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Revised Structure and Synthesis of Celastramycin A, A Potent Innate Immune Suppressor

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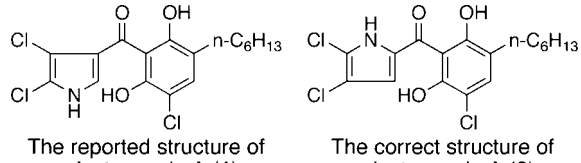
ABSTRACT



The reported structure of celastramycin A (**1**)

The correct structure of celastramycin A (**2**)

After searching for natural substances that regulate innate immunity using the ex vivo *Drosophila* culture system, a benzoyl pyrrole-type compound, celastramycin A, was identified and isolated as a potent suppressor. By synthesizing the previously reported structure 1 and another benzoyl pyrrole-type compound 2 reported in a Japanese patent, the correct structure of celastramycin A was confirmed to be 2. Compound 2 suppressed the production of IL-8 (IC_{50} 0.06 μg/mL) in human umbilical vein endothelial cells (HUVECs).

Innate immunity is the first line of defense against infectious microorganisms,[1,2] and the basic mechanisms of this process, including pathogen recognition and immune response activation, are evolutionarily conserved.[3] In mammals, innate immunity interacts with adaptive immunity and has a key role in regulating the immune response.[4] Therefore, innate immunity is a good target for the development of immune regulators that suppress unwanted immune responses, such as septic shock, inflammatory diseases, and autoimmunity. For example, eritoran, an LPS (lipopolysaccharide) antagonist,[5] and TAK-242, an inhibitor of the TLR4 (Toll-like receptor 4)-induced signaling pathway,[6] are in clinical trials for treatment of severe sepsis.

To screen pharmaceuticals that target innate immunity, we established an ex vivo culture system based on the innate immune response of *Drosophila*, which is highly useful for identifying immune regulators that act on human innate immunity.[7] We used this system to search for natural substances that regulate innate immunity and identified and isolated a benzoyl pyrrole-type compound from *Streptomyces* sp. as a potent suppressor. Interestingly, our isolated compound showed the same ^{1}H and ^{13}C NMR and mass spectra as those of both celastramycin A (**1**)[8] and another benzoyl pyrrole-type compound **2** reported in a Japanese patent.[9] It

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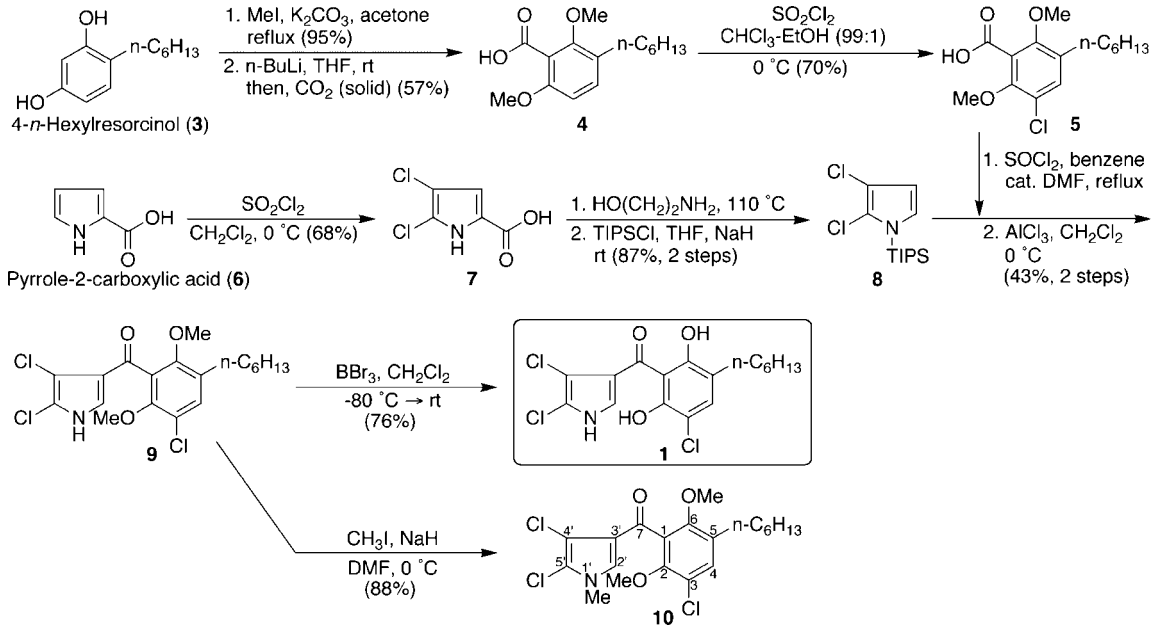
Scheme 1. Synthesis of Compound **1**



was difficult to confirm the structure of our compound by derivatization because we isolated a limited amount of the compound. Therefore, we decided to determine which structure, either **1** or **2**, was correct by synthesizing both compounds.

The synthesis of **1** is illustrated in Scheme 1. After *O*-methylation of commercially available 4-*n*-hexylresorcinol (**3**), *ortho*-lithiation followed by carboxylation gave compound **4**.[10] Treating **4** with sulfuryl chloride afforded 3-chloro derivative **5** as the sole product. In regard to the pyrrole moiety, pyrrole-2-carboxylic acid (**6**) was chlorinated with sulfuryl chloride to produce 4,5-dichloro compound **7**. Decarboxylation of **7** with heat in ethanolamine[11] and subsequent N-silylation gave the *N*-TIPS protected pyrrole **8**, which underwent Friedel−Crafts acylation with an acyl chloride derived from **5** to produce *β*-benzoylpyrrole **9**. *β*-Acylation of **8** was induced by its *N*-TIPS group,[12] which was cleaved during the reaction. In the HMBC spectrum of **10**, an *N*-methyl derivative of **9**, the correlation peak for H-2′ to the *N*-methyl carbon atom, and the *N*-methyl proton to C-2′ confirmed the position of a benzoyl group at C-3′ (Figure 1). Finally, demethylation of **9** with BBr_3 allowed us to complete the synthesis of **1**. However, the ^1H and ^{13}C NMR spectra of synthetic **1** were different from those of the compound we isolated and the reported spectra of **1**[8] (Table 1).

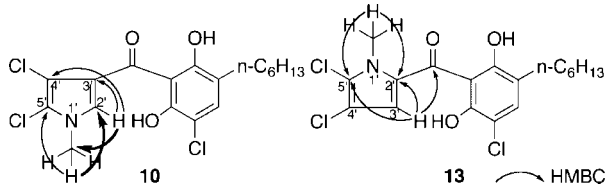


Figure 1. Selected HMBC correlations of **10** and **13**.

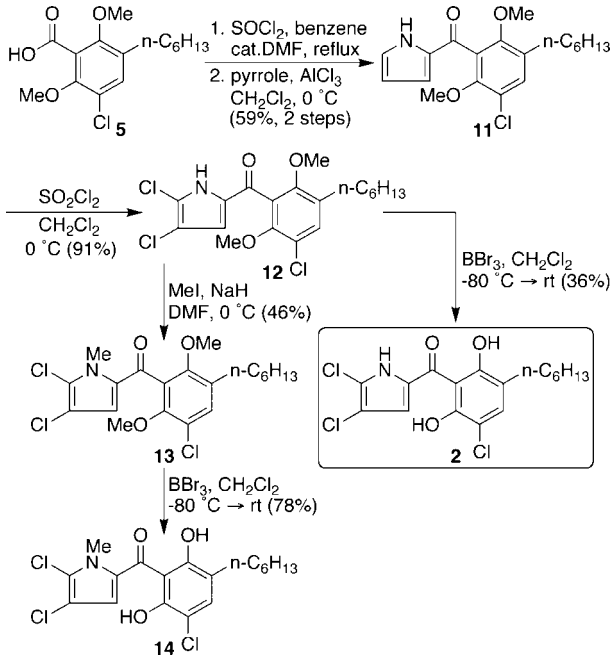
Table 1. ^{13}C NMR Spectral Data of Synthetic and Reported **1** and **2**[a]

	synthetic **1**	synthetic **2**	reported **1**[8]	reported **2**[9]
1	111.6	112.6	112.6	112.6
2	149.6	147.9	148.0	147.9
3	110.3	110.4[c]	110.3[e]	110.3[g]
4	134.2	133.7	133.7	133.8
5	124.3	124.8	124.8	124.8
6	157.2	157.3	157.3	157.3
7	190.2	182.8	182.8	182.8
2′	122.9	129.0	129.0	128.9
3′	123.9	119.7	119.6	119.8
4′	109.8	110.3[c]	110.3[e]	110.3[g]
5′	114.7	121.6	121.4	121.6
1″	29.4[b]	29.4[d]	29.4[f]	29.4[h]
2″	29.1[b]	29.2[d]	29.4[f]	29.3[h]
3″	29.1[b]	29.1[d]	29.1[f]	29.1[h]
4″	31.7	31.7	31.7	31.7
5″	22.6	22.6	22.6	22.7
6″	14.1	14.1	14.1	14.1

[a] 600 MHz for ^1H and 150 MHz for ^{13}C in pyridine-d_5. [b] These signals were indistinguishable. [c] These signals were indistinguishable. [d] These signals were indistinguishable. [e] These signals were indistinguishable. [f] These signals were indistinguishable. [g] These signals were indistinguishable. [h] These signals were indistinguishable.

The synthesis of **2** is illustrated in Scheme 2. Carboxylic acid **5** was converted into its acid chloride, and then a Friedel−Crafts reaction with pyrrole gave α-benzoylpyrrole **11**. Chlorination of **11** with sulfuryl chloride afforded 4,5-

Scheme 2. Synthesis of Compound **2**



dichloro compound **12** selectively. No correlation peak for H-3′ to the *N*-methyl carbon atom and the *N*-methyl proton to C-3′ in the HMBC spectrum of **13**, an *N*-methyl derivative of **12**, indicated the position of a benzoyl group at C-2′ (Figure 1). Finally, compound **2** was synthesized by treating **12** with BBr_3. The ^1H and ^{13}C NMR spectra of synthetic **2** were identical to those of our compound and to the spectra of **1**[8] and **2**[9] reported in the literature (Table 1). Therefore, the reported structure of celastramycin A (**1**) is incorrect, and the correct structure for **1** and the compound that we isolated is **2**. The chemical shift (δ_c 182.8) of a carbonyl carbon in synthesized **2** was significantly different from that in synthesized **1** (δ_c 190.2). This fact may be useful to distinguish between α-benzoylpyrrole and *β*-benzoylpyrrole. In addition, compound **14**, an *N*-methyl derivative of **2**, was synthesized by treating **13** with BBr_3.

The immunosuppressive effects of **1**, **2**, **12**, and **14** on the imd (immune deficiency) pathway in *Drosophila* innate immunity were evaluated using the ex vivo *Drosophila* culture system.[7] Compound **2** showed a potent immunosuppressive effect (IC_{50} 0.008 μg/mL), while **1**, **12**, and **14** had no effect. These results indicated that the α-benzoylpyrrole moiety, two phenolic hydroxyl groups, and the imino proton in the structure of **2** are crucial for the biological activity.

The TNF-α signaling pathway in humans plays a critical role in the inflammatory response, sepsis, and rheumatoid arthritis by producing costimulatory molecules, cytokines, chemokines, and adhesion molecules through the activation of NF-κB,[13] which shares some similarity with the imd pathway in *Drosophila* innate immunity. To examine whether compound **2** suppresses the mammalian TNF-α signaling pathway as well as the *Drosophila* imd pathway, we investigated the effect of **2** on TNF-α-stimulated production of IL-8, a neutrophil chemotactic factor, in human umbilical vein endothelial cells (HUVECs). Compound **2** showed a potent suppressive effect (IC_{50} 0.06 μg/mL) on the production of IL-8, like LL-Z-1640-2[14] (5*Z*-7-oxozeaenol) (IC_{50} 0.01 μg/mL). LL-Z-1640-2 is a highly potent inhibitor of TAK1,[15] which regulates the TNF-α signaling pathway.[16] This result indicates that compound **2** can be used as a lead compound for novel immunosuppressive agents. Further investigations on the structure−activity relationship of this compound and its mechanism of action are in progress.

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Supporting Information Available: Experimental methods and NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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