, 10S$ ) from the chemical conversion to the authentic sample.<sup>9</sup> The reduction product (**12a**) was then converted to the corresponding (*R*)- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenyl acetate ((*R*)-MTPA ester),<sup>10</sup> of which the optical purity was calculated as 95% ee. Then, the reduction of the  $\beta$ -keto ethyl ester (**13b**) using various yeasts was examined. Among them, *Candida albicans* was found to afford the *syn*- $\beta$ -hydroxy ethyl ester (**12b**) (35% yield) along with 10% of the corresponding *anti*-ester and 32% of starting material (**13b**). These are readily separable by simple chromatography. The structure of the main product was assigned as **12b** ( $9S, 10R$ ) and its optical purity (97% ee) was determined in the same way as described in the case of **12a**. The remarkable feature of the present reduction is that the absolute configuration of ( $9S,10R$ )-**12b** derived

from the ethyl ester (**13b**) is just opposite to that of (9*R*,10*S*)-**12a** derived from the methyl ester (**13a**), which shows that the stereochemistry of the reduction is strictly governed by the structure of the substrates and the species of yeasts. The (9*S*,10*R*)- $\beta$ -hydroxy ester (**12b**) thus obtained was converted to the  $\beta$ -methoxy silyl ether (**14**) (68% overall yield from (9*S*,10*R*)-**12b** in three steps (a-c). Conversion of **14** to the methoxy ester (**15**) was achieved by the standard procedure (five steps, d-h) in overall 34% yield. Formylation of **15** with LDA and methyl formate in THF at -78 °C to 0 °C, followed by treatment with CH<sub>2</sub>N<sub>2</sub>-MeOH produced the optically active oudemansin A (**1**) (26% yield) after purification by HPLC. The spectral data ( $[\alpha]_D$ , mp and NMR) of the synthetic (-)-**1** were identical with those of natural oudemansin A (**1**).<sup>3</sup> The 9*S*, 10*S* absolute configuration of natural oudemansin A (**1**) was thus established. An alternative synthesis of (9*S*,10*R*)-**17** corresponding to (9*S*,10*R*)-**12b** was achieved by a combination of a chemo-selective method and enzymatic resolution of the racemic  $\beta$ -acetoxy ester ( $\pm$ )-**17**. Zn(BH<sub>4</sub>)<sub>2</sub>-mediated reduction<sup>11</sup> of  $\beta$ -keto ester ( $\pm$ )-**13a** gave predominantly the ( $\pm$ )-*syn*- $\beta$ -hydroxy ester (**12a**), of which acetylation produced the corresponding ( $\pm$ )-acetate (**17**). This racemic acetate was subjected to the enantioselective hydrolysis using lipase Amano A-6 to provide (9*R*,10*S*)-**12a** (34%) and (9*S*,10*R*)-**17** (51%) possessing 90% ee and 79% ee, respectively<sup>12</sup> (Scheme 3).

## 2.2. Synthesis of oudemansin B<sup>13</sup>

Oudemansin B (**2**) is an antibiotic isolated from mycelial cultures of *Xerula mlanotricha* and inhibits the growth of a wide variety of saprophytic and phytopathogenic fungi at very low concentration.<sup>4</sup> The structure has been deduced by spectroscopic methods and relative structure has been confirmed by the synthesis of ( $\pm$ )-**2** by Kallmerten *et al.*<sup>14</sup> However, the absolute configurations of these two chiral centers remain unknown. The absolute structure of **2** was established as 9*S*, 10*S* by the total synthesis starting from the chiral intermediate of the known absolute structure. It has been reported that silyl acetylide attacks preferentially the C(3)-position of ( $\pm$ )-*trans*-(2,3)-epoxybutyrate **18a** producing ( $\pm$ )-*syn*-C(2)-OH, C(3)-Me ester **19**,<sup>15</sup> from which ( $\pm$ )-**2** is expected to be derived by following a similar route already used in oudemansin A (**1**) synthesis.<sup>6</sup> Thus, initially we focused our attention to the synthesis of optically active **18a**. We intended to synthesize (2*R*,3*S*)-epoxide **18b** by a microbial enantioselective reduction of ( $\pm$ )-2-chloro-3-oxobutanoate **20** followed by base-catalyzed epoxide formation.<sup>16</sup> The main drawback of this biological method is that along with the desired optically pure *anti*-compound **21**, the isomeric *syn*-compound **22** is presumed to be produced in almost equal quantity, because we have encountered this difficulty in all biological reductions producing two chiral centers. However, in this particular case, formation of the *syn*-isomer **22** can not be a serious obstacle, since Mukaiyama *et al.* have already shown that sodium ethoxide promoted epoxide formation of a mixture of ( $\pm$ )-*syn*- and *anti*-2-chloro-3-hydroxy ester affords ( $\pm$ )-*trans*-epoxide preferentially (*cis/trans* =



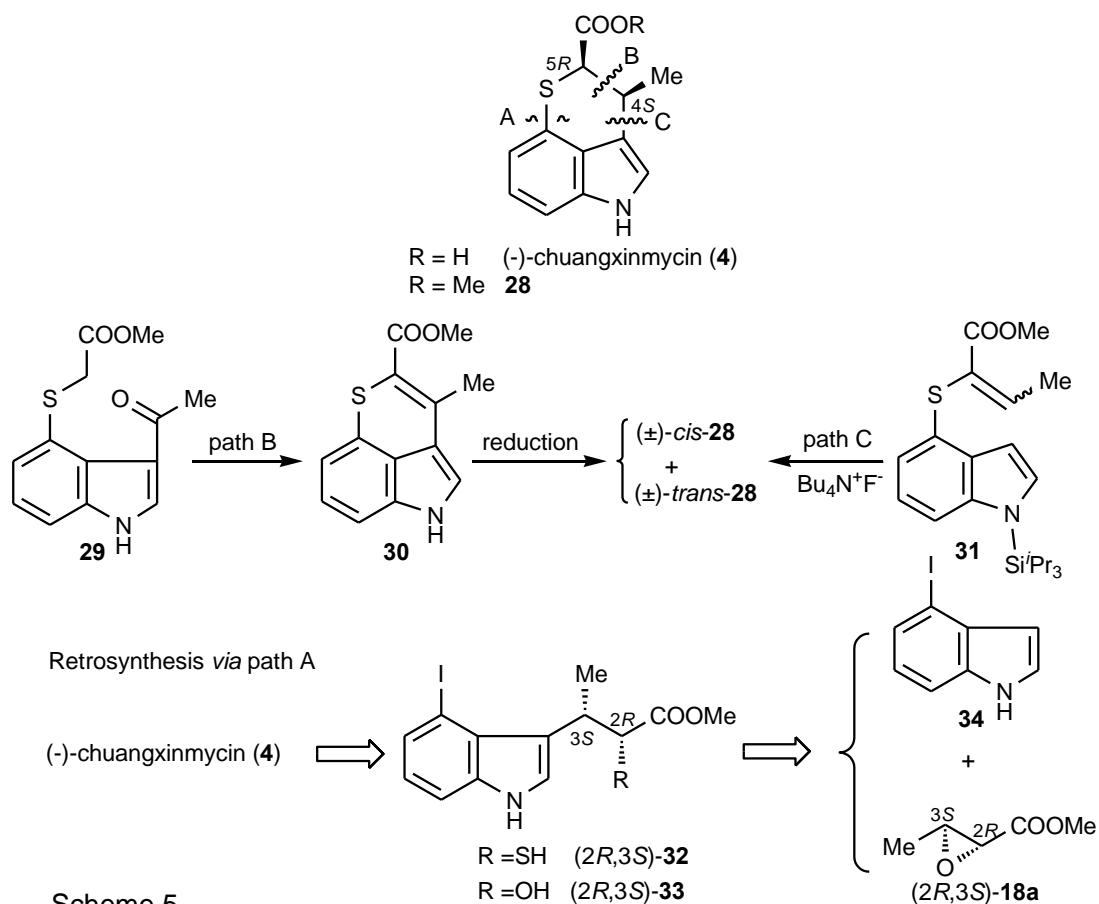
Scheme 4

15/85~1/99).<sup>17</sup> In fact, reduction of commercially available **20** with baker's yeast (*Saccharomyces cerevisiae*) afforded a 1:1 mixture of chlorohydrin **21** and **22** in 68% yield. The mixture was treated with NaOEt giving a mixture of *cis* and *trans* epoxy acid (**18b**) in 84% yield, whose ratio was, from NMR data, found to be 85:15, *trans* epoxide **18b** being predominant as expected. In order to eliminate the contaminated *cis*-epoxide, the 85:15 mixture was converted to brucine salt. On one recrystallization of the crude salt followed by base treatment, the isomer-free, optically pure ( $>99\%$  ee) (2*R*,3*S*)-epoxy carboxylic acid was obtained. This acid was treated with  $\text{CH}_2\text{N}_2$  to give the desired pure **18a** in overall 37% yield from a mixture of **21** and **22**. The absolute structure of (2*R*,3*S*)-**18a** was confirmed by the conversion to the known (2*R*,3*S*)-indolmycenic methyl ester.<sup>18</sup> By applying Roush's method,<sup>15</sup> **18a** was then converted to the acetylene (**19**) in 60% yield. Partial hydrogenation of **19** using Lindlar catalyst followed by consecutive methylation, hydroboration with disiamylborane in THF and silylation gave silyl ether (**23**) in 30% overall yield from **19**. DIBAL reduction of **23** afforded aldehyde **24**, which was allowed to react with phosphonium salt (**25**) in the presence of *n*-BuLi giving a 1:1 mixture of the condensation products (*cis*-**26** and *trans*-**26**) in 70% yield from **23**. After desilylation with the fluoride ion

( $\text{Bu}_4\text{N}^+\text{F}^- \cdot 3\text{H}_2\text{O}$ ), the mixture was separated by silica-gel column chromatography to *cis*-alcohol (45.2%) and *trans*-alcohol (35.3%). The *trans-syn*-alcohol was oxidized with  $\text{CrO}_3$  and the resulting acid was esterified with  $\text{CH}_2\text{N}_2$  giving methyl ester (**27**) in 75% yield. Formylation of **27** with methyl formate in the presence of LDA in THF at  $-78^\circ$  (then to  $0^\circ\text{C}$ ) followed by  $\text{CH}_2\text{N}_2$ -MeOH treatment afforded (-)-oudemansin B (**2**) in 31% yield after purification by HPLC. The physical data (CD,  $[\alpha]_D$ , MS, NMR and UV of the synthetic (-)-**2** were identical with those of natural oudemansin B (**2**).<sup>4</sup> The absolute configuration of natural oudemansin B (**2**) was thus established as shown in **2**. Chemoenzymatic synthesis of oudemansin X (**3**)<sup>19,20</sup> and formal syntheses of oudemansins A (**1**), B (**2**), X (**3**)<sup>21,22</sup> were also achieved (Scheme 4).

### 3. SYNTHESIS OF (-)-AND (+)-CHUANGXINMYCIN<sup>23,24,25</sup>

Chuangxinmycin (**4**), isolated from *Actinoplanes tsinanensis*. sp. in China, exhibits *in vitro* an antibacterial spectrum that includes a number of Gram-positive and Gram-negative bacteria. This material was reported to be active in mice against *Escherichia coli* and *Shigella dysenteria* infections *in vivo*, and effective in the treatment of septicaemia, urinary, and biliary infections caused by *E. coli* in preliminary clinical results.<sup>26</sup> The relative structure of **4** was confirmed by synthesis<sup>26</sup> and the absolute configurations were determined as 4*S*, 5*R* based on the degradation study of the natural product (**4**)<sup>26</sup> and



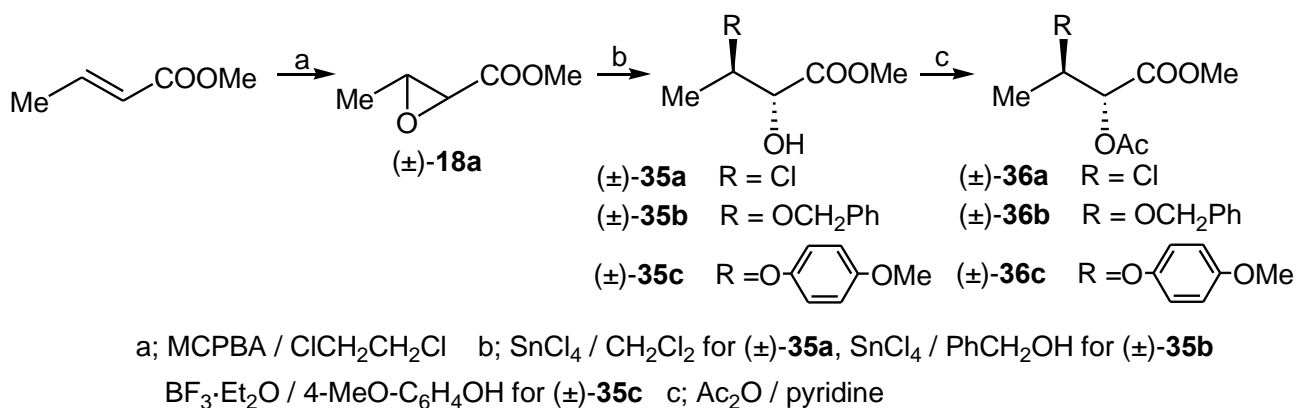
Scheme 5

optical resolution of ( $\pm$ )-**4** with *S*-(-)- $\alpha$ -phenylethyl amine.<sup>26</sup> Synthetic attempts were made using two published routes. One is an internal Knoevenagel condensation of 4-substituted-3-acetyl indole **29** and the subsequent reduction of **30** gave a mixture of ( $\pm$ )-*cis*-methyl ester **28** of **4** and *trans*-**28** via pathway B.<sup>26</sup> In the other route, treatment of **31** with the fluoride ion liberated the indol-1-yl anion by desilylation, and Michael addition of the ambident C-3 anion to the powerful  $\alpha$ -thioacrylate acceptor could bring about the required cyclization via pathway C.<sup>26</sup> (Scheme 5) But these routes were found to be unacceptable for the synthesis of the desirable optically active form of **4**. Stereoselective synthesis of (-)-**4** via pathway A was directed toward chiral synthesis starting with the requisite (2*R*,3*S*)-*syn*-mercapto ester (**32**) possessing two definite absolute configurations at the C(2)- and C(3)-positions. The synthesis of (2*R*,3*S*)-**32** could be performed from (2*R*,3*S*)-4'-iodoindolmycenate (**33**) along with retention at C(2) stereochemistry in **33**. The synthesis of indolmycenate, being an important intermediate for the synthesis of indolmycin,<sup>18</sup> was achieved by the reaction of indole and ( $\pm$ )-*trans*-(2,3)-epoxy butanoate **18a** in the presence of SnCl<sub>4</sub> along with nucleophilic displacement with inversion at the C(3) carbon of the coordinated epoxide.<sup>18</sup> This strategy appeared to be the most promising from a stereochemical standpoint for the stereoselective construction of C(2)- and C(3)-configurations of **33** by the reaction of 4-iodoindole **34**<sup>26</sup> and (2*R*,3*S*)-**18a** in the presence of SnCl<sub>4</sub> (Scheme 5).

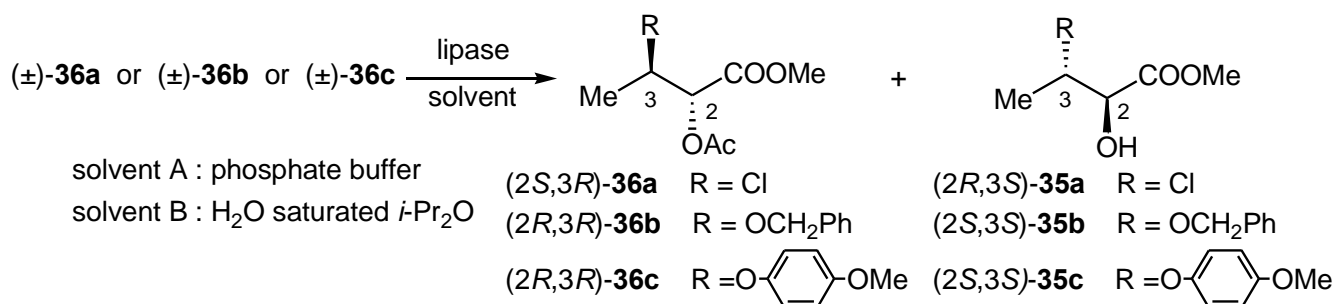
### 3.1. Synthesis of (2*R*,3*S*)- and (2*S*,3*R*)-epoxybutanoates<sup>18,24,25</sup>

The synthesis of (2*R*,3*S*)-**18a** based on the microbial reduction of  $\alpha$ -chloroacetoacetate was already described in 2.2. In this section, the synthesis of both enantiomers of the (2,3)-*anti*-2-hydroxy-3-substituted butanoate congener based on the lipase-catalyzed enantioselective hydrolysis and the synthesis of (2*R*,3*S*)- and (2*S*,3*R*)-**18a** were described as shown in Scheme 6. The reaction of ( $\pm$ )-*trans*-(2,3)-epoxy butanoate **18a** derived from methyl crotonate with the chloride ion, benzyl alcohol and *p*-methoxyphenol in the presence of Lewis acid gave (2,3)-*anti*-2-hydroxy-3-substituted butanoate (( $\pm$ )-**35a** (39%), ( $\pm$ )-**35b** (27%), ( $\pm$ )-**35c** (46%)), respectively, which were subjected to acetylation to afford the corresponding acetates (( $\pm$ )-**36a,b,c**), respectively<sup>18</sup> (Scheme 6). Initially, three kinds of ( $\pm$ )-acetates (**36a,b,c**) were subjected to screening experiments using several kinds of commercially available lipases in phosphate buffer (pH 7.25) or water-saturated isopropyl ether. The results are shown in Table 1. When the substrate ( $\pm$ )-**36a** was subjected to enzymatic hydrolysis using lipase "Amano P" from *Pseudomonas* sp. in water-saturated isopropyl ether, the (2*R*,3*S*)-**35a** (40%, 89% ee) and the unchanged (2*S*,3*R*)-**36a** (45%, 87% ee) were obtained (entry 4, Table 1). The *E*-value<sup>27</sup> of this resolution was estimated to be 16.2. The (2*S*,3*R*)-**36a** having 87% ee was again subjected to enzymatic hydrolysis to afford the enantiomerically pure (2*S*,3*R*)-**36a** in 84% yield (entry 5, Table 1). On the contrary, the 89% enantiomeric excess of (2*R*,3*S*)-**36a** was subjected to enzymatic hydrolysis to provide



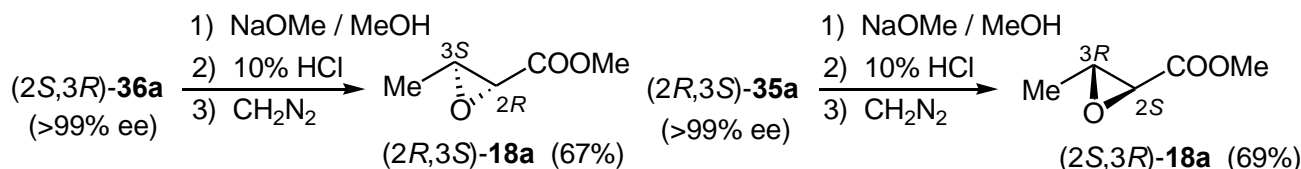


Scheme 6

Table 1 Enantioselective hydrolysis of (±)-**36a,b,c** using lipase

Entry	Substrate (g, %ee)	Lipase	Solvent	Time (hr)	Products (%) (%ee)			
1	(±)- <b>36a</b> (0.1)	Amano P	A	19	(2 <i>S</i> ,3 <i>R</i> )- <b>36a</b> (24) (>99)	(2 <i>R</i> ,3 <i>S</i> )- <b>35a</b> (12) (48)		
2	(±)- <b>36b</b> (0.1)	OF-360	A	62	(2 <i>R</i> ,3 <i>R</i> )- <b>36b</b> (28) (95)	(2 <i>S</i> ,3 <i>S</i> )- <b>35b</b> (63) (28)		
3	(±)- <b>36c</b> (0.1)	Amano P	A	40	(2 <i>R</i> ,3 <i>R</i> )- <b>36c</b> (43) (95)	(2 <i>S</i> ,3 <i>S</i> )- <b>35c</b> (42) (70)		
4	(±)- <b>36a</b> (14.0)	Amano P	B	120	(2 <i>S</i> ,3 <i>R</i> )- <b>36a</b> (45) (87)	(2 <i>R</i> ,3 <i>S</i> )- <b>35a</b> (40) (89)		
5	(2 <i>S</i> ,3 <i>R</i> )- <b>36a</b> (6.3, 87)	Amano P	B	72	(2 <i>S</i> ,3 <i>R</i> )- <b>36a</b> (84) (>99)	(2 <i>R</i> ,3 <i>S</i> )- <b>35a</b> (12) (0)		
6	(2 <i>R</i> ,3 <i>S</i> )- <b>36a</b> (4.4, 89)*	Amano P	B	72	(2 <i>R</i> ,3 <i>S</i> )- <b>36a</b> (12) (27)	(2 <i>R</i> ,3 <i>S</i> )- <b>35a</b> (68) (>99)		

\* The substrate (2*R*,3*S*)-**36a** (89% ee) was obtained by acetylation of (2*R*,3*S*)-**35a** (89% ee)

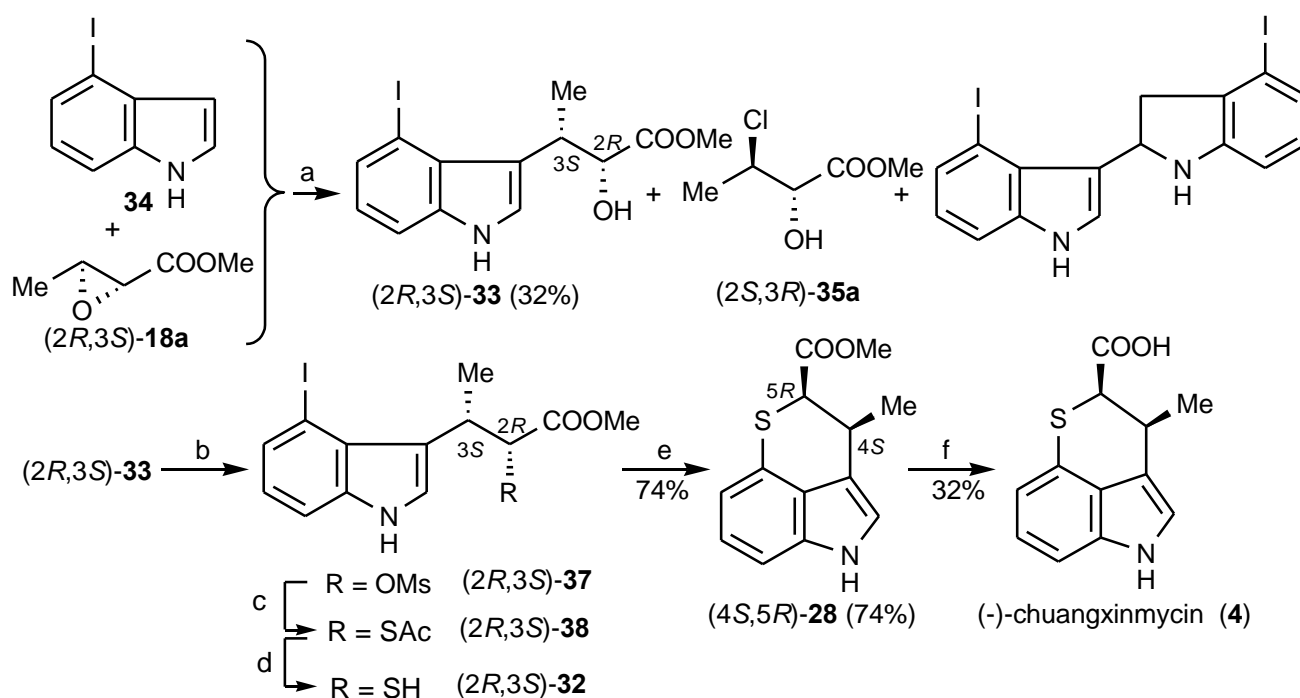


the enantiomerically pure (2*R*,3*S*)-**35a** in 68% yield (entry 6, Table 1). The enantiomeric excess of (2*S*,3*R*)-**36a** and (2*R*,3*S*)-**35a** was calculated based on NMR (400 MHz) data of the corresponding (*R*)-MTPA ester. The thus-obtained (2*S*,3*R*)-**36a** was treated with NaOMe followed by acid treatment and esterification with CH<sub>2</sub>N<sub>2</sub> to provide the enantiomerically pure (2*R*,3*S*)-epoxy butanoate (**18a**) in 67%

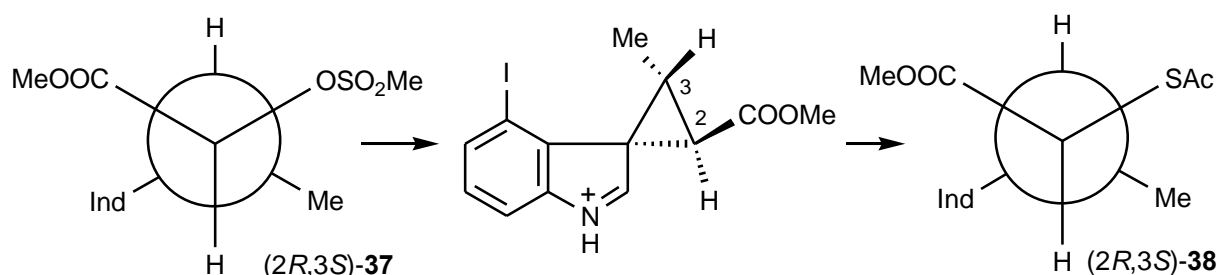
overall yield. Likewise, the enantiomerically pure (2*R*, 3*S*)-**35a** was also converted to (2*S*,3*R*)-**18a** in 69% overall yield.

### 3.2. Synthesis of (-)- and (+)-chuangxinmycins<sup>24,25</sup>

The reaction of 4-iodoindole (**34**) and (2*R*,3*S*)-**18a** in the presence of SnCl<sub>4</sub> afforded (2*R*,3*S*)-**33** (32% yield) along with a mixture of (2*S*,3*R*)-**35a** and 4'-iodoindole dimer. Treatment of (2*R*,3*S*)-**33** with MsCl in pyridine followed by treatment with CsSAc provided (2*R*,3*S*)-2-thioacetoxy ester (**38**) in 73%



a; SnCl<sub>4</sub> / CH<sub>2</sub>Cl<sub>2</sub> b; MsCl / pyridine c; CsSAc d; K<sub>2</sub>CO<sub>3</sub> / MeOH e; Pd(Ph<sub>3</sub>)<sub>4</sub> / Et<sub>3</sub>N f; NaOH / EtOH / H<sub>2</sub>O



Scheme 7

overall yield with complete retention of C(2)-stereochemistry. Deacetylation of (2*R*,3*S*)-**38** with K<sub>2</sub>CO<sub>3</sub> in MeOH provided (2*R*, 3*S*)-2-mercapto ester **32** (70% yield), which was subjected to Pd(PPh<sub>3</sub>)<sub>4</sub>-mediated cyclization in the presence of Et<sub>3</sub>N to give the (4*S*,5*R*)-*cis*-methyl ester (**28**) in 74% yield. An alkaline hydrolysis of (4*S*,5*R*)-**28** was carried out by the reported procedure<sup>26</sup> to provide the natural chuangxinmycin (4*S*,5*R*)-**4**, which is consistent with the reported (4*S*,5*R*)-**4**. Likewise, the

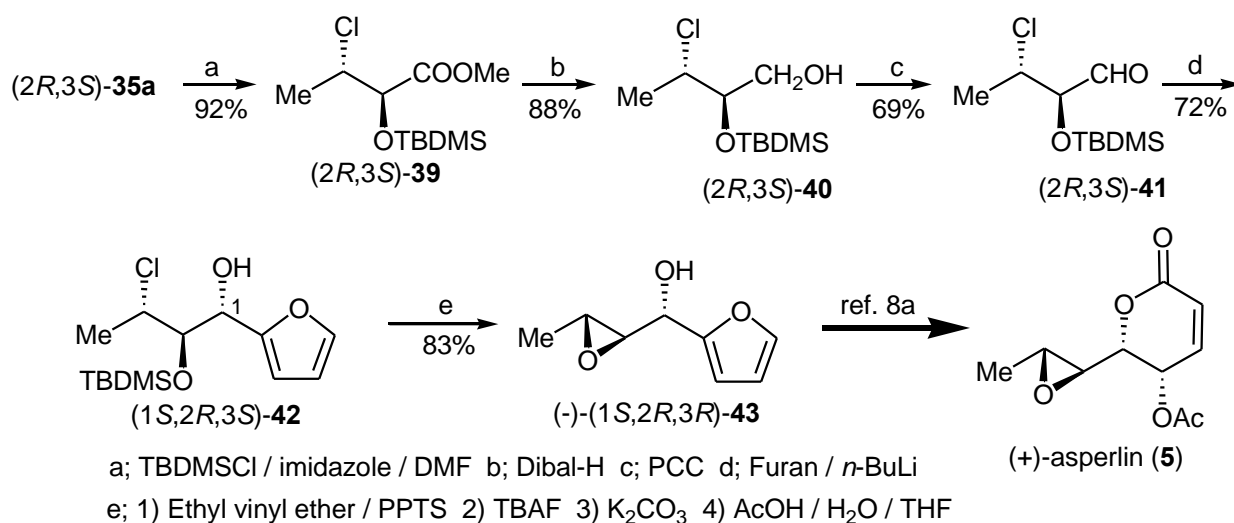
enantiomerically pure (2*S*,3*R*)-**18a** was converted to the (4*R*,5*S*)-**4** via (2*S*,3*R*)-**33**, (2*S*,3*R*)-**38**, (2*S*,3*R*)-**32** and (4*R*,5*S*)-**28**. The physical data of the present (4*R*,5*S*)-**4** was identical with those of the reported (4*R*,5*S*)-**4**.<sup>26</sup> As shown in Scheme 7, it is proposed that neighboring group participation involving the electron-rich C(3) of the indole ring accounts for the stereoselective conversion of (2*R*,3*S*)-**37** to (2*R*,3*S*)-**38**. The preferred conformation of the mesylate **37** derived from (2*R*,3*S*)-**33**, in which steric interactions are minimized, is shown in **37**. In this rotamer, the mesyloxy group is *trans* to the indol C(3), so that ready displacement can occur. Nucleophilic attack by the thioacetoxy ion takes place at the C(2)-position because the charge in the cyclopropylm ion intermediate is still predominantly centered on the C(2) carbon atom. Since this is essentially a double S<sub>N</sub>2 mechanism, the *syn*-stereochemistry of the (2*R*,3*S*)-*syn*-2-hydroxy ester (**33**) is retained in the (2*R*,3*S*)-*syn*-2-thioacetoxy ester (**38**) (Scheme 7).

#### 4. SYNTHESIS OF (+)-ASPERLIN<sup>28</sup>

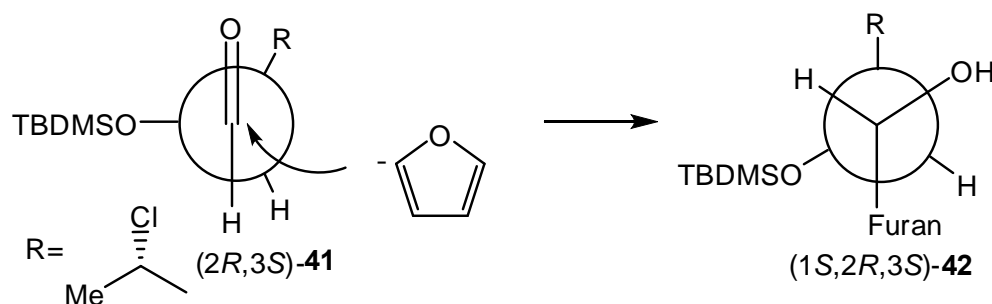
(+)-Asperlin (**5**), isolated from *Aspergillus nidulans* and *Aspergillus caespiyosus*, has been shown to exhibit antitumour and antibacterial activity. Its structure, including the absolute configuration, was determined by spectroscopic and chemical studies.<sup>29-31</sup> Because of its interesting bioactivity, the synthesis of natural product (**5**) and its related compounds has been reported by several groups.<sup>32</sup> The syntheses of (+)-**5** from natural products such as L-rhamnose,<sup>33</sup> (*S,S*)-tartaric acid,<sup>34</sup> and D-glucose<sup>35</sup> has been reported. Recently, the convenient syntheses of (+)-**5** based on the Sharpless asymmetric epoxidation of unsymmetrical divinylmethanol congeners have been reported.<sup>36</sup> On the other hand, we reported the lipase-catalyzed resolution of racemic  $\alpha$ -acetoxy ester ( $\pm$ )-**36a**, a key intermediate in the synthesis of (+)-asperlin, to give the optically pure (2*R*,3*S*)-**35a** (>99% ee) and (2*S*,3*R*)-**36a** (>99% ee) as shown in Table 1. The two concise syntheses of (+)-asperlin (**5**) based on the stereoselective addition of carbon-nucleophile to the chiral  $\alpha$ -silyloxy aldehyde, (2*R*,3*S*)-3-chloro-2-*tert*-butyldimethyl-silyloxybutanal (**41**), derived from (2*R*,3*S*)-**35a** was shown in Schemes 8 and 10.

##### 4.1. Formal synthesis of (+)-asperlin (**5**)

The formal synthesis of (+)-asperlin (**5**) from (2*R*,3*S*)-**35a** is shown in Scheme 8. Silylation of (2*R*,3*S*)-**35a** with *tert*-butyldimethylsilyl chloride (TBDMSCl) gave the corresponding silyl ether (2*R*,3*S*)-**39** (92%), which was reduced with diisobutylaluminum hydride (Dibal-H) to afford an alcohol (2*R*,3*S*)-**40** in 88% yield. Pyridinium chlorochromate (PCC) oxidation of (2*R*,3*S*)-**40** gave the desired aldehyde (2*R*,3*S*)-**41** (69%), which was reacted with  $\alpha$ -furyl anion to afford the (1*S*,2*R*,3*S*)-secondary alcohol (**42**) stereoselectively in 72% yield. To confirm the stereochemistry of (1*S*,2*R*,3*S*)-**42**, it was converted to the known chiral intermediate, epoxy-alcohol (-)-(1*S*,2*R*,3*R*)-**43**,<sup>36a,b</sup> for the synthesis of (+)-**5**. Protection of the secondary alcohol group of (1*S*,2*R*,3*S*)-**42** as tetrahydropyranyl (THP) group



Scheme 8

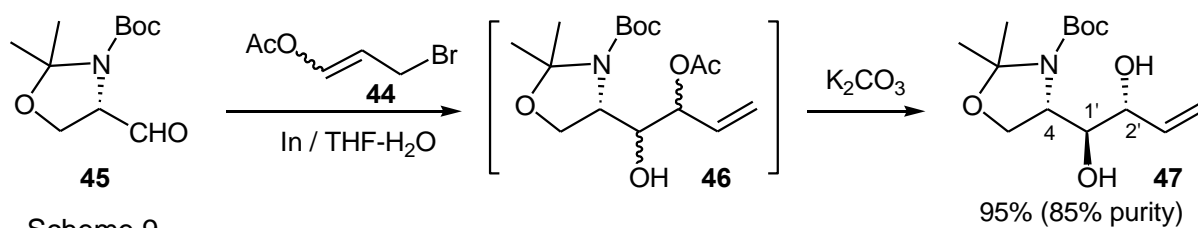
Figure 1 Felkin-Anh model for the preparation of *anti, anti*-**42**

followed by consecutive desilylation and K<sub>2</sub>CO<sub>3</sub> treatment gave an epoxide, which was subjected to deprotection of the THP group to provide the desired epoxy-alcohol (**43**) in 83% overall yield from **42**. Spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of the synthetic **43** were identical with those of the reported (-)-(1*S*,2*R*,3*R*)-**43**.<sup>36a</sup> The synthesis of (+)-**5** from (-)-(1*S*,2*R*,3*R*)-**43** was already achieved by Honda *et al.*<sup>36a</sup> The stereoselective formation of (-)-(1*S*,2*R*,3*S*)-**42** from (2*R*,3*S*)-**41** could be explained by Felkin-Anh model as shown in Figure 1.

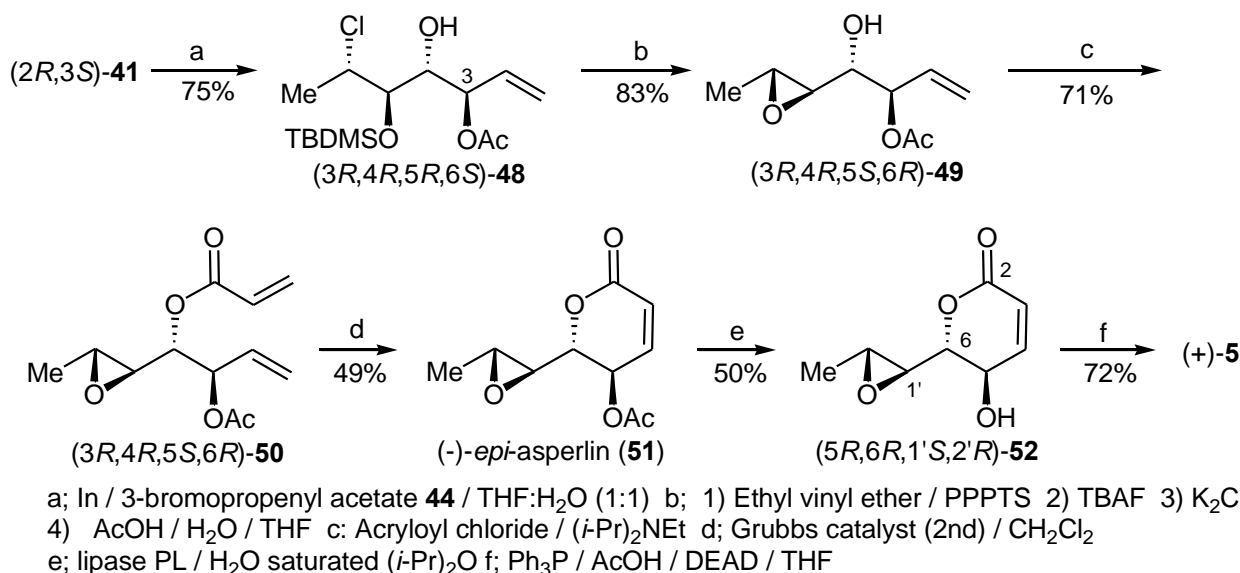
#### 4.2. Concise synthesis of (+)-asperlin (**5**)

A concise synthesis of (+)-asperlin (**5**) from (2*R*,3*S*)-**41** based on a combination of the indium (In)-assisted stereoselective addition of 3-bromopropenyl acetate (**44**)<sup>37</sup> to the  $\alpha$ -silyloxy aldehyde, (2*R*,3*S*)-**41** and ring closing metathesis (RCM) using Grubbs catalyst<sup>38</sup> is shown in Scheme 10. Recently, M. Lombardo *et al.* reported that  $\alpha$ -hydroxyallylation reaction of Garner aldehyde (**45**) using **44** in the presence of In gave predominantly a C(4)-C(1')-*anti*-C(1')-C(2')-*anti*-diol (**47**) *via* **46** as shown in Scheme 9.<sup>39</sup> This strategy could be promising for the construction of the four contiguous chiral centers

in asperlin (**5**) (Schemes 9 and 10).



The reaction of (2*R*,3*S*)-**41** with 3-bromopropenyl acetate (**44**) in the presence of In gave predominantly alcohol **48** in 75% yield, which was converted to the epoxyalcohol **49**. Protection of the secondary alcohol group of **48** as a THP group followed by consecutive desilylation and  $K_2CO_3$  treatment gave an epoxide, which was subjected to deprotection of the THP group to provide the desired epoxyalcohol (**49**) in 83% overall yield from **48**. Treatment of **49** and acryloyl chloride gave the corresponding acrylate (**50**) in 71% yield, which was subjected to RCM reaction using Grubbs catalyst 2nd generation<sup>40</sup> to afford (-)-*epi*-asperlin (**51**) in 49% yield. Spectral data ( $^1H$ - and  $^{13}C$ -NMR) of the synthetic (-)-**51** were identical with those of the reported (-)-**51**.<sup>32b</sup> Based on the conversion of **48** to (-)-**51**, the



stereochemistry of **48** was determined to be 3*R*, 4*R*, 5*R*, 6*S*-configurations. When hydrolysis of acetyl group in (-)-**51** was carried out using  $K_2CO_3$  in MeOH, the desired product (**52**) was not obtained. The lipase PL (from *Alcaligenes* sp.)-catalyzed hydrolysis of (-)-**51** in H<sub>2</sub>O saturated (*i*-Pr)<sub>2</sub>O gave the desired alcohol (**52**, 50%) along with the starting material (-)-**51** (32% recovery). Finally, the alcohol **52** was treated with AcOH in the presence of triphenylphosphine (Ph<sub>3</sub>P) and diethyl azodicarboxylate

(DEAD) to provide (+)-asperlin (**5**) in 72% yield. Spectral data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) of the synthetic (+)-**5** were identical with those of the reported (+)-**5**.<sup>36a</sup> Stereoselective formation of **48** from (2*R*,3*S*)-**41** could be explained by insights reviewed by Lombardo *et al.*<sup>41</sup> Among four possible twist-boat transition states (TSs), TS-A, -B, -C and -D as shown in Figure 2, TS-C (or TS-D) might be favored than TS-A (or

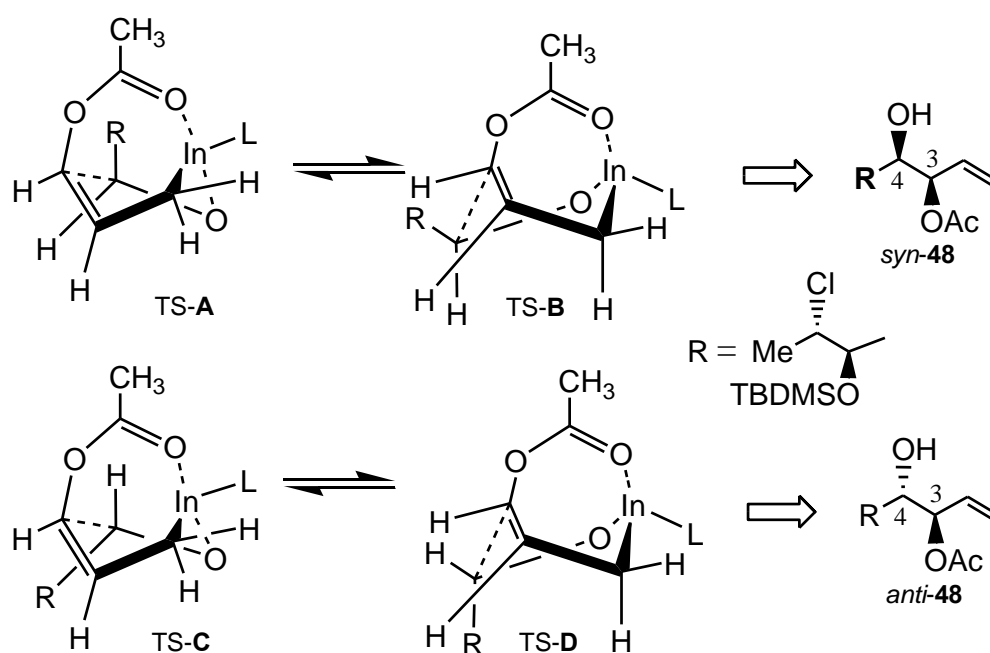


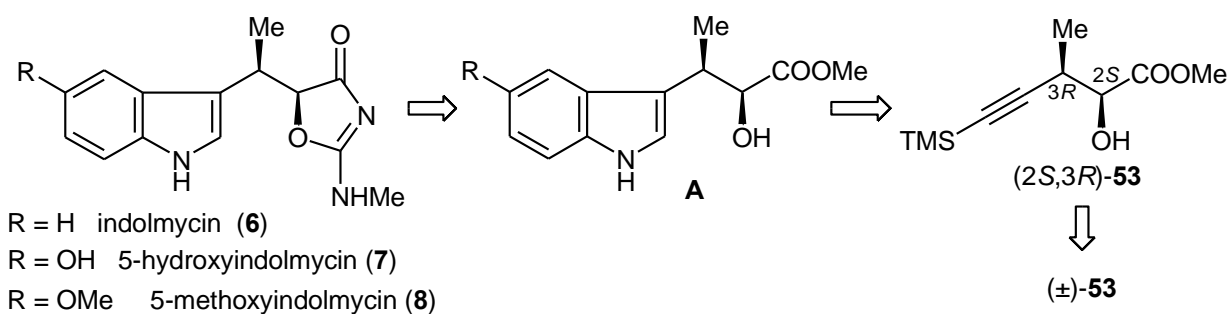
Figure 2 Chelation model for the preparation of *anti*-**48**

TS-B) because steric repulsion between the aldehyde substituent (**R**) and acetoxy group appears to be small. This insight might imply the formation of C(3)-C(4)-*anti*-**48**. On the other hand, the C(4)-C(5)-*anti*-stereoselection of **48** could be explained by Paquette *et al.*<sup>42</sup> who showed that 1,2-addition of the allylindium reagents to  $\alpha$ -oxygenated aldehydes gave the non-chelation-controlled product, which is corresponding to the 1,2-*anti* product by the Felkin-Anh model (Figure 2).

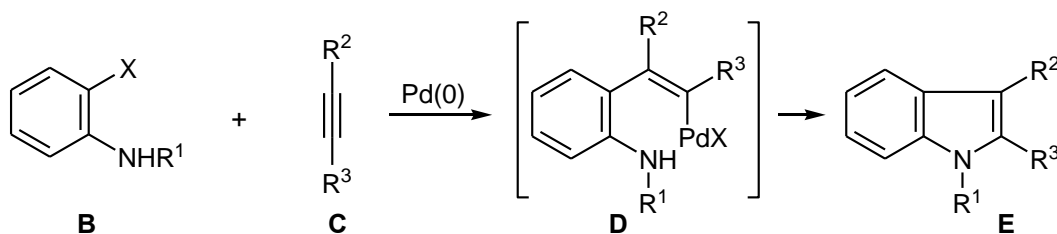
## 5. SYNTHESSES OF INDOLMYCINS (**6** AND **8**)<sup>43</sup>

(-)-Indolmycin (**6**), an antibiotic isolated from an African strain of *Streptomyces albus*, exhibits an antibacterial activity against *Staphylococci*.<sup>44</sup> Indolmycin congeners **7** and **8**, which possess a hydroxyl or methoxyl group in the 5'-position of the indole skeleton, have been obtained by the addition of indole precursors such as 5-hydroxy- and 5-methoxyindoles to growing cultures of *Streptomyces griseus* ATCC 12648.<sup>45</sup> These congeners show a moderate increase in antimicrobial activity compared to **6**. In a recent study, **6** was reported to exhibit potent antibacterial activity against *Helicobacter pylori* (*H. pylori*), and is thus a promising anti-*H. pylori* agent<sup>46</sup> (Scheme 11). Racemic syntheses of **6** have been developed by two groups.<sup>47</sup> The first asymmetric synthesis of (-)-**6** was achieved based on the reaction of

(2*R*,3*S*)-6-alkyliden-3,4-dimethyl-2-phenylperhydro-1,4-oxaazepine-5,7-dione with Grignard reagent.<sup>48</sup> Syntheses of optically active indolmycin (**6**) have been carried out using resolution method<sup>49</sup> or based on the reaction of indolyl magnesium bromide with (2*S*,3*R*)-epoxy butanoate, affording (2*S*,3*R*)-indolmycenate derivative (**A**).<sup>50</sup> Palladium-catalyzed heteroannulation of internal alkynes (**C**) using



Scheme 11



Scheme 12 Tandem Heck reaction and cyclization

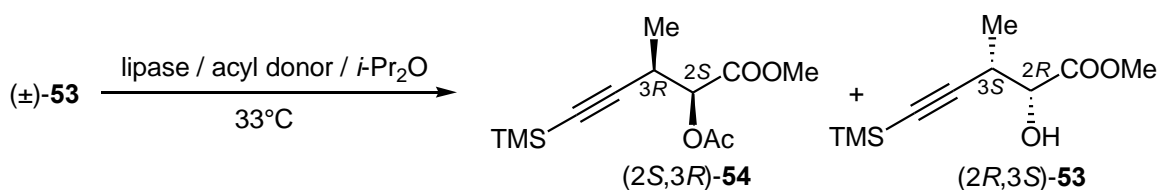
*o*-iodoaniline or its derivatives (**B**) has been reported to give indole derivatives (**E**) via a vinylpalladium intermediate (**D**) as shown in Scheme 12,<sup>51</sup> and this procedure (the Larock indole synthesis) seems to be promising for synthesis of optically active indolmycenate derivatives (**A**). The concise synthesis of (-)-indolmycin (**6**) and (-)-5-methoxyindolmycin (**8**) based on a palladium-catalyzed reaction of *o*-iodoaniline congeners and (2*S*,3*R*)-2-hydroxy-3-methyl-5-trimethylsilyl-4-pentynoate (**53**) or its acetate (**54**) was shown in Scheme 13. The optically active (2*S*,3*R*)-**53** or (2*R*,3*S*)-**53** could be obtained based on the enantioselective acetylation of (±)-**53** using lipase as shown in Table 2.

### 5.1. Lipase-catalyzed enantioselective acetylation of (±)-**53**

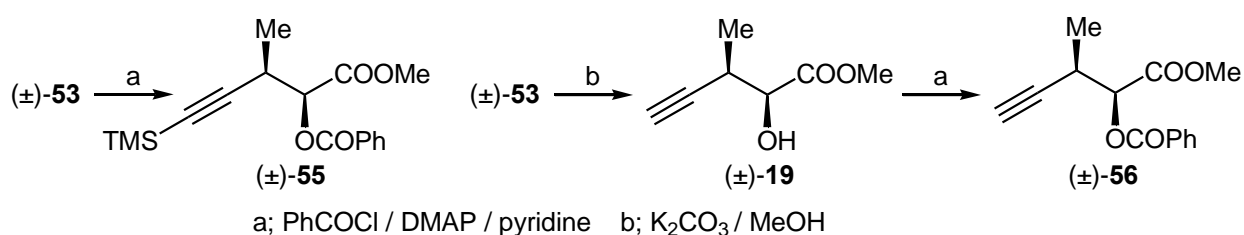
Using a previously reported procedure,<sup>15</sup> substrate (±)-**53** was obtained by reaction of (±)-*trans*-(2,3)-epoxy butanoate (**18**) (Scheme 6) and trimethylsilylacetylide. For the purpose of determining the enantiomeric excess (ee) of the enzymatic reaction products, racemate (±)-**53** was converted to two benzoates (±)-**55** and (±)-**56**, which gave two well separated peaks of enantiomeric isomers, respectively, in HPLC analysis, thus allowing determination of the ee of the enzymatic reaction products. A screening experiment for finding a suitable enzyme showed that the most effective lipase was found to be Amano P or Amano PS from *Pseudomonas* sp. When (±)-**53** was subjected to enantioselective

acetylation using “Amano P” in the presence of isopropenyl acetate as an acylating reagent for 7 days, acetate **54** (28%, 82% ee) and unreacted alcohol **53** (59%, 44% ee) were obtained (Table 2, entry 1). The 44% ee of unreacted alcohol **53** was subjected again to enzymatic acetylation using Amano PS in the presence of vinyl acetate for 3 days, giving acetate **54** (23%, 86% ee) and unreacted alcohol **53** (60%, 95% ee) (Table 2, entry 2). Enzymatic acetylation of ( $\pm$ )-**53** using Amano PS in the presence of vinyl acetate for 5 days gave acetate (+)-**54** (48%, 95% ee) and unreacted alcohol (-)-**53** (50%, 94% ee) (Table 2, entry 3). The enantiomeric excess (ee) for each enzymatic product was calculated by HPLC analysis after conversion of the enzymatic product to the corresponding benzoate. The *E*-value of this enzymatic reaction (entry 3) was estimated to be 139. The absolute structure of the enzymatic reaction product (-)-**53** was determined by direct comparison with previously reported sample<sup>52</sup> (*2R,3S*)-**53** (>99% ee), and thence the absolute configurations of acetate (**54**) were to be *2S, 3R* (Table 2).

Table 2 Lipase-catalyzed acetylation of ( $\pm$ )-**53**



Entry	Substrate	Lipase	Acyl donor	Time (days)	Products (% , % ee)
1	( $\pm$ )- <b>53</b> (5.012 g)	Amano P	isopropenyl acetate	7	( <i>2S,3R</i> )- <b>54</b> (28, 82) ( <i>2R,3S</i> )- <b>53</b> (59, 44)
2	( <i>2R,3S</i> )- <b>53</b> (44% ee) (2.980 g)	Amano PS	vinyl acetate	3	( <i>2S,3R</i> )- <b>54</b> (23, 86) ( <i>2R,3S</i> )- <b>53</b> (60, 95)
3	( $\pm$ )- <b>53</b> (5.150 g)	Amano PS	vinyl acetate	5	( <i>2S,3R</i> )- <b>54</b> (48, 95) ( <i>2R,3S</i> )- <b>53</b> (50, 94)

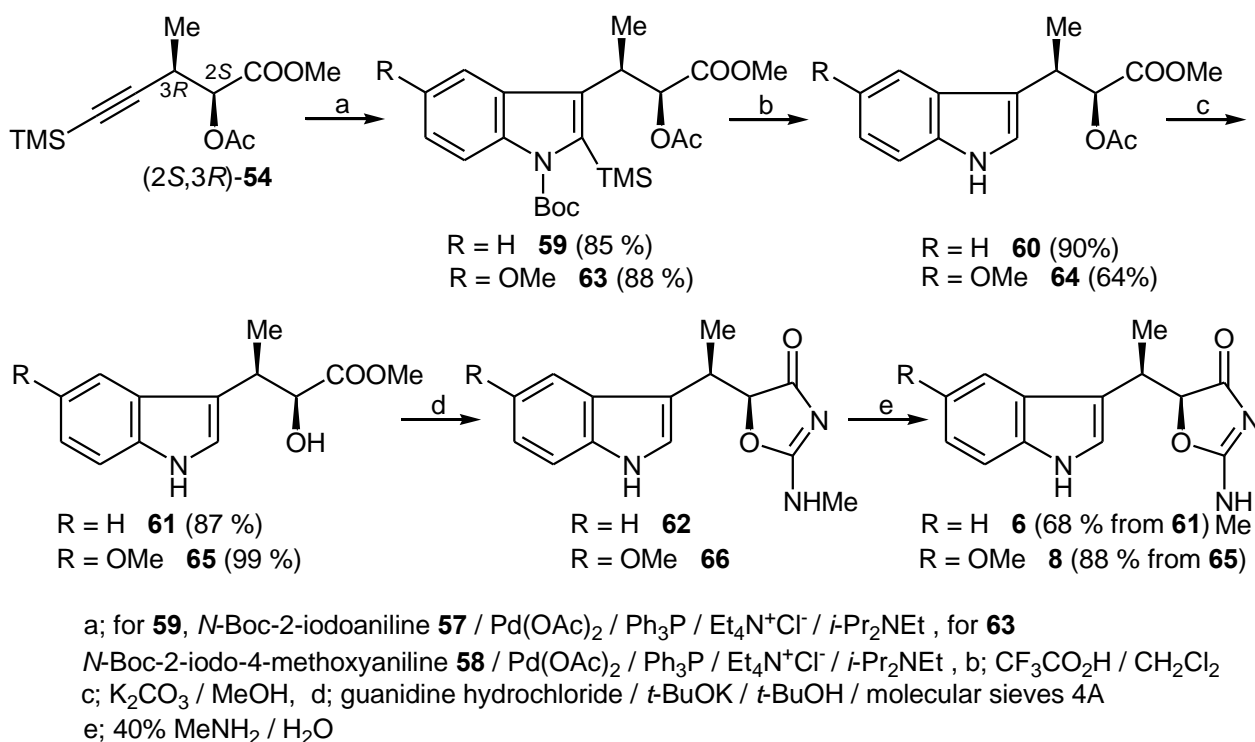


## 5.2. Synthesis of (-)-indolmycin (**6**) and (-)-5-methoxyindolmycin (**8**)

Syntheses of (-)-indolmycin (**6**) and (-)-5-methoxyindolmycin (**8**) from (*2S,3R*)-**54** are shown in Scheme 13. At first, the reaction of *o*-iodoaniline and ( $\pm$ )-**53** in the presence of [1,1-bis(diphenylphosphono)-ferrocene]palladium(II) dichloride dichloromethane complex, LiCl, and Na<sub>2</sub>CO<sub>3</sub> in DMF followed by desilylation did not give the desired indolmycenate ( $\pm$ )-**61** (Scheme 13). Then, two types of *o*-iodoaniline congeners, *N*-(*tert*-butoxycarbonyl)-2-iodoaniline (**57**) (Scheme 14) and *N*-(*tert*-Butoxycarbonyl)-2-iodo-4-methoxyaniline (**58**) (Scheme 14), were synthesized by *N*-*tert*-butoxycarbonylation of *o*-iodoaniline and the known 2-iodo-4-methoxyaniline,<sup>53</sup> respectively. Reaction of (*2S,3R*)-**54** and **57** using Pd(OAc)<sub>2</sub> in the presence of Ph<sub>3</sub>P, Et<sub>4</sub>N<sup>+</sup>Cl and *i*-Pr<sub>2</sub>NEt gave the indole congener **59** in 85% yield,

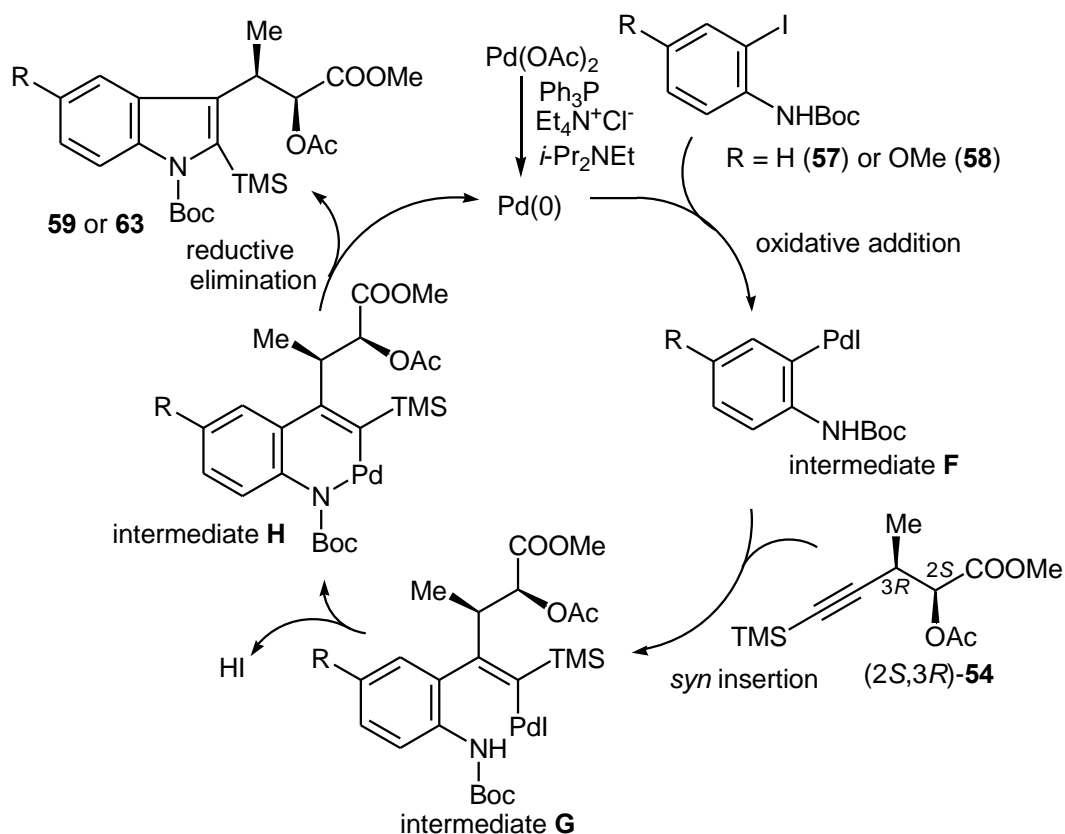


this was treated with  $\text{CF}_3\text{COOH}$  to afford **60** in 90% yield. Alcoholysis of **60** with  $\text{K}_2\text{CO}_3$  in MeOH gave (+)-indolmycenate (**61**, 87% yield), whose physical data were consistent with those of the reported (+)-**61**,<sup>18,54</sup> including the sign of the specific rotation. The reaction of (+)-**61** with guanidine hydrochloride in the presence of *tert*-BuOK using a previously reported procedure<sup>50</sup> gave 2-amino-4(5*H*)-oxazolone congener **62**, which was reacted with 40% methylamine to afford (-)-indolmycin (**6**, 68% yield from **61**). The physical data of synthetic (-)-**6** were consistent with those of previously reported indolmycin **6**.<sup>48</sup> From NMR study of synthetic (-)-**6**, (-)-**6** was found to be a 2:1 mixture of two tautomers **6** and its tautomer. The main tautomer was the desired (-)-**6**. Isolation of two tautomers was found to be difficult and this tendency was found in the previous case.<sup>50</sup> Similarly, the reaction of



Scheme 13

(2*S*,3*R*)-**54** and **58** using  $\text{Pd}(\text{OAc})_2$  in the presence of  $\text{Ph}_3\text{P}$ ,  $\text{Et}_4\text{N}^+\text{Cl}$  and *i*- $\text{Pr}_2\text{NEt}$  gave the indole congener **63** (88% yield), which was treated with  $\text{CF}_3\text{COOH}$  to afford **64** in 64% yield. Alcoholysis of **64** with  $\text{K}_2\text{CO}_3$  in MeOH gave (-)-5-methoxyindolmycenate (**65**) in 99% yield. The reaction of (-)-**65** with guanidine hydrochloride in the presence of *tert*-BuOK provided 2-amino-4(5*H*)-oxazolone congener (**66**), which was reacted with 40% methylamine to afford (-)-5-methoxyindolmycin (**8**, 88% yield from **65**). From NMR study of synthetic (-)-**8**, (-)-**8** was also found to be a 2:1 mixture of two tautomers **8** and its tautomer.



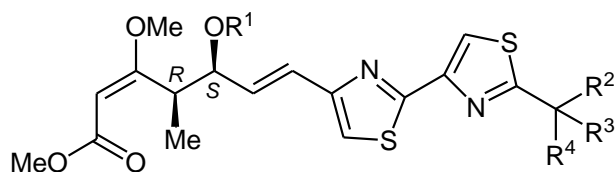
Scheme 14

The formation of indole congener (**59** and **63**) by a palladium(II)-catalyzed reaction of internal alkyne  $(2S,3R)$ -**54** with *o*-iodoaniline derivative **57** and **58**, respectively, and the corresponding catalytic cycle may be explained as shown in Scheme 14. First, palladium (II) is converted to palladium (0) under the specified reaction conditions. Oxidative addition of *o*-iodoaniline congener **57** or **58** to  $\text{Pd}(0)$  gives intermediate **F**, which undergoes *syn* insertion of internal alkyne  $(2S,3R)$ -**54** into the arylpalladium bond to afford intermediate **G**. Next, nitrogen displacement of the palladium in the resulting vinylpalladium intermediate **G** results in the formation of a six-membered-ring intermediate **H** along with elimination of hydrogen iodide (HI); finally, reductive elimination of palladium from intermediate **H** gives indole congener **59** or **63** and  $\text{Pd}(0)$ . Annulation of unsymmetrical alkynes has proved to be highly regioselective, providing only the appropriate regioisomer. The more sterically bulky group is located nearer the nitrogen atom in the indole product<sup>51</sup> (Scheme 14).

## 6. SYNTHESSES OF CYSTOTHIAZOLES AND MELITHIAZOLS

In 1998, Sakagami and co-workers reported the isolation of six secondary metabolites, named cystothiazoles A (**67**)-G (**72**) from a culture broth of the myxobacterium, *Cystobacter fuscus*.<sup>55</sup> On the other hand, in 1999, G. Höfle and co-workers reported the isolation of thirteen new  $\beta$ -methoxyacrylate (MOA) fungicides, melithiazols A-N related to myxothiazol A (**10**)<sup>56</sup> from cultures of *Melittangium*

*lichenicola*, *Archangium gephyra* and *Myxococcus stipitatus*.<sup>57</sup> They are related to the oudemansins A(1),<sup>3</sup> B(2),<sup>4</sup> X(3),<sup>5</sup> which are naturally occurring congeners of  $\beta$ -methoxyacrylic acid. Myxothiazol A (10), cystothiazoles and melithiazols possess a bis-thiazole skeleton as well as a  $\beta$ -methoxyacrylate moiety. Cystothiazoles have indicated potent antifungal activity against the phytopathogenic fungus, *Phytophthora capsici* (0.05-5  $\mu\text{g}/\text{disk}$ ), and have shown activity against a broad range of additional fungi with no effect on bacterial growth. Furthermore, cystothiazole A (67) was examined for the *in vitro* cytotoxicity using human colon carcinoma HCT-116 and human leukaemia K562 cells. The  $\text{IC}_{50}$  values of cystothiazole A (67) were 110~130 ng/ml, which were significantly higher than those of myxothiazol A. The fungicidal activity of these  $\beta$ -methoxyacrylate (MOA) inhibitors has been shown to be due to their ability to inhibit mitochondrial respiration by blocking electron transfer between cytochrome b and cytochrome c. The absolute structure of cystothiazole A (67) was established by a combination of spectroscopic analysis and chemical degradation of the natural product. The structures of melithiazols



- cystothiazole A (67) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$ ,  $\text{R}^4=\text{Me}$   
 cystothiazole B (68) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{OH}$ ,  $\text{R}^3=\text{Me}$ ,  $\text{R}^4=\text{Me}$   
 cystothiazole C (69) :  $\text{R}^1=\text{H}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$ ,  $\text{R}^4=\text{Me}$   
 cystothiazole D (70) :  $\text{R}^1=\text{H}$ ,  $\text{R}^2, \text{R}^3=-\text{CH}_2$ ,  $\text{R}^4=\text{Me}$   
 cystothiazole F (71) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{CH}_2\text{OH}$ ,  $\text{R}^4=\text{Me}$   
 cystothiazole G (72) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{H}$ ,  $\text{R}^4=\text{Me}$   
 melithiazol B (73) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2, \text{R}^3=-\text{CH}_2$ ,  $\text{R}^4=\text{Me}$   
 melithiazol E (74) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$ ,  $\text{R}^4=\text{Me}$  (cystothiazole A (67))  
 melithiazol F (75) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{H}$ ,  $\text{R}^4=\text{Ph}$   
 melithiazol G (76) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{Me}$ ,  $\text{R}^4=\text{Et}$   
 melithiazol H (77) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{H}$ ,  $\text{R}^4=\text{Me}$  (cystothiazole G (72))  
 melithiazol I (78) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2=\text{H}$ ,  $\text{R}^3=\text{H}$ ,  $\text{R}^4=i\text{-Pr}$   
 melithiazol M (79) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2, \text{R}^3=-\text{O}-$ ,  $\text{R}^4=\text{Me}$   
 melithiazol N (80) :  $\text{R}^1=\text{Me}$ ,  $\text{R}^2, \text{R}^3=-\text{CH}_2\text{O}-$ ,  $\text{R}^4=\text{Me}$

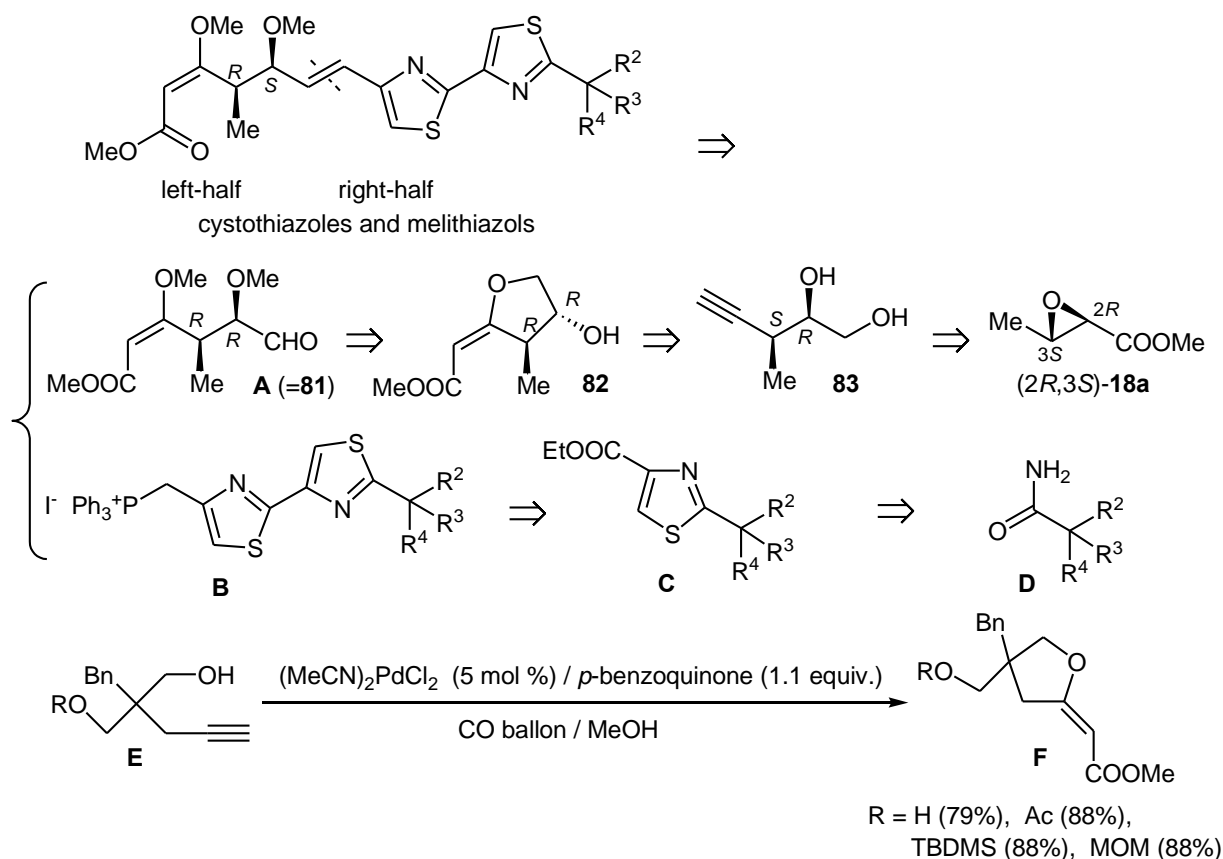
Scheme 15

were established on the basis of spectroscopic data, and confirmed in the case of melithiazol E (74), including its relative configuration, by an X-ray structure analysis. The absolute configuration of

melithiazols A and B (**73**) was determined by degradation and CD spectroscopy. Biological activities including antifungal and cytotoxic activities, inhibition of NADH oxidation, etc., were also examined (Scheme 15).

### 6.1. Retrosynthesis of cystothiazole and melithiazols<sup>58</sup>

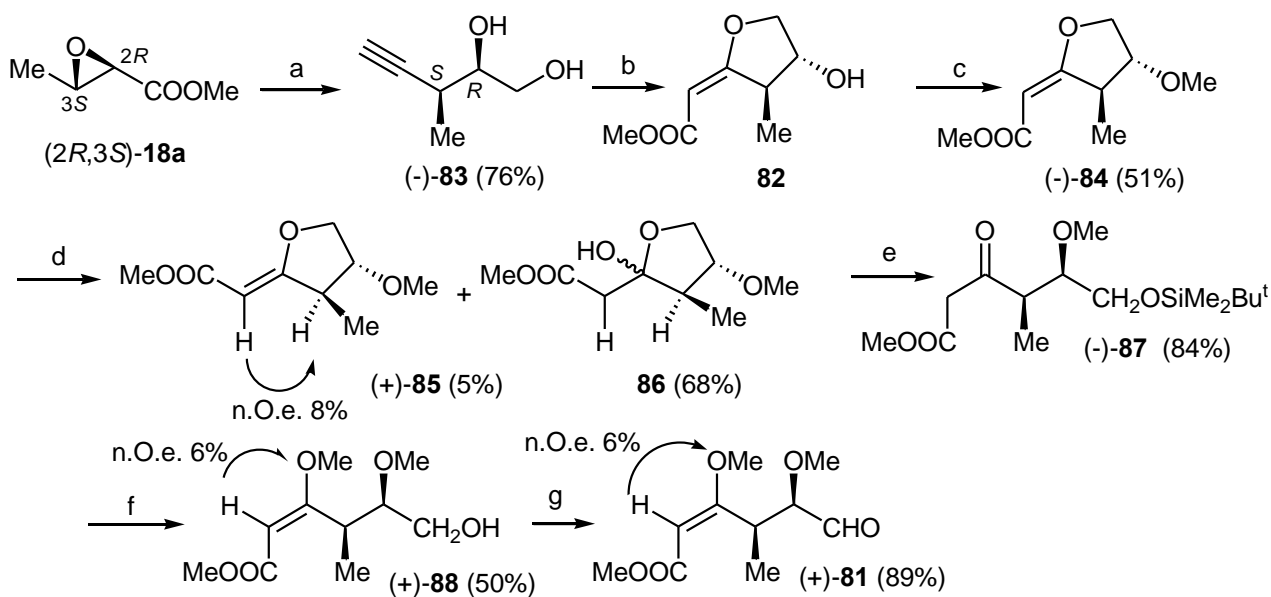
Total synthesis of a diastereomeric mixture of myxothiazol A (**10**) has been achieved,<sup>59</sup> but chiral synthesis of the left-half part possessing two chiral centers has not been carried out. Another racemic synthesis of the left-half part of **10** starting from benzyloxyacetaldehyde is also reported.<sup>60</sup> The first enantiocontrolled synthesis of cystothiazole A (**67**) was achieved based on the preparation of a bis-thiazole core for the application of the asymmetric Evans aldol methodology for the development of the C(4)/C(5) vicinal stereochemistry.<sup>61</sup> Semisynthesis of melithiazols B (**73**) and M (**79**) was achieved based on the chemical conversion from myxothiazol A (**10**) possessing (4*R*,5*S*,14*S*)-absolute configurations.<sup>62</sup> After our synthesis of cystothiazole A (**67**) and B (**68**) was reported, syntheses of cystothiazole A (**67**),<sup>63-67</sup> B (**68**),<sup>65</sup> C (**69**),<sup>61,63</sup> and E (**112**, see Scheme 20)<sup>68</sup> were reported. Our retrosynthetic strategy of cystothiazoles and melithiazols is illustrated in Scheme 16 (Scheme 16). Retrosynthetically, the synthesis of cystothiazoles and melithiazols can be achieved by Wittig condensation of the left-half aldehyde **A** (=81) and the right-half phosphonium iodide **B**. The aldehyde (**81**) can be derived from the tetrahydro-2-furylidene acetate (**82**), which can be obtained by oxidative cyclization-methoxycarbonylation of (2*R*,3*S*)-3-methylpenta-4-yn-1,2-diol (**83**) in the presence of Pd(II) /*p*-benzoquinone in MeOH under a carbon monoxide atmosphere. The synthesis of the chiral diol **83** can be achieved by the reaction of (2*R*,3*S*)-epoxy butanoate (**18a**)<sup>16,18,25</sup> and silyl-acetylide followed by reduction. An important chiral synthon (2*R*,3*S*)-**18a** has been synthesized based on the lipase-catalysed asymmetric hydrolysis of (±)-(2,3)-*trans*-3-acetoxy-2-chlor-butanoate (**36a**) as described in 3.1.<sup>18,25</sup> A catalytic conversion of **83** to **82** is a key step in this total synthesis, and this type reaction was previously reported as shown in Scheme 16.<sup>69</sup> The oxidative cyclization-methoxy-carbonylation of acyclic-4-yne-1-ols **E** affords (*E*)-cyclic-β-alkoxyacrylates **F** in good to excellent yields.<sup>69</sup> In this reaction, *p*-benzoquinone is found to be a very efficient reagent for trapping a proton of hydrogen chloride arising from the catalytic cycle and oxidative transfer of the generated Pd(0) species to a Pd(II) species. The synthesis of right-half phosphonium salt **B** could be achieved from an amide **D** as shown in Scheme 16. The thioamide derived from amide **D** was first subjected to Hantzsch reaction with ethyl bromopyruvate leading to the thiazole ester intermediate **C**. Following manipulation of the ester functionality in **C** to the corresponding thioamide, a second Hantzsch reaction with ethyl bromopyruvate then gave the bis-thiazole ester, which could be converted to the corresponding phosphonium salt **B**.



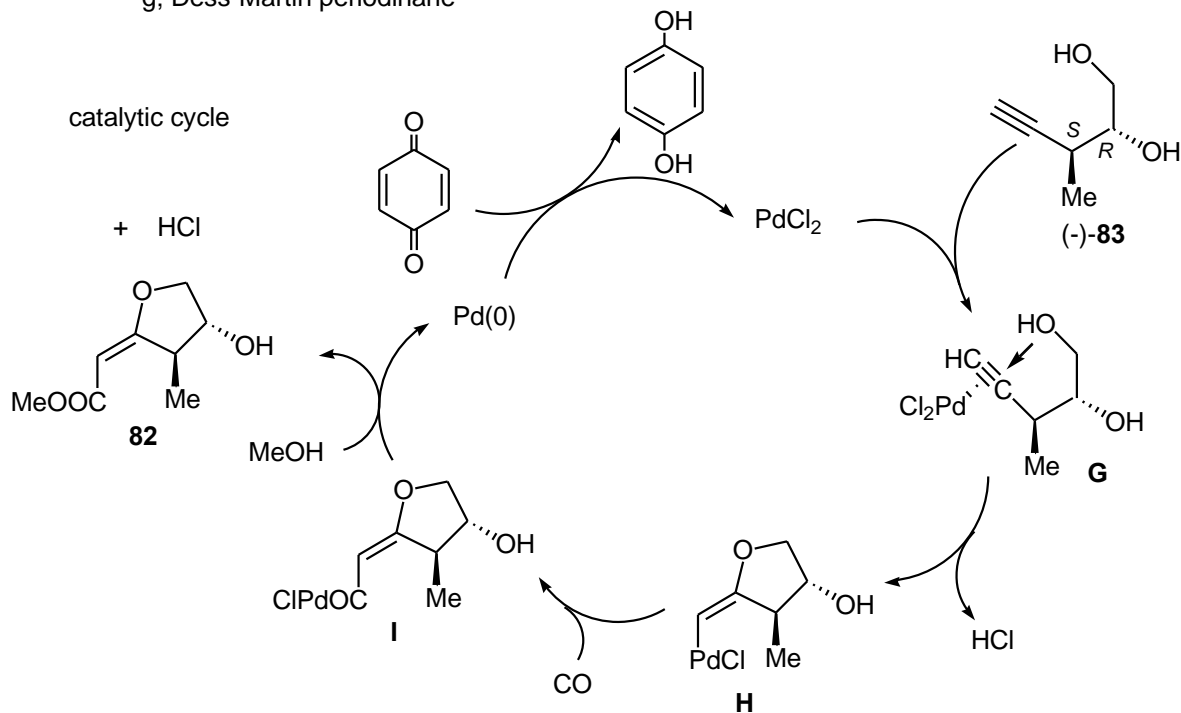
Scheme 16

## 6.2. Synthesis of left-half aldehyde (**81**)<sup>58</sup>

As we previously described the synthesis of oudemansin B (**2**) in **2.2**, the reaction of (2*R*,3*S*)-epoxy butanoate (**18a**) and lithium silyl-acetylide in the presence of Et<sub>2</sub>AlCl followed by consecutive desilylation and reduction gave (-)-**83** in 76% overall yield. The oxidative cyclization-methoxycarbonylation of (-)-**83** in the presence of PdCl<sub>2</sub> (5 mol %)/*p*-benzoquinone (1.1 equiv.) in MeOH at 0 °C under a carbon monoxide atmosphere (balloon) gave a crude secondary alcohol (**82**), which was immediately subjected to methylation using MeI in the presence of Ag<sub>2</sub>O to afford the methoxy compound (-)-**84** in 51% overall yield. Acid treatment of (-)-**84** provided a hemiketal **86** (68%) along with the isomerized product (+)-**85** (5%). The geometry of (+)-**85** was confirmed to be *Z*-form because of n.o.e. enhancement for the olefinic proton and methine proton (8%); thence, that of (-)-**84** was deduced to be *E*-form. Silylation of **86** in DMF at 80 °C gave a silyl ether (-)-**87** in 84% yield, which was subjected to consecutive methylation (Me<sub>2</sub>SO<sub>4</sub>/K<sub>2</sub>CO<sub>3</sub>) and desilylation (Et<sub>3</sub>N(HF)<sub>3</sub>) to afford an alcohol (+)-**88** in 50% overall yield. The (*E*)-geometry of (+)-**88** was confirmed by the n.o.e. enhancement for the olefinic proton and the methoxyl group (6%). Dess-Martin periodinane oxidation of (+)-**88** afforded the desired aldehyde (+)-**81** in 89% yield, whose NMR spectra were identical with those of the reported (±)-**81**.<sup>60</sup> As the reaction mechanism, the following path is plausible based on the precedents (Scheme 17). Coordination of the triple bond of the substrate (-)-**83** to the Pd(II) species



- a; 1)  $\text{Li}^+ \text{C}\equiv\text{C}-\text{SiMe}_3 / \text{Et}_2\text{AlCl}$  2)  $\text{Bu}_4\text{N}^+ \text{F}^-$  3)  $\text{LiBH}_4$   
 b;  $\text{PdCl}_2$  (5 mol %) / CO (balloon) / *p*-benzoquinone / MeOH  
 c; MeI /  $\text{Ag}_2\text{O}$  d; dil. HCl / THF e;  $^t\text{BuMe}_2\text{SiCl}$  / imidazole / DMF  
 f; 1)  $\text{Me}_2\text{SO}_4 / \text{K}_2\text{CO}_3$  / acetone 2)  $\text{Et}_3\text{N}(\text{HF})_3 / \text{CH}_2\text{Cl}_2$   
 g; Dess-Martin periodinane



Entry	Pd	Condition	Yield (%)
1	$\text{PdCl}_2$	0 °C, 2 h	76
2	$\text{PdCl}_2(\text{MeCN})_2$	0 °C, 1 h	81
3	$\text{PdCl}_2(\text{PhCN})_2$	0 °C, 1 h	45

Scheme 17

enables the inner primary hydroxyl group to attack the unsaturated bond, giving rise to  $\sigma$ -Pd complex **H**. It is well documented that the C-Pd bond undergoes CO insertion to generate intermediate **I**, which is reacted with MeOH to provide the product **82**. It is a well-established feature of the reactions initiated by intramolecular nucleophilic attack on the triple bond coordinated to Pd(II) that the attack is *anti* with respect to palladium. The exclusive occurrence of *E* stereochemistry in product **82** is clearly in agreement with this mechanistic hypothesis (Scheme 17).

### 6.3. Syntheses of cystothiazoles A (**67**),<sup>58</sup> B (**68**)<sup>70</sup> and melithiazol B (**73**)<sup>71</sup>

Antifungal substances named cystothiazoles A (**67**) and B (**68**) were isolated from the myxobacterium *Cystobacter fuscus* strain AJ-13278 by using an inhibition assay against the phytopathogenic fungus *Phytophthora capsici*.<sup>55</sup> The structure of cystothiazole B (**68**) was deduced based on similar <sup>1</sup>H-NMR data (H-4 and H-5) and specific rotations of cystothiazole A (**67**). Melithiazols B (**73**) and M (**79**) have been isolated from myxobacterium *Archangium gephyra*, strain Ar 7747, and exhibit antifungal and cytotoxic activity, inhibition of NADH oxidation. The structures of B (**73**) and M (**79**) were established on the basis of spectroscopic analysis and the absolute configuration of B (**73**) was determined by CD spectroscopy.<sup>57</sup> Semisynthesis of B (**73**) and M (**79**) was achieved based on the chemical conversion from myxothiazol A (**10**) possessing (4*R*,5*S*,14*S*)-absolute configuration.<sup>62</sup> The synthesis of three phosphonium iodides (**B=89, 90 and 91**) was achieved as shown in Scheme 18.

Treatment of isopropylamide (**92**) with P<sub>4</sub>S<sub>10</sub> gave an isopropylthioamide (**93**), which was reacted with bromopyruvate to afford a mono-thiazole ester (**94**) in 82% overall yield from **92**. Treatment of **94** with NH<sub>3</sub> / MeOH followed by thioamidation with Lawesson's reagent yielded a thioamide (**96**), which was reacted with bromopyruvate to afford a bithiazole ester (**97**) in 81% overall yield from **94**. LiBH<sub>4</sub> reduction of **97** followed by treatment with I<sub>2</sub>/Ph<sub>3</sub>P/imidazole provided an iodide (**99**) in 70% overall yield from **97**. The reaction of **99** and triphenylphosphine gave a phosphonium salt (**89**) in 80% yield. Treatment of **97** with *N*-bromosuccinimide (NBS) in the presence of 2,2'-azobisisobutyronitrile (AIBN) gave a bromo compound, which was treated with saturated NaHCO<sub>3</sub> aqueous solution to give an alcohol (**100**) in 91% overall yield from **97**. Diisobutylaluminium hydride (Dibal-H) reduction of **100** followed by treatment with I<sub>2</sub>/Ph<sub>3</sub>P/imidazole provided an iodide (**102**) in 76% overall yield. The reaction of **102** and triphenylphosphine gave a phosphonium salt (**90**) in 90% yield. Treatment of alcohol (**100**) with *p*-TsOH gave an olefin (**103**) in 88% yield, which was reacted with Dibal-H to afford a primary alcohol (**104**) in 65% yield. Treatment of **104** with I<sub>2</sub>/Ph<sub>3</sub>P/imidazole provided an iodide (**105**) in 86% yield. The reaction of **105** with triphenylphosphine gave a phosphonium salt (**91**) in 98% yield.





isomers were isolated by preparative silica-gel thin-layer chromatography to provide (+)-**67** and (*Z*)-isomer. The reaction of **81** and **90** in the presence of LHMDS in THF gave a mixture (*E/Z* = 3/1) of cystothiazole B (**68**) and (*Z*)-cystothiazole B in 69% yield. The condensation reaction conditions were not optimized at this stage. This mixture was subjected to preparative thin-layer chromatography (silica gel) to provide a colorless oil (+)-**68** and a colorless oil (*Z*)-isomer. The physical data of the synthetic (+)-**68** were identical with those of the natural cystothiazole B (+)-**68**.<sup>55</sup> The stereochemistry of cystothiazole B (**68**) was proved to be (4*R*, 5*S*, 6(*E*)). The reaction of **81** and **91** in the presence of LHMDS in THF gave a 3:1 (*E*)/(*Z*) mixture of olefins (**73**) in 39% yield. A part of this mixture was subjected to purification by means of preparative silica-gel thin-layer chromatography (CH<sub>2</sub>Cl<sub>2</sub>) to provide (+)-**73** as a colorless oil. The condensation yield was found to be unsatisfactory, but reaction conditions were not optimized at this time. The physical data of the synthetic (+)-**73** were identical with those of the reported melithiazol B (**73**).<sup>56</sup> Moreover, the physical data of the synthetic (+)-**73** were identical with those of melithiazol B (**73**) from the myxobacterium *Cystobacter fuscus* strain AJ-13278.<sup>71</sup>

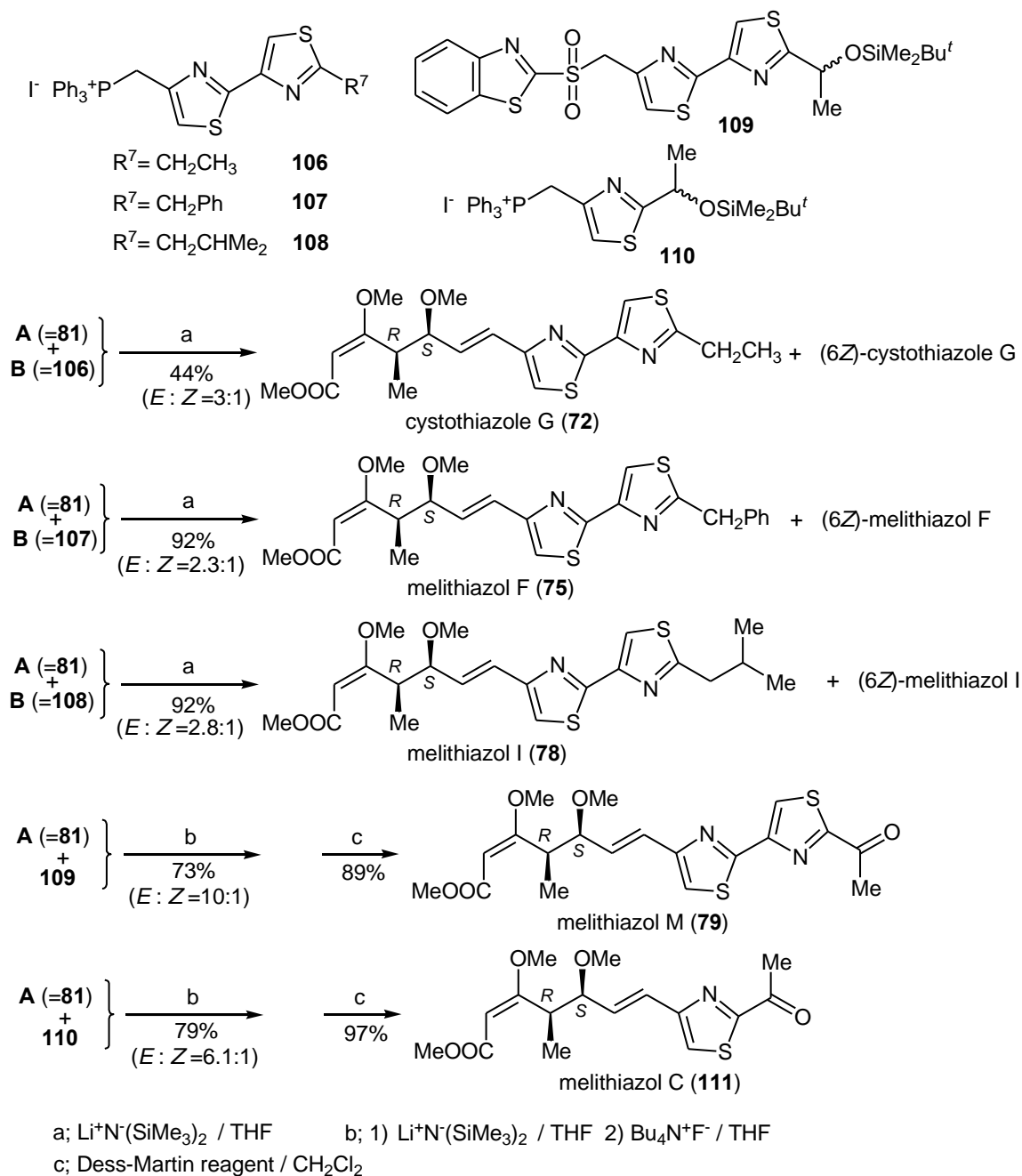
#### 6.4. Syntheses of cystothiazole G (**72**)<sup>72</sup> and melithiazols F (**75**),<sup>73</sup> I (**78**),<sup>73</sup> M (**79**)<sup>71</sup> and C (**111**)<sup>74</sup>

Relative structures of cystothiazole G (**72**) and melithiazols F (**75**), I (**78**), M (**79**) and C (**111**) were reported although the absolute structures of these natural products were not yet. For the purpose of determination of the absolute structures of these natural products, the reactions of (+)-**81** and phosphonium salts (**106**, **107** and **108**) in the presence of LHMDS was carried out to give cystothiazole G (**72**) and melithiazols F (**75**), I (**78**), respectively. Moreover, the reactions of (+)-**81** and phosphonium salt (**110**), and sulfone (**109**) in the presence of LHMDS afforded two condensation products, which were converted to melithiazols C (**111**) and M (**79**), respectively. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of the synthetic compounds (**72**, **75**, **78**, **79** and **111**) were identical to those of the natural products (**72**, **75**, **78**, **79** and **111**) including the sign of specific rotation, respectively, and the absolute structures of these natural products were determined as shown in Scheme 19.

### 7. SYNTHESIS OF CYSTOTHIAZOLE E (**112**)<sup>74</sup>

Cystothiazole E (**112**)<sup>55</sup> was isolated from *Cystobacter fuscus* strain AJ-13278. The absolute structure of cystothiazole E (**112**) was confirmed by the synthesis of antipodal **112**.<sup>68</sup> Retrosynthetically, the synthesis of **112** can be achieved by the double bond formation between the left-half aldehyde **A** and the right-half sulfone (**113**) as shown in Scheme 20. By applying the previously reported procedure,<sup>58</sup> the reaction of (2*R*,3*S*)-epoxy butanoate **18a** and lithium silyl-acetylide in the presence of Et<sub>2</sub>AlCl gave **53** in 71% yield. Desilylation of **53** followed by hydrolysis gave an acetylenic α-hydroxy-carboxylic acid **115**, which was treated with 5 mol % of Pd(MeCN)<sub>2</sub>Cl<sub>2</sub> and Et<sub>3</sub>N in MeOH to give methyl ketone (**116**) in

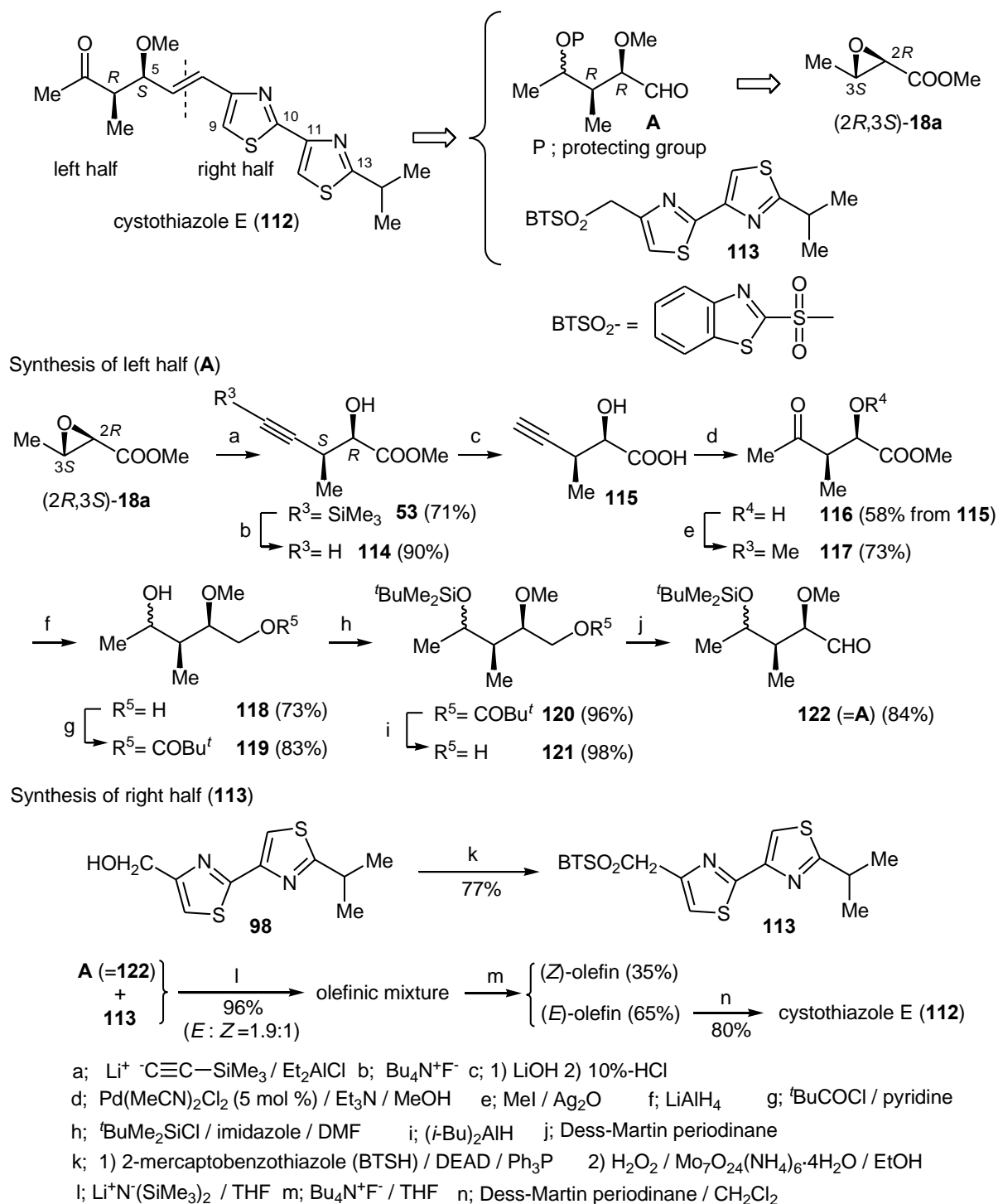
58% yield from **115**. Methylation of **116** gave  $\alpha$ -methoxy ester **117** (73%), which was subjected reduction to afford a diastereomeric mixture of diol (**118**) in 73% yield. Selective protection of primary



Scheme 19

hydroxyl group in **118** followed by silylation gave a diastereomeric mixture of **120** in 96% yield. Reductive deprotection of pivaloyl group in **120** gave a diastereomeric mixture of **121** in 98% yield, which was subjected to the Dess-Martin oxidation to afford a diastereomeric mixture of aldehyde (**122** = **A**) in 84% yield. The sulfone (**113**) corresponding left-half was obtained by treatment of alcohol (**98**) (see Scheme 18) with 2-mercaptobenzothiazole (BTSH)/ $\text{Ph}_3\text{P}$ /diethylazodicarboxylate (DEAD) followed by oxidation with 30%  $\text{H}_2\text{O}_2$  in the presence of  $\text{Mo}_7\text{O}_{24}(\text{NH}_4)_6 \cdot 4\text{H}_2\text{O}$  in 77% yield. The Julia's

coupling<sup>75</sup> of the sulfone(**113**) and aldehyde (**122**) in the presence of LHMDS in THF to afford a mixture of olefins in 96% yield.



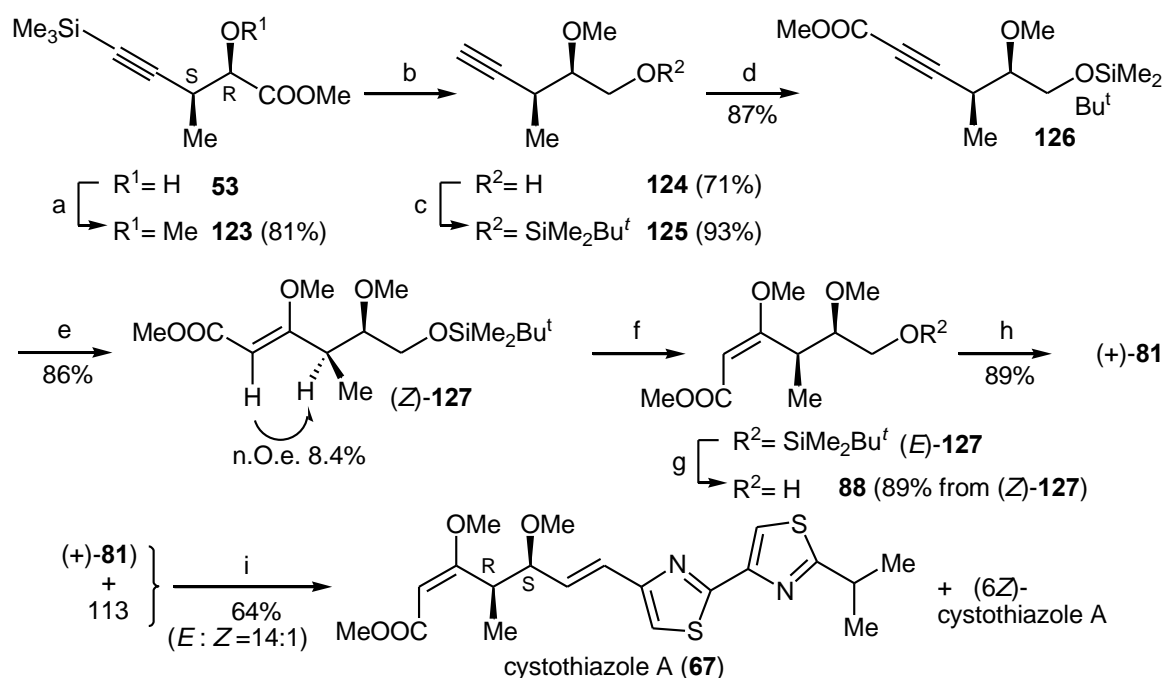
Scheme 20

Deprotection of silyl group of the olefinic mixture gave the less polar alcohol (35%) and the more polar alcohol (65%). The geometry of the less polar alcohol was found to be *Z*-form by the coupling constant ( $J=12.0$  Hz) due to olefinic proton and thence that of the more polar alcohol was deduced to be (*E*)-form. Dess-Martin oxidation of (*E*)-alcohol afforded methyl ketones (*E*)-**112** (80%). The specific rotation and

NMR data of the synthetic (*E*)-**112** were identical with those of natural cystothiazole E (**2**).<sup>55b</sup>

## 8. IMPROVED SYNTHESIS OF CYSTOTHAIAZOLE A (**67**)<sup>52</sup>

In this section, a new chiral synthesis of cystothiazole A (**67**) for the purpose of improvement of the overall yield and the (*E*)-selectivity of the C(6)/C(7) double bond was carried out as shown in Scheme 21. Methylation of **53** (Scheme 20) followed by consecutive reduction and silylation afforded the silyl ether **125** possessing the terminal acetylene group in 52% overall yield from **53**. By applying Tsuji's procedure,<sup>76</sup> the acetylenic compound **125** was converted to acetylenecarboxylate **126** in 87% yield under atmospheric pressure (balloon) of carbon monoxide at room temperature using 5 mol % of PdCl<sub>2</sub> and a stoichiometric amount of CuCl<sub>2</sub> in MeOH. By applying the reported procedures,<sup>77</sup> conjugate addition of MeOH to acetylenecarboxylate **126** in the presence of a catalytic amount of Bu<sub>3</sub>P afforded the corresponding (*Z*)- $\beta$ -methoxy- $\alpha,\beta$ -unsaturated ester **127** in 86% yield. The (*Z*)-geometry of **127** was confirmed by the n.O.e. enhancement for the olefinic proton and the methine proton (8.6%). The formation of (*Z*)-**127** from **126** could be explained as shown in Scheme 22. Addition of tributyl phosphine to **126** followed by MeOH attack would give intermediates **TS-A** and **TS-B**. Intermediate **TS-B** could lead to (*Z*)-**127** because **TS-B** is more stable than **TS-A** due to stereochemical repulsion in **TS-A**. Isomerization of (*Z*)-**127** to (*E*)-**127** was carried out by the following procedure. When a solution of

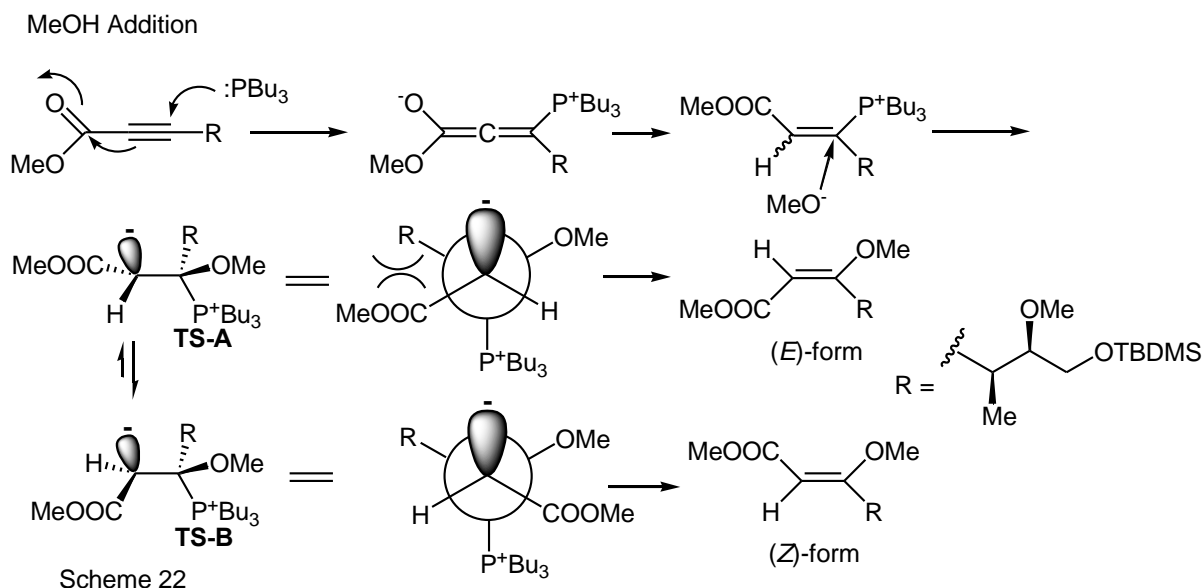
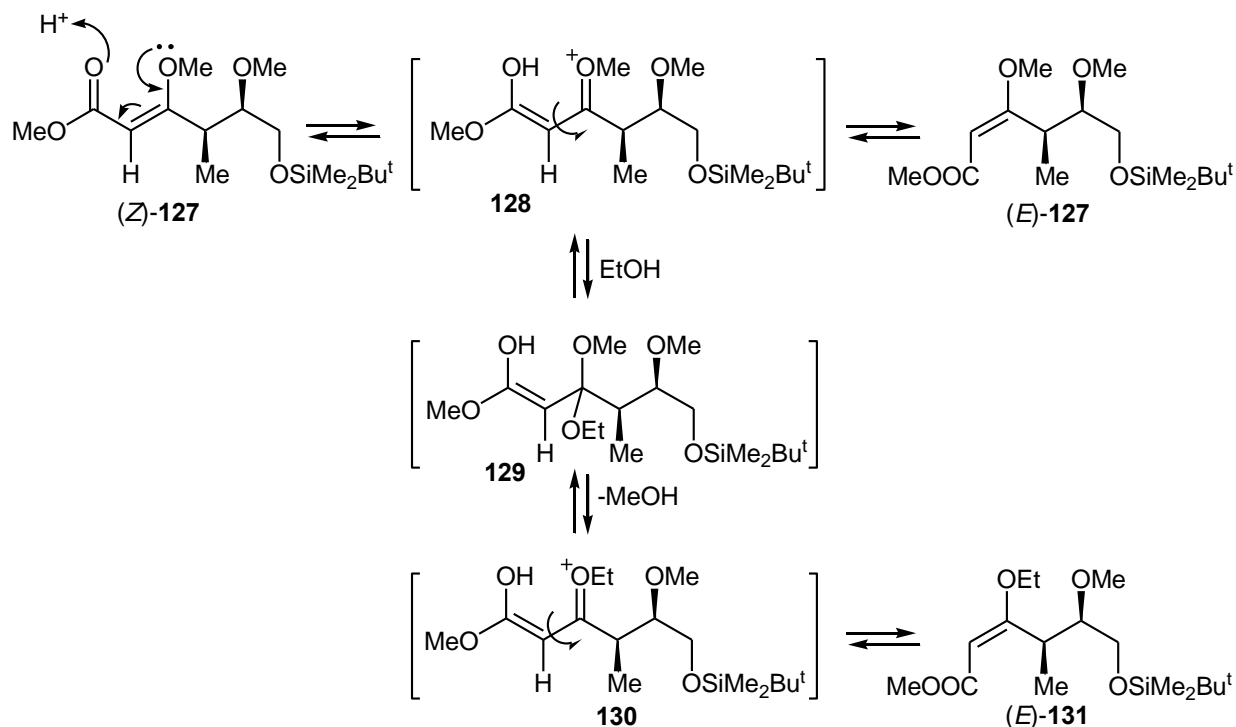


a; MeI / Ag<sub>2</sub>O      b; 1) Bu<sub>4</sub>N<sup>+</sup>F<sup>-</sup> 2) LiBH<sub>4</sub>      c; <sup>t</sup>BuMe<sub>2</sub>SiCl / imidazole / DMF

d; PdCl<sub>2</sub> (5 mol %) / CuCl<sub>2</sub> / NaOAc / CO (balloon) / CH<sub>2</sub>Cl<sub>2</sub>      e; Bu<sub>3</sub>P/MeOH

f; CDCl<sub>3</sub> (D; 99.8%)      g; Et<sub>3</sub>N·(HF)<sub>3</sub> / CH<sub>2</sub>Cl<sub>2</sub>      h; Dess-Martin periodinane      i; Li<sup>+</sup>N<sup>-</sup>(SiMe<sub>3</sub>)<sub>2</sub> / THF

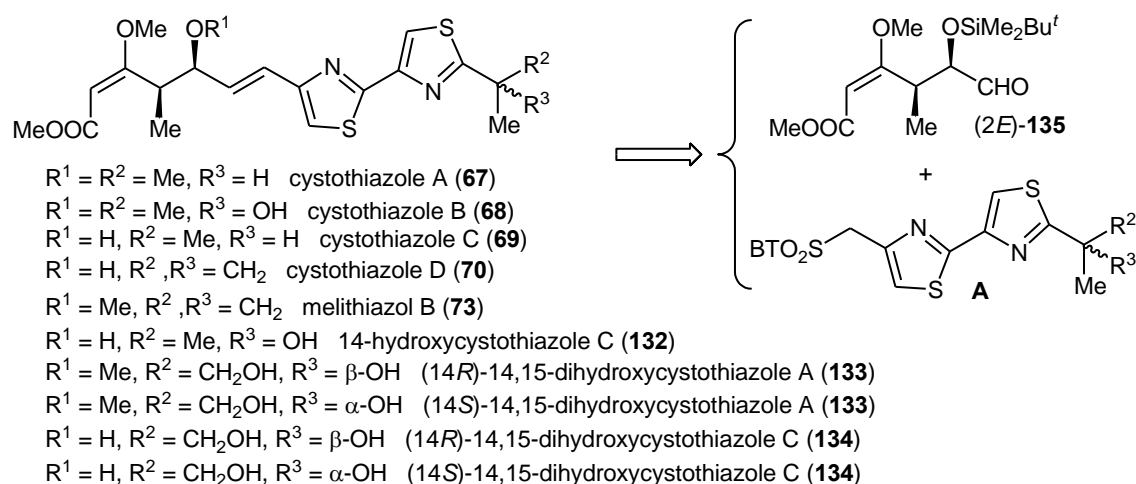
Scheme 21

Acid-catalysed isomerization (Synthesis of (*E*)-**127** from (*Z*)-**127**)

(*Z*)-**127** in  $\text{CDCl}_3$  (chloroform-*d* + 1% v/v TMS (D, 99.8%) + SILVER FOIL) from Cambridge Isotope Laboratories, Inc.) was stood for 3 d at room temperature, (*E*)-**127** was exclusively obtained. The crude (*E*)-**127** was treated with  $\text{Et}_3\text{N} \cdot (\text{HF})_3$  in  $\text{CH}_2\text{Cl}_2$  to give the primary alcohol (+)-**88** in 89% overall yield from (*Z*)-**127**, which was identical with the reported (+)-**88**.<sup>58b</sup> Another  $\text{CDCl}_3$  (99.8% atom % D from Aldrich) was also effective for this selective isomerization. On the other hand, the distilled  $\text{CHCl}_3$ ,  $\text{CH}_2\text{Cl}_2$  ( $\text{CF}_3\text{SO}_3$ ) $\text{Yb}/\text{CH}_2\text{Cl}_2$ , and silica gel/MeOH were inactive for this selective isomerization. Treatment of (*Z*)-**127** with pyridinium *p*-toluenesulfonate (PPTS)/MeOH/PhH, or

(CF<sub>3</sub>SO<sub>3</sub>)Sc/CH<sub>2</sub>Cl<sub>2</sub> or InCl<sub>3</sub>/CH<sub>2</sub>Cl<sub>2</sub> gave a complex mixture including a mixture of (*Z*)-**127** and (*E*)-**127**. The same type isomerization as conversion of (*Z*)-**127** to (*E*)-**127** in 99.8% CDCl<sub>3</sub> was observed in our previous case.<sup>69</sup> Meanwhile, a solution of (*Z*)-**127** in CHCl<sub>3</sub> containing EtOH as stabilizer was treated with a small amount of 4M HCl in dioxane to give a mixture of (*E*)-**127** and a trace amount of (4*R*,5*R*)-6-*tert*-butyldimethylsiloxy-3-ethoxy-5-methoxy-4-methyl-2(*E*)-hexenoate (*E*)-**131** as shown in Scheme 23. This experiment indicated proton (H<sup>+</sup>)-assisted isomerization of (*Z*)-**127** to (*E*)-**127** to seek for thermodynamically more stable (*E*)-**127** from (*Z*)-**127**. In the case of β-methoxy acrylate, it was reported that a trace amount of acidic impurities often was sufficient to bring about rapid equilibration toward the (*E*)-form from the (*Z*)-form at ordinary temperatures.<sup>78</sup> The thus obtained (+)-**88** was subjected to Dess-Martin periodinane oxidation to provide the desired aldehyde (+)-**81** in 89% yield. The overall yield (10 steps from (2*R*,3*S*)-**18a**; 23%) of (+)-**81** via the present route was improved in comparison to that (10 steps from (2*R*,3*S*)-**18a**; 10%) of the previously case (see **6.2**).<sup>58a,b</sup> Then, the modified Julia's coupling of the aldehyde (+)-**81** with sulfone (**113**) in the presence of LHMDS in THF gave a mixture (*E*/*Z* = 14/1) of cystothiazole A (**67**) and (*Z*)-cystothiazole A **67** in 64% yield. A part of this mixture was again subjected to chromatography to provide (*E*)-**67**, whose spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) were identical with those of the reported data.<sup>58b</sup> By applying the modified Julia's coupling method, selectivity (*E*/*Z*=14:1) of the (*E*)-form (cystothiazole A **67**) against the (*Z*)-form was improved in comparison to the Wittig method (*E*/*Z*=4:1<sup>58b</sup> ~ 6.9:1<sup>64</sup>).

## 9. SYNTHESIS AND STRUCTURE ELUCIDATION OF CYSTOTHAZOLE A METABOLITES<sup>79</sup>



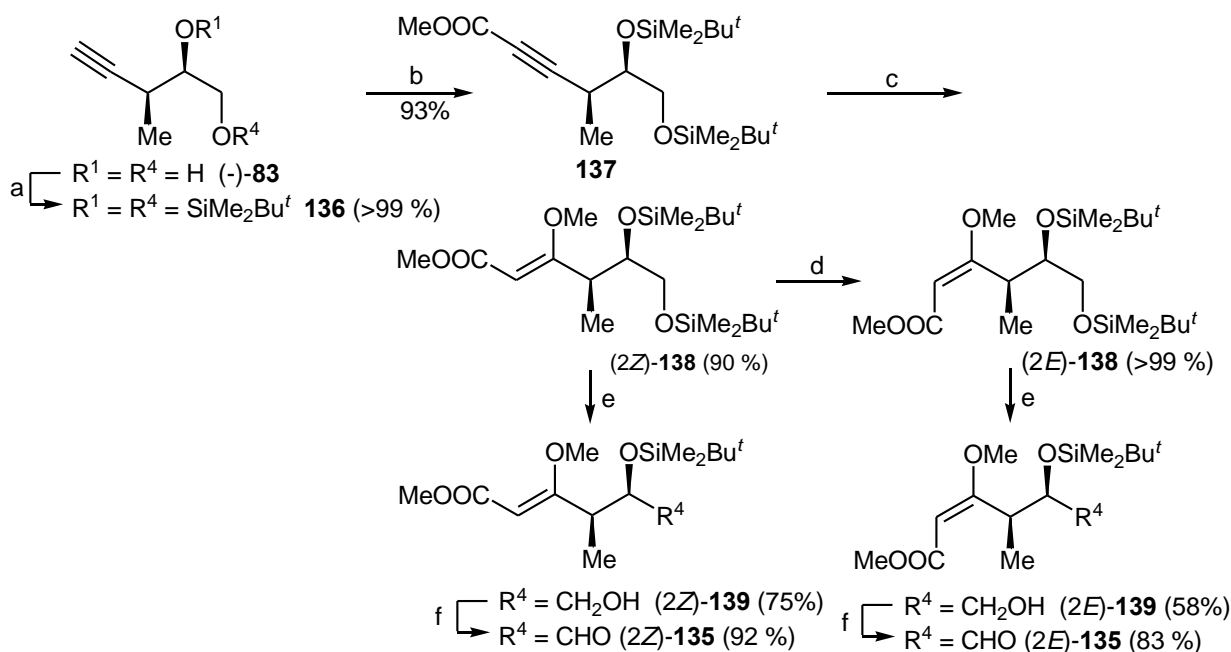
Scheme 24

External addition of cystothiazole A (**67**) to a culture of *C. fuscus* without adsorbent resin was reported to give a number of polar metabolic derivatives including cystothiazoles B (**68**), C (**69**), D (**70**), melithiazol

B (**73**) and the novel compounds 14-hydroxycystothiazole C (**132**), (14,15)-dihydroxycystothiazole A (**133**) and (14,15)-dihydroxycystothiazole C (**134**)<sup>80</sup> as shown in Scheme 24. The absolute structures of **132**, **133** and **135** were deduced by a spectroscopic analysis.<sup>80</sup> The structure elucidations of the novel compounds **132**, **133** and **135** based on their chiral syntheses was carried out. Retrosynthetic strategy for these natural products is illustrated in Scheme 24. It can be seen that these compounds can be synthesized by modified Julia coupling<sup>75</sup> between the left half aldehyde (*2E*)-**135** and the right half sulfone **A** possessing functionality in the side chain (Scheme 24).

### 9.1. Synthesis of left-half aldehyde (*2E*)-**135**

The synthesis of the left-half aldehyde (*2E*)-**135** is shown in Scheme 25. Bis-silylation of (-)-**83** (see Scheme 17) gave bis-silyl ether (**136**) in quantitative yield, which was treated with *n*-BuLi and methyl chloroformate to afford acetylenecarboxylate **137** in 93% overall yield. Conjugated addition of MeOH to acetylenecarboxylate **137** in the presence of a catalytic amount of Bu<sub>3</sub>P afforded a single isomer, (*2Z*)- $\beta$ -methoxy- $\alpha,\beta$ -unsaturated ester **138** in 90% yield. In spite of a lack of isomerization of the  $\beta$ -methoxyacrylate moiety in our previous synthesis, isomerization occurred accidentally in the present case and gave the isomerized product (*2E*)-**138** quantitatively when the MeOH addition product (**138**) was dissolved in aged CDCl<sub>3</sub> (available from Cambridge Isotope Laboratories Inc.) for several hours. The structure of the isomerized product (*2E*)-**138** was confirmed to be (*E*)-olefin due to NOESY observation between the C(2)-hydrogen and C(3)-methoxyl group in (*2E*)-**138**, and thence the geometry



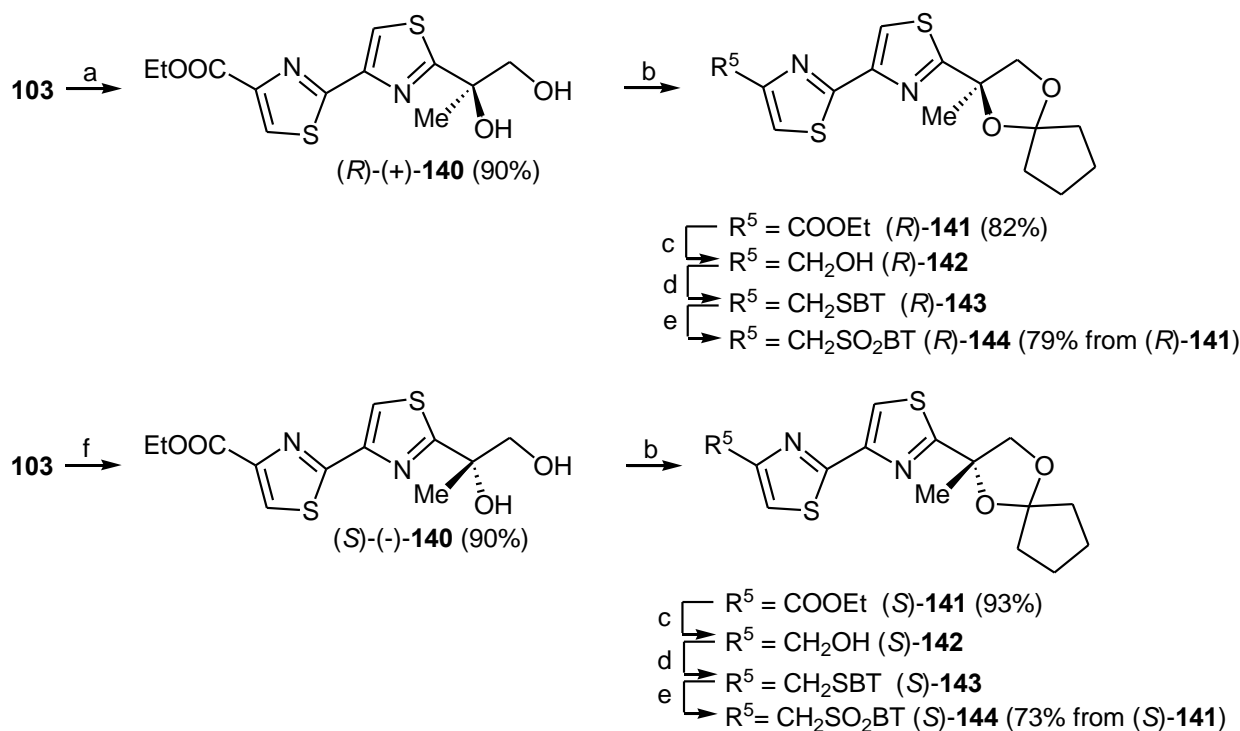
a; <sup>t</sup>BuMe<sub>2</sub>SiCl / imidazole / CH<sub>2</sub>Cl<sub>2</sub> b; *n*-BuLi / ClCO<sub>2</sub>Me / THF c; MeOH / Bu<sub>3</sub>P d; aged CDCl<sub>3</sub>  
 e; HF-pyridine / THF. 0°C f; Dess-Martin periodinane, CH<sub>2</sub>Cl<sub>2</sub>, rt

Scheme 25

of the MeOH addition product (2*Z*)-**138** could be the (*Z*)-geometry. Both (2*Z*)-**138** and (2*E*)-**138** thus obtained could be converted into the corresponding aldehydes (2*Z*)-**135** and (2*E*)-**135**, respectively. Deprotection of the silyl group attached to the primary alcohol group of both (2*Z*)-**135** and (2*E*)-**135** followed by Dess-Martin oxidation afforded the corresponding aldehydes (2*Z*)-**135** and (2*E*)-**135** in 69% and 48% overall yields, respectively (Scheme 25). Isomerization of (2*Z*)-**135** to (2*E*)-**135** in CDCl<sub>3</sub> might be explained by the energy difference between (2*Z*)-**135** and (2*E*)-**135** based on the Monte Carlo Multiple Minimum (MCMM) method.<sup>81</sup> Low-energy conformations were obtained for both (2*E*)-**135** and (2*Z*)-**135** using the MCMM method with OPLS2005 force fields implemented in MacroModel v. 9.5 software. Then, the global energy minima of (2*E*)-**135** and (2*Z*)-**135** were determined with geometry optimization at the HF/6-31G(d,p) level with NMChem v. 4.7 software starting from the low-energy conformers obtained with the MCMM method. The simulation indicated that (2*E*)-**135** is 7.5 kcal/mol more stable than (2*Z*)-**135**, which is consistent with the observed result.

## 9.2. Synthesis of right-half sulfone congeners (*R*)-**144** and (*S*)-**144**

Asymmetric dihydroxylation of bis-thiazole (**103**) with AD-mix  $\alpha$  afforded an 83% ee of chiral diol (+)-**140** in 90% yield, which was recrystallized from *i*-PrOH to give (+)-**140** (>98% ee). Likewise, asymmetric dihydroxylation of **103** with AD-mix  $\beta$  afforded a chiral diol (-)-**140** in 90% yield, which was



a; AD-mix  $\alpha$  / THF / *n*-BuOH / H<sub>2</sub>O, rt b; 1,2-dimethoxycyclopentane / *p*-TsOH·H<sub>2</sub>O / DMF, rt  
 c; LiBH<sub>4</sub> / THF, rt d; 2-mercaptobenzothiazole / DEAD / Ph<sub>3</sub>P / THF, rt  
 e; Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O / 35% aq. H<sub>2</sub>O<sub>2</sub> / EtOH, rt f; AD-mix  $\beta$  / THF / *n*-BuOH / H<sub>2</sub>O, rt

Scheme 26



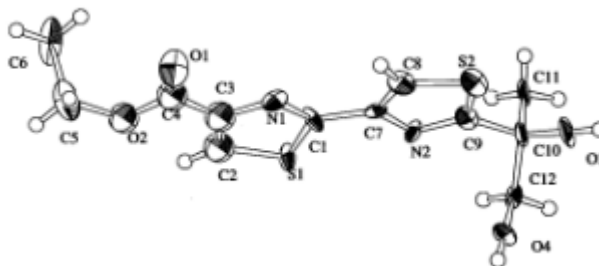


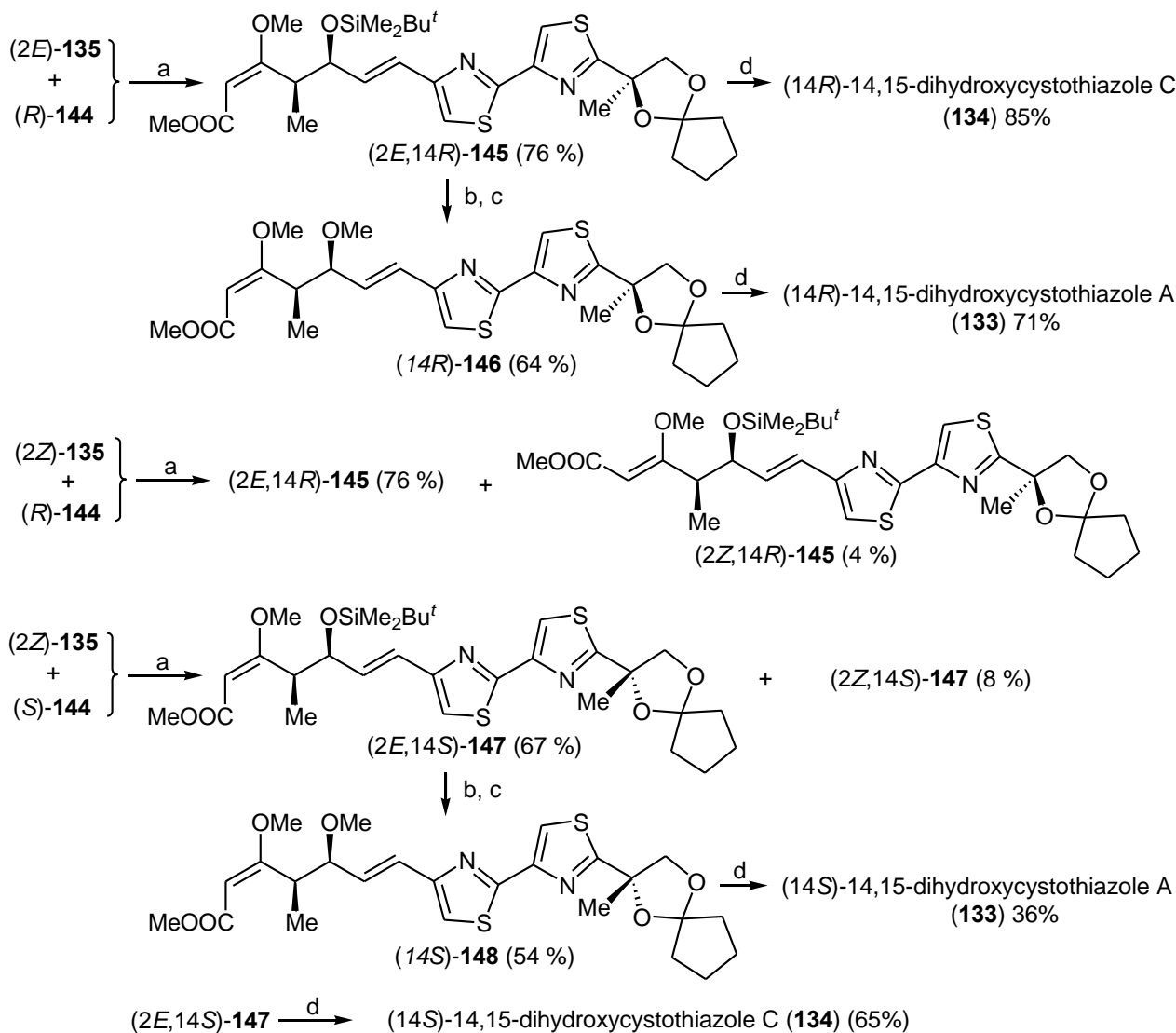
Figure 3 ORTEP diagram of (S)-(-)-140

recrystallized from *i*-PrOH to give optically pure (-)-140. The absolute configuration of (-)-140 was determined to be *S* by X-ray analysis. This assumption was confirmed by X-ray single crystal structure analysis of (S)-140 as shown in Figure 3. A crystal of (S)-140, obtained by recrystallization from *i*-PrOH by slow evaporation of the solvent, with an approximate size 0.2x0.3x0.1 mm<sup>3</sup>, was employed for X-ray analysis. The compound crystallizes in the orthorhombic space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> with a=5.497(2) Å, b=14.807(5) Å, c=17.658(6) Å, V=1437.2(8) Å<sup>3</sup>, Z=4, D<sub>c</sub>=1.453 g cm<sup>-3</sup>, μ=3.84cm<sup>-1</sup>. The intensities were measured on a Rigaku AFC7R/MSC Mercury CCD diffractometer. A total of 7338 reflections utilizing MoKα radiation (λ=0.71070 Å) and 1599 unique reflections were used for structure determination. The structure was solved by the direct method SIR92 and refined by full matrix least squares with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were obtained from difference Fourier maps and refined isotropically. The final R1-value was 0.075 for 5150 reflections with I>2.00σ (I). The absolute configuration was established using the Flack parameter (x=0.2(5)). Figure 3 shows an ORTEP of the refined molecular structure. Treatment of the thus-obtained chiral diols (*R*)-140 and (*S*)-140 with 1,2-dimethoxy-cyclopentane in the presence of *p*-toluenesulfonic acid gave the corresponding ketals (*R*)-141 and (*S*)-141 in 82% and 93% yields, respectively. LiBH<sub>4</sub> reduction of (*R*)-141 and (*S*)-141 afforded the corresponding primary alcohols (*R*)-142 and (*S*)-142, which were treated with 2-mercaptobenzothiazole (BTSH) in the presence of Ph<sub>3</sub>P and diethylazodicarboxylate (DEAD) to provide the corresponding sulfides (*R*)-143 and (*S*)-143. The sulfides (*R*)-143 and (*S*)-143 were then subjected to oxidation with 35% H<sub>2</sub>O<sub>2</sub> in the presence of Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O to give the corresponding sulfones (*R*)-144 (79% yield from (*R*)-141) and (*S*)-144 (73% yield from (*S*)-141), respectively.

### 9.3. Synthesis and structure elucidation of 14,15-dihydroxycystothiazole A (133) and 14,15-dihydroxycystothiazole C (134)

The coupling reaction of (2*E*)-135 and (*R*)-144 in the presence of LHMDS in THF gave (2*E*,6*E*,14*R*)-145 in 76% yield. Deprotection of the ketal and silyl groups in (2*E*,6*E*,14*R*)-145 using aqueous HF in

MeCN gave (14*R*)-14,15-dihydroxycystothiazole C (**134**) in 85% yield. Deprotection of the silyl group of (2*E*,6*E*,14*R*)-**145** followed by methylation of the secondary alcohol group using Meerwein's reagent



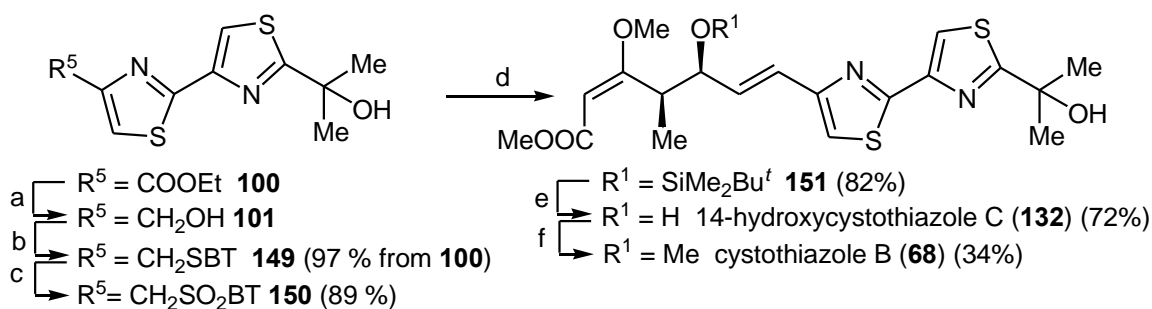
a; LiHMDS / THF, -78 to -30°C b; HF-pyridine / THF, 0°C c; Me<sub>3</sub>O<sup>+</sup>BF<sub>4</sub><sup>-</sup> / proton-sponge / CH<sub>2</sub>Cl<sub>2</sub>  
d; aq. HF / MeCN

Scheme 27

(Me<sub>3</sub>O<sup>+</sup>BF<sub>4</sub><sup>-</sup>) in the presence of a proton sponge provided (2*E*,6*E*,14*R*)-**146** (64% overall yield), which was treated with aqueous HF in MeCN to afford (14*R*)-14,15-dihydroxy-cystothiazole A (**133**) in 71% yield. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of the synthetic (14*R*)-14,15-dihydroxycystothiazole C (**134**) were identical to those of the natural product (14*R*)-**134** corresponding to the minor isomer,<sup>80</sup> including the sign of specific rotation. On the other hand, the coupling reaction of (2*Z*)-**135** and (R)-**144** in the presence of LHMDS in THF gave (2*E*,6*E*,14*R*)-**145** (76%) along with (2*Z*,6*E*,14*R*)-**145** (4%) in 76% yield. This experiment indicates the occurrence of isomerization of (2*Z*) to (2*E*) in the reaction process or work-up step, although the isomerization mechanism has not been clarified. The encouraging

result of this experiment prompted to carry out the reaction of (*2Z*)-**135** and (*S*)-**144** in the presence of LHMDS to give (*2E,6E,14S*)-**147** (67%) and (*2Z,6E,14S*)-**147** (8%). Deprotection of the silyl group of (*2E,6E,14S*)-**147** followed by methylation of the secondary alcohol group using Meerwein's reagent in the presence of a proton sponge provided (*2E,6E,14S*)-**148** (54% overall yield), which was treated with aqueous HF in MeCN to afford (*14S*)-14,15-dihydroxycystothiazole A (**133**) in 36% yield. Deprotection of ketal and silyl groups in (*2E,6E,14S*)-**147** using aqueous HF in MeCN gave (*14S*)-14,15-dihydroxycystothiazole C (**134**) in 65% yield. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of the synthetic (*14S*)-14,15-dihydroxycystothiazole A (**133**) and (*14S*)-14,15-dihydroxycystothiazole C (**134**) were identical to those of the natural products (*14S,15*)-**133**<sup>80</sup> and (*14S,15*)-**134**<sup>80</sup> corresponding to the major isomer, respectively, including the sign of specific rotation (Scheme 27).

#### 9.4. Synthesis of 14-hydroxycystothiazole C (**3**) and cystothiazole B (**2**)<sup>82</sup>



a; LiBH<sub>4</sub> / THF, rt b; 2-mercaptobenzothiazole / DEAD / Ph<sub>3</sub>P / THF, rt c; Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O / 35% aq. H<sub>2</sub>O<sub>2</sub> / EtOH, rt d; LiHMDS / (*2Z*)-**135** / THF, -78 to -30 °C e; HF-pyridine / THF, 0 °C f; Me<sub>3</sub>O<sup>+</sup>BF<sub>4</sub><sup>-</sup> / proton-sponge / CH<sub>2</sub>Cl<sub>2</sub>

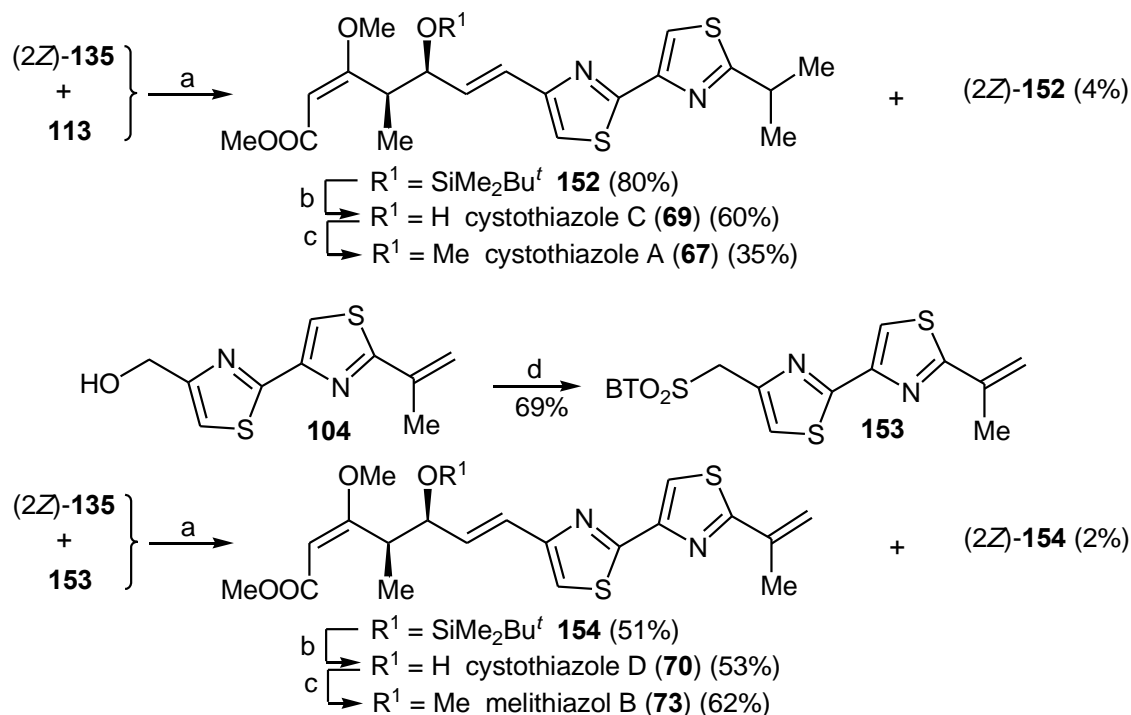
Scheme 28

The first chiral synthesis of 14-hydroxycystothiazole C (**132**) was achieved by the following procedure as shown in Scheme 28. LiBH<sub>4</sub> reduction of the hydroxy ester (**100**) (see Scheme 18) afforded the corresponding primary alcohol (**101**), which was treated with 2-mercaptobenzothiazole (BTSH) in the presence of Ph<sub>3</sub>P and diethylazodicarboxylate (DEAD) to provide the corresponding sulfide **149** in 97% yield from **100**. Oxidation **100** with 35% H<sub>2</sub>O<sub>2</sub> in the presence of Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O gave the corresponding sulfone **150** in 89% yield. The reaction of (*2Z*)-**135** and **150** in the presence of LHMDS gave (*2E,6E*)-**82** in 55% yield. Deprotection of the silyl group of (*2E,6E*)-**151** with aqueous HF in MeCN gave 14-hydroxycystothiazole C (**132**) in 72% yield. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of synthetic **132** were identical to those of the natural product,<sup>80</sup> including the sign of specific rotation. Then, methylation of the secondary alcohol group of **132** using Meerwein's reagent in the presence of a proton sponge provided cystothiazole B (**68**) in 34% yield along with the starting material **132** (25%

yield). The spectral data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) of synthetic **68** were identical to those of the natural cystothiazole B (**68**),<sup>55</sup> including the sign of specific rotation (Scheme 28).

### 9.5. Syntheses of cystothiazoles C (**69**) and D (**70**)<sup>82</sup>

Concise syntheses of cystothiazoles A (**67**), C (**69**), D (**70**),<sup>55</sup> and melithiazol B (**73**)<sup>57</sup> was shown in Scheme 29. The reaction of (2*Z*)-**135** and **113** (see Scheme 20) in the presence of LHMDS in THF gave



a;  $\text{LiN}(\text{SiMe}_3)_2 / \text{THF}$     b;  $\text{Bu}_4\text{N}^+\text{F}^- / \text{THF}$     c;  $\text{Me}_3\text{O}^+\text{BF}_4^- / \text{proton sponge} / \text{CH}_2\text{Cl}_2$

d; 1) 2-mercaptobenzothiazole (BTSH) / DEAD /  $\text{Ph}_3\text{P}$  / THF    2)  $\text{H}_2\text{O}_2 / \text{Mo}_7\text{O}_{24}(\text{NH}_4)_6 \cdot 4\text{H}_2\text{O} / \text{EtOH}$

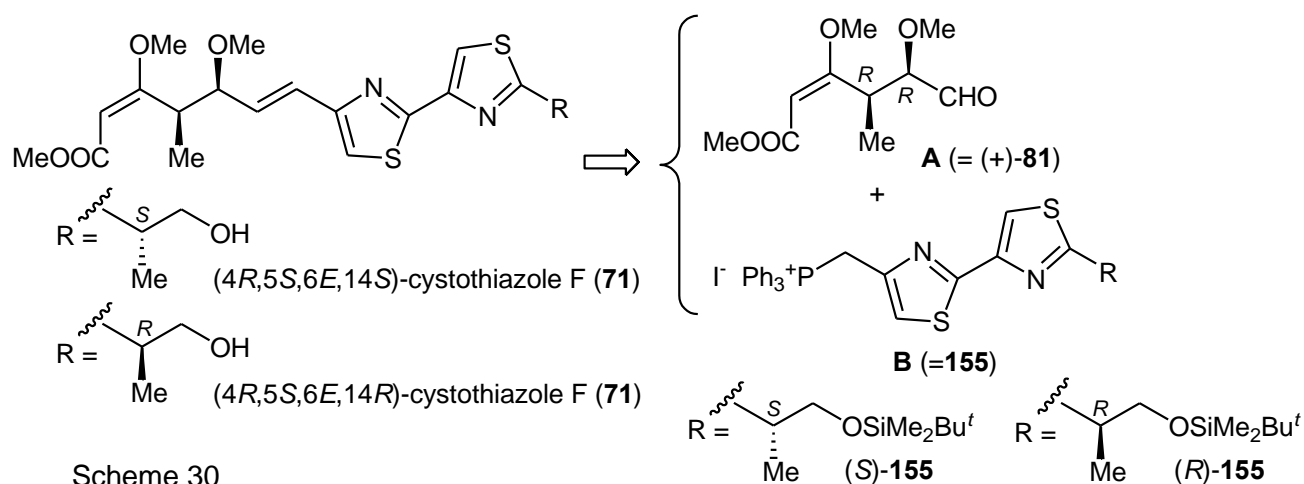
Scheme 29

a mixture (*E/Z* = 20/1) of coupled products, which were separated to give (2*E*)-**152** (80%) and (2*Z*)-**152** (4%). Deprotection of (2*E*)-**152** with tetrabutylammonium fluoride gave cystothiazole C (**69**) in 60% yield. The spectral data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) of synthetic **69** were identical to those of the natural cystothiazole C (**69**),<sup>55</sup> including the sign of specific rotation. Methylation of synthetic **69** using Meerwein's reagent ( $\text{Me}_3\text{O}^+\text{BF}_4^-$ ) in the presence of proton sponge provided cystothiazole A (**67**) in 35% yield along with the starting material **69** (37% recovery). The spectral data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) of synthetic **67** were identical to those of the natural cystothiazole A (**67**),<sup>55</sup> including the sign of specific rotation. Next the syntheses of cystothiazole D (**3**) and melithiazol B (**4**) were carried out. Treatment of the primary alcohol (**104**) (see Scheme 18) with 2-mercaptobenzothiazole (BTSH) in the presence of  $\text{Ph}_3\text{P}$  and diethylazodicarboxylate (DEAD) followed by oxidation with 30%  $\text{H}_2\text{O}_2$  in the presence of

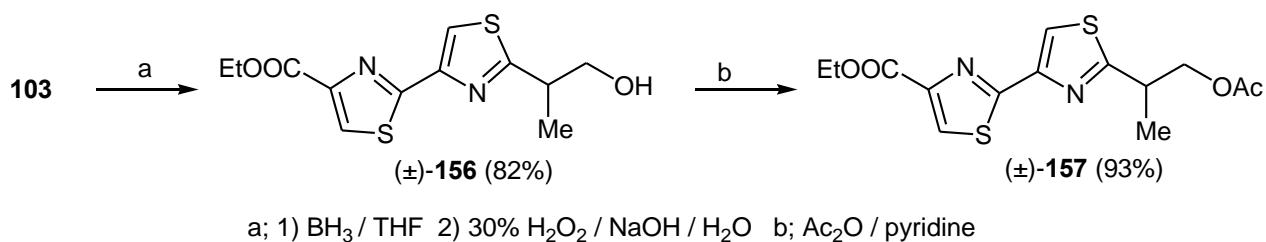
Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O gave the corresponding sulfone **153** in 69% overall yield. The reaction of (2*Z*)-**135** and **153** in the presence of LHMDS in THF gave a mixture (*E/Z* = 26/1) of coupled products, which were separated to give (2*E*)-**154** (51%) and (2*Z*)-**154** (2%). Deprotection of (2*E*)-**154** with tetrabutylammonium fluoride gave cystothiazole D (**70**) in 53% yield. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of synthetic **70** were identical to those of the natural cystothiazole D (**70**),<sup>55</sup> including the sign of specific rotation. Thus, the absolute structure of natural cystothiazole D (**70**) was confirmed by its first synthesis of **70**. Methylation of synthetic **70** using Meerwein's reagent (Me<sub>3</sub>O<sup>+</sup>BF<sub>4</sub><sup>-</sup>) in the presence of proton sponge provided melithiazol B (**73**) in 62% yield. The spectral data (<sup>1</sup>H- and <sup>13</sup>C-NMR) of synthetic **73** were identical to those previously reported melithiazol B (**73**),<sup>71</sup> including the sign of specific rotation (Scheme 29).

## 10. SYNTHESIS OF CYSTOTHIAZOLE F<sup>83</sup>

The structure of cystothiazole F (**71**) was deduced to be 15-hydroxycystothiazole A based on the spectral analysis. The relative stereochemistry (4*R*\*,5*S*\*) could be the same as that of cystothiazole A (**1**) because of the similar <sup>1</sup>H-NMR data (H-4 and H-5).<sup>55</sup> The absolute configuration of C(14)-carbon of **71** was not determined. The determination of the absolute structure of cystothiazole F (**71**) was carried out based on the total synthesis of a chiral form of **71** as shown in Scheme 30.

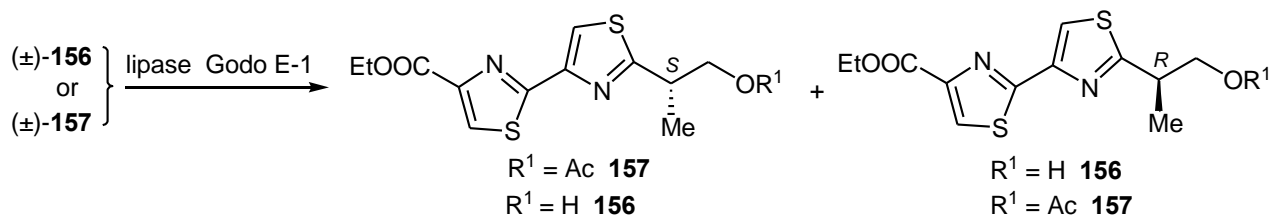


Retrosynthetically, the synthesis of **71** can be achieved by Wittig condensation of the left-half aldehyde **A** (= (+)-**81**) and the right-half chiral phosphonium iodide **B**. For the synthesis of a chiral form of **B** ((*S*)-**155** or (*R*)-**155**), lipase-catalyzed optical resolution of an alcohol [(±)-**156**] or an acetate [(±)-**157**] is thought to be the most effective method. The synthesis of substrate [(±)-**156** or (±)-**157**] for enzymatic reaction is shown in Scheme 31.



Scheme 31

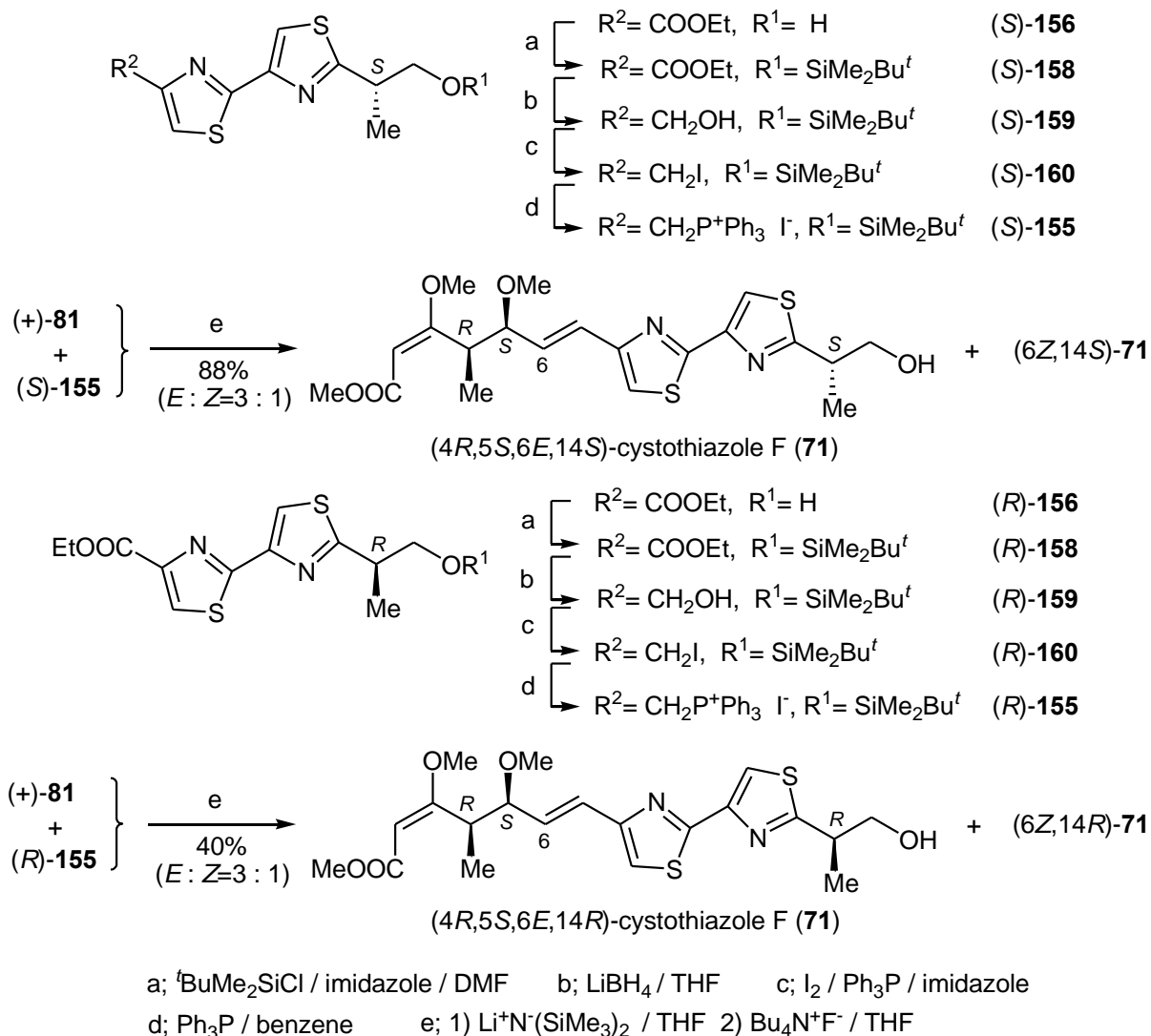
Hydroboration of the exo-olefin (**103**) (see Scheme 18) gave a primary alcohol ( $\pm$ )-**156** (82% yield), which was subjected to acetylation to afford the corresponding acetate ( $\pm$ )-**157** (93% yield). Initially, ( $\pm$ )-**155** was subjected to screening experiments using several kinds of commercially available lipases. Among them, lipase “Godo E-1” from *Pseudomonas* sp. was found to be effective. When ( $\pm$ )-**155** was subjected to enantioselective acetylation using “Godo E-1” in the presence of isopropenyl acetate as acylating reagent for 1 day, an acetate (*S*)-**157** (47%,  $[\alpha]_{\text{D}}^{25}$  -4.6 (c 1.28,  $\text{CHCl}_3$ ); corresponds to 83% ee) and unchanged alcohol (*R*)-**156** (50%,  $[\alpha]_{\text{D}}^{25}$  -3.7 (c 1.03,  $\text{CHCl}_3$ ); corresponds to 74% ee) were obtained

Table 3 Enantioselective acetylation or hydrolysis of ( $\pm$ )-**156** or ( $\pm$ )-**157** using lipase

Entry	Substrate (g)	Acylating reagent	Solvent	Time (d)	Products % (%ee)
1	( $\pm$ )- <b>156</b> (0.894)	isopropenyl acetate	<i>i</i> -Pr <sub>2</sub> O	1	( <i>S</i> )- <b>157</b> 47 (83) ( <i>R</i> )- <b>156</b> 50 (74)
2	( $\pm$ )- <b>156</b> (1.500)	isopropenyl acetate	<i>i</i> -Pr <sub>2</sub> O	2	( <i>S</i> )- <b>157</b> 70 (37) ( <i>R</i> )- <b>156</b> 28 (99)
3	( $\pm$ )- <b>157</b> (1.299)		<i>i</i> -Pr <sub>2</sub> O / H <sub>2</sub> O	1	( <i>R</i> )- <b>157</b> 62 (59) ( <i>S</i> )- <b>156</b> 37 (99)
4	( <i>S</i> )- <b>157</b> (1.198, 37% ee)		<i>i</i> -Pr <sub>2</sub> O / H <sub>2</sub> O	1	( <i>R</i> )- <b>157</b> 44 (16) ( <i>S</i> )- <b>156</b> 50 (98)

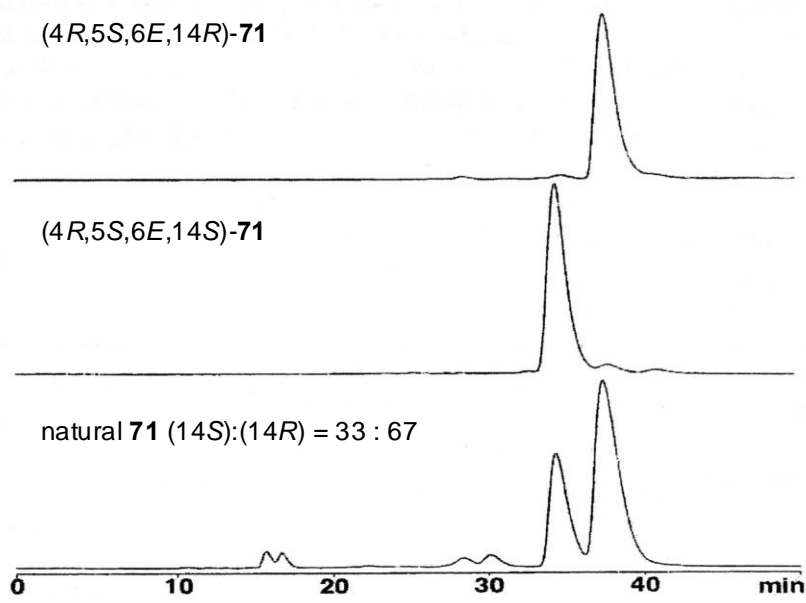
(entry 1, Table 3). The enantiomeric excess (ee) was calculated by means of HPLC analysis. The *E*-value of this enzymatic reaction was estimated to be 23.8. The absolute structures of enzymatic reaction products (*S*)-**157** and (*R*)-**157** were determined by the direct comparison of the reported samples<sup>84</sup> (*R*)-**157** ( $[\alpha]_{\text{D}}^{21}$  +5.0 (c 2.0,  $\text{CHCl}_3$ ); corresponds to 81% ee) and (*S*)-**156** ( $[\alpha]_{\text{D}}^{21}$  +4.36 (c 1.1,  $\text{CHCl}_3$ ); corresponds to 91% ee). When this reaction was carried out for a prolonged period (2 d), (*S*)-**157** (70%, 37% ee) and (*R*)-**156** (28%, 99% ee) were obtained (entry 2, Table 3). On the other hand, asymmetric hydrolysis of ( $\pm$ )-**157** for 1 d gave (*S*)-**156** (37%, 99% ee) and (*R*)-**157** (62%, 59% ee) (entry 3, Table 3). The acetate (*S*)-**157** with 37% ee was again subjected to enantioselective hydrolysis to afford (*S*)-**156** (50%, 98% ee) and (*R*)-**157** (44%, 16% ee) (entry 4, Table 3). The synthesis of (4*R*,5*S*,6*E*,14*S*)- and (4*R*,5*S*,6*E*,14*R*)-cystothiazole F (**71**) from the enzymatic productions, (*S*)- and

(*R*)-**156**, respectively, is shown in Scheme 32. Protection of the hydroxyl group of (*S*)-**156** as a silyl ether group followed by reduction with  $\text{LiBH}_4$  gave an (*S*)-alcohol (**159**) in 90% overall yield. Treatment of (*S*)-**159** with  $\text{I}_2$ /triphenylphosphine/imidazole provided iodide (*S*)-**160** (89% yield), which was treated with  $\text{Ph}_3\text{P}$  to give phosphonium salt (*S*)-**155** in 85% yield. Condensation of (*S*)-**155** with (+)-**81** (see Scheme 21) in the presence of LHMDS in THF followed by deprotection of silyl group afforded (*4R,5S,6Z,14S*)-**71** (22%,  $[\alpha]_{\text{D}}^{26} +206.0$  (c 1.36,  $\text{CHCl}_3$ )) and (*4R,5S,6E,14S*)-**71** (66%,  $[\alpha]_{\text{D}}^{26} +86.2$  (c 1.05,  $\text{CHCl}_3$ )). The synthesis of (*4R,5S,6Z,14R*)-**71** (10%,  $[\alpha]_{\text{D}}^{27} +208.8$  (c 0.3,  $\text{CHCl}_3$ )) and (*4R,5S,6E,14R*)-**71** (30%,  $[\alpha]_{\text{D}}^{26} +94.4$  (c 0.78,  $\text{CHCl}_3$ )) from (*R*)-**155** was carried out in the same way as for the synthesis of (*4R,5S,6Z,14S*)-**71** and (*4R,5S,6E,14S*)-**71**. The NMR data ( $^1\text{H}$ - and  $^{13}\text{C}$ -NMR) of the synthetic (*4R,5S,6E,14S*)- and (*4R,5S,6E,14R*)-cystothiazoles F (**71**) were found to be quite similar to those of natural product cystothiazole F (**71**),<sup>55</sup> respectively. Meanwhile, the sign of specific rotation of the natural **71** [ $[\alpha]_{\text{D}}^{23} +77.0$  (c 0.074,  $\text{CHCl}_3$ )]<sup>55</sup> was the same as that of the synthetic (*4R,5S,6E,14S*)-**71**



Scheme 32

and (4*R*,5*S*,6*E*,14*R*)-**71**, respectively. At this stage, identification of natural cystothiazole F (**71**) and synthetic **71** seemed to be difficult and direct comparison by means of a chiral HPLC analysis was carried out. The result is shown in the Fig. 4, and natural cystothiazole F (**71**) was found to be a 33:67 diastereomeric mixture [(4*R*,5*S*,6*E*,14*S*)-**71** : (4*R*,5*S*,6*E*,14*R*)-**71** =33:67] (Figure 4).



Conditions: Column; CHIRALCEL OD (4.6 x 250 mm), Solvent; hexane - *i*-PrOH (9 : 1), Flow rate; 0.5 ml/min, Detection; 310 nm, Sample; 10  $\mu$ g.

Figure 4 Chiral HPLC analysis of cystothiazole F (**71**)

## 11. TOTAL SYNTHESSES OF MYXOTHIAZOLS A AND Z<sup>85</sup>

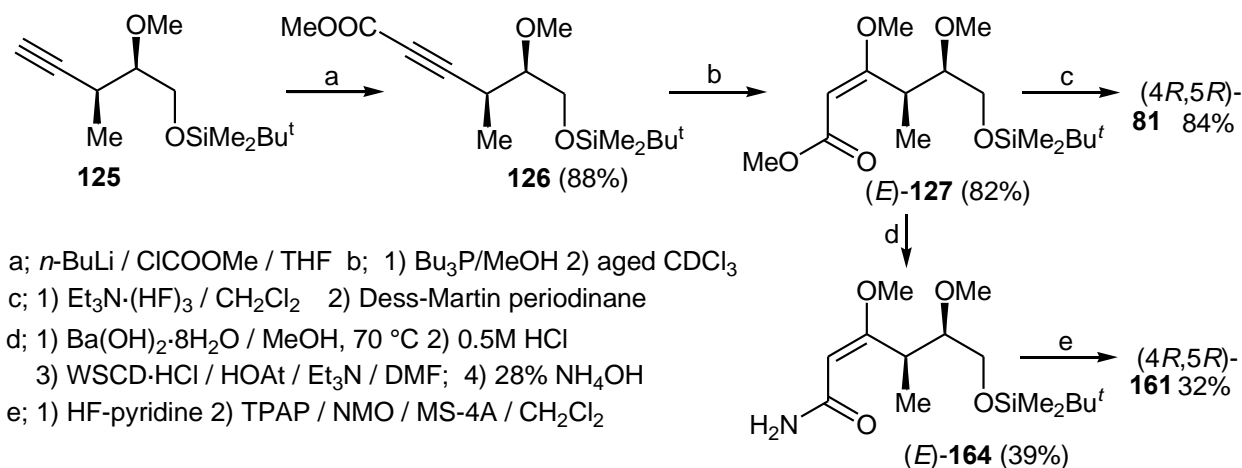
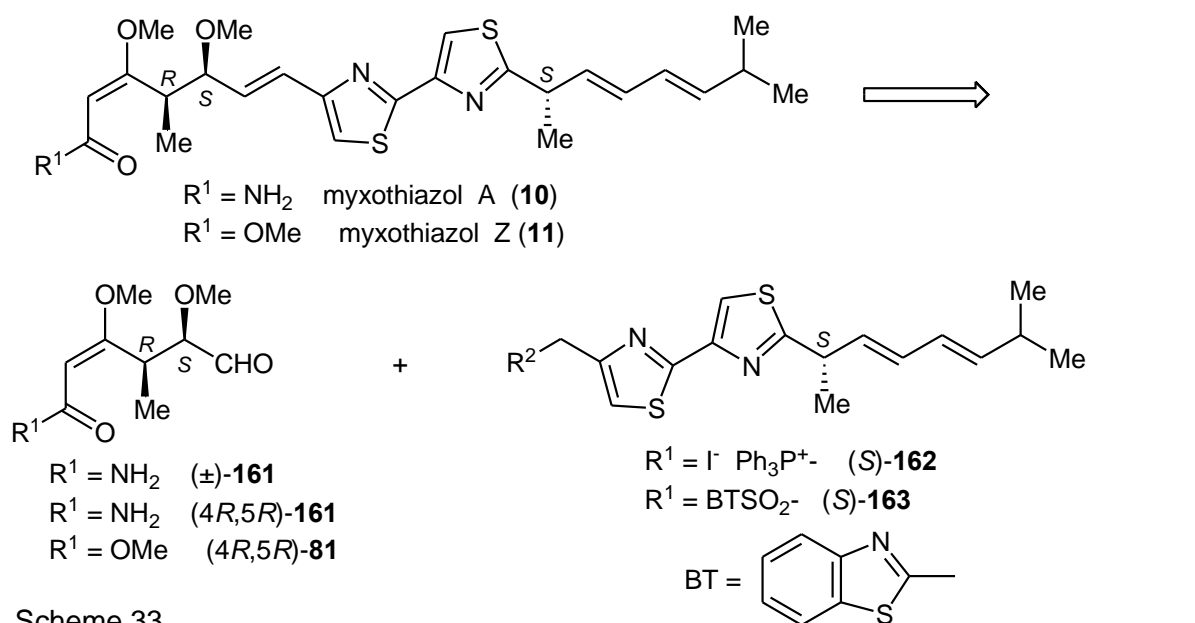
Myxothiazols A (**10**) and Z (**11**) possessing a bithiazole skeleton as well as a  $\beta$ -methoxyacrylate moiety were isolated from the myxobacterium *Myxococcus fulvus* strain Mxf16<sup>56</sup> and *Myxococcus fulvus*, respectively. Feeding experiments with labeled precursors established biosynthesis of **11** from **10**.<sup>86</sup> Myxothiazol A (**10**) is active against many filamentous fungi and completely inhibits growth of *Mucor hiemalis* at a concentration of 2  $\mu$ g/ml.<sup>56</sup> The fungicidal activity of the  $\beta$ -methoxyacrylate (MOA) inhibitors has been shown to be due to their ability to inhibit mitochondrial respiration by blocking electron transfer between cytochrome b and cytochrome c.<sup>87</sup> Myxothiazol Z (**11**) was reported to exhibit potent cytotoxicity against human tumor cell.<sup>88</sup> The structure of myxothiazol A (**10**) was established by a combination of chemical degradation and NMR study, and its absolute configuration at C(14)-carbon was determined by X-ray analysis of its degradation product.<sup>89</sup> The synthesis of a diastereomeric mixture of **10** was achieved based on a Wittig coupling between racemic aldehyde ( $\pm$ )-**161** corresponding to the left-half and chiral phosphonium salt (*S*)-**162** corresponding to the right half.<sup>59,90</sup> Meanwhile, the synthesis of (+)-**11** was reported by a Wittig coupling between chiral aldehyde (4*R*,5*R*)-**81** and (*S*)-**162**<sup>90</sup> as shown in Scheme 33. The chiral synthesis of **10** has not been achieved to date and the first synthesis



of (+)-**10** was obtained based on modified Julia olefination between the chiral aldehyde (4*R*,5*R*)-**161** and the chiral benzothiazole sulfone (*S*)-**163**. Furthermore, the synthesis of (+)-**11** was obtained based on modified Julia olefination between a (4*R*,5*R*)-**81** and (*S*)-**163** (Scheme 33).

### 11.1. Synthesis of left-half (4*R*,5*R*)-**81** and (4*R*,5*R*)-**161**

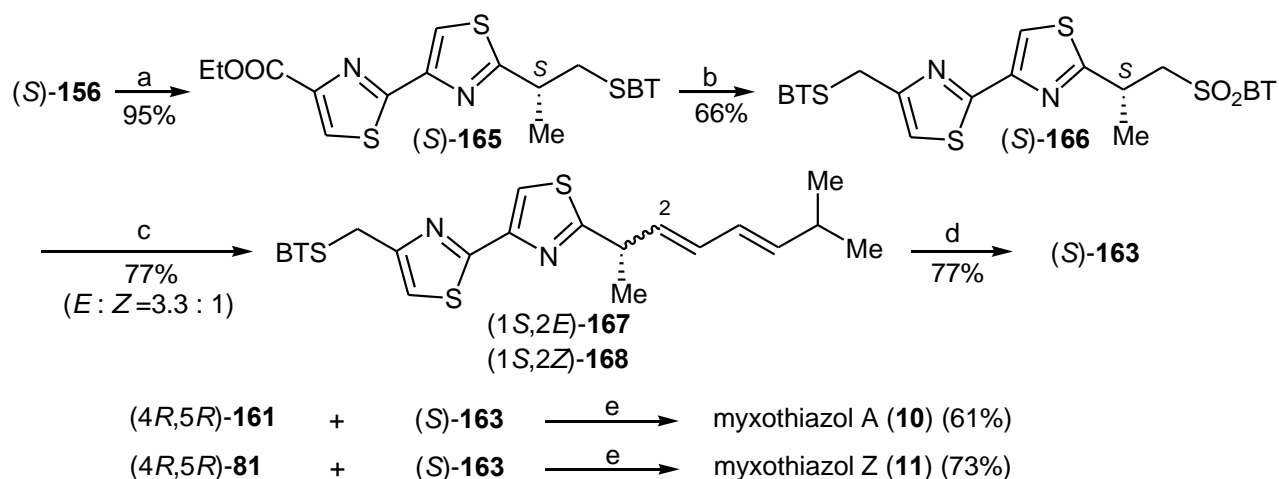
The synthesis of (±)-**161** was achieved in overall 1% yield (9 steps) based on a condensation reaction between cinnamaldehyde and the dianion derived from methyl 3-oxopentanoate followed by several synthetic steps<sup>59,90</sup> In this case, the preparation of chiral form of **161** could be possible due to the optical resolution of the intermediate. On the other hand, the synthesis of (4*R*,5*R*)-**81** was reported based on Evans asymmetric aldol condensation procedure.<sup>90</sup> The preparation of (4*R*,5*R*)-**161** was achieved by the following synthetic route as shown in Scheme 34.



The silyl ether **125** (Scheme 21) was treated with *n*-BuLi and methyl chloroformate to give an acetylenecarboxylate **126** in 88% yield. Conjugate addition of MeOH to **126** followed by isomerization under the previously mentioned procedure (Scheme 21) gave (*E*)-**127** in 82% overall yield. Desilylation of (*E*)-**127** with Et<sub>3</sub>N·(HF)<sub>3</sub> followed by oxidation with Dess-Martin reagent gave the desired aldehyde (4*R*,5*R*)-**81** in 84% yield. Conversion of ester group of (*E*)-**127** to amide was carried out by the following procedure. Alkaline hydrolysis of (*E*)-**127** followed by acid treatment gave a carboxylic acid. Treatment of this acid with water-soluble carbodiimide hydrochloric acid salt (WSCD·HCl) in the presence of 1-hydroxy-7-aza-benzotriazole (HOAt) followed by addition of aqueous NH<sub>3</sub> gave the desired amide (*E*)-**164** in 39% overall yield from (*E*)-**127**. Desilylation of (*E*)-**127** with HF·pyridine followed by oxidation with tetrapropylammonium perruthenate (TPAP) in the presence of 4-methylmorpholine *N*-oxide (NMO) and MS-4A afforded the desired aldehyde (4*R*,5*R*)-**161** in 32% overall yield. <sup>1</sup>H-NMR data of the synthetic (4*R*,5*R*)-**161** were consistent with those of the reported (±)-**161**.<sup>90</sup>

## 11.2. Synthesis of right-half (*S*)-**163**, myxothiazols A (**10**) and Z (**11**)

Treatment of chiral alcohol (*S*)-**156** (see Table 3) with 2-mercaptobenzothiazole (BTSH) in the presence of Ph<sub>3</sub>P and diethyl azodicarboxylate (DEAD) gave the corresponding sulfide (*S*)-**165** in 93% yield. LiBH<sub>4</sub> reduction of (*S*)-**165** followed by oxidation with 35% H<sub>2</sub>O<sub>2</sub> in the presence of Mo<sub>7</sub>O<sub>24</sub>(NH<sub>4</sub>)<sub>6</sub>·4H<sub>2</sub>O provided the corresponding sulfone-alcohol, which was again treated with 2-mercaptobenzothiazole (BTSH) in the presence of Ph<sub>3</sub>P and DEAD to afford the corresponding sulfide (*S*)-**166** in 66% overall yield. The reaction of (*S*)-**166** and (2*E*)-4-methylpentenal in the presence of LHMDS in THF gave a mixture (*E*/*Z* = 3.3/1) of coupled products, which were chromatographically separated to give (1*S*,2*E*)-**167** (59%) and (1*S*,2*Z*)-**168**. Oxidation of (1*S*,2*E*)-**167** under the same condition as preparation of (*S*)-**166** provided the desired (*S*)-**163** in 77% yield. The overall yield of (*S*)-**163** from (*S*)-**156** was 28% (4 steps). In contrast, the overall yield of (*S*)-**162** from the commercially available (2*R*)-3-hydroxy-2-methylpropanoate was 1% (19 steps).<sup>59,90</sup> Finally, modified Julia coupling between the chiral aldehyde (4*R*,5*R*)-**161** and the chiral benzothiazole sulfone (*S*)-**163** in the presence of LHMDS afforded (+)-myxothiazol A (**10**) in 61% yield. The spectral data of the synthetic **10** were identical with those of natural (+)-myxothiazol A (**10**)<sup>56</sup> including the sign of a specific rotation. Julia coupling between a chiral aldehyde (4*R*,5*R*)-**81** (see Scheme 21) and a chiral benzothiazole sulfone (*S*)-**163** in the presence of LHMDS afforded (+)-myxothiazol Z (**11**) in 73% yield. The spectral data of the synthetic **11** were identical with those of natural (+)-myxothiazol Z (**11**)<sup>88</sup> including the sign of a specific rotation.



a; 2-mercaptobenzothiazol (BTSH) / DEAD /  $\text{Ph}_3\text{P}$  / THF; b; 1)  $\text{LiBH}_4$  / THF 2)  $\text{H}_2\text{O}_2$  /  $\text{Mo}_7\text{O}_{24}(\text{NH}_4)_6 \cdot 4\text{H}_2\text{O}$  / EtOH 3) BTSH / DEAD /  $\text{Ph}_3\text{P}$  / THF c; LHMDS / (2E)-4-methylpentenal / THF d;  $\text{H}_2\text{O}_2$  /  $\text{Mo}_7\text{O}_{24}(\text{NH}_4)_6 \cdot 4\text{H}_2\text{O}$  / EtOH e; LHMDS / THF;

Scheme 35

## 12. CONCLUSION

Diastereoselective synthesis of ( $\pm$ )-(2,3)-*syn*-2-methyl-3-hydroxy ester **I** or ( $\pm$ )-(2,3)-*syn*-3-methyl-2-hydroxy ester **III** was achieved based on diastereoselective reduction of ( $\pm$ )-2-methyl-3-keto ester **IV** or the reaction of ( $\pm$ )-*trans*-epoxybutanoate **V** and carbon-nucleophile, respectively. These racemic alcohols were subjected to enzymatic resolution to afford the corresponding enantiomers. Each enantiomerically pure compound was converted to biologically active natural products such as oudemansins, chuangxinmycin, asperlin, indolmycin, cystothiazoles, melithiazols and myxothiazols possessing antifungal and cytotoxic activities, inhibition of NADH oxidation, etc.

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