Synthesis and Antifungal Activity of Novel 14-Membered Benzomacrolides, as Galbonolide Analogues

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Asymmetric total synthesis of benzene analogues of galbonolide, a 14-membered antifungal macrolide, possessing a benzene ring instead of a conjugated diene structure, was achieved starting from chiral 1-aryl-1-propanol obtained by enzyme-catalyzed kinetic resolution with high enantioselectivity. Representatively, a method for the introduction of a methylthio and chloride function at the vinyl position was also established. The resulting analogues unfortunately exhibited very little antifungal potency in comparison with galbonolide A.

Key words galbonolide; inositol phosphoceramide (IPC) synthase; benzene analogue; 14-membered macrolide antibiotic

The novel 14-membered macrolide antibiotics galbonolide A (rustmicin; **1**) and galbonolide B (neorustmicin; **2**) were discovered in a fermentation broth of *Micromonospara chalcea* by Otake *et al.*[1,2] Independently, Achenbach *et al.* also isolated galbonolide A and B from *Streptomyces galbas*.[3,4] In addition to the original potent activity against microorganisms that cause botanical disease, galbonolide A was shown to have good potency against clinically important fungi including *Cryptococcus* and *Candida* spp., and then a researching group at Merck found out that the fungicidal activity of galbonolide A was caused by an inhibitory activity against inositol phosphoceramide (IPC) synthase.[5,6] Galbonolide A itself is too difficult to develop for clinical application because of its poor stability under physiological conditions. However, due to their biological activities, galbonolide A and B remain interesting compounds in medicinal chemistry. The total synthesis of galbonolide B, a more stable analogue of galbonolide A, has already been achieved by Tse.[7]

In order to improve the stability and to simplify the reported total synthetic method, we attempted to prepare a benzene analogue corresponding to a conjugated diene system as shown in Fig. 1, focusing on the conversion of a methyl enol ether part of galbonolide A, which is the most important with respect to antifungal activity but also the most unstable part, to other vinyl type substitutes such as methyl thioenol ether or vinyl chloride. This letter reports the synthesis and antifungal activity of novel galbonolide analogues linked by a benzene ring.

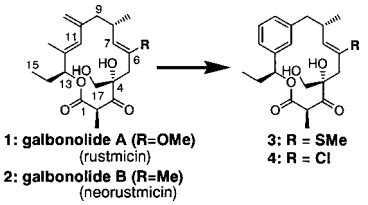


Fig. 1. Structures of Galbonolide A, B and Benzene Analogues

Our retrosynthetic analysis was shown in Chart 1. Following the report by Tse, we planned to achieve the formation of a 14-membered macrocyclic ring by C2–C3 bond formation *via* macro-Dieckmann condensation. The peculiar asymmetric quaternary carbon at C4 on **B** would be constructed by making good use of "contra-steric" enolate attack of **5** to allyl iodide **C**. The introduction of a trisubstituted double bond at C6–C7 would be achieved by employing an aldol or Horner–Emmons reaction of the aldehyde prepared from alcohol **6**. That is, the chiral alcohol **6** would be the key intermediate in the modification of the substituent on C6. The alcohol **6** was expected to be assembled from a benzaldehyde **7** through an optical resolution of a racemic secondary alcohol at C13 and coupling with a chiral side chain.

In the first place, we tried asymmetric synthesis of a chiral key intermediate **6** (Chart 2). Treatment of aldehyde **7** with EtMgBr in Et_2O gave racemic alcohol **8** in good yield (92%). Fortunately, enantioselective acetylation of (*rac*)-**8** using enzyme (Lipase LIP®)[8] provided (*S*)-**8** as a remaining alcohol, with a desirable configuration (42%, 95.7% ee).[9] Chiral propionyl amide **11** was prepared from commercially available optically active hydroxy ester **9** through protection by *tert*-butyldimethylsilyl (TBS) group (98%) and installation of a Weinreb amide in the presence of *i*-PrMgCl (98%).[10] The coupling reaction of a dianion prepared from (*S*)-**8** with Weinreb amide **11** was achieved successfully (quantitative yield), and the corresponding ketone was converted into alcohol **12** by protection of the secondary alcohol on C13 by TBS group (61%) followed by $NaBH_4$ reduction (92%). Deoxygenation on C9 by way of radical reduction *via* xanthate afforded **13** (89%), and subsequent selective deprotection using *p*-TsOH led to key intermediate **6** in reasonable yield

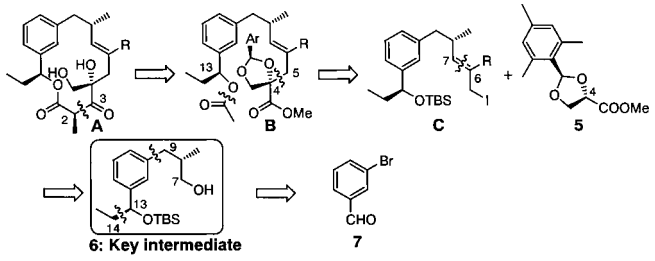
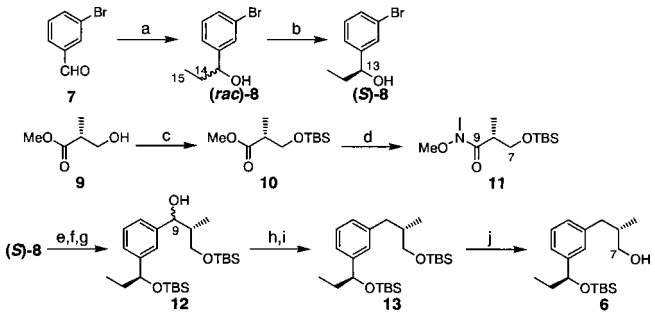


Chart 1



Reagents: a. EtMgBr, Et_2O, 92%. b. Lipase LIP, TEA, vinylacetate, 42%, 95.7%ee, c.TBSCl, imidazole, DMF, 98%, d. MeO(Me)NH-HCl, ^{i}PrMgCl, THF, 98%, e. *n*-BuLi, **11**, THF, quant., f. TBSCl, imidazole, DMF, 61%, g. $NaBH_4$, EtOH, 92%, h. CS_2, MeI, NaH, THF, 95%, i. *n*-Bu_3SnH, toluene, 94%, j. *p*-TsOH-H_2O, EtOH, 70%

Chart 2

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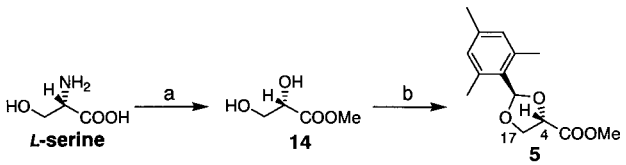
(70%).

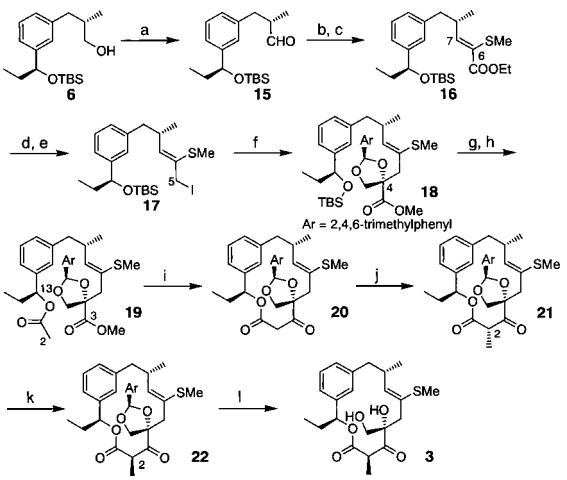
Another fragment **5** was prepared from the natural α-amino acid L-serine by changing the amino group to a hydroxyl group under the same configuration *via* Hirth's procedure[11] (Chart 3). Subsequent esterification gave dihydroxyl ester **14** in good yield (93%). Upon reaction with mesitaldehyde and camphorsulfonic acid (CSA) in the presence of $MgSO_4$ and 4A molecular sieves, **14** was converted to *trans*-dioxolane **5** and an undesired *cis*-isomer in a ratio of *ca.* 3 : 1 (62%). This ratio was increased to 12 : 1 by recrystallization from *n*-hexane (63%).

Synthesis of a benzene analogue having methylthio enol ether structure at C6–7 was actually attempted starting from key intermediate **6** (Chart 4). Alcohol **6** led to aldehyde **15** by conventional Swern oxidation (96%). The aldol reaction of **15** with lithium enolate of methylthioacetate afforded the corresponding hydroxyl esters as a mixture of **4** diastereomers. Fortunately, following dehydration provided α,β-unsaturated ester **16** with exclusively (*Z*) geometry (69% over 2 steps), probably because the dehydration reaction progressed to give thermodynamically favored product. The ethyl ester was then reduced by diisobutylaluminum hydride (DIBAL-H) (82%), and the resulting allyl alcohol was changed to the corresponding iodide **17** by the action of a combination of I_2, Ph_3P and imidazole (quantitative yield). The coupling reaction of allyl iodide **17** with ester **5** using lithium hexamethyldisilazide (LHMDS) in a THF-HMPA solvent system[12] gave adduct **18** in moderate yield (53%), although an excess amount of **5** was needed due to the very low stability of the lithium enolate of **5**. This reaction resulted in the formation of the peculiar asymmetric quaternary carbon at C4 with excellent selectivity. Deprotection of the TBS group at C13 was carried out by treatment of methyl ester **18** with tetrabutylammonium fluoride (TBAF) in THF. In this reaction, simultaneous cleavage of methyl ester to carboxylic acid occurred, and it was necessary to convert the resultant carboxylic acid to methyl ester by means of $TMSCHN_2$ (84% over 2 steps). The resulting secondary alcohol was then acetylated to yield a precursor of cyclization, **19** (85%). Because Tse reported in case of total synthesis of galobonolide B that a cyclization reaction was failed when a precursor having a propionyloxy group instead of a acetoxy group on C13 was used,[10] we decided to prepare the acetate **19** as a precursor of cyclization. And since the original procedures reported for the synthesis of galbonolide B gave poor reproducibility, the solvent was changed from THF to DME and HMPA. The macro-Dieckmann condensation of **19** took place very quickly, and compound **20** having a novel galbonolide skeleton was obtained in moderate yield (59%). Introduction of a methyl group at C2 using MeI and NaH gave α-methyl form **21** (42%) and the dimethyl form (14%). Inversion of the stereochemistry at the C2 position of **21** was carried out by treating **21** with *t*-BuOK in DMF followed by protonation to yield β-methyl form **22** (18%). Finally, the 2,4,6-trimethylbenzylidene acetal of **22** was removed by using a AcOH–H_2O system. This pro-



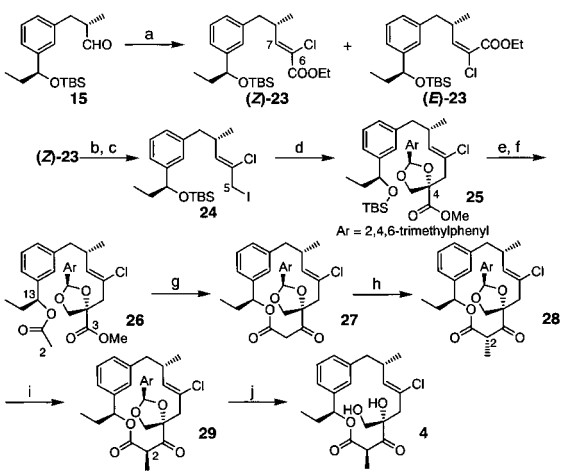
Reagents: a. i) 6N-H_2SO_4, 6N-$NaNO_2$, H_2O, ii) $HC(OMe)_3$, H_2SO_4, MeOH, 93%, b. mesitaldehyde, MS4A, $MgSO_4$, $CHCl_3$, 62% (trans/cis = 3/1), then recrystallization, 63% (trans/cis = 12/1)

Chart 3



Reagents: a. $(COCl)_2$, DMSO, TEA, CH_2Cl_2, 96%, b. ethyl methylthioacetate, LHMDS, THF, 88%, c. MsCl, TEA, DBU, CH_2Cl_2, THF, 78%, d. DIBAL-H, CH_2Cl_2, 82%, e. I_2, Ph_3P, imidazole, Et_2O, MeCN, quant., f. **5**, LHMDS, HMPA, THF, 53%, g. i) TBAF, THF, ii) $TMSCHN_2$, benzene, MeOH, 84%, h. Ac_2O, DMAP, pyridine, 85%, i. LHMDS, HMPA, DME, 59%, j. MeI, NaH, THF, 42%, k. *t*-BuOK, DMF, 18%, l. AcOH-H_2O (2:1), 80%

Chart 4



Reagents: a. triethyl phosphonoacetate, NaH, NCS, THF, 96% (*Z*/*E* = 3/7), b. DIBAL-H, CH_2Cl_2, 89%, c. I_2, Ph_3P, imidazole, Et_2O, MeCN, 77%, d. **5**, LHMDS, HMPA, THF, 57%, e. i) TBAF, THF, ii) $TMSCHN_2$, benzene, MeOH, 74%, f. Ac_2O, DMAP, pyridine, 88%, g. LHMDS, HMPA, DME, 74%, h. MeI, *t*-BuOK, DMF, 83%, i. *t*-BuOK, DMF, j. AcOH-H_2O (2:1), 85% over 2 steps

Chart 5

Table 1. Antifungal Activity of Galbonolide Derivatives

Organism	MIC (μg/ml)				
	Galbonolide A	Galbonolide B	**3**	**4**	Amphotericin B
Candida albicans ATCC90028	4	>64	>64	>64	0.5
Cryptococcus neoformans ATCC90112	<0.0031	16	>64	>64	0.5
Aspergillus funigatus TIMM1776	>64	>64	>64	>64	1

cedure afforded a novel galbonolide analogue **3** possessing a benzene ring and a methylthio enol ether part instead of the conjugated diene and methyl enol ether in 80% yield.[13)]

Next, the synthesis of another analogue having a vinyl chloride group at C6–C7 was attempted by a similar method (Chart 5). The Horner–Emmons reaction of aldehyde **15** using triethyl phosphonoacetate and NaH in the presence of *N*-chlorosuccinimide (NCS)[14)] was performed to assemble a trisubstituted double bond including a chlorine atom, to provide the desired isomer (*Z*)-**23** as a minor product (29%). (*Z*)-**23** led to iodide **24** by DIBAL-H reduction (89%) and subsequent iodination (77%). The allyl iodide was reacted with excess lithium enolate prepared from **5** to yield ester **25** (57%). TBS ether **25** was converted into acetate **26** by the same procedure as above (65% over 3 steps), and subsequent macro-Dieckmann cyclization of **26** using LHMDS in refluxing DME in the presence of HMPA gave compound **27** in good yield (74%). A methyl group was introduced at C2 by the action of MeI and *t*-BuOK in DMF. However, an undesired α-methyl form **28** was obtained exclusively yet again (83%) and a dimethyl form was not generated. After **28** was treated with *t*-BuOK followed by protonation to invert the stereochemistry at C2, removal of 2,4,6-trimethylbenzylidene acetal provided a novel galbonolide analogue **4** having a vinyl chloride function at C6–7 in good yield (85% over 2 steps).[15)]

The *in vitro* antifungal activities of the galbonolide derivatives obtained above were evaluated against *Candida albicans* (ATCC90028), *Cryptococcus neoformans* (ATCC90112) and *Aspergills fumigatus* (TIMM1776). Serial dilution techniques were employed for the minimum inhibitory concentration (MIC) determinations.[16)] Galbonolide A, B and amphotericin B were used as reference compounds. Unfortunately, the antifungal activities of galbonolide derivatives **3** and **4** were significantly lower than that of the original galbonolide A (Table 1).

In conclusion, we succeeded in the asymmetric total synthesis of novel galbonolide analogues possessing a benzene ring instead of a conjugated diene structure *via* the following transformations: optical resolution of benzylic alcohol (*rac*)-**8**, construction of a trisubstituted double bond with a methylthio ether or chlorine atom, assembly of an asymmetric quaternary carbon, and finally macro-Dieckmann cyclization. Unfortunately, the synthesized novel compounds **3** and **4** exhibited no significant antifungal activity. Although, it is not clear that which of transformations, conjugated diene to benzene ring and/or MeO to MeS or Cl, was the cause of such a loss of potency so far, we could establish a method for construction of galbonolide skeleton which possess replaced C6 functional group by this work. So, by applying the demonstrated method, further modification of this analogue to include compounds with a 1,3-diene system as in the natural product is currently under investigation, and will be reported in the near future.

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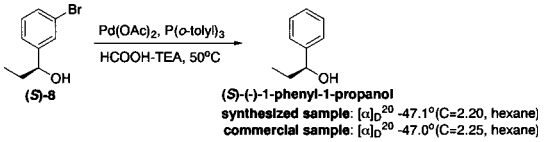
9) The configuration of (*S*)-**8** was determined by conversion to a chiral compound, (*S*)-(−)-1-phenyl-1-propanol by following procedure, then its specific rotation was compared with that of commercially available sample which was purchased from Aldrich.



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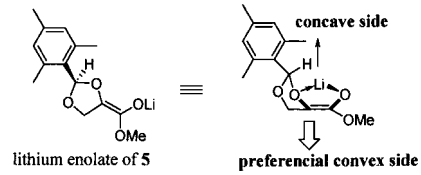

13) Data of compound **3**: ^{1}H-NMR (300 MHz, C_6D_6) δ: 0.82 (3H, t, J=7.3 Hz), 0.92 (3H, d, J=6.7 Hz), 1.41 (3H, d, J=7.0 Hz), 1.78 (3H, s), 1.88 (2H, m), 2.00 (1H, m), 2.25 (1H, dd, J=12.5, 8.8 Hz), 2.31 (1H, br s), 2.62 (2H, s), 2.73 (1H, dd, J=12.5, 4.6 Hz), 3.15 (1H, m), 3.55 (1H, q, J=7.0 Hz), 3.63 (1H, d, J=11.3 Hz), 3.72 (1H, m), 5.42 (1H, d, J=9.5 Hz), 5.50 (1H, t, J=7.6 Hz), 6.85 (1H, d, J=7.6 Hz), 7.02 (1H, d, J=7.6 Hz), 7.10 (1H, dd, J=7.6, 7.6 Hz), 7.25 (1H, s). FAB-MS *m/z*: 429 ($M+Na^+$).
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15) Data of compound **4**: ^{1}H-NMR (300 MHz, C_6D_6) δ: 0.80 (3H, t, J=7.7 Hz), 0.82 (3H, d, J=7.2 Hz), 1.40 (3H, d, J=7.5 Hz), 1.68—1.97 (2H, m), 2.12 (1H, dd, J=13.6, 9.4 Hz), 2.44 (1H, d, J=15.1 Hz), 2.65 (1H, dd, J=13.6, 5.7 Hz), 2.76—2.94 (1H, m), 2.86 (1H, d, J=15.1 Hz), 3.46 (1H, q, J=7.5 Hz), 3.55 (1H, d, J=9.8 Hz), 3.61 (1H, d, J=9.8 Hz), 5.28 (1H, d, J=9.1 Hz), 5.45 (1H, t, J=7.7 Hz), 6.82 (1H, d, J=7.8 Hz), 7.02—7.15 (3H, m). FAB-MS *m/z*: 417 ($M+Na^+$).
16) MICs were determined by microbroth dilution method using YNBP medium, comprising Yeast Nitrogen Base (Difco Laboratories, Detroit, Mich.), 1% glucose, and 0.25% KH_2PO_4. *Candida albicans* or *Cryptococcus neoformans* cells were suspended in YNBP liquid to give a final concentration of approximately 6—10×10^3 cells/ml. *Aspergillus fumigatus* conidia were suspended in YNBP agar (0.2%) to give a final concentration of approximately 2.5×10^3 conidia/ml. Test samples were dissolved and serially twofold diluted in dimethyl sulfoxide. Aliquots of 2 μl were distributed to a 96-well, flat-bottomed plate, then plates were filled with 200 μl of cell or conidia solution. The MIC was defined as the lowest concentration of samples that completely prevented visible growth was inhibited.