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Total Synthesis and Absolute Configuration of Simpotentin, a Potentiator of Amphotericin B Activity

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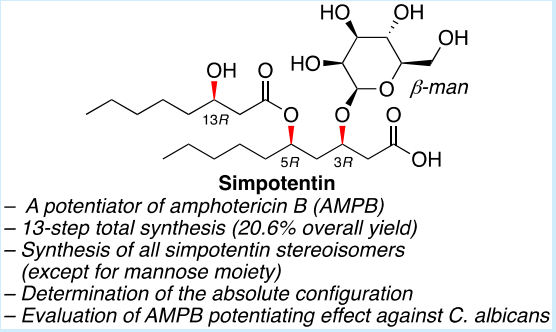
Supporting Information

ABSTRACT: The total synthesis of simpotentin (**1**), a new potentiator of amphotericin B activity against *Candida albicans*, was achieved. Our research results enabled the access of all stereoisomers of **1** and the elucidation of the unknown absolute configuration of **1**. Furthermore, one of the stereoisomers is a better amphotericin B potentiator than **1** and is an excellent lead compound for the development of a novel amphotericin B potentiator.



Over the past 30 years, the number of patients with mycoses has continued to increase, primarily due to highly advanced medical treatments, such as organ transplantation, the use of immunosuppressive drugs, radiotherapy, and antitumoral chemotherapy. Among them, *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and

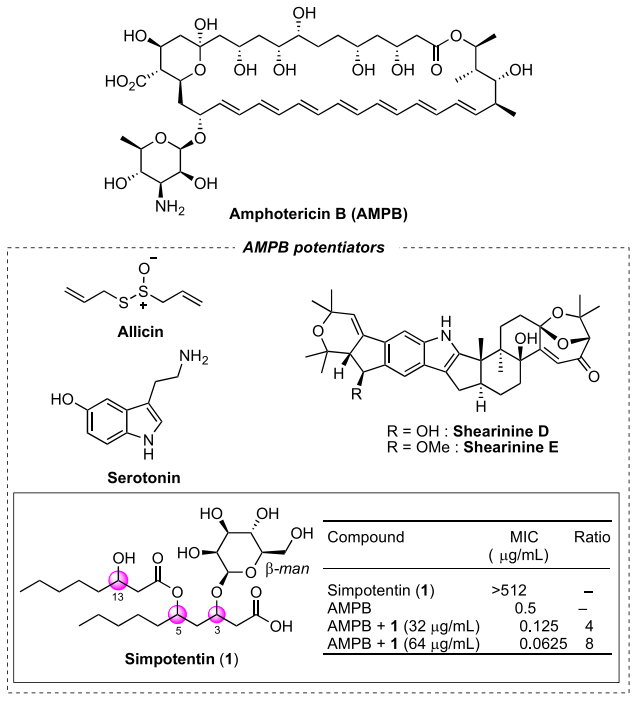
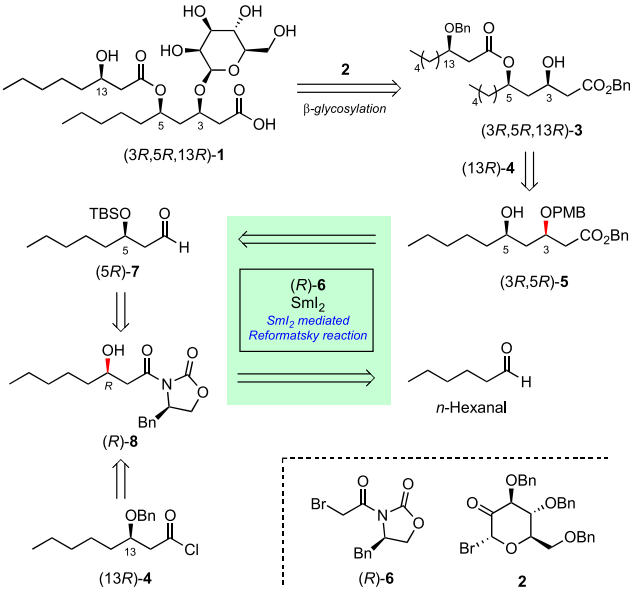


Figure 1. Structures of AMPB and its potentiators and the MIC values of **1**, AMPB, and their mixture against *C. albicans*.

Scheme 1. Retrosynthesis of (3*R*,5*R*,13*R*)-1



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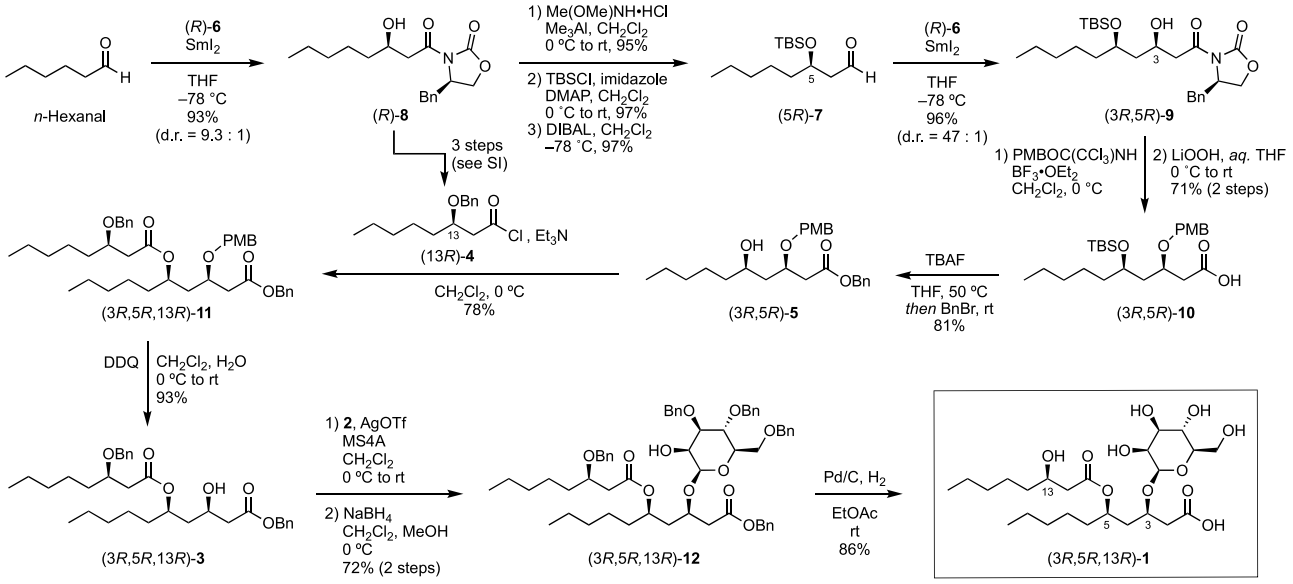
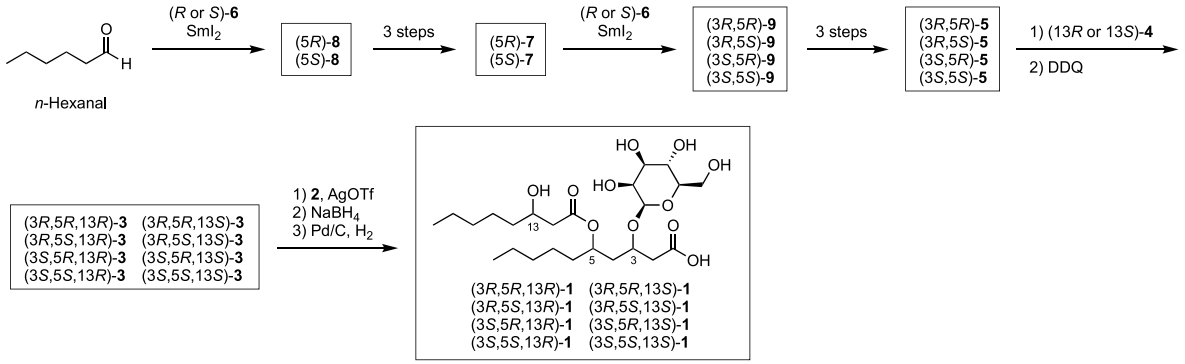
Scheme 2. Total Synthesis of (3*R*,5*R*,13*R*)-1



Scheme 3. Synthesis of All Stereoisomers of Simpotentin (1)



Rhizopus oryzae are pathogenic fungi that occur via invasive fungal infections.[1] *C. albicans* is especially implicated in approximately 50% of patients with candidasis.[2] Although several antifungal antibiotics are currently available for their treatment, amphotericin B (AMPB), a polyene macrolide antifungal antibiotic, has been the "gold standard" since its introduction into clinical practice in the 1950s.[3] Additionally, liposomal AMPB was developed to improve the tolerability profile of AMPB deoxycholate in the 1990s.[4] However, AMPB has serious side effects, such as nephrotoxicity, hypokalemia, fever, chills, and vomiting, which have led to discontinuation of this treatment. The development of potentiators for AMPB has reduced the dose of AMPB and the severe side effects. A few potentiators for AMPB have been reported thus far (Figure 1). Ogita et al. reported allicin, which enhances the membrane permeability of AMPB, as the first report of a potentiator for AMPB from garlic.[5] Serotonin has been reported by Hellar et al. to have a similar mechanism of action.[6] Recently, shearinenes D and E, alkaloid metabolites produced by a Alaskan soil-derived *Penicillium* sp., have shown an enhancement in the antifungal activity of AMPB, according to Cichewicz et al.[7] During our continuous screening for microbial AMPB potentiators against *C. albicans*, a novel compound, simpotentin (**1**), was isolated from the culture broth of *Simplicillium* sp. FKI-4981B (Figure 1).[8]

Simpotentin (**1**) is a β-mannoside, and its aglycone is 3-hydroxy-5-[(3-hydroxyoctanoyl)oxy]decanoic acid with three stereogenic centers. Simpotentin (**1**) is a nonantibiotic with no antifungal activity against *C. albicans* even at 512 μg/mL via the liquid microdilution method. On the other hand, the MIC_{90} value of AMPB against *C. albicans* was found to be 0.500 μg/mL. However, the MIC values decreased to 0.06 and 0.03 μg/mL in combination with 32 and 64 μg/mL of **1**, respectively. Thus, we found that **1** enhanced AMPB activity against *C. albicans* dose dependently.

Simpotentin (**1**) is a new potent potentiator of AMPB; however, the absolute configuration of the aglycone of **1** has not been clarified. To determine the absolute configuration of the natural **1**, a concise total synthesis of all simpotentin stereoisomers (total eight diastereomers) was required. Therefore, we performed the total synthesis of (3*R*,5*R*,13*R*)-**1** (Simpotentin numbering), which was selected as a primary target molecule among the eight diastereomers. The retrosynthetic analysis is shown in Scheme 1. Accordingly, (3*R*,5*R*,13*R*)-**1** could be synthesized via a stereoselective β-mannosylation of the key aglycone, (3*R*,5*R*,13*R*)-**3**, with ulosyl bromide **2**,[9] followed by the reduction of a carbonyl group and deprotection.[10] Aglycone (3*R*,5*R*,13*R*)-**3** could be produced from the acylation of (3*R*,5*R*)-**5** with acyl chloride (13*R*)-**4**. Then (3*R*,5*R*)-**5** could be derived from a commercially available *n*-hexanal via two sequential SmI_2-mediated stereo-

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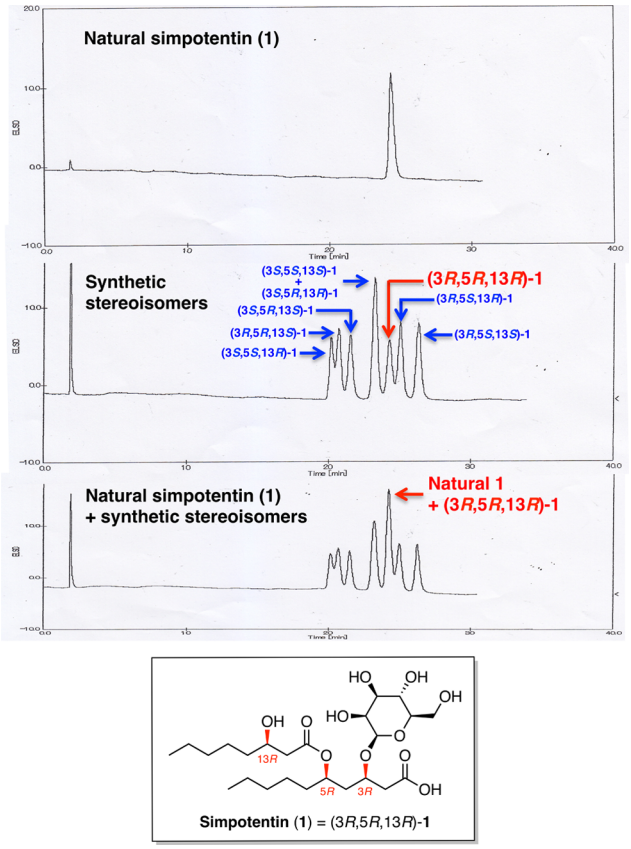

Figure 2. HPLC analyses of natural simpotentin (**1**), its synthetic stereoisomers, and the absolute structure of simpotentin (**1**). HPLC conditions: column, CHRALCEL OJ-RH (DAICEL) (ϕ 4.6 mm × 150 mm); mobile phase, 20−25% MeCN−0.1% HCO_2H (30 min gradient); flow rate, 1 mL/min; column temperature, 10 °C; detection, ELSD (temp 40 °C, gas flow; 1.5 L/min); injection, 5 μg.

selective Reformatsky reactions[11] with the chiral *N*-bromoacetyl oxazolidinone (*R*)-**6**[12] [(5*R*)-**7** → (3*R*,5*R*)-**5** and *n*-hexanal → (*R*)-**8**]. Then (13*R*)-**4** could be synthesized from a common intermediate, (*R*)-**8**. This synthetic strategy would also allow the synthesis of the other simpotentin stereoisomers by using the chiral oxazolidinone (*S*)-**6** instead of (*R*)-**6**.

The synthesis of (3*R*,5*R*,13*R*)-**1** is shown in Scheme 2. The first SmI_2-mediated Reformatsky reaction of *n*-hexanal with (*R*)-**6**[12] gave (*R*)-**8** in 93% yield (diastereomeric ratio (dr) of 9.3:1). The Conversion of (*R*)-**8** to the Weinreb amide[13] (95%), TBS protection (97%), and reduction with DIBAL afforded the aldehyde (5*R*)-**7**.[14] The second Reformatsky reaction of (5*R*)-**7** and oxazolidinone (*R*)-**6** gave (3*R*,5*R*)-**9**[15] in 96% yield with a high diastereoselectivity (dr of 47:1). PMB protection[16] of (3*R*,5*R*)-**9** followed by removal of the chiral auxiliary with LiOOH[17] afforded the carboxylic acid, (3*R*,5*R*)-**10**, in 71% yield in two steps. Subsequent TBS deprotection of (3*R*,5*R*)-**10** with TBAF afforded the desired 5-hydroxycarboxylic acid but was accompanied by formation of a six-membered lactone during workup after reaction and silica gel chromatography. Thus, after completing the TBS deprotection of (3*R*,5*R*)-**10** with TBAF, benzyl bromide was added to the reaction mixture in a one-pot manner. Esterification proceeded smoothly to yield the benzyl ester, (3*R*,5*R*)-**5**, in 81% yield without lactonization. Acylation of (3*R*,5*R*)-**5** with acyl chloride (13*R*)-**4**, which was easily derived from (*R*)-**8** in three steps,[18] in the presence of Et_3N gave (3*R*,5*R*,13*R*)-**11** in 78% yield. PMB deprotection via treatment with DDQ afforded the desired aglycone, (3*R*,5*R*,13*R*)-**3**, in 93% yield.

With the desired aglycone (3*R*,5*R*,13*R*)-**3** synthesized, β-mannosylation by PMB ether-mediated intramolecular aglycon delivery[19] was first investigated; however, it was unfruitful. Next, the β-glycosylation of (3*R*,5*R*,13*R*)-**3** with ulosyl bromide **2**[9] was attempted as a key reaction. β-Glycosylation in the presence of AgOTf furnished the desired β-mannoside (3*R*,5*R*,13*R*)-**12** in 72% yield in two steps as a single isomer after the reduction of the corresponding ketone with $NaBH_4$. Finally, deprotection of all benzyl groups via hydrogenolysis gave the (3*R*,5*R*,13*R*)-**1** in 86% yield. Because the total synthesis of (3*R*,5*R*,13*R*)-**1** was established, the other stereoisomers of simpotentin (**1**) were also synthesized in the same manner (Scheme 3).[18] This means that our total synthesis was very concise and practical.

For the determination of the absolute configuration of natural simpotentin (**1**), its physicochemical properties were compared with those of all the synthetic stereoisomers of **1**. Consequently, (3*R*,5*R*,13*R*)-**1** was completely identical to natural **1** in all respects ($[\alpha]_D$, ^1H, and ^{13}C NMR, IR, and ESIMS).[17] Furthermore, HPLC analyses of natural **1** and all synthetic stereoisomers were performed (Figure 2). Most of the stereoisomers, except for (3*S*,5*R*,13*R*)-**1** and (3*S*,5*S*,13*S*)-**1**, were separated. Then we found that the retention time of (3*R*,5*R*,13*R*)-**1** was completely identical to that of natural **1**. Additional HPLC analysis of the mixture of natural **1** with all synthetic stereoisomers showed that the only peak for (3*R*,5*R*,13*R*)-**1** was clearly amplified. These data also revealed that the absolute configuration of natural simpotentin (**1**) is 3*R*,5*R*,13*R*.

Next, the potentiating effects of all of the simpotentin stereoisomers on the AMPB activity against *C. albicans* were evaluated. The activities of most of the stereoisomers were similar to natural **1**; however, (3*R*,5*S*,13*S*)-**1** was found to be better than **1** with over 16-fold potentiation of AMPB activity

Table 1. MIC Values of Amphotericin B against *C. albicans* in the Presence of the Stereoisomers of 1[a,b]

	MIC (μg/mL) of AMPB								
conc of the stereoisomer (μg/mL)	+ natural **1**	+ (3*R*,5*R*,13*R*)-**1** = synthetic simpotentin (**1**)	+ (3*R*,5*S*,13*R*)-**1**	+ (3*S*,5*R*,13*R*)-**1**	+ (3*S*,5*S*,13*R*)-**1**	+ (3*R*,5*R*,13*S*)-**1**	+ (3*R*,5*S*,13*S*)- **1**	+ (3*S*,5*R*,13*S*)-**1**	+ (3*S*,5*S*,13*S*)-**1**
0[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2	0.25	0.25	0.5	0.25	0.25	0.25	0.125	0.25	0.25
8	0.25	0.25	0.25	0.25	0.25	0.25	0.0625	0.125	0.25
32	0.125	0.125 (x4)	0.125	0.125	0.125	0.125	<0.0313 (>×16)	0.0625	0.0625

[a]A smaller MIC value means that it has a more potent enhancing effect for AMPB activity against *C. albicans*. [b]The stereoisomers of **1** showed no antifungal activity against *C. albicans* even at 64 μg/mL. [c]The MIC_{90} value of AMPB alone for *C. albicans*.

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at 32 μg/mL (Table 1). The new stereoisomer, (3*R*,5*S*,13*S*)-**1**, has the potential to be an excellent lead compound for the development of a novel AMPB potentiator.

In summary, we achieved the concise total synthesis of simpotentin (**1**). Extension of this chemistry enabled the synthesis of all stereoisomers of **1** and the determination of the absolute configuration of natural **1**. Furthermore, we discovered a new stereoisomer, (3*R*,5*S*,13*S*)-**1**, which has a more potent activity as an AMPB potentiator than **1**. Application of our total synthesis would allow the synthesis of a variety of simpotentin analogues. The structure−activity relationship study of simpotentin (**1**) is currently underway and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.9b01945.

Experimental procedures and characterization of compounds including NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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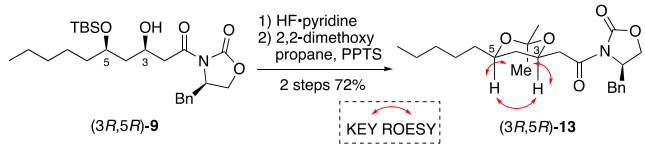

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