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Synthesis of BC-ring model of globostellatic acid X methyl ester, an anti-angiogenic substance from marine sponge

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ABSTRACT

Concise synthesis of BC-ring model compounds of 13*E*,17*E*-globostellatic acid X methyl ester, an anti-angiogenic triterpene derivative from Indonesian marine sponge, was achieved through ynolate olefination and allylic oxidation as key steps. The model compound **5**, which was synthesized within 10 reaction steps from commercially available Hajos–Parrish ketone, showed anti-proliferative activity against HUVECs with moderate selectivity.

Angiogenesis, a formation of new blood capillaries from pre-existing blood vessels, is critical for tumor growth and metastasis. A growing tumor needs an extensive network of capillaries to provide nutrients, oxygen, etc. In addition, the new blood vessels provide a way for tumor cells to enter in the circulation and to metastasize to another organ. Therefore, the substances that inhibit angiogenesis have a considerable potential to be novel therapeutic agents for the treatment of cancer.[1]

In the course of our study on the bioactive substances from marine organisms, we focused on a search for selective inhibitors of proliferation of human umbilical vein endothelial cells (HUVECs) as anti-angiogenic substances, and isolated novel iso-marabarican-type triterpenes named globostellatic acid X methyl esters (**1**–**4**, Fig. 1) from the Indonesian marine sponge *Rhabdastrella globostellata*.[2] We found that these compounds showed selective anti-proliferative activity against HUVECs and also inhibited migration and tubular formation of HUVECs induced by VEGF or bFGF. Structure–activity relationship studies of some isolated and chemically modified analogues suggested that the unfunctionalized conjugated penta-ene side chain with 13*E*-geometry would be important structural element for the potent and selective anti-proliferative activity against HUVECs, whereas the A-ring part had little contribution. These results prompted us to synthesize structurally simplified model compounds, in order to develop novel drug lead as anti-angiogenic agent. Here, we report the synthesis of the model compounds of globostellatic acid X methyl ester having BC-ring structure and conjugated penta-ene side chain, and the biological evaluation of them.

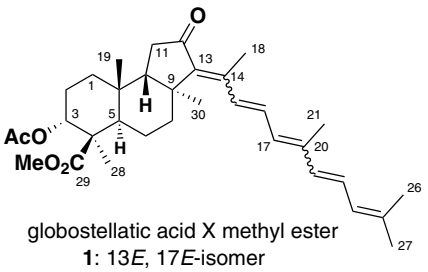

Figure 1. Globostellatic acid X methyl esters (**1**–**4**).

Compound **5**, shown in Figure 2, was designed for our target model compound. Synthetic strategy is as follows. The core structure part (**6**) and the side chain part (**7**) could be connected by Stille cross-coupling reaction. The *trans*-hydrindane skeleton of the core structure would be accessible using commercially available Hajos–Parrish ketone (**9**)[3] as a starting material. The key reaction steps of our strategy are the introduction of tetrasubstituted olefin and 5-membered keto-moiety.

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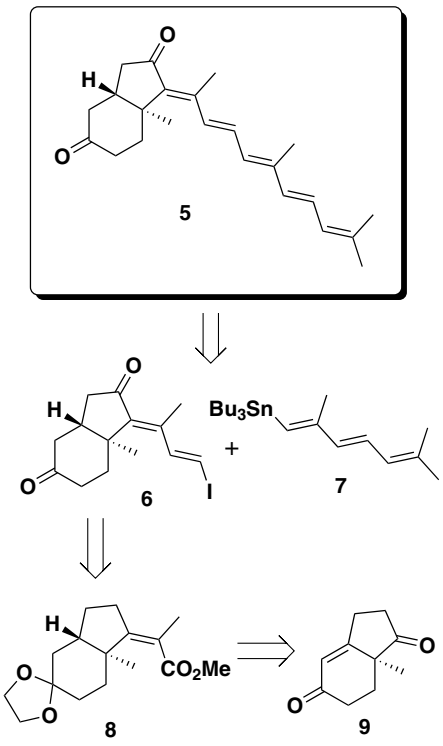
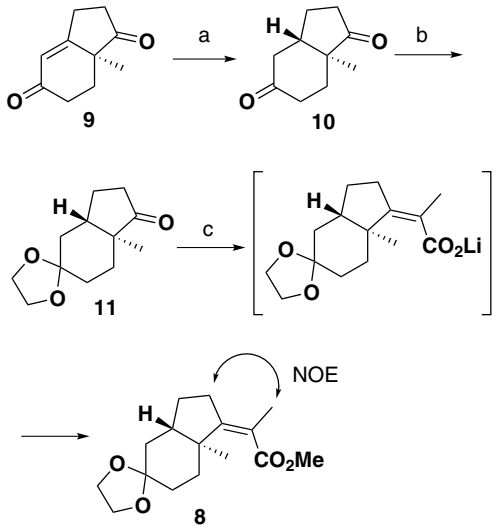


Figure 2. Retro-synthetic analysis of model compound (**5**).

Firstly, synthesis of the core structure was examined (Scheme 1). According to the literature,[4] stereoselective conjugate reduction of Hajos–Parrish ketone (**9**) using *tert*-butylmagnesium chloride, diisobutylaluminum hydride (DIBAL-H), and CuI in the presence of hexamethylphosphoramide (HMPA) gave a *trans*-hydrindanedione **10** in moderate yield. Subsequent protection of the less-hindered ketone gave an ethylene ketal **11**.

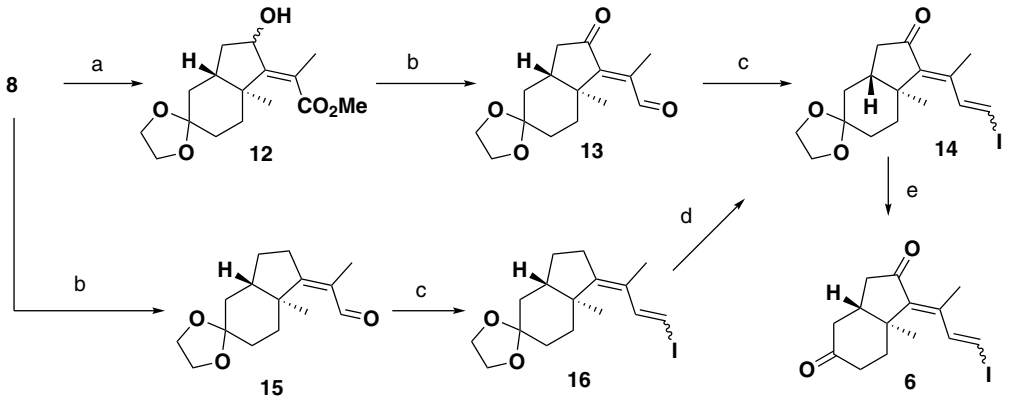
Next, we tried to introduce tetrasubstituted olefin to the sterically hindered carbonyl group in **11**. Conventional olefination methods, such as Wittig or Horner–Emmons reaction, did not give fruitful results, and then we utilized the ynolate olefination reaction developed by Shindo et al.[5] As we expected, ynolate anion, generated from α,α-dibromopropionate and *t*-BuLi, smoothly reacted with the hindered carbonyl group in **11** to afford a tetrasubstituted olefinic ester **8** as a single geometric isomer, after quenching the reaction mixture with iodomethane and HMPA.



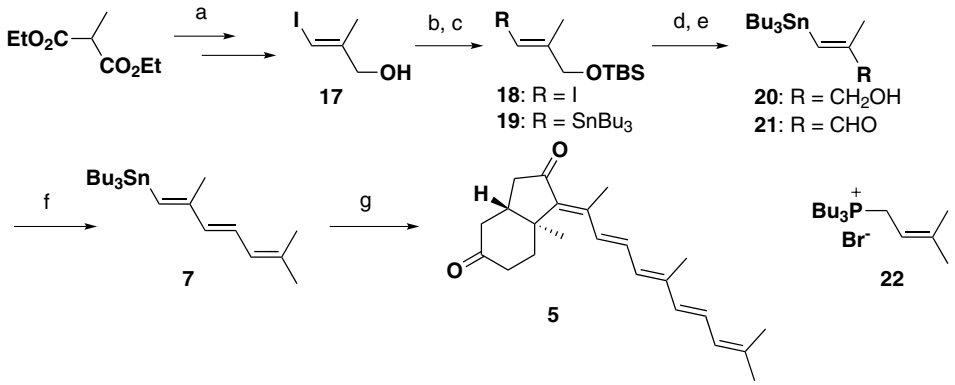
Scheme 1. Reagents and conditions: (a) DIBAL-H, *t*-BuMgCl, CuI, HMPA, THF, −78 °C, 60%; (b) ethylene glycol, $(COOH)_2$, CH_3CN, 95%; (c) ethyl α,α-dibromopropionate, *t*-BuLi, THF, −78 °C, MeI, HMPA, 48%.

The stereochemistry of the product was ascertained by NOE experiment.

Allylic oxidation of **8** by SeO_2 gave an allylic alcohol **12** in moderate yield. The ester moiety in **12** was reduced by DIBAL-H, and oxidation of the resulting diol provided a keto-aldehyde **13**. However, subsequent Takai olefination[6] ($CrCl_2$, CHI_3 in THF) did not proceed to give a desired vinyl iodide **14** at all. The reactivity of the aldehyde moiety in **13** was probably diminished by the conjugated keto-group. So, Takai olefination was executed before introducing carbonyl group at the allylic position. After reduction of the ester function in **8** and following Swern oxidation, the resulting aldehyde **15** was subjected to Takai olefination to give a vinyl iodide **16** in good yield, without stereoselectivity (*E*/*Z* = 1:1). It was known that the use of 1,4-dioxane as a cosolvent enhances *E*-selectivity in Takai reaction,[6b] but opposite selectivity was observed in this case. Successful introduction of carbonyl group was achieved by oxidation using CrO_3/3,5-dimethylpyrazole[7] leading to enone **14**. Allylic oxidation of **16** using SeO_2 gave a disappointing result (elimination of iodine). Deprotection of the ketal with 80% AcOH afforded a desired core structure fragment **6** (Scheme 2).



Scheme 2. Reagents and conditions: (a) SeO_2, 1,4-dioxane, 56%; (b) DIBAL-H, THF, $(COCl)_2$, DMSO, Et_3N, 82% from **12** to **13**, 87% from **8** to **15**; (c) CHI_3, $CrCl_2$, THF, 0% from **13** to **14**, 76% from **15** to **16**; (d) CrO_3, 3,5-dimethylpyrazole, CH_2Cl_2, 74%; (e) 80% AcOH aq, 77%.

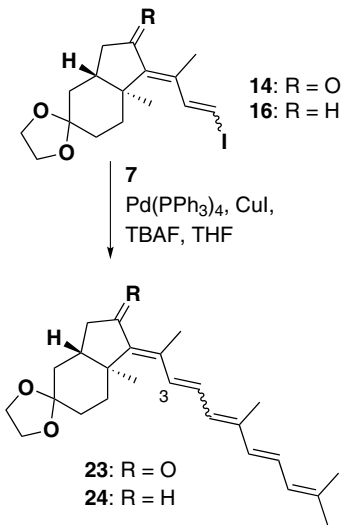


Scheme 3. Reagents and conditions: (a) Ref. 8; (b) TBSCl, Et_3N, THF, 97%; (c) *t*-BuLi, Bu_3SnCl, THF, 77%; (d) TBAF, THF, 92%; (e) $(COCl)_2$, DMSO, Et_3N, quant.; (f) **22**, *n*-BuLi, DMSO, toluene, 82%; (g) $Pd(PPh_3)_4$, CuI, TBAF, THF, 72%.

(*E*,*E*,*E*)-Triene side chain fragment (**7**) was also prepared, as shown in Scheme 3. Protection of the hydroxyl group of *E*-vinyl iodide **17**,[8] prepared from diethyl methylmalonate, provided a TBS ether **18**. Subsequent stannylation gave a *E*-vinylstannane **19** in moderate yield. Deprotection of the TBS group by tetrabutylammonium fluoride (TBAF) and following Swern oxidation of the alcohol **20** afforded an aldehyde **21**. Wittig reaction between **21** and tributylphosphonium salt **22** proceeded with high *E*-selectivity to give a desired side chain fragment (**7**).

Finally, Stille cross-coupling of both fragments **6** and **7** was examined. The reaction was sluggish in the case of using Pd-catalyst alone, while the combinatorial use of $Pd(PPh_3)_4$, CuI, and TBAF worked well to give an objective BC-ring model compound (**5**). The use of both copper (I) salt and fluoride ion was critical in this coupling reaction.[9] Geometric isomers were separated by reversed-phase HPLC (MeOH–H_2O) to isolate all-*trans* isomer. Two more analogues **23** and **24**[10] were also prepared from **14** and **16**, respectively, in the same way as that of **5** (Scheme 4).[11]

The anti-proliferative effect of the synthetic analogues against endothelial cells (HUVECs) and KB3-1 cells was evaluated (Table 1).[12] It revealed that the model compound **5** showed moderate anti-proliferative activity against HUVEC (IC_{50}: 2.6 μg/mL) with 6.5-fold selectivity over KB 3-1 cells (IC_{50}: 17 μg/mL), whereas two other compounds **23** and **24** having ketal moiety showed only a little selectivity. The results imply that further functional manipulation on the core structure would access to a promising anti-angiogenic drug lead.



Scheme 4.

Table 1
Growth inhibitory activity of synthetic model compounds

	IC_{50} (μM)		SI[a]
	HUVEC	KB3-1	
5	2.6	17	6.5
23	13	20	1.5
24[b]	8.8	21	2.3
1[c]	0.09	14	156

[a] SI, selective index. IC_{50} against testing cells/IC_{50} against HUVEC.
[b] *E*/*Z* mixture at C-3 in the side chain.
[c] SI values for **2**, **3**, and **4** were 200, 48, and 25, respectively.

In summary, short-step synthesis of BC-ring model compound of 13*E*,17*E*-globostellatic acid X methyl ester was achieved using stereoselective ynolate olefination and allylic oxidation as key steps. The model compound showed moderate anti-proliferative activity against HUVECs.

Acknowledgments

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10. Compound **24** was obtained and evaluated as a *E*/*Z* mixture (1:1) at C-3 in the side chain, because compound **24**, lacking the carbonyl group conjugated to the side chain, could not be subjected to HPLC separation due to its instability than two other analogues.
11. ^{1}H NMR data of analogues (500 MHz, $CDCl_3$). **5**: δ 7.11 (dd, 1H, J = 11.5, 15.0 Hz), 6.69 (d, 1H, J = 15.0 Hz), 6.61 (dd, 1H, J = 11.5, 15.0 Hz), 6.24 (d, 1H, J = 11.0 Hz), 5.95 (d, 1H, J = 11.0 Hz), 2.62–1.94 (m, 9H), 2.40 (s, 3H), 2.04 (s, 3H), 1.84 (br s, 6H), 1.28 (s, 3H). **23**: δ 7.01 (dd, 1H, J = 11.5, 15.0 Hz), 6.71 (d, 1H, J = 15.0 Hz), 6.55 (dd, 1H, J = 11.5, 15.0 Hz), 6.20 (d, 1H, J = 11.0 Hz), 6.20 (d, 1H, J = 15.0 Hz), 5.92 (d, 1H, J = 11.0 Hz), 3.96–3.91 (m, 4H), 2.32 (s, 3H), 2.19–1.65 (m, 9H), 1.98 (s, 3H), 1.74 (br s, 6H), 1.12 (s, 3H). **24**: δ 6.45 (dd, 1H, J = 11.0, 15.0 Hz), 6.25–6.13 (m, 4H), 5.91 (d, 1H, J = 11.0 Hz), 3.95–3.92 (m, 4H), 2.45–2.05 (m, 2H), 1.94–1.22 (m, 21H), 0.86 (s, 3H).
12. Growth inhibition assay method is as follows: each suspension of HUVECs or KB 3-1 cells in the culture medium (HuMedia-EG2 or RPMI 1640, respectively) with growth supplements was plated into each well of 96-well plate (2×10^3 cells/well/100 μl). After 24 h, test compounds were added, and then the plates were incubated for an additional 72 h in a humidified atmosphere of 5% CO_2 at 37 °C. The cell proliferation was detected by WST-8 colorimetric reagent. The IC_{50} value was determined by linear interpolation from the growth inhibition curve. We assessed selectivity of anti-proliferative activity [selective index (SI)] from the differences of IC_{50} values against HUVECs and KB 3-1 cells.