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# Total synthesis and biological evaluation of methylgerambullone

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ABSTRACT

First total synthesis of methylgerambullone (MGB, **1**) isolated from *Glycosmis angustifolia* was completed via a convergent route. The effect of MGB on the contractile responses of the isolated guinea-pig ileum induced by acetylcholine was investigated. As a result, it showed a potent relaxation rate (78.66 ± 4.30% at 100 mg/L) in a concentration-dependent manner on longitudinal smooth muscle contraction of isolated guinea-pig ileum induced by 1 μM acetylcholine.



Methylgerambullone (MGB, **1**) and methylisogerambullone (MIGB) were isolated from the methanolic leaf extract of *Glycosmis angustifolia* collected from Dambulla rock in Sri Lanka.[1] They have the same sulfone structures derived from methylthiopropenoic amide and also contain the phenethylamine moieties with 6́-oxogeranyloxy groups in *para*-position except for a position of a double bond as shown in Figure 1. Especially, MIGB was also isolated in Korean white radish root (*Raphanus sativus* L.) and reported to show stimulates the small bowel motility through the activation of acetylcholine receptors.[2] The intriguing structure, the low natural abundance, and the clinical potential of MGB, a constitutional isomer of MIGB, prompted us to develop a synthesis of MGB. Herein, we report a first convergent total synthesis and biological evaluation of MGB.

Our synthetic strategy is to divide a MGB molecule into three segments A, B, and C, as shown in Figure 2. We selected Mitsunobu reaction as a coupling reaction between the segment A (**2**) and the segment B (**3**), and amide coupling reaction between the segment B (**3**) and the segment C (**4**).

Synthesis of segment A (**2**) is shown in Scheme 1. Prenol (**6**) protected by TBDPS group was regioselectively oxidized using $SeO_2$/TBHP.[3] The reaction mixture containing over-oxidized aldehyde was reduced with $LiAlH_4$ to afford pure allylic alcohol **7** in overall 54% yield. The exact structure of **7** was confirmed by NOE experiment. Allylic alcohol **7** was easily converted to bromide **9** via tosylate **8**. Bromide **9** was coupled with dithiane anion of **10**[4] to provide **11**, which was treated with TBAF to give the desired segment A (**2**) in overall 80% yield.

Synthesis of segment B (**3**) and segment C (**4**) is shown in Scheme 2. *N*-Methyltyramine HCl salt (**3**) was obtained in high yield by indirect monomethylation of tyramine **12** as follows: introduction of $^t$Boc group, $LiAlH_4$ reduction and treatment with $SOCl_2$/MeOH. For the segment C (**4**), 1,4-addition of $CH_3SH$ (gas) to propiolic acid **13** by Ikegami method[5] afforded an unsatisfactory result, which showed the stereoisomeric mixture (**14** and **15**) as well as the side products derived from further addition of $CH_3SH$ due to the longer reaction time. However, the treatment of $CH_3SNa$ at 100 °C also afforded the mixture of *cis* and *trans*-isomers (1:1 ratio),[6] which was easily converted to all *trans*-isomer **15** on reflux in xylene. The oxidation of **15** with excess of *m*CPBA readily furnished the desired segment C (**4**) as a white solid in quantitative yield.

Segment B (**3**) was coupled with segment C (**4**) by using EDC to give amide **16** in 51% yield, which was also obtained by coupling reaction of segment B (**3**) with sulfide **15** and subsequent oxidation with *m*CPBA in higher yield as shown in Scheme 3. As a next step, the general Mitsunobu coupling reaction of segment A (**2**) with amide **16** was unexpectedly very sluggish (10% yield in 2 days reaction time). When ultrasound was applied to the reaction mixture,[7] however, the coupling reaction was completely gone within 3 h in 61% yield. As a final step, the deprotection of dithiane group of **17** with basic condition (EtI, $CaCO_3$, aq acetonitrile)[8] resulted in the cleavage of the corresponding MGB (**1**) into compounds **18** and **16** as shown in Figure 3.[9] In addition, the high temperature condition (TBHP/MeOH, reflux)[10] also afforded the same result. This result implies that MGB (**1**) cannot be simply converted into MIGB via a migration of double bond using basic or equilibrium condition. Eventually, the removal of dithiane group of **17** using Takano method ($CH_3I$, $CH_3CN/H_2O$, rt)[11] successfully afforded MGB (**1**) in

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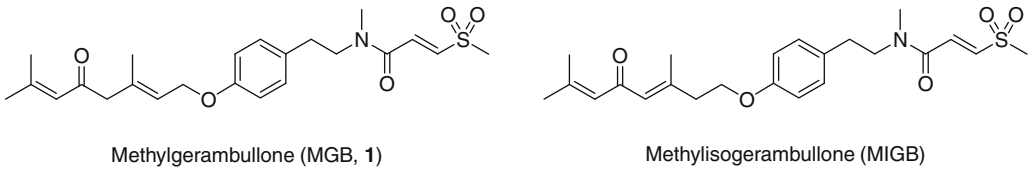



**Figure 1.** Structures of methylgerambullone (MGB, **1**) and methylisogerambullone (MIGB).

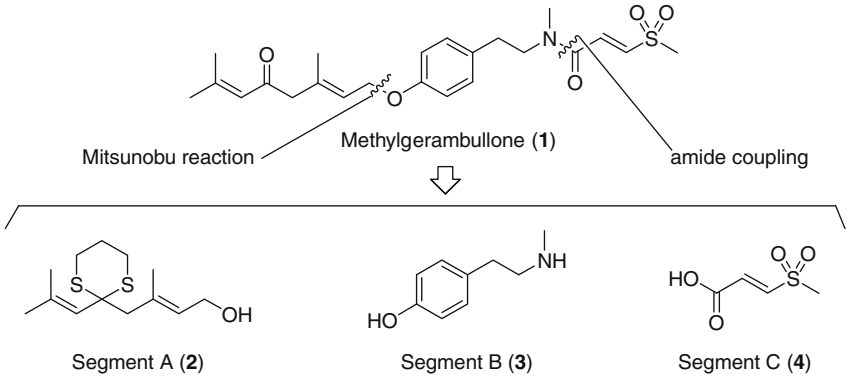
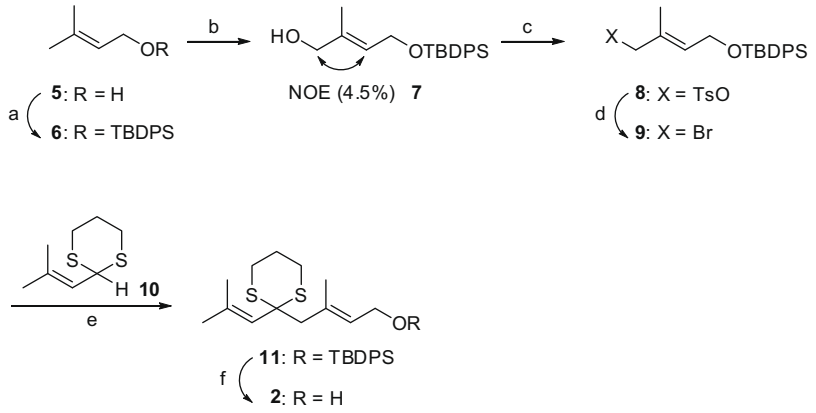
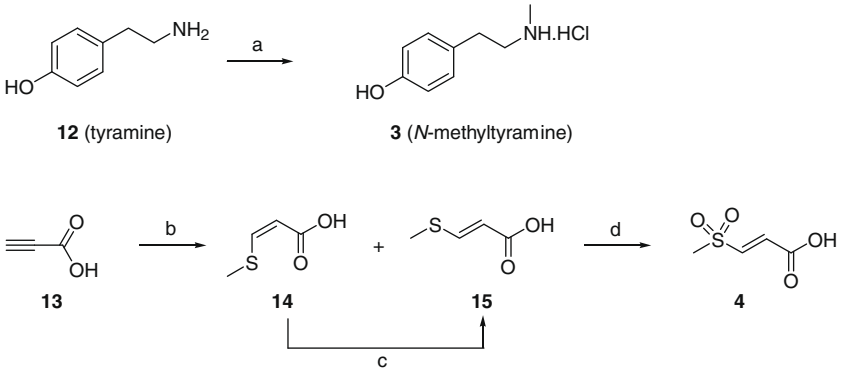


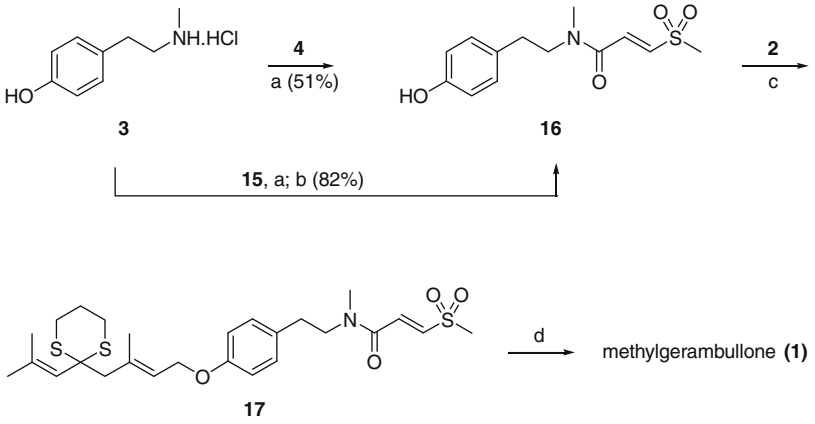

**Figure 2.** Synthetic strategy for methylgerambullone (MGB, **1**).




**Scheme 1.** Synthesis of segment A (**2**). Reagents and conditions: (a) TBDPSCl, imidazole, DCM, overnight; (b) TBHP, $SeO_2$ (cat.), then $LiAlH_4$, −78 °C, 3 h, 54% from **5**; (c) *p*-TsCl, DMAP, TEA, −20 °C, 2 h; (d) LiBr/ THF, −20 to 0 °C, 4 h; (e) **10**, *t*-BuLi, −78 to −40 °C, 4 h; (f) TBAF, THF, rt, overnight, 80% from **7**.




**Scheme 2.** Synthesis of segment B (**3**) and segment C (**4**). Reagents and conditions: (a) $(^tBoc)_2O$, DCM, rt; $LiAlH_4$, Dioxane, 0 °C to reflux, 48 h; $SOCl_2$/MeOH, 93% from **12**; (b) $CH_3SNa$, 100 °C, 12 h, then HCl, (c) xylene, reflux, 70%; (d) **15**, *m*CPBA (2.2 equiv), 0 °C to rt, >99%.




**Scheme 3.** Synthesis of methylgerambullone (MGB, **1**). Reagents and conditions: (a) EDC, DMAP, TEA, DMF, 48 h; (b) *m*CPBA, DCM, −5 °C, 12 h; (c) DIAD, $PPh_3$, THF, rt, ultrasound, 3 h, 61%; (d) $CH_3I$, $CH_3CN$, $H_2O$, rt, 1 day, 54%.

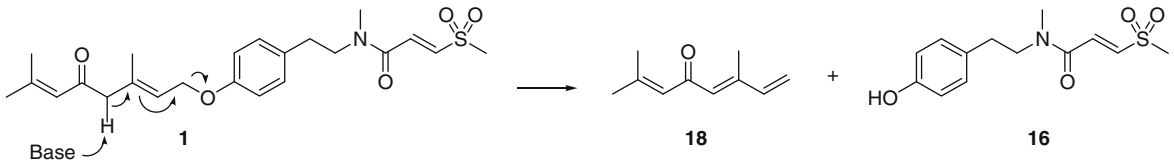



**Figure 3.** Supposed mechanism for cleavage of methylgerambullone (**1**) under basic condition.

54% without decomposition. The spectroscopic data ($^1H$, $^{13}C$ NMR, and HRMS) for synthetic MGB (**1**) were identical to those of the authentic natural product: the presence of two conformers due to restricted rotation about the amide C–N bond (s-*cis* and s-*trans*).[1,12]

Based on the traditional remedy that radishes help to aid digestion by stimulating the gut and the reported research about the increased effect of MIGB on gastrointestinal contraction and rat ileum motility,[2] we tested the biological effect of MGB (**1**) on the acetylcholine-induced contractions in the isolated longitudinal smooth muscle of guinea-pig ileum using the experimental protocol[13] as follows: The stable longitudinal smooth muscle of guinea-pig ileum was prepared and treated with 1 μM of acetylcholine in order to induce its contraction. After then, the precontracted smooth muscle of guinea-pig ileum was treated with MGB (**1**) at three concentrations, respectively. As shown in Table 1, the precontracted smooth muscle of guinea-pig ileum was relaxed in a concentration-dependent manner and showed a potent relaxation rate (78.66 ± 4.30%) at 100 mg/L concentration. Interestingly, this biological effect was contrary to that of MIGB, which caused a significant increase the isolated rat ileum contraction in a concentration-dependent manner.[2] Therefore, it seems that the combination treatment of MGB and MIGB could strongly stimulate a gastrointestinal mobility via complementary action each other and thus become a potential remedy for dysfunction of gastrointestinal mobility such as constipation.

**Table 1**
Relaxatory effect of methylgerambullone (**1**) on the isolated smooth muscle of guinea-pig ileum precontracted with 1 μM of acetylcholine

| Group | Number of animal (*n*) | Dose (mg/L) | Relaxation rate (%) |
|---|---|---|---|
| G1 | 8 | 30 | 45.31 ± 3.30 |
| G2 | 8 | 60 | 69.95 ± 4.18 |
| G3 | 8 | 100 | 78.66 ± 4.30 |

The results were statistically analyzed by *Student's t-test*; Data are expressed as Mean ± S.E; Relaxations induced by MGB (30–100 mg/L) were significant compared to equivalent volume of normal saline ($P$ <0.01).

In summary, we have successfully achieved the total synthesis of methylgerambullone (MGB, **1**) via a convergent route involving a coupling of three segments A, B, and C for the first time and MGB showed the relaxatory effect on guinea-pig isolated ileum precontracted with acetylcholine in a concentration-dependent manner. The exact mechanism of relaxatory action of MGB on the precontracted smooth muscle of guinea-pig ileum is in progress.

## Acknowledgements

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198.13, 162.29, 156.5, 157.5, 139.5, 135.2, 133.3, 130.5, 129.7, 124.3, 122.8, 114.9, 64.7, 55.0, 50.6, 42.5, 36.5, 32.5, 27.7, 20.8, 17.0. *s-tran conformer of methylgerambullone* (**1**): $^{1}$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.69–6.95 (2H, m), 6.85–6.78 (2H, m), 6.07 (1H, t, $J$ = 1.2 Hz), 5.53 (1H, m), 4.50 (1H, d, $J$ = 6.4 Hz), 3.55 (2H, t, $J$ = 6.4 Hz), 3.10 (2H, br s), 3.00 (3H, s), 2.76 (2H, t, $J$ = 6.4 Hz), 2.10 (3H, br s), 1.84 (3H, br s), 1.70 (3H, br s); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 198.11, 162.85, 157.9, 156.5, 138.6, 135.2, 132.4, 130.0, 129.2, 124.2, 122.8, 115.2, 64.7, 55.0, 52.2, 42.5, 34.2, 34.0, 27.7, 20.8, 17.0; HRMS (FAB): $m/z$ calcd for $C_{23}H_{32}NO_5S$ $[M+H]^+$: 434.2001, found 434.2007.

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