

Article

Development of an Advanced Synthetic Route to Macrophelides and Its Application to the Discovery of a More Potent Macrophelide Derivative

Yu Mi Heo ¹, Hunseok Lee ², Young Kee Shin ² and Seung-Mann Paek ^{1,*}

¹ College of Pharmacy and Research Institute of Pharmaceutical Sciences, Gyeongsang National University, Jinju daero, Jinju 660-701, Gyeongnam, Korea; E-Mail: ymh@gnu.ac.kr

² College of Pharmacy, Seoul National University, Seoul 151-742, Korea; E-Mails: ryanlee0@snu.ac.kr (H.L.); ykeeshin@snu.ac.kr (Y.K.S.)

* Author to whom correspondence should be addressed; E-Mail: million@gnu.ac.kr; Tel.: +82-55-772-2424; Fax: +82-55-772-2429.

External Editor: Richard A. Bunce

Received: 12 August 2014; in revised form: 22 September 2014 / Accepted: 24 September 2014 / Published: 29 September 2014

Abstract: The discovery of a more cytotoxic macrophelide derivative, including its total synthesis and bioassay are described. Application of the Koide protocol to a readily available propargylic alcohol allowed the rapid and practical synthesis of a macrophelide A skeleton. This strategy enabled the successful improvement of the cytotoxic activity of the macrophelide derivative.

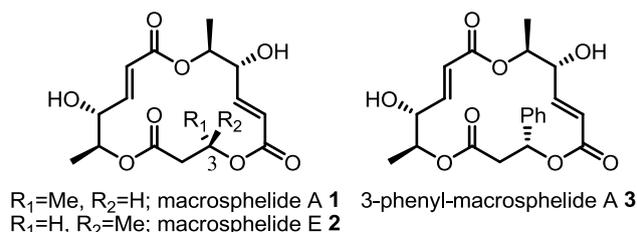
Keywords: macrophelides; natural product; total synthesis

1. Introduction

Natural products have been tapped as an endless supply of resources for new drug development, because of their potency and safety [1,2]. Actually, over the past three decades, approximately 50% of new drugs have been developed from natural products [3,4]. However, some natural products with low potency or high toxicity still need derivatization based on their structure-activity relationships (SAR) and chemical modifications [5].

Since its first isolation in 1995, macrospheptide A (**1**) has been expected to serve as a new lead compound because of its safety, anticancer activity and unique chemical skeleton [6–9]. However, its low potency still hampers further developments. To solve this limitation, a variety of approaches including total synthesis [10–20], medicinal chemistry [21–23], combinatorial chemistry [24], and fluorinated chemistry [25] have been carried out to establish the SAR and discover more promising derivatives of **1**. As a result, it has been possible to improve its various biological activities slightly after extensive efforts, including the confirmation of natural isomer structures and the discovery of potent derivatives such as aza-**1**, ring size modified-**1**, fluorinated-**1** and thiazoline linked-**1**. However, it is noteworthy that C3-modified derivatives of **1** without changing the ring skeleton have not been studied extensively [21,26]. Considering the similar potency of macrospheptide A (**1**) and E (**2**), it can be hypothesized that the C3 substituent can be altered without loss of activity (Figure 1) [27,28]. Moreover, modification of this carbon can be carried out to improve lipophilicity and cell permeability or suppress metabolism, such as by esterase mediated hydrolysis. With this possibility for chemical modification in mind, we sought to modify the C3 position of **1**, because this SAR data can be combined with other SAR information to develop more potent derivatives. For the purpose of this research, a practical synthetic route to the macrospheptide skeleton had to be established. Herein, we would like to report the discovery of 3-phenyl substituted macrospheptide A (**3**), which is much more potent than macrospheptide A **1**.

Figure 1. Structures of macrospheptide A, E and 3-phenylmacrospheptide A.

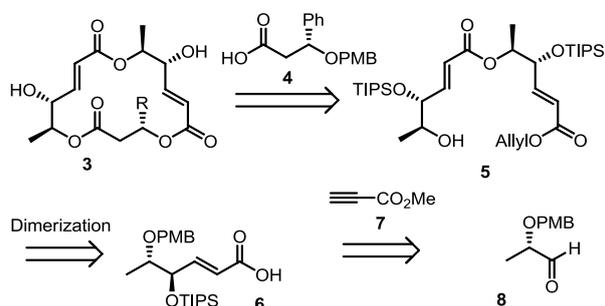


2. Results and Discussion

2.1. Retrosynthesis

The retrosynthesis is outlined in Scheme 1. Considering the convergent synthesis of **3**, it was envisioned that esterification of dimeric alcohol **5** with carboxylic acid **4** would produce a unique macrospheptide skeleton efficiently.

Scheme 1. Retrosynthesis to 3-phenyl-macrospheptides **3**.

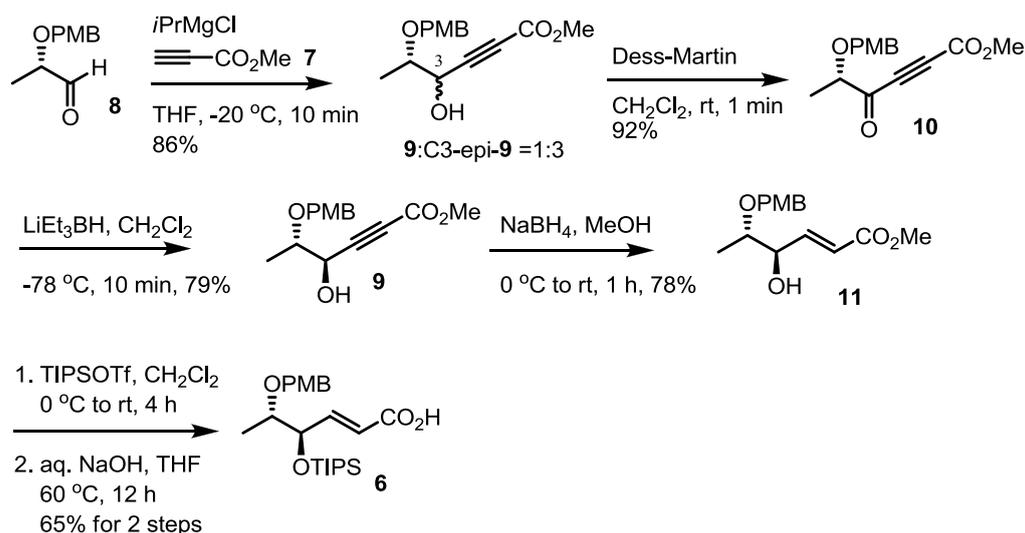


Dimeric alcohol **5** was expected to be transformed via a simple deprotection/esterification sequence of bis-protected unsaturated acid **6**. Finally, employment of the Koide protocol [29] to the PMB-protected aldehyde **8** was designed to yield the key monomeric acid **6** because this strategy can introduce a three-carbon unit directly using commercially available resources.

2.2. Synthesis of Monomer **6** of Macrosphelide *A*

Synthesis of monomer **6** commenced with the three-carbon homologation of the PMB-protected aldehyde **8** by reaction of **7** [30] (Scheme 2). Because use of normal bases such as LiHMDS or NaHMDS produced the desired addition product **9** in low yield, exploration of conditions for this conversion was carried out. After an intensive screening of bases and solvents, *i*PrMgCl was found to produce the corresponding propargylic alcohol **9**. Although the desired diastereomer **9** was produced as a minor isomer, this facial selectivity problem could be solved via an oxidation/stereoselective reduction sequence. Dess-Martin oxidation of the diastereomeric mixture **9**/C3-*epi-9* produced alkynone **10** in good yield. With alkynone **10** in hand, stereoselective reduction of the ketone moiety was executed. Based on the reaction pattern of similar systems [17,31], Super-Hydride was chosen to obtain a chelation-controlled reduction product. Gratifyingly, the desired secondary alcohol **9** was obtained exclusively as the desired isomer, which was subjected to the Koide procedure.

Scheme 2. Synthesis of monomer **6**.

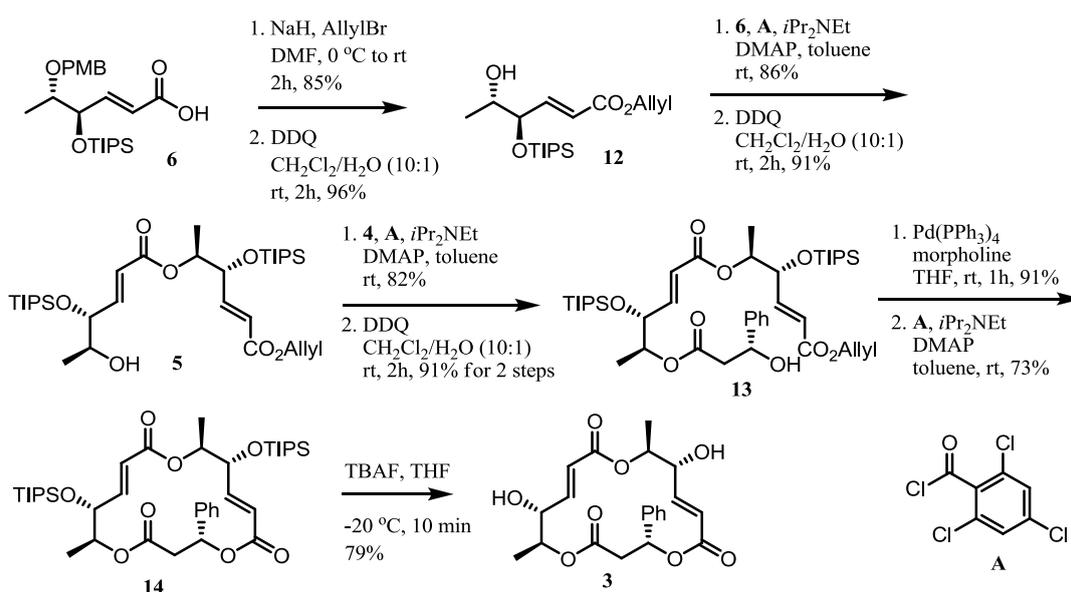


When the propargylic alcohol **9** in methanol was treated with NaBH₄, the desired *trans*-allylic alcohol **11** could be obtained in excellent yield, as was previously reported for a similar skeleton [32]. Moreover, this reaction could be performed at a more convenient temperature without loss of chemical yield. Considering the mildness of the reducing agent and ease of the reaction handling, this stereoselective reduction sequence is ideal for the efficient synthesis of macrosphelides and their derivatives. Finally, TIPS protection followed by hydrolysis of the allylic alcohol **11** produced **6** in excellent yield. Employing this synthetic route, the key building block **6** could be prepared on multi-gram scale.

2.3. Completion of the Synthesis

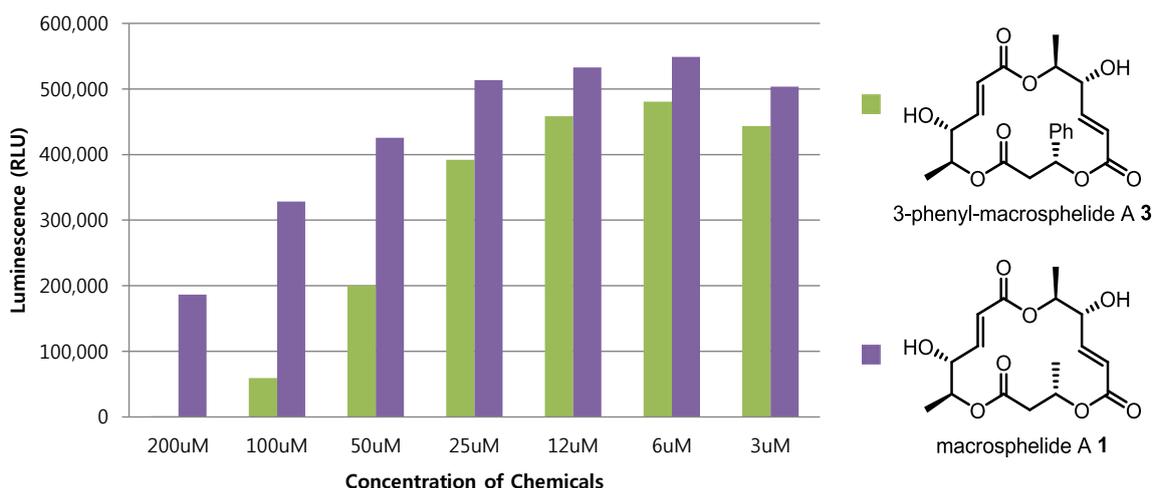
Completion of synthesis was performed by the iterative ligation of three hydroxy acid fragments (Scheme 3). *O*-Allylation and Yonemitsu PMB deprotection [33] of the bis-protected carboxylic acid **6** produced hydroxyester **12**, which was transformed into diester **5** by Yamaguchi esterification and PMB deprotection. Stepwise esterification of the known carboxylic acid **4** [34] with the alcohol **5**, followed by PMB deprotection produced trimeric alcohol **13** in excellent yield. Pd-catalyzed allyl deprotection and Yamaguchi lactonization produced the bis-TIPS protected 3-phenylmacrosphelide **14**. Finally, treatment of **14** with TBAF at a low temperature produced the target molecule **3**.

Scheme 3. Total synthesis of 3-phenylmacrosphelide A (**3**).



2.4. Cytotoxic Activity

The cytotoxic activities of **1** and **3** are shown in Figure 2. Cell viability was determined with the use of a luminescent cell viability assay kit. After 4×10^3 SKOV3 cells were seeded in an opaque-walled 96-well microplate, the cells were treated with **1** and **3** and incubated at 37 °C for 72 h. For equilibration of the microplate, it was incubated for 30 min at room temperature. Thereafter, 100 μ L CellTiter-Glo[®] reagent was added to 100 μ L of medium containing cells and the plate was mixed for 2 min on an orbital shaker to induce cell lysis. Finally, the plate was incubated for 10 min at room temperature and the luminescence was recorded by a GENios reader. From this assay, a remarkable improvement in cytotoxic activity could be observed. When cells were treated with **3** instead of **1**, cytotoxicity in the carcinoma cell line was increased (see Figure 2). This result means that C3 modification of the macrosphelide skeleton could contribute to an improvement of cytotoxic activity, which is strongly required for macrosphelide-related anticancer drug development.

Figure 2. Anticancer activity of **1** and **3**.

3. Experimental Section

3.1. General Information

Unless noted otherwise, all starting materials and reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran and Et₂O were distilled from sodium benzophenone ketyl. Dichloromethane was freshly distilled from calcium hydride. All solvents used for routine isolation of products and chromatography were reagent grade and glass distilled. Air and moisture sensitive reactions were performed under an argon atmosphere. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) with the indicated solvents. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). ¹H-NMR data were reported in the order of chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and/or multiple resonance), number of protons, and coupling constant in hertz (Hz).

(5*S*)-Methyl 4-hydroxy-5-(4-methoxybenzyloxy)hex-2-ynoate (**9**). To a solution of methyl propiolate (540 mg, 6.4 mmol) in THF (10 mL), iPrMgCl (2.0 M in THF, 3.2 mL, 6.4 mmol) was added at –20 °C. After stirring at the same temperature for 30 min, a solution of aldehyde **8** (340 mg, 1.8 mmol) in THF (5 mL) was added at –20 °C. The reaction mixture was stirred for 1 h at the same temperature and quenched with aq. NH₄Cl. The reaction mixture was extracted with EtOAc and the combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:5 to 1:2) to afford 420 mg (86%) of propargylic alcohol **9** as inseparable diastereomers: FT-IR (KBr) ν_{\max} 3424, 2953, 2236, 1715, 1512 cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz, 1:1 mixture of diastereomers) δ 7.30 (d, 2H, *J* = 8.5 Hz), 6.90 (d, 2H, *J* = 8.5 Hz), 4.63 (m, 1H), 4.50 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 3.48 (m, 2H), 3.73 (m, 1H), 1.37 (d, 3H, *J* = 6.5 Hz); LR-MS (ESI+) *m/z* 317 (M+K⁺).

(4*R*,5*S*,*E*)-Methyl 4-hydroxy-5-(4-methoxybenzyloxy)hex-2-enoate (**11**). To a solution of propargylic alcohol **9** (415 mg, 1.5 mmol, mixture of diastereomers) in CH₂Cl₂ (10 mL), Dess-Martin periodinane (760 mg, 1.8 mmol) was added at room temperature. After stirring for 10 min, the reaction mixture was quenched with saturated aq. NaHCO₃, and then extracted with CH₂Cl₂. The combined organic

layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:5 to 1:2) to afford 380 mg (92%) of slightly unstable ketone **10** as a yellow oil: $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 7.21 (d, 2H, $J = 8.5$ Hz), 6.81 (d, 2H, $J = 8.5$ Hz), 4.55 (d, 1H, $J = 10.5$ Hz), 4.36 (dt, 2H, $J = 10.5$ Hz), 3.77 (s, 3H), 3.72 (s, 3H), 1.34 (d, 3H, $J = 6.5$ Hz). To a solution of ketone **10** (280 mg, 1.0 mmol) in CH_2Cl_2 (10 mL), Super-Hydride (1.0 M in THF, 1.1 mL, 1.1 mmol) was added slowly at -78 °C. After stirring for 5 min, the reaction mixture was quenched with saturated aqueous NH_4Cl , and then extracted with CH_2Cl_2 . The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:2) to afford 260 mg (90%) of propargylic alcohol **9** as a colorless oil. A diastereoselectivity ($>10:1$) was confirmed by proton NMR analysis.

To a solution of propargylic alcohol **9** (210 mg, 0.76 mmol) in MeOH (3 mL), NaBH_4 (29 mg, 0.76 mmol) was added at 0 °C. After stirring for 2 h, the reaction mixture was quenched with saturated aq. NH_4Cl , and then extracted with EtOAc. The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:2) to afford 160 mg (75%) of allylic alcohol **11** as a colorless oil; $[\alpha]_{\text{D}}^{20} -18.9$ (c 0.76, CHCl_3); FT-IR (KBr) ν_{max} 2949, 1771, 1720, 1610 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 300 MHz) δ 7.26 (d, 2H, $J = 6.3$ Hz), 6.93–6.88 (m, 3H), 6.15 (dd, 1H, $J = 15.6, 1.8$ Hz), 4.65–4.44 (m, 3H), 3.83 (s, 3H), 3.76 (s, 3H), 3.70 (m, 1H), 2.37 (d, 1H, $J = 4.5$ Hz), 1.15 (d, 3H, $J = 6.5$ Hz); $^{13}\text{C-NMR}$ (CDCl_3 , 75 MHz) δ 166.7, 159.3, 145.9, 129.9, 129.3, 121.4, 113.9, 76.2, 72.6, 70.5, 55.2, 51.5, 14.0.

(4*R*,5*S*,*E*)-5-(4-Methoxybenzyloxy)-4-(triisopropylsilyloxy)hex-2-enoic acid (**6**). To a solution of allylic alcohol **11** (15 mg, 55 μmol) in CH_2Cl_2 (1 mL), *i* Pr_2NEt (14 mg, 110 μmol) and TIPSOTf (20 mg, 66 μmol) were added at 0 °C. After stirring for 5 h, the reaction mixture was quenched with aq. NH_4Cl , and then extracted with EtOAc. The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was used in the next step without further purification.

A solution of the crude mixture from the previous reaction in THF/ H_2O (3 mL/1 mL) was treated with NaOH (10% in H_2O , 0.2 mL) and warmed to 50 °C for 12 h. The reaction mixture was quenched with 1N HCl and then extracted with EtOAc (3 times). The combined organic layers were dried over MgSO_4 , filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:1 to EtOAc only) to afford 15 mg (65% for 2 steps) of carboxylic acid **6** as a colorless oil. $[\alpha]_{\text{D}}^{20} -9.0$ (c 1.0, CHCl_3); FT-IR (KBr) ν_{max} 2942, 2865, 2678, 1698, 1655 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ 7.26 (d, 2H, $J = 6.5$ Hz), 7.11 (dd, 1H, $J = 15.5, 5.5$ Hz), 6.88 (d, 2H, $J = 6.5$ Hz), 6.08 (dd, 1H, $J = 16.0, 1.0$ Hz), 4.55 (d, 2H, $J = 4.0$ Hz), 4.47 (m, 1H), 3.82 (s, 3H), 3.60 (m, 1H), 1.28 (d, 3H, $J = 6.6$ Hz), 1.17 (m, 21H); $^{13}\text{C-NMR}$ (CDCl_3 , 125 MHz) δ 159.5, 151.5, 130.9, 129.6, 114.2, 114.1, 78.4, 76.3, 71.7, 55.6, 18.4 (2C), 16.1, 13.1; LR-MS (ESI) m/z 461 ($\text{M}+\text{K}^+$).

(4*R*,5*S*,*E*)-Allyl 5-hydroxy-4-(triisopropylsilyloxy)hex-2-enoate (**12**). To a solution of carboxylic acid **6** (480 mg, 1.1 mmol) in DMF (5 mL), Cs_2CO_3 (740 mg, 2.3 mmol) and allyl bromide (0.15 mL, 1.7 mmol) were added at 0 °C. After stirring for 10 min at the same temperature, the reaction mixture was quenched with saturated aq. NH_4Cl . The reaction mixture was diluted with EtOAc and washed with H_2O (3 times) and the organic layers was dried over MgSO_4 , filtered and concentrated *in vacuo*.

The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:5, R_f 0.8) to afford 450 mg of allyl with small amount of impurities. To a solution of this ester in CH₂Cl₂/H₂O (20 mL/1 mL), DDQ (260 mg, 1.2 mmol) was added at ambient temperature. After stirring for 30 min, the reaction mixture was quenched with aq. NaHCO₃, and extracted with CH₂Cl₂. The organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:10 to 1:5) to afford 320 mg (81% for 2 steps) of monomeric alcohol **12** as a colorless oil: $[\alpha]_D^{20}$ -12.3 (c 0.8, CHCl₃); FT-IR (KBr) ν_{max} 2942, 2866, 1360, 1819, 1721 cm⁻¹; ¹H-NMR (CDCl₃, 300 MHz) δ 6.96 (dd, 1H, J = 15.6, 6.3 Hz), 6.04 (dd, 1H, J = 15.6, 1.2 Hz), 5.92 (m, 1H), 5.30 (m, 2H), 4.66 (d, 2H, J = 5.7 Hz), 4.35 (m, 1H), 3.92 (m, 1H), 1.13 (d, 3H, J = 6.6 Hz); ¹³C-NMR (CDCl₃, 75 MHz) δ 165.5, 146.4, 132.1, 122.6, 118.1, 76.1, 70.7, 65.1, 17.9, 17.4, 12.3; LR-MS (FAB+) m/z 343 (M+H⁺).

(4*R*,5*S*,*E*)-Allyl 5-((4*R*,5*S*,*E*)-5-hydroxy-4-(triisopropylsilyloxy)hex-2-enoyloxy)-4-(triisopropylsilyloxy)hex-2-enoate (**5**). To a solution of carboxylic acid **6** (410 mg, 0.97 mmol) in toluene (5 mL), *i*Pr₂NEt (0.34 mL, 1.9 mmol) and 2,4,6-trichlorobenzoyl chloride (0.18 mL, 1.2 mmol) were added at 0 °C. After stirring for 1 h, DMAP (240 mg, 1.9 mmol) was added at ambient temperature. After stirring for 10 min, a solution of monomeric alcohol **12** (320 mg, 0.97 mmol) in toluene (5 mL) was added and stirred for 1 h. The reaction mixture was quenched with aq. NH₄Cl and then extracted with EtOAc (3 times). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was filtered by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:5, R_f 0.7) to afford 610 mg (84%) of dimeric ester as a colorless oil.

To a solution of dimeric ester (610 mg, 81 mmol) in CH₂Cl₂/H₂O (30 mL/2 mL), DDQ (260 mg, 1.2 mmol) was added at ambient temperature. After stirring for 30 min and the reaction mixture was quenched with aq. NaHCO₃, and extracted with CH₂Cl₂. The organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:5) to afford 493 mg (97%) of dimeric alcohol **13** as a colorless oil: $[\alpha]_D^{20}$ -14.2 (c 0.76, CHCl₃); FT-IR (KBr) ν_{max} 2942, 2866, 1359, 1723, 1655 cm⁻¹; ¹H-NMR (CDCl₃, 500 MHz) δ 6.89 (m, 2H), 6.00 (dd, 1H, J = 15.3, 1.2 Hz), 5.96 (dd, 1H, J = 15.3, 1.2 Hz), 5.88 (m, 1H), 5.24 (m, 1H), 5.00 (dd, 1H, J = 8.5, 6.5 Hz), 4.58 (d, 2H, J = 3.3 Hz), 4.52 (m, 1H), 4.28 (m, 1H), 3.84 (dd, 1H, J = 6.5, 3.5 Hz), 1.16 (d, 3H, J = 6.6 Hz), 1.04 (d, 3H, J = 6.4 Hz), 0.98 (m, 21H); ¹³C-NMR (CDCl₃, 125 MHz) δ 166.0, 165.8, 147.5, 146.9, 132.5, 123.1, 122.6, 118.4, 76.4, 74.6, 73.6, 71.1, 65.5, 60.7, 18.3, 17.8, 14.5, 12.8, 12.7; LR-MS (ESI) m/z 649 (M+Na⁺).

(4*R*,5*S*,*E*)-Allyl 5-((4*R*,5*S*,*E*)-5-((*R*)-3-hydroxy-3-phenylpropanoyloxy)-4-(triisopropylsilyloxy)hex-2-enoyloxy)-4-(triisopropylsilyloxy)hex-2-enoate (**13**). To a solution of carboxylic acid **5** (130 mg, 0.45 mmol) in toluene (3 mL), *i*Pr₂NEt (0.13 mL, 0.75 mmol) and 2,4,6-trichlorobenzoyl chloride (0.070 mL, 0.45 mmol) were added at 0 °C. After stirring for 1 h, DMAP (91 mg, 0.75 mmol) was added at ambient temperature. After stirring for 10 min, a solution of dimeric alcohol **13** (240 mg, 0.38 mmol) in toluene (2 mL) was added and stirred for 1 h. The reaction mixture was quenched with aq. NH₄Cl and then extracted with EtOAc. The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:1) to afford 278 mg (82%) of trimeric ester as a colorless oil. To a solution of

trimeric ester (75 mg, 84 mmol) in CH₂Cl₂/H₂O (3 mL/0.3 mL), DDQ (21 mg, 0.092 mmol) was added at ambient temperature. After stirring for 30 min and the reaction mixture was quenched with aq. NaHCO₃ and extracted with CH₂Cl₂ (3 times). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:10 to 1:5) to afford 72 mg of trimeric alcohol **16**; [α]_D²⁰ −0.5 (c 0.86, CHCl₃); ¹H-NMR (CDCl₃, 500 MHz) δ 7.36 (m, 5H), 6.97 (dd, 1H, *J* = 15.5, 5.5 Hz), 6.92 (dd, 1H, *J* = 15.5, 5.5 Hz), 6.11 (m, 2H), 5.95 (m, 1H), 5.23 (m, 2H), 5.15 (dd, 1H, *J* = 8.0, 4.5 Hz), 5.00 (m, 2H), 4.68 (m, 2H), 4.63 (m, 1H), 4.58 (m, 1H), 2.75 (m, 2H), 1.45 (d, 3H, *J* = 6.6 Hz), 1.35 (d, 3H, *J* = 6.5 Hz), 1.07 (m, 42H); LR-MS (FAB+) *m/z* 775 (M+H⁺).

(4*R*, 7*E*, 9*R*, 10*S*, 13*E*, 15*R*, 16*S*)-10,16-Dimethyl-4-phenyl-9,15-bis(triisopropylsilyloxy)-1,5,11-trioxacyclohexadeca-7,13-diene-2,6,12-trione (**14**). To a solution of hydroxyl allyl ester **14** (72 mg) in THF (3 mL) were added Pd(PPh₃)₄ (30 mg, 0.028 mmol) and morpholine (0.10 mL, excess) at ambient temperature. The mixture was stirred for 10 min at the same temperature, quenched with 1 N HCl and extracted with EtOAc (3 times). The organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:2 to EtOAc only) to afford 62 mg of crude seco acid as a colorless oil. To a solution of crude acid (62 mg) in toluene (2 mL) were added *i*Pr₂NEt (94 μ L, 0.54 mmol) and 2,4,6-trichlorobenzoyl chloride (40 μ L, 0.27 mmol) at room temperature and the reaction was stirred for 2 h. To this reaction mixture, DMAP (66 mg, 0.54 mmol) was added and warmed to 80 °C. After stirring for 12 h, the mixture was quenched with aq. NH₄Cl and extracted with EtOAc (3 times). The combined organic layers were dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:10) to afford 35 mg (57% for 4 steps) of bis-TIPS protected phenyl macrospheptide **17** as a pale yellow oil.; [α]_D²⁰ −6.7 (c 0.76, CHCl₃); FT-IR (KBr) ν_{\max} 2942, 2866, 2359, 1721 cm^{−1}; ¹H-NMR (CDCl₃, 500 MHz) δ 7.36 (m, 5H), 6.88 (m, 2H), 6.22 (dd, 1H, *J* = 8.5, 2.5 Hz), 5.96 (d, 1H, *J* = 15.5 Hz), 5.93 (d, 1H, *J* = 15.5 Hz), 5.02 (m, 1H), 4.91 (m, 1H), 4.28 (t, 1H, *J* = 7.5 Hz), 4.24 (t, 1H, *J* = 7.5 Hz), 2.89 (dd, 1H, *J* = 15.5, 10.0 Hz), 2.75 (dd, 1H, *J* = 15.5, 2.5 Hz), 1.45 (d, 3H, *J* = 6.6 Hz), 1.35 (d, 3H, *J* = 6.5 Hz), 1.07 (m, 42H); ¹³C-NMR (CDCl₃, 125 MHz) δ 169.5, 164.9, 164.3, 148.5, 147.9, 139.5, 129.0, 128.6, 126.4, 123.0, 122.6, 76.4, 74.9, 74.0, 73.0, 72.4, 42.3, 31.9, 18.3, 18.2, 18.1, 12.9, 12.8; LR-MS (FAB+) *m/z* 717 (M+H⁺).

3-Phenylmacrospheptide **A** (**3**). To a solution of bis-TIPS protected macrospheptide **17** (18 mg, 25 μ mol) in THF (1 mL), TBAF (1.0 M in THF, 75 μ L, 75 μ mol) was added at −20 °C. After stirring for 10 min, the mixture was quenched with aq. NH₄Cl and extracted with EtOAc. The organic layers were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography on silica gel (EtOAc/*n*-hexane = 1:1) to afford 8.0 mg (79%) of phenyl macrospheptide **3** as a white solid: [α]_D²⁰ +34.2 (c 0.21, CHCl₃); ¹H-NMR (CDCl₃, 500 MHz) δ 7.39–7.31 (m, 5H), 7.02 (dd, 1H, *J* = 16.0, 4.0 Hz), 6.95 (dd, 1H, *J* = 16.0, 4.0 Hz), 6.31 (dd, 1H, *J* = 11.5, 2.0 Hz), 6.12 (dd, 1H, *J* = 16.0, 1.5 Hz), 6.10 (dd, 1H, *J* = 16.0, 1.5 Hz), 5.04–4.96 (m, 1H), 4.94–4.89 (m, 1H), 4.28 (m, 1H), 4.18 (m, 1H), 3.03 (dd, 1H, *J* = 16.0, 11.5 Hz), 2.94 (br, 1H), 2.75 (dd, 1H, *J* = 16.0, 2.0 Hz), 2.50 (br, 1H), 1.50 (d, 3H, *J* = 6.5 Hz), 1.41 (d, 3H, *J* = 6.5 Hz); ¹³C-NMR (CDCl₃, 125 MHz) δ 170.1, 166.3, 164.3, 146.6, 145.5, 139.3, 129.1, 128.8, 126.5, 122.7 (2C), 75.9, 75.1, 74.5, 73.6, 72.5, 42.0,

18.5, 18.4; FT-IR (KBr) ν_{\max} 3429, 2923, 2852, 1710, 1627; LR-MS (FAB) m/z 405 (M+H⁺); HR-MS (FAB) calcd for C₂₁H₂₅O₈ 405.1549 (M+H⁺) found 405.1569.

3.2. Cell Culture

The human ovarian carcinoma cell line SKOV3 was obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea) and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1× penicillin/streptomycin at 37 °C in 5% CO₂ atmosphere. Cells were routinely checked for mycoplasma contamination and authenticated using STR DNA technology. These culture check was carried out three times.

3.3. Cell Viability

Cell viability was determined by the use of a CellTiter-Glo[®] luminescent cell viability kit (Promega Corporation, Madison, WI, USA). After 4×10^3 SKOV3 cells were seeded in an opaque-walled 96-well microplate, cells were treated with chemicals and incubated at 37 °C for 72 h. For equilibration of a microplate, it was incubated for 30 min at room temperature (RT). Thereafter, 100 µL of CellTiter-Glo[®] reagent was added to 100 µL of medium containing cells and the plate was mixed for 2 min on an orbital shaker for inducing cell lysis. Finally, the plate was incubated for 10 min at RT to stabilize the luminescent signal and recorded by a GENios reader (Tecan, Männedorf, Switzerland). These tests were carried out three times.

4. Conclusions

The synthesis of a valuable C3-modified macrosphelide derivative was accomplished. The synthetic route includes a direct three-carbon introduction through propiolate addition and a sequential stereoselective reduction process. Employing this efficient synthetic route, biologically enhanced derivatives could be developed and utilized for macrosphelide-related anticancer drug discovery. Full disclosure of more advanced derivatives and the SAR studies of macrosphelides will be reported in due course.

Acknowledgments

This work was also supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, no. PJ0090882014).

Author Contributions

Y.M.H. made 3-phenyl-macrosphelide A and its synthetic intermediates. H.L. and Y.K.S. executed cytotoxic tests. S.M.P. made 3-phenyl-macrosphelide A and wrote this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Koehn, F.E.; Carter, G.T. The evolving role of natural products in drug discovery. *Nat. Rev. Drug Discov.* **2005**, *3*, 206–220.
2. Kong, D.-X.; Jiang, Y.-Y.; Zhang, H.-Y. Marine natural products as sources of novel scaffolds: Achievement and concern. *Drug Discov. Today* **2010**, *15*, 884–886.
3. Newman, D.J.; Cragg, G.M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **2007**, *70*, 461–477.
4. Li, J.W.-H.; Vederas, J.C. Drug discovery and natural products: End of an era or an endless frontier? *Science* **2009**, *325*, 161–165.
5. John, J.E. Natural products as lead-structures: A role for biotechnology. *Drug Discov. Today* **2010**, *15*, 409–410.
6. Hayashi, M.; Kim, Y.-P.; Hiraoka, H.; Natori, M.; Takamatsu, S.; Kawakubo, T.; Masuma, R.; Komiyama, K.; Ōmura, S. Macrophelide, a novel inhibitor of cell-cell adhesion molecule I. Taxonomy, fermentation, isolation and biological activities. *J. Antibiot.* **1995**, *48*, 1435–1439.
7. Ahmed, K.; Zhao, Q.-L.; Matsuya, Y.; Yu, D.-Y.; Feril, L.B.; Nemoto, H.; Kondo, T. Rapid and transient intracellular oxidative stress due to novel macrophelides trigger apoptosis via Fas/caspase-8-dependent pathway in human lymphoma U937 cells. *Chem.-Biol. Interact.* **2007**, *170*, 86–99.
8. Ahmed, K.; Matsuya, Y.; Nemoto, H.; Zaidi, S.F.H.; Sugiyama, T.; Yoshihisa, Y.; Shimizu, T.; Kondo, T. Mechanism of apoptosis induced by a newly synthesized derivative of macrophelides with a thiazole side chain. *Chem.-Biol. Interact.* **2009**, *177*, 218–226.
9. Tomprefa, N.; McQuilken, M.P.; Hill, R.A.; Whipps, J.M. Antimicrobial activity of *Coniothyrium minitans* and its macrolide antibiotic macrophelide A. *J. Appl. Microbiol.* **2009**, *106*, 2048–2056.
10. Sunazuka, T.; Hirose, T.; Harigaya, Y.; Takamatsu, S.; Hayashi, M.; Komiyama, K.; Ōmura, S.; Sprengeler, P.A.; Smith, A.B. Relative and absolute stereochemistries and total synthesis of (+)-macrophelides A and B, potent, orally bioavailable inhibitors of cell-cell adhesion. *J. Am. Chem. Soc.* **1997**, *119*, 10247–10248.
11. Nakamura, H.; Ono, M.; Shida, Y.; Akita, H. New total syntheses of (+)-macrophelides C, F and G. *Tetrahedron: Asymmetry* **2002**, *13*, 705–713.
12. Kobayashi, Y.; Wang, Y.-G. Synthesis of macrophelides H and G. *Tetrahedron Lett.* **2002**, *43*, 4381–4384.
13. Sharma, G.V.M.; Mouli, C.C. The total synthesis of macrophelides A and E from carbohydrate precursors. *Tetrahedron Lett.* **2002**, *43*, 9159–9161.
14. Matsuya, Y.; Kawaguchi, T.; Nemoto, H. New strategy for the total synthesis of macrophelides A and B based on ring-closing metathesis. *Org. Lett.* **2003**, *5*, 2939–2941.
15. Sharma, G.V.M.; Mouli, C.C. A total synthesis of macrophelides C and F from L-(+)-arabinose. *Tetrahedron Lett.* **2003**, *44*, 8161–8163.
16. Kusaka, S.-i.; Dohi, S.; Doi, T.; Takahashi, T. Total synthesis of macrophelide A by way of palladium-catalyzed carbonylative esterification. *Tetrahedron Lett.* **2003**, *44*, 8857–8859.
17. Paek, S.-M.; Seo, S.-Y.; Kim, S.-H.; Jung, J.-W.; Lee, Y.-S.; Jung, J.-K.; Suh, Y.-G. Concise Syntheses of (+)-Macrophelides A and B. *Org. Lett.* **2005**, *7*, 3159–3162.

18. Yun, H.; Paek, S.-M.; Jung, J.-W.; Kim, N.-J.; Kim, S.-H.; Suh, Y.-G. First total syntheses of (–)-macrophelides J and K and elucidation of their absolute configuration. *Chem. Commun.* **2009**, 2463–2465.
19. Prasad, K.R.; Gutala, P. Enantioselective total synthesis of macrophelides A and E. *Tetrahedron* **2011**, *67*, 4514–4520.
20. Sharma, G.V.M.; Reddy, P.S. Total synthesis of macrophelide M from diacetone glucose. *Eur. J. Org. Chem.* **2012**, *12*, 2414–2421.
21. Matsuya, Y.; Kawaguchi, T.; Ishihara, K.; Ahmed, K.; Zhao, Q.-L.; Kondo, T.; Nemoto, H. Synthesis of macrophelides with a thiazole side chain: New antitumor candidates having apoptosis-inducing property. *Org. Lett.* **2006**, *8*, 4609–4612.
22. Wang, B.-L.; Jiang, Z.-X.; You, Z.-W.; Qing, F.-L. Total synthesis of trifluoromethylated analogs of macrophelide A. *Tetrahedron* **2007**, *63*, 12671–12680.
23. Matsuya, Y.; Kobayashi, Y.; Kawaguchi, T.; Hori, A.; Watanabe, Y.; Ishihara, K.; Ahmed, K.; Wei, Z.-L.; Yu, D.-Y.; Zhao, Q.-L.; *et al.* Design, synthesis, and biological evaluation of artificial macrophelides in the search for new apoptosis-inducing agents. *Chem. Eur. J.* **2009**, *15*, 5799–5813.
24. Takahashi, T.; Kusaka, S.; Doi, T.; Sunazuka, T.; Ōmura, S. A combinatorial synthesis of a macrophelide library utilizing a palladium-catalyzed carbonylation on a polymer support. *Angew. Chem. Int. Ed.* **2003**, *42*, 5230–5234.
25. Curran, D.P.; Sinha, M.K.; Zhang, K.; Sabatini, J.J.; Cho, D.-H. Binary fluororous tagging enables the synthesis and separation of a 16-stereoisomer library of macrophelides. *Nat. Chem.* **2012**, *4*, 124–129.
26. Instead of methyl group in C3 position, introduction of a thiazole moiety was published. Matsuya, Y.; Nemoto, H. Artificial macrophelides as a novel apoptosis-inducing compound. *Heterocycles* **2010**, *81*, 57–66.
27. Numata, A.; Iritani, M.; Yamada, T.; Minoura, K.; Matsumura, E.; Yamori, T.; Tsuruo, T. Novel antitumor metabolites produced by a fungal strain from a sea hare. *Tetrahedron Lett.* **1997**, *38*, 8215–8218.
28. Yamada, T.; Iritani, M.; Doi, M.; Minoura, K.; Ito, T.; Numata, A. Absolute stereostructures of cell-adhesion inhibitors, macrophelides C, E–G and I, produced by a *Periconia* species separated from an *Aplysiasea* hare. *J. Chem. Soc. Perkin Trans. 1* **2001**, 3046–3053.
29. Meta, C.T.; Koide, K. Trans-selective conversions of γ -hydroxy- α,β -alkynoic esters to γ -hydroxy- α,β -alkenoic esters. *Org. Lett.* **2004**, *6*, 1785–1787.
30. Carr, K.; Greener, N.A.; Mullah, K.; Somerville, F.M.; Sutherland, J.K. A synthesis of (\pm)-demethoxydaunomycinone. *J. Chem. Soc. Perkin Trans. 1* **1992**, *15*, 1975–1980.
31. Faucher, A.-M.; Brochu, C.; Landry, S.R.; Duchesne, I.; Hantos, S.; Roy, A.; Myles, A.; Legault, C. Chelation-controlled reduction of α - and β -oxygenated ketones with lithium tri-*n*-butylborohydride. *Tetrahedron Lett.* **1998**, *39*, 8425–8428.
32. Formal synthesis of **1** using LiAlH₄ was reported. Srinivasa Rao, K.; Mukkanti, K.; Srinivasa Reddy, D.; Pal, M.; Iqbal, J. A simple procedure for the synthesis of γ -hydroxy- α,β -(*E*)-alkenoic esters: Formal synthesis of (+)-macrophelides A and B. *Tetrahedron Lett.* **2005**, *46*, 2287–2290.

33. Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. Specific removal of o-methoxybenzyl protection by DDQ oxidation. *Tetrahedron Lett.* **1982**, *23*, 885–888.
34. Eh, M.; Schomburg, D.; Schicht, K.; Kalesse, M. An efficient synthesis of radicinin analogues. *Tetrahedron* **1995**, *51*, 8983–8992.

Sample Availability: Samples of the compounds are available from the authors.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).