Synthesis of a Key Intermediate of Novel Galbonolide Analogues *via* Efficient Construction of a Conjugated Diene System

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The development of an efficient synthetic method enabled multi-gram synthesis of a key intermediate, which is useful for the modification at the C6-functional group of galbonolide analogues. The structure of a key intermediate including a conjugated diene was afforded by Horner–Emmons reaction, alkylation of Weinreb amide with alkyl lithium and a subsequent Wittig reaction.

Key words galbonolide; 14-membered macrolide antifungals; key intermediate; conjugated diene system

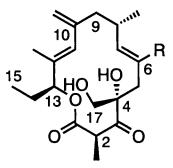
Galbonolide A (rustmicin; **1**) and galbonolide B (neorustmicin; **2**), the novel 14-membered macrolide antifungals, were discovered as fungal metabolites by Otake[1,2] and Achenbach,[3,4] independently. Galbonolide A exhibited potent activity against several clinically important microorganisms including *Candida* and *Cryptococcus* spp., which are known to cause human infections. Furthermore, the mechanism of the antifungal activity was determined to involve the unique inhibition of inositol phosphorylceramide (IPC) synthase.[5,6] Due to the poor stability of galbonolide A under chemical and physiological conditions, its development for clinical applications was not feasible. Therefore, we performed a chemical modification of galbonolide A to improve that problem by applying the synthetic method of galbonolide B reported by Tse.[7] The modification involved the conversion of the methyl enol ether part at C6-7 to other more stable functionalities, because this moiety was thought to be important to both the stability of the compound and its biological activity. Among them, a novel galbonolide analogue **3**, in which the methyl enol ether was converted to the methylthio enol ether functionality (Fig. 1), was found to retain its antifungal activity against *Cryptococcus neoformans*.[8,9]

The modified procedure for the syntheses of galbonolide analogues is summarized in Chart 1. Vinylic iodide **6** was prepared from methyl ketone **5** using the procedure developed by Takai.[10] Another constituent **9** was prepared from iodide **8** reacted with lithiated ethyl vinyl ether and subsequent hydrolysis with acid. A coupling reaction of lithiated **6** with ketone **9** afforded tertiary alcohol **10**, and subsequent dehydration using Martin's sulfurane reagent[11] gave desired diene **11** having *exo*-methylene and trace amount of *endo*-olefine product. From diene **11**, we could synthesize the novel galbonolide analogues possessing several vinylic functionalities at C6-7 through following three steps sequences, that is; 1) a construction of a trisubstituted double bond (**13**), a highly stereoselective alkylation (**15**)[12] and a macro-Dieckmann cyclization (**16**).

Compound **11** is a key intermediate, as it is also used to perform further substitution modification at the C6-position. Therefore, a large amount of **11** is needed not only for the modification, but also for multi-gram-scale preparation of a hopeful derivative such as **3**. But the synthetic method of **11**, shown above had some disadvantages. These disadvantages included poor reproducibility of the yields (**8**→**9**, **6**+**9**→**10**), low geometrical selectivity (**5**→**6**) and the use of harmful and expensive reagents ($CrCl_2$, Martin's sulfurane reagent). Thus, this route was not considered suitable for large-scale synthesis of **11**. More conventional synthetic reactions were employed in an effort to overcome these matters. Herein, we describe the improved synthesis of the key intermediate **11** by coupling of practical and convenient reactions.

In an effort to locate a new synthetic route, the cutting points of the intermediate **11** were reconsidered (Chart 2). The *exo*-methylene structure at C10 was introduced by Wittig reaction with enone compound (**i**). The C9-10 bond of the enone (**i**) was connected by a coupling reaction of carbonyl compound (**ii**) with the metalated **8**. Finally, (*E*)-double bond of compound (**ii**) was expected to construct preferentially in the conventional Horner–Emmons reaction of ketone **5**.

Contrastingly, the Horner–Emmons reaction of ketone **5**[13] using triethyl phosphonoacetate and NaH in THF yielded a mixture of (*E*)-α,β-unsaturated ester **17** and the undesired (*Z*)-isomer (80%, *E*/*Z*=7 : 1, Chart 3). Ester **17** was treated with MeO(Me)NH–HCl and *i*-PrMgCl to afford Weinreb amide **18** directly.[14] Subsequent purification by silica gel column chromatography gave pure (*E*)-α,β-unsaturated amide **18** (85%). A coupling reaction of the amide **18** with alkyl



1:Galbonolide A (R=OMe) (rustmicin)

2:Galbonolide B (R=Me) (neorustmicin)

3:C6-SMe Galbonlide (R=SMe)

Organism	MIC(μg/ml)		
	1	2	3
Candida albicans (ATCC90028)	4	>64	64
Cryptococcus neoformans (ATCC90112)	<0.0031	16	0.5
Aspergillus fumigatus (TIMM1776)	>64	>64	>64

Fig. 1. Structures of Galbonolide A, B and C6-SMe Analogue and Their Antifungal Activity

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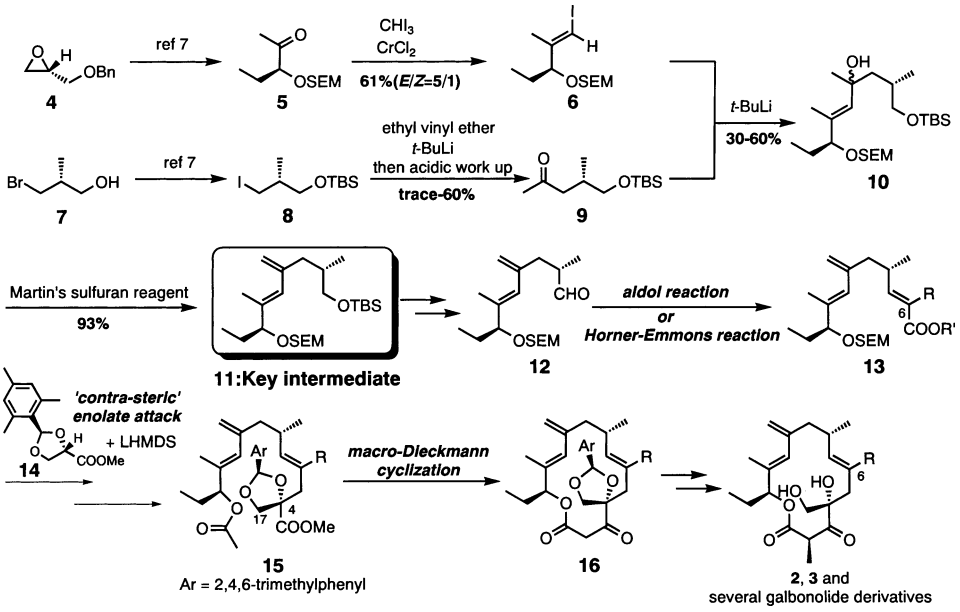

Chart 1

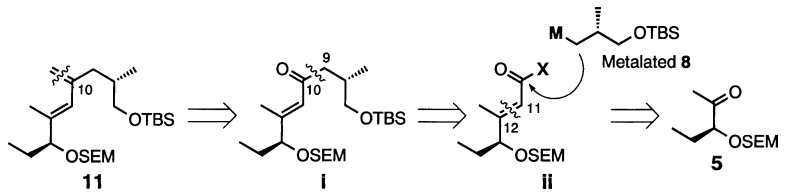

Chart 2

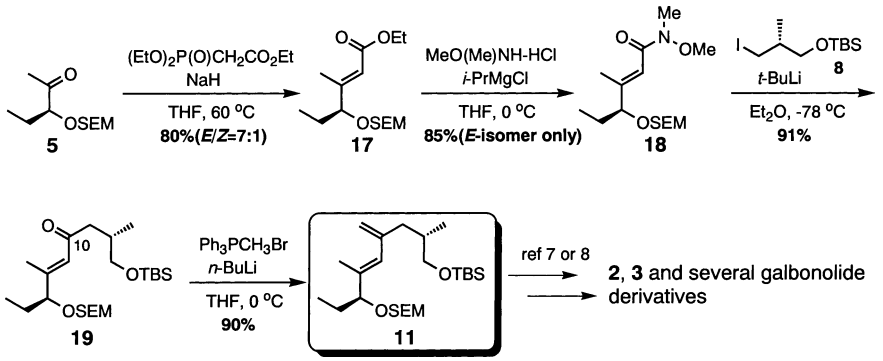

Chart 3

lithium, prepared by the action of the iodide **8** and *t*-BuLi in Et_2O, progressed smoothly. A high yield (91%) of corresponding ketone **19** was obtained. Finally, the conventional Wittig reaction utilizing methyltriphenylphosphonium bromide and *n*-BuLi in THF was performed to introduce an *exo*-methylene functionality on C10, affording the key intermediate **11** with desirable conjugated diene system (90%).

An improved synthesis of key intermediate **11** was accomplished by the assembly of an (*E*)-double bond at C11-12 by Horner–Emmons reaction, the connection of the C7-9 unit by direct transformation to the Weinreb amide followed by a coupling reaction with lithiated **8**, and construction of *exo*-methylene on C10 by conventional Wittig reaction. In comparison with the previous method, all these reactions and using reagents are employed usually well, and their handlings are safety and very easy. In conclusion, total yield from ketone **5** and its reproducibility were improved. Therefore, it became possible to synthesize multi-glam of the key intermediate **11**.

Experimental

General Methods The ^{1}N-NMR spectra were recorded on a Varian VXR-300 (300 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard. IR absorption spectra were recorded on a Horiba FT-200 spectrometer. Specific rotations were measured on a Jusco DIP-370 polarimeter. Mass spectra (MS) were measured on a JEOL JMS-SX102A spectrometer. The silica-gel TLC was performed with Merck Kieselgel F_{254} precoated plates. The silica gel used for column chromatography was WAKO gel C-300. All reactions involving air-sensitive reagents were performed under a nitrogen atmosphere using syringe-septum cap techniques.

Ethyl (2*E*,4*S*)-3-Methyl-4-(2-trimethylsilylethoxymethoxy)-hex-2-enoate (17) To a solution of triethyl phosphonoacetate (65.6 g, 293 mmol) in THF (525 ml) was added 60% NaH (11.7 g, 293 mmol) at 0 °C. After stirring at room temperature for 45 min, a solution of (*S*)-3-(2-trimethylsilylethoxymethoxy) pentan-2-one **5** (52.3 g, 225 mmol) in THF (150 ml) was added dropwise to the mixture. The reaction mixture was stirred at 60 °C for 2 h. The mixture was poured into ice water (200 ml) and the whole was extracted three times with EtOAc (900 ml). The organic layer was washed with brine (200 ml), dried over anhydrous $MgSO_4$ (200 g), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=30 : 1—10 : 1) to give the title compound **17** (54.6 g, 80%, *E* : *Z*=7 : 1) as a colorless oil. $[\alpha]_D^{25}$ −86.8° (*c*=1.0, $CHCl_3$); IR (KBr) ν_{max} 2967, 1718, 1219, 1030, 837 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.01 (9H, s), 0.90 (3H, t, *J*=7.3 Hz), 0.92 (2H, m), 1.28 (3H, t, *J*=7.2 Hz), 1.61 (2H, m), 1.85 (0.1H, s, *Z* isomer) 2.07 (0.9H, s, *E* isomer), 3.63 (2H, m), 3.92 (1H, t, *J*=6.6 Hz), 4.15 (2H, q, *J*=7.2 Hz), 4.59 (2H, q, *J*=6.9 Hz), 5.76 (0.1H, s, *Z* isomer), 5.84 (0.9H, s, *E* isomer); FAB-HR-MS Calcd for $C_{15}H_{30}O_4SiNa^+$: 325.1811. Found 325.1822.

(2*E*,4*S*)-*N*-Methoxy-*N*-methyl-3-methyl-4-(2-trimethylsilylethoxymethoxy)-2-hexenamide (18) To a mixture of α,β-unsaturated ester **17** (50.0 g, 165 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (32.2 g, 330 mmol) in THF (800 ml) was added *i*-PrMgCl (330 ml of a 2.0 M THF solution, 660 mmol) at −55 °C over 10 min. The reaction mixture was stirred at the same temperature and gently warmed to −25 °C over 2.5 h. The mixture was poured into a saturated solution of NH_4Cl (200 ml) and H_2O (300 ml) and the whole was extracted three times with EtOAc (900 ml). The organic layer was washed with brine (300 ml), dried over anhydrous $MgSO_4$ (200 g), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=20 : 1—10 : 1—5 : 1—3 : 1—2 : 1) to give the title compound **18** (44.4 g, 85%, pure *E*) as a colorless oil. $[\alpha]_D^{25}$ −99.4° (*c*=1.0, $CHCl_3$); IR (KBr) ν_{max} 2956, 1660, 1028, 837 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.01 (9H, s), 0.92 (3H, t, *J*=7.4 Hz), 0.93 (2H, m), 1.61 (2H, m), 2.04 (3H, s), 3.21 (3H, s), 3.64 (2H, m), 3.66 (3H, s), 3.96 (1H, t, *J*=6.6 Hz) 4.61 (2H, AB q, *J*=6.9 Hz), 6.29 (1H, s); FAB-HR-MS Calcd for $C_{15}H_{32}NO_4Si^+$: 318.2101. Found 318.2078.

(2*S*,5*E*,7*S*)-1-*tert*-Butyldimethylsiloxy-2,6-dimethyl-7-(2-trimethylsilylethoxymethoxy)-5-nonen-4-one (19) After *t*-BuLi (340 ml of a 1.48 M *n*-pentane solution, 503 mmol) was added to anhydrous diethyl ether (700 ml) at −78 °C using cannula technique, a solution of iodide **8** (86.7 g, 276 mmol) in diethyl ether (300 ml) was added dropwise to the *t*-BuLi solution over 8 min and the mixture was stirred at the same temperature for another 3 min. To the solution was added a solution of Weinreb amide **18** (43.7 g, 138 mmol) in THF (300 ml) at −78 °C over 5 min. The reaction mixture was stirred at the same temperature for 30 min. The mixture was poured into a saturated solution of NH_4Cl (300 ml) and H_2O (300 ml) and the whole was extracted twice with EtOAc (600 ml). The organic layer was washed with brine (300 ml), dried over anhydrous $MgSO_4$ (200 g), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=20 : 1—10 : 1) to give the title compound **19** (56.0 g, 91%) as a yellow oil. $[\alpha]_D^{25}$ −64.8° (*c*=1.0, $CHCl_3$); IR (KBr) ν_{max} 2956, 1695, 1250, 1030, 837 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.01 (9H, s), 0.02 (6H, s), 0.92 (17H, m), 1.61 (2H, m), 2.04 (3H, s), 2.19 (2H, m), 2.63 (1H, m), 3.47 (3H, m), 3.74 (1H, m), 3.89 (1H, t, *J*=6.4 Hz), 4.59 (2H, q, *J*=6.9 Hz), 6.21 (1H, s); FAB-HR-MS Calcd for $C_{23}H_{48}O_4Si_2Na^+$: 467.2989. Found 467.2969.

(2*S*,5*E*,7*S*)-1-*tert*-Butyldimethylsiloxy-2,6-dimethyl-4-methylene-7-(2-trimethylsilylethoxymethoxy)-5-nonene (11) To a solution of methyltriphenylphosphonium bromide (131.8 g, 369 mmol) in anhydrous THF (2.6 l) was added *n*-BuLi (241 ml of a 1.53 M *n*-hexane solution, 369 mmol) at 0 °C using cannula technique. After stirring for 15 min, a solution of ketone **19** (54.7 g, 123 mmol) in THF (300 ml) was added dropwise to the mixture at 0 °C over 8 min. The reaction mixture was stirred at the same temperature for another 2 h. The mixture was poured into ice water (500 ml) and the whole was extracted three times with EtOAc (1.1 l). The organic layer was washed with brine (300 ml), dried over anhydrous $MgSO_4$ (200 g), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc=50 : 1-EtOAc only) to give the title compound **11** (49.0 g, 90%) as a yellow oil. $[\alpha]_D^{25}$ −45.0° (*c*=1.0, $CHCl_3$); IR (KBr) ν_{max} 2956, 1097, 1028, 837 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.01 (9H, s), 0.02 (6H, s), 0.89 (17H, m), 1.68 (4H, m), 1.71 (3H, s), 2.28 (1H, m), 3.39 (2H, m), 3.51 (1H, m), 3.76 (1H, m), 3.86 (1H, t, *J*=7.0 Hz), 4.59 (2H, q, *J*=6.8 Hz), 4.88 (1H, d, *J*=1.2 Hz), 5.01 (1H, d, *J*=1.2 Hz), 5.76 (1H, s); FAB-HR-MS Calcd for $C_{24}H_{50}O_3Si_2Na^+$: 465.3196. Found 465.3196

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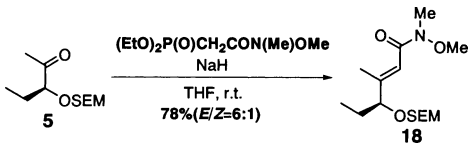
13) With regard to this reaction, we also tried a direct transformation from ketone **5** to amide **18** by utilizing a Horner–Emmons reagent which already had a Weinreb amide moiety. Since this reagent was too expensive for large-scale synthesis, we chose a stepwise procedure as mentioned above.



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