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Asymmetric Total Syntheses and Structure Revisions of Eurotiumide A and Eurotiumide B, and Evaluation of their Fluorescent Properties as Natural Probes

Atsushi Nakayama*[a], Hideo Sato[a], Sangita Karanjit, Naoki Hayashi[b], Masataka Oda[b], and Kosuke Namba*[a]

Abstract: Asymmetric total syntheses and structure revisions of dihydroisocoumarin-type natural products, eurotiumide A and eurotiumide B have been described. The key features of these total syntheses are the asymmetric Shi epoxidation, regio and stereo-selective epoxide opening, C1 insertion/lactonization cascade reaction for constructing 4-methoxyisochroman-1-one skeleton. We confirmed the structures and configurations of eurotiumide A and B on the basis of X-ray crystallographic analysis of the key intermediate, and revealed that eurotiumide A and B have *cis* and *trans* configurations at H3/H4 positions, which are the opposite relationship of the stereochemistry from the previous report, respectively. The absolute configurations of them are also determined. These natural products exhibited highly fluorescence in several solvents with large Stokes shifts involving the excited-state intramolecular proton transfer mechanism, which is supported by time-dependent density functional theory. Eurotiumide A also emitted fluorescence in *Bacillus cereus*.

Marine-derived fungi are an important source of diverse secondary metabolites possessing both unique structures and biological activities, such as antitumor,[1] antibacterial,[2a,b] antifouling,[3a-c] and other activities. These compounds are considered as important drug candidates and have attracted much attention in a variety of research fields. In 2014, Wang and co-workers reported the isolation of dihydroisocoumarin analogues as racemates, named eurotiumides, from a gorgonian-derived fungus, *Eurotium* sp. XS-200900E6 (Figure 1).[4] Among them, eurotiumide A (**1**), which was assigned *trans* configuration at H3/H4 positions on the basis of coupling constants between H3 and H4, displayed potent antibacterial activities especially toward *Vibrio anguillarum* and *Vibrio parahaemolyticus* (MIC = 0.39–0.78 μM), which are pathogens that cause vibriosis and gastrointestinal illness, respectively. Eurotiumide B (**2**), which was assigned *cis* configuration at H3/H4 positions by the same way as **1**, exhibited potent antifouling activity toward the larval settlement of the barnacle *Balanus amphitrite* (EC_{50} = 0.7–1.4 μg/mL) and also displayed a high therapeutic ratio (LC_{50}/EC_{50} = >68.5). These results suggest that **1** and **2** might be good candidates for a potent antimicrobial drug and an environment-friendly antifouling agent. To date, there is no report on the total syntheses of **1** and **2**, and their mode of actions are still unknown. Herein we report the first asymmetric total syntheses of the proposed structures of **1** and **2**, and clarified that their relative and absolute configurations on the basis of the X-ray crystallographic analysis, and their revised structures as **1'** and **2'**. Moreover, we found that **1'** and **2'** displayed the highly fluorescence in several solvents and demonstrated that it might be useful for studying the mode of action of **1'** toward *Bacillus cereus*.

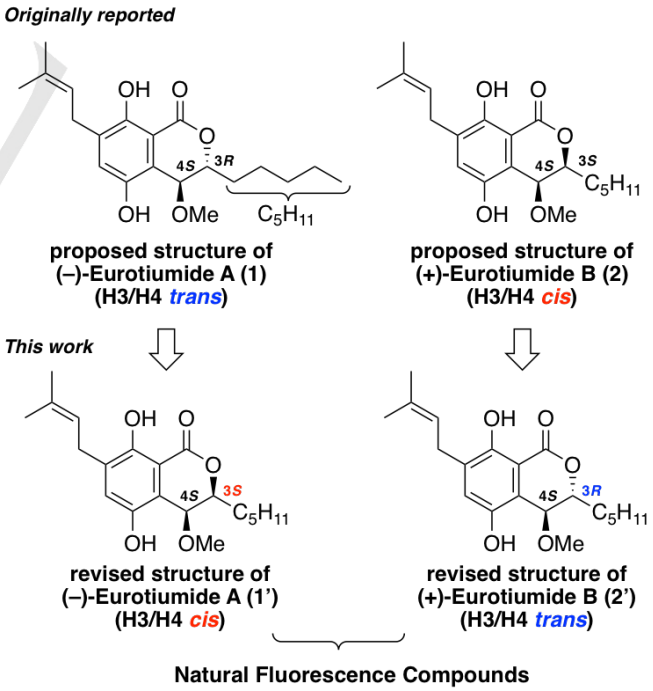

Figure 1. Structures of eurotiumide A and B.

Our synthetic plan toward proposed structure of (–)-eurotiumide A (**1**) and (+)-eurotiumide B (**2**), whose stereochemistry at C3-C4

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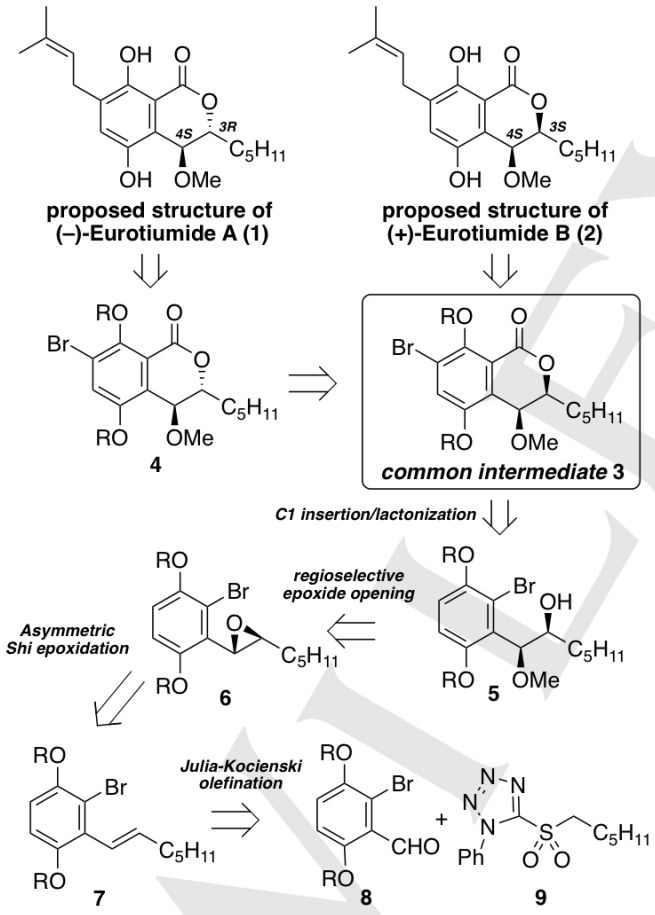
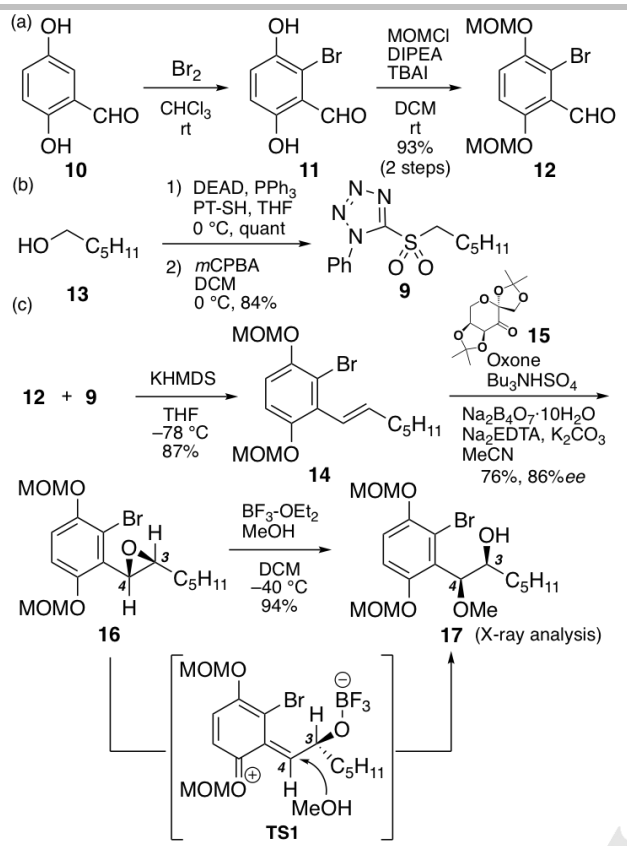
positions as (3*R*,4*S*) and (3*S*,4*S*), is outlined in Scheme 1. The difference between **1** and **2** is the stereochemistry at the C3 position. On the basis of this point, we considered that both **1** and **2** would be obtained from common intermediate **3**, from which **2** would be readily synthesized by introducing the prenyl side chain to **3**. The late stage introduction of the side chain by Pd-catalyzed cross coupling would make the structure-activity relationship (SAR) studies easier. On the other hand, **1** would be accessible by a similar cross coupling reaction from **4**, which would be obtained from **3** through inversion at the C3 position by sequential hydrolysis and intramolecular Mitsunobu reactions. We envisioned that the *cis* 4-methoxyisochroman-1-one skeleton, which is the key framework of **2**, could be constructed from alcohol derivative **5** via Pd-catalyzed C1 insertion/lactonization cascade reaction. Contiguous *cis* 3-hydroxy/4-methoxy groups would be obtained from chiral epoxide **6** through regio and stereoselective epoxide opening with MeOH, and **6** is expected to be obtained from alkene **7** by the asymmetric Shi epoxidation. Finally, **7** would be available by the Julia-Kocienski olefination between aldehyde **8** and 1-phenyl-1-*H*-tetrazol-5-yl (PT) sulfone **9**.



Scheme 1. Retrosynthetic analysis of proposed structures of (–)-eurotiumide A (**1**) and (+)-eurotiumide B (**2**).

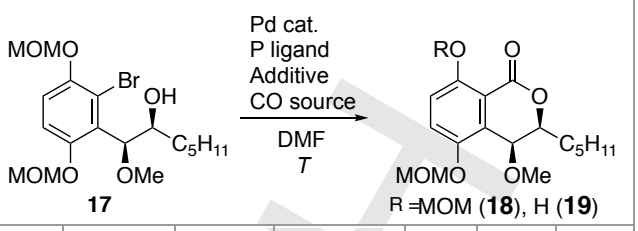
We initially prepared the aldehyde derivative and PT-sulfone as substrates of the Julia-Kocienski olefination (Scheme 2). Aldehyde **12**[5] was synthesized by a sequential operation of bromination of commercially available 2,5-dihydroxybenzaldehyde (**10**) and MOM protection of the two phenolic hydroxyl groups (**11**) (Scheme 2(a)). PT-sulfone **9** was obtained through the Mitsunobu reaction of 1-phenyl-1*H*-tetrazol-5-yl thiol (PT-SH) with *n*hexanol (**13**) followed by oxidation of the sulfide to sulfone (Scheme 2(b)). The Julia-Kocienski olefination between aldehyde **12** and PT-sulfone **9** proceeded smoothly to afford *trans* olefin **14** in 87% yield. Next, we examined the asymmetric epoxidation of **14** by the application of Shi's method.[6] First, the epoxidation of *trans* alkene **14** with a catalytic amount of chiral ketone **15** (30 mol%) was carried out under the general conditions of the asymmetric Shi epoxidation. However, the desired chiral epoxide **16** was obtained in only 37% yield. After several optimizations, we found that the use of 1.0 equivalent of **15** increased the isolated yield to 76% and the enantiomeric excess, which was determined by chiral HPLC analysis, was 86%. Then, we conducted the regio and stereoselective epoxide opening reaction with MeOH. Thus, treatment of epoxide **16** with BF_3-OEt_2 in DCM with an excess amount of MeOH (10 equiv) at –40 °C afforded desired product **17** in 94% yield as a single isomer. Interestingly, we found that **17** preferred to form a racemic crystal and the most of minor enantiomer of **17** was removed by simple filtration after recrystalization.[7] The enantiomeric excess of the resultant filtrate increased up to 94% *ee*. Moreover, its X-ray crystallographic analysis confirmed the relative stereochemistry of **17**.[8] We propose this high stereo selectivity as illustrated in Scheme 2. Ring opening, which is assisted by the lone pair of the oxygen atom at the *ortho*-position, occurs after activation of the epoxide with BF_3-OEt_2. The conformation of the opened intermediate should exist as **TS1** expected from the allylic 1,3-strain effect, and then MeOH attacks the C4 benzylic position from the opposite side of the bulkier *n*pentyl group.[9]



Scheme 2. Syntheses of (a) aldehyde derivative **12**, (b) PT sulfone **9**, (c) lactone precursor **17**.

Having prepared the precursor for the C1 insertion/lactonization, we turned our attention to construction of the *cis* 4-methoxyisochroman-1-one skeleton (Table 1). First, the general conditions used for Pd-catalyzed C1 insertion reaction under CO atmosphere did not give any products (entry 1). However, on screening phosphine ligands, we found that the use of Xantphos gave the desired lactone **18** in 8% yield (entry 2). Recently, Manabe *et al*. reported that *N*-formylsaccharin is an efficient CO source and could be applied to Pd-catalyzed fluorocarbonylation reactions of aryl halides in the presence of slight excess of potassium fluoride (KF).[10a-c] Following their procedure, the desired C1 insertion/lactonization cascade reaction proceeded to afford lactone **18** in 27% yield along with lactone **19** (5%), in which one MOM group was removed (entry 3). Encouraged by this result, we investigated further optimization of the reaction conditions. To our delight, increasing the amount of KF (5.0 equiv) significantly improved the conversion and afforded cyclized products in 65% combined yield (entry 4). The starting material was almost totally consumed at 115 °C to give **18** and **19** in excellent yields (entry 5). This result displayed that the C1 insertion reaction by using *N*-formylsaccharin is useful for natural product synthesis.

Table 1. C1 Insertion/lactonization cascade reaction for constructing *cis* 4-methoxyisochroman-1-one skeleton.



Entry	Pd cat[a] P ligand[b]	Additive (equiv)	CO source	*T* [°C]	Yield [%] **18**	Yield [%] **19**
1	$Pd(PPh_3)$ –	nBu_3N (2.0)	CO gas (1 atm)	80	0	0
2	$Pd(OAc)_2$ Xantphos	–	CO gas (1 atm)	80	8	0
3	$Pd(OAc)_2$ Xantphos	KF (2.5)	*N*-formyl-saccharin[c]	80	27	5
4	$Pd(OAc)_2$ Xantphos	KF (5.0)	*N*-formyl-saccharin[c]	80	60	5
5	$Pd(OAc)_2$ Xantphos	KF (5.0)	*N*-formyl-saccharin[c]	115	76	19

[a] 20 mol%. [b] 30 mol%. [c] 1.5 equiv.

Having established the method for the construction of the 4-methoxyisochroman-1-one skeleton, which is the key core of eurotiumides, we turned our attention to completion of the total synthesis of **1** and **2** (Scheme 3). The Pd-catalyzed C1 insertion/lactonization cascade reaction under the optimum conditions afforded the mixture of **18** and **19** with excellent conversion, and the MOM group of the salicylic acid moiety of **18** was subsequently selectively removed by treatment with magnesium bromide to afford **19** in a one-pot operation with 89% overall yield.[11,12] After the introduction of bromine to the *ortho*-position of the salicylic hydroxyl group, the phenolic hydroxyl group was reprotected with MOM in one-pot. It should be noted that bromination did not occur with MOM-protected **18**. At this stage, **20** was crystallized and its enantiomeric excess increased up to 99%. The relative and absolute configuration of **20** were confirmed by X-ray crystallographic analysis, and the stereochemistry at C3-C4 positions was determined as (3*S*,4*S*).[13] The Stille coupling reaction in the presence of $PdCl_2(PPh_3)_2$, CsF in DMF for the introduction of the prenyl group proceeded smoothly, and subsequent addition of 6 M HCl aq removed the two MOM groups to furnish the proposed structure for (+)-**2** in 55% yield in one-pot. Although we achieved the total synthesis of the proposed structure of (+)-**2**, its all spectral data did not match the reported (+)-**2**, instead matched the reported (–)-**1**. It indicates that the assignment of the relative configurations between C3-C4 positions was incorrect, and the correct configuration at the C3 position is opposite from the previous report. Toward the proposed structure of (–)-**1**, hydrolysis of **20** followed by intramolecular Mitsunobu reaction afforded the corresponding *trans* 4-methoxyisochroman-1-one compound **21** in good yield. The similar sequential operation of **21** afforded the proposed structure of (–)-**1**. We also compared the all spectral data of our synthetic **1** with the data of the reported (–)-**1**, however, our data were not identified with the reported (–)-**1**, and finally, the data of ours matched the data of the reported (+)-**2**. On the basis

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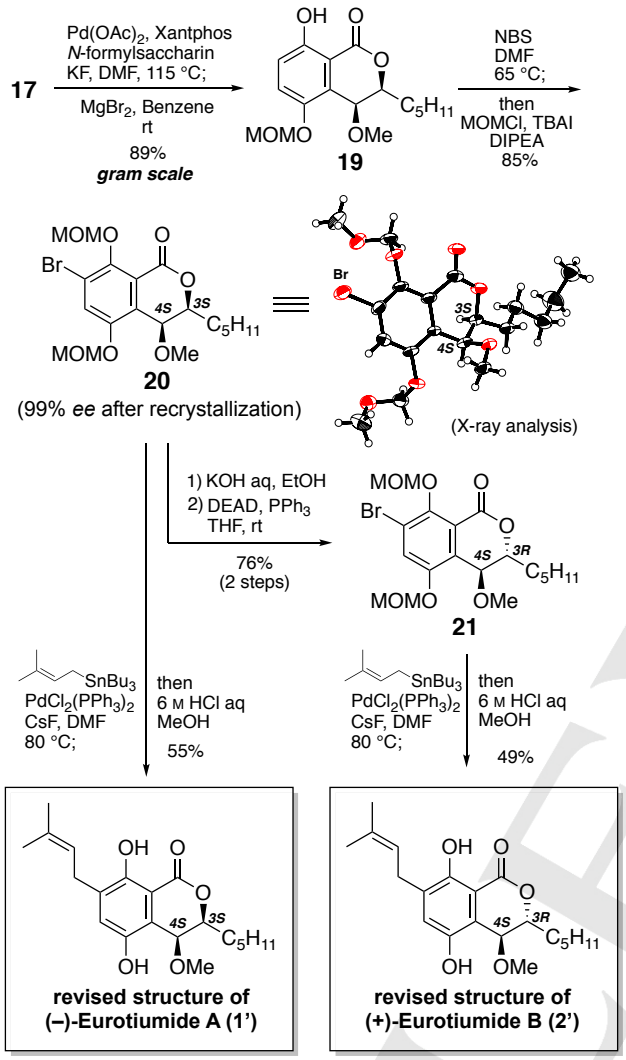
of these results, we revised the structures of the isolated natural eurotiumide A and eurotiumide B having contiguous *cis* and *trans* H3/H4 configurations, and the correct structures of (–)-eurotiumide A and eurotiumide B are **1'** and **2'** as displayed in scheme 3, respectively.



Scheme 3. Completion of total syntheses of (–)-eurotiumide A (**1'**) and (+)-eurotiumide B (**2'**).

Interestingly **1'** and **2'** emitted high fluorescence in dichloromethane after excitation at 365 nm, and thus, further investigation of their fluorescent properties was conducted. Figure 2 displays the fluorescence (a) spectra and (b) properties of **1'** in several solvents. The absorption maximum of **1'** is around 350 nm in dichloromethane, acetone, dimethyl sulfoxide, methanol, and water. Two peaks are observed in the fluorescence spectra at 486 nm/428 nm, 501 nm/439 nm, and 503 nm/439 nm in dichloromethane, acetone, and dimethyl sulfoxide, respectively, and the emission intensities of the longer wavelength peaks are stronger than those at the shorter wavelength region.[14] These observations suggest that the fluorescence of **1'** is derived from the excited-state intramolecular proton transfer (ESIPT).[15] Time-dependent density functional theory (TD-DFT) calculations support this observation (see the Supporting Information for details). Moreover, although two peaks are observed in the fluorescence spectrum in protic solvents such as methanol and water, the emission intensity of the longer wavelength peak substantially decreases in methanol and almost disappears in water. These experimental results suggest that the ESIPT of **1'** occurs readily in aprotic solvents. In contrast, protic solvents such as methanol and water facilitate the formation of intermolecular hydrogen bonds with the phenolic oxygen or carbonyl group of the lactone moiety preventing the ESIPT from occurring in these solvents.

Finally, we evaluated the utility of **1'** as a fluorescent probe for the investigation of the mode of action of antibiotics. Treatment of incubated *Bacillus cereus* with **1'** revealed an MIC 6.25 μM of **1'**, and the fluorescence of **1'** from bacteria was observed. As shown in figure 2 (c), the living bacteria treated with **1'** emitted blue fluorescence upon excitation at 345 nm, whereas no fluorescence was observed from the control treated with DMSO. On the other hand, rapid discoloration of the fluorescence of **1'** was observed from *Staphylococcus aureus*, which is treated with **1'** and inhibited the growth in MIC 12.5 μM of **1'**. This difference in the speed of the discoloration of **1'** indicates a possibility of different mode of actions or localization between each grampositive bacteria.

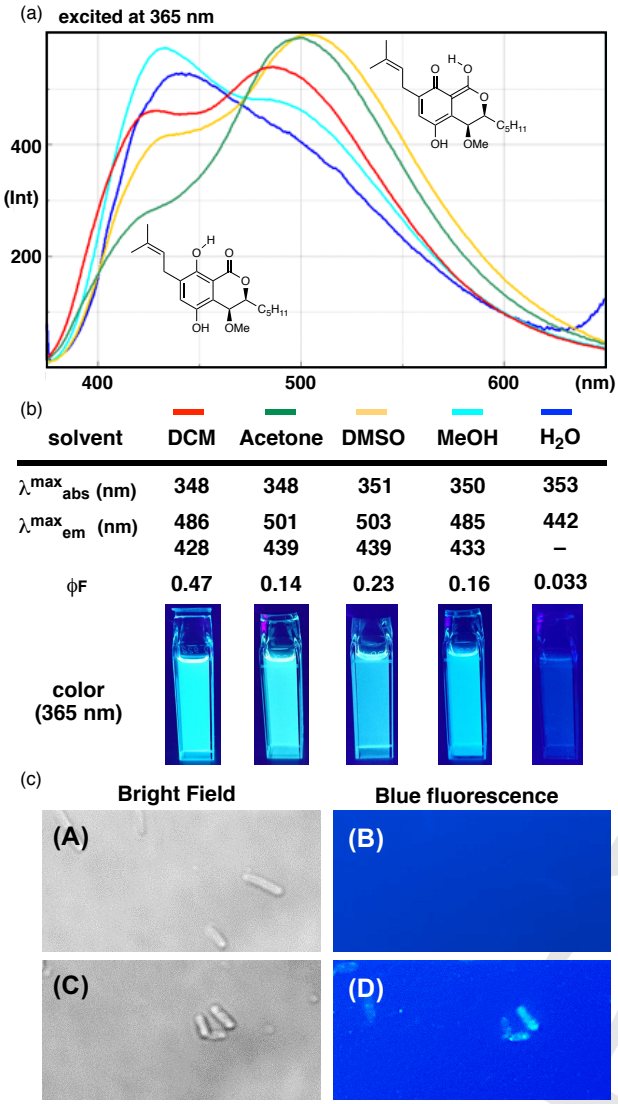


Figure 2. Fluorescent properties of eurotiumide A (**1'**). (a) Fluorescence spectra of **1'** in several solvents. (b) Fluorescent properties of **1'**. (c) Fluorescent labeling of *Bacillus cereus* with **1'**. (A) and (B) were treated with 1 μM DMSO. (C) and (D) were treated with 1 μM of **1'**.

In conclusion, we have achieved the first asymmetric total syntheses of (–)-eurotiumide A (**1'**) and (+)-eurotiumide B (**2'**) in longest linear 8 and 10 steps (totally 10 and 12 steps), respectively. We have also revised the relative configurations of H3/H4 of eurotiumide A and eurotiumide B. These syntheses involve the construction of a *cis* 4-methoxyisochroman-1-one skeleton by means of the asymmetric Shi epoxidation, region and stereoselective epoxide opening, and subsequent Pd-catalyzed C1 insertion/lactonization cascade reaction using *N*-formylsaccharin. This strategy is applicable to the synthesis of other related natural products that have a 4-oxoisochroman-1-one architecture. We have also discovered the high fluorescence property of **1'** and **2'**, and observed large Stokes shifts in several solvents. This fascinating property can be utilized for elucidating the mode of action of antimicrobial activities of **1'** against several bacteria by fluorescence imaging. Further investigations of the mode of action are underway in our laboratory.

Experimental Section

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.2018xxxxx.

Acknowledgements

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Keywords: natural products• total synthesis • fluorescent probes

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[7] Chiral **17**, whose enantiomeric excess is quite high, was not crystallized under several trials.

[8] CCDC 1821023.

[9] We also conducted the same reaction with the substrate which has methyl group instead of MOM group and observed the same selectivity. Thus, this selectivity does not come from the double inversion at C4 position involving the neighbouring effect of MOM group. Moreover, increasing amount of MeOH accelerates S_N2 reaction at C4 position and gave the mixture of *cis* and *trans* products.

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[12] This reaction proceeded in 76% even in 9.8 g scale with racemic **17**.

[13] CCDC 1821161.

[14] Detail of the fluorescence properties of **2'** is disclosed in the Supporting Information.

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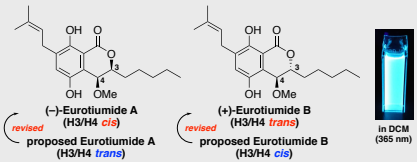

Atsushi Nakayama, Hideo Sato, Sangita Karanjit, Naoki Hayashi, Masataka Oda, and Kosuke Namba**

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Asymmetric total syntheses and structure revisions of dihydroisocoumarin-type natural products, eurotiumide A and eurotiumide B are disclosed. Key framework of these compounds was constructed by Pd-catalyzed C1 insertion/lactonization cascade reaction by using *N*-formylsaccharin as CO source. These compounds emitted highly fluorescence in several solvents.